ASSESMENT OF METHODS FOR THE DIAGNOSIS OF MALIGNANT CATARRHAL FEVER (MCF) AND KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING MCF AT KAPITI PLAINS RANCH

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DEDICATION

I wish to dedicate this thesis to the following:

To my fiancé Pharell Uwahimana.

My beloved children Vanessah Orono and Ethan Geraliah Mugisha.

My dear parents Johnston Damasus Orono and Bennadette Aluoch Omach.

My supervisors Professor Gitao Chege George and Dr. Elizabeth Annie Jessie Cook.

My younger brothers Joshua Papa, Robinson Aboke and Andrew Wanyama.

My dear nephew Lameck Myles Ratura.

My dear aunt Jane and my dear uncle Isaac Papa.

My dearest friends Valentine Uweziyemana, Jemima Uwingabiye, Dana Ineza Nzanira and Dr. Judy Wanjiru Kamau.

My late great grand mother Veronica Amun (1938-1994), late grand fathers Eli Esikuku Papa (1936-2011) and Martin Rabala Omach (1935- 2007).

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LIST OF ABBREVIATIONS

AlHV-1	Alcelaphine herpes virus 1
AlHV-2	Alcelaphine herpes virus 2
B.A.I	Bayesian agreement index
BBSK	Boran breeders society of Kenya
BBS	Boran breeders society
bp	Base pair
BVD	Bovine viral diarrhoea
BVDV	Bovine viral diarrhoea virus
BoHV	Bovine herpes virus
CBAHWs	Community based animal health workers
CBPP	Contagious Bovine Pleuropneumonia
cELISA	Competitive enzyme linked immunorsorbent assay
CF	Correction factor
CI	Confidence interval
CpHV-2	Caprine herpes virus 2
CPE	Cytopathic effect
DIVA	Distinguish between infected and vaccinated animals
DNA	Deoxyribonucleic acid
ECF	East Coast Fever
ELISA	Enzyme linked immunorsorbent assay
Fg	Femtogram
FMD	Foot and Mouth Disease
HRP	Horseradish peroxidase
IgG	Immunoglobulin gamma

IFA	Indirect fluorescent antibody
IPT	Immunoperoxidase test
ILRAD	International Laboratory for Research on Animal Diseases
ILRI	International Livestock Research Institute
IACUC	Institutional Animal Care and Use Committee
KAP	Knowledge, attitudes and practises
LSD	Lumpy skin disease
MCF	Malignant catarrhal fever
MCFV	Malignant catarrhal fever virus
MCFVs	Malignant catarrhal fever viruses
MS	Microsoft
μl	Microlitre
OD	Optical density
OIE	*Office International Des Epizooties*
ORF	Open reading frame
OR	Odds ratio
OvHV-2	Ovine herpes virus 2
PBLs	Peripheral blood leukocytes
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pH	Power of hydrogen ion concentration
RH	Relative humidity
RMNP	Rhodes Matopos National Park
RNA	Ribonucleic acid
PRA	Participatory rural appraisal

SA-MCF	Sheep associated malignant catarrhal fever
sdn	Single tube duplex nested
SSIs	Semi structured interviews
SSQs	Semi structured questionnaires
TCID	Tissue culture infectious dose
TMB	Tetramethylbenzidine
VN	Virus neutralization
WA-MCF	Wildebeest associated malignant catarrhal fever
WTD	White tailed deer

ABSTRACT

Malignant catarrhal fever (MCF) is a sporadic but severe viral disease that affects cattle and is transmitted from wildebeest calves following the wildebeest calving period. Up to now, an effective vaccine or treatment for this disease does not exist hence infected cattle often die causing huge economic losses to both small scale and large scale cattle farmers. The disease has also been associated with human wildlife conflicts and environmental degradation.

To date there has been no diagnostics based research nor qualitative assessment of the disease conducted at the Kapiti Plains Ranch Limited, Kenya. This study was therefore aimed to assess the methods for diagnosis and knowledge, attitudes and practises (KAP) of MCF on this ranch.

Archived samples (n=123) of clinically confirmed cases of wildebeest associated malignant catarrhal fever (WA-MCF) collected from 2014 to 2016 were tested by nested polymerase chain reaction (PCR) and indirect enzyme linked immunorsorbent assay (ELISA) to evaluate the performance in confirming the clinical cases in the laboratory. Evaluation of the performance of the tests was done in three ways. First, the sensitivity (Se) and specificity (Sp) of indirect ELISA was calculated using nested PCR as a gold standard. Second, the level of agreement between the two tests was assessed by the Cohen's kappa statistic. Third, the Bayesian agreement index (B.A.I) tested the agreement between these tests in the absence of a gold standard. A KAP study using semi-structured questionnaires (SSQs) with a mix of closed and open ended questions were issued to 60 staff of the ranch to identify various perceptions of WA-MCF in terms of recognition, clinical signs, seasonality of the disease and action taken during the manifestation of the disease. Also, a comparative approach of WA-MCF to Contagious Bovine Pleuropneumonia (CBPP), East Coast Fever (ECF), Foot and Mouth Disease (FMD) and Lumpy Skin Disease (LSD) using proportional piling was used to investigate the perception of mortality, annual cases and costs of WA-MCF to these diseases.

In addition the KAP study also sought the ways of controlling WA-MCF from the respondents at the ranch.

Of the 123 clinical samples, 62.6% (77/123) were positive by indirect ELISA while 94.3% (116/123) samples were positive by nested PCR. The sensitivity and specificity of the ELISA using nested PCR as a gold standard were 63.8% (95% C.I. 55 -72%) and 57.1% (95% C.I. 25 -85%) respectively. The kappa statistic revealed a value of 0.05 between these tests while the B.A.I. agreement for these assays was better in the positive than in the negative direction with B.A.I. of 76.7% (95% B.C.I. 70-83%) in the positive direction and 15.1% (95% B.C.I. 4-31%) in the negative direction. The KAP study revealed diverse views towards WA-MCF in terms of recognition, clinical signs, seasonality of the disease and action taken during the manifestation of the disease. The disease was revealed to be a very important disease with respect to cattle mortality while being the least important disease with respect to the number of annual cases as the respondents farms are away from wildebeest grazing areas and also in terms of management costs as it lacks an effective vaccine and treatment. A range of methods were suggested for controlling WA-MCF in the case that it could occur in the individual farm of the respondents with the most common being to chase the wildebeest away.

The results of this study suggest that when laboratory diagnosis of WA-MCF is needed, nested PCR performs better in confirming the clinical cases of WA-MCF than indirect ELISA. However the logistics of nested PCR make it an unsuitable tool for diagnosing WA-MCF as it is expensive, needs well trained personnel and specialized equipment. Future research on WA-MCF should focus on the development of cheap penside techniques for rapid diagnosis during outbreaks. The KAP study demonstrated that most respondents were able to recognize WA-MCF, which will assist in the management of the disease as they can report earlier and take action during outbreaks.

Kapiti Plains Ranch experiences considerable economic losses from WA-MCF, which requires a long-term solution for the wildebeest and cattle to coexist on this ranch. Development of a vaccine would be a long term solution as this will reduce cattle deaths and ensure coexistence between the wildebeests and cattle hence reducing human wildlife conflicts. Further work should be conducted in high risk areas to get a better insight of impacts of the disease. This will enable livestock researchers to collaborate with the cattle owners to give them updated information on new control methods for the disease.

CHAPTER 1: INTRODUCTION

1.1. Background Information

Conflicts between livestock and wildlife in pastoral lands can result because of predation, competition for basic resources and spread of infection. This is a common phenomenon in areas that border protected areas where livestock and wildlife often interact without restrictions. Wildebeest associated malignant catarrhal fever (WA-MCF) is a wildlife disease that is spread from wildebeest to cattle briefly after the wildebeest calving period (Bedelian *et al.*, 2007). Alcelaphine herpes virus 1 (AlHV-1) is the causative virus for WA-MCF. The natural hosts for AlHV-1 are the blue wildebeest (*Connochaetes taurinus taurinus*), black wildebeest (*Connochaetes gnou*) and white bearded wildebeest (*Connochaetes taurinus albojubatus*) (Plowright *et al.*, 1960). This disease occurs in sub Saharan Africa and is confined to geographical regions where cattle graze with wildebeest (Wambua *et al.*, 2016). It is therefore a major problem to pastoralists as wildebeest are endemic in sub Saharan Africa (Bedelian *et al.*, 2007) and cattle are grazed on pasture beside wildebeest (OIE, 2013). The disease is also a problem in zoological collections that hold wildebeest (Whitaker *et al.*, 2007).

In the field, diagnosis of MCF is by clinical signs, which include oculo-nasal discharge, fever, corneal opacity, swollen lymph nodes, conjunctivitis and erosive mucosal lesions in the upper respiratory tract (Quinn *et al.*, 2011). Depression (Russell, 2014) and in-appetence (Russell *et al.*, 2009) also characterize MCF clinically. Encrustation of the muzzle results from the profuse mucopurulent nasal discharges. Neurological signs including muscle tremors, incoordination and head pressing are present in some animals (Quinn *et al.*, 2011).

Potential differential diagnoses for MCF include rinderpest, bovine viral diarrhoea (BVD)/mucosal disease, foot and mouth disease (FMD), bluetongue and vesicular stomatitis (OIE, 2013) and infectious bovine tracheitis (Reid and Van Vuuren, 2004).

Whereas diagnosis on clinical and post mortem examination is possible in acute and prolonged cases, the wide range of clinical signs that develop means laboratory investigation is required to establish a definitive diagnosis (Reid and Van Vuuren, 2004). The most definitive diagnostic procedure in dead animals is histopathology (Pretorius *et al*., 2008). Histological findings in MCF affected animals include accumulation of lymphocytes in many organs some of which have vasculitis and necrotic lesions (Russell *et al.,* 2009).

Other diagnostic tests include serology to identify antibodies to AlHV-1 (Pretorius *et al*., 2008) or molecular assays identifying viral DNA (Wambua *et al.,* 2016). Molecular assays are more sensitive than serology and differentiate AlHV-1 and other macaviruses (Wambua *et al.,* 2016). In the absence of histopathological evidence, a positive PCR or serological test would provide support to clinical diagnosis (Russell, 2014).

Even though MCF has many sporadic outbreaks in cattle affecting one or few individuals in a herd sometimes severe epidemics occur affecting up to 40% of a herd and the cause for this is unknown (Russell *et al.,* 2009**)**. Between 95% to 100% of the sick cattle die within four to seven days (Wambua *et al.,* 2016) or up to several weeks after the onset of clinical signs (Russell *et al.,* 2009).

Presently, MCF lacks effective treatment or vaccine and the best approach to its prevention is separating the reservoir hosts (wildebeest and sheep) from susceptible species like cattle where possible (Russell, 2014). Separation excludes Maasai pastoralists in Kenya and Tanzania from crucial grazing sites and therefore MCF remains a very serious concern to

pastoralists in Maasai land (Bedelian *et al.*, 2007). During MCF outbreaks, the process of avoiding calving wildebeests results in additional costs that may worsen the problems of livestock production and cause environmental degradation (Cleaveland *et al.*, 2001).

Despite the impacts resulting from MCF, it is not considered one of the top 20 diseases of livestock in Eastern, Central and Southern Africa (Perry *et al.*, 2002). The World Organization for Animal Health (OIE) no longer classifies WA-MCF as a trans-boundary or notifiable disease despite it being of importance in Kenya where cattle cograze with wildebeest. The lack of information on the economic impact of WA-MCF (Bourn and Blench, 1999) has contributed to it being unrecognized as a significant disease. However, MCF is viewed as the most significant cattle disease by pastoralist communities in sub Saharan Africa with the highest perceived impact on cattle production and livelihoods (Bedelian *et al.*, 2007). The disease has direct impacts on both commercial and smallholder farmers whose economic livelihoods are affected by the death of their cattle resulting in loss of income from sale of milk and beef (Russell *et al.*, 2012; Mlilo *et al.*, 2015; Lankester *et al.*, 2015).

1.2. Hypothesis

- Nested PCR performs better as confirmatory test for diagnosing clinically confirmed cases of WA-MCF compared to indirect ELISA
- There is a good understanding of the epidemiology of MCF by workers at Kapiti Plains Ranch
- There is a good understanding of the control methods for MCF by workers at Kapiti Plains Ranch

1.3. Objectives

1.3.1. Broad Objective

To assess methods for the diagnosis of MCF and the knowledge, attitudes and practices regarding MCF at Kapiti Plains Ranch.

1.3.2. Specific Objectives

1. To assess the laboratory performance of indirect ELISA and nested PCR in confirming clinical cases of WA-MCF.

2. To explore the knowledge and perceptions of WA-MCF of respondents in the study area.

3. To document suggested ways of controlling WA-MCF by respondents in the study area.

1.4. Problem statement

Malignant catarrhal fever (MCF) has very severe consequences yet it still lacks an effective vaccine and treatment (Wambua *et al.*, 2016). Identification of the pathogen in field conditions requires a strong surveillance system and depends on diagnostics that are sensitive and fast, as up to 100% of the sick animals die within two weeks (Cleaveland *et al.*, 2001). Nested PCR and indirect ELISA have not been compared previously with respect to the diagnosis of WA-MCF. The complications of pathogenesis and epidemiology presents significant challenges in the diagnosis of malignant catarrhal fever viruses (MCFV) infection and/or disease (Li *et al.*, 2011). Nevertheless molecular diagnostic assays that have recently been developed have improved the recognition and distinction of MCFVs, thereby increasing precision of laboratory assays in confirming MCFV infection and/or disease for several species (Li *et al.*, 2011). The knowledge, attitudes and practices of WA-MCF have not been explored at the Kapiti Plains Ranch where wildebeests and cattle co grazing facilitating efficient spread of WA-MCF from wildebeests to cattle.

1.5. Justification

In Kenya, WA-MCF has a dramatic effect on pastoralists who lose up to 10% of their herds annually through cattle deaths and emergency sale of the affected cattle at half the value of the healthy cattle (Cleaveland *et al.*, 2001; Bedelian *et al.*, 2007). The disease directly affects commercial and smallholder farmers whose cattle die leading to a reduction of profits from selling milk and beef compromising the farmers economically and socioculturally (Bedelian *et al.*, 2007; Mlilo *et al.*, 2015). The losses attributed to WA-MCF have been partially quantified as travel and other management costs and livestock productivity losses (Lankester, *et al.*, 2015).

The currently available laboratory tests have not been evaluated before. This study explores how these tests perform in confirming the clinical cases of WA-MCF from archived samples. The diagnostic methods evaluated in this study will be a guiding tool for better diagnosis of the disease and consequently assist the management in Kapiti Plains Ranch in making decisions with regard to the management of affected stock.

This study also aims to explore and document the perceptions of WA-MCF by the workers in Kapiti Plains Ranch, as no such study has been carried out in the area before. The findings will aid in documenting the understanding of WA-MCF by the workers and their approach to the disease during outbreaks. The KAP study also aims to identify the ways of controlling WA-MCF and improve on control strategies for this disease. This study aims to collate the knowledge of all the related stake holders in combining efforts to manage the disease at Kapiti Plains Ranch.

CHAPTER 2: LITERATURE REVIEW

2.1. Malignant catarrhal fever

Malignant catarrhal fever (MCF) is a sporadic but frequently fatal viral illness of cows and a range of other ruminants comprising antelope (*Antilocarpa americana*), bison (*Bison bison*), captive and farmed species of deer (*Odocoileus virgianus*), water buffalo (*Bubalis bubalis*) and rarely domestic pigs (Reid and Van Vuuren, 2004). The disease is caused by either alcelaphine herpesvirus 1 (AlHV-1) or ovine herpesvirus 2 (OvHV-2) (Pretorius *et al*., 2008). The natural hosts for AlHV-1 are the blue wildebeest (*Connochaetes taurinus taurinus*), black wildebeest (*Connochaetes gnou*) and white bearded wildebeest (*Connochaetes taurinus albojubatus*) (Plowright *et al*., 1960). Domestic sheep are the natural hosts of OvHV-2 (Pretorius *et al*., 2008). These viruses do not cause clinical disease in their carrier species (wildebeest and sheep) (Reid and Van Vuuren, 2004; Pretorius *et al*., 2008; Li *et al*., 2014). However, MCF can develop when the viruses are transmitted to clinically susceptible species like cattle, bison, farmed deer, antelope, water buffalo to which they are poorly adapted (Plowright, 1990).

There are two forms of MCF with respect to geographical distributions, WA-MCF and sheep associated MCF (SA-MCF) (Russell *et al.,* 2009). WA-MCF results when cattle get infected with AlHV-1 while SA-MCF occurs following infection of cattle with OvHV-2 (Wambua *et al*., 2016). WA-MCF majorly occurs in Eastern, Central and Southern Africa while SA-MCF occurs worldwide as long as cattle and other vulnerable species are kept together with sheep (Russell *et al.,* 2009).

2.2. Aetiology of MCF

At least ten members of the malignant catarrhal fever viruses (MCFV) group are known, of which six are pathogenic in natural conditions (Li *et al.*, 2005). Alcelaphine herpesvirus 1 (AlHV-1) and ovine herpesvirus 2 (OvHV-2) are the major causative agents of MCF (Li *et*

al., 2011). AlHV-1, ibex-MCFV, OvHV-2, caprine herpesvirus 2 (CpHV-2), MCFV-white-tailed deer (WTD) and alcelaphine herpesvirus 2 (AlHV-2) comprise the six pathogenic viruses (Li *et al.*, 2014). The other four viruses by roan antelope (Reid and Bridgen, 1991), oryx, muskox and Barbary sheep (Li *et al.*, 2003) have not yet been linked with MCF (Li *et al.*, 2011). AlHV-1 has been finely characterized and is the only MCF causing virus that can be propagated *in vitro* (Russell, 2014).

2.3. AlHV-1 virus

2.3.1. Classification

AlHV-1 belongs to genus *Macavirus* of the order *Herpesvirales*, family *Herpesviridae* (Davison, 2010), in the subfamily *Gammaherpesvirinae* (Davison *et al.*, 2009). As a typical herpesvirus, AlHV-1 has an icosahedral capsid symmetry with a fairly huge double stranded, linear DNA genome of one hundred to two hundred genes (Sood *et al.*, 2013). Herpesviruses mainly infect lymphocytes (Flach *et al.*, 2002).

2.3.2. Properties of AlHV-1 virus

2.3.2.1. Physical and chemical properties

AlHV-1 is deactivated by detergents and lipid solvents and is also quite stable in culture media (Plowright, 1990). Ultraviolet radiation may lead to the rapid inactivation of AlHV-1. Virus from young wildebeest that remains unexposed to direct sunlight can persist for several days and may explain the transmission of AlHV-1 to cattle in the absence of close contact with wildebeest (Reid and Buxton, 1989).

2.3.3. Host range and susceptibility of AlHV-1

The black wildebeest (*Connochaetes gnou*), blue wildebeest (*Connochaetes taurinus taurinus*), and white bearded wildebeest (*Connochaetes taurinus albojubatus*) are the

reservoir hosts of AlHV-1 (Plowright *et al.*, 1960). These wildebeests carry the virus as an asymptomatic infection for their entire life (Li *et al.*, 2014).

The number of cases reported in zoological collections reveal the most vulnerable species to AlHV-1 to be Asiatic wild cattle, *Bos javanicus* (the banteng) and *Bos gaurus* (the Indian gaur) (Plowright, 1986). *Bos indicus* (eg Boran) and *Bos taurus* (eg Friesian) cattle are fairly resistant and experience an erratic disease, and may have a longer course of disease and show more obvious clinical signs, while the more vulnerable species like bison and water buffalo have less dramatic clinical signs as they have a shorter course of disease (Russell, 2014). MCF affected animals are dead end carriers as they do not pass infective virus to healthy individuals and this has the beneficial effect of reducing the spread of the virus in managing outbreaks (Russell *et al.,* 2009).

Even though susceptibility to MCF is shown by all age classes, a higher incidence has been shown by adults especially periparturient females but the high incidence in these females is not an obvious indication of a higher vulnerability but could also be a result of other unknown factors of potential significance in the epidemiology of WA-MCF (Barnard *et al.*, 1989). Calves show low incidence owing to the fact that most times they are kept in bomas and thus have less interaction with wildebeest (Cleaveland *et al.*, 2001)..

2.4. Epidemiology and transmission of WA-MCF

The epidemiology of MCF is relatively well defined for AlHV-1 (Li *et al.*, 2014). Generally sufficient spread through infected secretions of a reservoir host to a clinically susceptible host is favoured by neighbouring interaction and a cool, humid surrounding (Plowright, 1990). The timing and extent of virus secretion are also essential factors (Russell, 2014).

WA-MCF is usually a periodic illness in cattle and occurs in sub Saharan Africa during the wildebeest calving season due to the spreading of AlHV-1 from young wildebeest calves aged

about four months (Mushi *et al.*, 1981). The breeding season of wildebeest offers the greatest transmission of WA-MCF and the season is marked by relocation of many wildebeests to their favorite calving sites in savannah plains, thereby encountering herds of cattle feeding in the lush grasslands where the wildebeests transform these sites into crucial spots for the spread of WA-MCF (Wambua *et al.*, 2016). Clinically vulnerable species like cattle get the virus through consumption of virus-laden secretions, inhalation or probably through eating of contaminated foodstuff or water (Li *et al.*, 2014).

AlHV-1 occurs as a lifetime asymptomatic infection in all species of wildebeest (Li *et al.*, 2014). The spread of this virus in free living populations of wildebeest is very effective as newborn calves get infected during the first months of life (Mushi *et al.*, 1981). Newborn calves of wildebeest obtain the virus either perinatally through mingling with other sick calves that shed large quantities of the cell free virus in their oculo-nasal secretions (Lankester *et al.*, 2015) or prenatally by congenital transmission in the uterus by sick dams (Mushi and Rurangirwa, 1981). Infection of most calves appears to be perinatally from infected cohorts but the percentage of prenatal transmission has not yet been established (Li *et al.*, 2014). Wildebeest calves receive colostral antibody either *in utero* or during the first few months of life when the maternal antibody is present in colostrum (Reid and Van Vuuren, 2004). After infection, wildebeest calves develop neutralizing antibodies to the AlHV-1 thereby remaining latently infected throughout life (Hamblin and Hedger, 1984). Adult wildebeest may experience recrudescence of the virus when their immunological system is affected by factors like pregnancy and stressful conditions like food shortage or confinement thereby becoming a greater risk to cattle (Barnard *et al.*, 1989).

Close interaction between cattle and wildebeest calves that secrete the virus supports the spread of AlHV-1 (Mushi *et al.*, 1980). Wildebeest snort regularly generating aerosols thus

their close interaction with cattle favours droplet infection of AlHV-1 via the respiratory route (Whitaker *et al.*, 2007). Infected cattle are terminal dead end hosts since there is no reported proof of horizontal transmission from sick to healthy cattle (Wambua *et al.*, 2016). Like wildebeest, cattle also have vertical transmission of the virus whereby the infected dam passes on the virus via the placenta to her unborn foetus during gestation (Reid and Van Vuuren, 2004).

It has been proposed by Mushi *et al.* (1980) that cattle get AlHV-1 through interaction with placenta and fetuses and from birthing wildebeests and Lankester *et al.* (2015) in Tanzania showed AlHV-1 viral DNA in 50% of placentae from birthing wildebeest. Contact between cattle and foetal or placental material of calving wildebeests should be seen as a possible way by which cattle get the infection from wildebeests hence the actual presence of these materials in rangelands should be visual pointers of newly born calves of wildebeests which are a source of infection to cattle (Wambua *et al.*, 2016). Also wildebeest hair that is shed by wildebeest calves aged 3 to 4 months is indicative of the place the wildebeest have been lying down and hence more virus may be present (Reid and Van Vuuren, 2004).

To date, there exists no fundamental proof of an involvement of an intermediary invertebrate or vertebrate carrier in transmission of AlHV-1 to cattle from wildebeests. WA-MCF is limited to wildebeest areas that are mainly open grassland plains, which are the preferred pastures for wildebeests, livestock and other ungulates (Wambua *et al.*, 2016). Both cattle and wildebeest are herbivores showing more than 70% similarity in food niche (Ego *et al.*, 2003) which in turn contributes to close inter-species interfaces, promoting spread of the disease (Wambua *et al.*, 2016).

2.4.1. Occurrence of WA-MCF in Kenya

The south western region of Kenya forms the location of the three major wildebeest areas (Wambua *et al.*, 2016) which are Athi-Kaputiei, Maasai Mara and Amboseli-Kilimanjaro ecosystems (Talbot and Talbot, 1963; Western, 1982; Reid *et al.*, 2008). WA-MCF has two major outbreaks annually in Kenya. The first cases start appearing in January with peak being in March and April (Wambua *et al.*, 2016). A second outbreak when the calves of wildebeests are eight to ten months, occurs between September and November (Wambua *et al.*, 2016). However peak transmission of WA-MCF in Kenya occurs between the months of March and June **(**Cleaveland *et al.*, 2001).

2.5. The incidence of WA-MCF in Kenya

Highest incidences occur in zones where there is close association between cattle populations and wildebeest and has been positively correlated with the number of wildebeest (Cleaveland *et al.*, 2001; Bedelian *et al.*, 2007). In Maasai pastoral areas the yearly incidence of WA-MCF in Kenya is reported to vary from 1%-21%, other reports estimate the incidence to be 3%-12% (Bedelian *et al.*, 2007). The true incidence is higher than current approximations because of extensive underreporting and misdiagnoses. Other factors that contribute to the variation of the incidence include yearly rainfall amounts, closeness of cattle herds to wildebeest breeding grounds, number of wildebeest and host genetics (Wambua *et al.*, 2016).

2.6. Diagnosis of MCF

2.6.1. Clinical diagnosis

MCF has a variety of clinical signs (Russell *et al.*, 2009). The clinical changes of MCF caused by both AlHV-1 and OvHV-2 are similar in all susceptible animals and they cannot be distinguished readily (Reid and Van Vuuren, 2004). There are four reported forms of MCF which are peracute, alimentary, head and eye, and neurological (Russell *et al.*, 2009). The

length of the incubation period is difficult to know as the time of infection cannot be identified though there is substantive proof that it can range from two weeks to nine months (Reid and Van Vuuren, 2004).

All forms of MCF have initial signs being generally linked with fever (rectal temperature above 39°C), photophobia, inappetence, serous nasal exudate and lacrimation with the latter two changing to profuse mucopurulent discharges (Reid and Van Vuuren, 2004). The peracute form is shown by sudden depression and pyrexia, accompanied with diarrhoea which maybe bloody and death occurs within twelve to twenty four hours (OIE, 2013). The alimentary form of MCF is depicted by bloody diarrhoea (Quinn *et al.,* 2011).

Characteristically the head and eye form is depicted by ocular nasal discharges, corneal opacity, lesions of the skin on the muzzle and destruction of the gums, palate and tongue (Figure 1) (Russell, 2014). The ocular discharge may matt the facial hair while the nasal exudate could block the nares resulting in heavy breathing (Reid and Van Vuuren, 2004). There is also a reduction of milk yields from lactating cows (Bedelian et al., 2007). Bilateral corneal opacity often develops from the periphery to involve the whole cornea which may become oedematous accompanied by blindness (Figure 2) (Reid and Van Vuuren, 2004). Other early signs of MCF are swollen lymph nodes (Quinn *et al.,* 2011), salivation and hyperaemia of oral epithelium (Reid and Van Vuuren, 2004).

Figure 1: Classic head-and-eye signs of MCF with corneal opacity and mucopurulent nasal discharge (Russell et al., 2009).

Figure 2: Keratinitis in an animal affected by MCF (Whitaker et al., 2007).

Nervous signs depict the neurological form and include incoordination, nystagmus, hyperaesthesia and head pressing (OIE, 2013) and circling (Li *et al.,* 2014). Some animals get very aggressive (Reid and Van Vuuren, 2004).

2.6.2. Gross pathological findings

Gross pathological are usually extensive and involving many organ systems and are shown by erosions and haemorrhages that occur all over the gastrointestinal tract and in most severe cases can be linked to intestinal haemorrhage (OIE, 2013). Generally, lymph nodes swell, though the degree of lymph node association differs from one animal to another (OIE, 2013). The respiratory tract is often seen to have catarrhal buildups, erosions and the development of a diphtheritic film while the urinary tract frequently shows typical echymotic haemorrhages of the epithelial coating of the bladder (OIE, 2013).

2.6.3. Differential diagnosis

Rinderpest, BVD/mucosal disease, FMD, bluetongue and vesicular stomatitis (OIE, 2013) and infectious bovine tracheitis (Reid and Van Vuuren, 2004) are possible differential diagnoses.

2.6.4. Laboratory diagnosis

Many laboratory tests to identify specific antibody for AlHV-1 have depended on the attenuated isolate (WC11) which has undergone numerous passages in the laboratory as a source of viral DNA and antigen (Plowright *et al.*, 1960). Both frozen and fresh tissues can be used to acquire DNA for a definitive laboratory test for MCF (Li *et al.*, 2014).

2.6.4.1. Virus isolation

The limitations of virus isolation as a diagnostic technique is that it requires tissue culture laboratories, it takes several days to produce visible results, it is expensive and can only detect one sample at a given time. MCF viruses are not diagnosed quickly by conventional virus isolation procedures (Wambua *et al.*, 2016). Since AlHV-1 is strictly cell associated its isolation is only possible in cell suspensions of lymph nodes, peripheral blood leukocytes (PBLs) or other infected tissues (OIE, 2013). Since the virus can only be obtained from viable

cells, tissue samples for virus isolation must be obtained either before or briefly after the death of the animal and these tissues must be cooled in the laboratory until the time for processing since the virus cannot be obtained from frozen tissues (Reid and Van Vuuren, 2004).

2.6.4.2. Molecular diagnosis

Since PCR became a significant tool in MCF molecular diagnostics, several DNA based assays have been developed over the years (Li *et al.,* 2011). Naturally infected tissues have very little viral DNA that can be detected thus the viral genome has to be amplified by PCR or conventional culture (OIE, 2013). The publishing of the complete order of the C500 isolate of AlHV-1 has allowed the designing of primers from preserved parts of the genome for PCR reactions (Ensser *et al.*, 1997). The strength of PCR in detecting clinical MCF cases may reflect the pathology of MCF with infected cells located in blood as well as penetrating the lymphoid and nonlymphoid organs, enabling DNA of the virus to be found in virtually every tissue (Russell, 2014).

2.6.4.2.1. PCR by Hsu *et al.*, (1990)

This was the first PCR to be described for identifying AlHV-1 DNA. It used primer A situated at 1549 to 1578, GTACACGTCCTTATATCTGTTATAAGTAGT and primer B at 2547 to 2576, GATCTGTTAAGAAGGACTCTGAAATGCAGC which specify a DNA portion of 1,028 bp (Hsu *et al.*, 1990).

2.6.4.2.2. PCR by Katz *et al.*, (1991)

This nested PCR was established based on a piece of AlHV-1 DNA sub cloned to pUC 18 and sequenced (Katz *et al.*, 1991). The amplified AlHV-1 DNA products are 487bp and 172bp respectively and the identity of the amplified AlHV-1 DNA were unique Pvu II and Stu I restriction endonuclease cleavage regions (Katz *et al.*, 1991). This PCR detects very little 0.01

TCID$_{50}$ of AlHV-1 and may significantly boost the virologic diagnosis of clinically evident, persistent subclinical and latent AlHV-1 infections (Katz *et al.*, 1991).

2.6.4.2.3. Optimized PCR by Tham *et al.*, (1994)

This PCR used 20-base pair of primers created from the already available nucleotide order of gene A of WC11 isolate of AlHV-1. These primers amplified a fragment of DNA of 413 bp rendering this PCR to be highly sensitive by identifying 10 Femtogram (fg) of DNA.

2.6.4.2.4. Hemi nested PCR by Flach et al., (2002)

This PCR has been used to differentiate DNA of AlHV-1 from OvHV-2 and PCR amplifications used the non-specific herpesvirus primers POL1 and POL2 (Table 1) that target a section of the DNA polymerase gene that is preserved in both of these viruses amplifying a 386bp region (Flach *et al.*, 2002). The first amplification product size is 386 bp and the final amplification product is 172 bp (Flach *et al.*, 2002). AHVPol and OHVPol and are precise primers for AlHV-1 and OvHV-2 respectively which amplify 172bp products (OIE, 2013)

Table 1. Primers used in the hemi nested PCR

Primers	Length	Sequence*
Primer POL 1	24-mer	5'-GGC (CT)CA (CT)AA (CT)CT ATG CTA CTC CAC-3'
Primer POL 2	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, thus the oligonucleotide will have either of the two indicated bases at these positions (OIE, 2013).

2.6.4.2.5 Hybridization probe real time PCR by Traul et al., (2005)

This PCR was developed to improve the existing diagnostic tests for AlHV-1 and giving quantitative data of regarding the AlHV-1 biology in wildebeest (Traul *et al.*, 2005). It targets the AlHV-1 gene that codes for a tegument protein ORF 3 (Table 2) and can identify ten replicas of the target DNA (Traul *et al.*, 2005). Shedding patterns in wildebeest can be identified using this PCR thus enabling the development of control measures to limit the spread of the AlHV-1 virus since it is a fast and reliable method for detection of MCF viral DNA in clinical samples (Traul *et al.*, 2005). This PCR is valuable for studies that explain the shedding patterns and epidemiology of AlHV-1 in wildebeest and other animals with WA-MCF, however it less sensitive than nested PCR (Traul *et al.*, 2005).

Table 2. AlHV-1 primers and probes used in the real-time assay

Oligonucleotide	Sequence (5'-3')	Product
Forward primer (AlHVI-F)	GGGCTAATTTGTGCAGTTTGTGA	
Reverse primer (AlHVI-R)	AGGTGTTCTGAAAAGAGGGGAA	111
FAM probe (FAMAlHV1)	6FAMACAGGCTCCTCGTCCTCGTCGTGTCGTGT TAMRA[b]	

2.6.4.2.6. Multiplex real time PCR by Cunha et al., (2009)

This PCR was developed using one pair of primers and fluorescent probes precise for the five viruses AlHV-1, OvHV-2, CpHV-2, MCFV-WTD and MCFV-ibex and it uses only one reaction (Cunha *et al.*, 2009). The PCR targets a polymorphic section of the viral DNA polymerase gene having distinctive sequences for each virus (Table 3), which were used as probe markers bordered by preserved regions (Cunha *et al.*, 2009). The manifestation of the

bordering preserved sequences enabled one pair of primers to amplify the 80-bp piece from the DNA polymerase gene of the five viruses (Cunha *et al.*, 2009). This denoted a great advantage to optimization of the assay since primer interaction was reduced and it is fast, dependable and can differentiate five pathogenic MCFVs in clinical samples (Cunha *et al.*, 2009). It is convenient for the identification of the virus triggering clinical MCF in animals from game farms and zoos with mixed-species setups where the exact virus needs to be identified quickly and control strategies set up (Cunha *et al.*, 2009). Remarkably, this PCR is very flexible as it can be multiplexed i.e., the probes in the reaction mix may be adapted according to the interests of different laboratories or the thermocycler in use (Cunha *et al.*, 2009).

Table 3. Primers and probes used for the multiplex real-time PCR

Primer or probe	5'-3' sequence and label(s)
dpol771-F primer	CACACCCAACTGGAGTATGAC
dpol831-R primer	ATGTTGTAGTGGGGCCAGTC
OvHV-2 probe	FAM-ATGTGCGCTTCGACCTC-BHQ1
CpHV-2 probe	HEX-AGTTCCATTCTGAGCGGGT-BHQ1
MCFV-WTD probe	Texas Red- ACTTTAACCCCAACCGTCT-BHQ2
AlHV-1 probe	Cy5-TCGGTGGGTGACATTCAATA-BHQ2
MCFV-ibex probe	Cy5-CGTGCAGTTCCACCCCGAG-BHQ2
IPC-probe	Tye705-GACCGCCATCGCTCCAC-BHQ2

FAM, 6-carboxyfluorescein; BHQ, black hole quencher; HEX, hexachlorofluorescein

2.6.4.2.7. Single-tube duplex nested (sdn) PCR by Bremer et al., (2005)

This PCR was set to identify and distinguish AlHV-1 and OvHV-2. The first step used two primers with high annealing temperatures to target preserved parts of the tegument genes

while the second step used twofold primer sets found on variable areas of AlHV-1 and OvHV-2 genes with annealing temperatures 11°C lower than the first step (Table 4). The second step amplifies the inner areas with different sized amplicons and avoids two distinct steps in identification of both viruses hence reducing time, labour and cost (Bremer *et al.*, 2005).

Table 4. Primer sequences used in the single tube duplex nested PCR

Primer	Sequence	Nucleotide position	Primer length	T_m (°C)	Use	Amplicon size (bp)
MF	AAAAACAG(C/T) AGGCTCCAGGGGAGG	433-410 121325-121348	24 24	60	PCR 1	307
MR	CTCC(G/T)TGCTGGGTCCA GGGCAC	127-149 121631-121609	23 23	63	PCR 1	
OVF	TAAGTTGTTTATACTCAG G	406-388	19	42	PCR 2	180
OVR	ACGGTCAGTCCAAGAC	227-242	16	46	PCR 2	
WBN1	CGTACCCACTGGGTAAG	121361-121377	17	49	PCR 2	241
WR	GGCCTATCCTATAAGAC	121601-121585	17	44	PCR	

2.6.4.2.8. Nested PCR by Li et al., (2000)

This AlHV-1 specific PCR used primers that amplify part of the AlHV-1 gene that codes for the trans activator protein (ORF 50) (Ensser *et al.*, 1997). C500-1 and C500-2 are the primary amplification primers while C500-3 and C500-4 (Table 5) are the secondary amplification primers (Li *et al.*, 2000). The first and second amplification products are 405 bp and 274 bp respectively (Traul *et al.*, 2005). The nested PCR is highly sensitive over non-nested standard or real-time PCR tests (Russell, 2014). Also Traul *et al.* (2005) confirmed this PCR to be more effective than the hybridization probe real time PCR (Traul *et al.*, 2005).

Table 5. Primers, sequence and nucleotide position of the nested PCR

PCR and primer	Sequence (5'-3')	Nucleotide position
AHV-1		
C500-1	TACGGGAGCCCTGACATTTCATCTCTTTTG	73917-74014
C500-2	ATAACTGGTTGATGTGGCAGATGCATCTAT	74292-74321
C500-3	TCTGGCCCGTGCTGCAGCAAGACTCTCAG	73986-74014
C500-4	TATAGTAGAATCCCGTCTGAGTGGTAGCTG	74230-74259

2.6.4.2.9. SYBR Green real-time PCR by Pretorius *et al.*, (2008)

This PCR was developed and assessed for the precise identification of AlHV-1 (Pretorius *et al.*, 2008). This PCR was developed using earlier described AlHV-1-specific nested primers C500-3 and C500-4 (Li *et al.*, 2000). The assay could consistently detect dilutions of low concentrations of DNA equivalent to two hundred and fifty copies of the target DNA thus it proved to be specific in detecting AlHV-1 DNA (Pretorius *et al.*, 2008). This PCR was highly sensitive over the real time PCR developed by (Traul *et al.*, 2005).

2.6.4.3. Serological assays

Many serological tests have been advanced for identifying antibodies against MCFVs and all these tests use alcelaphine herpesviruses as antigens as it can be propagated *in vitro*. These tests can be placed into three classes namely: monoclonal antibody based tests i.e. competitive ELISA (cELISA); polyclonal antibody i.e. indirect fluorescent antibody (IFA) test, immuno-peroxidase test (IPT) and indirect ELISA (iELISA); and neutralizing antibody i.e. virus neutralization (VN) (Li *et al.*, 2011).

Serological results must always be interpreted with the pathological features of MCF in mind (Li *et al.*, 2014). Highly susceptible species, may fail to develop measurable levels of antibody before death (Li *et al.*, 2006). Similarly, a substantial proportion of cattle, bison and other clinically susceptible species can be sub clinically infected (Powers *et al.*, 2005; O'Toole *et al.*, 2002). Thus, a positive serological result signifies infection, and when combined with pathological proof are indicative of the disease (Li *et al.*, 2014).

2.6.4.3.1. Polyclonal assays

Polyclonal antibody based tests recognize antibodies for many epitopes of AlHV-1 (Li *et al.*, 2011).

2.6.4.3.1.1. Indirect ELISA (WC-11 ELISA)

This ELISA was established at the Moredun Research Institute using the WC-11 AlHV-1 virus. MCFV antibodies in sera are captured using crude lysate antigen from WC-11 AlHV-1. Antibodies are detected using a horseradish peroxidase-labeled rabbit-anti-bovine IgG conjugate.

The WC-11 ELISA results were compared to the commercially available cELISA (described in 2.7.4.3.2) as a gold standard and the sensitivity and specificity were reported to be 100%

and 91% respectively (Fraser *et al.*, 2006). The WC-11 ELISA detected more positive samples from clinically affected animals than the cELISA suggesting it may be a more sensitive test, since it depends on a polyclonal reaction and as such detects animals with inadequate or little antibody production (Fraser *et al.*, 2006). The ELISA was reported to be affordable, simple, reliable and fast, in recognizing MCF antibodies and showed no cross reactivity (Fraser *et al.*, 2006).

2.6.4.3.1.2. Indirect fluorescent antibody (IFA) test

WC-11 AlHV-1 positive cell suspensions and control negative suspensions are spotted onto multiwell slides, sera is added to both spots and rabbit anti-bovine IgG fluorescein isothiocyanate (FITC) conjugate used to detect antibodies (OIE, 2013). The differential diagnostic value of this test is reduced by the cross reactions with OvHV-2 and additional bovine herpesviruses (OIE, 2013).

2.6.4.3.2. Monoclonal assay-competitive inhibition ELISA (cELISA)

This ELISA was established to improve the specificity of identifying antibodies to MCF (Li *et al.*, 1994). The ELISA exists as a commercial kit (OIE, 2013) as a monoclonal-based test using the 15-A antibody for the epitope preserved in all MCF viruses (Li *et al.*, 1994). This test has enabled epidemiological studies in both domesticated and wildlife species and is effective at detecting MCF virus in reservoir species and subclinical infections (Russell, 2014).

Although the specificity of the cELISA is high (91–100%), it has lower sensitivity (56–87%) than histopathology or PCR tests (Muller-Doblies *et al.*, 1998). The sensitivity of this ELISA was improved by directly conjugating the monoclonal antibody with horseradish peroxidase (Li *et al.*, 2001). This ELISA is costly hence it is not commonly used in developing countries

and may be prone to incorrect negative results where antibodies to the 15A epitope do not develop in the sick animal (Fraser *et al.*, 2006).

The advantage of the cELISA is that samples from many species can be tested in the absence of species specific enzyme labelled conjugates (Li *et al.*, 2001). Also, fairly unsophisticated antigens may be used with this ELISA without decreasing the preferred specificity (Li *et al.*, 2011).

2.6.4.3.3. Virus neutralization (VN) test

This test has been used to determine the MCF status of a reservoir animal and for the confirmation of MCF in susceptible species (Fraser *et al.*, 2006). These tests are very precise and are appropriate for identification of infected wildebeest or other carriers like topi and hartebeests (Li *et al.*, 2011). The VN test is very laborious and is performed on microtitre plates using low passage cells or cell lines. This test identifies antibodies in both reservoir and clinically susceptible species and it uses the hartebeest isolate (AlHV-2), the cell-free virus of the WC11 strain or the weakened strain of AlHV-1 C500 (OIE, 2013).

2.7. Next generation diagnostics for WA-MCF

The diagnosis of WA-MCF cases using serological tests such as the cELISA (Li *et al.*, 1994) and the indirect ELISA (Fraser *et al.*, 2006) is slowed down by the rapid progression and high case fatality rates of AlHV-1 since many cattle die before generating an antibody response that can be detected (Cleaveland *et al.*, 2001). Serological tests also show substantial cross-reactivity with other macaviruses thus they need to be used carefully since they can fail to differentiate AlHV-1 and OvHV-2 infections (Wambua *et al.*, 2016). DNA tests distinguish AlHV-1 from other macaviruses and are more sensitive than ELISA tests. However, the existing range of diagnostic methods for WA-MCF need detailed procedures from skilled workers who use specialized equipment such as ELISA readers, PCR machines and

microscopes (Wambua *et al.*, 2016). Samples from suspected cases must be transported to veterinary laboratories where the essential amenities are provided for serological, and molecular tests and this is a lengthy and costly procedure and undoubtedly hinders diagnosis and mediation during epidemics (Wambua *et al.*, 2016). A vigorous surveillance scheme greatly depends on advanced diagnostics that are sensitive and fast in identification of the virus in the field since upto 100% of sick animals die in a fortnight (Cleaveland *et al.*, 2001). Development of affordable penside tests for quick detection of WA-MCF is urgently needed and these diagnostic assays should be highly sensitive and specific, fast and easy to use, since distribution and uptake of these tests would greatly improve national surveillance schemes for the disease in hard hit countries in sub Saharan Africa (Wambua *et al.*, 2016).

2.8. Control of WA-MCF

Since MCF lacks an effective treatment or vaccine the best approach in preventing it currently is separation of reservoir hosts like wildebeests and sheep from vulnerable species like cattle, and water buffalo (Russell, 2014). Cattle owners adopt disposal or avoidance as strategies (Wambua *et al.,* 2016). Disposal forces the pastoralists to sell sick cattle quickly for slaughter at a lower cost normally 30% or a lesser amount of that of healthy cattle (Cleaveland *et al.*, 2001; Bedelian *et al*., 2007). The distance of separating the vulnerable and reservoir species depends on their vulnerability, number and age of reservoir hosts and climate among other factors (Li *et al.*, 2008). Pastoralists should keep cattle away from wildebeest and grazing areas, especially nearing the time of wildebeest calving (OIE, 2013). Avoidance forces pastoralists to graze their cattle in thickets away from the wildebeest calving areas to prevent WA-MCF (Wambua *et al.,* 2016). Traditional medicines and spells have also been reported to treat or control MCF (Cleaveland *et al.*, 2001) though they have been largely unsuccessful and costly. Reduction of stress is also useful in reducing the number of cases, especially for

more vulnerable hosts with the more susceptible species (Li *et al.*, 2014).

2.9. General perceptions of WA-MCF

2.9.1. Recognition of WA-MCF

A study in Loliondo and Ngorongoro divisions in Tanzania showed that 99% of respondents recognized WA-MCF as a disease of cattle (Cleaveland *et al.*, 2001). Also in Zimbabwe, farmers near Rhodes Matopos National Park (RNMP) described WA-MCF as a disease transmitted between wildlife and livestock known in the vernacular as (*Ndebele*) and described through clinical signs and reservoir host as *Umkhuhlane wenkonkoni* (wildebeest disease), *Umkhuhlane wamehlo* (diseases of the eyes) and *Ubuphofu benkonkoni* (wildebeest blindness) (Mlilo *et al.*, 2015).

2.9.2. Clinical signs

Studies in the past have recorded several clinical signs reported by pastoralists. Blindness, heavy or open mouth breathing, dry and cracked muzzle, nasal and ocular discharges have been reported in Zimbabwe and Tanzania (Cleaveland *et al.*, 2001; Mlilo *et al.*, 2015). Maasai herders report early signs of the disease to be ocular and nasal lesions that increase and become mucopurulent with the progression of WA-MCF. Other clinical signs that have been reported include weight loss, salivation, coughing, constipation, anorexia, diarrhoea, muscle tremors, protruding eyes, loss of appetite, bloody urine, dry hair coat, desiccated muzzle, bloody eyes, bloody faeces, loss of appetite and yellowing of cornea (Cleaveland *et al.*, 2001). Neurological signs were reported by Mlilo *et al.*, (2015) though they did not specify the exact manifestations (Mlilo *et al.*, 2015).

2.9.3. Transmission of WA-MCF

The spread of MCF to cattle has been associated with the presence of wildebeest (Cleaveland *et al.*, 2001). Maasai refer to MCF as, *Emoyian oo engati,* meaning 'disease of wildebeest'.

Maasai also identified a number routes through which WA-MCF spreads from wildebeest to cattle such as eating grass or drinking water dirtied with afterbirth materials (Cleaveland *et al.*, 2001; Mlilo *et al.*, 2015). Also ingestion of hair from wildebeest calves and placental fluids, and smelling wildebeest calves and afterbirth materials (Cleaveland *et al.*, 2001). In Zimbabwe, respondents believed that WA-MCF spread was by insects and ticks, nasal secretions, feeding on dead wildebeests bones, saliva of wildebeest calves and after births (Mlilo *et al.*, 2015) and by birds in Tanzania (Cleaveland *et al.*, 2001) though there is no documented evidence of an intermediate host in spread of WA-MCF (Wambua *et al.*, 2016).

2.9.4. Seasonality of the disease

The pattern of MCF cases has been reported to have a clear period and was linked with the wildebeest calving season (Cleaveland *et al.*, 2001). The incubation period for WA-MCF is one to two months after contact with calves of wildebeests with the time taken between onset of clinical signs and death of the cattle reported by cattle holders in Tanzania to be 1-30 days (Cleaveland *et al.*, 2001).

2.9.5. Action taken in clinical cases

Slaughtering was reported by respondents in Tanzania, Kenya and Zimbabwe (Cleaveland *et al.*, 2001; Mlilo *et al.*, 2015)**.** However many animals die before the choice to slaughter is made (Cleaveland *et al.*, 2001). Some stated they would sell the meat though at lower prices (Cleaveland *et al.*, 2001; Bedelian, 2004). Respondents said they could eat meat of animals affected by MCF (Cleaveland *et al.*, 2001; Bedelian *et al.*, 2007). This meat was ideal if the animal had been slaughtered during the early phase of MCF and the meat would be consumed by family, friends and visitors (Cleaveland *et al.*, 2001).

Also some respondents would quickly sell the animals once it was clear they were affected by MCF (Cleaveland *et al.*, 2001; Bedelian, 2004). In Tanzania, selling would either be

disposing of the sick animals or exchanging them with goats (Cleaveland *et al.*, 2001). Most respondents could drink milk from an animal suffering from MCF, even though milk production decreased (Bedelian *et al.*, 2007). However some respondents knew no treatment exists for the WA-MCF and they would either let the sick animals die or try some traditional treatment (Cleaveland *et al.*, 2001; Mlilo *et al.*, 2015). Traditional medicine to treat eyes i.e. *Albizia antunensiana* (*Umnonjwana*) and *Synedenium cupulare* (*Umdlebe*) was used in Zimbabwe (Mlilo *et al.*, 2015) while in Tanzania a magic spell called *Zindiko* was used to treat some sick animals (Cleaveland *et al.*, 2001). Very few respondents in Zimbabwe used antibiotics like Hi-Tet 120 (Tetracycline) though it was not curative (Mlilo *et al.*, 2015).

2.9.6. Perception of WA-MCF in relation to other selected diseases in terms of mortality, annual cases and annual costs

In relation to mortality, MCF has previously been classified as the most important disease of cattle (Cleaveland *et al.*, 2001; Bedelian, 2004; Bedelian *et al.*, 2007). The case fatality rate of WA-MCF has been mentioned to be 100% for both adults and calves (Bedelian, 2004; Bedelian *et al.*, 2007). The high mortality rates together with lack of a curative or preventive medicine indicates that pastoralists suffer great economic losses when the disease occurs (Bedelian, 2004).

In terms of new cases annually, WA-MCF has previously been ranked the fourth most common disease behind ECF, FMD and anthrax respectively in Kenya (Bedelian, 2004; Bedelian *et al.*, 2007). In Tanzania respondents rated it the fifth disease in terms of veterinary costs behind CBPP, trypanosomiasis, bovine cerebral theileriosis and ECF respectively (Cleaveland *et al.*, 2001). In Southern Kenya, WA-MCF was mentioned to have least costs with all respondents being in agreement that it lacks no preventive or curative treatment (Bedelian *et al.*, 2007).

2.10. Perception on control of WA-MCF

It has been indicated that control of WA-MCF would increase herd productivity as it would directly reduce the number of deaths in cattle mainly in periparturient adults that are very susceptible (Cleaveland *et al.*, 2001; Bedelian, 2004).

Several methods for controlling WA-MCF have been suggested including: selling sick cattle, fencing, vaccination, avoidance, traditional medicine, chasing wildebeest, checking for signs of wildebeest that have started calving, killing wildebeests, legislative control, relocating wildebeests, educating farmers and combining efforts of both conservations and pastoralists.

Wildebeests control has been attempted through fencing of farms to block their pathways (Cleaveland *et al.*, 2001; Bedelian, 2004; Bedelian *et al*., 2007; Mlilo *et al*., 2015), although respondents in Southern Kenya stated that fencing could be expensive (Bedelian *et al.*, 2007). However, fencing has been found to be ineffective in controlling the spread of WA-MCF (Honiball *et al.*, 2008).

Vaccination has been suggested by farmers in Zimbabwe and pastoralists in Kenya and Tanzania (Cleaveland *et al.*, 2001; Bedelian, 2004; Bedelian *et al*., 2007; Mlilo *et al*., 2015). An effective vaccine for WA-MCF would reduce fatalities and improve the productivity and fecundity of cattle since they would graze on high-quality pasture at a critical period of year (Cleaveland *et al.*, 2001). A vaccine would also increase the number of cattle owned by the Maasai hence raising their standards of living (Bedelian *et al.*, 2007).

Traditional medicine has been used in both Kenya and Tanzania (Cleaveland *et al.*, 2001; Bedelian *et al.*, 2007). However traditional treatments have been considered ineffective and traditional healers accused of cheating and having very little knowledge (Cleaveland *et al.*, 2001).

Avoidance has been also been suggested as a control measure by almost all participants in Tanzania and some farmers in Zimbabwe (Cleaveland *et al.*, 2001; Mlilo *et al*., 2015). Cattle owners also have chased wildebeests from their farms to control WA-MCF (Cleaveland *et al.*, 2001; Bedelian *et al.*, 2007). In Kenya pastoralists used dogs to chase the wildebeests though the wildebeests would return at night to calve (Bedelian *et al.*, 2007). Relocating wildebeests to different areas far from farms of cattle owners has also been another way of controlling WA-MCF (Bedelian *et al.*, 2007; Mlilo *et al*., 2015). Confining cattle when wildebeests start to calve is another method used (Cleaveland *et al.*, 2001). Killing wildebeests was stated by some pastoralists though they recognized this was against the law (Bedelian *et al.*, 2007).

Legislative control and education of farmers have been suggested in Zimbabwe (Mlilo *et al*., 2015). Conservationists collaborating with pastoralists aim to generate methods that benefit both wildlife and people by giving appropriate veterinary and animal health services to boost the development of livestock output, development of water supply points and development of sustainable consumptive use of wildlife (Bedelian, 2004).

CHAPTER 3: ASSESSING THE LABORATORY PERFORMANCE OF INDIRECT ELISA AND NESTED PCR IN CONFIRMING CLINICAL CASES OF WA-MCF

3.1. Introduction

Between 2014 and 2016 WA-MCF has been occurring in the Kapiti Plains Ranch. The disease has caused economic losses to the ranch since the sick animals were disposed of immediately at an estimated cost of 50% of the value of a mature Boran animal (US$600). Though the disease has been manifested in Kenya before, a thorough study of the diagnosis of MCF at the ranch has not been conducted. This chapter describes the outbreaks of WA-MCF in this ranch and assesses an indirect ELISA and nested PCR in confirming the reported clinical cases of WA-MCF. First, the sensitivity (Se) and specificity (Sp) of indirect ELISA are evaluated using nested PCR as a gold standard. Second the agreement between these tests are evaluated in absence of a gold standard using the Cohen's Kappa statistic and the Bayesian agreement index (B.A.I).

3.2. Methodology

3.2.1. Ethical approval

The International Livestock Research Institute (ILRI) institutional veterinarian or a technician under instruction from the veterinarian collected samples from clinically affected animals and sample analysis was approved by the ILRI Institutional Animal Care and Use Committee (IACUC) 2016-02.

3.2.2. Study site

Kapiti Plains Ranch Limited is 33,800 acre ranch located in the Athi-Kaputiei plains 40 km east of Nairobi at 1.633333^0N and 37.145267^0E (Figure 3). It lies between 1,646 to 1,911 metres above sea level. The area experiences two short rainy seasons in March-May and again in October-December. Kapiti Plains Ranch comprises extensive open grassland and is

described as a semi-arid zone. Cattle and sheep are the main livestock species farmed on the land. The livestock graze together with multiple wild herbivore species, including: wildebeest, hartebeest, Thomsons and Grants gazelle, impala, zebra, ostrich, eland and giraffe. Historically, MCF cases in cattle have been documented during and shortly after the wildebeest calving season from February to July.

Figure 3: Map showing the location of Kapiti Plains Ranch

3.2.2.1. Kapiti Cattle

The cattle are grouped into two breeds i.e. crossbred dairy and pure-bred Boran. The crosses of exotic breeds, mainly Holstein-Friesian, Aryshire and Jersey with Boran comprises the

crossbred dairy. The Boran are kept for livestock experiments by ILRI and for sale of steers for beef (www://ilri-angr.wikispaces.com/file/view/Kapiti-20150218.pdf).

3.2.2.2. Cattle management

The Boran group is classified into several herds as:

1. Stud herd: Includes the best breeding herd of animals. These animals are listed in the Boran Breeders Society of Kenya (BBSK). The Boran Breed Society (BBS) maintains the pedigree and performance data of these animals.

2. Bull herd: This group is made of uncastrated male cattle. They are mainly kept as sires for mating.

3. Breeding herd: Four breeding herds exist with female animals that perform equally to the stud herd. Nonetheless, these animals are not registered in the Boran Breeders register.

4. Heifers: These are separated into three groups by age as, heifers 1: 8-<14 months, heifers 2: 14-27 months and heifers 3: >27 months (bulling heifers).

5. Steers: This group is made of castrated male animals which are kept to reach the mature market weights for sale thus they are purposely for beef production.

6. Sick herd: This comprises sick animals. The animal health technician attends to the animals in the morning and administers drugs according to diagnosed conditions.

7. Cull herd: They are for experimental purposes. Eventually they sold when a project terminates since all of them are non-breeders.

(www://ilriangr.wikispaces.com/file/view/Kapiti-20150218.pdf).

3.2.3. Study design

This was a retrospective study of WA-MCF outbreaks at the Kapiti Plains Ranch from 2014 to 2016. Historically cases of WA-MCF in cattle were reported during and shortly after the wildebeest calving season February to July. Management tools used in the past included culling wildebeest or chasing them from the farm. Presently there is the integrated management approach where wildlife and cattle coexist.

3.2.4. Sampling

This involved examining and collecting samples from sick animals as well as animals that died on the ranch. Other details recorded were animal identity, sex, age, sire and dam. Blood samples were opportunistically collected from 123 cases of clinically suspected WA-MCF. Blood samples were collected by jugular venipuncture directly into 10ml plain and 10ml Ethylenediaminetetraacetic acid (EDTA) vacutainer tubes (Becton Dickinson) (Figure 4). The tubes were placed in a cool box containing ice packs and transported to ILRI for further laboratory analysis.

Figure 4: A veterinarian drawing blood by jugular venipuncture. Courtesy Annie Cook.

3.2.5. Sample size calculation

A formula for detecting a difference in the proportion of positive ELISA results and the proportion of positive PCR results was used to calculate the sample size (Dohoo *et al.*, 2003).

$$n = \frac{[Z_\alpha\sqrt{2pq} - Z_\beta\sqrt{p_1q_1 + p_2q_2}]2}{(p_1 - p_2)2}$$

Where:

Z_α = 1.96, Z_β -0.84

α = 0.05 (confidence interval is 5%) β = 0.80 (power 80%)

p = proportion PCR positive 95%,

q = proportion ELISA positive 80%

The required sample size is 73 animals. This means that if 73 animals are studied a difference in the performance of the PCR and the ELISA test can be demonstrated with 95% confidence.

3.2.6. Laboratory analysis

The laboratory analysis was carried out at ILRI, Nairobi and involved indirect ELISA test, DNA purification and DNA amplification. The ELISA was performed at the ELISA laboratory while the DNA purification and amplification was performed at the molecular laboratory.

3.2.6.1. Indirect ELISA

The working benches and all equipment were decontaminated before preparation of each sample using 70% alcohol. All 123 samples were tested using an earlier described procedure (Russell *et al.*, 2012).

3.2.6.1.1. Procedure carried out before ELISA

- Coating buffer was made by dissolving 5μg/mL in 0.1 M carbonate buffer of pH 9.6. This buffer is used to coat the plates overnight with the ELISA antigen to ensure that the antigen is bound to the plate and does not get washed off.

- Washing buffer (PBS Tween 20) was made through a solution of 1X phosphate buffered saline (PBS) by dissolving five tablets of PBS in one litre of distilled water and adding 200μL of tween 20. The buffer was used to wash off any unbound material from the plates.

- Four per cent dried milk was made by dissolving 4g of non-fat dried milk into 100mL of 1X PBS. It is used in blocking non-specific binding.

- Two per cent dried milk was made by dissolving 2g of non-fat dried milk into 100mL of PBS Tween. This is used when incubating the test samples and the secondary antibody Immunoglobulin-horseradish peroxidase (IgG-HRP).

3.2.6.1.2. Procedure

Sera were separated by centrifugation at 3000rpm for 20 minutes at 4°C. Sera were screened for antibodies to MCF by indirect ELISA (Russell *et al.*, 2012). Ninety-six-well microtitre plates (Greiner Bio-One, Austria) were coated with 50μl of 5μg/mL of known virus positive serum or negative serum in 0.1 M carbonate buffer of pH 9.6. The plates were covered with parafilm and left overnight at 4°C. The plates were then washed six times with phosphate buffered saline (PBS) and 0.02% Tween 20. This was followed by blocking each well with 100μl of 4% non-fat dried milk/PBS, covering with parafilm for one hour at room temperature. The plates were then washed twice with PBS/Tween 20. 50μl of sample sera diluted in 2% non-fat dried milk/PBS/Tween 20 (1:500) was then added in duplicate in the layout demonstrated in Figure 5. The plates were covered with parafilm and left for 1.5 hours at room temperature. The plates were then washed six times with PBS/Tween 20. This was followed with the addition of 50μl per well of 1:1000 rabbit anti-bovine IgG-horseradish peroxidase (HRP) (Sigma Aldrich, USA) diluted in 2% non-fat dried milk/PBS/Tween 20 and left for one hour at room temperature after covering with parafilm. The plates were washed six times with PBS/Tween 20. Tetramethylbenzidine **(**TMB) substrate (50μl) was added to each well and colour change allowed to develop for five minutes. The reaction was stopped by adding 50μl of 0.1M hydrochloric acid in every well. The appearance of the final stopped plate is demonstrated in Figure 6. The plates were read by at 450nm for evaluation of the optical densities (OD) (Synergy HT, Biotek, USA). ELISA values were calculated by subtracting the mean of the negative antigen OD values from the mean of the positive antigen OD values for every sample. Inter-plate variations were adjusted by a correction factor (CF), which was applied to all of the plates that used the same positive and negative control sera over the screening period. The first plate run in a sequence with the same negative and positive controls was used in calculating the CF. The CF was calculated from the mean

ELISA value of the positive and negative controls from the first plate by the equation below, which was applied to all the subsequent plates.

Correction Factor $= (P_0-N_0)/P_t-N_t$

Where:

P_0 = Mean of the positive control sera from plate 1

N_0 = Mean of the negative control sera from plate 1

P_t = Mean of the positive control sera from plate on test

N_t = Mean of the negative control sera from plate on test.

The cutoff was calculated by taking the mean ODs of a plate of known negative samples plus three standard deviations.

	1	2	3	4	5	6	7	8	9	10	11	12
A	POS	POS	NEG	NEG	1	1	2	2	3	3	4	4
B	5	5	6	6	7	7	8	8	9	9	10	10
C	11	11	12	12	13	13	14	14	15	15	16	16
D	17	17	18	18	19	19	20	20	21	21	22	22
E	POS	POS	NEG	NEG	1	1	2	2	3	3	4	4
F	5	5	6	6	7	7	8	8	9	9	10	10
G	11	11	12	12	13	13	14	14	15	15	16	16
H	17	17	18	18	19	19	20	20	21	21	22	22

Figure 5: Layout of the ELISA plate showing the order of positive antigen, negative antigen and samples from 1-22 all in duplicates

Figure 6: A plate showing positive samples indicated by yellow colour in the microtitre wells

3.2.6.2. DNA extraction

The biosafety sample preparation cabinet, working benches and all equipment were decontaminated before preparation of each sample using 10% Sodium hypochlorite and 70% alcohol. DNA was extracted from blood samples using the Qiagen Flexigene kit according to the manufacturer's instructions. Extracted DNA was eluted in 100 μL elution buffer and stored at minus 20°C.

3.2.6.2.1. Procedure carried out before DNA purification

- The lyophilized QIAGEN Protease was resuspended Buffer FG3 (hydration buffer)

- The frozen blood was thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

- The blood to be processed was mixed with buffer FG2 and Qiagen protease mixture within an hour before being used

- The water bath was heated to 65°C for use in steps 5 and 13.

3.2.6.2.2. Procedure

1. A volume of 750µl of buffer FG1 was pipetted into a 1.5 ml centrifuge tube. A volume of 300µl whole blood was then added and mixed by inverting the tube 5 times.

2. The mixture was centrifuged for 20 seconds at 10,000g in a fixed-angle rotor.

3. The supernatant was discarded and the tube left inverted on a clean sheet of absorbent paper for 2 minutes, taking care that the pellet remained in the tube.

4. A volume of 150µl of buffer FG2/QIAGEN Protease was then added into the tube which was then closed and vortexing done immediately until the pellet was completely homogenized. The tube was inspected to check that homogenization was complete. Vortexing of each tube was done immediately after addition of Buffer FG2/QIAGEN Protease.

5. The tubes were centrifuged briefly for 3 to 5 seconds and then placed in a water bath and incubated at 65°C for 5 minutes. The sample changed colour from red to olive green, signifying protein digestion.

6. A volume of 150µl of 100% isopropanol was added into the tubes and mixed thoroughly by inversion until the DNA precipitate became visible as threads or a clump. Complete mixing with isopropanol is necessary to precipitate the DNA and must be checked by inspection.

7. The tubes were then centrifuged for 3 minutes at 10,000g. Where the resultant pellets were loose, centrifugation was prolonged or a higher gravitational force used.

8. The supernatant was discarded and the tube left inverted on a clean piece of absorbent paper for at least 5 minutes, taking care that the pellet remained in the tube.

9. A volume of 150µl of 70% ethanol was added and vortexed for 5 seconds.

10. Centrifugation was then done for 3 minutes at 10,000g. Where the resultant pellets were loose, centrifugation was prolonged or a higher gravitational force used.

11. The supernatant was discarded and the tube left inverted on a clean piece of absorbent paper for at least 5 minutes, taking care that the pellet remained in the tube.

12. The DNA pellet was left to air dry until all the liquid evaporated. This was done for at least 5 minutes. Over drying the DNA pellet was avoided as over dried DNA is very difficult to dissolve.

13. A volume of 200μl of buffer FG3 was added followed by vortexing for 5 seconds at low speed. The DNA was then dissolved by incubating in a water bath overnight at room temperature. The DNA was then stored at minus 80°C

3.2.6.3. Nested PCR

Amplification reactions employed available reagents and used previously described primers (Li *et al.*, 2000). The PCR reaction mix was 25μl and contained 12.5μl of oneTaq Universal Master Mix (Bio Labs, New England) containing OneTaq® DNA polymerase, deoxynucleoside triphosphates (dntps), buffer components, 0.5μl forward primer, 0.5μl reverse primer and 5μl of sample DNA. The initial round of nested PCR was performed using outer forward and outer reverse primers,

C500-1: TACGGGTGCCCTGACATTTCATCTCTTTTG; and

C500-2: ATAACTGGTTGATGTGGCAGATGCATCTAT respectively.

The second round amplification (274 basepair (bp) DNA fragments) used the first round product (2μl), with inner forward and inner reverse primers,

C500-3: TCTGGCCCGTGCTGCAGCAAGACTCTCAG; and

C500-4: TATAGTAGAATCCCGTCTGAGTGGTAGCTG.

Thermocycling conditions for the first and second round amplification were 95°C for 5 minutes, followed by 34 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes, and 72°C for 7 minutes. PCR products (6µl) were electrophoresed at 80 volts through 2.0% (w/v) agarose containing 4µl gel red (10,000x) per 100ml gel (Figure 7). Gels were photographed by ultraviolet light for PCR product analysis. In addition, DNA extracted from the blood sample of cattle that were confirmed to have died of WA-MCF served as positive control DNA. Sterile water served as negative controls.

Figure 7: Loading of samples into wells in the gel before electrophoresis

3.2.7. Statistical analysis

Data was managed in Microsoft (MS) Excel (Microsoft Corp., 2010). Descriptive statistics and graphs were produced in MS Excel. Prevalence estimates were calculated using the *trueprev* function in the *prevalence* package (Devleesschauwer *et al.*, 2015) in R environment

for statistical computing, version 3.4.0 (http://cran.r-project.org/). The logistic regression was conducted using the *glm* function in the base package of R to assess the association of gender and age groups on infection with WA-MCF. Odds ratios (OR) were calculated to measure the relationship between age and gender and WA-MCF and P values ≤ 0.05 were considered significant.

The estimate of financial losses were calculated as 50% of the value of a mature Boran animal (US$600) multiplied by the number of animals disposed/sold due to WA-MCF in each year. The epidemic curves were obtained using the number of cases reported in each week using January first as week one in the respective years. Incidence rates in all the different years were calculated by taking the total number of sick animals divided by the size of the herd at the beginning of the outbreak divided by time (one year).

For the diagnostic test performance comparison between the diagnostic tests was conducted using three methods:

1. The sensitivity and specificity indirect ELISA with their respective confidence intervals were calculated using nested PCR as a gold standard (https://www.medcalc.org/calc/diagnostic_test.php)

2. The Cohen kappa statistic was used to assess the agreement between PCR and indirect ELISA with scores divided into <0.2: slight agreement, 0.2-0.4:fair agreement, 0.4-0.6: moderate agreement, 0.6-0.8 substantial agreement and >0.8: almost perfect agreement (Dohoo *et al.*, 2003).

3. The Bayesian analysis index (B.A.I) was also performed in the R software package (Version 1.0.136) to determine the level of agreement between these two assays in the absence of a gold standard (Graham and Bull, 1998; Thomas, 2013) based upon an

assessment of the agreement between two independent operators (Feinstien and Cicchetti, 1990).

3.3. Results

3.3.1. Case history

In 2014, the largest recorded MCF outbreak for many years occurred at Kapiti Plains Ranch involving 215 animals. Clinical diagnosis was done by the ILRI institutional veterinarian Dr. Okoth or a technician under his direction who confirmed it to be WA-MCF. Clinical signs included pyrexia, bilateral corneal opacity and serosanguinous nasal discharges. In 2015, fences were put up and 78 animals were affected. In 2016, the wildebeest numbers decreased drastically possibly due to migration and only 32 animals were affected.

3.3.2. Clinical results

Most animals showed nasal discharge and corneal opacity as indicated in the Figure 8 below.

Figure 8: Animal affected by MCF demonstrating nasal discharge and corneal opacity. Courtesy Annie Cook.

3.3.3. Descriptive results

3.3.3.1. 2014 WA-MCF outbreak

The 2014 WA-MCF outbreak at Kapiti Plains Ranch started on May 7th (week 19 of 2014) and ended July 31st in (week 31) affecting 215 animals (Figure 9). The herd size at the beginning of the outbreak was 2,467 animals. The incidence of WA-MCF in 2014 was 8.7 (95% C.I. 9.5-12.3) cases per hundred animals per year (Table 6). The estimated direct losses to Kapiti Plains Ranch were estimated ~ US$64,500.

There was no significant difference in WA-MCF incidence between sexes, OR=1.00 (95% C.I. 0.71-1.37). However, there was a significant difference in WA-MCF incidence between age groups with steers, heifers and cows more likely to have WA-MCF compared to calves. The OR for steers was 4.53 (95% C.I. 2.29-10.03), heifers 3.85 (95% C.I. 2.00-8.37), and cows 2.79 (95% C.I. 1.44-6.07) respectively (Table 7).
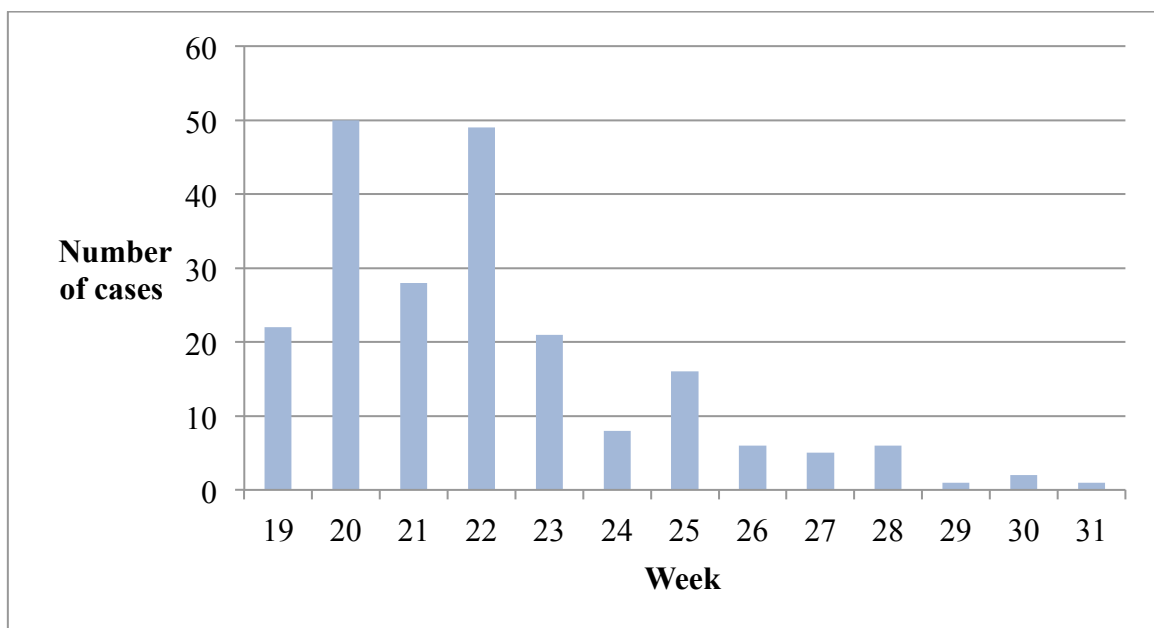
Figure 9: Epidemic curve of malignant catarrhal fever at Kapiti Plains Ranch, 2014

3.3.3.2. 2015 MCF outbreak

The 2015 outbreak started on April 2nd (week 14 of 2015) and ended June 30th (week 27) involving 78 animals (Figure 10). The herd size at the beginning of the outbreak was 2,106. The incidence in 2015 was 3.7 (95% C.I. 3.0-4.6) cases per hundred animals per year (Table 6). The estimated direct loses to Kapiti Plains Ranch were estimated ~US$23,400.

There was a significant difference in WA-MCF incidence between sexes, with males having an OR 0.38 (95% C.I. 0.16-0.78) compared to females. There was no significant difference between the incidence of WA-MCF cases within the age groups although there was a positive relationship with heifers and cows more likely to have WA-MCF compared to calves. The OR for heifers was 1.72 (95% C.I. 0.86-3.64) and cows 1.49 (95% C.I. 0.76-3.11) respectively (Table 7).
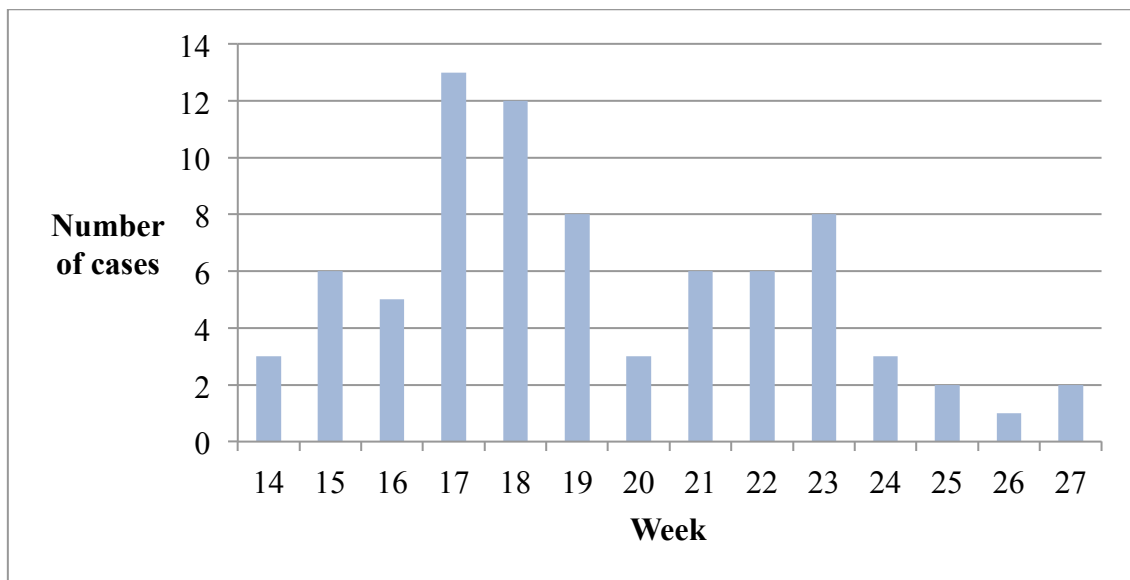
Figure 10: Epidemic curve of MCF at Kapiti Plains Ranch 2015

3.3.3.3. 2016 MCF outbreak

The outbreak in 2016 started on March 29th (week 13 of 2016) and ended September 19th (week 37) involving 32 animals (Figure 11). The herd size at the beginning of the outbreak was 2,069 animals. The incidence of WA-MCF in 2016 was 1.5 (95% C.I. 1.1-2.2) cases per hundred animals per year (Table 6). The estimated direct loses to Kapiti Plains Ranch were estimated ~US$9,600.

There was no significant difference between sexes with respect to WA-MCF incidence, males having an OR 0.45 (95% C.I. 0.13-1.15). There was positive but not a significant relationship between age group and MCF incidence with cows and steers being more likely to have WA-MCF compared to calves, OR=2.98 (95% C.I. 0.88-18.60) and 2.29 (95% C.I. 3.7-17.57) respectively (Table 7).
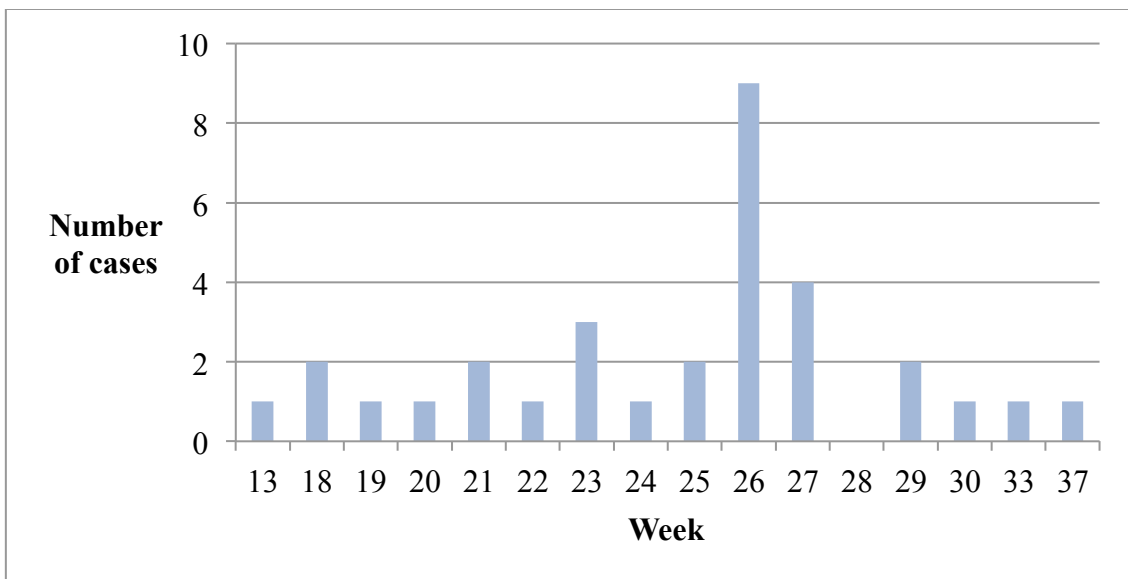
Figure 11: Epidemic curve of MCF on Kapiti Plains Ranch in 2016

Table 6. Descriptive results of MCF cases from 2014 to 2016

Year	Cases/ Susceptible % (95% C.I.)	Gender (excluding calves)		Breed		Age structure				
		Male	Female	Boran	Other breeds	Calves <10months	Steers 10-48 months	Heifers 10-36 months	Bulls	Cows >36 months
2014	215/1975	57/459	158/1332	200/1793	15/182	9/228	52/331	79/578	2/128	73/710
	10.9%	12.4%	11.9%	11.2%	8.2%	3.9%	15.7	13.7	1.6%	10.3%
	(9.5-12.3)	(9.6-15.8)	(10.3-13.7)	(9.8-12.7)	(5.0-13.2)	(2.1-7.4)	(12.2-19.9)	(11.2-16.7)	(0.5-5.4)	(8.3-12.7)
2015	78/2106	12/398	66/1328	77/1872	1/180	11/380	6/254	28/574	1/144	32/754
	3.7%	3%	5.3%	4.1%	0.6%	2.9%	2.4%	4.9%	0.7%	4.2%
	(3.0-4.6)	(1.7-5.3)	(4.2-6.8)	(3.3-5.1)	(0.2-3.0)	(1.7-5.1)	(1.1-5.1)	(3.4-6.9)	(0.2-3.8)	(3.0-5.9)
2016	32/2069	5/481	27/1406	32/1842	0/227	2/182	1/360	1/625	3/121	25/781
	1.5%	1%	1.9%	1.7%	0%	1.1%	0.3%	0.2%	2.5%	3.2%
	(1.1-2.2)	(0.4-2.4)	(1.3-2.8)	(1.2-2.5)	(0.0-1.6)	(0.3-3.9)	(0.1-1.5)	(0.0-0.9)	(0.9-7.1)	(2.2-4.7)

Table 7. Results of association between MCF infection & gender and age group 2014-2016							
Year	Variable		Totals	Positives	Odds ratio	95% C.I	P value
2014	Sex	Male	459	54	1	(0.71-1.37)	0.98
		Female	1288	152	1		reference
	Age group	Calves	228	9	1		reference
		Cows	710	73	2.79	(1.44-6.07)	0.005
		Heifers	578	79	3.85	(2.00-8.37)	0
		Steers	331	52	4.53	(2.29-10.03)	0.00005
		Bulls	128	2	0.39	(0.05-1.53)	0.228
2015	Sex	Male	398	7	0.38	(0.15-0.77)	0.016
		Female	1328	60	1		reference
	Age group	Calves	380	11	1		reference
		Cows	754	32	1.49	(0.76-3.11)	0.264
		Heifers	574	28	1.72	(0.86-3.64)	0.134
		Steers	254	6	0.81	(0.27-2.16)	0.685
		Bulls	144	1	0.23	(0.01-1.22)	0.167
2016	Sex	Male	481	4	0.45	(0.13-1.15)	0.134
		Female	1406	26	1	-	reference
	Age group	Calves	182	2	1	-	reference
		Cows	781	25	2.98	0.88-8.60)	0.14
		Heifers	625	1	0.14	0.01-1.51	0.115
		Steers	360	1	0.25	(0.01-2.63)	0.26
		Bulls	121	3	2.29	(0.37-7.57)	0.369

3.3.4. Laboratory results

A total of 123 samples were collected from 325 clinically affected animals between 2014-2016. These were tested by nested PCR and indirect ELISA. Of the 123 clinically diagnosed samples, 62.6% (77/123) were positive by indirect ELISA while 94.3% (116/123) were positive by nested PCR (Table 8).

Using these samples, a comparison of indirect ELISA to nested PCR as the gold standard revealed its sensitivity and specificity to be 63.8% (95% C.I. 55-72%) and 57.1% (95% C.I. 25-85%) respectively. A boxplot demonstrates the difference between OD values for PCR negative and PCR positive animals (Figure 12). An example of the PCR results is shown in Figure 13.

Table 8. Results of PCR and ELISA on samples collected from MCF cases between 2014-2016

	PCR positive	PCR negative	Total
ELISA positive	74	3	77
ELISA negative	42	4	46
Total	116	7	123

k=0.05, n=123
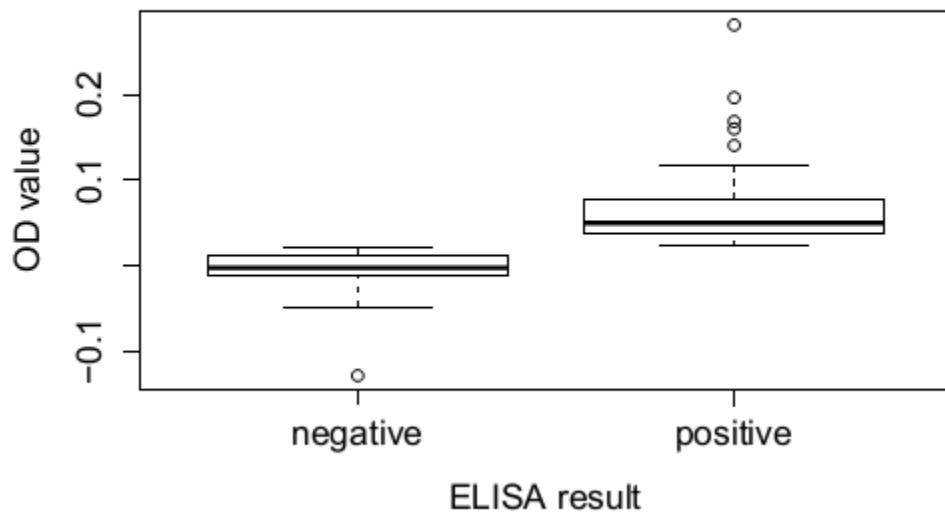
Figure 12: A Box plot of Corrected OD Values from MCF ELISA against PCR Result for MCF affected animals at Kapiti Plains Ranch 2014-2016
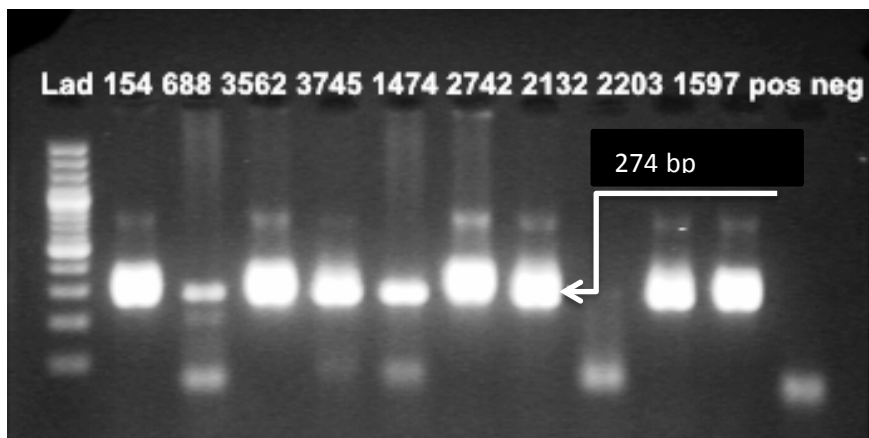
Figure 13: Results of nested PCR. Positive results are indicated by band at 274bp on agarose gel.

Lane 1: Ladder, Lanes 2 to 9 animals 154, 5688, 3562, 3745, 1474, 2742, 2132, 2203 and 1597.

Lane 10: Positive control. Lane 11: Negative control

3.3.5. Statistical analysis results

3.3.5.1. Kappa

A kappa value of 0.05 was obtained which revealed very little agreement between these two tests.

3.3.5.2 Bayesian Agreement Index

This revealed agreement for these two assays to be better in the positive than in the negative direction with a Bayesian Agreement Index of 76.7% (95% B.C.I. 70-83%) in the positive direction and 15.1% (95% B.C.I. 4-31%) in the negative direction.

3.4. Discussion

3.4.1. Clinical results

The clinical presentation of MCF diagnosed was the typical head and eye form which is a common feature of MCF with the nasal discharge and corneal opacity in this study consistent with documented reports of this form of this disease (Russell, 2014).

3.4.2 MCF outbreaks from 2014 to 2016

The outbreaks of WA-MCF in 2014 and 2015 occurred between the months of April and July. This period coincided with the time of the highest spread of WA-MCF in Kenya between the months of March and June (Wambua *et al.*, 2016). The extension to July in 2014 could have been from adult wildebeests which had the AlHV-1 reactivated as a result of their immunological competence being compromised by harsh conditions like starvation and pregnancy or a later calving season due to differing rainfall and pasture availability across years (Wambua *et al.*, 2016). The incidence of MCF in 2014 and in 2015 was 8.7% and 3.7% respectively. This agrees with previous estimates estimated to be 3%-12% (Bedelian *et al.*, 2007).

According to age group, steers, heifers and cows were more likely to develop WA-MCF as compared to the calves in 2014 while heifers and cows were more likely to develop WA-MCF as compared to the calves in 2015. This is similar to a previous study in Tanzania where 36% of respondents indicated that the disease attacked mainly adults and five respondents specifying that the adult females were mainly affected (Cleaveland *et al.*, 2001). The results of this study confirm earlier reports that all age classes are susceptible to MCF though other studies have also shown a higher incidence in adults especially in periparturient females (Barnard *et al.*, 1989). In addition, calves tend to be kept near the boma thus not spending as much time as adults in the wildebeest grazing areas hence a low incidence of the disease in the calves (Cleaveland *et al.* 2001) and calves are also suckling rather than grazing. In terms of sex the males seemed to be at less risk of getting WA-MCF. This agrees with earlier documented literature that periparturient females often are very prone to this disease (Barnard *et al.*, 1989).

The outbreak in 2016 was the longest and occurred between the months of March to September. As in the previous year the outbreak occurred at the time when peak transmission of WA-MCF was expected. However the extension of the outbreak through to September could be attributed to environmental factors such as rainfall or pasture distribution or delayed wildebeest calving due to drought (Wambua *et al.*, 2016). In Kenya outbreaks that occur from September to November are due to cattle undergoing trauma because of the unusually cold weather in the winter period thus they become prone to infection (Wambua *et al.*, 2016). The incidence in 2016 was 1.5%. The reduced size of the outbreak at Kapiti Plains Ranch compared to previous years and was likely due to lower numbers of wildebeest observed on the ranch in 2016.

3.4.3. Laboratory findings

This study demonstrated that when laboratory confirmation of clinical diagnosis is required, nested PCR is a more sensitive tool for diagnosing WA-MCF than the indirect ELISA. Nested PCR has been reported previously to be sensitive (Russell, 2014). However, it is expensive, requiring specialized equipment and trained personnel, which means it is not easily accessible. The poor performance of the ELISA is likely due to the lack of antibody production in some cattle with WA-MCF since susceptible animals may fail to develop measurable levels of antibody before death (Li *et al.*, 2006; Russell *et al.*, 2012). This is also supported by the B.A.I. which revealed a reasonable agreement between the indirect ELISA and the nested PCR in detecting positive cases and poorer agreement between the tests in detecting negative cases (Calle-Alonso and Sánchez, 2015).

3.4.4. Economic losses at Kapiti Plains Ranch from 2014-2016

Kapiti Plains Ranch incurred direct estimated costs of US$97,500 between 2014-2016. The lower proceeds consequently affects the budgetary plans of the ranch and the data on economic impact can also indicates that WA-MCF is a serious disease that has to be addressed to curb the financial losses associated with it even though it is sporadic in cattle.

The future of diagnosing WA-MCF should focus on cheap penside tests to assist this ranch, pastoralists and other ranches in WA-MCF endemic areas in Kenya to ease the process of confirming diagnosis in the field. This will also reduce the time, resources and personnel required for laboratory diagnosis as there will be no need to transport the samples to the laboratory for further analysis. Also the report on financial costs incurred by the ranch demonstrated the economic impacts of WA-MCF and highlights the urgent need for control

measures. A vaccine which is effective would be one of the long lasting solutions not only for Kapiti Plains Ranch but also for pastoralists and other ranches in the WA-MCF affected areas.

CHAPTER 4: EXPLORING PERCEPTIONS OF WA-MCF IN KAPITI PLAINS RANCH

4.1. Methodology

4.1.1. Study site

This was as earlier described in section 3.1.2.

4.1.2. Study design

The methods employed in this study were designed from past participatory disease studies (Cleaveland *et al.*, 2001; Bedelian *et al.*, 2007). The methods were tried before the actual study to assess their suitability, and necessary adjustments made. The study employed a mix of quantitative and qualitative methods with formal interviews conducted between the respondent and the interviewer. Semi structured questionnaires composed of both closed and open-ended questions were used. A quantitative method of proportional piling was used to determine the impact of WA-MCF and relate this to other endemic diseases such as ECF, FMD CBPP and LSD. Employing this method in disease surveys is advantageous as it avoids asking personal questions about herd size and a comparative assessment of important diseases can also be investigated to limit the focus on one particular disease. Interviews were carried out mainly in English and Kiswahili languages but in case where local names were needed the Kamba language was appropriate. Before carrying out any interview, the interviewer introduced herself to the interviewee and the purpose of the study. The interviewee then signed a consent form before giving answers to the questions.

4.1.3. Sampling unit

This comprised the workers of Kapiti Plains Ranch. At the time of the study, the ranch had 86 workers of which 25 were on leave and efforts were made to get information from the workers that were on duty. Also the timing of the interview process was in such a way that it targeted all informants either early in the morning before workers had moved to the field or when they returned in the evening.

4.2. Data collection

4.2.1. Semi structured interviews (SSIs)

Questions regarding disease recognition, clinical signs, how cattle get WA-MCF, seasonality (onset, incubation period and end of outbreak) and action taken for the clinical cases were asked from each respondent. During questionnaire administration, efforts to maintain interviews in the form of open discussions, using the questions as prompts for further discussion and following up interesting responses with further questions and probing were made. The questions related to common perceptions of WA-MCF and experiences.

4.2.2. Proportional piling

This was used to compare the impact of WA-MCF in Kapiti Plains Ranch and also in the respondents' farms. First mortality rate of WA-MCF was compared to ECF (*ngai*), FMD (*muthingithu*), CBPP (*mavui*) and LSD (*ikundu*) in 2017 at the Kapiti Plains Ranch. For mortality scores, the participants were issued with a piece of cardboard paper with the names of the five diseases written in an individual square drawn on the paper. They were then given fifty tokens and asked to put the number of tokens to represent cows that would die from each disease. The disease that causes the higher number of mortalities was given more tokens. Secondly the annual cases and costs of WA-MCF were relatively compared to the other diseases but this was

assessed for the respondent's farm. The local names were availed in order for the respondents to quickly familiarize themselves with the disease. The process of scoring both cases and costs was similar to the scoring for mortality. For both of these questions, tokens were used to rank the diseases. The disease with a highest impact was given a higher score of 5 and ranked number one with the one having least impact having few tokens and ranked number 5. The process of proportional piling is demonstrated in Figure 14.

Figure 14: A participant during the KAP study showing proportional piling

4.3. Data analysis

All results recorded from the KAP study were entered in MS Excel data sheet. The data was later cleaned and managed in MS Excel to obtain descriptive graphs.

4.4. Results

4.4.1. Demographic and socio-economic characteristics

All of the 60 respondents were male (100%). The majority of respondents were Kamba 85% (51/60), followed by the Meru 8.3% (5/60), Kikuyu 3.3% (2/60), Taita 1.7% (1/60) and Embu 1.7% (1/60) (Figure 15).
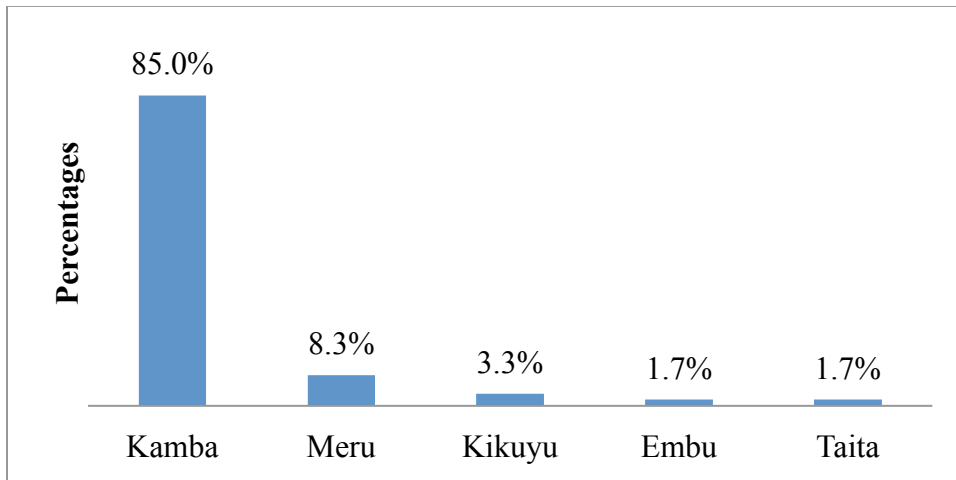
Figure 15: Ethnic origin of the respondents

The different respondents had different responsibilities in ranch. The workers were predominantly herders 38% (23/60); security officers 17% (10/60); general workers 12% (7/60) and other roles 33% (20/60) including technician, accountant, welders, shepherds, clerk and driver.

Most respondents 22% (13/60) were in the age group of 31 to 35 years. The other age groups were as follows 18% (11/60) between 41 to 45 years, 17% (10/60) between 36-40 years, 15% (9/60) between 18 to 25 years, 12% (7/60) above 50 years, 10% (6/60) between 26 to 30 years and 7% (4/60) between 46-50 years (Figure 16).
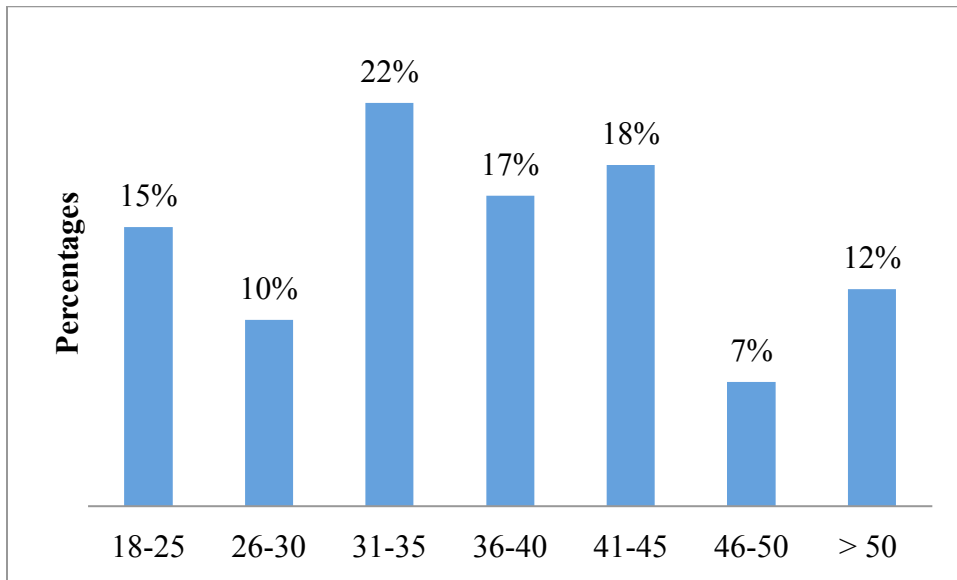
Figure 16: The different age groups of the respondents

All of the respondents had some formal education with 50% (30/60) of the respondents having attained primary education, 33% (20/60) having studied up to secondary level, 15% (9/60) had studied to tertiary level and 2% (1/60) had preprimary education (Figure 17).
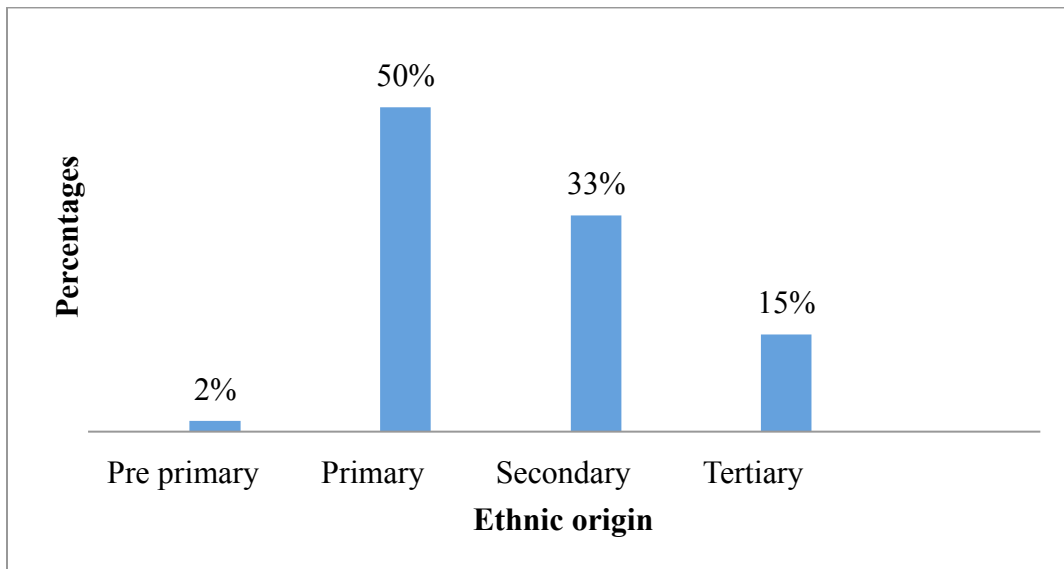
Figure 17: Level of education of respondents

4.5. Perceptions of WA-MCF

4.5.1. Recognition of WA-MCF

Almost all respondents 96.7% (58/60) knew WA-MCF to be a cattle disease. Most of them called the disease *ngatata* meaning wildebeests. Of the 58, one did not know much about the disease, one knew through informal means and one knew it from looking at the clinical signs on the head of the cattle.

4.5.2. Clinical signs of WA-MCF

The most common signs associated with MCF were cloudy eyes 68% (41/60) followed by nasal discharges 63% (38/60), not eating 63% (38/60), becoming wild 48% (29/60), heavy breathing 45% (27/60), ocular discharge 43% (26/60), weakness 25% (15/60), salivation 17% (10/60) and coughing 12% (7/60) (Figure 18). Other signs that were reported included bloody diarrhoea 2% (1/60), bloating 2% (1/60), bloody eyes 3% (2/60), weight loss 5% (3/60), dry coat 3% (2/60), darkening of fur 5% (3/60), raised fur 3% (2/60), dry muzzle 3% (2/60), high temperature 7% (4/60) and swollen lymph nodes 2% (1/60) (Figure 18).

Cloudy eyes, coughing blood and bloody diarrhoea were signs reported in the later stages of the disease. Ocular discharge was reported by one respondent who described it to begin with one eye but gets to affect both eyes when the disease progresses. Also one of the respondents who reported high temperature stated the temperature would reach $39^{o}C$ to $40^{o}C$.
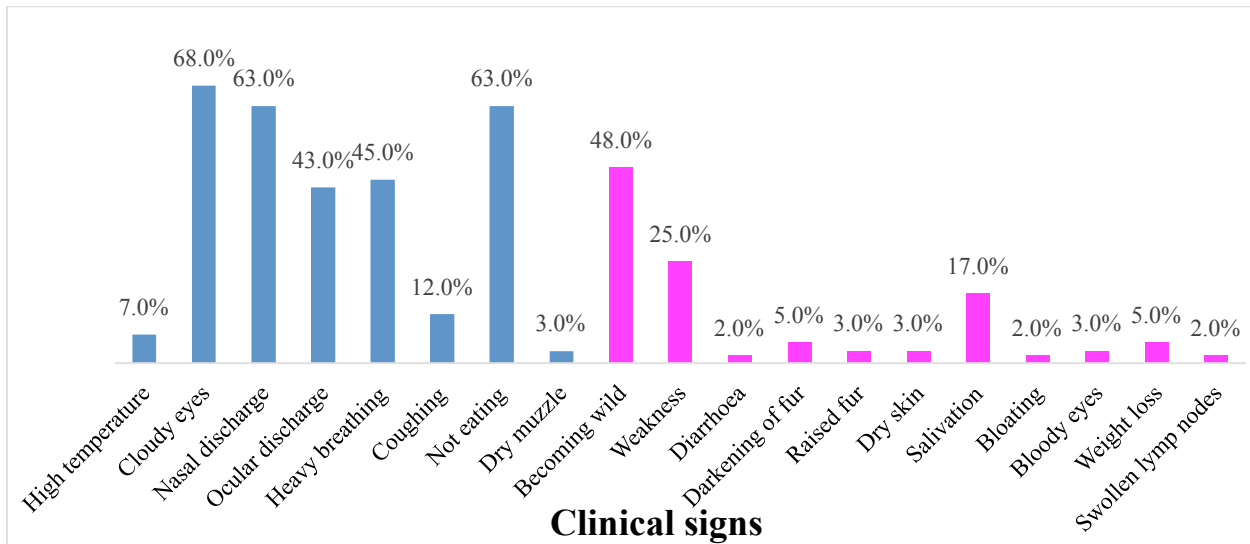
Figure 18: Clinical signs of WA-MCF at Kapiti Plains Ranch. The responses coded in blue are commonly reported signs, those in pink are additional clinical signs reported by the workers.

4.5.3. How cattle get WA-MCF

Many of the respondents associated spread of the disease to cattle from the wildebeests (Figure 19). Most of the respondents 84 % (49/58) linked the spread through contaminated pasture with 45% (22/49) stating the grass gets contaminated with placental fluid, 31% (15/49) reporting the grass gets contaminated with the placenta and 8% (4/49) saying that the grass gets contaminated by the wildebeests excrements. Almost half of respondents 45% (26/58) said the cattle get WA-MCF from calving sites of wildebeests. Other ways mentioned included hair from wildebeests 3% (2/58), rainy season 2% (1/58) but did not know how, calving time 5% (3/58), zebra 2% (1/58), gases emitted by wildebeests 2% (1/58), ticks 2% (1/58) and sleeping sites of wildebeests calves 2% (1/58). A small proportion 5% (3/58) of the respondents that knew the disease did not know how the cattle get WA-MCF.
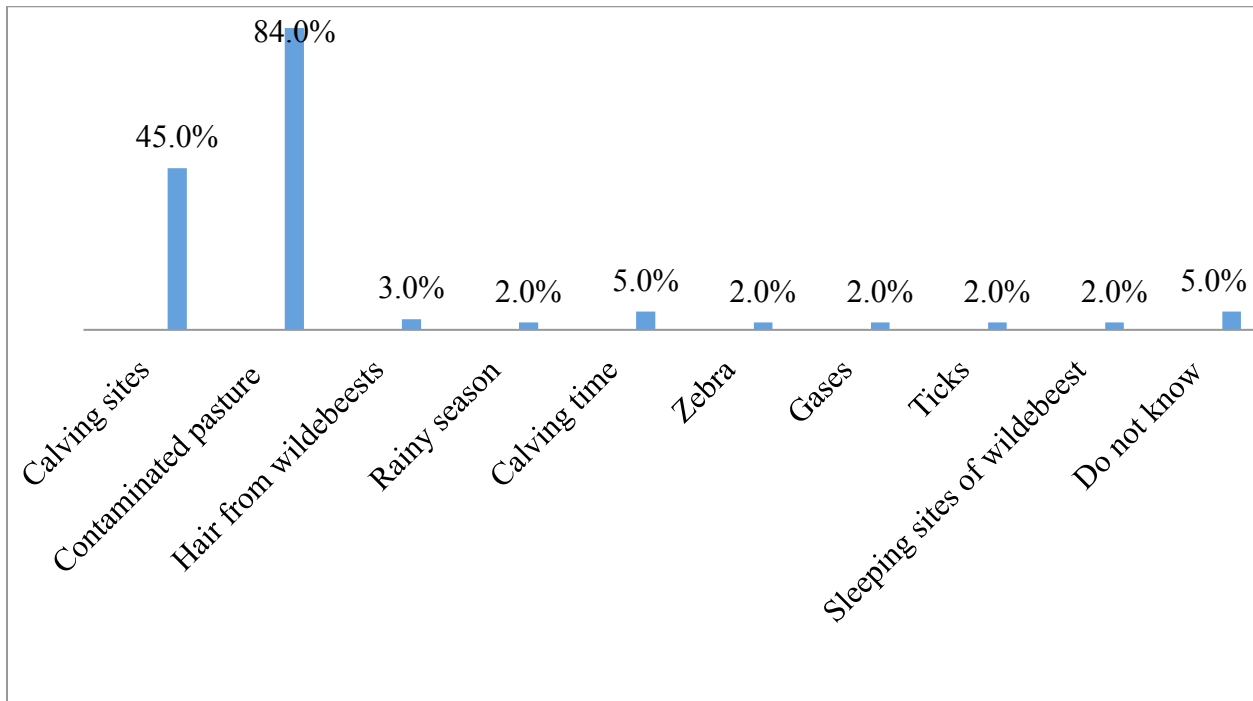
Figure 19: The different ways cattle get WA-MCF as reported by respondents at Kapiti Plains Ranch

4.5.4. Seasonality of the disease

4.5.4.1. Calving time of wildebeests

Respondents were asked when the wildebeests calved. Different months were mentioned by the respondents to indicate the period when the wildebeests gave birth (Figure 20). The month of December was reported to have many wildebeests that calved while the months of August and October had the least number of births.
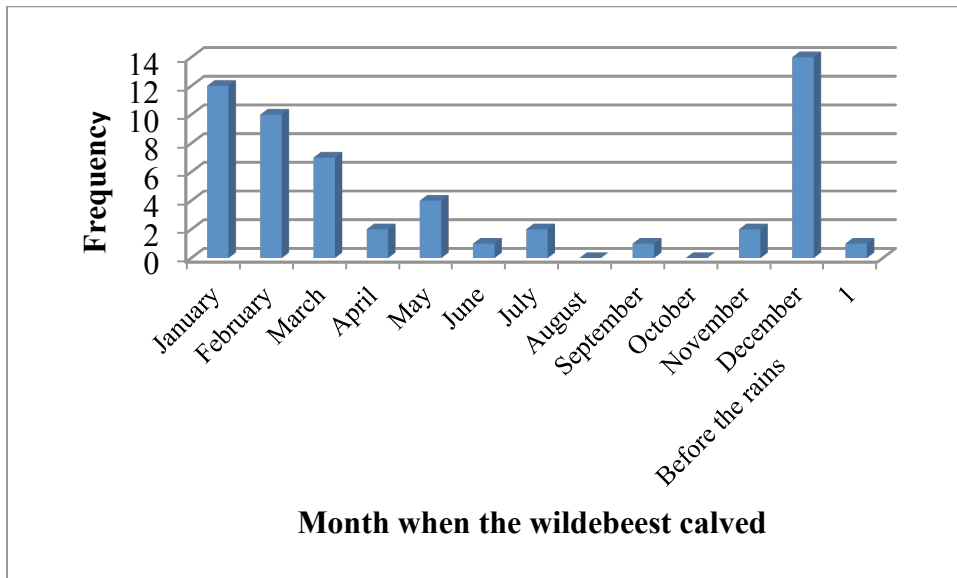
Figure 20: The time when the wildebeests calved as reported by workers at Kapiti Plains Ranch

4.5.4.2. Onset of the WA-MCF in cattle

Respondents reported a wide range from mid-February to October for when the cattle began showing clinical signs of WA-MCF. The month of April was recognized by most (29%) of the respondents for when the outbreaks began while September was the month with the least number of responses (Figure 21). One respondent could not tell when these outbreaks began while another mentioned the outbreaks began after the rains.
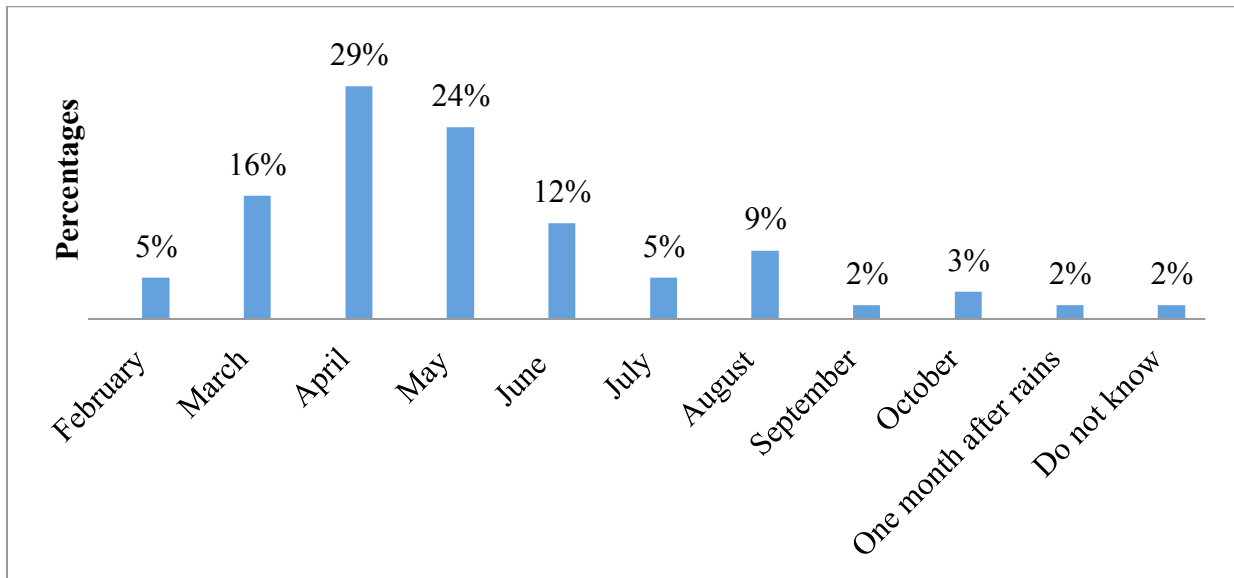
Figure 21: Onset of WA-MCF outbreaks in Kapiti Plains Ranch

4.5.4.3. End of the outbreaks in cattle

Different months were mentioned by respondents for the end of the WA-MCF outbreak in the cattle. The months of January and April were mentioned by 2% (1/58) of the respondents. A few respondents 7% (4/58) stated June while July and August had an equal representation of 29% (17/58) each. September, October and November were stated by 9% (5/58), 7% (4/58) and 3% (2/58) of the respondents respectively. Some respondents 10% (6/58) could not tell a specific month and 2% (1/58) said between four to five months after the beginning of the outbreak (Figure 22).
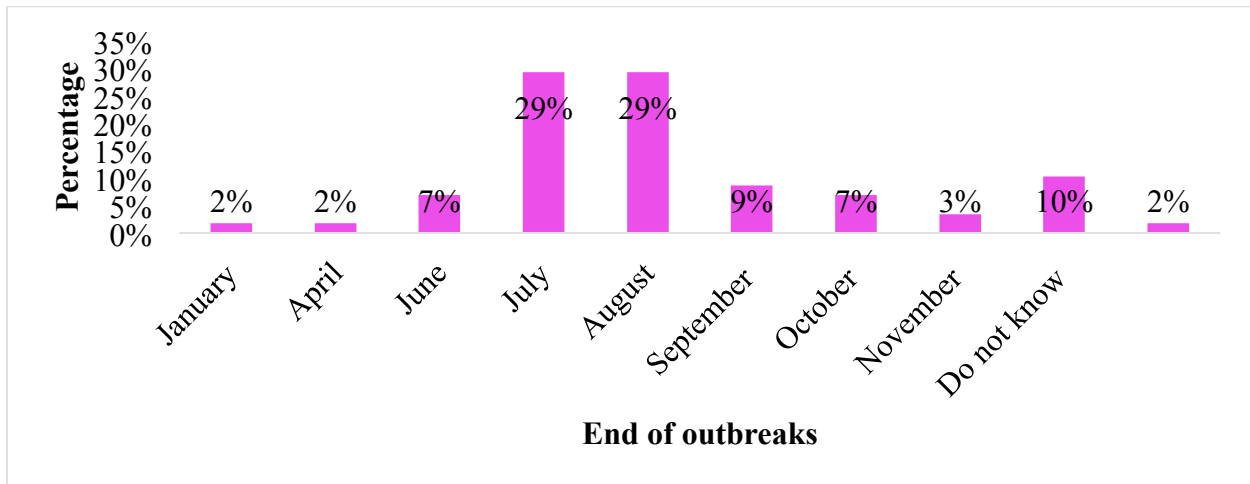
Figure 22: Months when the outbreak ended as reported by respondents at Kapiti Plains Ranch

4.5.5. Action taken in clinical cases

Many respondents 60% (35/58) said they would seek veterinary interventions (Figure 23). The majority of respondents who would seek veterinary care 66% (23/35) said they would seek help from veterinary doctors while 34% (12/35) of them said they would seek help from community based animal health workers (CBAHWs). Very few 6% (2/35) of these respondents said that they would seek this help though WA-MCF has no effective cure. The veterinary services offered included treating the animal, giving advice for slaughter and preventing future cases, and collecting blood samples for further analysis. However, 16% (9/58) respondents said they would not seek veterinary interventions with a majority of them explaining that this was due to ineffective drugs (78%; 7/9); the absence of a vaccine (11%; 1/9); and that a veterinary doctor cannot treat a disease from a wild animal (11%; 1/9).

Few respondents 12% (7/58) said they would slaughter the animal. The majority of these respondents 86% (6/7) of them said the meat would be consumed by family members while 14% (1/7) said the meat would be consumed by neighbour adding that he would not allow the head of

the sick animal to be consumed. A few respondents 12% (7/58) said they sell the animal with

71% (5/7) of them selling to butcher and 29% (2/7) of them selling to traders.
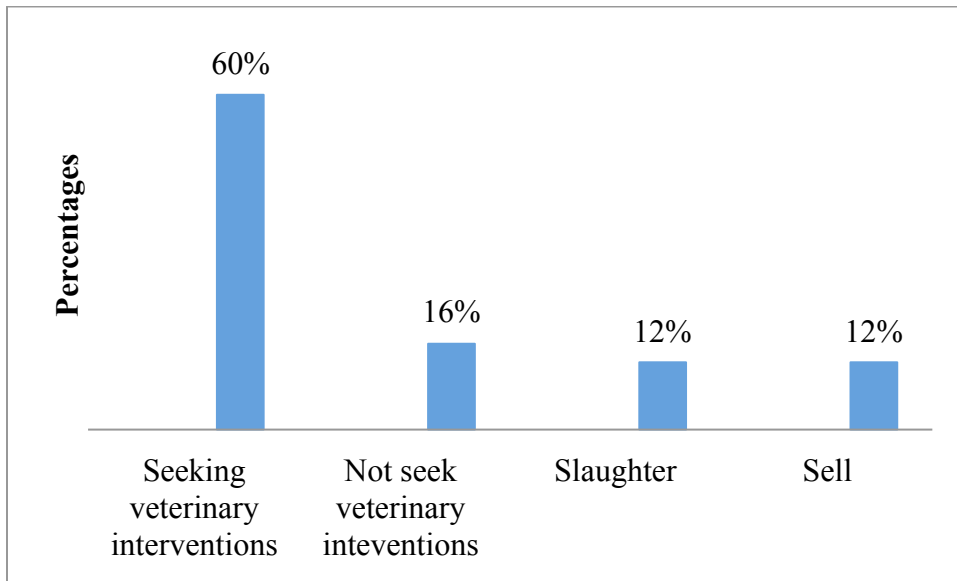
Figure 23: Action taken during clinical cases

4.5.6. Perception of disease impact

MCF was ranked to cause highest mortality among these diseases (Table 9). It was followed by

LSD and ECF respectively. CBPP and FMD were ranked fourth and fifth respectively.

MCF was ranked fifth with the least annual cases from the respondents' own farms (Table 9).

CBPP was however considered the first most important with highest cases in a year.

MCF was ranked fifth with least costs while CBPP was ranked the first with higher costs (Table

9).

Table 9. Ranks of MCF in comparison to other diseases from proportional piling

Proportional score	MCF	ECF	FMD	CBPP	LSD
Mortality (rank)	1	3	5	4	2
Annual cases (rank)	5	4	3	1	2
Annual costs (rank)	5	4	2	1	3

4.6. Discussion

4.6.1. Demographic and socio economic characteristics

There was no representation of the female gender since all the respondents were male. The main activities at Kapiti Plains Ranch are rearing of sheep and cattle for commercial and research purposes with these roles predominantly occupied by men. The livestock activities on the ranch also explains why most of the respondents were herders. Security officers were the second most dominant roles, which could be due to issues of patrolling and guarding the ranch especially from incursions from pastoralists, land grabbing and attack of wild animals.

The Kamba community was represented by a majority of the respondents. The location of the ranch in Machakos County means most of the workers come from nearby areas and are mostly of Kamba origin. All people on the ranch were of working age with a range of age from 18 years to above 50 years with most being between 31 to 35 years.

4.6.2. Perceptions of MCF

4.6.2.1. Recognition of MCF

Most of the respondents recognized WA-MCF and associated it with wildebeests demonstrating their awareness of the disease. This is consistent with similar findings in Tanzania where 99% of the respondents knew MCF as a cattle disease transmitted from wildebeests calling it *Emoyian oo engati*, which means 'disease of wildebeest' (Cleaveland *et al.*, 2001). Similar findings have also been reported in Zimbabwe where WA-MCF was reported by respondents as a disease transmitted between wildlife and livestock who called it *Ndebele* to mean disease of wildebeests (Mlilo *et al.*, 2015). Pastoralists in East Africa commonly refer to WA-MCF as *ugonjwa wa nyumbu* which is a Kiswahili translation for disease of the wildebeests (Wambua *et al.*, 2016).

4.6.2.2. Clinical signs

Many of the respondents could recognize a variety of clinical signs of WA-MCF. This is consistent with already published findings (Reid and Van Vuuren, 2004; Quinn *et al.,* 2011; OIE, 2013; Li *et al.,* 2014; Russell, 2014). The three forms of MCF were reported in this study. The head and eye form was described by respondents as oculo-nasal discharges, heavy breathing, blindness and dry muzzle, which is similar to findings in Tanzania (Mlilo *et al.*, 2015; Cleaveland *et al.*, 2001). These signs are consistent with documented literature (Reid and Van Vuuren, 2004; Russell, 2014). Bilateral corneal opacity is also a recognized sign of WA-MCF (Reid and Van Vuuren, 2004).

The general forms of MCF was recognized by respondents with reports of fever, inappetence, salivation, swollen lymph nodes, raised fur, coughing, bloody diarrhoea, bloating, dry coat, bloody eyes and weight loss. These findings are similar to reports in Tanzania. The reporting of

swollen that swollen lymph nodes was not mentioned in the study conducted in Tanzania (Cleaveland *et al.*, 2001). Swollen lymph nodes has been stated to appear during the earlier course of MCF (Quinn *et al.,* 2011).

The intestinal form represented by bloody diarrhea was reported to occur in the later stages of MCF by the respondents in this study and this contrasts to the findings in Tanzania where it was mentioned to have a rare occurrence (Cleaveland *et al.*, 2001). This study also differs to findings in Tanzania where respondents stated constipation to be a common feature of MCF and adding that it occurred during the initial phase and persisted throughout the course of the disease (Cleaveland *et al.*, 2001). These signs are consistent with the clinical signs of MCF reported in the literature (Reid and Van Vuuren, 2004; Quinn *et al.,* 2011).

The neurological form was described by animals becoming wild in this study with many saying the animals would get aggressive especially when approached by the workers. This is consistent with previous reports (Reid and Van Vuuren, 2004). However it contrasts to findings in Tanzania where circling was the only neurological sign reported (Cleaveland *et al.*, 2001).

The description of clinical signs by respondents in this ranch can aid in the management of outbreaks of WA-MCF on the ranch. Herders can report cases early, which will aid in managing the affected stock.

4.6.2.3. How cattle get MCF

It was clear that many respondents linked the spread of WA-MCF to the presence of wildebeests as in the Tanzanian study (Cleaveland *et al.*, 2001). Respondents reported the wildebeest calving season as a time when cattle get WA-MCF which shows they have knowledge of when the cattle get sick (Mushi *et al.*, 1981). Contaminated pasture by calving wildebeests was reported as the

source of MCF, in this study which is similar to findings in Tanzania (Cleaveland *et al.*, 2001). Contamination of pasture was reported to be by placental fluid, placenta and wildebeests' excrements. This is consistent with findings by Mushi *et al.* (1981) who proposed that cattle get AlHV-1 through interaction with placental fluids and fetuses and from birthing wildebeests. It has also been argued that the actual existence of placental material of wildebeests in rangelands should be seen as visual signs of newborn wildebeest calves that pose the risk of infection of WA-MCF in cattle (Wambua *et al.*, 2016).

A number of respondents mentioned hair molted by wildebeest calves as a source of disease transmission which is consistent with reports from Tanzania (Cleaveland *et al.*, 2001). The sleeping sites of the calves were also mentioned, as sources of infection, and these areas as well as areas with wildebeest hair are likely to have higher amounts of virus where wildebeest have congregated (Li *et al.,* 2014). Wildebeest calves aged 3 to 4 months of age are the biggest source of virus as they are highly viraemic and shed high quantities of the infective virus in the nasal and ocular fluids (Cleaveland *et al.*, 2001; Bedelian, 2004; Wambua *et al.*, 2016). Susceptible species like cattle acquire the virus through ingestion of contaminated foodstuffs or inhalation and ingestion of secretions that are heavily loaded with the virus (Li *et al.,* 2014). These findings also show the respondents are knowledgeable on the possible materials that can make cattle get the AlHV-1 from wildebeests.

Zebra and ticks were mentioned to spread WA-MCF. In Zimbabwe ticks were mentioned to transmit WA-MFC by farmers close to Rhodes Matopos National Park (RMNP) (Mlilo *et al*., 2015). Birds have been mentioned to spread the disease in Tanzania (Cleaveland *et al.*, 2001). However these arguments lack evidence since there is no fundamental proof of a transitional

vertebrate or invertebrate host involved in the spread of AlHV-1 from wildebeest to cattle (Wambua *et al.*, 2016).

Rain has not been previously mentioned as a direct route by which cattle get WA-MCF rather it is a factor since the wildebeests migrate to better pastures after the rains to augment their nutritional requirements and breeding (Holdo *et al.*, 2011).

4.6.2.4. Seasonality of MCF

4.6.2.4.1. Calving time of wildebeests

The month of December was recognized by many respondents to be a time for the wildebeests calving. This agrees with documented literature of when the wildebeests begin to calve with cases start to be reported early in the year (Wambua *et al.*, 2016).

4.6.2.4.2. Onset of WA-MCF

The disease was mentioned to begin between mid-February to October with outbreaks in April stated by the majority of respondents. This is similar to reports in Tanzania (Cleaveland *et al.*, 2001) and is consistent with previous reports that WA-MCF outbreaks begin in January with the greatest number of cases in March and April in Kenya occurring shortly after the wildebeests begin to calve (Wambua *et al.*, 2016).

September has been documented to have outbreaks till November when the wildebeest calves are eight to ten months old (Wambua *et al.*, 2016). This outbreak is linked to stress in cattle due to abnormally cold rainy weather in the winter months and bad grazing conditions (Reid and Van Vuuren 2005).

4.6.2.5. Action taken in clinical cases

Many respondents would seek veterinary interventions with many seeking help from veterinary doctors and few from CBAHW's. This contrasts to earlier studies where none of the respondents showed efforts to seek veterinary help instead resorting to some traditional treatments (Cleaveland *et al.*, 2001; Mlilo *et al.*, 2015).

A few respondents said they would not seek veterinary services which demonstrates knowledge that MCF lacks an effective vaccine or treatment (Russell, 2014; Wambua *et al.*, 2016). Few respondents said they would slaughter the animal and family members would eat the meat. This is consistent with previous findings although the convenience of getting meat during the WA-MCF outbreaks is likely to surpass the demand for home consumption (Cleaveland *et al.*, 2001). Very few respondents would sell the animals to the butchery. This is similar to previous findings where respondents stated they would be able to sell the meat though at lower prices usually half the normal price (Cleaveland *et al.*, 2001; Bedelian, 2004). The nature of MCF-related outbreaks makes is difficult for pastoralists to plan an effective strategy for commercial off take (Cleaveland *et al.*, 2001).

4.6.2.6. Perception on disease impact

WA-MCF was rated as the most important disease in causing mortality in cattle at Kapiti Plains Ranch. The results of WA-MCF mortality are comparable with previous findings where the case fatality of WA-MCF was reported to be 100% (Cleaveland *et al.*, 2001; Bedelian, 2004; Bedelian *et al.*, 2007). FMD was ranked the least in causing animal deaths at the ranch and this was attributed to the frequent vaccinations that are given to the animals.

However in reference to the individual respondents farms, it was considered the least important disease in causing annual cases with respondents stating it is because of absence of wildebeests

on their farms. The disease is largely reported in areas close to wildebeests (Cleaveland *et al.*, 2001; Bedelian, 2004; Bedelian *et al*., 2007). CBPP was reported to have a higher impact in the farms of these workers.

The reporting of few costs of WA-MCF also are comparable to previous findings due to lack of a preventive or curative medicine (Cleaveland *et al.*, 2001; Bedelian, 2004; Bedelian *et al*., 2007) and there is no vaccine (Cleaveland *et al.*, 2001).

CHAPTER 5: DOCUMENTING SUGGESTED METHODS OF CONTROLLING WA-MCF IN KAPITI PLAINS RANCH

5.1. Methodology

5.1.1. Study site

This was as earlier described in section 3.1.2.

5.1.2. Study design

This was as described in section 4.1.2.

5.1.3. Sampling unit

This was earlier described in section 4.1.3. The number of respondents was 60.

5.2. Data collection

This was as described in chapter 4.2. An open ended question on how to control WA-MCF was put to participants.

5.3. Data analysis

This was as described in section 4.3.

5.4. Results

Several methods of controlling WA-MCF were suggested by the 58 participants who were familiar with WA-MCF as a cattle disease. They are presented in Table 10 below.

Table 10: Methods of controlling WA-MCF by respondents

Method of controlling WA-MCF	Frequency
Chasing wildebeests	39
Zero grazing cattle	5
Moving cattle away	13
Fencing farms	21
Vaccinating cattle	5
Removing the wildebeests	5
Cooperating with livestock agents	1
Selling and buying other cattle	1
Finding origin and avoiding grazing cattle there	1
Informing others who do not know about WA-MCF	1
Practicing cleanliness	1
Killing wildebeests using a gun	1

5.5. Discussion

All the participants felt it was important to control WA-MCF. The method most suggested to control WA-MCF was chasing wildebeests. This is comparable to a previous study where participants across Isinya Division would chase the wildebeests away from their cattle with some using dogs to chase them even though the wildebeests would return at night to calve (Bedelian *et al.*, 2007). However this contrasts to findings in Tanzania where few respondents said they would chase wildebeests (Cleaveland *et al.*, 2001). Chasing wildebeests can be laborious and to

some extent can force the respondents to neglect other duties and be watchful over the wildebeests presence.

Avoidance was also suggested which included zero grazing cattle and moving cattle away This is similar to participants in Tanzania where they moved the cattle to woodland and highland areas during the calving season of wildebeests (Cleaveland *et al.*, 2001) and in Zimbabwe where the farmers grazed away from the RMNP and also grazed the cattle in paddocks (Mlilo *et al.*, 2015). Zero grazing cattle was suggested in Tanzania and Zimbabwe to ensure wildebeests and cattle do not get close contact (Cleaveland *et al.*, 2001; Mlilo *et al.*, 2015). Zero grazing cattle entails buying pasture, which also increases the cost of raising cattle. Also moving away from wildebeest calving sites has been stated to have detrimental effects on livestock productivity and the environment. The effects of moving cattle include an increase of infection of vector borne diseases, reduced intake of salt, reduction of milk production and body condition as the cattle travel large distances, overgrazing, depreciation in quality of the pasture, deforestation, bush encroachment and soil erosion (Cleaveland *et al.*, 2001). Also reduction of the livestock production contributes to land cultivation which is incompatible with wildlife conservation an pastoralism (Cleaveland *et al.*, 2001).

Fencing of farms (5%) was also mentioned with one respondent saying he would use the chain link fence. This is consistent with previous reports (Cleaveland *et al.*, 2001; Bedelian, 2004; Bedelian *et al.*, 2007). However fencing has been documented to be ineffective in controlling transmission of WA-MCF (Honiball *et al.*, 2008).

Vaccinating cattle was suggested to by few respondents. This is similar to previous findings where a vaccine has been seen as a very effective solution since it would increase the herd productivity of cattle (Cleaveland *et al.*, 2001; Bedelian *et al.*, 2007; Mlilo *et al.*, 2015).

Removing of wildebeests was mentioned by few of the respondents similarly to findings in Zimbabwe (Mlilo *et al.*, 2015). However this requires contacting the wildlife officials to come and transport the wildebeests. This may take a long time from the report to the action of transferring them.

Cooperation with livestock researchers was suggested by some participants, similarly to previous reports that conservationists and pastoralists need to work together to identify ways of benefiting both wildlife and livestock by provision of adequate animal health services to encourage livestock productivity thereby reducing the human wildlife conflicts (Cleaveland *et al.*, 2001; Bedelian *et al.*, 2007).

Disposing of all sick animals and buying others was suggested by one respondent. Selling symptomatic animals has economic disadvantages as often the animals are sold at half the price (Bedelian *et al.*, 2007). However, for this respondent it may be that he is aware that animals do not recover from WA-MCF, which is why he would opt to remove sick animals from the farm. Finding the origin and avoiding these sites was mentioned. This is similar to findings in Tanzania where one respondent could ask young boys to go and check if the wildebeests have started to calve in a bid to evade these areas (Cleaveland *et al.*, 2001).

Other methods suggested were practicing cleanliness, shooting wildebeests and informing people who are unaware of WA-MCF. However, shooting wildebeests is illegal and the respondent should be sensitized to the implications of killing wild animals.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- Nested PCR performs better in confirming clinical cases of WA-MCF than indirect ELISA. This is in line with hypothesis one as many samples tested positive by the nested PCR as compared to the indirect ELISA.

- The majority of workers at Kapiti Plains Ranch had knowledge of the epidemiology of WA-MCF. This is in agreement with hypothesis two of this study. These were in terms of recognition, clinical signs, spread to cattle, seasonality, action taken when WA-MCF manifests and disease importance. WA-MCF was also considered a very fatal disease by the workers at Kapiti Plains Ranch because of high rate of mortality. However it was considered the least important cause of disease in their own farms because of absence of wildebeests where the workers come from. In addition, in terms of costs it was considered least because of the absence of a vaccine and drugs that can effectively treat it.

- Different methods of controlling WA-MCF were outlined in this study and these were consistent with hypothesis three of this study. Due to the severity of this disease all the respondents saw it important to control WA-MCF. However some of the methods were costly and could be difficult to implement in the control of WA-MCF.

6.2. Recommendations

- The future of diagnosing WA-MCF should focus on cheap pen-side techniques to facilitate the diagnosis in the field during outbreaks. This is because the logistics

of nested PCR make it inaccessible. This will also reduce the resources and time required for the current elaborate laboratory diagnosis.

- Increased awareness on the losses incurred by cattle owners by selling animals at half price due to WA-MCF could create more focus on the need for research to enhance control.

- Communities living at the livestock-wildlife interface should be sensitized on the work of veterinary doctors as they are trained to treat both livestock and wildlife diseases.

- Controlling WA-MCF should be a priority in affected areas. Apart from efforts by farmers, livestock researchers should work hand in hand with the cattle holders to ensure they get upto date information especially on better ways to control WA-MCF.

- Concerted efforts should continue to develop a vaccine for WA-MCF as this would provide a long term solution for Kapiti Plains Ranch and other areas where WA-MCF occurs in Kenya. A vaccine would also encourage a better attitude by cattle owners towards wildebeest since they would be assured that their cattle are not at risk of getting WA-MCF.

- Better compensation schemes should be put in place to benefit farmers when WA-MCF occurs.

- The KAP study was limited to only Kapiti Plains Ranch because of logistic issues hence the sample size was small and only involved use of questionnaires and proportional piling. Future studies should be expanded to the ranches that neighbour Kapiti Plains Ranch and even the local communities and the use of

other rural participatory appraisal methods. This would enable a wider concept of WA-MCF to be captured than this study.

- Future studies should also target risk assessment studies from wildlife to livestock and damage assessment studies i.e. loss in cattle productivity and death for each calving wildebeest in a spatially defined area.

- Participatory surveillance involving communities in disease prevention using a range of social, behavioural and technical approaches should be promoted to reduce negative interactions with wildlife.

REFERENCES

Barnard, B. J. H., Bengis, R. G., Griessel, M. D. and **De Vos, V. (1989)**: 'Excretion of alcelaphine herpesvirus-1 by captive and free-living wildebeest (Connochaetes taurinus)', *Onderstepoort Journal of Veterinary Research*, **56**, pp. 131–134.

Barnard, B. J. H., Van De Pypekamp, H. E. and **Griessel, M. D. (1989):** 'Epizootology of wildebeest-derived malignant catarrhal fever in an outbreak in the north-western transvaal: indications of anintermediate host', *Onderstepoort Journal of Veterinary Research*, **56**, pp. 135–139.

Bedelian, C., Nkedianye, D. and **Herrero, M. (2007):** 'Maasai perception of the impact and incidence of malignant catarrhal fever (MCF) in southern Kenya', *Preventive Veterinary Medicine*, **78**, pp. 296–316. doi: 10.1016/j.prevetmed.2006.10.012.

Bedelian, C. (2004): *The Impact of Malignant Catarrhal Fever on Maasai Pastoral Communities in Kitengela Wildlife Dispersal Area, Kenya*. University of Edinburgh.

Bourn, D. and **Blench, R. (1999):** *Can Livestock and Wildlife Co-exist?* Edited by D. Bourn and R. Blench.

Bremer, C. W., Swart, H., Doboro, F. A., Dungu, B., Romito, M. and **Viljoen, G. J. (2005):** 'Discrimination between sheep-associated and wildebeest-associated malignant catarrhal fever virus by means of a single-tube duplex nested PCR.', *Onderstepoort Journal of Veterinary Research*, **72**(4), pp. 285–291.

Calle-Alonso, F. and **Sánchez, C. J. P. (2015):** 'A Monte Carlo – Based Bayesian Approach for

Measuring Agreement in a Qualitative Scale', *Applied Psychological Measurement*, **39**(3), pp. 189–207. doi: 10.1177/0146621614554080.

Cleaveland, S., Kusiluka, L., ole Kuwai, J., Bell, C. and Kazwala, R. (2001): *Assessing the impact of malignant catarrhal fever in Ngorongoro district, Tanzania*.

Cunha, C. W., Otto, L., Taus, N. S., Knowles, D. P. and Li, H. (2009): 'Development of a Multiplex Real-Time PCR for Detection and Differentiation of Malignant Catarrhal Fever Viruses in Clinical Samples', *Journal of Clinical Microbiology*, **47**(8), pp. 2586–2589. doi: 10.1128/JCM.00997-09.

Davison, A. J. (2010): 'Herpesvirus systematics', *Veterinary Microbiology*. Elsevier B.V., **143**(1), pp. 52–69. doi: 10.1016/j.vetmic.2010.02.014.

Davison, A. J., Richard, E., Bernhard, E., Gary, H. S., Duncan, M. J., Anthony, M. C., Philip, P. E., Bernard, R., Michael, S. J. and Etienne, T. (2009): 'The order of Herpesvirales', *Archives of Virology*, **154**(1), pp. 171–177. doi: 10.1007/s00705-008-0278-4.The.

Devleesschauwer, B., Torgerson, P., Charlier, J., Levecke, B., Praet, N., Roelandt, S., Smit, S., Dorny, P., Dirk, B. and Niko, S. (2015): 'CRAN - Package prevalence'.

Dohoo, I., Martin, W. and Stryhn, H. (2003): *Veterinary Epidemiologic Research*. Edited by M. M. S. Charlottetown: AVC Inc.

Ego, W. K., Mbuvi, D. M. and Kibet, P. F. K. (2003): 'Dietary composition of wildebeest (Connochaetes taurinus) kongoni (Alcephalus buselaphus) and cattle (Bos indicus), grazing on a common ranch in south-central Kenya', *African Journal of Ecology*,

41(1), pp. 83–92. doi: 10.1046/j.1365-2028.2003.00419.x.

Ensser, A., Pflanz, R. and **Fleckenstein, B. (1997):** 'Primary Structure of the Alcelaphine Herpesvirus 1 Genome', *Journal of Virology*, **71**(9), pp. 6517–6525.

Feinstien, A. R. and **Cicchetti, D. V (1990):** 'High agreement but low Kappa: I. The problems of two paradoxes', *Journal of Clinical Epidemiology*, **43**(6), pp. 543–549. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84936084977&partnerID=tZOtx3y1.

Flach, E. J., Reid, H., Pow, I. and **Klemt, A. (2002):** 'Gamma herpesvirus carrier status of captive artiodactyls', *Research in Veterinary Science*, **73**(1), pp. 93–99. doi: 10.1016/S0034-5288(02)00094-2.

Fraser, S. J., Nettleton, P. F., Dutia, B. M., Haig, D. M. and **Russell, G. C. (2006):** 'Development of an enzyme-linked immunosorbent assay for the detection of antibodies against malignant catarrhal fever viruses in cattle serum', *Veterinary Microbiology*, **116**, pp. 21–28. doi: 10.1016/j.vetmic.2006.03.002.

Graham, P. and **Bull, B. (1998):** 'Approximate Standard Errors and Confidence Intervals for Indices of Positive and Negative Agreement', *Journal of Clinical Epidemiology*, **51**(9), pp. 763–771.

Hamblin, C. and **Hedger, R. S. (1984):** 'Neutralising antibodies to wildebeest-derived malignant catarrhal fever virus in African wildlife', *Comparative Immunology, Microbiology & Infectious Diseases*, **7**(3–4), pp. 195–199.

Holdo, R. M., Fryxell, J. M., Sinclair, A. R. E., Dobson, A. and **Holt, R. D. (2011)** 'Predicted

impact of barriers to migration on the Serengeti wildebeest population', *PLoS ONE*, **6**(1), pp. 1–7. doi: 10.1371/journal.pone.0016370.

Honiball, E. J., Van Essen, L. D. and **Du toit, J. G. (2008):** *A review of malignant catarrhal fever in the Republic of South Africa*.

Hsu, D., Shih, L. M., Castro, A. E. and **Zee, Y. C. (1990):** 'A diagnostic method to detect alcelaphine herpesvirus-1 of malignant catarrhal fever using the polymerase chain reaction', *Archives of Virology*, **114**, pp. 259–263.

Katz, J., Seal, B. and **Ridpath, J. (1991):** 'Molecular diagnosis of alcelaphine herpesvirus (malignant catarrhal fever) infections by nested amplification of viral DNA in bovine blood buffy coat specimens.', *Journal of Veterinary Diagnostic Investigation*, **3**, pp. 193–198. doi: 10.1177/104063879100300301.

Lankester, F., Russell, G. C., Lugelo, A., Ndabigaye, A., Mnyambwa, N., Keyyu, J., Kazwala, R., Grant, D., Percival, A., Deane, D., Haig, D. M. and **Cleaveland, S. (2016):** 'A field vaccine trial in Tanzania demonstrates partial protection against malignant catarrhal fever in cattle', *Vaccine*. Elsevier Ltd, **34**(6), pp. 831–838. doi: 10.1016/j.vaccine.2015.12.009.

Lankester, F., Lugelo, A., Kazwala, R., Keyyu, J., Cleaveland, S. and **Yoder, J. (2015):** 'The economic impact of malignant catarrhal fever on pastoralist livelihoods', *PLoS ONE*, **10**(1), pp. 1–21. doi: 10.1371/journal.pone.0116059.

Lankester, F., Lugelo, A., Mnyambwa, N., Ndabigaye, A., Keyyu, J., Kazwala, R., Grant, D. M., Relf, V., Haig, D. M., Cleaveland, S. and **Russell, G. C. (2015):**

'Alcelaphine Herpesvirus-1 (Malignant Catarrhal Fever Virus) in Wildebeest Placenta : Genetic Variation of ORF50 and A9.5 Alleles', *PLoS ONE*, **10**(5), pp. 1–14. doi: 10.1371/journal.pone.0124121.

Li, H., Cunha, C. W., Taus, N. S. and **Knowles, D. P. (2014):** 'Malignant Catarrhal Fever: Inching Toward Understanding', *Annual Review of Animal Biosciences*, **2**, pp. 209–233. doi: 10.1146/annurev-animal-022513-114156.

Li, H., Cunha, C. W. and **Taus, N. S. (2011):** 'Malignant catarrhal fever: Understanding molecular diagnostics in context of epidemiology', *International Journal of Molecular Sciences*, **12,** pp. 6881–6893. doi: 10.3390/ijms12106881.

Li, H., Karney, G., O'Toole, D. and **Crawford, T. B. (2008):** 'Lond distance spread of malignant catarrhal fever from feedlot lambs to ranch bison', *Canadian Veterinary Journal*, **49**, pp. 183–185.

Li, H., Taus, N. S., Jones, C., Murphy, B., Evermann, J. F. and **Crawford, T. B. (2006):** 'A devastating outbreak of malignant catarrhal fever in a bison feedlot.', *Journal of Veterinary Diagnostic Investigation*, **18**(1), pp. 119–123. doi: 10.1177/104063870601800120.

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. and **Crawford, T. B. (2005):** 'A novel subgroup of rhadinoviruses in ruminants', *Journal of General Microbiology*, **86**, pp. 3021–3026. doi: 10.1099/vir.0.81238-0.

Li, H., Gailbreath, K., Bender, L. C., West, K., Keller, J. and **Crawford, T. B. (2003):**

'Evidence of Three New Members of Malignant Catarrhal Fever Virus Group in Muskox (Ovibos moschatus), Nubian ibex (Capra nubiana), and Gemsbok (Oryx gazella)', *Journal of Wildlife Diseases*, **39**(4), pp. 875–880. doi: 10.7589/0090-3558-39.4.875.

Li, H., McGuire, T. C., Müller-Doblies, U. U. and Crawford, T. B. (2001): 'A simpler, more sensitive competitive inhibition enzyme-linked immunosorbent assay for detection of antibody to malignant catarrhal fever viruses', *Journal of Veterinary Diagnostic Investigation*, **13**(4), pp. 361–364.

Li, H., Dyer, N., Keller, J. and Crawford, T. B. (2000): 'Newly Recognized Herpesvirus Causing Malignant Catarrhal Fever in White-Tailed Deer (Odocoileus virginianus)', *Journal of Clinical Microbiology*, **38**(4), pp. 1313–1318.

Li, H., Shen, D. T., Jessup, D. a, Knowles, D. P., Gorham, J. R., Thorne, T., O'Toole, D. and Crawford, T. B. (1996): 'Prevalence of antibody to malignant catarrhal fever virus in wild and domestic ruminants by competitive-inhibition ELISA.', *Journal of wildlife diseases*, **32**(3), pp. 437–443.

Li, H., Shen, D. T., Knowles, D. P., Gorham, J. R. and Crawford, T. B. (1994): 'Competitive inhibition enzyme-linked immunosorbent assay for antibody in sheep and other ruminants to a conserved epitope of malignant catarrhal fever virus', *Journal of Clinical Microbiology*, **32**(7), pp. 1674–1679.

Mlilo, D., Mhlanga, M., Mwembe, R., Sisito, G., Moyo, B. and Sibanda, B. (2015): 'The epidemiology of malignant catarrhal fever (MCF) and contribution to cattle losses in farms around Rhodes Matopos National Park, Zimbabwe', *Tropical Animal*

Health and Production, **47**(5), pp. 989–994. doi: 10.1007/s11250-015-0821-8.

Mlilo, D., Mhlangaa, M., Moyoc, B., Sisitob, G., Mwembeb, R., Sibandaa, B., Dagab, O. and **Ncubeb, S. (2015):** 'Perceptions on Malignant catarrhal fever (MCF) by farmes close to Rhodes Matopos National Park, Zimbabwe', *International Journal of Development Research*, **5**(12), pp. 6314–6319.

Muller-Doblies, U. U., Li, H., Hauser, B., Adler, H. and **Ackermann, M. (1998):** 'Field Validation of laboratory Tests for clinical diagnosis of sheep-associated malignant catarrhal fever', *Journal of Clinical Microbiology*, **36**(10), pp. 2970–2972.

Mushi, E. Z. and **Rurangirwa, F. R. (1981):** 'Epidemiology of bovine malignant catarrhal fever', *Veterinary Research Communications*, **5**, pp. 127–142.

Mushi, E. Z., Rurangirwa, F. R. and **Karstad, L. (1981):** 'Shedding of malignant catarrhal fever virus by wildebeest calves', *Veterinary Microbiology*, **6**, pp. 281–286.

Mushi, E. Z., Rossiter, P. B., Karstad, L. and **Jessett, D. M. (1980):** 'The demonstration of cell-free malignant catarrhal fever herpesvirus in wildebeest nasal secretions', *Journal of Hygiene (Cambridge)*, **85**(2), pp. 175–179. doi: 10.1017/S0022172400063208.

O'Toole, D. ., Li, H., Sourk, D. L., Montgomery, T. B. and **Crawford, T. B. (2002):** 'Malignant catarrhal fever in a bison (Bison bison) feedlot, 1993-2000', *Journal of Veterinary Diagnostic Investigation*, **14**, pp. 183–193.

O'Toole, D. ., Li, H., Miller, D., Williams, W. R. and **Crawford, T. B. (1997):** 'Chronic and recovered cases of sheep-associated malignant catarrhal fever in cattle', *Veterinary*

Record, **140**, pp. 519–525.

OIE (2013): 'Malignant catarrhal fever', in *Manual of Diagnostic Tests and Vaccines for Terrestial Animals (mammals, birds and bees)*. Paris: Office International Des Epizooties, pp. 1–12.

Ottichilo, W. K., de Leeuw, J. and **Prins, H. H. T. (2001):** 'Population trends of resident wildebeest [Connochaetes taurinus hecki (Neumann)] and factors influencing them in the Masai Mara ecosystem, Kenya', *Biological Conservation*, **97**(3), pp. 271–282. doi: 10.1016/S0006-3207(00)00090-2.

Perry, B. D., Randolp, T. F., McDermott, J. J., Sones, K. R. and **Thornton, P. K. (2002):** *Investing in animal health research to alleviate poverty*.

Plowright, W. (1990): 'Malignant catarrhal fever virus', in Dinter, Z. and Morein, B. (eds) *Virus infections of Ruminants*. Newyork: Elsevier Science Publishers, B.V, pp. 123–150. doi: 10.1016/0378-1135(87)90022-8.

Plowright, W. (1986): 'Malignant Catarrhal Fever', *Revue scientifique et technique (International Office of Epizootics)*, **5**(4), pp. 897–918.

Plowright, W., Ferris, R. D. and **Scott, G. R. (1960):** 'Blue wildebeest and the aetiological agent of bovine malignant catarrhal fever', *Nature*, **188**(4757), pp. 1167–1169.

Powers, J. G., VanMetre, D. C., Collins, J. K., Dinsmore, R. P., Carman, J., Patterson, G., Brahmbhatt, D. and **Callan, R. J. (2005):** 'Evaluation of ovine herpesvirus type 2 infections, as detected by competitive inhibition ELISA and polymerase chain reaction assay, in dairy cattle without clinical signs of malignant catarrhal fever.',

Journal of the American Veterinary Medical Association, 227(4), pp. 606–611. doi: 10.2460/javma.2005.227.606.

Pretorius, J. A., Oosthuizen, M. C. and **Vuuren, M. van (2008):** 'Gammaherpesvirus carrier status of black wildebeest (Connochaetes gnou) in South Africa', *Journal of the South African Veterinary Association*, **79**(3), pp. 136–141. doi: 10.4102/jsava.v79i3.260.

Quinn, P. J., Markey, B. K., Leonard, F. C., FitzPatrick, E. S., Fanning, S. and **Hartigan, P. J. (2011a):** 'Herpesviridae', in *Veterinary Microbiology and Microbial Disease*. 2nd edn. West Sussex: Wiley-Blackwell, pp. 1479–1524. Available at: www.wiley.com/wiley-blackwell.

Quinn, P. J., Markey, B. K., Leonard, F. C., FitzPatrick, E. S., Fanning, S. and **Hartigan, P. J. (2011b): '**Malignant catarrhal fever', in *Veterinary Microbiology and Microbial Disease*. 2nd edn. Chichester: Wiley-Blackwell, pp. 1492–1496.

Reid, H. W. and **Van Vuuren, M. (2005):** Bovine malignant catarrhal fever. In: *Infectious diseases of livestock,* 2nd edn. Oxford University Press.

Reid, H. W. and **Van Vuuren, M. (2004):** 'Malignant catarrhal fever', in Coetzer JAW, T. R. C. (ed.) *Infectious diseases of livestock*. 2nd edn. Cape Town: Oxford University Press, pp. 895–908.

Reid, H. W. and **Bridgen, A. (1991):** 'Recovery of a herpesvirus from a roan antelope (Hippotragus equinus)', *Veterinary Microbiology*, **28**, pp. 269–278.10.1007/978-1-4613-1587·2

Reid, H. W. and **Buxton, D. (1989)** 'Malignant catarrhal fever and the gammaherpesvirinae of Bovidae', in Wittmann, G. (ed.) *Herpesvirus diseases of cattle , horses and pigs*. 1st edn. Massachusetts: Kluwer Academic Publishers, pp. 116–162. doi:

Reid, R., Gichohi, H., Said, M., Nkedianye, D., Ogutu, J. and **Kshatriya, M. (2008)** 'Fragmentation of a peri-urban Savanna, Athi-Kaputiei Plains, Kenya.', in *Fragmentation in Semi-Arid and Arid Landscapes Consequences for Human and Natural Systems*. Netherlands: Springer, pp. 195–224. doi: doi: 10.1007/978-1-4020-4906-4_9.

Russell, G. C. (2014): 'Malignant Catarrhal Fever Virus (Alcelaphine Herpesvirus Type 1)', in Liu, D. (ed.) *Manual of Security Sensitive Microbes and Toxins*, pp. 679–688.

Russell, G. C., Benavides, J., Grant, D., Todd, H., Deane, D., Percival, A., Thomson, J., Connelly, M. and **Haig, D. M. (2012**): 'Duration of protective immunity and antibody responses in cattle immunised against alcelaphine herpesvirus-1-induced malignant catarrhal fever', *Veterinary Research*, **43(**1), pp. 1–11. doi: 10.1186/1297-9716-43-51.

Russell, G. C., Stewart, J. P. and **Haig, D. M. (2009):** 'Malignant catarrhal fever: A review', *Veterinary Journal*, **179**(3), pp. 324–335. doi: 10.1016/j.tvjl.2007.11.007.

Sood, R., Hemadri, D. and **Bhatia, S. (2013):** 'Sheep associated malignant catarrhal fever : an emerging disease of bovids in India', *Indian Journal of irology*, **24**(3), pp. 321–331. doi: 10.1007/s13337-013-0163-y.

Talbot, L. M. and **Talbot, M. H. (1963**): 'The Wildebeest in Western Masailand, East Africa',

in *Wildlife Monographs*. Newyork: Wiley, pp. 3–88.

Tham, K. M., Ng, K. and **Young, L. W. (1994):** 'Polymerase chain reaction amplification of wildebeest-associated and cervine-derived malignant catarrhal fever virus DNA', *Archives of Virology*, **135**, pp. 355–356.

Thomas, L. F. (2013): *Epidemiology of Taenia solium Cysticercosis in western Kenya*. Universtity of Edinburgh. Available at: https://www.era.lib.ed.ac.uk/handle/1842/8849.

Traul, D. L., Elias, S., Taus, N. S., Herrmann, L. M., Oaks, J. L. and **Li, H. (2005):** 'A real-time PCR assay for measuring alcelaphine herpesvirus-1 DNA', *Journal of Virologial Methods*, **129**, pp. 186–190. doi: 10.1016/j.jviromet.2005.05.021.

Wambua, L., Wambua, P. N., Ramogo, A. M., Mijele, D. and **Otiende, M. Y**. **(2016):** 'Wildebeest-associated malignant catarrhal fever: perspectives for integrated control of a lymphoproliferative disease of cattle in sub-Saharan Africa', *Archives of Virology*. Springer Vienna, 161, pp. 1–10. doi: 10.1007/s00705-015-2617-6.

Western, D. (1982): 'Amboseli National Park: Enlisting Landowners to Conserve Migratory Wildlife', in *Ambio*. Berlin: Springer, pp. 302–308. Available at: http://www.jstor.org/stable/4312824.

Whitaker, K. A., Wessels, M. E., Campbell, I. and **Russell, G. C. (2007):** 'Outbreak of wildebeest-associated malignant catarrhal fever in Ankole', *Veterinary Record*, **161**, pp. 692–695. doi: 10.1136/vr.161.20.692.

https://www.medcalc.org/calc/diagnostic_test.php). Viewed on 1st August 2017

(www://ilriangr.wikispaces.com/file/view/Kapiti-20150218.pdf). Viewed on 15th July 2016

Appendix 1. IDENTIFICATION OF THE KNOWLEDGE, ATTITUDES AND PRACTISES OF MALIGNANT CATARRHAL FEVER IN KAPITI PLAINS RANCH

Good morning/afternoon, I am **Sheillah Ayiela Orono**, a Master's student from the University of Nairobi, *College of Agriculture and Veterinary Sciences,* Faculty of Veterinary Medicine in the Department of Veterinary Pathology, Microbiology and Parasitology. We are conducting a research on "**Assessing the knowledge, attitudes and practises of Malignant Catarrhal Fever in Kapiti".** The results from this study will help us to identify the major problems of MCF and possibly help in finding solutions from relevant stake holders in combating the disease. I would like to assure you that the information you will provide is entirely for research purpose and therefore it will be kept confidential.

Date of interview:……………….

Qno:………………

GENERAL INFORMATION

Interview: start time   [_____:_____]

Interview: end time   [_____:_____]

1. Farm code:………………………...

2. County:……………………………………………………………………………..

3. Sub county……………………………………………………………………………….

4. Village…………………………………………………………………………………….

PART A: PARTICULARS OF RESPONDENT

   1. Name of respondent   ………………………………….

   2. Role of the respondent

        a) Farm owner

        b) Family member _____

        c) Spouse

        d) Farmhand/herder…………………………………………………………………

      e) Other_____

  3. Gender: (a) Male [____]   (b) Female [____]

4. Age (years)

a) 18 – 25 yrs ☐

b) 26 – 30 yrs ☐

c) 31-35 yrs ☐

d) 36-40 yrs ☐

e) 41-45 yrs ☐

f) 46-50 yrs ☐

g) More than 50 yrs ☐

h) Don't know or prefer not to say ☐

5. Which highest level of education have you attained?

a) Non formal education ☐

b) Pre-primary education ☐

c) Primary level ☐

d) Secondary level ☐

e) Tertiary level ☐

f) Other………………………….. ☐

6. Which cattle type and what is your herd size?

Animal class	Number
Male	
Female	
Calves (<10months)	

7. What breeds?

Breed	Number owned
Grade (Friesian, Jersey, Ayrshire)	
Short horn Zebu	
Boran	
Cross breed	
Other	

8. What are the main reasons for rearing cattle? (Tick all that apply)

Products	Yes	No
Milk (*yiiya*)		
Beef (*nyama*)		
Labour (*wia*)		
Prestige (*uthwii*)		
Other		

9. Which other livestock do you keep? (Tick all that apply)

Livestock group	Yes	No
Sheep (*malunga*)		
Goats (*mbui*)		
Pigs (*ngulue*)		
Donkeys (*mang'oi*)		
Poultry (*nguku*)		
Camels (*ngamile*)		
Other		

PART B – 1: ASSESSMENT OF KNOWLEDGE

1. Do you know malignant catarrhal fever (MCF) (local name)?   Yes   No

 If yes, what are the main signs of this disease (tick all that apply)?

Main signs of the disease	Yes	No
a) High temperature		
b) Cloudy eyes		
c) Discharge from the nose		
d) Discharge from the eyes		
e) Heavy breathing /open mouth		
f) Coughing		
g) Not eating		
h) Hard muzzle		

2. How do the cattle get MCF (record all comments)?

_____

_____

_____

_____

PART B – 2: LOSSES RELATED TO MCF

1. Have you experienced the disease in your farms/ranch? YES   NO

If yes, specific questions about the last outbreak (2017)

    a) When did the wildebeest calve? Month_____

    b) When did the outbreak start? Month_____

    c) How long did the outbreak last?_____

    d) How many animals did you lose?_____

Animal class	Number died
Male	
Female	
Calves (<10months)	

Breed	Number died
Grade (Friesian, Jersey, Ayrshire)	
Short horn Zebu	
Boran	
Cross breed	
Other	

2. What costs did you incur during the last outbreak?

Livestock management costs	Costs
Emergency slaughter (cost per animal)	
Emergency sale (cost per animal lost)	
Exchanging sick animals for other livestock	
Animals deaths (cost per animal lost)	
Buying extra feeds	
Salt licks	
Mitigation costs	
Fencing	
Fuel vehicles	
Labour costs (farm hands)	
Migration	
Moving animals (total costs)	
Costs of grazing (total costs)	
Rehoming the household (cost)	

3. Rate MCF losses in relation to other animal diseases. (proportional piling)

Disease	Rank
ECF (*Ngai*)	
MCF (*Uwau wa Ngatata*)	
BVD ()	
FMD (*Muthingithu*)	
CBPP (*Mavui*)	
LSD (*Ikundu*)	

PART B – 3: CONTROL OF MCF

1. How do you handle the MCF affected cattle (tick all that apply)?

    a) Call for veterinary interventions?   YES   NO

        1) If yes from whom do you seek the interventions?

Intervention	Tick where appropriate
Veterinary Doctors	
Community based animal health workers	
Agro veterinary shops	

2) If no, why?

Reason	Tick where appropriate
Expensive drugs	
Distance to farm	
Drugs being ineffective	

b) Self-treatment　　　YES　　　　　　　NO

If yes which treatment?

Type of treatment	Tick where appropriate
Traditional medicine	
Antibiotics (e.g. Oxytet /Penstrep)	
Anti-inflammatories (e.g. Dex/Flunixin)	

c) Quarantine　YES　　　NO

d) Slaughter for home consumption　YES　　　NO

If yes who eats

Who eats	Tick where appropriate
Family	
Friends	
Neighbours	

e) Sell   YES   NO

If yes to who?

Sell to who	Tick where appropriate
Butcher	
Trader	
Neighbours	
Other	

f) Exchange with other livestock   YES   NO

g) Other (specify)

_____

2. What are the methods you employ in preventing MCF?

Methods	Tick where appropriate

Chasing the wildebeest	
Zero grazing the cattle	
Fencing the ranches	
Moving the animals away	
Removing the wildebeest	
Others (specify):	

Asanteni