

**EFFICACY OF THE MUGUGA COCKTAIL EAST COAST FEVER VACCINE AT A
CATTLE-BUFFALO INTERFACE IN LAIKIPIA COUNTY, KENYA.**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
in Veterinary Epidemiology and Economics.

Zaverio N. Kiraithe (BVM).

Department of Public Health Pharmacology and Toxicology,

Faculty of Veterinary Medicine

University of Nairobi

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DECLARATION

I declare that this thesis is my original work and has not been presented for a degree in any other University and that all sources of material that are used for this thesis have been duly acknowledged.

Signature _____ Date _____

Dr. Zaverio N. Kiraithe (BVM - UON)

This thesis has been submitted with our approval as University Supervisors:

Signature _____ Date _____

Prof. Jackson N. Ombui (BVM, MSc, PhD)

Signature _____ Date _____

Prof. Philip M. Kitala (BVM, MSc, PhD)

Signature _____ Date _____

Dr. John M. Mugambi (BVM, MSc, PhD)

DEDICATION

This Thesis is dedicated to my beloved soul mate and wife Jane M. Kiraithe; Our Children: Ann M. Kiraithe, Gerald M. Kiraithe, Late Anthony G. Kiraithe and Dennis M. Kiraithe.

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ABSTRACT

The study was conducted to assess the efficacy of Muguga Cocktail East Coast Fever (ECF) vaccine at a cattle-buffalo interface in some ranches in Laikipia County.

It is not known with certainty how the current vaccine for cattle against East Coast Fever, that is, Infection and Treatment Method (ITM) using the Muguga Cocktail would perform in areas where buffalos exist with cattle. This is because of the suspicion that buffalo strain of *T. parva* circulating at the cattle-buffalo interface would lower the efficacy of the ITM with *T. parva* strains sourced from cattle. The specific objectives of the study were to;

1. Assess the efficacy of ECF vaccine in cattle at a cattle-buffalo interface in Laikipia County.
2. Determine the viability of the ECF vaccine..

The study was conducted in two ranches which were conveniently selected including Mutara and Ole Naishu with herd sizes of 986 and 2,796 heads of cattle, respectively. A controlled trial was conducted on the two ranches whereby calves were recruited for the study. The calves had no previous history of East Coast Fever (ECF) infection. In both the treatment and control group, a total of 65 immunized and control calves were recruited in each of the ranches. The calves were randomly selected and allocated to immunized and control groups. Calves in the treatment group were injected with Muguga cocktail using a standard protocol developed at the Veterinary Research Institute, Muguga. Prior to the injection of the vaccine, a 30% long acting oxytetracycline was administered at a dose of 30mg per body weight by deep intramuscular injection. Any immunized calf that developed clinical signs of ECF with fever and presence of macroschizonts (7-10 days post immunization) in lymph node smears for at least three days was designated as “ECF reactor”. Calves were bled pre-immunization at day 0 and then again 35 days post immunization to determine the antibody response using an Indirect ELISA test, this was to test for the viability of the vaccine. Daily clinical surveillance was kept on all the calves in both the treatment and control groups for twelve months.

The sero-conversion in the vaccinated group was 83% in Ole Naishu and 69% in Mutara. The sero-conversion among the control groups was 3.1% and 6.2% in Ole Naishu and Mutara respectively.

The efficacy of the vaccine was established at 97.8% for Ole Naishu, and 78.4% for Mutara. The ECF vaccine (Muguga cocktail) was not as efficacious in Mutara ranch as in Ole Naishu ranch. This may be attributed to sharing of grazing of cattle with buffalos in Mutara which was not the case in Ole Naishu.

CHAPTER ONE

1.0 Introduction

1.1. Background

Approximately 80% of the human population in Africa is largely dependent on agriculture. Kenya is estimated to have a total of 13.5 million cattle of which 3.2 million are exotic breeds of dairy and beef in both large and small scale holdings in the country's high potential areas . About 10.5 million cattle are the indigenous zebu found in arid and semi arid areas (ASALS) which embody 80% of the country's landscape. The livestock sector contributes to slightly over 10% of the country's national income and employs over 50% of the total agricultural sector.

East Coast Fever (ECF) is a major challenge to the growth of Kenya's cattle industry as 76% of the cattle population is at risk of succumbing to the disease. The distribution and the concentration of the tick vector *Rhipicephalus appendiculatus* that carries the affiliate pathogen *Theileria parva* correlates to a large extent with the highest concentration of cattle of either indigenous or exotic classification. The disease is responsible for annual mortality rates of 40-80% in cattle (Homewood *et al.*, 2006; Di Guillo *et al.*, 2009).

Although several strategies have been used in the past for the control of ECF with varying results, the use of a vaccine against the disease has been advocated by several authors as the most cost effective (Minjauw and McLeod, 2003). It is estimated that an effective ECF vaccine for cattle could save the countries where the disease is endemic at least 3 million US dollars a year (ILRI, 2010).

The vaccines currently in use in Kenya are : 1). Muguga cocktail : a preparation of the Muguga strain, Kiambu 5 strain isolate both derived from cattle *T. parva* and buffalo- derived *T. parva* strains isolated from Serengeti. This vaccine is used in areas where cattle have shared grazing with buffalos). 2. Marikebuni is used in the dairy cattle and where there is no shared grazing of cattle and buffalos and is derived from *T. parva* from Marikebuni strain in the coast region.

A number of studies were conducted to establish the efficacy of the ECF vaccine in the Central, Coastal, South and North Rift Valley regions of Kenya and in the Ngorongoro region of Tanzania (Young *et al.*, 1981; Radley, 1981; Mutugi *et al.*, 1989; Mutugi *et al.*, 1991; Wesonga *et al.*, 2000; Wanjohi *et al.*, 2001; Babo Martins *et al.*, 2010). All these studies demonstrated a

significant difference between the numbers of cases of ECF observed among immunized compared to the non-immunized cattle.

Although the efficacy of Muguga Cocktail vaccine has been demonstrated, there have been concerns that it does not work so well in areas where wildlife and domestic animals interact (Rashid *et al.*, 2009). In these interfaces it is believed a strain of the causative agent of ECF (*T. parva*) adapted to buffalos may make the vaccine less efficacious (Katzer *et al.*, 2009). This phenomenon is referred to as ‘break through’, that is cattle that have sero-converted after vaccination, come down with ECF.

1.2 Objectives

1.2.1 Overall objective

The overall objective of the study was to assess the efficacy of the ECF vaccine at a cattle-buffalo interface in cattle in ranches in Laikipia.

1.2.2 The specific objectives were to:

1. Assess the efficacy of East Coast Fever (ECF) vaccine in cattle at a cattle-buffalo interface in Laikipia County.
2. Determine the viability of the ECF vaccine.

CHAPTER TWO

2.0. Literature Review

2.1 Definition of East Coast Fever

East Coast fever (ECF), a form of bovine theileriosis, is a tick-transmitted protozoal disease of cattle characterized by high fever and lymphadenopathy. The tick responsible for the transmission of the disease is the brown ear tick, *Rhipicephalus appendiculatus*. The disease causes high mortalities in breeds not indigenous to the endemic areas, and is confined to Eastern, Central, and parts of Southern Africa (Young *et al.*; 1989, Norval *et al.*, 1992).

2.2 Etiology

The causative agent of classical ECF is *Theileria parva*. Some previously recognized separate species and subspecies have been combined with *T. parva* as a result of studies on their DNA (Conrad *et al.*, 1989). The life cycle of *T. parva* is complex in its tick and mammalian hosts (Norval *et al.*, 1992). Sporozoite stages, produced in large numbers in the acinar cells of the salivary glands of the infected *Rhipicephalus appendiculatus* tick vector, are inoculated along with saliva during feeding and rapidly enter target lymphocytes, which become transformed after the *Theileria* schizont is formed. The infected lymphocyte is transformed into a lymphoblast and divides in conjunction with the schizont, giving rise to two infected daughter cells. This process has been termed "parasite-induced reversible transformation" because, if the cells are treated with antitheilerial drugs, the transformed cells revert to quiescent lymphocytes (Ole Moiyo, 1989). Within the infected lymphocytes, schizonts are associated with microtubules involved in spindle formation during host cell division (Norval *et al.*, 1992). Clonal expansion of infected cells occurs with an approximate tenfold increase of schizonts every three (3) days. Schizonts, traditionally called macroschizonts or Koch's blue bodies vary in size and in the number of nuclei. Early detectable forms are small with nuclei that, when Giemsa-stained, appear as chromatic granules. From day 14 after tick infection of cattle, individual schizonts undergo merogony to produce merozoites (traditionally called microschizonts).

Merozoites invade the erythrocytes to become piroplasms, which may subsequently undergo limited division also by merogony (Conrad *et al.*; 1986). Piroplasm-infected erythrocytes are ingested by ticks of the larval or nymphal stages and undergo a sexual cycle in the gut of the

replete tick to produce zygotes, which in turn develop into motile kinete stages that infect the salivary glands of the next instar, the nymph or adult (Mehhorn, and Schein, 1984, Fawcett *et al.*; 1985).

2.3 Host Range type

Cattle in endemic areas, particularly the zebu (*Bos indicus*) type, appear less susceptible to ECF than exotic cattle. In addition, introduced cattle, whether of a taurine, zebu, or sanga breed, are much more susceptible to theileriosis than cattle from endemic areas. The Indian water buffalo (*Bubalis bubalis*) is as susceptible to *T. parva* infection as cattle. The African buffaloes (*Syncerus caffer*) are reservoirs of *T. parva* infection, and it has been proved that waterbucks (*Kobus* spp.) are also reservoirs (Norval *et al.*;1992). Buffaloes may suffer clinical disease from *T. parva* infection, but its effects on waterbuck are unknown. Organisms isolated from buffalo, on repeated passage in cattle, result in a parasite that produces disease characteristics indistinguishable from those associated with ECF (Norval *et al.*; 1992). Hence, the organism causing ECF is assumed to be a cattle-adapted form of the buffalo parasite causing Corridor disease. Piroplasms can be demonstrated in most wild antelopes in East Africa, but the relationship of most of them to *T.parva* is unclear.

2.4. Occurrence and impact of East Coast Fever (ECF)

The disease is prevalent across the Eastern, Central, and Southern parts of Africa, covering 11 countries in the region: Kenya, Uganda, Tanzania, Burundi, Rwanda, Malawi, Mozambique, Southern Sudan, Democratic Republic of Congo (DRC), Zambia and Zimbabwe (Lawrence *et al.*, 1992). East Coast fever was also reported in Comoros between 2003 and 2004 for the first time (De Deken *et al.*, 2007). The latter incident was suggested to result from importation of immunized cattle from Tanzania, which were fed upon by naïve ticks that subsequently transmitted the infection to a susceptible local cattle population (De Deken *et al.*, 2007). About 28 million cattle in the region are at risk and the disease kills at least 1 million cattle per year (Gachohi *et al.*, 2012). Economic losses are concentrated on small-scale resource-poor households (Minjauw and McLeod, 2003).

In Kenya, *Theileria parva* infection poses a significant threat to the livestock sector in two ways: through the economic impact of the disease from cattle morbidity and mortality and production

losses as well as from the costs of the measures taken to control ticks and the disease. The costs of acaricide application, which is the primary means of tick control, was estimated to range between US\$6 and US\$36 per animal in Kenya, Tanzania and Uganda (Minjauw and McLeod, 2003). The disease further prevents the introduction of the ECF susceptible but more productive exotic breeds of cattle, considerably hampering the development of the livestock sector. This loss is termed “lost potential” (Gachohi *et al.*, 2012).

2.5. Transmission

East Coast fever (ECF) is a tick-borne disease (TBD) of cattle whose etiological agent is a protozoan parasite called *Theileria parva*. The parasite is transmitted cyclopropagatively and transtadially by three-host ticks called *Rhipicephalus appendiculatus*, which have dropped from infected cattle during the preceding stage of the life cycle (Norval *et al.*, 1992). In cyclopropagative and transtadial transmission, the *T. parva* parasite multiplies and undergoes cyclical changes within two developmental stages (nymphs and adult) of the vector. The epidemiological implication of this kind of transmission is the amplification of the vector’s competence in parasite transmission and the ability to infect more than one host during the vector’s life cycle (Gachohi *et al.*, 2012).

Rhipicephalus appendiculatus in Kenya is found from sea level to over 8,000 feet in areas where there is annual rainfall of over 500 mm (Norval *et al.*, 1992). Areas that are more suitable for ticks are warmer and more humid with landscapes characterized by a mixture of grass and tree cover (savannah woodland) (Norval *et al.*, 1992). These are found in the Lake Victoria basin (in Nyanza and parts of Western provinces), the Kenyan coastal region and some parts of the Central and Eastern highlands, representing agro-ecological zones II, III and IV. In these areas, antibody prevalence is high. Indeed, after ECF was first reported in Kenya in 1904, reports indicate that the disease spread fast from some of these foci (Lake Victoria basin and the Kenyan coastal region) (Norval *et al.*, 1992).

2.6. Morbidity and Mortality

Morbidity and mortality depend on, among other factors, the magnitude of the infected tick challenge and susceptibility of the host and strain of parasite. East Coast fever in susceptible cattle, which are not indigenous to the enzootic area, is very severe with a mortality approaching 100 percent. Animals that recover are often unthrifty and sickly. Zebu cattle residing for many

generations in endemic areas become infected (100 percent morbidity), but only a minor proportion succumb; however, many become carriers, and early infection with *T. parva* can affect their growth and productivity (Moll *et al.*, 1986).

2.7. Clinical Signs

The first clinical sign of ECF in cattle appears 7 to 15 days after attachment of infected ticks. Under experimental conditions, using either ticks of known infection or sporozoite stabilate, the incubation period has a medium range of 7 to 12 days. The incubation period may be much more variable in the field owing to differences in challenges experienced by the cattle and may extend to beyond 3 weeks after attachment of infected ticks.

The first sign is seen as a swelling of the draining lymph node, usually the parotid, for the ear is the preferred feeding site of the vector. This is followed by a generalized lymphadenopathy in which other superficial subcutaneous lymph nodes such as the, prescapular, and prefemoral lymph nodes, can easily be seen and palpated. Fever ensues and continues throughout the course of infection (Norval *et al.*, 1992). This rise in temperature is rapid and is usually in excess of 103° F (39.5° C) and may reach 106° F (42° C). Anorexia develops, and loss of condition follows.

Other clinical signs may include lachrymation, corneal opacity, nasal discharge, terminal dyspnea, and diarrhea. Before death the animal is usually recumbent, the temperature falls, and there is a severe dyspnea due to pulmonary edema that is frequently seen as a frothy nasal discharge. Death usually occurs 18 to 30 days after infestation of susceptible cattle by infected ticks. Mortality in fully susceptible cattle can be nearly 100 percent. The severity and time course of the disease depend on, among other factors, the magnitude of the infected tick challenge, for ECF is a dose dependent disease and on the strain of parasites. Some stocks of parasites cause a chronic wasting disease. A fatal condition called "turning sickness" is associated with the blocking of brain capillaries by infected cells and results in neurological signs (Irvin A.D. and Mwanachi, 1983). In recovered cattle, chronic disease problems can occur that result in stunted growth in calves and lack of productivity in adult cattle (Moll *et al.*, 1986). However, this syndrome tends to be in the minority of recovered clinical cases; majority is asymptomatic carriers with apparently little or no loss in productivity (Moll *et al.*, 1986).

2.8. Gross Lesions

A frothy exudate is frequently seen around the nostrils of an ECF-infected animal (Norval *et al.*, 1992). Signs of diarrhea, emaciation, and dehydration may be seen. Lymph nodes are greatly enlarged and may be hyperplastic, hemorrhagic, and edematous. In acute cases of ECF, lymph nodes are edematous and hyperemic but often become necrotic and shrunken in more chronic disease. Generally, muscles and fat appear normal but, depending on relative acuteness of infection, fat may become greatly depleted; serosal surfaces have petechial and ecchymotic hemorrhages, and serous fluids may be present in body cavities. Hemorrhages and ulceration may be seen throughout the gastrointestinal tract — particularly in the abomasum and small intestine, where necrosis of Peyer's patches can be observed. Lymphoid cellular infiltration appears in the liver and kidney as white foci that have been referred to as pseudoinfarcts. The most striking changes are seen in the lungs. In most cases of ECF, interlobular emphysema and severe pulmonary edema appear, the lungs are reddened and filled with fluid and the trachea and bronchi are filled with fluid and froth.

2.9 Diagnosis

Cases of ECF are tentatively diagnosed in the field based on clinical assessment/ signs. A Giemsa stained thin blood smear is used for examination of haemoparasites/piroplasms to confirm suspected disease cases (Lawrence *et al.*, 1992). Each smear is examined for theilerial piroplasms in the red blood cells using the oil immersion x 100 objective of the light microscope. At least three fields are thoroughly examined for each smear. The disease is confirmed by detection of macroschizonts (Koch's blue bodies) in the lymphocytes on stained lymph node smears (Lawrence *et al.*, 1992). In case of death, a post-mortem examination is carried out whenever possible.

East Coast Fever is only found in association with its known tick vectors, *Rhipicephalus appendiculatus*, *R. zembeziensis* and possibly *R. duttoni* and *R. nitens*. A febrile disease with signs of enlarged lymph nodes associated with infestation by tick vectors is suggestive of ECF. An acute disease with high mortality on farms, where tick control is not effectively applied, also is suggestive of ECF. In many epidemiological situations, high mortality occurs only in calves; the adult cattle represent immune survivors.

2.10. Differential diagnosis

Identification of schizonts in lymphoid cells is considered to be pathognomonic of ECF. However, it must be realized that in an area such as Kenya, five species of *Theileria* have been recognized in cattle (*T. parva*, *T. mutans*, *T. velifera*, *T. taurotragi* and *T. buffeli*) and it is possible for an individual animal to harbor all these parasites at once (Norval *et al.*, 1992). Also, all these species produce schizonts which, except for those of *T. mutans*, are not morphologically distinct (Norval *et al.*, 1992). Piroplasms of *Theileria* spp. have similar morphology and thus are difficult to differentiate on blood slides. In addition, recovered animals, particularly in areas with endemic stability, become carriers of parasites and may show both *T. parva* schizont and piroplasm stages without clinical ECF (Norval *et al.*, 1992).

Theileria parva derived from African buffalo (*Syncerus caffer*), which causes Corridor disease in cattle, is characterized by production of low parasitosis and parasitaemia in cattle although it can result in high fatality rates (Norval *et al.*, 1992). Other species tend to be of low pathogenicity (*T. mutans*, *T. taurotragi*, *T. buffeli*) or avirulent (*T. velifera*) in cattle. *T. annulata* is the cause of Mediterranean or tropical theileriosis, which is also a severe disease of cattle; although it is endemic in Northern Africa, there is no evidence that its distribution overlaps with that of *T. parva* (Norval *et al.*, 1992).

2.11. Treatment

Drugs are available to treat ECF, but are expensive and require an early diagnosis to be effective (Katzer *et al.*, 2009). Animals with ECF are treated with buparvaquone and supportive antibiotic drugs and antihistamines. Treatment with haluluginone (Terit®) has been attempted in the past.

2.12. Control

Efforts to control ECF are largely based on the use of acaricides to prevent infestation with infected ticks, but this approach is increasingly being compromised by the emergence of acaricide resistance in the vector tick populations (Katzer *et al.*, 2009), food-safety concerns and environmental contamination resulting from toxic residues (George *et al.*, 2004). In addition, dipping facilities are frequently not operational because of lack of financial resources for maintenance, particularly in pastoral systems, which depend to a substantial degree on the 'informal economy'. After nearly a century of acaricide utilization, it is widely believed that

acaricides alone do not provide a sustainable solution to control tick and tick-borne diseases (Norval *et al.*, 1992).

2.13 Infection and Treatment Immunization

An alternative method of ECF control is infection and treatment immunization (ITM) involving inoculation of cattle with an estimated 5.9×10^4 sporozoites, using appropriately diluted stabilate, combined with treatment using a long-acting formulation of oxytetracycline (OTC). The dose estimate, which is lethal based on simultaneous inoculation of control cattle, is determined upon quantification of the number of *Theileria parva*-infected salivary gland acini from ticks fed on infected animals. However, it is not known to what extent the number of infective sporozoites might be reduced during the process of stabilate production, storage and delivery. The method was developed in the 1970s (Radley *et al.*, 1975) and was based on (i) the ability to harvest *Theileria parva* sporozoites and cryopreserve material as stabilates (Cunningham *et al.*, 1973) and (ii) induction of protective immunity by injecting cattle with *Theileria parva* stabilate combined with OTC treatment (Radley, 1981). Immunized animals usually undergo an asymptomatic or mild ECF reaction. The mechanism by which the OTC works to control infection and enhance immunity is not fully understood, but the drug seems to affect the degree of maturation of sporozoites to schizonts after infection of lymphocytes (Spooner, 1990). One mechanism of protective immunity induced by ITM is thought to be based on major histocompatibility complex (MHC) class-I-restricted CD8⁺ cytotoxic T cells (McKeever *et al.*, 1994).

2.14. Efficacy of the vaccine

Ideally efficacy should be conducted for a minimum period of 12 months in order to capture the possible effects of factors such as season of the year, tick dynamics (which vary with season), livestock management practices and tick control frequency (Rashid *et al.*, 2009). These factors have been shown to affect the efficacy of the ECF vaccine and greatly influenced by climate (Ochanda *et al.*, 2003.; Olson and Patz.; 2010). The parasite thrives in the tick vector within the environmental temperatures range of 18 – 28⁰ C (Ochanda *et al.*; 2003). The sporozoite stage of the parasite multiplies rapidly within the salivary gland of the tick vector under high environmental temperatures. High tick infection rates with *T. parva* can result in exposed animals particularly calves, developing the disease before the vaccine has had time to stimulate

the body's immunity (James, 1999; Rashid *et al.*, 2009). The implication of this is that the efficacy of the ECF vaccine can be influenced by prevailing weather conditions. The efficacy of the vaccine can further be affected by stressful conditions such as drought and poor livestock management practices. (Clement *et al.*, 2004; Rashid *et al.*, 2009). Besides, cattle immunized against ECF require a minimum period of 2 months for the vaccine to confer "adequate" immunity against the disease. Sero-conversion following immunization is a useful tool to monitor the viability of the ECF vaccine. Thus, it is necessary to determine seropositivity to *Theileria parva* on the day that the animals are immunized (day 0) and on the 35th day (day 35) post immunization Radley,(1981).

It has been shown that high tick challenges can precipitate immunosuppression (Bock and De Vos 2001) in the infested animals resulting in clinical ECF. This inevitably lowers the efficacy of the vaccine. Tick challenge in turn is influenced by the season of the year. High tick populations on pastures and livestock are observed soon after the rainy season.

CHAPTER THREE

3.0 Materials and Methods

3.1 Description of the study area

The study was conducted in two ranches in Laikipia County where there was shared grazing between cattle and wildlife in one ranch and no shared grazing in the other. The ranches conveniently selected were Mutara, and Ole Naishu. There was shared grazing of cattle and buffalos in Mutara ranch but none in Ole Naishu ranch. As at November 2013 Mutara had 986 heads of cattle mostly crosses of local Zebus and Borans. The ranch also had 800 sheep. Ole Naishu had 2,796 heads of cattle mostly crosses of local Zebus and improved Borans and a flock of 260 sheep. Wild animals were found in the two ranches that included buffalos, elephants, zebras, gazelles, impalas among others. The sizes of the two ranches were 4,000 and 30,000 hectares for Mutara and Ole Naishu, respectively. Figure 3.1 shows the location of Laikipia County within Kenya and Figure 3.2 the location of the study ranches within the County.

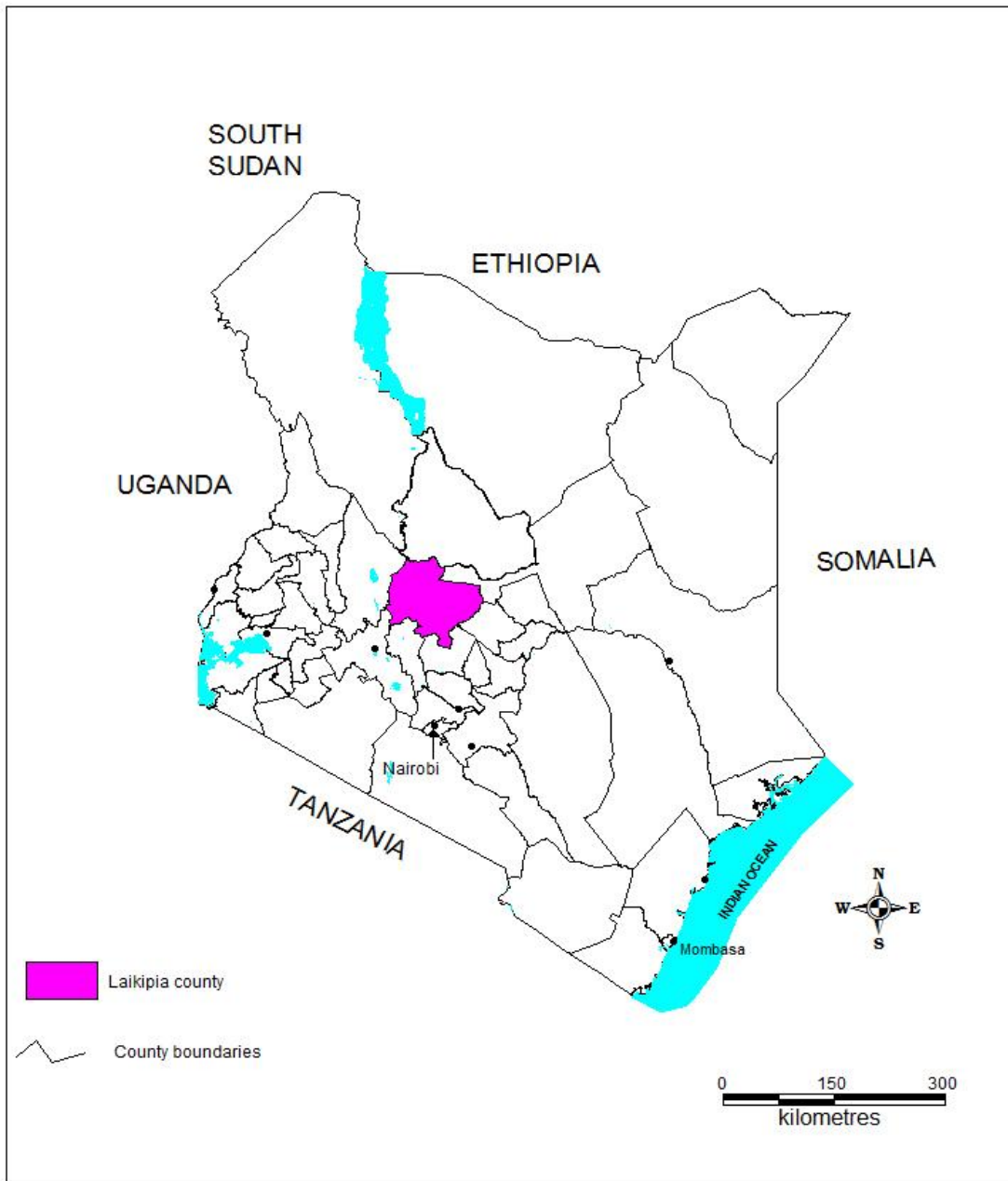


Figure 3.1: A map of Kenya showing location of Laikipia County

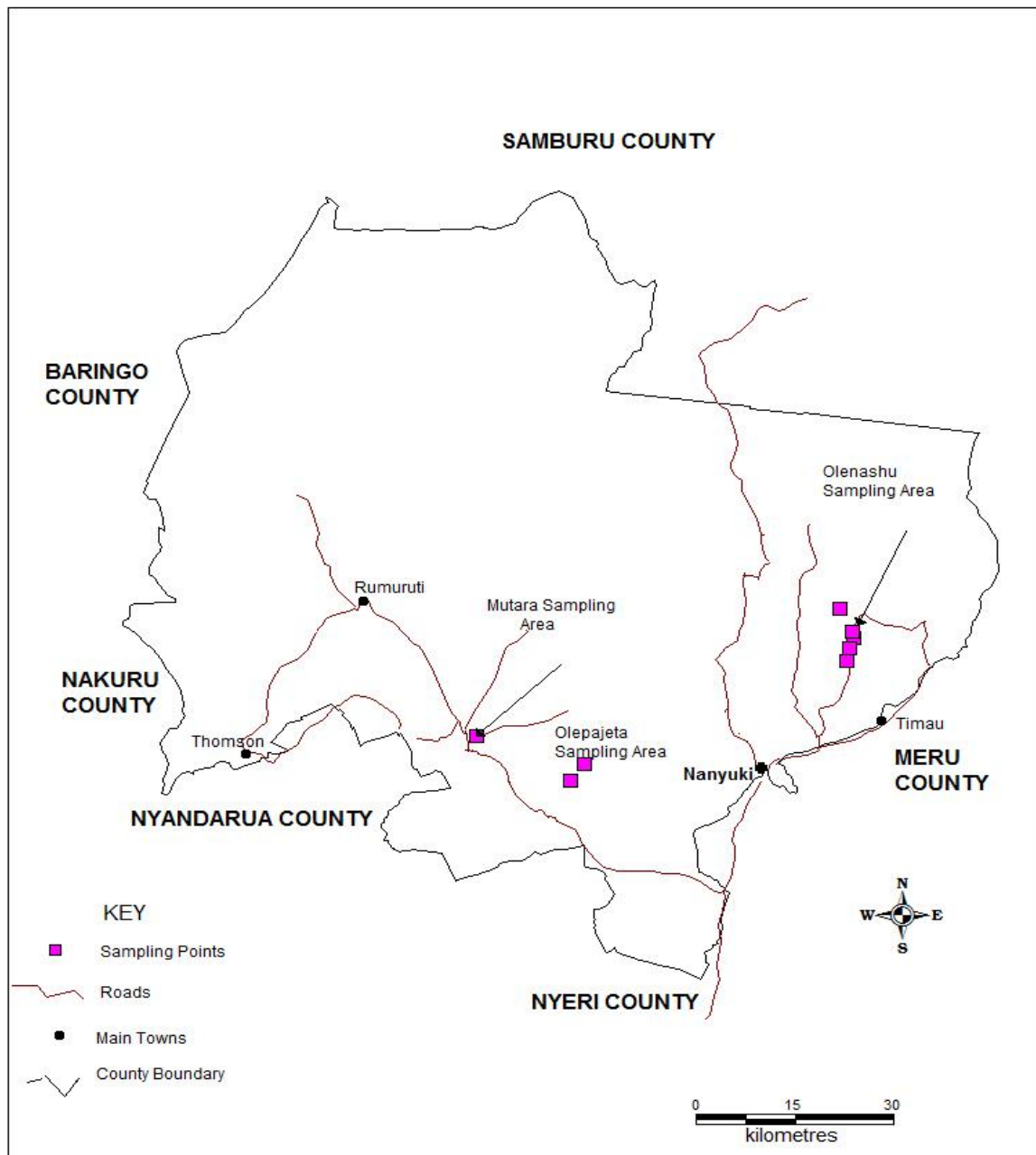


Figure 3.2: A map of Laikipia County showing the location of the study ranches.

3.2 Sample size determination

The minimum number of calves that were immunized (assuming that immunizing against ECF will result in 50% reduction in incidence of ECF) was derived from the formula of Dohoo *et al.* (2003) for comparing two proportions.

$$n = [Z_{\alpha} (2PQ)^{1/2} - Z_{\beta} (P_e Q_e + P_c Q_c)^{1/2}]^2 / (P_e - P_c)^2$$

n = estimated sample size for each of the groups (treatment) and the (control) group required.

Z_{α} = Value of Z (1.96) which provides $\alpha/2$ in each tail of a normal curve for a two-tailed test or α in one tail if a one tail test is used.

Z_{β} = Value of Z (-0.84) which provides β in lower tail of a normal curve (Z_{β} negative if $\beta < 0.5$).

P_e = Estimate of response rate in vaccinated group assuming prevalence of ECF to be (25%), it is assumed that the vaccination will reduce the prevalence by 50%.

P_c = Estimate of response rate in non-vaccinated group (50%), this prevalence was chosen because the actual prevalence of ECF in the area was unknown.

$$P = (P_e + P_c) / 2 = (0.25 + 0.5) / 2 = 0.375$$

$$Q = 1 - P = 1 - 0.375 = 0.625$$

Thus minimum number of calves needed to be vaccinated for the trial was;

$$\begin{aligned} &= \frac{[1.96(2 \times 0.375 \times 0.625)^{1/2} + 0.84(0.25 \times 0.75 + 0.5 \times 0.5)^{1/2}]^2}{(0.25 - 0.5)^2} \\ &= 58 \end{aligned}$$

with a minimum number of 58 calves as control.

Based on this criterion, sixty five (65) calves were selected and immunized on each of the two ranches. Another sixty five (65) non-immunized calves on each of the ranches were selected to act as the “controls” for the follow-up study. The sample was increased to cater for losses during the follow-up.

3.3 Selection of calves for immunization

Calves between one and six months of age were selected for immunization. Only calves with no previous history of ECF disease were eligible for inclusion into the study. This was determined

as described below (Section 3.4). To block the effects of herds, control calves were also selected within the same herds.

3.4 Immunization procedure

Calves were randomly allocated to the “treatment” (immunized) group and control (non-immunized) groups using a random number table. Clinical examination of the animals was undertaken just prior to the immunization. Only animals with a rectal temperature of no more than 39.4⁰C were selected for immunization. However irrespective of whether the body temperature was normal or not, animals with enlarged superficial lymph nodes were excluded on suspicion of having been recently infected with ECF. Animals that appeared malnourished (weakness with protrusion of bones of the shoulders, ribs, backbone, hips and sunken eyes) were also excluded from the study.

Immunization was carried out using a standard immunization protocol developed at Muguga. The *T. parva* (Muguga cocktail) stabilate from ILRI stored in 0.5ml aliquots in plastic straws kept under liquid nitrogen was thawed by rubbing between the palms and their contents dispensed into universal bottles. A 1:40 dilution of the stabilate was undertaken using Eagles Minimum Essential Medium with 3.5% w/v bovine plasma albumin and 7.5% glycerol. After 30 minutes of equilibration, 1ml of the diluted stabilate was inoculated subcutaneously in front of the pre-scapular lymph node. A 30% long acting oxytetracycline (Tetroxy L.A, Bimeda) was administered at a dosage rate of 30 mg per kg body weight by deep intramuscular injection prior to inoculation with stabilate. The weight of the calves was estimated with a weighing band. A 10% error was taken into consideration by adding 10% of weight when computing the dose of oxytetracycline.

Any immunized animal that would develop clinical signs of ECF with fever and macroschizonts in lymph node smear (7-10 days post-immunization) for at least three days would be designated “ECF reactor” (that is the animal got the ECF due to vaccination).

3.5 Serology and follow-up data

Sero-conversion following immunization was used as a tool to monitor the viability and correct administration of the ECF vaccine. An indirect ELISA test as described by Katende *et al.*(1998) was used to monitor the immune response, by recording the change in antibody levels of the study animals before and after immunization. Since the tick vector of *T. parva* was prevalent in

the study area, it was expected that some of the calves could have already have been exposed to *T. parva* at the time of immunization by the time they are one month old. So all the calves were screened for prior exposure to *T. parva* at the time of immunization and only those not showing signs of exposure were selected for the study.

Sero-positivity to *T. parva* was determined on the day of immunization (day 0) and again on the 35th day after immunization.

Surveillance for ECF in both the vaccinated and the control groups was determined by antibody titres in serum and the incidence rate of the disease. Daily clinical surveillance was kept on all the calves in both treatment groups by the farm management who were trained by the investigator on how to detect signs of ECF. Information collected included:

Pre and post-immunization serological status, tick challenge levels, cases of ECF and other tick-borne diseases, mortality due to ECF, tick control frequency, meteorological data.

Cases of ECF were tentatively diagnosed in the field based on clinical assessment. The key clinical signs of disease included malaise, lacrymation or corneal opacity, petechial hemorrhages on gums, tongue and vulva, anorexia, acute respiratory distress, parotid and pre-scapular lymph node enlargement and a fever (rectal temperature of more than 39.4^oC).

3.6 Confirmation of ECF

Stained thin blood smears were used for examination of haemoparasites. Each smear was examined for theilerial piroplasms in the red blood cells using the oil immersion x 100 objective of the light microscope. The disease was confirmed by detection of macroschizonts in the lymphocytes of stained lymph node smears (See Section 2.9.). Only confirmed cases of ECF were included in the analysis. Animals found to be suffering from ECF were treated with buparvaquone and supportive antibiotic drugs. All information was recorded in a field book.

3.7 Tick challenge

A tick count, done by counting the number of ticks attached on half the body of the animal and multiplying by two, to estimate the total tick infestation, was undertaken on a monthly basis.

Tick challenge was recorded on a four-point scale:

None=0 Low = < 10 Moderate = 10-20 High= > 20 ticks

Ticks were also collected from the grass for identification and also for attempt to isolate the *T.parva* from the acini of the salivary glands of the *Rhipicephalus appendiculatus*.

3.8 Viability of the vaccine

To test whether the vaccine was viable Chi square values of sero-conversion of the treatment group and the control group before immunization (day 0) and again after immunization (day ≥ 35) were calculated. The hypothesis tested was: H_0 : There was no difference in the sero-conversion between the immunized calves (treatment) group and the non-immunized (control) group. H_A : There was a difference in the sero-conversion between the immunized calves (treatment group) and the non-immunized (control) group calves. If the calculated value of chi square was greater than the critical value of 3.84 at significant level of $p < 0.05$, then the null hypothesis was rejected and the alternate hypothesis was accepted. In this case the vaccine was deduced to be viable. Likewise if the calculated value of chi square was less than the critical value of 3.84 at significant level of $p < 0.05$, then the null hypothesis was accepted and the vaccine was deduced to be not viable.

3.9 Data handling and analysis

Data collected were entered in an excel spread sheet. The data was also analyzed using the same spread sheet.

The incidence rate of ECF was computed as described in Dahoo *et al.* (2003):

$$\text{Incidence Rate (IR)} = \frac{\text{Number of events during the observation period.}}{\text{Animal-months at risk.}}$$

Vaccine efficacy was calculated as described by Babo Martins (2010):

Efficacy =

$$\frac{(\text{Incidence rate in control group} - \text{Incidence rate in immunized group}) \times 100\%}{\text{Incidence rate in control group}}$$

Other measures of association between vaccination and ECF were computed according to Dohoo *et al.* (2003) including:

Attributable Risk (AR): The rate of disease (ECF) in the non-vaccinated group that was attributed to being non-vaccinated ; Attributable Fraction (AF): The proportion of disease in the non-vaccinated cattle that was due to being non-vaccinated; Population Attributable Risk (PAR): The rate of ECF in the calf population that was attributable to being non-vaccinated.; and The Population Attributable Fraction (PAF): The proportion of East Coast Fever (ECF) in the whole calf population that was attributed to being non-vaccinated and could be avoided if the calves were vaccinated.

CHAPTER FOUR

4.0 Results

4.1 Farm Characteristics

4.1.1 Mutara Ranch.

The GPS reading taken were (N00.06705, E036.68145) and (N00.065566, E036.68141). The size of the ranch was 4,000 hectares. The farming system used in the ranch was extensive free range system. Wildlife animals such as buffalos, zebras, antelopes and others were observed in the ranch. The livestock breeds kept were Borans and crosses between the Borans and the local Zebus in the area. The livestock in the ranch usually had contact with other livestock from other herders in the area.

4.1.1.1 Livestock diseases and tick control

Tick born diseases commonly observed in the ranch were ECF, anaplasmosis, and also babesiosis with the highest incidences recorded in the months of April-June and October-December. These are the wettest months of the year when the area experiences the long rains and the short rains respectively. During these seasons tick challenge was the highest. Reported incidences of tick-born diseases also increased when wildlife especially buffalos grazed in areas with the cattle. The tick control methods used in the ranch was application of acaricide to the animals using a spray race. The acaricide used was tixfix[®] (an amitrax group). The interval of spraying was usually seven days but during the wet season it was reduced to an interval of five days. The mixing of the acaricides was done according to the manufacturer's instructions. There were stringent tick control measures in the ranch and hence tick challenge was very low, majority of the animals had less than five ticks attached. ECF cases reported were seventeen (17), three cases of babesiosis and no cases of anaplasmosis and heart water were reported in the ranch during the follow up period from October 2013 to September 2014.

The management of the ranch had no prior knowledge of the ECF vaccine, this was their first time and sixty five (65) calves were vaccinated. Ticks were collected from the ranch for identification, a total of five hundred and fifty (550) ticks were collected during the study period (October 2013- September 2014). The main ticks found in the ranch were *R. appendiculatus* 30%

(165/550), *R. evertsi* 20% (106/550), *B. decorolatus* 30% (165/550), and *Amblyomma spp*s 20% (114/550).

4.1.2 Ole Naishu Ranch

The GPS readings were (N00.177838, E037.18574) and (N00.22139, E037.19474). The size of the ranch was 30,000 hectares. The livestock population was 2,796 heads of cattle that were mostly Borans with a few Sahiwals and Crosses. There were also 260 sheep in the ranch. The ranch also had wildlife animals such as buffalos, zebras, giraffes, antelopes, gazelles and other animals but were not mixing with the cattle when grazing. The production system practiced was extensive free range grazing system. The wildlife animals including buffalos were not sharing grazing with the cattle, there was separation of wildlife and cattle with the game rangers controlling the wildlife. There was no livestock from outside the ranch which got in contact with the ones in the ranch as it was fenced and illegal grazing was not allowed.

4.1.2.1 Livestock diseases and tick control

During the follow up period (October 2013 –September 2014), a number of tick born diseases were confirmed: 54 cases of ECF, 51 cases of anaplasmosis, 2 cases of babesiosis and 1 case of heartwater. The tick control method used was spraying with a spray race and the acaricide used was bovitrax® (an amitrax group). The mixing of acaricide was done according to the manufacturer's recommendations and it was done correctly by trained personnel. The spraying of the cattle was done after seven (7) days but this interval was reduced during the wet seasons to five (5) days due to increased tick challenge. The tick challenge was very low with most of the animals having less than five (5) ticks attached. During the wet seasons of the months of April-June and October-December there was increased number of tick-borne diseases. Ticks collected from the ranch were one hundred and seventy five (175) during the study period. The common species encountered in the ranch were *R. appendiculatus* 35% (61/175), *B. decorolatus* 35% (62/175), *R. evertsi* 15% (26/175), and *Amblyomma spp*s 15% (27/175). The management of the ranch had no prior knowledge of ECF vaccine.

4.2 Sero-conversions following immunization against East Coast Fever

The higher sero-conversion (83%) following immunisation against ECF was observed on Ole Naishu ranch (Table 4. 1). In Mutara (69%) sero-conversion was recorded. The sero-conversion for the control were 3.1% for Ole Naishu and 6.3% for Mutara (Table 4.1). The difference in

sero-conversion between the vaccinated and the control groups were significantly different ($p \leq 0.05$).

Table 4.1: Serological reactions of calves immunized against East Coast Fever with the Muguga cocktail vaccine and the controls in 2 ranches in Laikipia County 2013/2014.

Ranch	Status	Days	No of calves +ve	No of calves -ve	Total	% +ve
Mutara	Immunized (Treatment group)	Day 0	2	63	65	3.0 %
		Day \geq 35	45	20	65	69.2 %
	Non-immunized (Control group)	Day 0	3	62	65	4.7%
		Day \geq 35	4	61	65	6.2 %
Ole Naishu	Immunized(Treatment group)	Day 0	1	64	65	1.5 %
		Day \geq 35	54	11	65	83.1 %
	Non-Immunized(Control group)	Day 0	2	63	65	3.1 %
		Day \geq 35	2	63	65	3.1 %

4.3 Viability of the vaccine

The chi square values of sero-conversion calculated were:

- i). Mutara Ranch chi-square value was 61.62 with 1df and p value < 0.001 for the treatment group and 0.15 with 1 df and p value = 0.698 for the control.
- ii). Ole Naishu chi- square value was 88.52 with 1 df and p value < 0.001 for the treatment group and 0.00 for the control group.

4.4 Efficacy of East Coast Fever (Muguga Cocktail) vaccine

4.4.1 In Mutara

Total number of East Coast Fever (ECF) cases in the immunized group were four (4) whereas in the non- immunized was seventeen (17), for incidence rate of 6.2% (4/65) and 26.2% (17/65) respectively (Table 4.2). Three of the four calves died from the immunized group. They died from ECF for a mortality rate of 4.6% (3/65). Most of the cases were reported in the wet season of April – June and October – December in both ranches (Table 4.2). True rate of ECF in the

immunized group was $0.065 (4 / ((65+58) \div 2))$ and for control group $0.301 (17 / ((65+48) \div 2))$. Three animals were withdrawn from the ranch during the study period.

4.4.2 In Ole Naishu

Total number of ECF cases in the immunized group was two (2) whereas in the non-immunized was fifty four (54), for incident rate of 3.1% (2/65) and 83.1 (54/65) respectively (Table 4.2). True rate of ECF in the immunized group was $0.031 (2 / ((65+63) \div 2))$ per animal year and 1.42 ($54 / ((65+11) \div 2)$) per animal year. No East Coast Fever (ECF) specific deaths were reported in this ranch.

Table 4.2 East Coast Fever (ECF) cases confirmed in the ECF vaccination trial in Mutara and Ole Naishu ranches from October 2013 to September 2014.

Ranch	Month Year	Oct 13	Nov 13	Dec 13	Jan 14	Feb 14	Mar 14	Apr 14	May 14	Jun 14	July 14	Aug 14	Sep 14	Total
Mutara	Immunized	1	0	0	0	0	0	2	1	0	0	0	0	4
	Not Immunized	2	4	1	0	0	0	2	3	1	2	0	2	17
Ole Naishu	Immunized	0	0	0	0	0	0	0	0	1	1	0	0	2
	Not Immunized	10	6	3	3	3	3	5	4	10	4	1	2	54

4.5 Vaccine efficacy

Efficacy of Vaccine was computed using the formula:

$$= \frac{(\text{Incidence rate in the control group} - \text{Incidence rate in the treatment group}) \times 100\%}{\text{Incidence rate the control group}}$$

Hence:

- i. Incidence Rate in the treatment group in Ole Naishu was $2 / ((65+63) \div 2) = 0.031$ per animal year and for the control was $54 / ((65+11) \div 2) = 1.42$ per animal year.
- ii. Efficacy of vaccine in Ole Naishu was therefore $(1.42 - 0.031) / 1.42 = 0.978 \times 100 = 97.8\%$.
- iii. Incidence Rate in the treatment group in Mutara was $4 / ((65+61) \div 2) = 0.065$ per animal year and for the control was $17 / ((65+48) \div 2) = 0.301$ per animal year.

iv. The efficacy of vaccine for Mutara was $(0.301 - 0.065)/0.301 = 0.784 \times 100 = 78.4\%$ (See Table 4.2 for the data used to compute this).

4.6 Measures of association of East Coast Fever (ECF) and vaccination

4.6.1.1 Association of vaccination and development of East Coast Fever (ECF) in Mutara ranch.

The incident rate of ECF in the vaccinated calves was 0.061 and 0.262 in the non-vaccinated calves (Table 4.3). There was strong association between being vaccinated and non-vaccinated ($X^2 = 9.6$, OR= 5.4).

Attributable Risk (AR) was 0.2 which means that the rate of ECF cases in the non-vaccinated calves in the ranch attributed to not being vaccinated was 20%. Attributable Fraction (AF) was 0.7647 which means 76.47% of the ECF cases in the ranch were attributed to not being vaccinated. Population Attributable Risk (PAR) was 0.09999 which means that the rate of ECF in the population of the calves in the ranch that was attributed to not being vaccinated was 10%. Population Attributable Fraction (PAF) was 0.61902 which means that 61.9% of all ECF cases in calf population in the ranch were attributed to not being vaccinated.

Table 4.3 Association between vaccination and development of East Coast Fever in Mutara ranch.

		ECF Status						
Ranch	Treatment	Yes	No	Total	Rate	OR	X^2	
Mutara	Vaccinated	4	61	65	0.061538	5.4	9.6	
Mutara	Not vaccinated	17	48	65	0.261538			
	Total	21	109	130	0.161538			

4.6.1 Association between vaccination and development of East Coast Fever in Ole Naishu ranch.

The incidence of East Coast Fever in the non-vaccination calves (83.1%) was much higher than that marked in the vaccinated calves (3.1%). Indeed there was a very strong association between non-vaccinated and development of the disease ($X^2 = 88$, OR = 155) (Table 4.4)

Attributable Risk (AR) was 0.8 meaning that the rate of ECF in the non-vaccinated calves in the ranch attributed to being non-vaccinated was 80%. Attributable Fraction (AF) was 0.96296 meaning that 96.3% of the ECF cases in the non-vaccinated calves in the ranch was attributed to non-vaccination. Population Attributable Risk (PAR) was 0.4 meaning that the rate of ECF in calf population in the ranch attributed to being non-vaccinated was 40%.

Population Attributable Fraction was (PAF) 0.92857 meaning that 92.9% of ECF in the calves population in the ranch was attributed to not being vaccinated.

The above calculations show that the stratum specific ORS were different and therefore confounding was irrelevant. The different ORS for the two ranches strata indicate that there was interaction within the ranches and hence the ranch was a modifier variable.

Table 4.4 Association between vaccination and development of East Coast Fever in Ole Naishu ranch.

		ECF Status						
Ranch	Treatment	Yes	No	Total	Rate	OR	χ^2	
Ole Naishu	Vaccinated	2	63	65	0.030769	154.6312	88.12	
Ole Naishu	Not vaccinated	54	11	65	0.830769			
	Total	56	74	130	0.430769			

4.7 Interaction by ranch

From the table 4.5, the calculated Chi square of interaction was 55.54 which was greater than the critical value of 3.84 at 0.05% significant level. The interaction was therefore significant, and hence reporting the specific stratum ORS was appropriate. Thus ranch modified the effect of the vaccination.

Table 4.5 2×2 table showing the effect of interaction by ranch.

		ECF							
Interaction		Yes	No	Total	Rate	AR	OR	Joint effect	Compare with observed
+	+	4	61	65	0.061538	0.76923	0.185149	-1.36923	-0.76923
+	-	17	48	65	0.261538	-0.56923			
-	+	2	63	65	0.030769	-0.8	0.006467		
-	-	54	11	65	0.830769	0			
Total		77	183	260					

4.8 Tick challenge

Tick challenge on the animals was very low throughout the observation period; rarely was more than 10 attached ticks of any species seen on any of the trial animals.

4.9 East Cost Fever (ECF) Reactors

There were no ECF reactors observed.

CHAPTER FIVE

5.1 Discussiono A

The results show that vaccinated animals sero-converted after 35th day in both Mutara and Ole Naishu ranches. Sero-conversion in Mutara (69%) was however lower than that in Ole Naishu (83%). The non-vaccinated groups in both of the ranches did not have significant sero-conversion indicating that the vaccine was viable. Studies done in Machakos Wesonga., *et al.*, (2013) showed that sero-conversion varied from division to division but overall it was found to be higher (92%) than what was found in the Laikipia study. The sero conversion in Mutara ranch was even lower than the expected range of a good viable vaccine.

The efficacy of the vaccine was lower in Mutara ranch (78.4%) than in Ole Naishu (97.8%). The efficacy of the vaccine for Ole Naishu compares favourably with those obtained by ILRI (2010) (95%) in studies conducted in Central, Coastal, South and North Rift regions of Kenya. The efficacy for Ole Naishu was similar to that obtained by Babo Martins *et al.*, (2010) of 97% in studies conducted in the Ngorongoro region of Tanzania. In a study carried in a buffalo-livestock interface in Narok County, Mukholi (2015) reported an efficacy of 89% which was lower than that obtained in Ole Naishu (97.8%) but higher than the one obtained in Mutara. The efficacy reported in Mutara was similar to the one reported by Wesonga *et al.*, (2013) in studies conducted in a mixed crop-livestock production system in Machakos County of 82%. The differences in the efficacies might have been caused by differences in the tick challenge levels, vector infection rates with *T. parva*, environmental conditions and also interactions with the wildlife. In Mutara ranch the efficacy was lower (78.4%) and this may have been as a result of the observed interaction of cattle with buffalos. Cattle in Mutara might have been exposed to the buffalo *T.parva* which rendered the vaccine less effecacious. The viability of the vaccine in Mutara ranch was rather low and may also have explained the low efficacy of the vaccine observed in that ranch. The association of the development of East Coast fever with vaccination with the Muguga cocktail vaccine was strong especially in Ole Naishu but lower in Mutara. The association values obtained in Mutara (AR= 20%; AF = 76%; PAR = 9%; PAF =62%) compares favourably with those values obtained in the Machakos study (AR = 24%; AF = 77%; PAR = 14%, PAF = 58%) but contrasts sharply with the values obtained in Ole Naishu (AR = 80%; AF = 96%; PAR = 40%; PAF = 92). The difference in the proportion between Ole Naishu and the

two other places may be attributed to the good management practices including tick control observed in Naishu. The different odds ratios (ORS) for the two ranches strata indicated that there was interaction within the ranches. The ranches modified the effect of the vaccine. This might have been due to factors within the ranches such as management levels in the ranches, tick control measures taken, level of interaction with wildlife especially the buffalos. In Ole Naishu, contact of cattle with the buffalos was minimal as there were delineated areas for the buffalos and cattle. In Mutara, the wildlife including the buffalos were grazing in the same areas and this could have exposed cattle to buffalo *T.parva* infections which could have rendered the vaccine less efficacious.

5.2 Conclusions

The following conclusions can be drawn from this study:

1. The viability of the Muguga cocktail vaccine was high in Ole Naishu ranch (83.1%) where good management practices were observed and comparatively low (69.%) in Mutara ranch.
2. In this study the efficacy of the vaccine was very high in Ole Naishu (97.8 %) where there was no sharing of grazing between cattle and buffalos and low in Mutara (78.4%) where there was sharing of grazing between cattle and buffalos.

5.3 Recommendations

The following recommendations can be derived from the study:

- 1.The Muguga cocktail vaccine for East Coast Fever is recommended for use in areas where cattle do not interact with buffalos.
2. More studies need be undertaken in those buffalo-cattle interfaces to isolate the buffalo *T.parva* strain for characterization to find out whether it is different from the strains in the vaccine and if so then it can be used to improve the vaccine.
- 3.Tick control may be relaxed in the farms which have done East Coast Fever immunization to cut down on the cost of acaricides.

CHAPTER SIX

6.0 References

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CHAPTER SEVEN

7.0 Appendices

Appendix 1: QUESTIONNAIRE ON RISK FACTORS FOR OCCURRENCE OF ECF.

Section 1. Ranch Identification.

- 1) Ranch Code
- 2) Name of Ranch
- 3) Location of Ranch
- 4) Date of interview -
- 5) Name of interviewer
- 6) GPS Reading

Section 2. Ranch characteristics and livestock production

- 1. Total acreage of the ranch
- 2. Ranching system characteristics
 - i. Mainly livestock
 - ii. Mixed livestock and crops
- 3. i. Which of the following livestock species do you keep and how many of them are on the ranch presently?

Type Of Livestock	Cattle	Sheep	Goats	Others
Number Of Livestock				

ii. Which other animals are the cattle in contact with?

- a) Other Cattle
- b) Sheep
- c) Goats
- d) Wild Animals

iii. Do the animals graze where wild animals (buffallos) graze? 1. Yes----- 2.No-----

-

iv. During which period of the year (specify months) are animals grazed close to where wild animals (buffaloes) graze?

4. What type of cattle (breed) are kept in the ranch?

Section 3. Animal health

5. Disease history

No of cases	ECF		Anaplasmosis		Babesiosis		Heartwater	
	Immunized	Control	Immunized	Control	Control Immunized	Control	Immunized	Control
Sept 2013								
Oct 2013								
Nov 2013								
Dec 2013								
Jan 2014								
Feb 2014								
March 2014								
April 2014								
May 2014								
June 2014								
July 2014								
August								

2014								
September 2014								

- 6.
- a. What are the main tick borne diseases control methods used on the ranch?
- i.
- ii.
- iii.
- iv.
- b. Which month of the year do you encounter a lot of ticks in the ranch?
- c.
7. Which is the main type of acaricide(s) used to control the ticks?.....
8. What is the tick control frequency?.....
- i. Are there sometime when tick when tick control frequency is changed?.....
- ii. If so state the period of the year
9. State the reason for changing the tick control frequency.

Section 4 Immunization against East Coast Fever

10. How many animals were immunized against ECF on the ranch? Total number of animals immunized.....
-
11. ECF disease history among immunized and non immunized calves

Month	Immunized	Control
October 2013		
November 2013		
December 2013		

January 2014		
February 2014		
March 2014		
April 2014		
May 2014		
June 2014		
July 2014		
August 2014		
September 2014		

12. Tick control system
 - a. What are the main tick species found in the ranch.....
 - b. What are the tick control methods used in the ranch
 - c. What is the frequency of tick control
 - d. What type of acaricide is used in the ranch

13. Any other information found necessary.....
14.

