AN ASSESMENT OF THE EFFICACY OF INFECTION AND TREATMENT METHOD AGAINST EAST COAST FEVER AND THE FINANCIAL AND ECONOMIC BENEFITS IN NAROK COUNTY OF KENYA.

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DECLARATION

This thesis is my original work and to the best of my knowledge, has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as the University supervisors:

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DEDICATION

TO

My caring parents

Rose Mukoya and the late Sebastiano Wakuma.

My loving Wife

Elizabeth Achungo Atemi

My dear daughter and sons

Cintah Rozah Kimono

Cabral Otindo

Sydney Mutoro

Gabriel Suva

Ambrose Wanyonyi

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ABBREVIATIONS AND ACRONYMS

AEZ	Agro-ecological zones
ASALs	Arid and semiarid lands
BQ	Black quarter
BVM	Bachelor of Veterinary Medicine
BVSc	Bachelor of Veterinary Science
CAHWs	Community- based animal health workers
CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
DFID	Department for International Development
DNA	Deoxyribonucleic acid
DVS	Directorate of Veterinary Services
ECF	East Coast fever
ECFiM	East Coast fever infection and treatment method
ELISA	Enzyme- linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot and Mouth Disease
GIS	Geographical information system
GPS	Geographical positioning system
GTZ	German Technical Cooperation
IFAT	Indirect fluorescent antibody test
IFAD	International Fund for Agricultural Development
ILCA	International Livestock Center for Africa
ILRAD	International Laboratory for Research on Animal Diseases
ILRI	International Livestock Research Institute
ISVEE	International Society for Veterinary Epidemiology and Economics
ITCZ	Inter-tropical convergence zone
ITM	Infection and treatment method
KALRO	Kenya Agricultural Livestock Research Organization
LPEC	Livestock productivity efficiency calculator
ME	Metabolizable energy
MSc	Master of Science
M.O.U	Memorandum of Understanding

MCF	Malignant catarrh fever
NGO	Non-governmental organization
NVRC	National Veterinary Research Centre
OTC	Oxytetracycline
PCR	Polymerase chain reaction
PRA	Participatory rural appraisal
T.A.R	Tick attachment rate
TBD	Tickborne diseases
TLU	Tropical livestock unit
U.O.N	University of Nairobi
RRA	Rapid rural appraisal
VSF	Veterinarie sans frontier

ABSTRACT

A 5-month longitudinal controlled trial was carried out in two sub-counties of Narok County, Kenya to assess safety and efficacy of the Infection and Treatment Method (ITM) of East Coast fever immunization, using the Muguga cocktail stabilate, and to quantify the financial and economic viability of ITM.

Four herds were purposively selected, based on accessibility and willingness of the farmers, in three contrasting study sites. In each herd there were both experimental calves and control calves that were randomly selected. One-hundred-twenty-three calves were vaccinated and another one-hundred-nineteen calves were followed as controls. The two groups were followed for a period of 5 months from August, 2004 to December, 2004. There were no reactors. The antibodies to *Theileria parva* were 46.2% on day 0 and peaked up to 93.3% on day 35 following immunization with Muguga cocktail stabilate. The difference in antibody titres in the two periods was significant (p= 0.000). In the control herd the sero-prevalence changed from 46.5% down to 41%. The difference in antibody titres in the two periods was not significant (p=0.442107). The morbidity rate of clinical ECF was 15.1% in the control group and 1.6% in the immunized group. The difference in the two incidence rates was statistically significant (p=0.000101). The cause specific mortality of ECF was 87.5%. The vaccine efficacy was 89%. The conclusion of the study was that Muguga cocktail stabilate was safe and efficacious.

Partial budgeting analysis recorded positive net returns an indication of profitability of the ITM technology. The ITM realized a net return of Ksh.1, 559.59 per immunized calf. This was significant in the study area since the average price of a calf was relatively low (Ksh.4, 700.00). High net returns are indicators of high profitability of immunization. Thus, it can be concluded from the study that it is economically worthwhile to immunize calves against ECF in the Narok County. If immunization against the disease is integrated with reduced acaricide usage, then accrued returns are even much higher.

CHAPTER ONE: INTRODUCTION

1.1 Importance of theileriosis

Theileriosis is caused by several species of *Theileria*, the most important of which are *Theileria parva* and *Theileria annulata* in Africa. *Rhipicephalus appendiculatus* is the tick vector that transmits *Theileria parva*, the causative agent of East Coast fever (ECF). *Theileria parva* infection was first recognized in southern Africa as ECF when it was introduced at the beginning of the 20th century through cattle imported from eastern Africa, where the disease is thought to have been endemic for centuries (Norval *et al.,* 1992). Theileriosis causes dramatic losses with high cattle mortality of more than 75% (Mukhebi *et al.,* 1992a**)**. A surviving cow suffers from weight loss and decreases in milk production. The estimated milk loss is about 25%, and 10% and 5% loss in beef production for surviving ECF- infected immature cattle and calves, respectively (Mukhebi et *al*., 1992a*).* Theileriosis has persisted in eastern Africa, where, despite continued and often intensive control efforts, it remains probably the most important cattle disease in terms of economic losses and restriction of livestock development, due to the high susceptibility of the productive Taurine breeds of cattle**.**

1.2 Control of theileriosis

In Africa, strategies for tick control were first implemented in southern Africa and gradually spread to the entire continent, particularly in tick-infested areas of sub-saharan and northern parts of Africa. The initial stages of these strategies involved a combination of pasture spelling, control of cattle movement, acaricides application and cattle killing (Norval *et al.,* 1992). Ticks were effectively controlled through intensive application of acaricides to cattle (Norval et *al.*,1992). However, this was not sustainable due to acaricides resistance, environmental pollution, high costs and production losses such as reduced growth (Dipeolu *et al*., 1992; William, 1995). Immunizing cattle against *T.parva* and subsequently treating them appears more appropriate for the changing livestock production systems due to its ability to select individual animals to be protected. However, it has been successfully applied on a large scale only in a few circumstances (Mutugi, *et al;* 1991; Kariuki *et al*., 1995). Infection and Treatment method (ITM) appears to work, but considerable field research in areas to be covered by immunization is required before scientists and governments can confidently apply it.

1.3 Justification for the Study

Kenya has a system of immunization of cattle against ECF in smallholder dairy farms referred to as East Coast fever infection and treatment method (ECFiM) (Radley, 1981). The ECFiM is a trade mark name and refers to ITM based on Marikebuni stabilate.The Muguga cocktail stabilate has not been used on cattle in pastoral production systems in Kenya. It has a wide use and successful penetration only in Tanzania. Impact assessment studies have found ECFiM feasible under smallholder dairy farms (Mukhebi *et al*.,1990). The ITM-based on Marikebuni stabilate has a narrow spectrum and was therefore thought not feasible in pastoral areas due to the buffalo strain missing in the Marikebuni stabilate. Immunization of cattle based on the Muguga cocktail stabilate in pastoral systems has been successfully carried out in Tanzania (IFAD, 2002; DFID, 2003; Lynen *et al*., 2012). There is therefore need to assess whether the Tanzania experience is localized or can be adopted elsewhere. There is hence the need to carry out an efficacy and financial viability assessment study of ECF immunization under pastoral systems in Kenya to determine whether it is feasible or not.

1.4 Objectives

Broad objective

To assess the efficacy of Infection and Treatment method against East Coast fever and the financial and economic benefits in Narok County of Kenya.

Specific objectives:

(i) To assess safety and efficacy of the Muguga cocktail stabilate against ECF in Narok County.

(ii) To assess the morbidity of other tick-borne diseases and trypanosomiasis that are prevalent in Narok County.

(iii) To asses and quantify the financial and economic viability of applying the Muguga cocktail stabilate in cattle in Narok County.

CHAPTER TWO: LITERATURE REVIEW

2.1 Pastoral production systems in Kenya

More than 46 million hectares or 80% of the landmass in Kenya is unsuitable for rain-fed arable farming and intensive livestock production because there is too little rain and/ or insufficient water **(**HerLocker *et al.,* 1977; Hopkins and Jones, 1983). Commercial livestock production in this area is possible using cattle, sheep, goats, and camels. Kenyan pastoral areas are densely populated relative to pastoral areas in other African countries but their production density is low (Behnke, 1984; Angelo *et al*., 1992; Swift *et al*., 1996).

Two forms of pastoralism are evident in Kenya, namely, sedentary and migratory. Sedentary pastoralists live in more fixed locality within a tribal framework and cover approximately 25% of the range areas (Coppock, 1994; Herlocker, 1996). Migratory pastoralism involves a lot of movement for livestock and the herders in search of livestock feed and water. Although pastoralist production, as a way of life, is bound to persist in the foreseable future, there is plenty of evidence that pastoralists in Kenya have been operating under constant changing circumstances (Audru *et al.,* 1989 ; Frakin, 1994).

Pastoral areas generally fall under agro-ecological zones (AEZ) 4-7, and they are referred to as arid and semi-arid lands (ASALs **(**Coppock, 1994; Herlocker, 1996)**)**. Agricultural technology in ASALs is low and reliance is exclusively on rainfall with exception of few areas where irrigation is carried out. The productive capacity of ASALs resources is dependent on forage quality and availability and is a major factor influencing carrying capacity measured in Tropical Livestock Units Km^{-2} (TLU). The carrying capacity also influences the production systems and degree of mobility of livestock (Coppock, 1994; Herlocker, 1996). The ASALs of Kenya include dry land ecosystems and they provide home to 7.5 million Kenyans as well as 54% of the country's livestock and 65% of its wildlife (Brown, 1994). Most inhabitants are pastoralists, although agro-pastoralists and farming communities exist. The common feature is the low and variable rainfall of 800 mm per year with most areas receiving 250 mm. Water resources may be limited, poorly distributed and hardly conserved when available. There are seasonal streams, which are usually dry throughout the year. Vegetation ranges from dry bush-land to grassland / shrubs**.**

2.2 Cattle productivity in the Tropics

Livestock production is aimed at improving the welfare of the owner. Usually, but not always, the owners' welfare is improved by some form of output (e.g. milk, traction power of

animals) for consumption or for sale. In order to maintain the livestock and to produce output, various inputs, the most important of which is feed, are required. Therefore, livestock production is seen as a system for converting various inputs into various forms of outputs as illustrated below:

INPUTS ⟶	LIVESTOCK PRODUCTION SYSTEM ⟶	OUTPUTS
e.g. Feed Labour Buildings Drugs	e.g. Migratory Sedentary	e.g. Animals, milk, traction power, manure, wool/hair

According to the nature of the livestock production system, the inputs and outputs of the system are variable. Of particular interest to the owner will be the rate of input and output, i.e. the quantity per unit time.

Productivity is usually taken to mean the efficiency of the production system. Efficiency is defined as the quantity of output divided by the rate of input. Because a livestock production system uses many different kinds of inputs (e.g. feed, labour, medicines) and produces several kinds of outputs (e.g. milk, meat, traction, manure) a common unit is required. This is usually the economic value of each type of input and output. Thus, the productivity of a livestock production system could be defined as (LPEC, 1991):

$$= \frac{\text{Total value of outputs per unit of time}}{\text{Total value of inputs per unit of time}}$$

2.3 East Coast fever

2.3.1 Aetiology of East coast fever

East Coast fever (ECF), a form of bovine theileriosis, is a tick-transmitted protozoal disease of cattle characterised by high fever and lymphadenopathy. The disease causes high mortalities in non- indigenous breeds in the endemic areas, and is confined to Eastern, Central, and parts of southern Africa (Irvin, 1987; FAO, 1997). The causative agent of classical ECF is the protozoan parasite, *Theileria parva*.

The brown ear tick, *Rhipicephalus appendiculatus* is the main field vector of ECF, although in certain areas other vectors play a role such as *R. zambeziensis* in drier areas of Southern

Africa and *R. duttoni* in Angola (Norval *et. al.,* 1992). East Coast fever is not maintained in the absence of these field vectors. The Rhipicephalid vectors are three-host ticks, and transmission occurs from stage to stage; transovarian transmission is not known to occur (Norval *et. al.,* 1992). Ticks can remain infected in pasture for up to 2 years depending on the climatic conditions and the stage of infection (Norval *et al.,* 1992). The parasite dies out faster in hot climates in nymphs than in adults (Norval *et al.,* 1992). Normally for transmission to occur, the infected tick has to attach for several days to enable sporozoites to mature and be emitted via the saliva of the feeding tick. However, under high ambient temperatures, ticks on the ground can develop infective Theileria sporozoites, which can be transmitted within a few hours after attachment (Norval *et. al.,* 1992). Unlike other Theileria species, *T. parva* is not easily transmitted experimentally by blood. Schizont-infected lymphoid tissues have been used to initiate infection with variable results.

2.3.2 Life cycle of *Theileria parva*

The life cycle of *T. parva* is complex in the 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 tick vector, are inoculated along with saliva during feeding and rapidly enter target lymphocytes, which become transformed after 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 anti-theilerial drugs, the transformed cells revert to quiescent lympocytes (Ole-Moiyoi, 1989). 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 by merogony (Conrad *et al.,* 1986). Piroplasm-infected erythrocytes are ingested by ticks of the larval or nymphal stages and undergo 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 (Mehlhorn and Schein,1984 *;* Fawcet *et al*., 1985)**.**

2.3.3 Clinical signs of East coast fever

The first clinical sign of ECF in cattle appears 7 to 15 days after attachment of infected ticks (Aiello and Mays, 1998). This is seen as a swelling of the draining lymph node, usually the parotid, for the ear is the preferred feeding site of the tick vectors. This is followed by a

generalised lympadenomegally in which superficial subcutaneous lymph nodes such as the parotid, prescapular, and prefemoral lymph nodes are enlarged and can easily be seen and palpated. Fever ensues and continues throughout the course of infection. This rise in temperature is rapid and is usually in excess of 39.5^0 C but may reach 42^0 C. Anorexia develops, and loss of condition follows. Other clinical signs may include petechial hemorrhages on mucus membranes, lacrimation, corneal opacity, coughing, nasal discharge, terminal dyspnea, and diarrhoea. Before death, the animal is usually recumbent, the temperature falls, and there is severe dyspnea due to pulmonary edema that is frequently seen as a frothy nasal discharge.

Death usually occurs 8 to 30 days after infection of susceptible cattle by infected ticks. Mortality in fully susceptible cattle can be nearly 100 percent. The severity and time course of the disease depends on, among other factors, the magnitude of the infected tick challenges, 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 (Norval *et al.,* 1992).

2.3.4 Epidemiology of East coast fever

Cattle in endemic areas, particularly the Zebu type (*Bos indicus*), appear less susceptible to ECF. In addition, introduced cattle, whether of a exotic, zebu, or sanga breed, are much more susceptible to theileriosis than cattle from endemic areas. The Indian water buffalo (*Bulbalis bulbalis*) 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 with 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 a disease commonly referred to as Corridor disease.

Piroplasms have been demonstrated in most wild antelopes in East Africa, but the relationship of most of them to *T. parva* is unclear. The distribution of ECF is strictly associated with the distribution of the vector tick species, although, the range of *T. parva* is less than the tick vector. In the case of ECF, the area extends from southern Sudan to South Africa and as far West as the Democratic Republic of Congo.

Free populations of *R. appendiculatus* free of *T. parva* occur in Zambia and Kenya (Yeoman, 1966a**)**. *Rhipicephalus appendiculatus* is found from sea level to over 8,000 feet in areas where there is annual rainfall of over 500mm. Up to three generations of the tick vector can occur per year in favorable areas of East Africa (Lake Victoria Basin), but there is only one generation a year in Southern and Central Africa because of a behavioural diapause, controlled by photoperiod, in the adult tick (Norval *et al.,* 1992). This results in a strict seasonal occurrence of the different tick stages on cattle and a seasonal occurrence of ECF. This behavioural diapause allows the tick to survive during the long hot dry season occuring in the Southern parts of Africa.

2.3.5 Species and breed susceptibilities by ticks

Among domestic animals, cattle are the major hosts and become heavily infested with larva, nymphs and adults. Sheep, goats, horses, donkeys and mules are also parasitized but not to the same extent (Norval *et al.*, 1982). Several wild ungulates species such as buffalo (S*yncerus caffer*), giraffe (*Giraffa cameloparadalis*), eland (*Taurotragus oryx*), greater kudu (*Tragelaphus strepsiceros*), bush-buck (*Tragelaphus scriptus***),** can also become infested (Dinnik *et al*., 1963). *R. appendiculatus* do not exist in abundance in the large wildlife reserves of Eastern and Southern Africa as these are generally situated in areas that are climatically unsuitable or only marginally suitable for the tick and mean host densities remain relatively low (Perry *et al*., 1990b).

Whereas many wild species are known to be susceptible to the tick, there are others such as Warthog (*Phacochoerus aethiopicus*), wildbeast (*Connochaetes taurinus*) and tsessebe *(Damalisces lunatus*) that appear to be highly resistant (Lightfoot and Norval, 1981). Among cattle, Taurine breeds are considerably more susceptible to *R.appendiculatus* than Zebus (Rechav and Zeederberg, 1986) or the Southern African Sanga breeds (Norval *et al;* 1988b). Herefords are particularly susceptible and may lose ears if infestations of adults are not controlled. The losses caused by infestations of adults are more of a problem in Taurine than Sanga or Zebu cattle because these animals become heavily infested in the absence of effective tick control.

2.4 Diagnosis of East Coast fever

2.4.1 Clinical signs

A febrile disease with signs of enlarged lymphnodes, rise in temperature in excess of 39.5^0C but may reach 42^0C, anorexia develops, loss of condition, petechial hemorrhages on mucus membranes, lacrimation, corneal opacity,coughing, nasal discharge, terminal dyspnea, and diarrhoea 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, is also suggestive of ECF. In many epidemiological situations, high mortality occurs only in calves; the adult cattle represent immune survivors (Norval *et al.*, 1992**)**.

2.4.2 Blood smears

Thin and thick blood smears are made from marginal ear vein, on the slide. The slide is air-dried, and the thin smear is fixed in Methanol. The thick smears are not fixed in methanol (Malmquist *et al.,* 1970). The smears are stained with Giemsa at a dilution of 1:10 for 30 minutes and left to dry on a wooden rack, before being examined. The microscope objective lens (X40) is used for scanning through the slide and the objective (X100) is used for identification of the piroplasms. Small piroplasms in erythrocytes are suggestive of ECF but not confirmatory (Malmquist *et al.,* 1970).

2.4.3 Lymphnode smears

Lymphnode smears are made by puncturing the prescapular lymphnode with a needle (gauge 18 or 19) and extruding the aspirate onto a slide to make a thin smear. The slide is air-dried and fixed in methanol (Malmquist *et al.,* 1970). The smears are stained with Giemsa at a dilution of 1:10 for 30 minutes and left to dry on a wooden rack, before being examined. The microscope objective lens (X40) is used for scanning through the slide and the objective (X100) for identification of the schizont-infected cells. The schizont-infected cells in lymphnode samples are diagnostic of ECF (Malmquist *et al.,* 1970).

2.4.4 Serological tests

The most commonly used serum antibody assay in recent past for *T.parva* is the schizont antigen indirect fluorescent antibody (IFAT) test (Burridge and Kimber, 1972*;* Goddeeris *et al*., 1982). The IFA employs a cell culture schizont antigen. Because of the often acute nature of the disease, serological tests are useful in detecting a changed immune status of recovered animals within an exposed herd (Norval et *al.,* 1992). Enzyme-linked immunoassays have

been developed using whole parasite lysates or specific antigens isolated by monoclonal antibodies (Katende et *al.,* 1990). Evaluation of the levels of antibodies to *T.parva*, *B.bigemina* and *A.marginale* were carried out using ELISA tests described in Nielsen *et al*., (1996) and Katende *et al., (*1998). The tests have been used successfully in field studies in Kenya and elsewhere (Muraguri *et al*.,2000).

Now DNA technologies can be applied to material from cattle and ticks, including the use of probes and polymerase chain reaction (Conrad *et al.,* 1989; Chen *et al.,* 1991; Norval *et al.,* 1992; Bishop *et al.,* 1992), for the diagnosis of ECF.

2.5 Control of East Coast fever

2.5.1 Tick control

Tick control has been used as a means of controlling the diseases caused by *T.parva* since Lounsbury (1904) discovered that ECF was transmitted by the tick *R. appendiculatus*. Tick control measures for ECF were initially implemented in Southern Africa and consisted of various combinations of pasture spelling (the keeping of pasture free of cattle for 15 months), the control of cattle movement and acaricide application (Norval, 1977a). Pasture spelling was found to be impractical because of the length of periods involved, which were based on survival periods of the infected ticks, and this method was soon abandoned. Cattle movement control and acaricide application were retained and made compulsory through veterinary legislation in the Southern African countries to which *T.parva* had spread.

Similar measures were adopted in Kenya, where theileriosis caused by *T.parva* was a serious problem in the Taurine cattle that were being brought in by European settlers (Swynnerton, 1954; Perry *et al*., 1990a; Maloo *et al*., 2001). In Kenya, it was implemented on compulsory weekly dipping in communal dips. Intensive tick control is still widely practised in the Eastern and Southern African countries that have been affected by *T.parva* since the turn of the last century. Methods of biological control of ticks using predators and pathogens, tick killing or repelling plants, habitat modification and resistant hosts have been discussed by veterinary scientists and in some instances tested, but have not as yet been implemented practically on a large scale (Samish and Glazer, 1990; 1992**)**. While it is known that indigenous breeds of cattle in Africa tend to be tick-resistant, this resistance has not been exploited to any extent. Similarly, while habitat modification caused by overstocking is known to be detrimental to the survival of some tick species such as *R. appendiculatu*s and may be the cause of their absence from larger areas, stocking rates are seldom or never altered for purposes of tick control.

In recent years the rapidly rising costs of acaricides and their applications, as well as the growing problem of tick resistance to acaricides, have stimulated research into new and innovative methods of tick control. This research is beginning to yield benefits with several new control methods emerging, including vaccines against ticks, slow release acaricide devices, more efficient means of topical application of acaricides, manipulation of hybrid sterility between closely related tick species and the use of pheromones to disrupt mating or to attract ticks and so improve the efficiency of acaricide treatment (Norval *et al*., 1991c). Tick population models are also being developed and used to simulate the effects of control strategies, enabling veterinary authorities to select the most appropriate and cost-effective strategies for given circumstances in the field (Norval *et al*., 1992).

2.5.2 Infection and Treatment method

Infection and Treatment method procedure was developed over 40 years ago at the former East African Veterinary Research Organization and now the National Veterinary Research Centre (NVRC), KARI-Muguga (Radley *et al.,* 1978). The system depends on the observation that cattle, which undergo an episode of ECF, develop strong immunity against subsequent infection. The immunity has been demonstrated for at least three years, and is probably life-long. In the ITM procedure, cattle are injected with a precise dose of live sporozoites of a virulent isolate of *T. parva*, and simultaneously with a dose of long-acting oxytetracycline to limit the development of the infection. In most cases, the resultant infection is asymptomatic and it stimulates strong immunity. However, in a small proportion of immunized cattle, clinical ECF of varying severity develops. In about 5% of the cases, this may be severe enough to require specific curative therapy. Such clinical reactions are more common in calves and malnourished (stressed) animals than in older cattle (Moll *et al*., 1986). Cattle, which recover following these clinical reactions, also develop strong immunity. Because of the risk of clinical reactions after ITM, all cattle must be observed at least once every three days for a period of 14 days after injection, so that any reactions can be detected and, if necessary, treated. This period of monitoring of immunized cattle was an integral and essential part of the ITM system of immunization (Young *et al*., 1990d).

There are many immunologically different strains of *T. parva.* Immunity developed as a result of infection with one strain may not give full protection against other strains, and there is no simple test to predict the spectrum of immunity, which has been developed. Recovery from some strains, however does give a relatively broad spectrum of protection against other

strains. The strain for ITM (the Marikebuni strain, originally isolated in the Coast Province of Kenya) was selected because it gave a broader spectrum of protection than any other strain tested (Mutugi *et al*., 1990a, 1990b). The ECFiM does not protect against *T.parva* transmitted by ticks that have derived their infection from buffalo (Young *et al*., 1990a). However, the ITM based on Muguga cocktail stabilate has been found to protect against some *T. parva* transmitted by ticks that have derived their infection from buffalo (IFAD,2002 ; DFID,2003). The Muguga cocktail stabilate as the name suggests is a cocktail vaccine from three seed stocks(stabilates) inluding *T. parva* Muguga stabilate, *T. parva* Kiambu 5 stabilate, and *T. parva* Serengeti transformed stabilate. *T. parva* Serengeti transformed stabilate was initially isolated from buffalo and was included to try and protect against buffalo-derived parasites.

2.6 Economic and financial viability of Infection and Treatment method in Kenya

Theileriosis causes economic losses to individual farmers and governments by lowering cattle production, costs incurred for controlling the disease, research, training, and extension services pertaining to the disease (Norval *et al*., 1992). Such economic losses vary widely within and among countries, due to differences in livestock production systems, cattle types, level of disease risk, disease control policies and programmes, cost and price structures**.**

2.6.1 Production losses

2.6.1.1 Direct production losses

Direct production losses are those that are directly attributable to the presence of the disease in cattle population through morbidity and mortality. Cattle, which become severely infected usually, die unless treated. Morbidity and mortality in certain breeds of susceptible cattle may approach 100% (Hooke, 1981**).**

2.6.1.2 Indirect production losses

Indirect production losses result from the disease acting as a constraint to livestock production and improvement. In the affected areas, farmers face a substantial risk if they try to keep taurine or crossbreed cattle due to their high susceptibility to the disease. Many farmers are, therefore, constrained or prohibited from improving livestock productivity and efficiency (Callow, 1983).

2.6.2 Control costs

2.6.2.1 Tick control

The conventional method of controlling tick vectors in Africa is by close interval application of acaricides to the surface of an animal through dipping, spraying or hand washing, to kill the ticks. In conditions of heavy tick infestation or high disease incidence, the frequency of acaricide applications can be as often as twice a week. Depending on the frequency of applications, annual costs of acaricide to those farmers who bear the full cost of control can range from US$ 2 to US$ 20 per animal (Perry *et al.,* 1990c). The real costs of tick control to the farmer who uses public dip tanks include loss of animal traction time and human labour for the time spent in trekking animals to and from the dip tanks, which are often several kilometres away from the farm. Other losses include stress- induced abortions, drowning and physical injury.

2.6.2.2 Treatment

Effective treatment of theileriosis requires identification of the disease through surveillance and diagnosis, and treatment of infected animals with drugs. Costs to the farmer who pays fully can be over US$20 per animal (Young *et al.,* 1988**).**

2.6.2.3 Infection and Treatment method

There is a practical, though not yet widely applied, method of immunizing cattle against ECF. It is based on the infection of animals with live *T. parva* parasites and simultaneous treatment with an antibiotic. The ITM has been field-tested in several countries in Eastern and Southern Africa (Radley, 1981). Technical results demonstrate that it is very effective if properly administered, and provides a life- long strain-specific immunity (Mutugi *et al.,* 1988b).

A cost assessment under specified circumstances in Kenya indicated that it could cost US$ 0.45-0.90 to produce one dose of the infection and treatment vaccine and a further US$ 1.50 to deliver the dose to the field, for a total of about US$ 1.95-2.40 per immunized animal (Mukhebi *et al.,* 1990). Clearly, this cost will vary depending on the circumstance of production and delivery. Cost estimates of up to US$ 20 per immunized animal have been made (Radley, 1981). In contrast, the costs of acaricide application to the farmer per animal per year are in the range of US$ 2-20. Immunization with infection and treatment vaccine would therefore appear to represent a substantial cost saving over the lifetime of an animal.

Immunization of calves and yearlings in smallholder farms could reduce the cost of ITM by over 30% as a result of the reduced amount of both blocking and therapeutic drugs (Muraguri *et al.,* 1996). Acaricide application frequency under ITM was reported to drop from twice a week to once in three weeks (Rumberia *et al.***,** 1998**;**Wesonga *et al.***,** 1998). Results from the simulated study showed that the greater the reduction in the use of acaricides after immunization, the higher the profitability achieved (Nyangito *et al.,* 1994). In addition, cattle productivity in terms of surplus females and males, milk, beef, and replacement stock increases (Young *et al.,* 1992). In Kenya, ITM has been commercialized based on the preliminary results of this study and comprehensive provision of ex-ante financial viability assessment on ITM in the pastoral production systems is most appropriate.

2.7 Partial budgeting

Partial budgeting analysis refers to the financial or economic analysis of only those parts of a production system that would be affected by the decision to be made (Sloan and Arnold, 1970). It is thus, a decision-making tool, assisting in arranging information in such a way that the economic implications are clear. It is time saving since analyzing only the relevant parts of the production system will take less time than analyzing the whole production system with and without the implementation of the decision.

The basic framework for partial analysis is: (Brown, 1978; Putt *et al*., 1983**)**

Table 2.1: The basic framework for partial budget analysis

Costs	Benefits
a) Extra costs	c) Costs saved
b) Revenue loss	d) Extra revenue

Partial analysis can be undertaken for one year, or for a period of several years. If the analysis only covers one year, benefits and costs can be compared as shown:

Table 2.2: The partial budget analysis computation for one year

a + b = Total costs and c + d = Total benefits
Net benefit = Total Benefits - Total Costs = (c + d) – (a + b)
Benefit-Cost ratio = Total Benefit / Total Costs = (c + d) / (a + b)

When looking at several years the costs and the benefits should be quantified separately for each year, using the basic partial analysis framework. However, they cannot simply be added up as shown immediately above. The comparison of costs and benefits should then be done according to the rules of discounting **(**Gittinger, 1973).

The four categories of benefits or costs provide a checklist for ensuring that all areas of cost and benefit resulting from the decision under consideration have been covered. If the decision is whether or not to implement a given livestock project, then the four components of the basic framework are some of the items that might be identified. It should be noted that all four categories will not always be needed. Many projects will not involve any revenue lost or cost saved. All projects will involve extra revenue (hopefully, unless the project is a failure) and extra costs (Brown, 1978; Gittinger, 1973; World Bank, 1981b)

2.7.1 Extra costs

Extra costs consist of the basic costs of the livestock project. These could involve pasture improvement, housing improvement, extension inputs, nutritional supplements, disease control inputs such as veterinary interventions, drugs, disinfectants, fees for vaccinations and dipping (Brown, 1978; Gittinger, 1973; World Bank, 1981b). They also include extra time invested by the producer in implementing the project, although this may be difficult to value. Where livestock numbers increase as a result of the project, extra costs will also include the extra cost of maintaining the animals.

2.7.2 Revenue lost

Revenue lost refers to revenue lost as a result of the type of project implemented. For many projects, there may not be any items to fill in revenue lost. Animal disease control provides some examples: a reduction in emergency slaughtering due to a reduction in mortality rates, or a reduction in the value of the herd due to slaughtering of diseased stock (Brown, 1978; Gittinger, 1973; World Bank, 1981b).

2.7.3 Costs saved

Projects do not always involve cost savings, but these do occur where the project makes it possible to produce livestock products at a lower cost. Again, livestock disease control provides a useful example. Where a disease has been present in the livestock population, a comprehensive control programme should lead to a reduction in the incidence or severity of the disease. This should lead to a saving in the costs of measures previously used to deal with

the disease, especially in treatment costs and in time spent caring for the sick animals (Brown, 1978; Gittinger, 1973; World Bank, 1981b).

2.7.4 Extra revenue

Extra revenue is usually the ultimate goal of a livestock project. In order to estimate it correctly, it is necessary to go through all the items included in the output calculation. Often, it is calculated as: (Brown, 1978; Gittinger, 1973; World Bank, 1981b).

Extra revenue = output with the project minus output without the project

This works very well, but in this case, any revenue lost will usually be automatically accounted for in the above calculation and should not be estimated separately. For example, if there is a reduction in mortality due to disease control, the extra revenue or difference between output with disease control and output without disease control will reflect: a reduction in home consumption of animals due to emergency slaughter; an increase in the final herd value due to presence of these animals. Estimating the reduction in home consumption again separately under the heading revenue lost would thus not be correct in this case (Brown, 1973; Gittinger, 1973; World Bank, 1981b).

2.8 Financial viability studies

The aspect of ITM financial viability using the cost/financial analysis of ITM can be observed from studies carried out by different scholars as outlined below. Mbogo *et al*. (1994) carried out a study in Limuru and Kikuyu sub-counties of Kiambu County to assess morbidity and mortality amongst immunized and non-immunized calves. Twenty-three calves were immunized and compared to 24 controls over a 7- month period. Results obtained from the study showed that the annual mortality risk in immunized calves was 45% compared to 84% in the non-immunized group. The annual incidence rate for ECF amongst immunized calves was 9.1% compared to 61.7% amongst the non-immunized. However, the differences in the incidence rates were not statistically significant (p=0.21).

Muraguri *et al.* (1998) carried out a cost analysis of immunization against ECF on smallholder dairy farms in central Kenya. Data from an immunization trial carried out on 102 calves and yearlings on 64 farms in Githunguri Sub-county of Kiambu County was used in the analysis. A reference base scenario of a mean herd size of five animals, a 10% rate of

reaction to the immunization and a 2-day interval monitoring regimen (a total of 10 farm visits) was simulated. Under these conditions, they showed that the mean cost of immunization per animal was US$ 16.48 (Ksh.955.78 at the 1998 exchange rate); this was equivalent to US$82.39 (Ksh. 4,778.90) per five-animal farm. They noted that under the commonly reported reactor rate of 3%, the cost per animal would decrease to US$14.63 (Ksh.848.29). Reducing the number of farm monitoring visits from 10 to 7 would further reduce the total cost by 10%, justified if farmers were trained to undertake some of the monitoring work. The fixed costs were 53% of the total cost of immunization per farm. They further noted that the cost of immunization decreased with increasing number of animals per farm, showing economies of scale.

Mukhebi *et al*. (1992) estimated that the benefit-cost ratio of immunization against ECF was in the range of 9-17, thus indicating a high level of economic returns. Data obtained from a trial site in Kitale showed that tick control by means of acaricide application could be reduced by 83% (from weekly dipping to only nine times a year) without increasing the risk of cattle to contract ECF under mixed crop-livestock production systems typical of Kitale (Kiara et *al.,* 2000). Observations by Wesonga et *al*. (1998) and Rumberia *et al*. (1998) during trial studies in Nakuru and Trans-Nzoia counties showed that dipping interval could be relaxed from once weekly to once every three weeks following ECFiM without exposing animals to increased risks of contracting ECF or other tick-borne diseases. A similar study by the Tick-borne Diseases Division (TBD) at Muguga on 30 farms in Limuru and Kikuyu sub-counties of Kiambu County showed that the mean acaricide application frequency reduced from 3.03 times a month to twice a month thus representing a 34% reduction in a acaricide use or a 34% reduction in cost of tick control as no other TBDs were reported during the study period (Mbogo *et al*., 1996). The age at which calves were treated against ticks rose from a mean of 2.5 months to 3 months, thus representing a 20% increase. While this had the potential of increasing the incidence of ECF, it was, however, advantageous because it created a chance for immunity against other TBDs such as babesiosis and heartwater to develop. However, no financial viability assessment study on ITM has been carried out in pastoral systems.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was conducted in Narok County in the period from August 2004 to December 2004.The County is situated in the South western tip of Kenya and lies in the southern part of the former Rift Valley Province (Figure 3.1). It borders the Republic of Tanzania to the South, Bomet and Nakuru County s to the North and Kajiado County to the East (Figure 3.2). It lies between Latitudes $O^0$50' and $2^0$50' South and Longitudes $35^0$58' and $36^0$5' East. The County occupies an area of over 15,074.8 km^2 and is divided into three regional areas as shown in Figure 3.2.

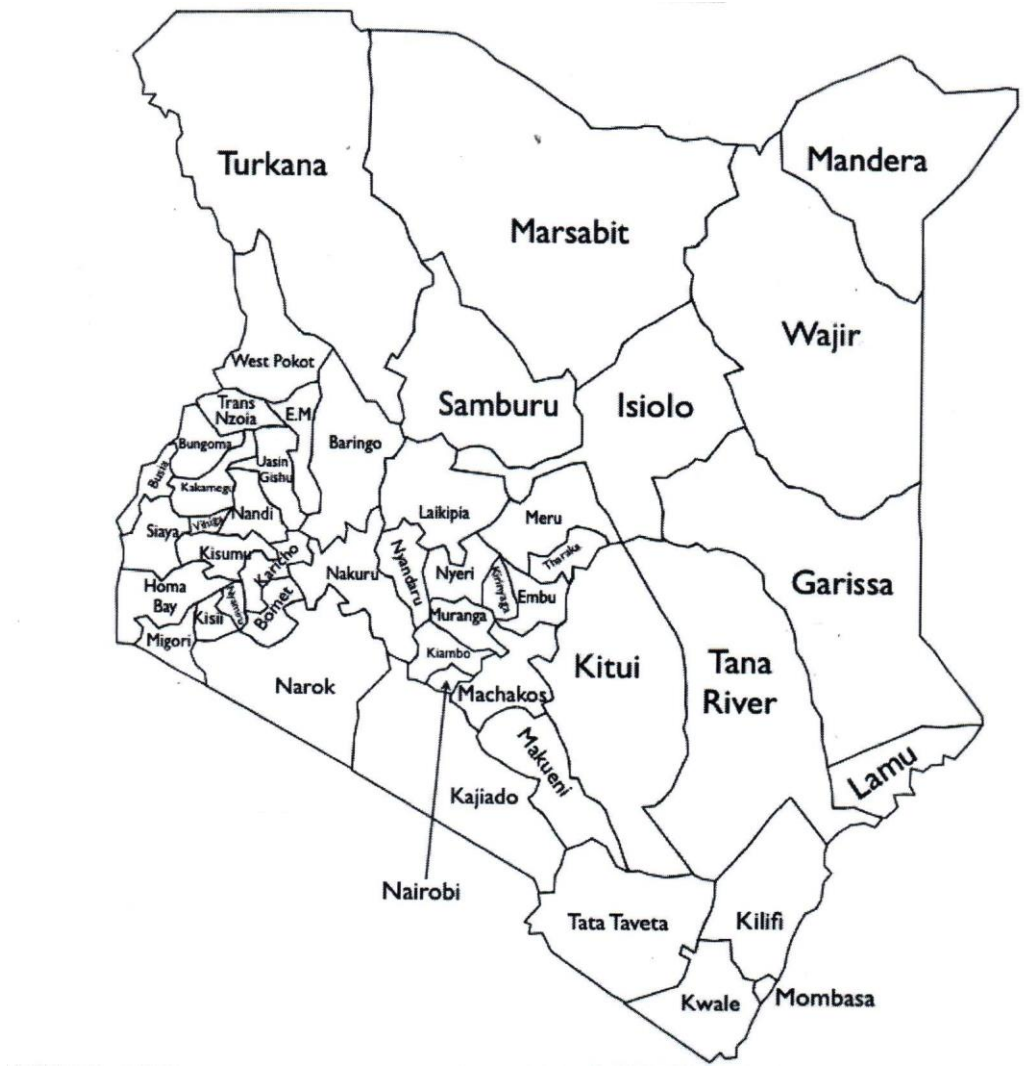

Figure 3.1: Map of Kenya showing the location of Narok County.

Figure 3.2: Map of Narok County showing regional administrative boundaries

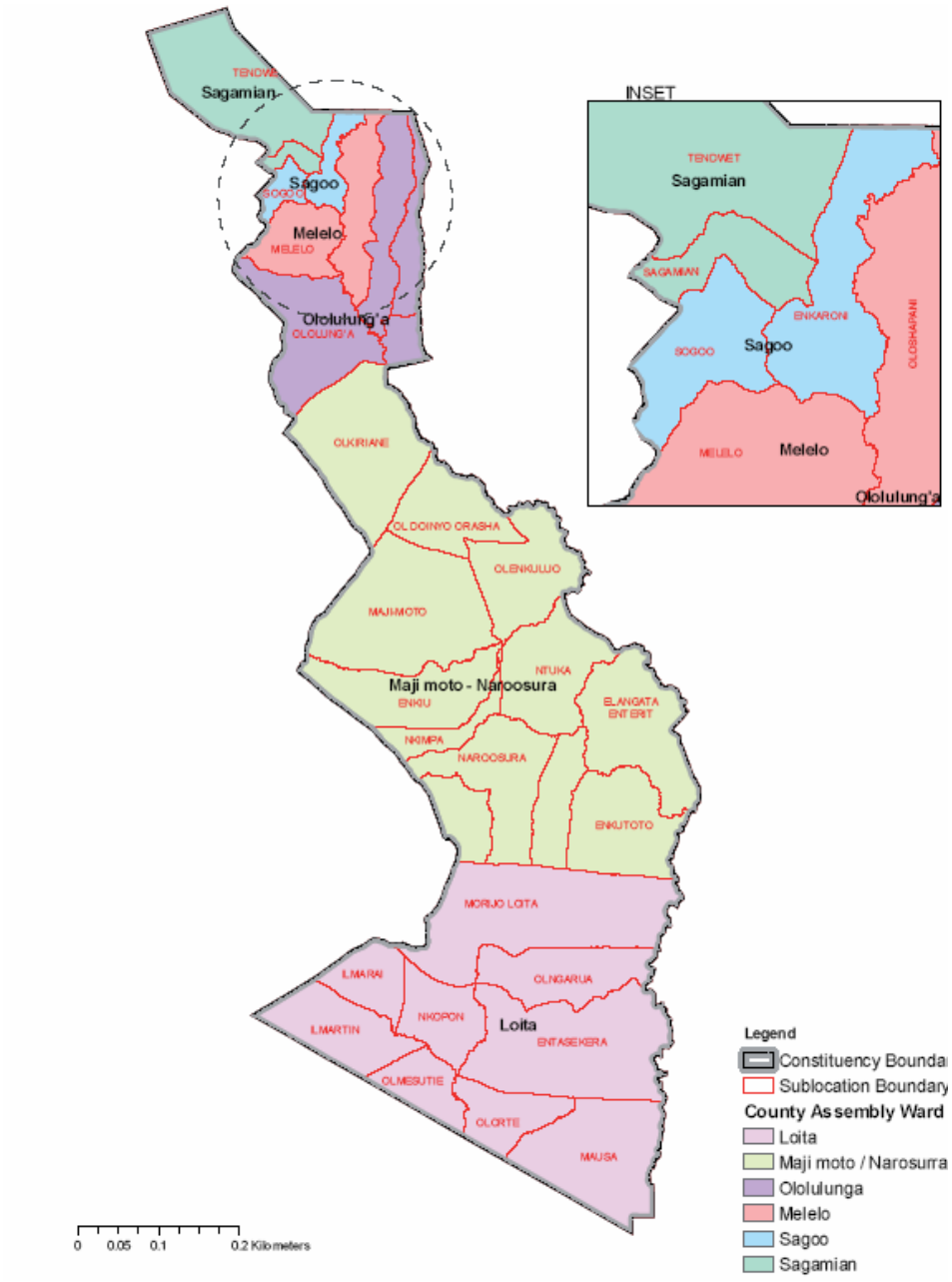

Figure 3.3: Map of Narok South showing areas of controlled trial.

3.1.1 Topography and Climate

Narok County has a varying topography with altitude ranging from 3,098m above sea level in the highlands to 620 metres above sea level in the lowlands that is Mausa area in Loita Subcountty (Narok County Development plan, 2004-2008). The highlands consisting of Mau, Olokurto and Mulot sub-counties, have a high potential for wheat, barley, maize, beans and potato growing. This is attributable to fertile soils, reliable rainfall ranging from 1200-1800mm per annum and temperatures ranging from 10^0 and 15^0 C. The lowland areas, consisting of Ololunga, Mara, Loita and Osupuko sub-counties have a high potential for livestock rearing. The highlands have rich volcanic soils suitable for intensive agricultural production. The major rivers are Ewaso Ng'iro and its tributaries Siapei and Narok. The river drains southwards into Tanzania. The Ewaso Ng'iro is perennial due to the heavy rainfall at its sources.

Narok County lies within the tropics and experiences sunshine throughout the year but the effect of Inter-Tropical Convergence Zone (ITCZ) plays a greater role in determining seasons (Narok County Development plan, 2004-2008). The sun is on the Equator in March and September resulting in conventions during the day, while the temperatures are relatively low at night. The bi-annual passage of ITCZ over the County gives rise to pressure area, which attracts southerly and northeasterly winds, which converge and give a bimodal pattern of rainfall.

Narok County experiences high rainfall caused by southeasterly winds during the months of March to June. Between October and March, the area is influenced by northeasterly winds, which are relatively dry and cause low to moderate rainfall. Some parts of the County experience a modified type of climate due to the influence of the altitude on the highlands. Breezes from Lake Victoria cause high rainfall in the western part of the County and along the Mau Escarpment. Thunderstorms and hailstorms are common phenomena in the County.

The rainfall is unevenly distributed with an annual average rainfall ranging from 500 mm to 1,800 mm. The long rains start from mid-March to June with the peak in April while the short rains occur between the months of September and December. The northern and western parts of the Narok County are the wettest. These, however, decrease towards Central Narok where the lowest amount recorded is about 50 mm on the Loita plains. The temperatures range from

8^0C-28^0 C with low temperatures reaching 8^0C in June – September, while the maximum reach 28^0C in November – February.

Most of the land in the Narok County falls under the category of trustland. Less than half of it is available for cultivation and is either individually or communally owned. In the high potential areas, land is individually owned. Mixed farming (crop:livestock) is practised here while large-scale companies and wealthy individuals have introduced large-scale cultivation of barley, wheat and rapeseed.

The rangelands are used as group ranches. Land on group ranches is communally owned and used mainly for grazing purposes. The present Government policy is to have group ranches sub-divided into individual plots (Herlocker, 1999). This will ensure that land is used to optimal level as land in Narok is not optimally used due to lack of individual ownership and title deeds. Figure 3.3 shows the patterns of land use in the County. Most of the land is still under trustland.

Tropical Alpine Zone: This zone covers an altitude of 2,980 to 3,040m above sea level with an annual average rainfall of 1,200mm – 1,400 mm. Temperatures range from 10.0^o C to 10.4 ^{o} C making it too cold for growing crops. It is used for sheep and cattle rearing.

Upper Highland Zone: This zone covers an altitude of 2,150m to 2,970m above sea level and receives rainfall of 1,000mm to 1,800mm. Temperature ranges from 10.5 ^{o}C to 10.15 ^{o}C. The zone is suitable for sheep, dairy cattle rearing as well as wheat, pyrethrum and barley and forestry production.

Lower highland zone: This zone covers an altitude of 1,800m above sea level with an annual average rainfall of 750mm. Temperature ranges from 14.8^{0}C – 17.5 ^{o} C. The land here is used for growing tea, maize, sunflowers and sorghum and livestock is also reared here.

Upper midland zone: It covers an altitude of 1,460m above sea level and has an annual rainfall of 600mm – 1,000mm. Temperature ranges from 17.7^{o}C -20^o C. Land here is used for coffee, tea and maize production.

Lower midland zone: It covers an altitude of 1,450m above sea level and has an annual rainfall of 600mm – 1,000mm. Temperature ranges from 20^{o}C –23^o C. Land here is used for growing sorghum, millet, sweet potatoes and livestock production.

3.1.2 Human population

Population size

The human population of Narok County according to the population census was 576,388 in 2009 with a population density of 38 persons per km^2 up from 24 persons per km^2 in 1999, indicating a significant increase over the last 10 years. The highland areas have the highest population densities in the County due to favorable agro climatic conditions. There are relatively high poverty incidence levels in the County with 52% of the rural population living below the national poverty line (KNBS, 2004).

Labour- force (Age group15-64)

There were 242,170 people in this group in 2004 with males and females almost equal. Most of this labour-force is unskilled which necessitates investment in institutions for training them in practical skills. The increase in this population also requires creation of more employment opportunities to absorb them. The dependency ratio stood at 100:142 in 2004 and is expected to stay so in the plan period. This implies that every 100 working people support 142 dependants.

Distribution and density

In 2004, Mau Subcounty had the highest population followed by IImotiok and Olokurto while Ololunga and Central sucounties had the least. The high populations for Mau, IImotiok and Olokurto are attributed to favourable weather and soil, which support agricultural activities and thus enabling more people to settle there. The high population for IImotiok is attributed to immigration from the neighbouring County s especially Bomet. The low population of Ololunga Subcounty is attributed to the fact that this Sub-county comprises of large farms.

Urban population

Narok County does not have many large urban centres other than Narok Town and Nairagie Enkare. This is due to the fact that the Maasai people have not so much embraced urban lifestyles. Narok town had a population of 40,283 persons in 2004 while Nairagie Enkare had 5,383 persons.

3.1.3 Livestock industry

The estimated livestock population in the Narok County is as follows: 488,424 cattle, 558,914 goats, 732,563 sheep, 120 camels, and 171 pigs as per year 2004 (Narok County Livestock production office, 2004). The main livestock and their products in the County are displayed in Table 3.1. Mau Sub-county has the largest number of households keeping livestock followed by former IImotiok and Osupuko sub-counties. Ilmotiok has the highest land carrying capacity of 52.56 livestock units/hectare, followed by Olokurto while Osupuko has the least. Most of the County is a ranching zone and can support 265,303, 172 livestock units. However, there are 675,017,095 livestock unit all over the County. This implies that there is overgrazing on ranches thus causing environmental degradation.

Table 3.1 Distribution of Livestock species and their products in Narok County, 2004 (Source: Narok County Livestock Production office).

Division	Area (Km^2)	No.of households	Main Livestock kept	Livestock production	Livestock carrying unit per hectare(Lu/h)
Osupuko	5,469	8,524	Cattle, sheep, goats, bees, camel	Meat, milk, hides & skins, honey wax	7.8
Ololunga	3,966	6,938	Cattle, sheep, goat, poultry, camel, donkeys	Meat, milk, hides & skins, eggs	8.75
IImotiok	952	10,008	Cattle, sheep, goats, poultry, bees	Milk, meat, hides & skins, eggs, honey, wax	52.56
Central	538	4,013	Cattle, sheep, goats	Wool, meat, milk, hides & skin	8.5
Olokurto	1,365	8,901	Cattle, sheep, goats, donkey	Meat, milk, wool, hides & skins, eggs	45.4
Mau	2,838	14,028	Cattle, sheep, goat, donkeys	Meat, milk, hides & skin	24.7

3.2 Study design

The study comprised of a controlled experimental trial and financial analysis of the Muguga cocktail stabilate on cattle productivity. The controlled experimental trial was carried out from August 2004 to December 2004 and the financial analysis was conducted from October to December 2004.

3.3 Controlled trial of the Infection and Treatment method

Three sites were selected for the controlled trial, namely, Entasikira, Enkutoto and Olntulele locations. The three were selected conveniently based on known ECF risk and buffaloes risk. The Entasikira is a buffalo zone all year round and with a heavy tick infestation. Enkutoto is a seasonal buffalo zone and during drought, cattle are taken to the highlands for grazing hence they come in contact with the buffaloes. During rainy seasons, cattle contact with buffalo is rare. Olntulele is non-buffalo zone and tick vector transmission is seasonal. The three selected locations were in Osupuko and Loita sub-counties in Narok County and had the highest ECF incidence rate of between 30% and 50% in the County (Narok County Veterinary office, 2004).

3.4 Sample size determination

The minimum number of cattle that needed to be immunized by the end of the study (assuming that immunizing against ECF will result in 50% reduction in incidence of ECF) was derived from the formula (Dohoo *et al.*, 2009):

$n= [Z_{\alpha} (2PQ)^{1/2}-Z_{ß} (P_eQ_e + P_cQ_c)^{1/2}]^2/ (P_e -P_c)^2$

Where,

Z_{α} = Value of Z (1.96) which provides α/2 in each tail of a normal curve for a two- tailed test,

$Z_{ß}$ = Value of Z (-0.84) which provides ß in lower tail of a normal curve ($Z_{ß}$ is negative if ß < 0.5),

P_c = Estimate of response rate in non-vaccinated group assuming prevalence of ECF of 40%

P_e= Estimate of response rate in vaccinated group = 20% (Ngumi *et al.*, 2005).

$P = P_e + P_c / 2$

$Q = 1 - P$

The minimum number of animals for the vaccine trial was

$$= \frac{[1.96 (2 \times 0.30 \times 0.70)^{1/2} + 0.84 (0.20 \times 0.80 + 0.40 \times 0.60)^{1/2}]^2}{(0.20 - 0.40)^2}$$

$$(1.270 + 0.563)^2 / 0.20^2 = 81$$

Thus, a minimum of 81 calves needed to be immunized with 81 controls. The number recruited for vaccination was 123 calves and 119 calves were followed up as controls.

3.5 Immunization procedure

A simple random method was used to allocate calves to the treatment and control groups. The study population was selected by herd and within each herd calves and yearlings were randomly (using a random number table) allocated to each of the two groups. A total of four herds were selected. The selection of the herds was based on the required number of calves, which were 32 calves (1 vial of the vaccine had 32 doses) and the willingness of the farmer to participate in the trial. Calves selected ranged from 1-month to 12-months in age and yearlings were from 12-months and above but before first parturition. Cattle selected had no history of ECF.

Clinical examination of the selected animals was undertaken just prior to the inoculation of the vaccine. Animals with a rectal temperature > 39.4^0 C were excluded. 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 and hips and sunken eyes) were also excluded from the trial. Based on this criterion, a total of 123 calves were initially vaccinated against ECF while 119 others served as controls.

The immunization procedure was carried out using the method described by Radley (1978). The *T. parva* (Muguga cocktail) stabilate was stored in 0.5ml aliquots in plastic straws kept under liquid nitrogen canisters. The straws were rapidly thawed by rubbing between the palms and contents of required number dispensed into cryovials (Nalgene$^{(R)}$). A 1:40 dilution of the stabilate was done using Eagles Minimum Essential Medium with 3.5% w/v bovine

plasma albumin and 7.5% glycerol. After 30 minutes of equilibration, the stabilate was inoculated subcutaneously in front but next to the pre-scapular lymph node. A 30% long acting oxytetracylines (Tetroxy L.A, Bimeda) was administered at a dosage rate of 30 mg per kg body weight by deep intramuscular injection. Any immunized animal developing clinical signs of ECF with fever and macroshizonts in lymph node smears for at least three days was designated as an "ECF reactor" (Wanjohi *et al*., 2001). However, inspite of using the 30% oxytetracylines formulation, there was regular communication with the farmers just in case of the odd reactors. Surveillance for ECF in both the vaccinated group and controls was by determination of antibody titres and the incidence rate of the disease.

3.6 Enzyme-Linked Immunosorbent Assays

Immunity to ECF is cell-mediated (McKeever, 2007), however sero-conversion following immunization was used as a tool to monitor the viability of the ECF vaccine. Seropositivity to *T. parva* was determined on the day the animals were immunized (day 0) and on the 35th day post immunization. Evaluation of the levels of antibodies to *T. parva*, *B. bigemina* and *A. marginale* were carried out using ELISA tests as described in Katende *et al*. (1998) and Nielsen *et al*. (1996).

Briefly, the specific antigens (the polymorphic immunodominant antigen for *T. parva*, p32 kilodalton, antigen for *A. marginale* and p 200 kilo-dalton antigen for *B. bigemina*) were coated in Starwell polysorp micro-ELISA plates (Polysorp, Nunc, Denmark). The coated plates were incubated with sera at the correct dilution for 2 hours at 37^{o} C in an Insel incubator/ shaker. At the end of the incubation, excess antigen was discarded by flicking out the contents into a sink. Any remaining antigen was drained by slapping the inverted plate onto hand paper towels, then leaving the inverted plate on the towels for 15 minutes. Casein 0.25% was used as the blocking agent. The test sera was diluted 1:200 for *T. parva* and 1:100 for *B. bigemina* and 1:40 for *A. marginale* in Dulbeco's phosphate buffered saline (DPBS) (pH7.4), containing 0.1% Tween 20 and 5% skimmed milk. Positive and negative sera were used for each parasite. The control sera were reconstituted using sterile distilled water. The presence of antibodies to specific parasite antigens was tested by addition of the test sera into wells of antigen-coated plates in duplicates. The plates were then incubated for 25 min at 25^{0}C to allow antibodies if present to bind to specific antigens. The plates were washed 5 times with washing buffer. To detect the antigen–antibody reaction an anti-bovine IgG monoclonal antibodies conjugated to Horse-Radish Peroxidase (HRP) were added. The plates were then incubated at 37^{0} C for 30 minutes. The plates were then washed 5 times with

washing buffer. The reaction was then revealed by addition of 1% hydrogen peroxide as substrate and 40nM 2, 2'-amino-bis (3-ethylbenz-thiazoleline-6-sulphuric acid), diammonium salt (ABTS) as chromogen in sodium citrate buffer pH 4.0. The plates were incubated at room temperature in the dark for one hour for colour development.

The intensity of the colour developed (optical density; OD) was determined using an ELISA reader. Optical density (OD) readings from the reference highly positive control sera were used to compute the percent positivity (pp) for the test sera (Wright *et al*., 1993), expressed as: pp= (OD of the test serum/ OD of strong positive) X 100). For ease of interpretation and comparison, animals were classified as seropostive if the pp was 20% for *T. parva* and 15 % for *A. marginale* and *B. bigemina* (Katende *et al*., 1998; Morzaria *et al*., 1999).

3.7 Diagnosis of Tick-borne diseases

Animals were followed up for a period of 5-months post vaccination. Each farm was visited after every third day for the first thirty-five days and every ten days beyond the thirty five day and the infection status of each animal determined by clinical and laboratory examination of blood and lymph node smears. Clinical surveillance was kept on all the cattle in both groups on daily basis by trained community based animal health workers. To ensure rapid reporting of diseases, all clinical cases of TBDs and other infectious disease conditions in cattle on the selected farms were treated free of charge throughout the trial period. Early signs of ECF looked for included pyrexia, enlargement of superficial lymph nodes and dyspnoea. Blood smears were made from marginal ear vein and needle biopsies were made from prescapular lymph nodes of all animals reported ill especially when accompanied by a rectal temperature of $\geq 39.4^{o}$ C. The smears were fixed in methanol and taken to the laboratory at the Narok County Veterinary Office, for staining in Giemsa and examination under a light microscope. The lymph node smears were examined for the presence of schizonts and the blood smears for *Theileria parva* piroplasms, anaplasma and babesia and any other haemoparasites. Animals found to be suffering from ECF were treated with buparvaquone (Butalex®, Pitman Moore, UK) and supportive antibiotic drugs while cases of anaplasma were treated with either imidocarb diproponiate® (Pitman, Moore, UK) or a long- acting tetracycline.

3.8 Tick infestation

Using the technique of Muraguri (2000), tick challenge was assessed by observing tick infestation on at least five randomly selected cattle on each herd. The species of the infesting

ticks were recorded. Ticks collected were stored in methanol and transported to VRC laboratories at Muguga for identification up to species level according to Kaiser *et al*. (1988) whenever identification *in-situ* was doubtful

3.9 Financial analysis of Infection and Treatment method based on Muguga cocktail stabilate.

The financial analysis of Muguga cocktail stabilate against ECF in cattle was carried out in the months of October, November and December 2004. The study covered the four trial farms and was assumed to be representative of the County in terms of clinical ECF and other tick-borne diseases. The herd data were collected from the respondents of the four trial herds. Narok County data were collected from the Narok County Veterinary and Livestock production officers. The other data were collected from the existing reports.

3.9.1 Partial budget analysis

Partial farm budget analysis was used to estimate the profitability level of herd immunization against ECF by the infection and treatment method (ITM) in Narok County. Partial budgeting provides a simple economic description and comparison of different disease control measures (Dijkhuizen *et al.,* 1995). The partial budget framework and the components and parameters used are as shown in Tables 3.2 and 3.3, respectively.

Table 3.2: Partial farm budget framework.

1. Additional returns
2. Costs no longer incurred
3. Subtotal: 1 + 2
4. Foregone returns
5. Additional costs
6. Subtotal: 4+5
7. Difference: 3 – 6: Derived net return. If net return is negative, then the procedure is not recommended and vice versa.

Table 3.3: Parameters and components of Partial budget analysis in Infection and Treatment method in Narok County.

Parameters	Components considered
Additional returns	Extra Calves Sold =ECS x (CP NI Group- CP I Group)
Additional costs incurred	1.Cost of vaccination = VC x NoA I Group 2.Cost of treatment of reactors= TC x (R x NoI) 3.Cost of treatment of infected calves= TC x ECFInc group I x No animals group I 4. Tick control (NI Group and I Group)
Costs No longer incurred	1.Costs with treatment of diseased calves= TC x ECFInc GroupNI x No animals GroupI 2. Tick control. It is envisaged that tick control costs will be reduced by 50% among immunised animals (GPI).
Foregone returns	None since calves that died had no salvage value

Key: CP= Cost per head; ECFInc= East Coast fever Incidence; ECS =Extra calves sold; I = immunised group; NI= Non-immunized group; NoA= Number of calves R= percentage of reactors to vaccination; TC= Treatment cost; VC = Vaccine cost

3.10 Data management and analysis

The data on the controlled experimental trial were entered into Ms Access and exported to SAS for statistical analysis. The safety and efficacy of the Muguga cocktail stabilate immunization of pastoralists' cattle against ECF was evaluated.

The vaccine safety was determined between day 0 and day 35. On day 0, prevaccination, blood was collected for ELIZA antibody prevalence estimation in both the treatment and control groups. On day 35, blood was collected from the vaccinated and the non-vaccinated cattle and ELIZA antibody prevalence determined. The sero-conversion levels recorded were in the two groups. The antibody prevalence was computed for *T. parva, T. mutans, B. bigemina, A. marginale* at day 0 and day 35. The ECF reactors were recorded between day 1 and day 35.

The vaccine efficacy was determined between day 35 to day 180 and it captured the recording of the ECF disease incidence in both the treatment group and control group. As by Babo Martins *et al*. (2010),

$$\text{Efficacy} = \frac{\text{Incidence of ECF cases in the control} - \text{Incidence of ECF cases in the vaccinates}}{\text{Incidence of ECF cases in the control}}$$

The incidence rate (IR) of ECF was computed as described by Dohoo *et al*. (2009).

$$IR = \frac{\text{Number of events during observation period}}{\text{Animal-days at risk}}$$

Also captured were the incidence of other tick-borne diseases and trypanosomiasis. Ticks were collected, counted and identified.

The endemic stability and instability to cattle-derived *Theileria parva* infection on the basis of theileriosis occurrence was classified as per guidelines issued by Norval *et a*l. (1992) as shown in Table 3.4 below.

Table 3.4: Epidemiological states for endemic stability and instability

Epidemiological state	Antibody prevalence affected	Disease incidence	Age group	Case-fatality
Very Unstable	Low	Low, medium or high	All	High
Unstable	Low to medium	Medium to high	All but predominantly immature	High
Stable	Medium to high	Low	Calves	Low
Very Stable	High	Very low or zero	Young calves	Low

The Fisher exact test at 5% significance level was used to determine the differences between the two groups.

The partial budget analysis was computed based on the partial budget framework (Table 3.2) and parameters and components of partial budget analysis in infection and treatment method in Narok County (Table 3.3).

CHAPTER FOUR: RESULTS

4.1 Controlled trial of Infection and treatment method

4.1.1 Antibody response in vaccinated and non-vaccinated calves.

The prevalence of antibodies to *T. parva* in the vaccinated calves was 46.2% before vaccination and this increased to 93.3% post-vaccination (Table 4.1). The difference in antibody titres in the two periods was significant (p=0.000). Antibody titres in the control group did not change significantly (p=0.442107) between the two periods (46.4% vs 42.3%) (Table4.1).The fact that both groups had detectable antibodies was an indication of past exposure to *T. parva*. In addition, the dramatic change in antibody response after vaccination was an indication that the vaccine was viable.

Table 4.1: The Antibody prevalence to *Theileria parva* in pre-immunization (day 0) and post -immunization period (day 35).

Pre-immunization				Post immunization	
Comparison group of calves	No of calves	No of calves positive	Proportion (%) positive	No positive	Proportional (%) positive
Vaccinated	119	55	46.2	111	93.3
Unvaccinated	123	59	46.4	52	42.3

4.1.2 Screening for other Tick borne diseases

Antibodies to other tick-borne diseases were also assessed as shown in Table 4.2. The majority of the tested cattle had detectable antibodies against the three parasites with the highest prevalence (86.7%) been recorded for *Theileria mutans.* Thus, these cattle had been exposed to three parasites previously.

Table 4.2: The prevalence of antibodies to tick-borne diseases

Parasite	Number tested	Number positive	Proportional (%) positive
A. Marginale	233	73	31.3
B. Bigemina	233	46	18.5
T. Mutans	233	202	86.7

4.1.3 East Coast fever disease incidence and Reactor rate

The incidence of ECF in the control and vaccinated calves was 15.1% (18/119), and 1.6% (2/123), respectively. The difference in disease incidence between the vaccinates and the controls was significant (p=0.000101). There were no reactors. There were no allergic reactions or anaphylactic shock following immunization. On the other hand 18 cases of ECF were observed during the study period for an incidence of 15.1%.

4.1.4 Efficacy of Muguga cocktail stabilate as a vaccine against East Coast fever in cattle

The ECF incidence in control and vaccinated herd was 15.1% and 1.6% respectively.

The efficacy was 89.4%.

During the follow up period from day 35 to 150, a total of 8 ECF cases were observed in the control group and two in the vaccinated group. Cause-specific mortality rate for ECF in the control group was 87.5% (7/8). None of the two cases of ECF in the vaccinated group died.

4.2 Intercurrent infection

During the follow up period both groups of calves suffered from other diseases including anaplasmosis and trypanosomiasis. The incidence of anaplasmosis was 8.9% (11/123) in the vaccinated group and 16.0% (19/119) in the control group. The difference in disease incidence between the vaccinates and the controls was not significant (p=0.11925). The incidence rate of trypanosomiasis was 11.4% (14/123) and 16.8% (20/119) in the vaccinate and control groups respectively. The difference in disease incidence between the vaccinates and the controls was not significant (p= 0.268326) .

4.3 Other tick vectors

The following tick species were collected in the three sites including *Rhipicephalus* appendiculatus(R.A), *Rhipicephalus decoloratus*(R.D), *Ambylomma spp* and existed in the following proportions: 90.0% (694/771), 6.7% (52/771) and 3.2% (25/771) respectively as shown in Table 4.3. The 3 tick species were responsible for ECF, anaplasmosis and /or babesiosis and heart water transmissions respectively.

4.4 Partial budget analysis of infection and treatment method

Partial farm budget analysis was used to estimate the profitability level of herd immunization against ECF by the infection and treatment method (ITM) in the Narok County.

4.4.1 Cost of immunization

The mean herd size was 32 calves ranging between the age of 1-month and 12-months.

The immunization costs are as shown in Table 4.4. The consumable items included syringes, hypodermic needles, microscopic slides and staining reagents.

The estimates of the cost of an immunizing dose of stabilate are based on the current production costs of 100,000 doses at VRC Muguga. The current total cost of producing the stabilate (100,000 doses) was USD 113,300. This included the cost of quality control processes (cross-immunity trials, titration and screening for pathogens).

The total cost of a dose of the vaccine (inclusive of all costs) was 7.50 USD (Table 4.3) (equivalent to Ksh. 600 at the average exchange rate Ksh.80 to the dollar at the time of the trial in 2004).

Based on the data collected from the 4 trial farms, the average cost of treating a calf (up to 12 month of age) for ECF was Ksh.300 while average annual cost of application of acaricides per animal was Ksh.260 (Table 4.4).

Table 4.3: Estimated cost of the various components in ECF immunization in Kenya, 2004.

Item	Category*	Cost in USD.		Percentage of total cost
		Per farm	Per animal	
Stabilate production	Variable	36.16	1.13	15.07
Blocking drugs	Variable	15.36	0.48	6.40
Consumable items	Variable	51.2	1.60	21.33
†Labour (monitoring)	Fixed	-	-	-
Transportation	Fixed	25.28	0.79	10.53
Professional charges	Fixed	112.0	3.50	46..67
Total		240.00	7.50	100.00

*Parameters costed per animal (animal-dependent) were termed as "variable" while those costed per whole farm were termed as fixed.
†No reactors were expected when 30% oxytetracylines formulation is used. This eliminates the need for monitoring.

Table 4.4: Inputs used in partial farm budget analysis of the financial benefits of East Coast Fever immunisation by the infections and treatment method in Narok County, 2004.

Parameter	Value in Number and Kshs.		Source
	Immunized	Non immunized	
No of calves (NoA)	123	119	Study data
Market value of a calf (CP)	*Ksh.4,700	Ksh.4,700	Study data
ECF cumulative incidence (CumInc)	16.7	155.6	Study data
ECF cumulative mortality (CumMort)	0	14.1	Study data
Vaccine Cost (Ksh) VC	Ksh.600		Study data
Cost of treatment (Ksh) TC	Ksh.300	Ksh.300	Study data
Percentage of reactors to vaccination (R)	0	-	
Cost of tick control Annual basis per animal (TCA)	Ksh.260	Ksh.260	Study data

*Based on field data from elsewhere, the price of immunized calves is expected to increase by at least 50% (Babo Martins *et al.*, 2010).

Immunization of calves against East Coast fever generated a net output of Ks 377,420.00 which translated into a mean marginal return of Ksh.1, 559.59 per vaccinated calf (Table 4.5).

Table 4.5: Net return of immunization against ECF in Narok County.

Parameter
*Additional returns
Additional costs Cost of vaccination Ksh.73,800.00 Cost of treatment of infected calves-immunized group Ksh. 59,040.00 Tick control Ksh.62,920.00
Costs no longer incurred Treatment of diseased cattle Ksh.557,190.00. (Non-immunized calves) Tick control Ksh. 15,990 Net return = Ksh (557,190.00 + 15,990.00) –(73,800.00 +59,040.00 + 62,920.00) = 377,420 Average net return per calf = Ksh. 1,559.59

* Accurate records of extra calves sold as a result of immunization not available.

The number of animals immunized per farm had a major influence on the mean cost per animal, with the total cost of immunization decreasing as the number of cattle per herd increased. In this analysis, the cost of monitoring, the professional fees and transportation costs were termed as fixed costs, since they were charged uniformly, irrespective of the number of animals on the farm. These cost contributed 57.2% of the total cost, hence the high cost when few animals were immunized on the farm.

The ITM realized a net return of Ksh.4, 261.45 per immunized calf. If immunization against the disease is integrated with reduced acaricide usage, then accrued returns are even much higher. If the tick control frequency is reduced to once every two weeks, this will result into a 50% reduction in acaricide costs. The annual cost of tick control per animal (cattle) dropped from Ksh.260 to Ksh. 130.

CHAPTER FIVE: DISCUSSION

The results of the study showed dramatic increase in sero-prevalence of antibodies to *Theileria parva* from 46% to 93% in the immunized calves clearly indicated that the viable parasites would provide immunity to homologus challenge. The drop in sero-prevalence from 46% to 41% in the control herd indicated that some of the calves may have had maternal antibodies and after 35 days they could not be detected. This 93% rate of sero-conversion following immunization was within the range (85% to 100%) considered acceptable for a viable vaccine (Muraguri *et al*., 2003). The sero-conversion rates were similar to those observed in similar studies (Wesonga *et al*., 2000; Wanjohi *et al.,* 2001; Oura, 2004; Babo Martins *et al.,* 2010).

The two calves in the vaccinated group that developed ECF may have been incubating the disease at the time of recruitment. The two cases were detected on the 5^{th} day post-vaccination. Other studies have observed reactor rate of 0.9% and 3.0% obtained in Tanzania (DFID, 2009) and Kenya (Muraguri *et al.,* 2003) (Marikebuni strain), respectively. The ECF incidence in the vaccinated calves was 1.6% compared to 15.1% in the control calves. This was statistically significant (P=0.000101). There was also resistance in the vaccinated calves towards other tick-borne diseases such as anaplasmosis where disease incidence dropped from 16.0% to 8.9%. This therefore means Muguga cocktail vaccine was safe and efficacious to use in the cattle in the pastoralists region in Kenya.

The efficacy of the vaccine in this study was 89.4% which compares favourably with that reported by ILRI (2010) of 95% and by Babo Martins *et al*.(2010) of 97%. The efficacy was however higher than that obtained by Wesonga *et al*. (2014) in a trial conducted in a mixed livestock: crop production system in Machakos County of 82%. The low efficacy observed in this trial was attributed to the adverse drought conditions during the trial which may have been a major source of stress to the animal. Stress conditions such as drought and poor livestock management practices are known to have adverse effects on the efficacy of vaccines (Clement *et al.,* 2004; Rashid *et al.,* 2009).

The ECF incidence in the study was high in calves and low in adult cows. The epidemiological status of cattle in this region to *Theileria parva* was unstable endemicity due to high case-fatality of 87.5% and the disease was predominantly affecting immature. This could be as a result of seasonal activity of tick instars that follow the bimodal rainfall and combined with ineffective acaricide application results into ECF epidemics in the adult cows.

The seroprevalence to other tickborne diseases is low in this study except for *T.mutans* and this could be the reason for epidemics of these diseases in the adult cows. The low seroprevalence of these other tick-borne diseases could be attributed to low population of *Boophilus decolaratus* which could be as a result of unfavourable climatic conditions. The results for babesia and anaplasma parasites show low sero-conversion. This is an indication that animals could suffer from these diseases even in adult age. Muguga cocktail stabilate was able to indicate efficacy in the wide geographical region covered in the two sub-counties of Osupuko and Loita. Also of most importance was the ability of the Muguga cocktail stabilate to contain ECF in cattle that freely mixed with buffalo. The Muguga cocktail stabilate has a component known as *T. parva* Serengeti which protects cattle against ECF transmitted by buffaloes.

Partial budgeting analysis results of the study showed that ITM technology was financially profitable even when the extra calves sold as a result of reduced mortality and the expected increase in the price of immunized calves were not taken into consideration. The ITM realized a net return of Ksh.1, 559.59 per immunized calf. This was significant in the study area since the average price of a calf was relatively low (Ksh.4,700.00). High net returns are indicators of high profitability of immunization (Dijkhuizen *et al.*,1995). Thus, it can be concluded from the study that it was economically worthwhile to immunize cattle against ECF in Narok County. If immunization against the disease is integrated with reduced acaricide usage, then accrued returns are even much higher. If the tick control frequency is reduced to once every two weeks, this will result into a 50% reduction in acaricide costs. The annual cost of tick control per animal (cattle) will drop from Ksh.260 to Ksh. 130. Another benefit that can be derived from immunization is the increased value of the immunized cattle. For instance, among the Masaai pastoralists of Tanzania immunized calves are sold at a price 50% higher than the non-immunized calves (Babo Martins *et al.,* 2010).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- The vaccine against ECF was found to be safe and efficacious against the disease in the study area as it reduced the incidence of ECF by 89.4%.
- The vaccinated calves were able to resist infection by other tick-borne diseases and trypanosomiasis.
- The partial costs and partial benefits showed partial net benefits when Muguga cocktail stabilate was integrated with reduced acaricide application**.**
- The results showed that the vaccine was efficacious and economically feasible.

6.2 Recommendations

There should be:

- More and widespread trials on Muguga cocktail stabilate, particularly for a minimum of two years, to fine tune safety and efficacy levels
- Comprehensive financial and economic analysis needs to be taken for financial viability assessment of the ITM.
- Strategic tick control, tick infection dynamics and tick attachment rate experiments need evaluation and validation to optimize on the technology

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APPENDICES

Appendix 1a- ELIZA reading

T. PARVA (indirect) Plate status: WITHIN LIMITS										
Plate name: Narok04 8 Test date: 10/07/04										
Created by: EDI V2.1.1 Test time: 12:56:49										
Filter: 405										
Technician: MO										
Blanking value: 0.046 Kit Batch#: NDE 5										
CONTROLS: Acceptable OD range C++: 0.850 - 1.750										
Threshold: PP >= 20 %										
outside control limits:										
OD(#), PP(*)										
ID	**STATUS**	**OD1**	**OD2**	**OD3**	**OD4**	**PP1**	**PP2**	**PP3**	**PP4**	**LCL-UCL**
C++	**In**	**1.214**	**1.12**	**1.075**	**1.089**	**110**	**101**	**97**	**99**	**80 120**
C+	**In**	**0.651**	**0.585**	**0.699**	**0.492**	**59**	**53**	**63***	**45**	**35 60**
C-	**In**	**0.069**	**0.016**	**0.051**	**0.006**	**6**	**1**	**5**	**1**	**-2 10**
Cc	**in**	**0.011**	**0.009**	**0.008**	**0.007**	**1**	**1**	**1**	**1**	**-5 5**
The average of the two intermediate C++ OD values is 1.105										
TEST SAMPLES:										
ID	**WELLS**	**DESCRIPTION**	**STATUS**	**OD1**	**OD2**	**ODav**	**PP1**	**PP2**	**Var**	**PPav**

1	**A3/B3**	**3-56 Post 23/9/04**	**POS**	**0.771**	**0.794**	**0.783**	**70**	**72**	**2**	**71**

2	C3/D3	3-57 " "	N	0.07	0.057	0.064	6	5	1	6
3	E3/F3	3-58 " "	N	0.149	0.148	0.149	14	13	0	13
4	G3/H3	3-59 " "	POS	0.599	0.616	0.608	54	56	2	55
5	A4/B4	3-60 " "	N	0.138	0.155	0.147	13	14	2	13
6	C4/D4	3-61 " "	POS	0.734	0.691	0.713	66	63	4	65
7	E4/F4	3-62 " "	N	0.175	0.185	0.18	16	17	1	16
8	G4/H4	3-63 " "	POS	0.957	1.028	0.993	87	93	6	90
9	A5/B5	3-64 " "	N	0.088	0.076	0.082	8	7	1	7
10	C5/D5	3-65 " "	POS	0.857	0.921	0.889	78	83	6	80
11	E5/F5	3-66 " "	POS	0.605	0.532	0.569	55	48	7	51
12	G5/H5	3-67 " "	POS	0.693	0.604	0.649	63	55	8	59
13	A6/B6	3-68 " "	POS	0.803	0.745	0.774	73	67	5	70
14	C6/D6	3-69 " "	POS	0.649	0.666	0.658	59	60	2	60
15	E6/F6	3-70 " "	POS	1.309	1.292	1.301	119	117	2	118
16	G6/H6	3-71 " "	N	0.196	0.178	0.187	18	16	2	17
17	A7/B7	3-72 " "	N	0.18	0.169	0.175	16	15	1	16
18	C7/D7	3-73 " "	retest	0.235	0.215	0.225	21	19	2	20
19	E7/F7	3-74 " "	N	0.14	0.127	0.134	13	12	1	12
20	G7/H7	3-75 " "	N	0.115	0.085	0.1	10	8	3	9
21	A8/B8	3-76 " "	N	0.145	0.106	0.126	13	10	4	11
22	C8/D8	3-77 " "	N	0.08	0.102	0.091	7	9	2	8
23	E8/F8	3-78 " "	N	0.052	0.072	0.062	5	7	2	6
24	G8/H8	3-79 " "	N	0.103	0.104	0.104	9	9	0	9
25	A9/B9	3-80 " "	POS	1.062	1.107	1.085	96	100	4	98
26	C9/D9	3-81 " "	POS	0.416	0.408	0.412	38	37	1	37
27	E9/F9	3-82 " "	POS	0.641	0.636	0.639	58	58	0	58
28	G9/H9	3-83 " "	POS	0.308	0.332	0.32	28	30	2	29
29	A10/B10	3-84 " "	POS	0.982	1.065	1.024	89	96	8	93
30	C10/D10	3-85 " "	N	0.149	0.169	0.159	14	15	2	14
31	E10/F10	26346 " "	POS	0.89	0.909	0.9	81	82	2	81
32	G10/H10	26336 " "	POS	0.28	0.256	0.268	25	23	2	24
33	A11/B11	26337 " "	POS	0.93	0.952	0.941	84	86	2	85
34	C11/D11	26338 " "	POS	1.245	1.303	1.274	113	118	5	115
35	E11/F11	26339 " "	POS	0.696	0.725	0.711	63	66	3	64
36	G11/H11	26340 " "	POS	0.446	0.476	0.461	40	43	3	42
37	A12/B12	26341 " "	POS	0.788	0.867	0.828	71	79	7	75

38	**C12/D12**	**26342 " "**	**POS**	**0.732**	**0.722**	**0.727**	**66**	**65**	**1**	**66**
39	**E12/F12**	**26343 " "**	**POS**	**0.422**	**0.476**	**0.449**	**38**	**43**	**5**	**41**
40	**G12/H12**	**26344 " "**	**POS**	**0.709**	**0.77**	**0.74**	**64**	**70**	**6**	**67**

Appendix 1b - ELIZA Reading

A. MARGINALE (Indirect) Plate status: WITHIN LIMITS										
Plate name: **NAROK048** Test date: 10/12/04										
Created by: EDI V2.1.1 Test time: 17:26:07										
Filter: 405 Technician: AN										
Blanking value: 0.046 Kit Batch#: MSP54										
CONTROLS: Acceptable OD range C++: 0.850 - 1.850 Threshold: PP >= 15 %										
outside control limits: OD(#), PP(*)										
ID	**STATUS**	**OD1**	**OD2**	**OD3**	**OD4**	**PP1**	**PP2**	**PP3**	**PP4**	**LCL-UCL**
C++	**in**	**1.068**	**1.107**	**1.031**	**1.048**	**101**	**105**	**97**	**99**	**80 120**
C+	**in**	**0.556**	**0.531**	**0.552**	**0.499**	**53**	**50**	**52**	**47**	**35 60**
C-	**in**	**0.04**	**0.031**	**0.043**	**0.036**	**4**	**3**	**4**	**3**	**-2 10**
Cc	**in**	**0.007**	**0.009**	**0.009**	**0.008**	**1**	**1**	**1**	**1**	**-5 5**
The average of the two intermediate C++ OD values is 1.058										
TEST SAMPLES:										
ID	**WELLS**	**DESCRIPTION**	**STATUS**	**OD1**	**OD2**	**ODav**	**PP1**	**PP2**	**Var**	**PPav**

1	A3/B3	3-56 Post 23/9/04	POS	0.364	0.407	0.385	34	38	4	36
2	C3/D3	3-57 " "	N	0.123	0.121	0.122	12	11	0	12
3	E3/F3	3-58 " "	retest	0.207	0.14	0.173	20	13	6	16
4	G3/H3	3-59 " "	POS	0.191	0.163	0.177	18	15	3	17
5	A4/B4	3-60 " "	N	0.138	0.148	0.143	13	14	1	14
6	C4/D4	3-61 " "	POS	1.002	0.978	0.99	95	92	2	94
7	E4/F4	3-62 " "	N	0.094	0.103	0.098	9	10	1	9
8	G4/H4	3-63 " "	N	0.043	0.06	0.051	4	6	2	5
9	A5/B5	3-64 " "	N	0.096	0.104	0.1	9	10	1	9
10	C5/D5	3-65 " "	N	0.046	0.041	0.043	4	4	0	4
11	E5/F5	3-66 " "	N	0.077	0.074	0.075	7	7	0	7
12	G5/H5	3-67 " "	POS	0.248	0.287	0.267	23	27	4	25
13	A6/B6	3-68 " "	POS	0.469	0.634	0.551	44	60	16	52
14	C6/D6	3-69 " "	N	0.085	0.07	0.077	8	7	1	7
15	E6/F6	3-70 " "	POS	0.905	0.898	0.901	86	85	1	85
16	G6/H6	3-71 " "	POS	0.213	0.205	0.209	20	19	1	20
17	A7/B7	3-72 " "	N	0.057	0.047	0.052	5	4	1	5
18	C7/D7	3-73 " "	POS	0.482	0.355	0.418	46	34	12	40
19	E7/F7	3-74 " "	POS	0.292	0.517	0.404	28	49	21	38
20	G7/H7	3-75 " "	N	0.095	0.1	0.097	9	9	0	9
21	A8/B8	3-76 " "	POS	0.182	0.189	0.185	17	18	1	18
22	C8/D8	3-77 " "	N	0.135	0.103	0.119	13	10	3	11
23	E8/F8	3-78 " "	retest	0.138	0.193	0.165	13	18	5	16
24	G8/H8	3-79 " "	N	0.073	0.076	0.074	7	7	0	7
25	A9/B9	3-80 " "	N	0.04	0.036	0.038	4	3	0	4
26	C9/D9	3-81 " "	N	0.045	0.028	0.036	4	3	2	3
27	E9/F9	3-82 " "	N	0.126	0.129	0.127	12	12	0	12
28	G9/H9	3-83 " "	POS	0.27	0.288	0.279	26	27	2	26
29	A10/B10	3-84 " "	POS	0.856	0.737	0.796	81	70	11	75
30	C10/D10	3-85 " "	N	0.064	0.061	0.062	6	6	0	6
31	E10/F10	26346 " "	N	0.044	0.043	0.043	4	4	0	4

32	G10/H10	26336 " "	N	0.046	0.051	0.048	4	5	0	5
33	A11/B11	26337 " "	N	0.048	0.062	0.055	5	6	1	5
34	C11/D11	26338 " "	N	0.083	0.077	0.08	8	7	1	8
35	E11/F11	26339 " "	POS	0.375	0.329	0.352	35	31	4	33
36	G11/H11	26340 " "	N	0.112	0.101	0.106	11	10	1	10
37	A12/B12	26341 " "	N	0.099	0.068	0.083	9	6	3	8
38	C12/D12	26342 " "	POS	0.386	0.399	0.392	36	38	1	37
39	E12/F12	26343 " "	N	0.052	0.032	0.042	5	3	2	4
40	G12/H12	26344 " "	N	0.021	0.015	0.018	2	1	1	2

Appendix 1c- ELIZA Reading

B. BIGEMINA (Indirect) Plate status: WITHIN LIMITS										
Plate name: Narok04 8 Test date: 10/12/04										
Created by: EDI V2.1.1 Test time: 17:26:26										
Filter: 405 Technician: AN										
Blanking value: 0.046 Kit Batch#: EP1b 5										
CONTROLS: Acceptable OD range C++: 0.800 - 1.650 Threshold: PP >= 15 %										
outside control limits: OD(#), PP(*)										
ID	**STATUS**	**OD1**	**OD2**	**OD3**	**OD4**	**PP1**	**PP2**	**PP3**	**PP4**	**LCL-UCL**
C++	**in**	**1.274**	**1.362**	**1.336**	**1.332**	**96**	**102**	**100**	**100**	**80 120**
C+	**in**	**0.778**	**0.756**	**0.662**	**0.802**	**58**	**56**	**50**	**60***	**35 60**
C-	**in**	**0.01**	**0.013**	**0.014**	**0.013**	**1**	**1**	**1**	**1**	**-2 10**
Cc	**in**	**0.007**	**0.01**	**0.018**	**0.011**	**1**	**1**	**1**	**1**	**-5 5**
The average of the two intermediate C++ OD values is 1.334										
TEST SAMPLES:										
ID	**WELLS**	**DESCRIPTION**	**STATUS**	**OD1**	**OD2**	**ODav**	**PP1**	**PP2**	**Var**	**PPav**
1	**A3/B3**	**3-56 Post**	**N**	**0.052**	**0.059**	**0.055**	**4**	**4**	**1**	**4**

		23/9/04								
2	C3/D3	3-57 " "	N	0.04	0.042	0.041	3	3	0	3
3	E3/F3	3-58 " "	N	0.076	0.055	0.065	6	4	2	5
4	G3/H3	3-59 " "	N	0.044	0.033	0.038	3	2	1	3
5	A4/B4	3-60 " "	POS	0.344	0.269	0.306	26	20	6	23
6	C4/D4	3-61 " "	retest	0.199	0.238	0.218	15	18	3	16
7	E4/F4	3-62 " "	N	0.093	0.08	0.086	7	6	1	6
8	G4/H4	3-63 " "	N	0.044	0.074	0.059	3	6	2	4
9	A5/B5	3-64 " "	N	0.061	0.052	0.056	5	4	1	4
10	C5/D5	3-65 " "	N	0.041	0.033	0.037	3	2	1	3
11	E5/F5	3-66 " "	POS	0.583	0.649	0.616	44	49	5	46
12	G5/H5	3-67 " "	POS	1.101	1.061	1.081	83	80	3	81
13	A6/B6	3-68 " "	N	0.075	0.055	0.065	6	4	1	5
14	C6/D6	3-69 " "	POS	1.064	1.112	1.088	80	83	4	82
15	E6/F6	3-70 " "	N	0.114	0.111	0.112	9	8	0	8
16	G6/H6	3-71 " "	N	0.029	0.036	0.032	2	3	1	2
17	A7/B7	3-72 " "	N	0.053	0.056	0.054	4	4	0	4
18	C7/D7	3-73 " "	N	0.067	0.06	0.063	5	4	1	5
19	E7/F7	3-74 " "	N	0.134	0.128	0.131	10	10	0	10
20	G7/H7	3-75 " "	N	0.137	0.131	0.134	10	10	0	10
21	A8/B8	3-76 " "	N	0.078	0.109	0.093	6	8	2	7
22	C8/D8	3-77 " "	N	0.096	0.08	0.088	7	6	1	7
23	E8/F8	3-78 " "	N	0.064	0.1	0.082	5	7	3	6
24	G8/H8	3-79 " "	N	0.027	0.045	0.036	2	3	1	3
25	A9/B9	3-80 " "	N	0.042	0.035	0.038	3	3	1	3
26	C9/D9	3-81 " "	N	0.016	0.012	0.014	1	1	0	1
27	E9/F9	3-82 " "	retest	0.296	0.194	0.245	22	15	8	18
28	G9/H9	3-83 " "	POS	0.393	0.567	0.48	29	42	13	36
29	A10/B10	3-84 " "	POS	0.282	0.312	0.297	21	23	2	22
30	C10/D10	3-85 " "	N	0.041	0.039	0.04	3	3	0	3
31	E10/F10	26346 " "	N	0.028	0.029	0.028	2	2	0	2
32	G10/H10	26336 " "	N	0.024	0.024	0.024	2	2	0	2

33	**A11/B11**	**26337 " "**	**N**	**0.077**	**0.103**	**0.09**	**6**	**8**	**2**	**7**
34	**C11/D11**	**26338 " "**	**N**	**0.043**	**0.037**	**0.04**	**3**	**3**	**0**	**3**
35	**E11/F11**	**26339 " "**	**POS**	**0.697**	**0.838**	**0.767**	**52**	**63**	**11**	**58**
36	**G11/H11**	**26340 " "**	**N**	**0.079**	**0.079**	**0.079**	**6**	**6**	**0**	**6**
37	**A12/B12**	**26341 " "**	**N**	**0.173**	**0.172**	**0.172**	**13**	**13**	**0**	**13**
38	**C12/D12**	**26342 " "**	**retest**	**0.196**	**0.206**	**0.201**	**15**	**15**	**1**	**15**
39	**E12/F12**	**26343 " "**	**N**	**0.065**	**0.033**	**0.049**	**5**	**2**	**2**	**4**
40	**G12/H12**	**26344 " "**	**N**	**0.099**	**0.099**	**0.099**	**7**	**7**	**0**	**7**

Appendix 1d- ELIZA Reading

T. MUTANS (Indirect) Plate status: WITHIN LIMITS										
Plate name: Narok04 8 Test date: 10/12/04										
Created by: EDI V2.1.1 Test time: 17:26:54										
Filter: 405 Technician: AN										
Blanking value: 0.046 Kit Batch#: TPM32 3										
CONTROLS: Acceptable OD range C++: 0.850 - 1.600 Threshold: PP >= 20 %										
outside control limits: OD(#), PP(*)										
ID	**STATUS**	**OD1**	**OD2**	**OD3**	**OD4**	**PP1**	**PP2**	**PP3**	**PP4**	**LCL-UCL**
C++	**in**	**1.353**	**1.381**	**1.363**	**1.357**	**99**	**102**	**100**	**100**	**80 120**
C+	**in**	**0.587**	**0.555**	**0.621**	**0.631**	**43**	**41**	**46**	**46**	**35 60**
C-	**in**	**0.025**	**0.026**	**0.027**	**0.028**	**2**	**2**	**2**	**2**	**-2 10**
Cc	**in**	**0.009**	**0.008**	**0.005**	**0.007**	**1**	**1**	**0**	**1**	**-5 5**
The average of the two intermediate C++ OD values is 1.360										
TEST SAMPLES:										
ID	**WELLS**	**DESCRIPTION**	**STATUS**	**OD1**	**OD2**	**ODav**	**PP1**	**PP2**	**Var**	**PPav**
1	**A3/B3**	**3-56 Post**	**POS**	**0.293**	**0.343**	**0.318**	**22**	**25**	**4**	**23**

		23/9/04								
2	C3/D3	3-57 " "	N	0.054	0.052	0.053	4	4	0	4
3	E3/F3	3-58 " "	POS	1.11	1.121	1.115	82	82	1	82
4	G3/H3	3-59 " "	POS	0.594	0.632	0.613	44	46	3	45
5	A4/B4	3-60 " "	POS	0.773	0.696	0.734	57	51	6	54
6	C4/D4	3-61 " "	POS	0.366	0.398	0.382	27	29	2	28
7	E4/F4	3-62 " "	N	0.246	0.217	0.231	18	16	2	17
8	G4/H4	3-63 " "	POS	0.559	0.557	0.558	41	41	0	41
9	A5/B5	3-64 " "	POS	1.242	1.152	1.197	91	85	7	88
10	C5/D5	3-65 " "	POS	0.349	0.329	0.339	26	24	1	25
11	E5/F5	3-66 " "	POS	1.152	1.175	1.163	85	86	2	86
12	G5/H5	3-67 " "	POS	0.679	0.652	0.665	50	48	2	49
13	A6/B6	3-68 " "	POS	0.87	0.752	0.811	64	55	9	60
14	C6/D6	3-69 " "	POS	1.26	1.294	1.273	93	95	4	94
15	E6/F6	3-70 " "	POS	0.563	0.62	0.591	41	46	4	43
16	G6/H6	3-71 " "	POS	1.357	1.382	1.369	100	102	2	101
17	A7/B7	3-72 " "	retest	0.3	0.253	0.276	22	19	3	20
18	C7/D7	3-73 " "	POS	0.71	0.741	0.725	52	54	2	53
19	E7/F7	3-74 " "	POS	1.387	1.397	1.392	102	103	1	102
20	G7/H7	3-75 " "	POS	0.694	0.657	0.675	51	48	3	50
21	A8/B8	3-76 " "	N	0.196	0.201	0.198	14	15	0	15
22	C8/D8	3-77 " "	POS	0.328	0.314	0.321	24	23	1	24
23	E8/F8	3-78 " "	POS	1.359	1.423	1.391	100	105	5	102
24	G8/H8	3-79 " "	POS	0.807	0.784	0.795	59	58	2	58
25	A9/B9	3-80 " "	POS	0.664	0.548	0.606	49	40	9	45
26	C9/D9	3-81 " "	POS	1.346	1.35	1.348	99	99	0	99
27	E9/F9	3-82 " "	N	0.248	0.251	0.249	18	18	0	18
28	G9/H9	3-83 " "	POS	1.011	0.953	0.982	74	70	4	72
29	A10/B10	3-84 " "	POS	1.296	1.326	1.311	95	97	2	96
30	C10/D10	3-85 " "	POS	0.998	0.988	0.993	73	73	1	73
31	E10/F10	26346 " "	POS	1.105	0.731	0.918	81	54	28	67
32	G10/H10	26336 " "	POS	0.9	0.826	0.863	66	61	5	63

33	**A11/B11**	**26337 " "**	**POS**	**1.123**	**1.215**	**1.169**	**83**	**89**	**7**	**86**
34	**C11/D11**	**26338 " "**	**POS**	**0.922**	**0.838**	**0.88**	**68**	**62**	**6**	**65**
35	**E11/F11**	**26339 " "**	**POS**	**1.167**	**1.26**	**1.213**	**86**	**93**	**7**	**89**
36	**G11/H11**	**26340 " "**	**POS**	**1.087**	**1.044**	**1.065**	**80**	**77**	**3**	**78**
37	**A12/B12**	**26341 " "**	**POS**	**0.468**	**0.512**	**0.49**	**34**	**38**	**3**	**36**
38	**C12/D12**	**26342 " "**	**POS**	**0.565**	**0.546**	**0.555**	**42**	**40**	**1**	**41**
39	**E12/F12**	**26343 " "**	**POS**	**0.55**	**0.545**	**0.547**	**40**	**40**	**0**	**40**
40	**G12/H12**	**26344 " "**	**POS**	**0.765**	**0.674**	**0.719**	**56**	**50**	**7**	**53**