EPIDEMIOLOGICAL STUDIES OF VECTOR-BORNE DISEASES WITH CONSEQUENT DEVELOPMENT OF PREVENTIVE MEDICINE PROGRAMMES FOR SMALL-HOLDER DAIRY FARMERS IN COASTAL KENYA

BY

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DECLARATION

The work presented in this thesis is original and was carried out solely by the author, except where collaboration with others has been acknowledged

Seiffuddin Hatimali Maloo
August 1993

DEDICATION

TO

MY MUM,

MY WIFE,

RASHIDA

AND

MY CHILDREN,

KHADIJA AND MUSTAFA

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ABBREVIATIONS

ABTS	2,2'-azino bis (3-ethyl)-benzthiazoline-6 sulfonic acid di-ammonium salt
AEZ	Agro-ecological zones
Ag-ELISA	Antigen ELISA
AHA	Animal health assistant
AI	Artificial insemination
ANOVA	Analysis of variance
CA	Capillary agglutination
CAT	Card agglutination test
CBPP	Contagious bovine pleuro-pneumonia
CFT	Complement fixation test
ci	Confidence interval
CL	Coastal lowland
CL3	Coconut-cassava agro-ecological zone
CL4	Cashew-nut-cassava agro-ecological zone
CL5	Livestock- millet agro-ecological zone
cm	Centimetre (s) (1×10^{-2} metre
DDT	dichlorodiphenyltrichloroethane
DEAE	Diethylaminoethyl cellulose
DG	Darkground/phase contrast buffy coat technique
DMY	Daily milk yield
DNA	Deoxyribonucleic acid
DTP	Days to trypanosome parasitaemia
DVO	District Veterinary Officer
e.p.g.	Eggs per gram
EC	European Commission

ECF	East Coast fever
ELISA	Enzyme linked immunosorbent assay
F1	Filia one - First generation offspring
FAO	Food and Agriculture Organisation of the United Nations
FITC	Fluorescein isothiocyanate conjugate
g	Gravitational force
gm	Gramme(s)
ha	Hectare(s)
hr	Hour(s)
HRPO	Horseradish peroxidase
I&T	Infection and treatment
i.m.	Intramuscular
IBAR	International Bureau of Animal Resources of the Organisation of African Unity
IFAT	Indirect fluorescent antibody test
IgG	Immunoglobulin G
IHA	Indirect haemagglutination ()
ILCA	International Livestock Centre for Africa
ILRAD	International Laboratory for Research on Animal Diseases
KARI	Kenya Agricultural Research Institute
kDa	KiloDalton
KETRI	Kenya Trypanosomiasis Research Institute
kg	Kilogramme(s)
km	Kilometre (s)
Ksh	Kenya shilling
LO	Livestock Officer

m	Metre(s)
mg	Milligramme (s) (1 x 10^{-3} gramme
mm	Millimetre (s) (1 x 10^{-3} metre
MoAb	Monoclonal Antibodies
MOLD	Ministry of Livestock Development
NaCl	Sodium chloride
NDDP	National Dairy Development Project
nm	Nanometre (s) (1 x 10^{-9} metre)
NVRC	National Veterinary Research Centre
^{o}C	Degrees Celsius
OIE	Office International des Epizooties
PBS	Dulbecco's 0.1M phoshate buffer saline pH 7.4
PBST	Phosphate buffer saline pH 7.4 containing 0.1% Tween 20
PCR	Polymerase chain reaction
PCV	Packed red cell volume
PG	Prophylactic group
pg	picogramme
PT	Prophylactic treatment
PTFE	Polytetrafluroethylene
RELP	Restriction fragment length polymorphism
RRC	Regional Research Centre
SAS	Statistical Analysis Systems
SD	Standard deviation
se	Standard error
SIT	Sterile insect technique
SSA	Sub-Saharan Africa

STDM	Standard Trypanosome Detection Methods
TBD	Tick-borne diseases
TP	Trypanosome parasitaemia
TPS	*Theileria parva* schizont
ul	Microlitre(s) 1 x 10^{-6} litre
ULV	Ultra-low-volume techniques
VAT	Variable antigen types
VIL	Veterinary Investigation Laboratory
VO	Veterinary Officer
VSG	Variant surface glycoprotein
w\v	Weight\volume
WHO	World Health Organisation

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SUMMARY

This thesis describes a series of epidemiological investigations carried out between 1989 and 1991 in small-holder dairy cattle in coastal Kenya, with the aim of estimating prevalence, incidence and mortality rates of major diseases limiting dairy production. In addition, the studies assessed the on-going disease control measures and identified appropriate alternative interventions to reduce disease risk and minimise production losses.

Chapter One comprises a background introduction with general objectives of a multidisciplinary approach to research on identifying and resolving biological and socio-economic constraints affecting small-holder dairy development in coastal Kenya carried out by the KARI/ILCA project. The disciplines involved were animal health, animal breeding, animal nutrition, forage agronomy, socio-economics and milk marketing.

Chapters Two and Three reviews the literature on the subject of epidemiology of tick-borne diseases and tsetse-transmitted trypanosomiasis, the major diseases encountered in coastal Kenya, and specify the objectives of studies reported in the thesis.

Chapter Four gives a comprehensive description of the three study areas: Kaloleni, Kwale and Mtwapa, together with the farming systems, animal husbandry and health practices carried out in small-holder and research cattle herds. In addition, the general sampling and diagnostic methods are included.

In Chapter Five, two epidemiological investigations are described using on-farm small-holder cattle herds in Kaloleni Division, Kilifi District. The first investigation a stratified cross-sectional study of disease prevalence (Section 5.1) was designed based on cattle census data from the area. The study sampled 734 cross-bred dairy cattle and 205 local Zebu cattle in 132 dairy and mixed (dairy and Zebu cattle) herds found in the three agro-ecological zones (AEZ): coconut-cassava (CL3), cashew-nut-cassava (CL4) and livestock-millet (CL5).

In CL3, dairy herds were managed in two grazing systems, namely zero- and free-grazing. The sero-prevalence results showed that exposure to *Theileria parva*, *Babesia bigemina* and *Anaplasma marginale* infections occurred in over 70% of the cattle population indicating tick-borne diseases to be highly prevalent in the area. Exposure to *T. parva*, the causative agent of East Coast fever (ECF), was influenced by AEZ, grazing system and animal age. In the wetter CL3 and CL4, *T. parva* antibody prevalence in cattle was significantly higher than in those found in the drier CL5. At the same time, cattle kept in the free-grazing system had a significantly higher prevalence compared to those in the zero-grazing system. In addition, antibody prevalence increased with age. Sero-prevalence of other TBDs were not affected by AEZ, but cattle kept in the zero-grazing system had a significantly lower *B. bigemina* antibody prevalence than those in the free-grazing system.

With regard to tsetse-transmitted trypanosomiasis, a relatively lower trypanosome antigen and antibody prevalence was observed in comparison with the sero-prevalence of TBDs. Helminthiasis and brucellosis appeared to be of minor importance during the study period.

The next stage in the investigation was a 19 month longitudinal study (Section 5.2) involving 195 cross-bred dairy cattle in 30 herds, half of them zero-grazing herds and the remaining free-grazing, all located in CL3 AEZ. The study aimed at estimating disease incidence and mortality rates. East Coast fever was the most important disease, contributing 62% of all clinical cases, while only a few cases of anaplasmosis, babesiosis and trypanosomiasis were encountered. The prevalence, incidence and risk rates of ECF estimated were two-fold higher in free-grazing herds compared to zero-grazing herds. Irrespective of the grazing system, the majority of *T. parva* infections were diagnosed in young stock (less than 18 months old), with a two-fold significantly higher occurrence of ECF in males compared to females. Overall, a 32%

mortality rate was estimated, with ECF accounting for two thirds of all calf deaths, three quarters of young stock mortality and more than a third of all adult losses. Case-fatality due to ECF approached 60%, with proportionally more deaths from ECF occurring in free-grazing than in zero-grazing herds. The studies showed that ECF, besides being the major cattle disease, caused substantial production losses through mortalities. At the same time, the results highlighted that current preventive and curative measures, mainly tick control and chemotherapy, implemented by small-holder farmers for controlling ECF were not effective. An alternative prevention measure, infection and treatment (I&T) method of immunisation which was economically and environmentally acceptable was proposed.

In Chapter Six, experimental studies were carried out to identify target population and age-window for immunisation against ECF. This was followed by a pilot immunisation trial of small-holder dairy herds. In the first study (Section 6.1) six sentinel groups of susceptible calves exposed to natural tick challenge contracted ECF within 1 to 4 months of age, identifying the age-window for immunisation to be within this age range. The second investigation (Section 6.2) evaluated the immune status of 19 exposed local Zebu cattle and 8 exposed cross-bred dairy calves, all over 6 months, to needle challenge with *T. parva* Marikebuni, the immunising parasite stock. All local Zebu and most cross-bred calves withstood challenge, and were immune. Zebu cattle were excluded from the target population, but exposed dairy cattle were included as vaccinating them was considered to be beneficial.

In the pilot immunisation trial (Section 6.3) a logistical approach was developed for the delivery of immunisation to small-holder dairy farms which paved way for large-scale immunisation of dairy herds in the Division.

The principle behind I&T method was first, to artificially create an endemically stable population, and subsequently to immunise new animals introduced by birth or by purchase to sustain endemic stability.

In other areas of coastal Kenya, where tsetse-transmitted trypanosomiasis proved to be the major disease, studies were carried out to evaluate the efficacy of trypanocidal drugs for controlling the disease. Chapter 7 describes two studies, the first involving on-farm small-holder cross-bred dairy cattle in Kwale District, and the second with an on-station dairy herd of Jersey cattle at Regional Research Centre, Mtwapa. In the first study which lasted 7 months (Section 7.1), the chemoprophylactic drug isometamidium chloride (Samorin) given at 0.5 mg kg^{-1} every 6 weeks to 75 dairy cattle, failed to protect against trypanosomiasis in areas of known high tsetse challenge, An overall prevalence of 17.5% in chemoprophylactically-treated cattle indicated the likelihood of drug-resistant trypanosome strains. On the other hand, curative treatments with diminazene aceturate (Berenil) at 7.0 mg kg^{-1} successfully cured parasitaemic cattle.

The second 18 month study (Section 7.2), two drug treatment groups, one receiving isometamidium chloride at 0.5 mg kg^{-1} every 3 months, and the other treated with diminazene aceturate at 7.0 mg kg^{-1} only when detected parasitaemic, were monitored for occurrence of trypanosome parasitaemias and their effect on liveweight changes and daily milk yield. No significant differences were observed between the isometamidium prophylactic group and the non-prophylactic group in the occurrence of trypanosome parasitaemias, liveweight changes and daily milk yields. However, parasitaemic cattle showed a significant drop in mean PCV and in daily milk yield for 2 weeks after curative treatment. Both studies showed that isometamidium chloride failed to afford adequate protection and at the same time highlighted the importance of

regular blood-testing and early curative intervention for the control of trypanosomiasis in these areas of coastal Kenya.

Finally, Chapter Eight presents an overview of the epidemiological investigations undertaken and consequently describes preventive medicine programmes for small-holder dairy farmers with the emphasis on the role of private sector in providing cost-effective and sustainable animal health services. The thesis ends by highlighting the significance of the blueprint of epidemiological methods developed with the view to them being used in various countries of sub-Saharan Africa. These methods will support the intensification of livestock production by small-holder farmers, reckoned to be the main producers of meat and milk in the future.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

In sub-Saharan Africa, domestic livestock have a multiple role; they not only provide meat and milk, but also manure for fertilizer and fuel; hides, skins and wool and animal traction. They are used as a means of transport. Livestock are assets, in that they provide social security, have cultural functions within the structure of rural communities and are also a form of savings in areas where investment opportunities are rare (Jahnke, 1982).

Sub-Saharan African farmers raise approximately 162 million cattle and 274 million small ruminants, representing 11% and 17% of the world's population, respectively (FAO, 1986a,b, 1989). However, they contribute only 5% of the world's beef, a reflection of their low productivity. Currently, the human population in sub-Saharan Africa has been estimated at 500 million, up from 250 million in 1965 (Winrock Report, 1992). According to World Bank, the population is expected to reach 676 million in the year 2000 and 1,294 million in 2025, an increase of 2.6 times in 35 years with a current growth rate of 3.1% per year. In the past decade, increase in food production have not kept pace with the rapid growth in human population (Pritchard, 1988). With regard to livestock production, an annual growth of 2.6% for meat and 3.2% for milk which was estimated between 1962 and 1987 was inadequate, and relying on this level of production will result in massive deficits in supplies of meat and milk by 2025. Therefore, clear strategies must be formulated that will enhance the expansion of food production to feed the growing population in a sustainable manner, support the economic development of the region, increase incomes and promote the welfare of rural communities as well as protect the environment.

Accelerating the growth of livestock industry offers one alternative to alleviate food shortage in sub-Saharan Africa. The demand for meat and milk will be influenced by population growth, urbanisation and income growth.

With these factors taken into consideration, the World Bank has set a goal for sub-Saharan Africa of a 4% annual increase in food production which would be sufficient to meet the demands for feeding the rapidly growing population and progressively reduce dependency on food imports.

Increasing livestock production implies either a major expansion of livestock numbers or intensification of production, or both. From the perspective of environmental degradation, and resource sustainability, intensification is preferable to expansion. Intensification will alleviate the need to increase stocking rates on marginal or fragile rangelands. It will also probably lead to industrialisation through stimulation of agro-industrial development and allow concentration of improved livestock in the more productive highland and sub-humid regions of sub-Saharan Africa.

Mixed crop-livestock systems have the greatest potential for intensification and for contributing to overall agricultural productivity and sustainability. As the recent Winrock Report (1992) has pointed out, animals increase overall net productivity and reduce environmental degradation as they form an alternative to crops for the less agriculturally productive areas and can utilise crop residues as feed. The need for animal feed often broadens the crop base to include forage and tree crops that prevent soil erosion. Livestock provide biophysical opportunities to manage nutrient cycling among areas of cropped land and to utilise land that is continuously idle or under-used. They also play an important role by providing socio-economic stability and sustainability from enhanced income through diversification, opportunities for crop intensification through traction, better use of family labour, gender equity in a farming enterprise, income to invest in alternative crop production, and as a hedge against inflation.

Milk production is one livestock enterprise which can be improved through, and which can contribute to, agricultural intensification, particularly

in mixed farming systems. In the 1980s, milk production in developing countries was estimated to be on average 90 litres per head of cattle per year compared to 900 litres in industrialised nations (Tacher, 1982a). The current production level in sub-Saharan Africa is thought to be still lower.

In East and Southern Africa, dairy production is mainly concentrated in the subtropical highlands where the majority of marketed milk is produced by small-holder farmers (Mbogoh, 1984; Ashimogo and Kurwijila, 1992). This contrasts with the coastal lowlands where dairy development has been limited, especially in the small-holder sector. Currently, milk production in the coastal lowland region is mainly for subsistence from local Zebu herds and it contributes little towards meeting the demand for milk and dairy products from the rapidly increasing urban population. The milk deficit in the coastal region is reflected in the total amount of liquid milk sales in the region relative to the milk produced locally. In 1990 in the Coast Province of Kenya, less than one million litres of fresh milk were produced locally out of about 20 million litres of recorded milk sales (Kenya Cooperative Creameries, 1991). Similarly, the Tanzanian Dairies Limited, processing plant in the urban centre, Tanga, averaged only 30% fresh milk intake over the period 1981-87 (Ngigwana, 1992) and in Madagascar fresh milk represented 34% of total milk processed in 1988 (Ranaivoson, 1992).

These figures should not be interpreted as showing that dairy production possibilities are inherently low as informal dairy marketing channels predominate, a reflection of the high local demand relative to current supply (Thorpe, Chabari, Maloo, Muinga, Mukhebi, Mullins, Mureithi, Mussukuya, Nyambaka, Ole-Maki, Otieno, Perry, Rugema and Wekesa, 1993). In the higher rainfall areas of the coastal lowlands, there is considerable potential to satisfy the demand for fresh milk. This can be achieved through peri-urban dairy production by integrating dairy production into small-holder

mixed farming systems to increase and stabilise farm income and create employment opportunities, thereby catalysing agricultural development and contributing towards improved standards of living.

As well as the unsatisfied demand for milk, other factors contributing to the small-holder interest in dairying in coastal Kenya, include, poor returns from the major cash crops, coconuts and cashew-nut, and increasing pressure on land resulting from rapidly growing human population. Small-holder interest in dairying has been supported in coastal Kenya since 1980 by the Ministry of Livestock Development's National Dairy Development Project (NDDP) (van der Valk, 1990). The NDDP advocates intensified dairy production through a zero-grazing management system. As yet in coastal lowland Kenya, the number of small-holder farmers adopting the zero-grazing package is relatively small, and extensive (free) grazing systems sustain the major part of the small-holder dairy population (Thorpe *et al.*, 1993).

The productivity of existing small-holder dairy herds is affected by a number of factors ranging from lack of infrastructure for milk marketing; unfavourable government price policies; restricted access to credit for the purchase of dairy cattle; the limited supply of dairy cattle; inadequate feed supplies; and high risk of disease (Thorpe *et al.*, 1993). Similarly, the adoption of dairying by the small-holder sector will have been inhibited by these policy and technical factors. Therefore, for small-holder dairy development to be adoptable on a wide scale and for it to be sustainable, technical innovations must be appropriate to small-holder resources and matched by input services, including veterinary care and milk marketing channels, all within a policy environment conducive to dairy production.

In early 1988, the Kenya Agricultural Research Institute (KARI) and International Livestock Centre for Africa (ILCA) signed a memorandum of understanding for the establishment of a collaborative programme of

multidisciplinary and inter-institutional on-station and on-farm research to support small-holder dairy development in the high rainfall coastal lowland zone of Kenya. The broad objectives of the programme were to identify and resolve biological, social and economic constraints to the development, adoption and productivity of sustainable small-holder dairy systems with the target group being the peri-urban mixed crop-livestock small-holders in the medium rainfall areas of coastal lowland Kenya.

Broad research protocols covered five major interrelated studies. These were:

- Characterisation of the farming systems in the high rainfall coastal lowlands.
- Estimation of current and long-term demand for milk and dairy products.
- Studies on the production and utilisation of year-round feed supplies.
- Evaluation of the comparative health and performance of dairy cattle genotypes for small-holder production.
- Identification and estimation of disease risk and the development, testing and delivery of alternative technologies for control of these diseases

In particular, epidemiological research was initiated to address those issues relevant to the fifth protocol.

1.2 DISEASE CONSTRAINTS TO SMALL-HOLDER DAIRY PRODUCTION

Animal diseases constitute major constraints to the improvement of animal production in sub-Saharan Africa (Winrock Report, 1992). Animal diseases cause direct production losses, for example, mortalities, and reduced growth and milk yields, and indirect losses due to the costs of prophylactic and therapeutic control programmes. Further indirect losses are incurred because the risk of disease inhibits farmers from adopting livestock production or from substituting breeds of low production potential with breeds of higher production potential.

In the tropics of sub-Saharan Africa, control measures, usually vaccinations, are available against most common viral and bacterial diseases, and their delivery is generally carried out effectively. By contrast, the practice of controlling major vector-borne parasitic diseases, particularly in small-holder herds, is limited by inadequate knowledge of the epidemiology of the diseases and often inappropriate control recommendations. In most African countries, diagnostic and epidemiological services are poor, mainly due to inadequate field operations, lack of well-equipped diagnostic facilities, failure and constraints in submitting specimens for confirmation of disease aetiology, poor disease surveillance and monitoring, and generally insufficient operating budgets (Provost, 1991). Quantitative epidemiological data are rare, preventing the estimation of the economic assessment of losses due to specific diseases (Msellati and Tacher, 1991).

It was against this background of disease, and in particular the major vector-borne diseases that limit small-holder dairy development in coastal lowland Kenya, that the KARI/ILCA collaborative programme embarked upon epidemiological research:

1. to identify the major diseases affecting small-holder dairy production in coastal lowland Kenya,
2. to assess current control methods,
3. and to identify, test and deliver alternative methods to reduce disease risk in small-holder dairy cattle where necessary, and
4. through these studies to design a systematic approach to epidemiological studies for the small-holder sector.

This thesis reports the results of the epidemiological findings that point towards approaches that can minimise disease constraints to small-holder dairy production.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

The recent Winrock report on African animal agriculture (Winrock International Institute for Agricultural Development, 1992) highlights the role that animal diseases play as major biological constraints to the improvement of animal production in sub-Saharan Africa (SSA). Diseases have been categorised into three groups (Provost, 1991). In cattle, the major diseases in these groups were as follows:

- Group I diseases include diseases such as rinderpest that can be controlled effectively by existing technology (vaccination) and provision of appropriate services. Control campaigns have gradually brought the disease under control through the combined efforts of national veterinary services, pan-African organisations and international agencies. In spite of these collaborative efforts, outbreaks of rinderpest have been reported in the last decade in several countries of western central and eastern Africa (de Haan and Nissen, 1985).
- Group II such as tick-borne diseases, trypanosomiasis, and in the case of West Africa, dermatophilosis are presently the most important disease constraints limiting livestock development. Methods of control in most parts of SSA depend mainly on the use of acaricides for tick control, and the use of drugs for the control of trypanosomiasis.
- Group III are diseases whose importance increases as production systems are intensified. These include, both infectious and non-infectious diseases and are not generally associated with significant mortalities, except for those in neonatal animals. However, they cause economic losses through reduced productivity. Among the most important diseases are bacterial diseases, ,e.g., anthrax, brucellosis, and those causing mastitis, and gastrointestinal parasites. Helmithiasis in SSA, is more of a problem in calves where about one third of them die before weaning due to interaction between worm burden and nutritional stress (de Haan and Nissen, 1985).

My review concentrates mostly on Group II diseases of importance in eastern Africa. These are tick-borne diseases, with emphasis on East Coast fever (ECF), and tsetse-transmitted trypanosomiasis. The review also describes some of Group III diseases on the basis that small-holder dairy production in SSA is considered as an intensified livestock production system.

2.2 TICK-BORNE DISEASES

Ticks transmit parasites that can cause devastating, often fatal diseases in cattle throughout the tropics. The major tick-borne diseases include: anaplasmosis, caused by the parasite *Anaplasma marginale*; babesiosis, caused by *Babesia* species; cowdriosis, caused by *Cowdria ruminantium*, which is of considerable economic importance in small-ruminants as well as in cattle; and theileriosis, caused by *Theileria* species

Tick-borne diseases result in severe economic losses. Throughout the tropics, an estimated 600 million cattle are exposed to anaplasmosis and babesiosis, and 200 million to theileriosis (ILRAD, 1991). In Africa, 175 million cattle and small ruminants are exposed to cowdriosis (ILRAD, 1991). However, precise economic losses from ticks and most tick-borne diseases, are difficult to quantify from the majority of African countries (Pergram, Lemchie, Chizyuka, Sutherst, Floyd, Kerr and McCosker, 1989) due to inadequate data on their impact on livestock productivity.

2.2.1 THEILERIOSIS: EAST COAST FEVER CAUSED BY *THEILERIA PARVA*

Theileriosis is a complex of diseases caused by several species of *Theileria*. The following species of *Theileria* are known to infect cattle in sub-Saharan Africa:

Theileria parva

Theileria mutans

Theileria taurotragi

Theileria velifera

Theileria orientalis/sergenti/buffeli

Theileria annulata (observed in areas of southern Sudan).

This review concentrates mainly on *T. parva*, a parasite known to be pathogenic in cattle. The presence of the disease in the continent closely follows the distribution of the main tick vector.

Theileria parva, is transmitted in the field by a three-host tick, *Rhipicephalus appendiculatus*, commonly known as the African brown ear tick. The parasite infects cattle and Cape buffalo (*Syncerus caffer*) in 11 African countries which include parts of Kenya, Tanzania (including Zanzibar and Pemba), Uganda, Rwanda, Burundi, Zaire, southern Sudan, Malawi, Zambia, Zimbabwe, and Mozambique (Mukhebi, Perry and Kruska, 1992).

It is estimated that of the 63 million cattle in the region, 24 million are at risk of infection and in 1989 alone approximately one million died from East coast fever (ECF) (Mukhebi *et al.*, 1992). Using a computer spreadsheet model to estimate annual economic impact of theileriosis in the sub-Saharan region, Mukhebi *et al.* (1992) reported total losses of US$ 168 million, as a result of losses of beef, milk, traction and manure, and from treatments, acaricides, research and extension costs.

Potentially, the importance of the disease is greater as there are areas inhabited by the tick vector where the disease has yet to appear or has been eradicated. In addition, there are areas of Africa which are suitable for multiplication of the tick vector should it be inadvertently introduced (Dolan, 1989; Norval, Perry, Gebreab and Lessard, 1991).

Not only do financial losses result from transmission of pathogens causing disease, but tick infestation *per se* can cause milk and weight loss,

reduce hide quality, and predispose animals to bacterial and fungal infections, as well as screw-worm attack (Bram, 1983).

Economic losses due to theileriosis not only effects the individual farmer, but also national governments through costs incurred for controlling the disease and providing research, training and extension services in relation to the disease (Mukhebi, 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, and costs and price structure.

The disease in affected areas is known to be a constraint to successfully raise improved cattle breeds for dairy and beef production and has prohibited many farmers from improving the productivity of their cattle (Callow, 1983). Exclusion of such cattle from potentially high producing areas, contributes to indirect production losses.

Theileria parva is a parasite of Cape buffalo which is also infective to cattle and the Asiatic buffalo. Recently, there is evidence to suggest that it is infective to waterbuck (*Kobus* spp.) (Stagg, Young, Leitch, Grootenhuis and Dolan, 1983). While the main vector is *R. appendiculatus*, *R. zambesiense* and *R. duttoni* have also been implicated in its transmission (da Graca and Serrano, 1971; Lawrence, Norval and Uilenberg, 1983). Transmission of *T. parva* by the three-host *R. appendiculatus* tick is transstadial, i.e., a larva that picks up parasites by feeding on an infected mammalian host can transmit infection as a nymph; similarly ticks infected as nymphs can transmit infection after moulting to adult stage.

Sporozoites are the infective stages of the parasite which when inoculated by nymphs or adult ticks into cattle during the course of feeding, enter the lymphocytes where they undergo differentiation to form schizonts. This stage induces the host cells to transform to lymphoblasts and proliferate

with the parasite dividing synchronously with the host cell. This results in clonal expansion of parasitised cells. Schizonts differentiate into merozoites which upon release following rupture of the host cells, rapidly invade the erythrocytes and develop into piroplasms. It appears that *T. parva* piroplasm division in the erythrocytes is limited (Conrad, Denham and Brown, 1986; Fawcett, Conrad, Grootenhuis and Morzaria, 1987) and the pathogenic effects of *T. parva* are normally associated with the schizont stage and rather than the piroplasm stage.

Until recently, *T. parva* was classified into three sub-species, namely *T.parva parva*, *T.parva lawrencei* and *T. parva bovis* causing ECF, Corridor disease and January disease, respectively (Uilenberg, 1976; Lawrence, 1979). This trinomial system of nomenclature was based on certain epidemiological and behavioural characteristics of the parasites and was used for convenience and had no biological validity (Uilenberg, 1981; Young, 1981). Lately, new methods of studying the parasites using monoclonal antibodies (Minami, Spooner, Irvin, Ocama, Dobbelaere and Fujinaga, 1983; Conrad, Stagg, Grootenhuis, Irvin, Newson, Njamuggeh, Rossiter and Young, 1987b; Maritim, Young, Lesan, Ndungu, Stagg and Ngumi, 1992) and studies on deoxyribonucleic acid (DNA) characterisation have shown that these three subspecies are not genetically distinct from *T. parva* derived from cattle and causing ECF (Conrad, Iams, Brown, Sohanpal and Ole-Moi Yoi, 1987a; Allsopp, Carrington, Baylis, Sohal, Dolan and Iams, 1989). In a workshop on theileriosis in Eastern, Central and Southern Africa, it was recommended that *T. parva* parasites should therefore be classified based on their host species of origin, for example, cattle-derived or buffalo-derived *T. parva* (Anon, 1989).

2.2.1.1 DISEASE SYNDROME IN CATTLE

The clinical aspects of ECF have been reviewed by Jura and Losos (1980) and Irvin and Mwamachi (1983). In susceptible cattle, ECF caused by cattle-derived *T. parva*, in its early stages, is a lympho-proliferative disease with noticeable enlargement of several superficial lymph nodes, particularly those draining the head region. In acute cases, the disease is characterised by pyrexia, anorexia, drop in milk yield and deterioration of body condition and rapid weight loss. Once animals become pyrexic, a lympho-destructive phase occurs which is normally associated with a marked leucopenia in fatal cases. As the disease progresses, lacrimation accompanied by photophobia, ocular and nasal discharge may become pronounced. In prolonged cases, corneal opacity may develop, sometimes resulting in blindness. Diarrhoea commonly occurs, which may be bloody. In terminal cases, pulmonary oedema, clinically manifested as acute dyspnoea and frothing at the nostrils, is often the predominant sign. Occasionally, the central nervous system may be involved. Cattle finally become recumbent, cachectic and hypothermic, and eventually die. Case-fatality rate can reach 90%; however, cattle undergoing severe infection can recover dramatically with schizonts disappearing within a few days.

Corridor disease, caused by the buffalo-derived *T. parva*, has a more pronounced lympho-proliferative phase, but a less prominent lympho-destructive stage than in ECF. The disease is characterised by enlargement of the lymph nodes and the development of pyrexia. Subcutaneous oedema particularly under the jaw is common, whilst pulmonary oedema may or may not be severe. Corneal opacity is observed more often. A prominent feature of the disease is the involvement of gastrointestinal tract where widespread ulceration results in bloody diarrhoea. Petechiation on all mucous membranes

is also present. As a result of loss of blood from intestinal lesions, anaemia can be a prominent clinical feature.

In susceptible cattle the course of ECF is normally about 3 weeks. The prepatent period after entry of sporozoites range from 5 to 10 days before detection of the schizonts. Once they are detectable, the clinical signs and pyrexia develop rapidly and death may follow in 5 to 6 days. In general, the clinical phase lasts about 10 to 14 days, but may be prolonged in older animals (Irvin and Mwamachi, 1983).

2.2.1.2 DIAGNOSIS

In cattle, the occurrence of ECF and Corridor disease in the field is confined to the geographical distribution of its vector *R. appendiculatus*. Manifestation of the clinical signs of ECF and the presence of the tick feeding on cattle contribute towards diagnosis of the disease. However, the absence of such ticks does not rule out ECF, as ticks may have dropped off the host following engorgement, nymphal ticks may have gone undetected or the animal may recently have been treated with acaricide.

Confirmation and presence of the disease therefore depends on diagnostic tests for the detection of *Theileria* parasites and the antibodies to them (Irvin and Mwamachi, 1983; FAO, 1984; Young, 1987a). Recently, the application of immunological and molecular diagnostic tools demonstrating the presence of parasite have been introduced and are reviewed by Stiller (1990) and Williamson, Lesan, and Awich (1990).

a) Detection of parasites in mammalian hosts

Examination of Giemsa-stained lymph node biopsy smears for schizonts is considered to be the most effective and practical detection method. Schizonts to *T. parva* can be demonstrated within a week following tick-transmitted

infection and may persist throughout the clinical episode. At necropsy, impression smears of cut lymphoid organs such as lymph nodes and spleen can be examined for schizonts (Irvin and Mwamachi, 1983). However, this detection technique has its limitations as it cannot differentiate between schizonts of *T. parva*, *T. taurotragi* or *T. annulata* on morphological grounds (Young, 1987a). It is important to note that *T. annulata* is unlikely to occur in the same geographical area as *T. parva* and *T. taurotragi*, except where their distribution may overlap, as in southern Sudan. In cattle-derived *T. parva* infections, large number of schizonts may be observed, whereas with *T. taurotragi, T. mutans* and buffalo-derived *T. parva* their numbers are usually very low.

In most instances, piroplasms can be detected in blood smears 5 to 8 days after detection of schizonts (Irvin and Mwamachi, 1983). This may be a useful adjunct to the clinical assessment and detection of schizonts in lymph node smears. However on their own, piroplasms are of no diagnostic value as they may only reflect carrier status (Section 2.2.1.3 c) in otherwise healthy animals (Young, Mutugi, Kariuki, Maritim, Linyonyi, Mining, Kwena, Ngumi, Ndungu, Lesan, Lampard, Awich, Stagg, Leitch, Williamson and Grootenhuis, 1990c; Kariuki, 1991). Piroplasms of theilerial species occur in a variety of forms, including rod, oval and comma shapes, with comma and bacillary being the predominant forms for *T. parva* infections (FAO, 1984).

Estimating the incidence and prevalence of theilerial infection in a cattle population based on schizont identification and piroplasm detection cannot be accurate and specific enough. Therefore, alternative methods such as serological tests have been developed to demonstrate exposure to *T. parva* infections by detecting antibodies to them.

b) Serological tests

Schizont antigen indirect fluorescent antibody test (IFAT) has been used for the detection of antibodies against *T. parva* infections (Lohr and Ross, 1969; Burridge and Kimber, 1972; Goddeeris, Katende, Irvin and Chumo, 1982).The schizont antigen is prepared by *in vitro* infection of culture suspension of lymphoid cells with *T. parva* macroschizonts using the method described by Malmquist, Nyindo and Brown (1970).

The IFAT has been widely applied in epidemiological studies to estimate the prevalence of *T. parva* infections in African countries (Norval, Perry and Young, 1992). The advantages of the test are that it allows assays of large number of samples and can determine whether cattle in any given area have been exposed to the theilerial infections. Beside epidemiological studies, the test has also been used in monitoring experimental infections (Norval *et al.*, 1992).

However, the application of the diagnostic test has been limited by the cumbersome nature of the technique, and depends on subjective observation of the degree of fluorescence, requiring the need for well-trained technicians. Although the *T. parva* schizont IFAT does not cross-react with other haemoparasites, it lacks specificity, in that, it cannot distinguish between *T. annulata* and *T. taurotragi* infections (Burridge, Brown, Crawford, Kirimi, Morzaria, Payne and Newson, 1974a; Burridge, Brown and Kimber, 1974b). The test, however, shows no cross-reactivity with *T. mutans* (Burridge and Kimber, 1972). In areas where the distribution of *T. parva* and *T. taurotragi* overlap, which is throughout much of eastern, central and southern Africa, cross-reactions with *T. taurotragi* become highly significant. Generally, antibody positive reactions are determined at titre values of 1/40 for *T. parva* antigens. This increases the specificity of the test as it minimises false positive cross-reactions with other haemoparasites, except with *T. taurotragi*. On the

other hand, test sensitivity is decreased due to failure to detect samples with low antibody titre.

Other serum antibody assays include the piroplasm indirect haemagglutination test (IHA) (Duffus and Wagner, 1974) which was used in the serological surveys carried out in Kenya in the 1970s (FAO, 1975). The capillary agglutination (CA) test (Ross and Lohr, 1972) and complement fixation (CF) test using *T. parva* piroplasm antigen, have been used in the past, but have been found to be of limited value due to the problem of cross-reactivity with antibodies to other haemoparasites.

There is considerable variation in the duration of antibody responses to theilerial infections. In the case of *T. parva* infections, in the absence of rechallenge, antibodies to schizont antigen can be demonstrated for longer periods then antibodies to piroplasm antigen (Burridge and Kimber, 1973). Generally, antibodies can be detected within 2 to 4 weeks after infection, with titres rising for a further 2 to 4 weeks, after which they stabilise. In the absence of rechallenge, titres start to decline and schizont antibody titres have been known to drop to 1/40 or less by 6 months post infection (Burridge and Kimber, 1973).

Since the development, two decades ago, of the schizont IFAT for detection of antibodies to theilerial infections, only one other diagnostic test has become available. This test is an enzyme linked immunosorbent assay (ELISA) for *T. mutans* (Katende, Gooddeeris, Morzaria, Nkonge and Musoke, 1990). At present, similar ELISA tests for *T. parva*, *T. annulata* and *T. taurotragi* are undergoing development and field testing.

c) Detection of parasite DNA

With the advent of parasite-specific DNA probes which allow the detection of parasite DNA sequences in samples, it has been possible to identify species,

strains and stocks of pathogenic protozoan parasites, which otherwise are difficult or impossible to differentiate morphologically or serologically. *Theileria parva* specific repetitive DNA probes have been developed and are useful for the distinguishing *T. parva* from other *Theileria* species and for determination of stocks of *T. parva* by detection of restriction fragment length polymorphism (RFPL) (Conrad *et al.*, 1987a; Allsopp and Allsopp, 1988; Morzaria Spooner, Bishop Musoke and Young., 1990).

Presently, the application of these probes provides useful tools for laboratory-based work, but their practical application in routine field work is as yet unsuitable.

2.2.1.3 EPIDEMIOLOGY OF EAST COAST FEVER

Epidemiology is defined as the study of diseases in populations and of factors that influence their occurrence (Thrusfield, 1986). Very often, the terms endemic and epidemic are used to describe the frequency of occurrence of disease with time (Thrusfield, 1986; Lessard and Perry, 1988). Thrusfield (1986) defined endemic situations where the constant presence of disease occurs at a predictable level. On the other hand, epidemic is described as the occurrence of disease in excess of its expected frequency.

The term endemic stability is often used to imply a complex relationship between host, causative agent, vector and environment, which all co-exist, with little or no clinical disease. In the case of ECF, endemic stability has been defined by Norval *et al.* (1992) as the state in a cattle population where the large majority of the population becomes infected and immune by 6 months of age, and little or no clinical disease occurs. Endemic instability describes a situation in which only a proportion of the cattle in the population become infected and immune by 6 months of age, and clinical disease is observed.

Epidemiological terms used as useful indicators of endemic stability and instability are the prevalence of infection, which can be assessed in part by antibody prevalence, the incidence of disease, and the case-fatality rate. Incidence is defined as the occurrence of the number of new cases expressed over a specific period of time (Thrusfield, 1986; Martin, Meek and Willeberg, 1987), whereas prevalence is the total number of cases, whether new or old. In endemic stable areas, prevalence of infection, measured as antibody prevalence, rather than prevalence of disease is preferred as infection occurs with virtually no clinical disease. Case-fatality rate measures mortalities due to ECF over a specified time-period. This measure together with the incidence of disease are of significance in endemically unstable situations where clinical disease is seen and often results in mortalities. Norval *et al.* (1992) suggests that in endemically stable areas, epidemiological parameters for ECF are best measured in animals under 6 months of age, whereas in endemically unstable situations all age groups need to be monitored.

To understand the epidemiology of theileriosis, a knowledge of the distribution of the vector and the mammalian host and the ability of the parasite to maintain itself within the host and the tick populations is essential in assessing the extent and potential of the disease problem (Young, 1981; Lessard, L'Eplanttenier, Norval, Kundert, Dolan, Croze, Walker, Irvin and Perry, 1990). Factors affecting the occurrence and epidemiology of ECF are multiple in nature and are expressed at the vector, host and parasite level.

a) Distribution of *Rhipicephalus appendiculatus*

Recently, Lessard *et al.* (1990), using spatial databases and in a computerised geographical information system, were able to plot the distribution of *R. appendiculatus* in Africa. Several factors were found to influence the distribution and abundance of *R. appendiculatus*, the most important being

climate (Yeoman and Walker, 1967; Tatchell and Easton, 1986; reviewed by Perry, Lessard, Norval, Kundert and Kruska, 1990), vegetation cover (Yeoman, 1967; Walker, 1974; reviewed by Perry *et al.*, 1990), and presence, density and movement of cattle and wild ungulates (Minshull and Norval, 1982; Norval and Lightfoot, 1982; Tatchell and Easton, 1986). Other determinants were the ability of the host to resist tick infestations and tick-borne diseases (Young, 1981; Norval, Sutherst, Kurki, Gibson and Kerr., 1988), policies governing the use of acaricides (Young, 1981; Tatchell, 1987) and the development of resistance to acaricides (Baker, 1978).

i) Climate

Several authors have described the importance of ideal climatic conditions for the development, survival and abundance of *R. appendiculatus* (Branagan, 1973a,b; Newson, Chiera, Young, Dolan, Cunningham and Radley, 1984; Punya, 1984; Short, Floyd, Norval and Sutherst, 1989). In general, temperature appeared to be the decisive factor controlling the duration and development periods of the ticks. In cooler climates, with mean maximum and minimum temperatures of 20.2^{o}C and 9.8^{o}C, respectively, only one life cycle per year was possible, whereas two life cycles were possible in warmer areas with mean maximum and minimum temperatures of 24^{o}C and 10.7^{o}C, respectively. (Branagan, 1973a). The climatic requirements of the tick have been documented and incorporated into a climate matching model CLIMEX (Sutherst and Maywald, 1985) for predicting the distribution of the vector in Africa (Maywald and Sutherst, 1987; Lessard *et al.*, 1990).

Yeoman (1966) and Tatchell and Easton (1986) in Tanzania, and Norval and Perry (1990) in Zimbabwe reported on the dynamic nature of the distribution of *R. appendiculatus*. During the years of relatively high rainfall,

the distribution of the tick extended into dry areas and subsequently disappeared when conditions became climatically unfavourable.

ii) Vegetation

Rhipicephalus appendiculatus depends upon vegetation cover which helps in creating a suitable microclimate, and allows the tick to seek a suitable host (Newson, 1978; Minshull and Norval, 1982; Short *et al.*, 1989). The tick occurs most commonly in savannah and woodland-savannah habitats, and tends to be absent from open plains and heavy forests (Perry, Kruska, Lessard, Norval and Kundert, 1991).

iii) Host availability

To complete the different stages of its life-cycle, *R. appendiculatus* has to seek mammalian hosts, namely cattle and the wild ungulates. The species becomes very abundant particularly in the presence of hosts that have a low level of resistance (Kaiser, Sutherst and Bourne, 1982; Lightfoot and Norval, 1981). On the other hand, resistance of the host to tick infestation can influence the tick species present (Norval *et al.*, 1988). Several studies in Africa have shown that indigenous Zebu and Sanga breeds of cattle are more resistant than *Bos taurus* breeds and their crosses to a variety of tick species, including important vectors of theileriosis (Barnett and Bailey, 1955, Norval et al.,1988). However, in Uganda, Kaiser *et al.* (1982) found that local Zebu cattle were infected with more *R. appendiculatus* ticks than any other tick species, and concluded that the degree of resistance to this species was poor.

b) Distribution of mammalian hosts

i) African buffalo

This wild bovidae is the reservoir host for the buffalo-derived *T. parva* which causes Corridor disease in cattle (Neitz, 1955; Barnett and Brocklesby, 1966). Although buffalo-derived *T. parva* does not cause disease in the parent host, it is highly pathogenic to cattle resulting in high mortality rates (Grootenhuis and Young, 1981). Therefore, the presence of buffalo in areas where their distribution overlaps with cattle, plays a significant role in the epidemiology of Corridor disease (Young, Grootenhuis, Mutugi, Maritim, Kariuki and Lampard, 1990a).

ii) Cattle

Cattle are the principal hosts of *T. parva* (reviewed by Young, 1981) and their probable current distribution and density on the continent have been mapped by Lessard *et al.* (1990). These authors, using the data on cattle distribution prepared by Adeniji (IBAR 1989) and tsetse distribution maps revised by Katondo (1984), were able to identify areas of high and low cattle densities. Several other factors influence the outcome of disease in the cattle population. These include the cattle breed and type, cattle management systems and cattle movement.

Variation in the susceptibility to *T. parva* infections between the exotic taurine breeds such as Guernsey and Jersey/Nganda (Zebu) crosses and Zebu cattle have been shown by Guilbride and Opwata (1963) Losses from morbidity and mortality in breeds of susceptible cattle may reach 100% (Cunningham, 1977; Hooke, 1981). Mortality can be particularly high in the *Bos taurus* cattle, their crosses and improved *Bos indicus* cattle, raised endemically unstable ECF areas. On the other hand, mortality rates of indigenous cattle raised in endemic areas, where tick control practices are

virtually non-existent, are usually low (Moll, Lohding and Young, 1984). In general, case-fatality rates range from zero to 50% in endemic areas (Staak, 1981; Moll *et al.*, 1984; Moll, Lohding, Young and Leitch, 1986; Ngulo, 1985; Ngulube, Ellwood and Radley, 1985; Berkvens, Geysen and Lynen, 1989; Otim, 1989) to as high as 80% to 100% in epidemic situations (Julla, 1985). Even though deaths in indigenous cattle in endemic areas can be low, calves are often stunted (Moll *et al.*, 1984).

Stobbs (1966) demonstrated a difference in ECF incidence rates over a 3 year period between East African Zebu raised in an endemic area of Uganda, and Boran cattle introduced to that area. Calf mortality rates of 23% in local Zebu, 43% in local Zebu-Boran crosses and 77% in Boran, most of them to be due to ECF, were observed. However, to date there have been few studies on the susceptibility of cattle breed and type to *T. parva*, and there is clearly a need for well-defined experimental studies to clarify the situation.

Cattle management systems in Africa are diverse (Jahnke, 1982) and have been categorised into crop-livestock production systems in the highlands, crop-livestock production systems in the lowlands, ranching production systems and pastoral range-livestock production systems. East Coast fever occurs mainly in highland crop-livestock systems, and ranches, and to a certain extent, in the lowlands crop-livestock systems (Norval *et al.*, 1992). Generally, cattle kept in the pastoral range systems are not usually affected, as conditions are often unsuitable for the survival of the vector. In the event that pastoralists take their cattle to graze in areas which are suitable for the vector, and in close proximity with the buffaloes and other wild ungulates, the risk of theileriosis can be high (Moll *et al.*, 1984).

In areas favourable for the survival of the vector, various management practices carried out can influence exposure of cattle to the tick. In the communal grazing system where exposure of cattle of all ages to *R.*

appendiculatus occurs throughout the year, endemic stability to theileriosis develops (Moll *et al.*, 1984; Young, Leitch, Newson and Cunningham, 1986; Morzaria, Musoke and Latif, 1988b). In endemic situations described by Moll *et al.* (1984), morbidity in calves reached up to 100%, but case-fatality was low, 2.6%. In these areas, communal grazing and watering points generally allow high rates of acquisition of the infection at a young age (less than 6 months), which in turn results in majority of surviving cattle in the herd being immune to the disease.

Management systems that interfere with the equilibrium by restricting the natural exposure of the herd to ticks, create endemically unstable conditions. This usually arises due to frequent use of acaricides, and imposing restrictions to limit the movement of calves by housing them, as observed in Pemba (J. deBoorder, cited by Norval *et al.*, 1992), leading to a delay in the acquisition of *T. parva* infection.

Cattle movements have historically contributed to the spread of *T. parva* infections in sub-Saharan Africa through the dispersion of infected ticks and the movement of carrier animals. These factors probably were important in the introduction of ECF into southern Africa. However, in most cases, the occurrence of ECF attributed to cattle movements has been limited to localised areas mainly as a result of cattle trekking to slaughter markets or in search of pasture and water. Long-distance trekking of cattle through unfavourable habitats does not usually result in the establishment of the disease in new areas, although a source of infection in carrier cattle can be introduced (Norval *et al.*, 1992).

c) Maintenance of infection in the field

In the field, the theilerial parasite is maintained at two levels; by the development and survival of the parasite within the tick vector and the carrier state within the mammalian host (Young, Leitch and Newson, 1981).

i) Carrier state of *Theileria parva* in cattle

In theileriosis caused by *T. parva*, the role of carrier state of the parasite in their mammalian hosts needs to be fully understood, particularly in assessing the implications of immunising cattle with live parasite stocks. The carrier state of *Theileria* has been defined as the ability of an infected and recovered host to infect ticks, which are then able to transmit the parasite to susceptible cattle. (Levine, 1973; Young *et al.*, 1986).

Immunity to ECF in cattle following infection with the cattle-derived *T. parva* was generally considered to be sterile (Neitz, 1957; Barnett, 1968). However, studies carried out by Young *et al.* (1981) have demonstrated the existence in cattle of cattle-derived *T. parva* carrier state, while the buffalo-derived *T. parva* carrier states are known to have existed in naturally recovered buffaloes and cattle for long periods (Barnett and Brocklesby, 1966).

In an ECF endemic area of South Nyanza District, Kenya, Young *et al.* (1986) reported that carrier state of *T. parva* in adult cattle reached up to 100% and suggested that carrier cattle were primarily responsible for maintenance of infection in endemic areas. Further studies have elucidated the widespread presence of *T. parva* carriers in cattle populations in the field in Kenya, in areas such as Uasin Gishu, Kajiado, and Muranga Districts (Kariuki, 1990), despite the twice a week recommendation of acaricide application.

ii) Development and survival of *Theileria parva* within the tick

Temperature appears to be the most important factor influencing the development of the theilerial parasite in the tick. Optimum temperatures which were best suited for *T. parva* development were found to range between 23°C and 28°C (Young and Leitch, 1981). However, in field conditions there is diurnal fluctuation of temperatures compared to controlled conditions in the laboratory. Ochanda, Young, Mutugi, Mumo and Omwoyo, (1988) have shown that sporozoites will develop in unfed ticks in laboratory and field conditions under higher ambient temperatures, and these ticks can transmit fatal infections within 24 hours of attachment to cows. *Rhipicephalus appendiculatus* and the parasites appeared to survive well at altitudes between 1,000m to 2,000m. Studies carried out in the field in Kenya showed that up to 2,100 m altitude adult ticks remained infected for about 20 months (Newson, Chiera, Young, Dolan Cunningham and Radley 1984; Young, Leitch, Dolan, Newson, Ngumi and Omwoyo, 1983), at 1,500m altitude, *R. appendiculatus* adults maintained their infection for 15 months (Young, Leitch, Morzaria, Irvin, Omwoyo and de Castro, 1987) and for 9 months at sea level (S.P.Morzaria, unpublished data, 1986, cited by Norval *et al.*, 1992).

2.2.1.4 EPIDEMIOLOGICAL SURVEYS AND STUDIES

The main features of the epidemiological surveys and studies are shown in Table 2.1. To date, epidemiological information from work carried out on theileriosis, can generally be obtained from retrospective surveys, retrospective studies and prospective studies.

a) Surveys and studies estimating antibody prevalence

Serological surveys in eastern and southern Africa have been used to estimate the prevalence of antibodies to *T. parva* in Burundi (Kiltz, 1985), Kenya (FAO,

Table 2.1 A summary of the descriptions of epidemiological surveys and studies
Source: Modified from Norval *et al.* (1992).

	Retrospective survey	Retrospective study		Prospective study
Name	Survey	Cross-sectional study	Case-study	Cohort study
Disease frequency unit measured	Prevalence (sometimes incidence)	Prevalence	Prevalence	Incidence
Comparison group	None (counting cases)	Non-diseased animals in the study	Selected controls matched with cases for factors such as breed, sex, age, etc.	Selected animals not exposed to potential causal determinants

1975), Rwanda (FAO, 1982), Uganda (Otim, 1989), Zanzibar (Flach, 1988) and Zimbabwe (Norval, Fivaz, Lawrence and Brown, 1985).

In Kenya, a national serological survey carried out tested approximately 18,000 serum samples using the IHA test (FAO, 1975). The survey covered 36 districts of the country. Serum samples were collected from 20 clusters of 25 animals each for every district. The clusters were ideally four to five herds found within close proximity and were classified as calves, immatures, young cattle and adults. Results showed the highest antibody prevalence rates were found in western Kenya, particularly towards the shores of Lake Victoria. The survey also demonstrated that antibody prevalences increased with age and tick burden. Overall, the survey indicated the importance of the ECF in Kenya.

Apart from studying the distribution of antibody prevalence at national levels, serological studies provide valuable baseline information for planning and implementation of disease control programmes. Serological studies, however, can have certain weakness in their design. One of them is the cattle number sampled in relation to the population at large. This means that the prevalence rate observed may only relate to those animals sampled and not to any larger population if the population methodologies sampling procedures used were incorrect (reviewed by Thrusfield, 1986; Perry, 1988). Another weakness of these studies is that if factors influencing differences in the antibody prevalence rates were not recorded, then the results may provide information of little value to decision makers.

A number of serological studies of theileriosis at the local-level have been carried out. In Kenya, these include work done at Mbita, on the shores of Lake Victoria, where of the 90 cattle sampled, approximately 90% had antibodies to *T. parva* on the schizont IFAT (Young *et al.*, 1986). In another study on Rusinga island in Lake Victoria, where *R. appendiculatus* is present throughout the year, samples from a total of 80 cattle (cows and their calves

within 48 hours of birth) from 13 small-holder farms were screened for antibodies using the schizont, sporozoite and piroplasm IFAT (Morzaria *et al.*, 1988b). On all tests, a 100% antibody prevalences were observed for cows whilst the prevalence rates in the calves ranged from 56% to 84%, depending on the antigen used. The fact that no acaricides were used for tick control on the island, suggests an endemic stable area for ECF with a high proportion of calves having maternal antibodies, as they were sampled within 48 hours after birth (Morzaria *et al.*, 1988b).

b) Disease occurrence - retrospective surveys and studies

Retrospective studies can be extremely valuable in assessing the changing patterns of theileriosis over time. Thus, in Kenya, since 1981, there has been a steady increase in the ECF cases (Kariuki, 1990). This increase has been thought to be partially due to improved reporting, diagnosis and treatment by field staff, and at the same time in the decline in the recommended practices of acaricide application.

c) Prospective (longitudinal) studies of disease occurrence

The advantage of prospective studies is that they allow the measurement of disease incidence and case-fatality rates (Thrusfield, 1986; Perry, 1988), and provide data for assessing the economic importance of the disease. The disadvantage of such studies is the need for close supervision of monitored herds, which depend on adequate logistic support, thereby limiting its geographical representation.

In Kenya, examples of prospective studies have been performed by Moll *et al.* (1984, 1986), where the dynamics of theilerial infections in Maasai Zebu cattle in an endemic area were monitored. These studies quantified the relationship between age, tick burden, presence of schizonts and piroplasms,

and case-fatality. In their earlier study, Moll *et al.* (1984) reported a morbidity to theileriosis of 100% with all calves showing *Theileria* piroplasms infections by 5 months of age and a schizont parasitosis prevalence of 44% by 6 to 7 months. Although overall mortality of 25% was recorded by 18 months of age, case-fatality due to ECF was only 2.6%. The majority of the mortalities were attributed to malnutrition, while the low case-fatality from ECF suggested an existence of an endemically stable state. In addition, most of the calves had antibody responses against *T. parva* and *T. mutans* by 6 months of age, indicating exposure to these theilerial infections early in life. These responses were as a result of parasite infections and not due to maternal antibodies as peak antibody responses to *T. parva* and *T. mutans* were observed following detection of schizont and piroplasm of the respective parasite.

2.2.1.5 CONTROL OF THEILERIOSIS

Prevention of losses in susceptible cattle due to theileriosis can be achieved by means of tick control, by chemotherapeutic intervention, by immunisation of cattle populations, and possibly by a combination of the foregoing methods.

a) Tick control

Various methods have been used since the discovery that ECF was transmitted by the tick *R. appendiculatus*. These can be categorised as either ecological, biological or chemical methods of tick control (Young, Groocock, and Kariuki, 1988). This review focuses on the use of chemicals, the current recommended control method in most areas of eastern, central and southern Africa.

Chemical control methods rely on the application of acaricides to the body of the livestock through dips and sprays. The development of acaricides in tick control has been reviewed by several authors (Henning, 1956; Barnett, 1961; Nolan, 1981; Keating, 1983; Matthewson, 1984).

The most common method of acaricide application involves the animals plunging into and swimming through dip tanks filled with an acaricide which is diluted to a recommended concentration. This technique provides almost complete coverage of the animal and ensures adequate exposure of the attached ticks to the acaricide. However,the major problems associated with dip tanks is their high cost of construction, the need for regular supply of large volumes of water, the cost of acaricides when filling the dip tanks, and, of most importance, the use of correct acaricide concentration for effective tick control.

Another method of acaricide application, commonly used on large commercial beef and dairy farms, is the spray race. Aqueous suspension of acaricide is sprayed under pressure on to cattle passing through a race. Although the method can be very effective in controlling ticks, it needs regular maintenance of the spraying equipment, a factor which has limited its wider application (Young *et al.*, 1988).

In Africa, for many small-holders, where communal functional dips or power spray races may not be easily accessible, the use of hand-spraying, whereby, animals are treated with diluted acaricide using hand pumps is quite common. This is the method of choice for most small-holder dairy farmers, particularly those keeping a few cattle in zero- grazing units; when correctly and routinely applied it is effective in controlling ticks. However, in practice, this method of manual spraying, beside being wasteful and expensive, is often not carried out adequately, resulting in poor tick control.

Other acaricidal treatments involve control of ticks at specific body sites such as the ears or perineum using hand-dressing preparations, e.g., tick grease (grease containing acaricide). Lately, new formulations, such as spot-on and pour-on have become available; these contain solvents/propellants that spread readily over the surface of skin and hair providing partial or total cover of the

body of the animal. Slow-release devices such as acaricide-impregnated eartags have been shown to be effective in the control *R. appendiculatus* for varying periods (Young, de Castro, Burns and Murphy, 1985). Also, systemic acaricidal treatments using ivermectin (Ivomec, Merck Sharp Dome) have been found to be effective under field conditions in Africa (Schroder, Swan, Soll and Hotson 1985). However, none of the slow-release devices tested has as yet been marketed for commercial use in Africa.

Since the use of arsenious oxide as the first acaricide, from the early part of the century until the 1960s and 1970s, a series of acaricides have been marketed. The organochlorines were introduced in the 1960s and 1970s, followed by the organophosphates and carbamates in the 1970s and 1980s, with the launching of amidines and synthetic pyrethroids in the 1980s. The development of acaricide resistance to the various chemical groups was primarily responsible for the introduction of newer products, although marketing forces and the increasing awareness of undesirable levels of organochlorine residues in cattle by-products also contributed.

Resistance of ticks to acaricides has been a problem in Africa and elsewhere (Baker, 1978; Nolan, 1981, 1990; Keating, 1983; Mathewson, 1984). Development of resistance is usually first seen in the one-host tick (*Boophilus* spp.) followed later in the two- and three-host ticks (*Rhipicephalus, Amblyomma* and *Hyalomma* spp). In Africa, the problem of tick resistance to acaricide has not been as marked as in Australia. Nevertheless, because of extensive acaricide use due to the threat of disease, resistance to a wide range of acaricides has occurred in *B. decolaratus* and to a lesser extent, in *R. appendiculatus, R. eversti* and *Amblyomma* species (reviewed by Baker, 1978; Keating, 1983; Chema, 1984).

In eastern Africa, development of resistance in *B. decolaratus* has been mainly responsible for the introduction of new and invariably expensive

acaricides (Tatchell, 1984). Recently, it has been felt that resistance to *B. decolaratus* does not economically justify the need for a change of acaricides (Tatchell, 1984); even though *B. decolaratus* can cause significant production losses and mortalities in susceptible cattle breeds (Sutherst and Wharton, 1971), it has recently been shown that in undipped indigenous cattle, the number of *B. decolaratus* was too low to cause major production losses (Kaiser *et al.*, 1982; Tatchell and Easton, 1986). In areas where infestations of *B. decolaratus* occur, endemic stability to *Babesia bigemina* is usually known to prevail (de Vos and Every, 1981; de Vos and Potgieter, 1983; Norval, Fivaz, Lawrence and Daillecourt, 1983), creating a situation generally considered to be desirable as the risk of losses due the parasite can be minimal.

One of the major factors that necessitated the adoption of intensive tick control programmes in eastern Africa was the importation of tick-and disease susceptible *Bos taurus* breeds of cattle. In the event that acaricidal control programmes are relaxed, epidemics are likely to occur resulting in reduced cattle survival and productivity. However, in the future, the maintenance of ECF-free areas in Africa by use of acaricidal control strategies, will be influenced by several factors. These factors include the increasing cost of acaricides in relation to economic returns, the development of acaricide resistance, the concern over residues in milk and meat and availability of alternative methods for control of tick-borne disease.

b) Chemotherapy

Major advances in chemotherapy against theileriosis have occurred in the last 15 years and have been reviewed by several authors (Dolan, 1981; McHardy, 1984a, 1989). Currently, parvaquone (Boehm, Cooper, Hudson, Elphick and McHardy, 1981), buparvaquone (McHardy and Wekesa, 1985) and

halofuginone lactate (Schein and Voigt, 1981) are available as specific anti-theilerial drugs.

McHardy, Hudson and Rae, (1979) showed that parvaquone given intramuscularly was very effective in treating experimentally-induced clinical infections. Following experimental field studies with parvaquone in susceptible cattle, a clinical trial was carried out in several parts of Kenya to test the efficacy of the drug (Chema, Waghela, James, Dolan. Young, Masiga, Irvin, Mulela and Wekesa, 1986). Treatment was recommended as two intramuscular injections, each at a dosage of 10 mg kg^{-1}, given 48 hours apart.

Another naphthoquinone compound, buparvaquone was found to be effective in both *in vitro* and *in vivo* infections of *T. parva* (McHardy, Wekesa, Hudson and Randall, 1985). Buparvaquone is recommended for treatment against *T. parva* at a dose of 2.5 mg kg^{-1}, repeated after 48 hours, as a single dose does not cure some strains of *T. parva* (McHardy, 1989).

Halofuginone, a quinozoline compound was identified as being effective against *T. parva* infections when given orally at 1-2 mg kg^{-1}. (Schein and Voigt, 1979; 1981). Further clinical trials carried out in Kenya (Chema, Chumo, Dolan, Gathuma, James, Irvin and Young, 1987) led to the registration of the drug for commercial use. The recommended dose of halofuginone is 1.2 mg kg^{-1} given orally in two doses at 48 hours interval.

With the advent of these drugs, chemotherapy has become the method of choice for treatment of clinical theileriosis particularly in dairy cattle. However, for effective cure, early diagnosis is essential so as to allow treatment in the initial stages of the clinical disease. Once the respiratory signs develop, treatment with these chemotherapeutic agents is usually ineffective. One limiting factor for wider application of these drugs, is their cost. At present, the cost of treatment in Kenya is over KShs. 2,400 (US $40) for an adult animal. In addition, the drugs may not be always available.

The successful use of anti-theilerials has resulted in the creation of more immune animals in the cattle population than they were before the introduction of these drugs. On the other hand, cattle cured with either parvaquone or halofuginone develop into carrier animals (Dolan, 1986a,b) in contrast to those treated with buparvaquone (Mutugi Young, Maritim, Linyonyi, Mbogo and Leitch, 1988a). In general, widespread use of chemotherapy may increase the number of carriers, thereby increasing the risk of infection to susceptible (in-contact) cattle, particularly calves.

Chemotherapy is effective as a therapeutic control measure on individual clinically sick animals, but due to cost, its use is generally limited to animals of high productivity. For controlling an outbreak of the disease in larger populations an alternative approach is needed. One such approach is immunisation by the infection and treatment (I&T) method.

2.2.1.6 IMMUNISATION AGAINST *THEILERIA PARVA* INFECTIONS

Since the identification of the causative agent for ECF, several attempts have been made to immunise cattle. The first method to be tested by Theiler (1911) used a suspension of spleen and enlarge lymph node cells from *T. parva*-infected cattle. This approach was, however, abandoned, firstly because of high mortalities, and secondly due to failure to confer immunity in 30% to 40% of inoculated cattle.

Consequently, it was found that infection of cattle with tick-derived sporozoite stabilates of *T. parva* followed by treatment with various anti-theilerial drugs was the effective method of immunising cattle against theileriosis in East Africa (Radley, 1978, 1981; Cunningham, 1977; Mutugi, Young, Kariuki, Ole-Tameno and Morzaria, 1991b) This method has shown promise for use in the field (Mutugi, Ndungu, Linyonyi, Maritim, Mining, Ngumi, Kariuki, Leitch, d'Souza, Maloo and Lohr, 1991a).

The principle of this test involves artificial infection using optimal dilutions of tick-derived sporozoite stabilate, followed by simultaneous treatment with oxytetracycline or buparvaquone. Buparvaqoune has been known to suppress the proliferation of schizont-infected lymphocytes (McHardy and Wekesa, 1985; Mutugi *et al.*, 1988a). Oxytetracycline is thought to have a cytostatic effect on schizont-infected lymphoblasts. This was observed when treatment of *T. parva* sporozoite infected bovine peripheral blood leucocytes with oxytetracyclines in *in vitro* studies inhibited the development of sporozoites to mature schizonts in infected cell lines (Spooner, 1990).

One of the major problems limiting the use of I&T method on a larger scale has been the existence of different *T. parva* strains, which were not cross-protective (Young, Radley, Cunningham, Musisi, Payne and Purnell 1977; Young, Brown, Cunningham and Radley, 1978; Radley, Brown, Burridge, Cunningham, Kirimi, Purnell and Young, 1975a; Radley, Young, Brown, Burridge, Cunningham, Musisi and Purnell, 1975b; Irvin, Dobbelaere, Mwamachi, Minami, Spooner and Ocama, 1983). These studies showed that cattle immunised against one strain of *T. parva* were not necessarily protected against challenge with a heterologous strain. These results indicated the need for a better understanding of the various *T. parva* parasites and their cross-protection patterns. Therefore, studies on the characterisation of theilerial parasites were carried out to identify appropriate *T. parva* stock or stocks for effective ECF immunisation programmes.

a) Characterisation of *Theileria parva* stocks and strains

With the advent of new biochemical, molecular and immunological methods, the task of characterising *Theileria* stocks and strains has been made possible. The details of these various techniques have been reviewed by Irvin (1987) and

Morzaria (1989b). Most modern characterisation methods have been developed as *in vitro* tests with the aim of identifying markers that will correlate with immunity observed in *in vivo* tests. However, to date, none of these new techniques simulate the results of cross-immunity patterns observed with conventional *in vivo* studies.Thus, stocks must still be characterised by a cross-immunity test.

The principle behind the cross-immunity test involves immunising cattle with a stock of *T. parva* by infection and treatment method and challenging the immune animals with a different stock. Parasite stocks that breakthrough on challenge are classified as immunologically distinct (Morzaria, 1989b). Thus, this test enables the identification of parasite stock or stocks that will provide broad-spectrum immunity. For example, the two stocks commonly used in immunisation studies and trials, the "Muguga cocktail" and the "Marikebuni" stocks were selected on the basis of cross-immunity tests (Radley, 1981; Morzaria, Irvin, Taracha, Spooner, Voigt, Fujinanga and Katende, 1987; Mutugi, Young, Maritim ,Mining, Linyonyi, Ngumi, Leitch, Morzaria and Dolan, 1989b).

b) Identification of protective *Theileria parva* stocks for immunisation

To be able to carry out ECF immunisation on a larger scale in the field, it is essential to identify *T. parva* stock ("master stock") capable of conferring a wide protection against other *T. parva* parasites. Alternatively, several theilerial parasite stocks can be incorporated into the immunisation inoculum to form a "cocktail" of parasites providing a wide protective cover. One such example is the Muguga cocktail (Radley, 1978, 1981), comprising of three stocks (*T. parva* Muguga, *T. parva* Kiambu 5, and buffalo-derived *T. parva* Serengeti transformed) which has been shown to afford satisfactory protection

against *T. parva* challenge in many eastern and central African countries (Radley, 1981; Musisi, 1990).

In Kilifi District, Coast Province of Kenya, a *Theileria* parasite, referred to as *T. parva* Marikebuni was isolated and characterised (Irvin, Chumo, Dobbelaere, Goddeeris, Katende, Minami Ocama and Spooner, 1981). This stock was shown to provide good protection against severe challenge from other stocks isolated from Kilifi District (Irvin *et al.*, 1983; Minami *et al.*, 1983; Morzaria *et al.*, 1987).

Further studies were carried out to evaluate the extent of broad-spectrum immunity afforded by the *T. parva* Marikebuni stock (Mutugi *et al.*, 1989b; Mutugi, Young, Linyonyi, Mining, Maritim, Ngumi, Lesan, Stagg, Ndungu and Leitch, 1990). A total of 28 *T. parva* stocks from geographically separate areas of Kenya were used in the immuno-protection studies. Results showed there was widespread cross protection to *T. parva* Marikebuni immune cattle when challenged with 16 heterologous parasite isolates from the Coast (mainly Kilifi District), Rift Valley, Nyanza and Central Provinces. Furthermore, cattle immune to 12 other *T. parva* stocks from Central and Rift Valley Provinces did not breakthrough when challenged with lethal doses of *T. parva* Marikebuni stock (Mutugi *et al.*,1990). This study confirmed the immuno-protective properties of *T. parva* Marikebuni and indicated its potential as a "master" stock for immunisation against cattle-derived theileriosis, particularly in Kilifi District, and probably in all those parts of the country where buffalo-derived theileriosis is not a threat (Mutugi *et al.*, 1990).

A safe optimal sporozoite inoculum, which is also immunogenic, was determined as a dilution ranging from $10^{-0.7}$ and $10^{-1.7}$. This dose range in combination with one of three drugs; a short-acting oxytetracycline, given at 10 mg kg^{-1} on days zero and four, or a long-acting oxytetracycline given at 20 mg kg^{-1} simultaneously with the stabilate inoculation, or treatment with

buparvaquone at 2.5 mg kg^{-1} given at the same time of inoculation, was found to be effective for immunisation (Mutugi *et al.*, 1988a; Mutugi, Young, Maritim, Ndungu, Stagg, Grootenhuis and Leitch, 1988b) This stabilate dose range with either of the oxytetracycline treatments has been used in various field immunisation trial carried out on the Kenya coast.

c) Immunisation against East Coast fever using infection and treatment method in coastal Kenya

Using *T. parva* Marikebuni as the immunising stock in the I & T several successful immunisation trials have been carried out in the Kilifi District, Coast Province of Kenya (Morzaria *et al.*, 1987, Morzaria, 1989a; Mutugi *et al.*, 1991a).

There were several reasons why Kilifi District was selected for pilot field immunisation trials. There have been several systematic investigations of the epidemiology of ECF and characterisation of *T. parva* isolates from the area (Irvin *et al.*, 1981; Minami *et al.*, 1983; Morzaria *et al.*, 1987; Morzaria, 1989b). The parasite selected as the immunising stock *T. parva* Marikebuni (Irvin *et al.*, 1983) has undergone extensive laboratory evaluation and cross-protection characterisation studies for use in field immunisation trials (Mutugi *et al.*, 1989b). Furthermore, in Kilifi District, the threat from buffalo-derived theileriosis is minimal as buffalo, the reservoir host, is absent from the area (Irvin *et al.*, 1981; Morzaria, 1989a, Mutugi *et al.*, 1990).

Pilot trials were carried out on three Government farms, two research and one commercial parastatal farms in Kilifi district where over 1,500 head of cattle of varying ages and breed type were immunised with *T. parva* Marikebuni stock (Mutugi *et al.*, 1990).

On two farms, the Animal Production Research Station, Mariakani and Coast Agricultural Research Station, Mtwapa, 373 cattle (271 Zebu-exotic crosses and 102 pedigree Jersey) were immunised using a $10^{-0.7}$ and 10^{-1} stabilate dilution and simultaneously treated with long-acting oxytetracycline (Mutugi *et al.*, 1991a). Results showed that over 95% of the immunised animals developed antibodies to *T. parva* and majority (92.7%) of the animals seroconverted without any clinical reactions to the I&T method. However, ECF reactors, those that develop clinical disease and require anti-theilerial treatments, varied from 0.6% in Zebu/exotic cross-breds to 14.7% in the pedigree Jersey cattle.

Furthermore, all 37 calves immunised on both the farms seroconverted without any adverse theilerial reactions (Mutugi *et al.*, 1991a), indicating that calfhood immunisation can be effectively carried out between 1 to 4 months of age. One of the advantages of immunising younger animals is the reduced cost; the smaller the animal the less oxytetracycline required (Mutugi *et al.*, 1990).

On the commercial parastatal farm, at Kiswani near Malindi, belonging to the Agricultural Development Corporation, over 1,200 head of cattle kept on two separate areas of the farm namely the Top farm and the Home farm were immunised. Seroconversion to *T. parva* antigens were observed in 86.4% and 95% of the cattle kept at the Top and Home farms, respectively, with 22 clinical reactors, of which only 6 were at the Top farm (Mutugi *et al.*, 1990).

Earlier studies carried out by Morzaria *et al.* (1987) on fewer cattle on the same farm showed that cattle immunised with *T. parva* Marikebuni survived natural challenge, whilst 87% of control unimmunised cattle died from ECF. Moreover, acaricidal treatments could be extended from twice a week to once every 3 weeks, for immunised cattle, without any adverse effects on their productivity. In contrast, most unvaccinated cattle on these control

regimes died from ECF, including four of six cattle that were sprayed twice a week (Morzaria *et al.*, 1987).

Further studies compared the effect of immunisation against ECF on beef productivity together with the effect of varying the interval of acaricidal treatments on productivity in immunised and unimmunised beef cattle (Morzaria, Irvin, Wathanga, d'Souza, Katende, Young, Scott and Gettinby, 1988a). Results showed that during the 9-month exposure, overall weight gain in immunised cattle, irrespective of the tick control regime, was better than the weight gains in cattle which were not immunised. Maintaining unimmunised cattle on intensive twice a week acaricidal regime was found to be uneconomical, whilst immunised cattle on twice a week dipping recorded the highest weight gains (Morzaria *et al.*, 1988a).

At present in coastal Kenya, small-holder dairy farmers depend on the use of acaricide application for the control of ticks and tick-borne diseases. Studies carried out by Ochanda *et al.* (1988) have shown that despite weekly acaricide application, *R. appendiculatus* can attach on cows by the sixth day and can transmit fatal *T. parva* infections within 24 hr. Therefore, the best possible tick control method has less than 100% efficiency in controlling ECF. Acaricide application must be carried out at regular intervals. Failure to do so can lead to serious outbreaks of ECF, especially in areas where the tick challenge is high. Moreover, cost of acaricides is escalating and there is always the possibility of tick resistance to acaricide developing. At the same time, chemotherapy using anti-theilerial drugs is expensive and not all small-holder dairy farmers can afford the treatment. Furthermore, availability of drugs can be unreliable.

Following the successful implementation of large-scale field immunisation trials on institutional farms, there is a strong justification for controlling ECF by I & T method in small-holder dairy herds in the coastal

lowland region of Kenya. This alternative approach is most likely to benefit the increasing numbers of smallholder dairy farmers and help to develop the confidence of more smallholder farmers to venture into the dairy enterprise.

Although, successful immunisation against ECF will prevent major losses due to this disease, it will not justify relaxation or cessation of tick control as animals still remain susceptible to other tick-borne diseases (TBD).

2.2.2 OTHER TICK-BORNE DISEASES

Babesiosis, anaplasmosis and cowdriosis are other diseases which are of importance in cattle kept under endemically unstable tick-infested environments. For all TBD, there is certain uniformity in their epidemiology; the ability of hosts to acquire immunity, the role of carrier status and to survive with virtually no clinical disease in areas which are endemically stable (Dalwitz, Young Mahoney and Sutherst, 1986). Most of the principles for ECF epidemiology apply to these other diseases, one exception being age-related resistance. Early exposure of calves to *Anaplasma* and *Babesia* parasites will prime their immune system and allow natural immunisation; however, in the case of ECF, this only applies to indigenous calves in ECF endemic areas, but does not appear to be applicable in endemically unstable situations (Young *et al.*, 1990c).

2.2.2.1 BABESIOSIS

The *Babesia* species are widely distributed in Africa with *Babesia bovis* and *B. bigemina* being closely associated with particular tick vectors; *Boophilus microplus* responsible for transmitting *B. bovis* and *B. bigemina*, and *B. decolaratus* for *B. bigemina.* These are one host ticks and transovarian transmission of the parasites occurs through the next generation of larval or nymphal stages (Young and Morzaria, 1986; Young 1988).

The epidemiology of babesiosis in Africa has been reviewed by Young (1988). In Kenya, at the district level, babesiosis is only reported as the number of cases diagnosed and attended (Anon, 1991a). This information does not help in understanding the epidemiological significance of the disease at the district level. Mulei and Rege (1989) reported on the incidence of TBD diagnosed over a period of 8 years in Kiambu District, Kenya, where of the 1,472 cases of TBD, only 8.7% were diagnosed as babesiosis.

The disease causes most problems in *Bos taurus* dairy breeds where endemic stability does not occur due to implementation of tick control practices. In susceptible cattle, clinical disease is characterised by an acute onset of fever, anorexia, depression, weakness, cessation of rumination and fall in milk yield. The main clinical symptom is anaemia and haematuria and in terminal stages there can be severe jaundice.

The disease is diagnosed by clinical signs, but confirmation by examination of Giemsa-stained blood smears is essential. Serological diagnostic tests help in detecting exposure to the disease. The commonly used tests are complement fixation test (CFT) (Callow, Emmerson, Parker and Knott, 1976), IFAT (Ross and Lohr, 1968), IHA and ELISA (Bidwell, Turp, Joyner, Payne and Rurnell 1978). These tests are not species-specific as cross-reaction to *B. bigemina* is observed (Callow 1979). In Kenya, where *B. bigemina* is the only *Babesia* parasite reported, a national serological survey using IHA gave an antibody prevalence of 50.4%, with a range from 12.4% to 93.4% (FAO, 1975). The results indicated widespread exposure to the parasite and areas of high antibody prevalence were suggestive of endemic stablility In a more localised cross-sectional study performed in coastal Kenya, antibody prevalence, using an ELISA test in Zebu cattle in the three agro-ecological zones (AEZ) that occur in the region (Jaeztold and Schmidt, 1983), ranged

from 79% to 94% (Deem, Perry, Katende, McDermott, Mahan, Maloo, Musoke and Rowlands, 1993).

In endemically unstable areas, control of the disease is achieved by tick control; however, exposure of calves from immune dams to the parasites helps to boost the preimunnity status. Vaccination against babesiosis has been achieved by inoculation of infective blood, and has been extensively used in Australia and in southern Africa. (Callow, 1977; FAO, 1984). This method has its disadvantages (Mahoney, 1983). Beside the risk of transmitting other haemo-pathogens, the degree of virulence of the parasite varies, causing efficacy problems when used as a vaccine. In eastern Africa, immunisation against babesiosis is not the method of choice, and clinical cases of babesiosis are treated therapeutically using diamidine derivatives. The most widespread compounds used are diminazene aceturate and amicarbalide diisothionate which are very effective against *B. bigemina* (Barnett, 1965).

2.2.2.2 ANAPLASMOSIS

Anaplasma marginale and *A. centrale*, the less pathogenic of the two, are rickettsial organism which infect erythrocytes of cattle in Africa (Ristic, 1977). In susceptible cattle, severe debility, emaciation anaemia and jaundice are the major clinical signs. The parasites are transmitted by ticks, biting flies and accidentally as in vaccination campaigns where the same needles are repeatedly used on different animals. *Boophilus* species are the main tick species involved in transmission in Africa. In Kenya, anaplasmosis is the second most important TBD causing high losses particularly in European breeds of cattle where conditions favouring endemic stability do not prevail (Young 1987b). In an area in Kiambu District, Kenya, anaplasmosis was diagnosed in 21.7% of the cases of TBD, although only 38.2% of the clinically

diagnosed cases were confirmed on examination of Giemsa-stained blood smears (Mulei and Rege, 1989).

A number of serological tests, CF, CA, card agglutination test (CAT) (Ristic, 1977), IHA (FAO, 1975) and ELISA (Dunzgun, Schuntner, Wright, Leatch and Waltisbuhl, 1988) have been used for antibody detection.

Results of a serological survey carried out in 14 districts of Kenya (FAO, 1975) estimated an *A. marginale* antibody prevalence of 20% using IHA, with a range from 4.3% to 48.5%. In a recent cross-sectional survey performed in the three AEZs of coastal lowlands Kenya, antigen prevalence in Zebu cattle using an antigen ELISA was estimated to range from 81% to 94% across the zones, suggesting an area of endemic stability (Deem *et al.*, 1993).

In eastern Africa, tick control is used for controlling the disease in susceptible cattle and in endemically unstable areas. Several methods of immunisation against anaplasmosis have been attempted in the field, but none has proved ideal (McHardy, 1984b). As *A. centrale* is reported to be less pathogenic in cattle and produces an immunity against *A. marginale* infection, it has been used as a vaccine strain in southern Africa and Australia (FAO, 1984). However, Potgieter (1979) and Wilson, Parker and Trueman (1980) have shown that *A. centrale* can cause severe clinical disease. In Kenya, vaccination against anaplasmosis is not practised. Clinical cases are treated chemotherapeutically, and the most commonly drug used is oxytetracycline.

2.2.2.3 COWDRIOSIS

This disease, commonly called heartwater, is caused by the rickettsia *Cowdria ruminantium*. It is widespread in Africa, but its distribution is limited by the occurrence of various species of Amblyomma (Uilenberg and Niewold, 1981). In cattle, pathogenesis of the disease is caused by the proliferation of the organism in the endothelial cells of the blood vessels of the brain. In acute

cases, the damage caused results in the characteristic nervous symptoms, such as high-stepping stiff gait, exaggerated blinking of eyes, and chewing movements terminating in convulsions, prostration and death.

Confirmatory diagnosis is by examination of Giemsa-stained smears of the cerebral cortex. Until recently, no reliable serological tests were available to be able to provide information on the exposure of animals to the disease. The use of an IFAT developed by Semu, Mahan, Yunker and Burridge *(in press)* shows promise. Antibody prevalence ranged from 73% to 80% in Zebu cattle which were sampled across the three agro-ecological zones (AEZ) in coastal lowlands, Kenya. The results were indicative of an endemic stable situation occurring for cowdriosis in coastal Kenya (Deem *et al.*, 1993).

Once again control in susceptible populations is aimed at tick control. Immunisation against cowdriosis relies on the use of chilled blood from *Cowdria*-infected sheep to infect sheep or cattle followed by treatment with oxytetracycline when a febrile response develops or even before that stage arises (Bezuidenhout, 1981). This method has its limitations as an infective blood stabilate is used. Clinical cases, if diagnosed correctly, respond to treatment with oxytetracylines (Uilenberg, 1983).

In many parts of sub-Saharan Africa, TBDs are not the only vector-borne disease constraints to livestock production. Another disease of equal or even greater importance in cattle is tsetse-transmitted trypanosomiasis.

2.3 AFRICAN ANIMAL TRYPANOSOMIASIS

Tsetse-transmitted African animal trypanosomiases are disease complexes of domestic livestock caused by trypanosomes, which are flagellate haemoprotozoan parasites belonging to the Genus *Trypanosoma.*

One of the most significant factors responsible for limiting the expansion of cattle producing areas in Africa is the tsetse fly, the vector

responsible for transmitting trypanosomes (FAO,1961; Jordan,1986; Holmes and Torr, 1988). The result is that large areas of prime arable and the best watered lands of sub-Saharan Africa cannot be utilised for livestock production (MacLennan, 1980).

Currently, tsetse infest 11 million km^2 of the Africa, about 37% of the continent affecting 40 countries (FAO/WHO/OIE, 1982) and one half of the arable land. It has been estimated that 7 million km^2 of this area would be suitable for livestock and agricultural development without any detrimental effects to the environment if trypanosomiasis could be eliminated (Finelle, 1974, MacLennan, 1980). This would support an extra 120 million cattle and at least an equivalent number of small ruminants (FAO, 1987; FAO/WHO/OIE, 1982).

Moreover. in areas where cattle are kept in association with tsetse, losses in livestock production and performance include, poor growth, weight loss, lowered milk yield, reduced animal traction output, infertility and high abortion rates, and mortalities from acute fatal infections (FAO/WHO/OIE, 1963; McDowell, 1977). Losses in terms of food production from mixed agriculture due to lack of manure, draught power and cash income are incalculable (Stewart, 1986). This disease complex, costs millions of dollars annually in trypanocidal drug treatments and vector control programmes. Currently, of a total population of approximately 173 million cattle, only about 25% is located in the tsetse-infested zone (IBAR, 1989).

Consequently, trypanosomiasis is regarded economically as the most important disease of livestock in Africa (Jawara,1990). It is therefore, estimated that controlling trypanosomiasis in the tsetse-infested areas would lead to the development of animal agriculture which could generate an additional US$ 750 million annually (Finelle, 1980).

In order to fulfil the need of the growing African population, and to alleviate livestock pressure on tsetse-free regions, the fly-infested lands must be better utilised (Trail *et al.*, 1985). Therefore, there is an urgent need to control tsetse and trypanosomiasis using cost-effective and sustainable control programmes (FAO, 1979).

2.3.1 EPIDEMIOLOGY OF TRYPANOSOMIASIS

The epidemiology of trypanosomiasis is complex and requires consideration to be given to many factors such as the location of infected and potentially infected animals, their susceptibility to infection and disease, and the distribution and dynamics of the vectors of trypanosomiasis. An understanding of these relationships requires sound assessment of these factors, by being able to successfully detect trypanosomes in animals and in vectors, and to determine in any given area, the risk of contracting trypanosomiasis.

Tsetse-transmitted trypanosomes have a wide range of mammalian hosts which include domestic livestock, wild game and man (Hoare, 1972). This review focuses mainly on the importance of the disease in cattle.

a) The parasite

The genus *Trypanosoma* is divided into two main groups, the stercoraria and the salivarian groups, depending on the site of development of the parasite in the vector and its mode of transmission. The stercorria develop in the alimentary tract of the vector with the production of metacyclics, the infective stage of trypanosomes, occurring in the hind gut and cause infection either by contamination of the skin or oral ingestion by the host. *Trypanosoma cruzi* is the most important in this group. On the other hand, the salivarian group complete their development in the anterior station, i.e., the salivary glands and the proboscis, and so transmission is by inoculation of metacyclics. In general,

trypanosomes inhabit plasma, body fluids and tissues of a wide range of host (Hoare, 1970). The salivarian group consists of trypanosome species of major medical and veterinary importance in tropical Africa. This group of trypanosomes are divided into four subgenera according to their morphological and biological characteristics (Table 2.2).

b) Transmission

Tsetse flies, the main vectors for transmitting animal trypanosomiasis from one vertebrate host to another, belong to the genus *Glossina* (Glasgow, 1970). There are some 36 species and sub-species which are classified into three taxonomic groups according to their ecological habitats; these are the *fusca, morsitans* and the *palpalis* groups (Jordan, 1986). The *fusca* group flies are largely confined to humid forest areas, the *morsitans* inhabit the savanna woodland and the *palpalis* group are primarily found in riverine habitats (Jordan, 1986). The distribution of tsetse in Africa has been reviewed by Katondo (1984) and Moloo (1985) and recently updated by IBAR (1989). In eastern and southern Africa, flies of the *palpalis* and the *morsitans* are predominant, with *G. morsitans, G. pallidipes, G. brevipalpis, G. longipennis* and *G. austeni* being mainly responsible for transmission of trypanosomiasis.

The tsetse ingests trypanosomes when it feeds on an infected host. Within the fly, bloodstream forms of trypanosomes called trypomastigotes undergo several development stages, where they multiply, migrate and finally mature into infective metacyclics. The development cycle in the fly varies from 5 days to 5 weeks depending on the trypanosome species (Vickerman, Tetley, Hendry and Turner, 1988).

Biting flies such as *Tabinidae* and *Stomoxys* are also believed to transmit trypanosome infections mechanically (reviewed by Wells, 1972), particularly *T. vivax* infections, where haematophagus flies feeding on infected hosts are

Table 2.2 Classification of trypanosomes pathogenic to domestic animals in Africa

Subgenus	Species	Subspecies	Major hosts
Duttonella	*T. vivax*		Cattle, goats, sheep
Nannomonas	*T. congolense*		Cattle, goats, sheep
	T. simiae		Pigs
Pycnomonas	*T. suis*		Pigs
Trypanozoon	*T. equiperdum*		Horses, donkeys
	T. evansi		Camels, horses, donkeys
	T. brucei	*T. b. brucei*	Cattle, sheep, goats, dogs

interrupted and immediately seek other feed sources to complete their bloodmeals. In the process, they inoculate the live parasites in their mouthparts "mechanically" into the new hosts. Non-tsetse transmitted trypanosomiasis due to *T. vivax* and *T. evansi* is known to occur in South America and Asia (Wells, Bentacourt and Raminez, 1982; Mahmoud and Gray, 1980) and in the Indian Ocean island of Mauritius (Jordan, 1986).

c) Host susceptibility

Before the introduction of cattle in sub-Saharan Africa, the primary vertebrate hosts for the tsetse fly were the wild mammals. Today, trypanosome infections in wildlife rarely manifests as clinical disease unless stressed, and these animals act as carriers and constitute an important reservoir of infection. Wild animals have been shown to play a major role in the epidemiology of trypanosomiasis in East and Central Africa (Ashcroft, 1959; Karstard, Grootenhuis and Mushi, 1978).

Trypanosomes have successfully established themselves as parasites of importance largely due to their ability to undergo antigen variation, i.e., change a single glycoprotein termed variant surface glycoprotein (VSG) (Cross, 1975) which covers the pellicular surface, thereby evading host immune responses and establishing persistent infection. In addition to the complexity of multiple variable antigen types (VAT) expressed during a single infection, each trypanosome species comprises an unknown number of different serodemes, all capable of giving rise to a different repertoire of VAT (van Meirvenne, Magnus and Vervoort, 1977). As a result, no effective vaccine has been produced for use in the field.

In cattle, susceptibility of breeds to the disease varies. Those that are able to limit the effects of trypanosome infection and remain productive under tsetse challenge without the aid of chemotherapy are referred to as being

trypanotolerant (Murray, Morrison, and Whitelaw, 1982). Breeds of cattle in West and Central Africa known to be trypanotolerant are the N'Dama and the West African Shorthorn (Roberts and Gray, 1973; Murray, Clifford, Gettinby, Snow and McIntyre, 1981). The capacity of the N'Dama to control anaemia following trypanosome infection is significantly correlated with trypanotolerance and is believed to be a heritable trait. (Murray, Stear, Trail, d'Ieteren, Agyemang and Dwinger, 1991; Trail, d'Ieteren and Teale, 1989). However, genetic markers for this trait have yet to be identified. In East Africa, there are reports of some East African Zebu breeds, namely the Galana Orma and the Maasai Zebu, expressing some degree of trypanotolerance (Njogu, Dolan, Wilson and Sayer 1985; Dolan, Njogu, Sayer, Wilson and Alushula, 1985; Ishmael, 1988; Mwangi, 1993). In contrast, other breeds of cattle and, particularly, most *Bos indicus* types are much more susceptible to trypanosomiasis (Roberts and Gray, 1973). Imported Friesian and 2/3 Ayrshire X 1/3 Sahiwal were very susceptible and require regular treatment with trypanocidal drugs to thrive in tsetse-infested areas. (Mwongela, Kovatch, and Fazil, 1981; Monirei, Murray, Whitelaw, Trail, Wissocq and Chema, 1982; Paling, Leak, Katende, Kamunya and Moloo, 1987).

d) The disease in cattle

Bovine trypanosomiasis is caused by three trypanosome species, namely *T. congolense, T. vivax* and *T. brucei*, the latter considered to be the least pathogenic. (Fiennes, 1970; Morrison, Murray and McIntryre, 1981). All three species are found throughout the tsetse-infested areas with mixed infections commonly observed in the field (reviewed by Stephen, 1970).

The clinical disease varies in severity depending on host susceptibility and species, stock and virulence of the trypanosome (Stephen, 1970; Losos and

Ikede, 1972; Soltys and Woo, 1977; Morrison *et al.*, 1981). Physiological and environmental factors such as age, sex, pregnancy, nutritional status, degree of tsetse challenge, increased work output resulting in stress, previous exposure to trypanosomiasis and intercurrent infections can also influence the course of clinical trypanosomiasis (Murray, 1989).

Trypanosome species are capable of producing a range of clinical responses in the host. Infections can be acute and often fatal, mild or subclinical, chronic or even asymptomatic. The acute form of the disease is generally characterised by pyrexia following infection, with high persistent parasitaemia, lasting 2 to 6 weeks which terminates either in death, or chronic disease. In animals where infection persists longer than 3 months, a chronic form of the disease develops.

Occasionally, a hyperacute form of the disease can cause high mortalities within 2 weeks of infection (Wellde, Chumo, Adoyo, Kovatch, Mwongela and Opiyo, 1983). Outbreaks of such a fulminating septicaemia-like haemorrhagic syndrome in cattle infected with certain *T. vivax* strains in the field have been described in Kenya and Somalia (Hudson, 1944; Mwongela *et al.*, 1981; Dirie, Wallbanks, Moleneux, Borstein and Omer, 1988). This haemorrhagic infection is characterised by pyrexia, high persistent parasitaemia, extensive haemorrhages on the conjunctival and vulval mucous membranes, epistaxis, and diarrhoea with frank blood. On gross pathology, ecchymotic and petechial haemorrhages are extensive on all visceral organs and the gastro-intestinal tract and "blood splashed" appearance of the skeletal muscles is prominent (Wellde *et al.*, 1983; Mwongela *et al.*, 1981; Gardiner, Assoku, Whitelaw, and Murray 1989).

The most common clinical feature of bovine trypanosomiasis is progressive anaemia (Hornby, 1921; Murray, 1974; reviewed by Murray and Dexter, 1988) associated with persistent undulating parasitaemia (Morrison *et*

al., 1981; Murray, 1979), due to the phenomenon of antigenic variation. The first detectable lesion is the development of a cutaneous swelling measuring several centimetres in diameter at the site where an infected tsetse fly has successfully fed on the host. The term chancre is used to describe this inflammatory reaction. Chancres have been studied extensively in experimental infections (Emery and Moloo, 1980, 1981; Akol and Murray, 1982, Dwinger, Murray, and Moloo, 1987), but are rarely detected in natural infections. Enlargement of the superficial lymph nodes occurs early in the infection and with the onset of parasitaemia, infected cattle develop intermittent fever, tachycardia, with reduced intake of food and, as the disease progresses weight loss, loss of condition and stunted growth in young animals become obvious. Pallor of mucous membranes develops as the packed red cell volume (PCV), a measure of anaemia, continues to decline. As the disease progresses into the chronic form, a phase associated with diminishing parasitaemia, cattle become more emaciated even though they continue to eat. During prolonged chronic infection, cachexia develops, body condition continues to deteriorate with bony protuberances and scruffy rough hair coat becoming prominent.

Reproduction is frequently impaired by trypanosomiasis (reviewed by Ikede, Elhassan and Alpavie, 1988). Infertility and long calving intervals are common features of trypanosomiasis in the field. Infected pregnant cows are likely to abort or give birth to stillborns or small weak calves. Neonatal mortality in these calves may be high. Calves may be unthrifty and stunted. Poor reproductive performance may be attributed to trypanosome-infected bulls which have poor semen quality when infected with *T. vivax* or *T. congolense*. Experimentally it has been shown that infected bulls were unfit for breeding by the sixth week of infection due to poor semen and lack of libido (Sekoni, Kumi-Diaka, Saror and Njoku, 1988). Disruption of oestrous cycles

has been reported in trypanosome-infected Boran cows with most infected cows becoming acyclic (Llewelyn, Munro, Luckins, Jordt, Murray and Lorenzini, 1988;).

In endemic areas, animals can be repeatedly challenged with different trypanosome antigenic strains as they trek longer distances for food and water. In such situations, animals continue to deteriorate until death. Chronically-infected cattle finally die of congestive heart failure which is due to a combination of anaemia, myocardial damage and circulatory disturbances (Morrison *et al.*, 1981).

2.3.2 DIAGNOSIS

In understanding the epidemiology of animal trypanosomiasis in any geographical location and for assessing the need for, and efficacy of treatment, it is important that accurate diagnosis of the disease is carried out. The completeness of the epidemiological picture, also depends on the ability to determine with accuracy trypanosome infections in the tsetse flies and to identify animal species providing reservoirs of infection (Tarimo, Snow, Butler and Dransfield, 1985).

Diagnosis of trypanosomiasis still remains a major problem as clinically the disease cannot always be differentiated from other anaemia-causing infections. Therefore, the specific diagnosis of the disease still depends on the demonstration of the parasite in blood or tissue fluids by light microscopy or by immunological techniques (Nantulya, 1990).

a)Parasitological diagnostic techniques

The Standard Trypanosome Detection Methods (STDM) were for many years the only direct methods available for confirmatory diagnosis (reviewed by Killick-Kendrick, 1968; Wilson, 1969). These consists of thick, thin and wet

blood films, and or inoculation of blood into susceptible mice. Trypanosomes in peripheral blood can be detected by microscopic examination of wet blood film or Giemsa-stained thick and thin blood smears. Direct microscopy,though useful in screening large cattle herds, may miss about 50% of the infections (Barnett, 1947).

Inoculation of laboratory animals with blood from suspected host is more sensitive for the diagnosis of *T. brucei* infections, but not for other species of trypanosomes, mainly the East African *T. vivax* and some *strains of T. congolense*, as they do not infect laboratory rodents (Paris, Murray and McOdimba, 1982;). Wider application of this method is also limited, as diagnosis is not immediate since inoculated mice have to be examined for a minimum of 30 days before being ruled as non-infected. Diagnosis is routinely made using a combination of these above methods as any one alone is not good enough to detect parasitaemic cases.

More recently, the sensitivity of direct microscopy has been improved through trypanosome concentration methods consisting of haematocrit centrifigation (Woo, 1970), the darkground/phase contrast (DG) technique (Murray, Murray, and McIntyre, 1977), and the miniature anion exchange technique (Lumsden, Kimber, Evans and Doig, 1979). Centrifugation of unclotted blood in microhaematocrit capillary tubes concentrates trypanosomes on the buffy coat, thereby increasing its sensitivity over the STDM (Woo, 1970; Murray *et al.*, 1977). In addition, the DG buffy coat technique (Murray *et al.*, 1977 allows the estimation of intensity of parasitaemia and identification of trypanosome species (Paris *et al.*, 1982).

At present, the DG technique is widely used as the diagnostic method to estimate the prevalence of trypanosomiasis in domestic livestock (ILCA, 1986; Connor, 1990). Besides on the spot diagnosis, it also allows for treatment

of individual cases. Nevertheless, the sensitivity of this method is limited when detecting low or intermittent parasitaemia as often occurs in chronic disease.

Although a number of parasitological detection techniques are available for diagnosis of trypanosomiasis, infections can go undetected as many of them are chronic with fluctuating parasitaemias. Therefore, there is a need for methods of increased sensitivity and specificity, which are readily applicable and allow assay of large number of samples.

b)Immunological Techniques: Detection of trypanosome antigens

Initial attempts at detecting trypanosome antigens in infected animals showed low sensitivity and specificity (Araujo, 1982; Rae and Luckins, 1984). Recently, trypanosome species-specific monoclonal antibodies (MoAbs), which are able to demonstrate trypanosome antigens in infected host, have paved the way for improved diagnostic tests for trypanosomiasis (Nantulya, 1981; Nantulya, Musoke, Rurangirwa, Saigar and Minja, 1987; Richardson, Jenni, Beecroft and Pearson, 1986;).

Using *T. brucei* group-specific MoAbs, a sandwich ELISA was developed for the diagnosis of *T. brucei brucei* infections in cattle and *T. b. rhodesiense* and *T. b. gambiense* in man (Nantulya, 1989). Species-specific MoAbs against *T. congolense* and *T. vivax* have also been produced for use in an antigen capture sandwich ELISA (Nantulya and Lindqvist, 1989).

Results of field studies in cattle from an endemic area in Kenya show that antigen ELISA (Ag-ELISA) detected infection in 96% of the parasitologically positive cases and 52.6% of the parasitologically negative animals (Nantulya, Lindqvist, Stevenson and Mwangi, 1992). In another field study, Trail, d'Ieteren, Maille, Yangari and Nantulya, (1992) observed that of the 28% of parasitaemic N'Dama cattle kept under natural tsetse challenge, 90% were antigen positive. Moreover, 40% of the samples tested negative on

DG technique, were found to be antigen positive. Nevertheless, a few animals detected parasite positive tested negative on Ag-ELISA. Recent laboratory studies (Masake and Nantulya, 1991) showed that in goats and cattle experimentally infected with different clones of *T. congolense* and left to develop into chronic form of disease, antigens could be detected using Ag-ELISA in over 94% and 82% of the caprine and bovine samples tested, respectively. On the other hand, only about 11% and 20% of goat and cattle blood, respectively, had demonstrable trypanosomes as revealed by the DG technique. The Ag-ELISA showed a four-fold increase in sensitivity compared to the DG method in monitoring experimental *T. congolense* infections (Masake and Nantulya, 1991). However, the test misses a proportion of the early parasitologically positive infections and shows persistence of antigens for varying periods after successful treatment.

The advantage of the Ag-ELISA is that a single assay technique can be applied to a variety of animal host species for the diagnosis of disease. The test is easy to perform, allows the analysis of large numbers of serum samples, the positive colour reactions can be read visually and is reported to be more sensitive than the current parasitological detection methods (Nantulya, 1990). The major drawbacks of this test are that it requires access to specialised reagents and the small number of false-negative results.

For future epidemiological studies, the diagnostic strategy is likely to require a combination of one of the more sensitive parasitological techniques such as the DG technique with the Ag-ELISA.

However, the significance of detecting more antigenaemic animals even though they showed no clinical and pathological effects of trypanosome infections, particularly under field conditions, definitely needs further investigation.

c) Detection of trypanosome DNA

Recent developments in molecular biology have created new avenues for a major improvement in parasite detection and characterisation. The technology uses recombinant DNA for cloning and expression of genes responsible for encoding specific parasite antigens needed in designing highly specific and sensitive diagnostic tests, while nuclear hybridization techniques allow for improved diagnosis in the identification of parasites in the tissue specimens of infected hosts (reviewed by Nantulya, 1990).

The limitations of morphological identification of all trypanosome species, and particularly those in the tsetse, has to some extent been resolved by using trypanosome species-specific DNA probes for repetitive DNA sequences (Kukla, Majiwa, Young, Moloo and ole-Moi Yoi, 1987; Majiwa and Webster, 1987; reviewed by Ole-Moi-Yoi, 1987; Gibson, Dukes and Gashumba, 1988; Majiwa and Otieno, 1990). For example, trypanosomes of the subgenus *Nannomonas* have been shown to be genetically diverse. Identification on the basis of isoenzyme patterns and repetitive DNA sequences have shown that there are at least three distinct subspecies of *T. congolense*, savanna, riverine-forest and Kenya coast type, which differ from *T. simiae* (Majiwa, Hamers, van Meirvenne and Matthyssens, 1986; Majiwa and Webster, 1987; Gibson *et al.*, 1988).

With the advent of polymerase chain reaction (PCR), it is possible to amplify minute amounts of parasite DNA which can then be exposed to a hybridization probe. Moser, Cook, Ochs, Bailey, McKane and Donelson, (1989) were able to demonstrate amplified nuclear DNA of *T. congolense* and *T. brucei* from parasitized mouse blood. Amplification of 10% of the DNA (0.01pg) of a single parasite of *T. congolense* or *T. brucei* produced sufficient product to be visible as a band on an agarose gel stained with ethidium

bromide. This level of detection is 100 times more sensitive than the repetitive sequence probes.

The application of these molecular diagnostic tests provide useful new tools not only for the understanding of the complexities of the taxonomy of the trypanosomes, but also as sensitive techniques for conducting epidemiological surveys. However, the drawbacks of such tests is their practical use in the field where appropriate facilities are lacking.

d) Immunological techniques: Detection of trypanosome antibodies

The presence of antibodies does not indicate active infection, but for epidemiological studies, it provides useful information in assessing degree of exposure in a given population in a region. At present, the two most commonly used immunological methods for the detection of antibodies in cattle are the indirect immunofluorescent antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA).

i) Indirect immunofluorescent antibody test

The development and use of IFAT for the diagnosis of bovine trypanosomiasis has been reported by several authors (Wilson 1969; Wilson and Cunningham, 1971; Zwart, Peerie, Keppler and Goedbloed, 1973; Luckins and Mehlitz, 1978). The antigens used are prepared by fixing smears of parasitized blood using a number of fixatives (Zwart *et al.*, 1973). The disadvantages of the method are; difficulty in standardisation of the antigen preparation, ultra-low temperatures required for preservation of antigen slides, and the problem of nonspecific reactions However, improvements in the preparation of antigen have since involved separation of trypanosomes from blood using diethylaminoethyl (DEAE) cellulose 52 columns (Lanham and Godfrey, 1970) and fixation and storage of separated trypanosomes using a mixture of acetone

and formaldehyde suspension (Katende, Musoke, Nantulya and Goddeeris, 1987). This technique has enhanced the specificity of the test by differentiating, to a limited extent, between *T. vivax*, *T. congolense* and *T. brucei* infections in cattle (Katende *et al.*, 1987), Although IFAT have been extensively used, the method depends on skilled operators, sophisticated equipment which is not suitable for field use and relies on subjective interpretation of the results.

ii) Enzyme-linked immunosorbent assay (ELISA)

In order to overcome the cumbersome IFAT, enzyme immunoassays have been developed. The technique is cheaper, easy to perform, requiring simple equipment, interpretation of results is easier and excludes subjecti bias. Furthermore, the test can be used for screening large number of samples. The test was first used in the human disease (Voller, Bidwell, Barlett, 1975). It was later employed by Luckins (1977) for the diagnosis of bovine trypanosomiasis under natural and experimental infections. Luckins and Mehlitz (1978), when comparing the sensitivity of IFAT and ELISA, found that more serologically positive cattle were detected using ELISA, but both test were equally sensitive in detecting antibodies in cattle in which trypanosomes were demonstrated by examination of peripheral blood.

The major limitations of this technique are that the antigens used are crude lysates and their quantity ill-defined, thus causing difficulty in standardisation of the assay, with respect to specificity and sensitivity. However, Ijagbone, Staak and Reinhard (1989) were able improve the assay by using fractionated trypanosome species-specific antigens, but the test is yet to be widely used in the analysis of field samples mainly due to the cumbersome process of antigen preparation..

The IFAT and the ELISA are useful tools in seroepidemiological surveys where prevalence of trypanosomiasis in given localities needs to be

estimated (Luckins and Mehlitz, 1978). These tests are, however, not specific, and cannot differentiate between *T. brucei*, *T. vivax* and *T. congolense* infections (Luckins, 1977). Likewise, the tests may fail to pick antibodies in early infections as the host immune response in the initial stages of infection may be below their sensitivity level.

Antibody-detection systems are unable to distinguish between current and past infections. Weisenhutter (1969) found that animals treated with the trypanocidal drug diminazene aceturate (Berenil^{R} Hoechst) were tested positive for 40 days post-treatment, while Wilson (1969) reported antibody titres for as long as 112 days after treatment. Luckins (1977), found that antibodies in cattle infected with *T. vivax* could be demonstrated for 83 days following treatment with Berenil. The persistence of antibodies makes its difficult to interpret the status of an animal ,i.e., whether it is clinically infected or recovered from an infection after chemotherapy or self-cured. These immunodiagnostic test for antibody detection, therefore, are less definitive than parasitological tests when used alone, and lack reliability in the diagnosis of individual cases of trypanosomiasis. However, for epidemiological purposes, they provide data on exposure to the disease.

2.3.3 CONTROL OF TRYPANOSOMIASIS

To date, no major breakthrough has been made in search for a vaccine against trypanosomiasis. This has been hampered by the phenomenon antigenic variation whereby the trypanosome evades the host's immune system (Murray and Urquhart, 1977). Therefore, at present, control of trypanosomiasis in livestock, particularly in trypanosusceptible cattle, depends upon treatment of infected host with trypanocidal drugs, and control of the tsetse population.

2.3.3.1 CHEMOTHERAPY

Treatment with trypanocidal drugs is the most widespread and commonly used method of controlling trypanosomiasis (reviewed by Williamson, 1970; Leach and Roberts, 1981). Successful cures using drugs were first demonstrated by Plimmer and Thompson, (1908) who administered tartar emetic (potassium antimony tartrate) to mice infected with *T. brucei* and *T. evansi*, and as a result the treatment was subsequently introduced for the control of bovine trypanosomiasis. In Tanganyika, Rhodesia and Zululand, regular intravenous application in cattle against *T. congolense* and *T. vivax* infections in the field demonstrated the efficacy of the compound (Bevan, 1928). Subsequently, compounds belonging to chemical classes of acid naphthylamines, diamidines, quinalidines and phenanthridines have been used as trypanocides.

However, until the recent launching of melarsenoxide cysteamine (MelCy, Cymelarsen^{R}, Rhone Merieux) for the treatment of *T. evansi* in camels (Raynaud, Sones and Friedheim, 1989), no new trypanocidal drug has been released for the last 30 years mainly due to prohibitive costs of drug development and the limited trypanocide market (Murray *et al.*, 1991). Although the market for trypanocidal drugs is potentially large, it is made up of countries with unpredictable economies and political stability (Williamson, 1976).

At present, the situation regarding use of trypanocidal drugs is likely to deteriorate not only from lack of new drugs, but also due to development of resistance to the ones currently in use. To maximise the effectiveness of the current drugs, well co-ordinated programmes for drug administration, surveillance and screening for resistance need to be implemented (Leach and Roberts, 1981).

The delivery of such programmes is limited by logistical and financial problems which include high drug costs, lack of operational funds, poor

infrastructure for drug administration, inadequate diagnostic facilities, irregular supply of drugs, syringes and needles, and lack of well trained personnel (Holmes and Scott, 1982; Murray and Gray, 1984).

This creates an increasing gap between treatment demand and actual treatment given (MacLennan, 1981). As a result, only 25 million doses of trypanocides are estimated to be administered annually (Tacher, 1982b), whilst at least 50 million cattle and 70 million small ruminants are at risk. Even if the animals were to be treated twice per year, 240 million doses would be required (Murray *et al.*, 1991).

Chemotherapy and chemoprophylaxis can be effective and economically justified if managed in an efficient and organised system (Leach and Roberts, 1981; Trail *et al.*, 1985). Jahnke (1974) reported that it was relatively cheaper to use trypanocidal drugs as opposed to tsetse control under certain conditions. This applied to areas where stocking density and incidence of trypanosomiasis were both high and tsetse control relatively expensive (Finelle, 1976).

The decision to carry out practical and effective therapy or prophylaxis depends on the level of tsetse challenge and the type of animal husbandry practiced. Generally, curative treatments and strategically timed prophylaxis with simultaneous monitoring of herds for infections have been recommended, the frequency of which depends on the level of tsetse challenge (MacLennan, 1981).

a) Trypanocidal drugs

The commonly used drugs in animal trypanosomiasis are classified into four chemical groups (reviewed by Leach and Roberts, 1981). The drugs currently used for the treatment of animal trypanosomiasis are shown in Table 2.3. Depending on phamacokinetics of these drugs, they have been referred to as

Table 2.3 Drugs used for the treatment of trypanosomiasis in domestic animals

Compounds	Generic name	Action	Susceptible trypanosomes
Diamidines	Diminazene aceturate[1]	Curative	*Trypanosoma vivax*, *T. congolense* (*T. brucei*, *T. evansi*).
Phenanthridiums	Homidium bromide[2]	Curative	*T. vivax*, *T. congolense*
	Homidium chloride[3]	Curative	*T. vivax*, *T. congolense*
	Isometamidium chloride[4,5]	Prophylactic	*T. vivax*, *T. congolense* (*T. brucei*)
Quinoline Pyrimidine group	Quinapyramine sulphate	Curative	*T. evansi*, *T. equiperdum*, *T. brucei* (*T. vivax*, *T. congolense*)
	Quinapyramine sulphate:chloride (3:2 w/v)	Prophylactic	As for sulphate
Napthalidines	Suramin	Curative	*T. evansi*, *T. brucei* , *T. equiperdum*

1 Berenil ,Hoechst, Germany
2 Ethidium, CAMCO, U.K
3 Novidium RMB, U.K
4 Samorin , RMB. U.K
5 Trypamidium, Rhone Meriux, France
6 Trypacide Rhone Merieux, France
7 Trypacide Prosalt, Rhone Merieux, France
8 Naganol, RMB UK
9 Cymelarsan, Rhone Merieux, France

Parenthesis indicates species less susceptible to the drug

either therapeutic or prophylactic. Examples of therapeutic drugs commonly used in cattle are diminazene aceturate, homidium chloride, and homidium bromide, On the other hand, isometamidium chloride is currently the only drug used in cattle for prophylaxis. Where properly managed, the use of trypanocidal drugs can be extremely cost-effective in controlling bovine trypanosomiasis (Trail *et al.*, 1985).

A chemotherapeutic strategy is indicated for animals in an area of low tsetse challenge or in cattle no longer exposed to the disease risk following complete withdrawal from an endemic area (MacLennan, 1980). Thus, the use of strategic therapeutic treatment for dairy cattle kept on a ranch under low tsetse challenge has allowed successful maintenance of the herd at Kilifi Plantations, Kenya. Animals were bled regularly and those with PCV below 30% were treated with diminazene aceturate (Berenil^{R}, Hoechst) (Wissocq, Trail, Wilson, and Murray, 1983). This method of control for trypanosomiasis was observed to be cost-effective and resulted in increased productivity of the dairy herd. Before this approach was adopted, cattle on the ranch had previously experienced abortion storms associated with trypanosomiasis.

Slaughter cattle travelling on hoof through tsetse-infested stock routes, should be prophylactically protected for the entire period of movement. Similarly, prophylaxis is advisable where transhumance is practised and should be given to cattle before they move into tsetse-infested areas, while curative treatment should be administered as soon as cattle return to tsetse-free areas (FAO,1979). Such curative treatments are important in preventing mechanical transmission of the infection (Tacher, 1982b; Gray, 1983).

Susceptible cattle and draught oxen residing in endemic areas, require frequent monitoring, correct choice and dosage of trypanocide and constant surveillance for drug resistance. In Ethiopia, some 400 draught oxen in a settlement scheme were successfully raised in a high tsetse challenge by use of

prophylaxis under close veterinary supervision (Bourn and Scott, 1978). Likewise, it was possible to carry out prophylactic control programmes in sedentary Zebu cattle kept under traditional village management in the medium challenge area of Muhaka, Kenya coast; the programme reduced the disease incidence by 39%,and achieved a 20% increase in the overall herd productivity (Maloo, Chema, Connor, Durkin, Kimotho, Maehl, Mukendi, Murray, Rarieya and Trail, 1988a, b).

Under ranching conditions, strategic application of trypanocidal drugs has allowed successful maintenance and economic productivity of cattle herds in tsetse-infested areas. In East Africa, this has been reported in Kiburine ranch, where liveweight gains of Boran cattle raised in moderate tsetse infested areas were about 75% of the gains recorded by the same breed kept in non-tsetse areas (Wilson, LeRoux, Paris, Davidson and Gray, 1975). Similarly, at Mkwaja ranch in Tanzania, an area of high challenge, it was found impractical to successfully raise cattle without use of chemoprophylaxis (Blaser, Jibbo and McIntyre, 1979; Trail *et al.*, 1985). Strategic intervention with chemoprophylaxis achieved herd productivity of cattle on the ranch close to that of Boran cattle reared in tsetse-free areas of Kenya (Trail *et al.*, 1985). Studies carried out on Galana ranch have demonstrated that implementation of organised chemoprophylactic regimes reduced therapeutic drug costs significantly by up to 60% in the low to medium tsetse challenge areas (Wilson Njogu, Gatuta, Mgutu and Alushula, 1983).

Similarly, studies carried out along the coastal regions of southern Tanzania and Mozambique have also shown that using prophylactic drugs in cattle afforded protection against trypanosomiasis under high and medium risk (Connor, Mukangi and Halliwell, 1988; Takken, Taylor-Lewis and Woodford, 1988).

b) Duration of prophylaxis

The prophylactic period that isometamidium chloride confers on cattle appears to vary widely; a dosage of 1mg kg^{-1} bodyweight has been shown to afford protection for 2 to 22 weeks (Kirkby, 1964; Pinder and Authie, 1984; Whitelaw, Bell, Holmes, Moloo, Hirumi, Urquhart and Murray, 1986). Factors that may influence the prophylactic period include drug dosage (Boyt, Lovemore, Pilson and Smith, 1962; Ogunyemi and Illemobade, 1989), vector density or trypanosomiasis risk (Davey, 1957; Whiteside, 1962), relapses from tissue sites impenetrable by the drug (Jennings, Whitelaw and Urquhart, 1977), plane of nutrition and stress (Boyt *et al.*, 1962; Ogunyemi and Illemobade, 1989), acquisition of immunity following the use of trypanocides (Fiennes, 1953; Wilson *et al* 1975; Bourn and Scott, 1978) and differing levels of drug sensitivity between trypanosome populations (Peregrine, Moloo and Whitelaw, 1991).

Observation by field workers have shown that higher drug dosage conferred longer period of protection compared to lower dosages under varying trypanosomiasis risk areas (reviewed by Ogunyemi and Illemobade, 1989). Generally, it has been suggested that the duration of prophylaxis is directly related to the intensity of tsetse challenge (Fiennes, 1953; Davey, 1957; Whiteside, 1962). In studies carried out in the high challenge areas of Mozambique, Takken *et al.*, (1988), reported that protection afforded by prophylactic drugs was shorter during the rainy season, when trypanosomiasis risk was high, than during the dry season. Similar evidence was also observed at Mkwaja Ranch in Tanzania, where cattle kept in areas of high tsetse densities required more frequent isometamidium treatments than those kept on other areas of the ranch (Trail *et al.*, 1985).

Recent work in experimental animals has indicated that the duration of prophylaxis is a function of the dose of drug but is not apparently dependent

on the intensity of metacyclic *T. congolense* challenge (Whitelaw *et al* 1986; Peregrine, Ogunyemi, Whitelaw, Holmes, Moloo, Hirumi, Urquhart and Murray, 1988). A group of 24 Boran cattle treated intramuscularly with 1.0 mg kg^{-1} isometamidium chloride and challenged monthly with five tsetse infected with *T. congolense*, were completely protected for 5 months (Whitelaw *et al.*, 1986). In a similar study, Peregrine *et al.* (1988) reported duration of prophylaxis for 4 months against *T. congolense* challenge. Despite the fact that isometamidium chloride is recommended as a prophylactic drug against *T. congolense* and *T. vivax* infections in cattle, comparative studies on the prophylactic cover afforded in the field are lacking. Under experimental conditions, recent studies have shown vast differences between the prophylactic and therapeutic effect of 0.5mg kg^{-1} of isometamidium chloride on fly-transmitted Kenyan *T. vivax* infections in cattle (Peregrine, Moloo and Whitelaw, 1991). Prophylaxis was afforded for less than one month for the Galana *T. vivax* populations and for only a month against the Likoni *T. vivax*, whereas therapeutic treatments with isometamidium chloride, 11 days after infection, resulted in all animals being cured. These finding demonstrate that the two Kenyan *T. vivax* populations showed lowered sensitivity to the prophylactic action of isometamidium, yet were highly sensitive to the therapeutic action of the drug, possibly due to the drug concentration levels achieved in plasma. The results also indicate that differences in drug sensitivity between different trypanosome isolates play a major role in determining the apparent period of prophylaxis afforded by isometamidium chloride (Peregrine, Moloo and Whitelaw, 1991).

c) Drug resistance

The extensive use and abuse of the limited numbers of commercially available trypanocides, particularly for the control of bovine trypanosomiasis, have been

responsible for the appearance of drug-resistant trypanosomes in many parts of Africa (Williamson, 1979; Githata, 1979; Leach and Roberts, 1981; Kupper and Wolters, 1983; Pinder and Authie, 1984; Rottcher and Schillinger, 1985; Schoenfeld, Rottcher and Moloo, 1987; Rowlands, Woudyalew-Mulatu, Authie, d'Ieteren, Leak, Nagda and Peregrine, 1993). Drug resistance has been associated with numerous factors such as, prolonged under-dosing due to incorrect body weight estimation, failure to calculate adequate dosage, deliberate under dosing, poor drug preparation and administration, high incidence of trypanosomiasis and erratic treatments with prophylactics, or withdrawal of prophylactic drug whilst animals are still at risk (Davey, 1957; Whiteside, 1962). All the above factors allow exposure of trypanosome to sub-therapeutic drug levels, thereby favouring the selection of drug-resistant subpopulations (Leach and Roberts, 1981).

It is generally believed that drug resistance is more likely to occur with trypanocides with prophylactic activity rather than those with only therapeutic effects (Holmes and Torr, 1988). Diminazene aceturate, a curative trypanocide, when administered, is fast acting, rapidly broken down and excreted (Bauer, 1958), thereby allowing subcurative concentrations to persist for only short periods. This view was supported by the absence of resistance to diminazene aceturate until the 1960s (Holmes and Torr, 1988). However, since then a few reports have described the appearance of diminazene resistant trypanosome strains in cattle that were refractory to at least 3.5mg kg^{-1}. In East Africa, diminazene resistant *T. vivax* strains have been reported by several authors (Mwambu and Mayende, 1971a,b; Mbwambo, Mella and Lekaki, 1988; Njau, Mkonji and Kundy, 1983; Rottcher and Schillinger, 1985) whilst reports of resistance in field isolates of *T. congolense* to the diminazene at 3.5mg kg^{-1} and at 7.0 mg kg^{-1} are few (Gitatha, 1979; Codija, Woudyalew-Mulatu, Majiwa, Leak, Rowlands, Authie, d'Ieteren and Peregrine, 1993; Rowlands *et al.*,

1993,). One possible explanation for the differences between the two species may be the ability of *T. vivax* to express a higher degree of innate resistance to diminazene than *T. congolense* (Williamson, 1960).

In contrast, resistance to homidium appears to occur more commonly in *T. congolense* than in *T. vivax*. Widespread resistance to *T. congolense* with homidium chloride has been described in eastern Africa (Gadir, Tahir, Razig and Osman, 1972; Gitatha, 1979; Scott and Pegram, 1974). However, recently there has been evidence to show that resistance to *T. vivax* also occurs, as the recommended dose of 1.0mg kg^{-1} failed to cure a few isolates of *T. vivax* under experimental infections (Rottcher and Schillinger, 1985; Schoenfeld *et al.*, 1987).

Early breakthroughs of trypanosome infection following prophylactic treatment with isometamidium chloride in the field are usually attributed to drug resistance. Such observations have been reported in Zimbabwe (Lewis and Thompson, 1974), in Ethiopia (Scott and Pegram, 1974), in Ivory Coast (Kupper and Wolters, 1983), in Burkina Faso (Pinder and Authie, 1984; Clausen, Sidibe, Kabore and Bauer, 1992), and in Kenya (Dolan, Stevenson, Alushula and Okech, 1992; Munstermann, Mbura, Maloo and Lohr, 1992). On the other hand, at Mkwaja Ranch, in Tanzania, where isometamidium has been used prophylactically for over 20 years, problems of drug resistance were not encountered probably due to good management and the use of correct drug dosage. In addition, the long-term drug did not have any detrimental effect on performance as it did not lower the productivity of the Boran herd (Trail *et al.*, 1985).

d) Cross resistance

Besides the problem of drug resistance, trypanocidal drugs were observed to develop cross resistance. Cross resistance is a situation where a strain of

trypanosome resistant to one trypanocide is also observed to be resistant to a second chemically unrelated trypanocide, even though it might not have been exposed to the latter.

Extensive cross-resistance studies were conducted by Whiteside (1960) using strains of *T. congolense* and *T. vivax*. He showed cross resistance was not necessarily related to the chemical structure of the compounds, e.g., trypanosomes resistant to quinapyramine were resistant to diminazene and homidium. Whiteside (1958) introduced the term "sanative pair" to refer to pairs of drugs that do not induce resistance to one another. Currently, the two sanative pairs are homidium/diminazene and isometamidium/diminazene. However, recently, some *T. congolense* strains have been reported in West Africa to show cross resistance between isometamidium and diminazene (Pinder and Authie, 1984) and between isometamidium, homidium and diminazene (Clausen *et al.*, 1992).

With increasing reports of failure of chemotherapy due to drug resistance, alternative means are needed to reduce the risk of trypanosomiasis. One such measure is the control of the vector.

2.3.3.2 TSETSE CONTROL

A variety of methods have been applied to control or even eradicate tsetse populations for over the last 60 years (reviews by MacLennan, 1981; Jordan, 1986; Holmes and Torr, 1988). Initially, they included elimination of wildlife, clearing and maintaining of fly barriers to prevent the advance of the vector, and indiscriminate clearing of bush and forests to destroy breeding habitats. Since then, the use of insecticides has been the principle method employed to combat tsetse.

a) Insecticide application

The insecticides used fall into two categories, residual and non-residual and their application can be achieved either by ground or aerial spraying. Residual insecticides such as DDT and dieldrin, delivered in a single application mostly by hand-operated spray pumps, aim at depositing insecticide to the tsetse resting sites (Jordan, 1986). Reclamation of tsetse-infested areas has been successfully achieved by ground spraying campaigns in Northern Nigeria where about 200,000 sq km of land was cleared of tsetse (MacLennan, 1981; Jordan, 1986). Non residual insecticides due to their lack of persistency, require several applications and are delivered by aerial spraying using fixed winged aircraft or helicopters (Lee, Parker, Baldry and Moleneux, 1978; MacLennan, 1981). Currently, endosulphan is the insecticide most commonly used in aerial spraying and is applied using ultra-low-volume techniques (ULV).

Control attempts have been successful where proper implementation of insecticide application has been carried out, e.g., Nigeria, Zimbabwe, Botswana and Zambia (MacLennan, 1980; Allsopp, 1984). Although insecticide application can be effective in tsetse control, the approach has several limitations. These include high operational costs, both in ground and aerial spraying programmes, and lack of trained personnel to carry out control programmes (Holmes and Torr, 1988). Besides implementation of control measures, natural or man-made barriers are needed to prevent reinvasion of tsetse into the sprayed area, and constant surveillance is required for early detection of any reinvasion. Moreover, there is increasing pressure for restricted use of insecticides because of possible environmental effects on the flora and fauna. However, the effects of anti-tsetse spraying on non-target species appear to be transient and do not last for more than a year (Jordan, 1986). The search for alternative potent compounds with possibly low toxic

effect on the environment has lead to the identification of synthetic pyrethroids.

b) Trap and target technology

More recently a new concept to tsetse control has been conceived. In the past, sampling of tsetse populations was done by the use of traps and screens. Lately, the principle of attracting tsetse to traps and targets has been greatly improved with advances in design, identification of colours and odours which attract tsetse. This approach has been given increasing attention in the deployment of traps and targets as a method of tsetse control .

The use of traps and targets, most of them impregnated with insecticides, are an attempt to reduce the tsetse population over an extended period of time.

Trials conducted in *palpalis* riverine habitat of Cote d'Ivoire, Burkina Faso and Nigeria, using deltamethrin impregnated biconical traps and screens, reduced tsetse populations (Laveissiere and Couret, 1981; Laveissiere, Couret and Kienon, 1981; Allsopp, 1984;).

A series of experiments carried out in Zimbabwe has led to the development of a simple insecticide-impregnated target incorporating chemicals such as carbon dioxide, acetone and 1-octen-3ol (octenol) which attract tsetse, particularly the *morsitans* group (Vale and Hall, 1985 a,b; Vale, 1987). Buffalo and cow urine were also observed to have potent natural olfactory attracting properties for some tsetse species (Owaga, 1984, 1985). These developments were of significance with respect to introducing a simple, and environmentally safe method of control of tsetse.

Odour-baited visually attractive insecticide impregnated targets have now been used successfully in tsetse control programmes. In Zimbabwe, targets were deployed at $4/km^{-2}$ to completely eradicate *G. m. morsitans* and

G. pallidipes from an island in Lake Kariba (Vale, Bursell and Hargrove, 1985; Vale, Hargrove and Cockbill, 1986). This technique implemented over a larger area, 600km^{-2} in the Rifa Triangular, Zimbabwe, reduced the tsetse population drastically (Vale, Flint and Hall, 1986). In East Africa, a similar approach adopted at Galana Ranch, in Kenya, resulted in the reduction of tsetse catches (Opiyo *et al.*, 1987). Recently, the same methodology has been tried out in Lambwe Valley, Western Kenya, and shows promise in the integrated approach towards the control of tsetse and trypanosomiasis (KETRI, 1991). Lately, the deployment of an newly designed odour-baited NG2B trap (Brightwell, Dransfield, Kyorku, Golder, Tarimo and Mungai, 1987) suppressed *G. pallidipes* by over 90% at Nguruman, Kenya (Dransfield, Williams and Brightwell, 1991).

However, this approach has not been successful with all species of tsetse and the long-term maintenance, service and repairs of odour-baited traps and targets can lead to cost accruing with time (Holmes and Torr, 1988).

c) Insecticidal use on cattle

Dipping of cattle in insecticides has been practised for controlling ticks However, observations have been made by workers in Zambia, where the use of deltamethrin in dips, besides controlling ticks, reduced the incidence of trypanosomiasis in cattle from 40% to 5% (Chizyuka and Luguru, 1986). More recently, pour-on preparations of synthetic pyrethroids (deltamethrin and flumethrin) have been applied on cattle in several areas of Africa to assess their efficacy as mobile targets. Results were promising as marked decrease in the incidence of disease and higher body weight gains were achieved using flumethrin in high tsetse challenge areas in Kenya (Lohr, Omukuba, Njogu, Maloo, Gisemba, Okedi and Mwongela, 1991) and in Burkina Faso (Bauer, Kabore, Liebish, Meyer and Petrich-Bauer, 1992) and under low tsetse

challenge in Zanzibar (Schoenfeld, 1988). Likewise, similar results with deltamethrin have been reported from several parts of southern Africa with varying tsetse challenge (Thompson, Mitchell, Rees, Shereni, Schoenfeld and Wilson, 1991) and under high challenge in Kenya (Stevenson, Munga, Makumi, Baylis and Alushula, *in press*).

d) Other methods

Besides the use of insecticides and attractants, various other methods aimed at biological and genetic control have been tested. One such technique is the use of sterile insect technique (SIT). The principle of this method is to control *Glossina* populations by decreasing significantly the number of fertile females in the wild. As females mate only once in a lifetime, the release of sterilised males to mate with the wild females should significantly effect the reproductive rate of flies, and consequently reduce tsetse population. The success of SIT depends on, the number of males released, their ability to compete with wild males in the released environment and to disseminate effectively in a given habitat (reviewed by Knippling, 1982). However, in field trials this technique was only able to achieve local eradication of riverine species in Burkina Faso (Politzar and Cuisance, 1982) and in Tanzania on savanna species (Williamson, Dame, Gates, Cobb, Bakuli and Warner, 1983) when used in combination with insecticidal application. Apart from the technique being species-specific, i.e., sterile males of one *Glossina* species will mate with only the respective females and not with other female tsetse species, it requires significant capital investment and the exercise is unlikely to be sustainable under the present economic climate prevailing in most African countries.

In conclusion, various methods have been attempted for tsetse control, but no single method has been found to be ideal. The use of insecticide-impregnated traps and targets may provide a simple and environmentally safe

method for controlling some tsetse species, while the cattle population in a given area subjected to insecticidal applications in the form of a dipwash or pour-on can possibly be used to reduce the tsetse population and subsequently the disease incidence. A combination of these approaches coupled with strategic use of trypanocidal drugs may be the answer towards control of trypanosomiasis. Before employing any tsetse control method, feasibility studies must be carried out to evaluate the economic justification and sustainability of the control programme.

2.3.3.3.TRYPANOTOLERANCE: GENETIC RESISTANCE

Because of the limitations of the current control methods and the likelihood that a vaccine will not become available in the immediate future, increasing considerations is now being given to the use of trypanotolerant breeds of domestic animals as a sustainable approach to livestock development in tsetse-infested areas (Murray *et al.*, 1991). The ability of certain cattle breeds to survive and be productive in tsetse-infested areas without the aid of treatment where other breeds rapidly succumb to the disease, is termed trypanotolerance (Pierre, 1906; Murray *et al.*, 1982). This trait is generally attributed to the *Bos taurus* breeds of cattle in West and Central Africa, namely the N'Dama (Roberts and Gray, 1973) and the West African Shorthorn (Roelants, 1986). In Africa, only 6% of the 173 million cattle population are of the trypanotolerant type (Murray *et al.*, 1991). Failure to exploit these breeds can be attributed to the belief that they are not productive because of their small size (Stephen, 1966) and to the view that their trypanotolerance was restricted to resistance to the local trypanosome populations. Also, the limited availability of the breeds has prevented them from being used in wide-scale programmes to stock tsetse-infested areas. Nevertheless, N'Dama cattle have been imported into countries such as Cote d'Ivoire, Central African Republic, Gabon, and Zaire,

and are now reared successfully in large ranching schemes (ILCA, 1979; Murray, Trail and Grootenhuis, 1984; Shaw and Hoste, 1987). In eastern Africa, there is evidence that significance differences in resistance to trypanosomiasis occur among *Bos indicus* breeds, (Cunningham, 1966; Njogu *et al.*, 1985) with some of them considered to be trypanotolerant. However, in East Africa the extent of trypanotolerant breeds has not been adequately investigated, though recent studies reported by Mwangi, (1993) showed that in addition to the Orma cattle, Maasai Zebu cattle demonstrated the phenomenon of trypanotolerance. Further studies need to be carried out to assess the productivity of these breeds and their ability to thrive in different parts of tsetse-infested Africa.

2.3.4 EPIDEMIOLOGY OF TRYPANOSOMIASIS: KENYA COAST.

Although trypanosomiasis is endemic in most parts of the Coast Province of Kenya, the incidence of the disease varies from area to area depending on a number of factors which influence the distribution of the tsetse (Heckalau, 1986). One of the factors is the rapidly increasing human population, particularly in the vicinity of major urban centres, causing disruption of the tsetse habitat due to settlement (Heckalau, 1986). As a result, tsetse distribution along the coastal region is mainly limited to the sparsely populated regions, areas of and adjacent to the national game reserves and the remains of the original forest areas (Heckalau, 1986). *Glossina pallidipes, G. austeni, G. brevipalpis* and *G. longipennis* with *G. pallidipes* are the most common and widespread tsetse species throughout the region (Heckalau, 1986).

a) Prevalence of trypanosomiasis

In the high tsetse challenge area at Witu, Lamu District, relative tsetse densities during 1989 ranged from 53 to 163 flies/trap/ day, and the weekly

trypanosome prevalence ranged from 17% to 34% in Boran/Orma cattle (Lohr *et al.*, 1991). On a nearby ranch at Kipini, the weekly trypanosome prevalence ranged from 11% to 18% (Munstermann *et al.*, 1992). In these cattle, 60% to 75% of the parasitaemias were identified as *T. congolense,* just under 20% were *T. vivax* and rest were mixed *T congolense/T. vivax* infections (Lohr *et al.*, 1991; Munstermann *et al.*, 1992).

In the densely human populated areas along the coastal strip of Kilifi District, where *G. pallidipes* and *G. austeni* occur in varying intensities, a small isolated focus of *G. austeni* was responsible for transmitting trypanosomiasis in Ayrshire X Sahiwal X Brown Swiss crossbred dairy cattle managed on a ranch near the Kilifi creek. On this ranch, a routine chemotherapeutic strategy developed over the years consists of treatment of all cattle with PCV of 30% or less with a trypanocide. Although the trypanosome prevalence over the 9 months study period was 0.8%, 32% of the samples which had PCVs of 30% or less, were subsequently treated with either diminazene aceturate, homidium chloride or homidium bromide (Paling, *et al.*, 1987). In a sentinel herd of 20 cattle, exposed for 182 days on the same ranch, all cattle became infected with *T. congolense*; these infections were treated with diminazene aceturate (Paling *et al.*, 1987).

Further south of the creek, on a commercial veal producing ranch at Vipingo, *G pallidipes was* the only species trapped in the area (Dowler, Schillinger and Connor, 1989). In 1985, the monthly trypanosome prevalence ranged from 1.9 to 22% in the 3,000 Boran cattle which were regularly blood tested on the ranch. Those detected parasitaemic or considered as 'thin cows' were treated using isometamidium intravenously at 0.6mg kg^{-1} bodyweight (Dowler *et al.*, 1989).

Along the southern Kenyan coast, in Kwale District, trypanosomiasis was responsible for major losses in productivity. An outbreak of haemorrhagic

T. vivax infection in two dairy farms over a period of 2 months caused severe losses due to mortalities, when up to 30% of the herd on one farm died and about 60% of the cows aborted. On an investigation carried out on the farms, about 30% of the sampled cattle were positive for *T. vivax* on thin and thick blood smears (Mwongela *et al.*, 1981). In another study carried out from April 1988 to March 1989, weekly monitoring of the dairy herd on one of the farms gave a trypanosome prevalence ranging from 5 to 54% (22% overall mean), with most infections coinciding with the onset of rains. *Trypanosoma vivax* accounted for 79% of the infections, while the rest were *T. congolense* (13%) and mixed infections (8%) (Gaturaga, Maloo and Lohr., 1990).

A much lower trypanosome prevalence was observed in East African Zebu cattle kept in traditionally managed village herds at Muhaka, in the south coast of Kenya. In a baseline study, where health and performance of 700 Zebu cattle were monitored monthly, the trypanosome prevalence ranged from 2 to 8%. Infection rates of tsetse flies ranged from 2% to 10%, with a rise in the fly density just after the onset of rains (Maloo, Chema, Koskey. Kimotho, Trail and Murray, 1985). Although the tsetse density was relatively low, trypanosome infections were detected in all months indicating that transmission of the disease occurred throughout the 12 months study period (Maloo *et al.*, 1985).

b) Control of trypanosomiasis

Control of trypanosomiasis in the Province depends mainly on the use of trypanocidal drugs and the importance of the disease is reflected on the large quantity of trypanocides used to combat the disease (Anon, 1990b). Due to the varying degree of trypanosomiasis risk within the coastal belt, cattle are maintained either by chemoprophylaxis with isometamidium, by chemotherapy with curative trypanocides or by a combination of both.

Livestock owners keeping local Zebu cattle in low to medium challenge areas generally rely on curative treatments, though prophylactic cover using isometamidium is also used from time to time. In order to assess the effectiveness of a chemoprophylaxis and a chemotherapeutic programme in sedentary village East African Zebu cattle at Muhaka, Kenya Coast, 2/3 of the adult and 2/3 of the young stock out of some 700 cattle within 17 herds were treated with 0.5mg kg^{-1} isometamidium at three monthly intervals for a period of 30 months. Simultaneous collection of matching health and performance data every month showed that prophylactic cows had 39% fewer detectable trypanosome parasitaemias, required 64% fewer diminazene therapeutic treatments and gave 24% more extracted milk resulting in an overall increase in the productivity of 20% (Maloo *et al.*, 1988b). The increase in productivity achieved by the implementation of a chemoprophylaxis regime was found to be highly cost-effective (Itty, Chema, d'Ieteren, Durkin, Leak, Maehl, Maloo, Mukendi, Nagda, Rarieya, Thorpe and Trail, 1988), demonstrating the biological and economical advantages of delivering proper and acceptable control programmes for village cattle under trypanosomiasis risk.

In other situations as at Kilifi Plantations, an area of low tsetse challenge, a series of abortions storms observed were eventually associated with trypanosomiasis. To prevent future losses, a chemotherapeutic strategy was introduced whereby routine blood testing of the dairy herd was performed four to five times per year. Parasitaemic cattle or those with PCV less than 30%, were treated with diminazene (3.5mg kg^{-1}) or homidium (1.0mg kg^{-1}) This strategy was successful in resolving the trypanosomiasis problem (Wissocq *et al.*, 1983; Paling *et al.*, 1987). Using this control method it was possible to achieve an average lactation yield of 2833 kg and a calving interval of 402 days resulting in a mean annual milk yield of 2589 kg (cited by Murray and Trail, 1982).

When a therapeutic approach using diminazene aceturate failed to control trypanosomiasis on a beef ranch at Vipingo, a prophylactic regime of 1.0 mg kg^{-1} isometamidium was introduced. Intramuscular injection of isometamidium can cause severe tissue reactions at the injection sites if not properly administered. In order to prevent these reactions and also to control the "thin cow" syndrome attributed to chronic trypanosomiasis, isometamidium was given intravenously at 0.6mg kg^{-1} to individual trypanosome-infected cattle (Dowler *et al.*, 1989). By weekly blood testing and treating parasitaemic and suspected cases over a period more than two years, the number of trypanosome-infected cattle declined ten-fold, even though there was continued presence of tsetse. The early detection and treatment of infected cattle may have possibly led to the reduction of cattle reservoirs of trypanosome infections, consequently leading to reduced infection rates in flies, thus lowering the risk of trypanosomiasis (Dowler *et al.*, 1989; Munstermann, *et al.*, 1992).

Although the intravenous administration of isometamidium technique resulted in improved control of trypanosomiasis, implementation of this regime requires organised and well managed team. The need for a high standard of management will limit its application elsewhere (Dowler *et al.*, 1989).

In the higher challenge areas of Lamu District, despite routine chemoprophylactic treatments, effective control of trypanosomiasis could not be achieved. Thus, cattle in poor body condition with low PCV values were frequently observed on these ranches. On a large ranch at Kipini, supporting 4,500 beef cattle, a study, incorporating two groups of 50 Boran weaner cattle, was conducted to evaluate the efficacy of isometamidium administered at 1.0 mg kg^{-1} either intramuscularly as a prophylactic or intravenously to individual parasitaemic animal. Another group of 50 weaners kept as controls were

treated with diminazene on detection of trypanosome infection (Munstermann *et al.*, 1992). Curative intravenous application of isometamidium at 1.0mg kg^{-1} appeared to be superior to the same dosage given as an intramuscular prophylaxis. Besides reduction in drug costs and over 30% higher weight gains in the 30 week study period, the weekly infection rates in the intravenous treated group declined over time even though the tsetse catches were constantly high.

Of concern, using this approach is whether there might be development of toxicity following prolonged use. Since acute systematic reactions, although transient, do occur following intravenous administration (Schillinger, Maloo and Rottcher, 1985; Dowler *et al.*, 1989), caution is necessary with this route. Moreover, the long-term effects of intravenous application of isometamidium are unknown.

These studies concluded that trypanosomiasis control was difficult to achieve regardless of the method used. The high tsetse challenge and the probability of drug resistant strains could account for the detection of early breakthroughs observed in the prophylactic group (Munstermann *et al.*, 1992).

Evidence of drug resistance to *T. vivax* isolates from coastal regions Kenya and Somalia has been reported by Schoenfeld, Rottcher and Moloo, (1987), while drug resistance to *T. congolense* isolates collected from Shimba Hills Settlement Scheme, Kwale District has been described by Gitatha, (1979).

In some areas of high tsetse challenge in the Coast Province, Kenya, there appears to be increasing evidence of failure to control of trypanosomiasis by use of trypanocidal drugs (Gitatha, 1979; Rottcher and Schillinger, 1985; Schoenfeld, *et al.* 1987; Dolan *et al.*, 1992; Munstermann *et al.*, 1992), and with no prospects of new animal trypanocides becoming available in the near future, alternative control measures preferably directed against the vector,

need to be tested and adopted. Such attempts have been made on Galana Ranch, where the use of odour-baited deltamethrin impregnated targets significantly reduced populations *Glossina* species in the suppression zone (Opiyo *et al.*, 1987). Recently, the use of synthetic pyrethroid pour-on preparations on beef cattle under high tsetse challenge areas of Galana and Witu resulted in significance decreases in the incidence of trypanosomiasis and in tsetse catches (Stevenson *et al., in press*; Lohr *et al.*, 1991).

Due to the varying risk of trypanosomiasis on the Kenya coast, no single control programme can resolve the problem of trypanosomiasis. In areas where the risk is high, effective control can only be achieved with an integrated approach where combinations of suitable and cost-effective methods targeted towards both the vector and the parasite need to be implemented. However, in low to medium challenge areas strategic intervention with trypanocides in individual cattle or on herd basis can control trypanosomiasis. For any effective control, the epidemiology of the disease must be understood at the local or regional level.

2.4 OTHER DISEASES

Although tick-borne diseases and trypanosomiasis are considered to be major constraints to increased productivity in most parts of sub-Saharan Africa, other diseases, such as, rinderpest, contagious bovine pleuro-pneumonia (CBPP), foot-and-mouth disease and brucellosis are also of significance. As production systems intensify ,e.g., in small-holder dairy units, other diseases and conditions become increasingly apparent. Such diseases include helminthiasis, mastitis, neonatal diseases and nutritional deficiencies. (Walshe, Grindle, Nell and Bachmann, 1991). This review briefly describes the diseases which are likely to be of particular importance in small-holder dairy production systems in coastal Kenya.

Brucellosis in cattle caused *by Brucella abortus*, results in abortions in late pregnancy and a subsequent high rate of infertility. Besides causing economic losses in cattle through loss of calf and the subsequent lactation, the disease give rise to serious humans health problems. It is thought that the incidence of disease can be expected to increase with intensification of dairying (Walshe *et al.*, 1991). Control can be effectively carried out using a relatively low-cost vaccine. In Kenya, brucellosis is controlled by vaccination, which is mostly carried out in dairy cattle and in improved beef herds.

Foot-and-mouth disease is endemic in most parts of SSA (Walshe *et al.*,1991) and has a major impact on milk production. In Kenya, the disease is controlled by twice a year vaccination in the compulsory areas; in other non-compulsory areas the disease is contained by ring vaccination in the face of an outbreak (Anon 1991a). On the Kenya coast, small-holder dairy farmers are advised to vaccinate their herds bi-annually.

Helminthiasis and coccidiosis are more of a serious problem in small-ruminants (Allonby and Urquhart, 1975), although these diseases can be important in calves and young stock. In Kenya, helminthiasis is believed to be widespread (Allonby, 1975; Gatongi, Gathuma and Munyua, 1987; Omara-Opyene, 1985; Ndarathi, Waghela and Semenye, 1989; Maingi and Gichigi, 1992). However, there are limited epidemiological studies describing the prevalence, distribution and epidemiology of helminths in cattle and other domestic animals. Studies have been carried out in the highlands (Maingi and Gichigi, 1992; Gatongi *et al.*, 1987) and in the semi-arid areas of Kenya (Omara-Opyene, 1985). In these two different ecological zones, strongylid larvae were found to be the dominant helminth parasites. This information is of importance in the formulation of parasite control strategies. At present, control depends on the use of anthelmintics, particularly in dairy cattle belonging to small-holder farmers.

Epidemiological data on helminthiasis in coastal Kenya are the restricted to reports from the Kenya Veterinary Department. Therefore there is a need for studies to assess the importance of helminthiasis in cattle, particularly in dairy cattle, as the effects of helminth infections can contribute significantly towards economic losses.

In many parts of East Africa, particularly in the high agricultural potential areas of Kenya, majority of the milk is produced by the small-holder dairy farmer (Mbogoh, 1984). As the intensified production system is adopted, occurrence of acute and subclinical mastitis becomes widespread, particularly in the small-holder sector (Walshe *et al.*, 1991). The predisposing factors, include, poor hygiene, inadequate housing and most important of all lack of knowledge about the cause and importance of regular monitoring for mastitis. Control depends largely on effective education of farmers regarding hygiene and milking/suckling methods. These, supplemented by use of suitable antibiotics can be effective in treatment of clinical mastitis. In less intensive systems, partial suckling by calves helps to reduce the incidence of mastitis (Walshe *et al.*, 1991).

In conclusion, a variety of diseases contribute towards animal health constraints. The possibilities and mechanism of control of these diseases vary considerably. Vaccination against the principle infectious diseases such as rinderpest, CBPP, is necessary to prevent fatalities; vaccines are available, but requires operational funds for mass vaccination campaigns. In small-holder dairy herds in coastal Kenya, vector-borne parasitic disease form one of the major biological constraints for improved dairy production. In general, prevention of tick borne diseases depends on effective control of ticks using acaricides. Control of tsetse-transmitted trypanosomiasis relies upon use of trypanocidal drugs. However, because of managemental problems in implementing control programmes, including, the high cost of curative and

prophylactic drugs, effective control of disease in the field is limited. Therefore, alternative approaches need to be developed, tested and implemented.

Before such interventions are carried out, it is important to understand the epidemiology of these vector-borne disease at local, and at regional levels. It was against this background that methodologies were designed in my thesis studies towards a systematic approach for identification and control of the major vector-borne diseases effecting small-holder dairy production systems.

CHAPTER THREE

OBJECTIVES OF THE THESIS

3.1 GENERAL OBJECTIVES

The research reported in this thesis is a series of investigations to identify and quantify major disease constraints limiting small-holder dairy production in coastal lowland Kenya, and to develop possible control methods for the major diseases. The general objectives of the epidemiological research were therefore:

1. to determine the major diseases affecting small-holder dairy production,
2. to assess current control methods,
3. and where necessary, to test and deliver alternative methods to reduce the effect of the major diseases on the production of small-holder dairy cattle, and
4. through these studies to develop and test a systematic approach to epidemiological and control methods for the small-holder sector.

3.2 SPECIFIC OBJECTIVES

To achieve these general objectives, the series of epidemiological studies and experiments were carried out with dairy cattle in small-holder and research herds. The specific objectives of the individual studies were as follows:

1. To identify the major diseases and factors affecting their prevalence in small-holder dairy herds,
2. To estimate disease incidence and case-fatality rates, and factors influencing them in small-holder herds,
3. To evaluate the efficacy of current control methods, and
4. To define target populations for methods aimed at controlling the major diseases affecting small-holder dairy production.

The first objective was addressed in a cross-sectional study of disease prevalence and is reported in Chapter 5.1. The second objective, the estimation of disease incidence and case- fatality rates, was achieved through a longitudinal cohort study described in Chapter 5.2. Studies to identify target

populations for the control of East Coast fever are presented in Chapter 6, while in Chapter 7 a field study and an experiment are reported which assessed the efficacy of chemoprophylaxis for controlling trypanosomiasis in dairy cattle.

Chapter 8, discusses the systematic epidemiological approach developed and tested through this research for identifying and resolving disease constraints to small-holder dairy production and reviews the results of the research in relation to preventive medicine programmes for small-holder dairy production.

CHAPTER FOUR

GENERAL MATERIALS AND METHODS

4.1 INTRODUCTION

Various field studies and experiments form the research reported in this thesis. Many of the same materials and methods are relevant to each of the experiments. This chapter therefore, reports those materials and methods common to two or more studies/experiments. It also describes the agro-ecological zones and farming systems, including the cattle management practices in the high rainfall coastal lowland (CL) and of the specific experimental sites. Finally, detailed descriptions are given of the sampling and diagnostic methodologies.

4.2 AGRO-ECOLOGICAL ZONES

In Kenya, the coastal lowland region lies between latitudes 0 - 4 ^{o}S and longitudes 38 - 41 ^{o}E stretching inland from sea level and rising to about 300 m above sea level. It is classified into three major agro-ecological zones (AEZ) based broadly on the climate, soil type and number of plant growing days of leading crops (Jaetzold and Schmidt, 1983) (Table 4.1).

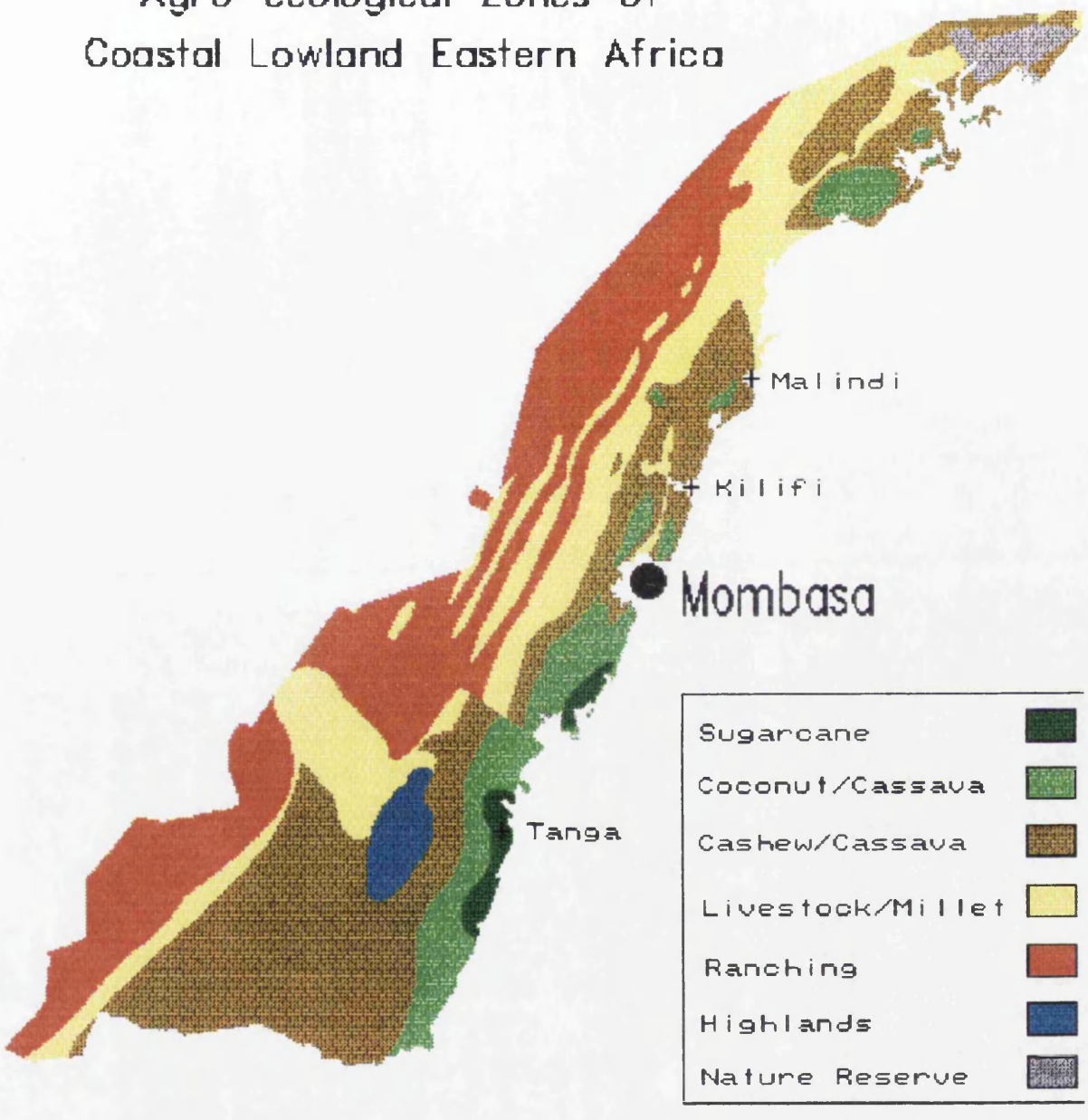
These semi-humid (coconut-cassava, CL3), transitional (cashew-nut-cassava, CL4,) and semi-arid (livestock- millet, CL5) AEZs extend southwards from the Kenya/Somalia border through Tanzania into Mozambique an area of approximately 40,000 km^{2}. They also cover all or part of the Indian Ocean islands. The region is characterised by marked similarities in climate, soils and farming systems, particularly in Kenya and Tanzania (Figure 4.1).

Mean annual rainfall in the three AEZs is between 800 and 1,350mm, generally distributed bimodally. Rainfall is highly variable, both in annual quantity and seasonal distribution; the first rainy season from April to June, with the less reliable second rainy season from October to November (Table 4.1). Mean monthly maximum temperatures range between 28^{o}c and 33^{o}c

Table 4.1 Agro-ecological zones and the range of average annual rainfall (mm) with 60% probability in the two rainy seasons for an area in coastal lowland Kenya (Kilifi District) (modified from Jaetzold and Schmidt, 1983)

Agro-ecological zone	Average annual rainfall (mm)	60%- probability rainfall (mm) 1st rains	2nd rains
CL3 Coconut-cassava	1000-1230	400-800	50-220
CL4 Cashew-nut-cassava	800-1100	200-600	50-180
CL5 Livestock-millet	700-880	170-270	150-170

Figure 4.1 The agro-ecological zones of coastal lowland of eastern Africa


Agro-ecological Zones of
Coastal Lowland Eastern Africa
Malindi
Kilifi
Mombasa
Tanga
Sugarcane
Coconut/Cassava
Cashew/Cassava
Livestock/Millet
Ranching
Highlands
Nature Reserve

and mean monthly minimum temperatures from 14°C to 21°C. Relative humidity is generally high, ranging from 60 - 80% throughout the year.

The soils of the coastal belt show a very wide range of depth, physical properties, including organic matter content, and soil fertility. In general, soils are mainly sandy, free draining and low in organic matter.

4.3 STUDY AREAS

Epidemiological studies and experiments reported in this thesis were carried out in three different sites in Kilifi and Kwale Districts, Coast Province, Kenya (Figure 4.2). The agro-ecological zones of the two districts are shown in Figure 4.3. The first site was Kaloleni Division, Kilifi District, the second in Kwale District, and the third was at the Regional Research Centre (RRC), Mtwapa. These study areas were within a radius of 50 km from the urban town of Mombasa. Table 4.2 gives an overview of the studies carried out in the respective areas. A cross-sectional study was first carried out from July to September, 1989 in Kaloleni Division involving small-holder cattle herds in three AEZs. This was followed by a longitudnal study over a 19 month period with small-holder dairy herds in the coconut-cassava AEZ. In addition, two experimental on-farm and on-station studies were undertaken using cross-bred dairy calves and local Zebu cattle.

At the same time two longitudinal studies were carried out, one an on-farm with small-holder dairy herds in Kwale District, and the other using an on-station dairy herd belonging to a research centre at Mtwapa. The objectives of the specific studies are described in Chapters 5, 6 and 7.

4.3.1 KALOLENI

Kaloleni Division is one of six administrative Divisions of Kilifi District. It lies between longitude 39° 25' - 39° 35'E and latitude 3° 40' - 3° 58'S covering

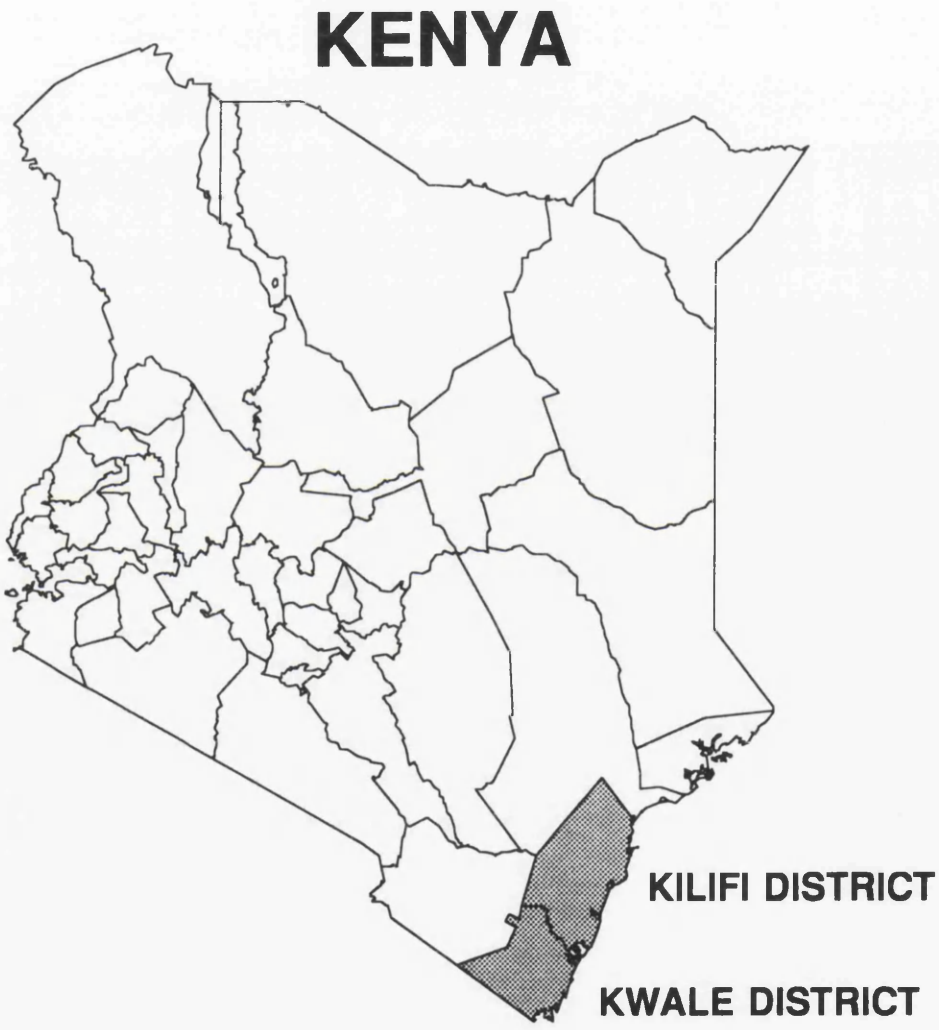


Figure 4.2 Administrative districts of Kenya showing Kilifi and Kwale Districts

Figure 4.3. Agro-ecolocical zones of Kilifi and Kwale Districts

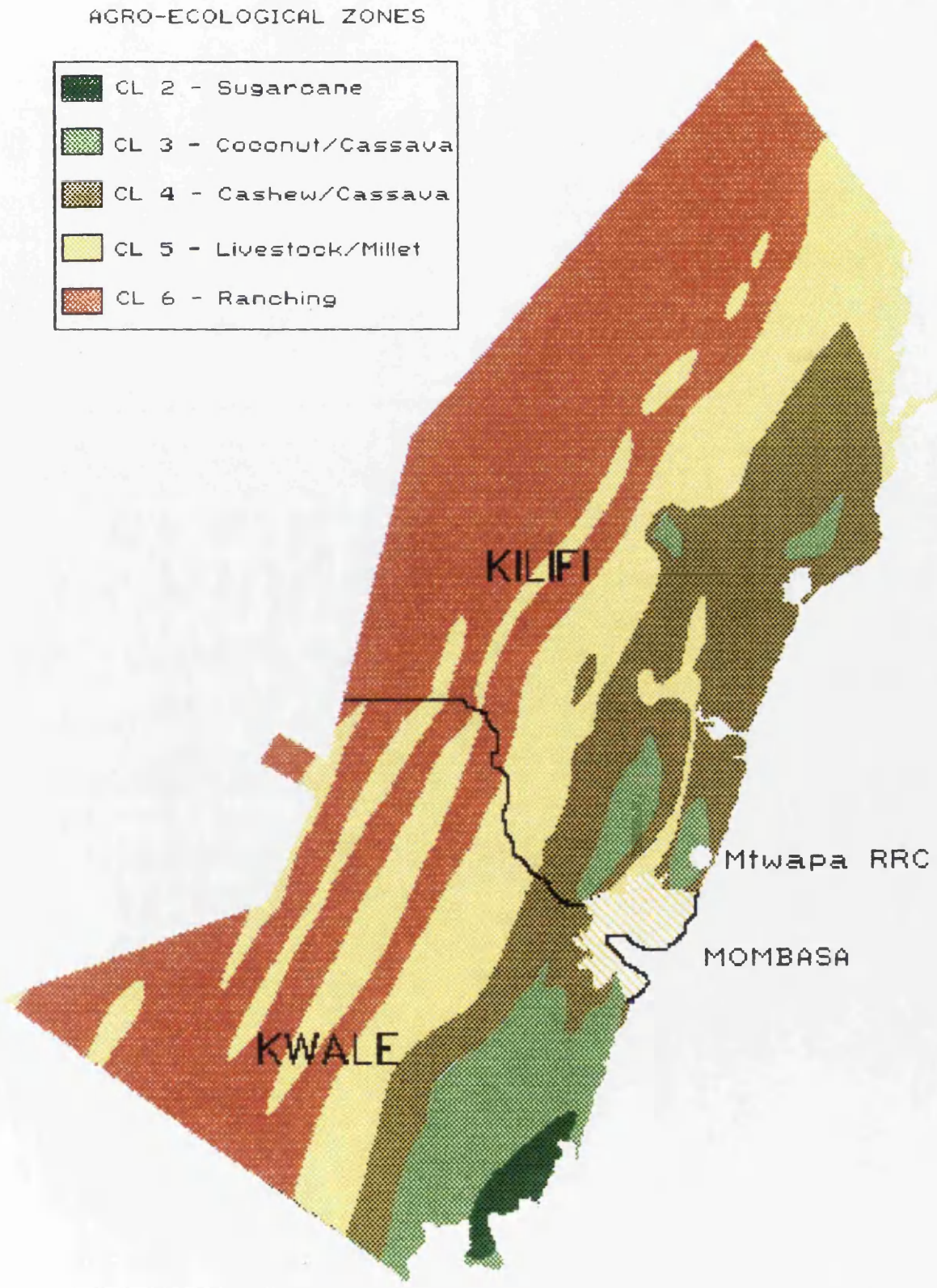

AGRO-ECOLOGICAL ZONES
CL 2 - Sugarcane
CL 3 - Coconut/Cassava
CL 4 - Cashew/Cassava
CL 5 - Livestock/Millet
CL 6 - Ranching
KILIFI
Mtwapa RRC
MOMBASA
KWALE

Table 4.2 Design of epidemiological studies and experiments carried out in coastal lowland, Kenya

Study area	Epidemiological study design	Study animals	AEZ*	Time-period
Kaloleni	Cross-sectional	Small-holder cattle herds	CL3, CL4 and CL5	July - September 1989
	Longitudinal	Small-holder dairy herds	CL3	June 1990 - December 1991
	Experimental (on-farm)	Sentinel calves	CL3	May1990 - June 1991
	Experimental (on-station)	Zebu/ Cross-bred calves	CL5	November 1990
Kwale	Longitudinal	Small-holder dairy herds	CL3	June - December 1990
Mtwapa	Longitudinal (on-station)	Dairy herd	CL3	June 1990 - December 1991

* Agro-ecological zones

approximately 750 km^2 at an altitude of 100 - 250 m above sea level. The Division has three AEZs as shown in Figure 4.4.

Mean annual rainfall ranges from 1,000 - 1,200 mm in the wetter areas of CL3 to 700 - 900 mm in the drier zone of CL5. Rainfall generally decreases from the coastline towards the interior. The distribution of the rainfall is bimodal, with the long rainy season from April to July and the short rains falling in October and November. The second rainy season is unreliable, especially in the drier areas.

Natural vegetation varies from lowland woodland dominated by *Brachystegia* and *Afzelia* species in the drier areas to lowland forests of *Sterculia, Chlorophora* and *Memecylon* species in the wetter areas (Moomaw, 1960). Deforestation of the lowland forest, due to increasing human population pressure in the semi-humid zone has limited the forested areas to a few 'sacred sites' (Kayas) and protected forests.

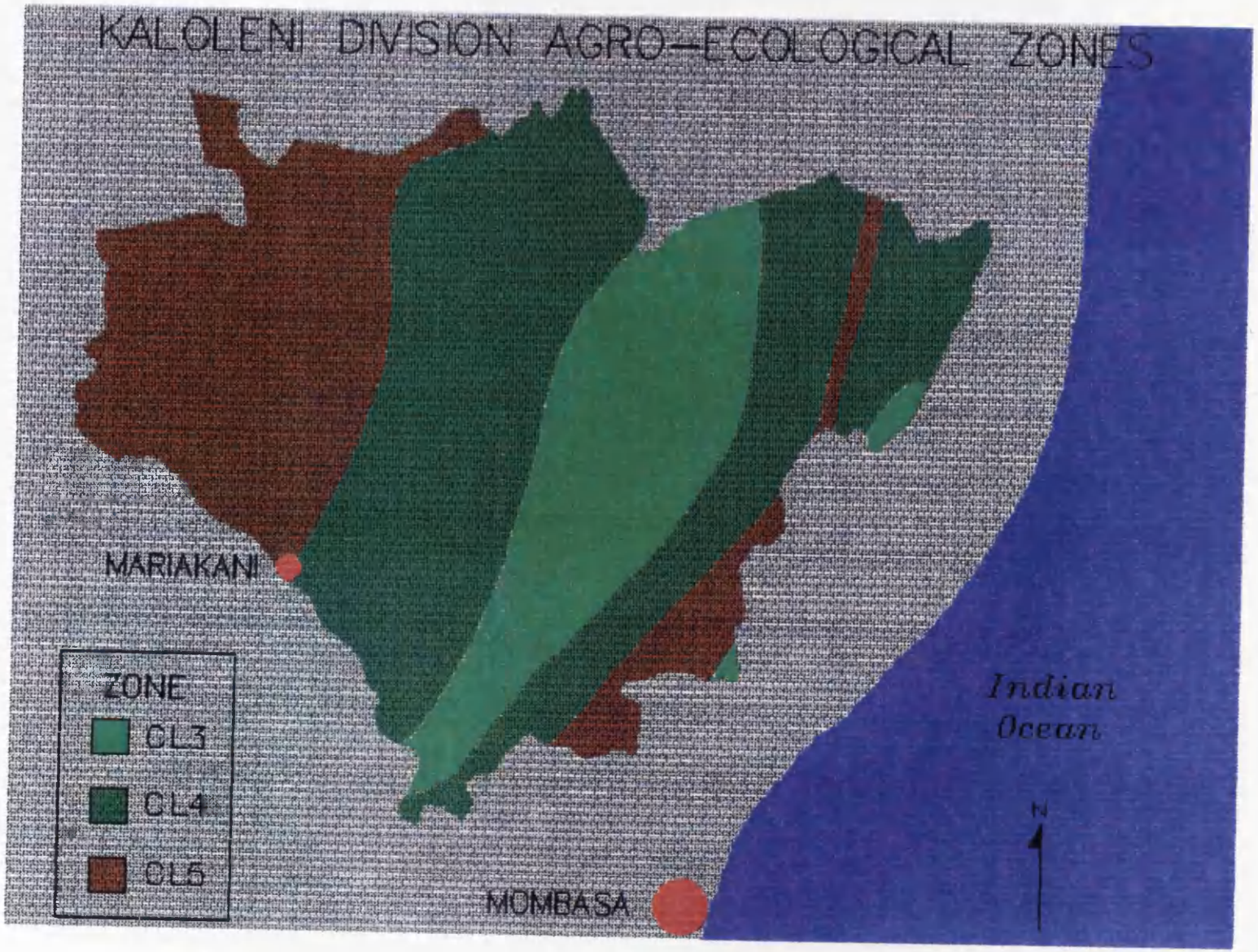
The Division is generally thought to be devoid of the bigger wild game. The forested areas, however, serve as the main habitats of the few remaining wildlife: suids (mainly wild pigs), small antelopes (dik dik and duikas), baboons and monkeys.

Local inhabitants comprise of seven of the nine distinct tribes of the Mijikenda ethnic group. These tribes are the Giriama, Chonyi, Jibana, Kambe, Kauma, Ribe and the Rabai, who by tradition are agro-pastoralists.

a) Farming systems

Subsistence crop agriculture forms the main activity of the people residing in CL3 and CL4. A farming systems survey of some 1,800 small-holder households in CL3 and CL4 in Kaloleni showed that 35%, of the households had no ruminant livestock and less than 20% of the households kept any cattle (Thorpe, *et al.*, 1993). The major land use practices depended on cropping

Figure 4.4 Agro-ecological zones of Kaloleni Division, Kilifi District


KALOLENI DIVISION AGRO-ECOLOGICAL ZONES
MARIAKANI
MOMBASA
ZONE
CL3
CL4
CL5
Indian
Ocean
N

systems which were based on cash tree crops, predominantly coconut and cashew-nut. Subsistence staples, maize and cassava, and other food crops, peas, beans and rice were grown by shifting cultivation. Intercropping was commonly practiced. Most farmers did not use any form of fertiliser or manure on their subsistence crops.

In general, household families were large, averaging 10.8 per household with over 70% of the residents being permanent members of the household. Despite the large number in the family, household plot sizes were small, the mode being 2 ha with 75% being 6 ha or less. However, ownership of one or more additional nearby plots was reported by nearly two thirds of the households.

Off-farm income has become increasingly important as pressure on land has increased due to high human population density and decline in agricultural productivity. In a farming systems survey, over 40% of the households reported having off-farm income (Thorpe *et al.*, 1993).

On the other hand, in the semi-arid zone (CL5), the traditional livestock farming forms the main enterprise. This was reflected in a livestock census carried out during 1989 in Kaloleni Division (Thorpe *et al.*, 1993).

b) Livestock population

In order to describe the livestock production sub-systems in the three agro-ecological zones, a census of all cattle herds and associated small-ruminant flocks was carried out in the Division (Thorpe *et al.*, 1993). In this thesis, only information on cattle is reported.

The census recorded over 57,500 head of cattle in 1,552 herds, of which more than half were found in the semi-arid livestock-millet zone (Table 4.3). The dominant breed in all the three AEZ was the small East African Zebu

Table 4.3 Total cattle population, the proportion which were dairy cattle, and the number of herds and the proportion of herds with dairy cattle by agro-ecological zone in Kaloleni Division

Agro-ecological zone	Percent total area	No. of cattle	Proportion which were dairy cattle	No. of herds	Proportion of herds with dairy cattle
Coconut-cassava (CL3)	17	7,345	0.062	491	0.175
Cashew-nut- cassava (CL4)	47	20,780	0.013	608	0.095
Livestock-millet (CL5)	36	29,391	0.006	453	0.029
Total	100	57,516	0.015	1552	0.101

with dairy cattle comprising only 1.5% (889) of the total population. Only 157 herds, amounting to 10% of all herds, were recorded as having dairy cattle.

Dairy refers to either *Bos taurus* cattle (mainly Ayrshire, Friesian, Guernsey and Jersey breeds), or the crosses of these breeds with the *Bos indicus* Sahiwal breed, indigenous Zebu, or Boran. Although the number of herds having dairy cattle were less than 3% in CL5, the proportion of herds in CL4 and CL3 having all or some dairy cattle increased to 10% and 17%, respectively.

Herd size varied by herd type and AEZ with the mean of the Zebu herds increasing four-fold from 15.6 in the CL3 to 65.6 in CL5. As for 'mixed' herds, where both Zebu and dairy cattle (mainly Zebu-*Bos taurus* crosses) were present in a herd, similar increases in the mean herd size of Zebu component were observed (Table 4.4).

Generally dairy herds were small, with over 90% having 10 or fewer cattle. Although the herd structures of both the dairy and Zebu herds were similar, the dairy herds had twice as many lactating as compared to dry cows, whilst in the Zebu herds the proportions were reversed. The majority of the farms (84%) keeping exclusively dairy cattle reported having purchased some of their animals in their herds, but only 34% of the mixed herds had bought any dairy cattle. Most purchases were females (94%) and over half of them were supplied by a large commercial dairy ranch in Kilifi District.

In the census, there were various dairy breeds; a third of the dairy cattle were recorded as either Sahiwal X Ayrshire crossbreds with the Friesian, Guernsey, Jersey and Brown Swiss breeds represented in decreasing proportions. Figure 4.5 shows some examples of the dairy cattle found in the area.

Table 4.4 Mean herd size of herds classified by herd type and agro-ecological zone in Kaloleni Division

Agro-ecological zone	Herd type			
	Zebu	Zebu + Dairy (Mixed herd)		Dairy
Coconut-cassava (CL3)	15.6	11.5	3.9	7.2
Cashew-nut-cassava (CL4)	36.0	20.6	4.0	5.3
Livestock-millet (CL5)	65.6	43.3	9.2	22.5*
Total	39.4	17.9	4.5	7.4

* the mean herd size is inflated due to inclusion of a large dairy herd belonging to a research centre.

Zebu cattle are the local Small East African Zebu

Figure 4.5 The commonly observed cross-bred dairy cattle belonging to small-holder farmers in Kaloleni

Jersey/ Zebu F1 cross

2/3 Ayrshire 1/3 Sahiwal cross

About 96% of the Zebu herds were grazed on natural pasture by herders. Of those herds managed otherwise, for example by tethering, most were in the coconut-cassava (CL3) and the cashew-nut-cassava zones (CL4).

As for the dairy herds, 23% of them were kept in zero-grazing systems, concentrated in CL3 and CL4, whilst the rest were managed as free-and semi-zero grazing herds (Table 4.5).

c) Management practices of dairy herds

This section describes the grazing management systems, breeding and animal husbandry and health practices.

i) Grazing systems

a) Zero-grazing

The zero-grazing management system was introduced into Kilifi District in 1981 by the National Dairy Development Project (NDDP) of the Ministry of Livestock Development (MOLD). By 1989, this management practice had been adopted in Kaloleni Division by 22 farmers, over 90% being in CL3, where the increasing human population has resulted in limited grazing land and created a high demand for milk. Figure 4.6 shows feeding management practice of dairy cattle in a zero-grazing unit built by small-holder farmers.

In this system, cows were confined in stalls, fed on cut and carried natural pasture or cultivated fodder (usually Napier grass), and supplemented with maize bran and copra cake concentrates. For the small-holders, stalls were designed to accommodate three to five cows with a resting place, a calf pen with a slatted floor, and a concrete floor walking area adjacent to the feeding and watering troughs. Faeces and urine from the concrete floor were collected in a pit. It was recommended that the slurry be used as fertiliser for the Napier grass. Generally cultivated fodder was not available all the year

Table 4.5 Number of herds with dairy cattle in zero, semi-zero, and free range grazing system by herd type and agro-ecological zone in Kaloleni Division

Agro- ecological zone	Herd type	Grazing system			Total
		Zero	Semi-zero	Free	
Coconut-cassava	Dairy	15	13	13	41
(CL3)	Mixed	6	-	39	45
Cashew-nut- cassava	Dairy	1	13	12	26
(CL4)	Mixed	-	4	28	32
Livestock-millet	Dairy	-	2	-	2
(CL5)	Mixed	-	1	10	11
Total	Dairy	16	28	25	69
	Mixed	6	5	77	88
Proportion of total		0.14	0.21	0.65	

Figure 4.6 Cut and carry feeding practices for cattle managed in a zero-grazing unit built by a small-holder farmer


AVA

round. Therefore, during the dry spells, most farmers fed their animals on natural pasture, harvested either from nearby fallow land or along roadsides.

Supplementation with concentrates such as maize bran and or copra cake was a common practice. Animals were watered in the unit, with the principal water source being piped (reticulated) supply or well water. On a number of occasions, water from the regular source was unavailable, requiring farmers to seek alternative means of water supply (rivers, or distant wells) with resultant increased labour costs.

b) Free- and semi-zero- grazing

Two thirds of the dairy herds in CL3 and CL4 were kept in the free-and semi-zero-grazing systems (Table 4.5). In these systems, herds generally grazed on fallow or communal lands near the river banks and roadsides under the supervision of herdsmen. A few were left to graze in paddocks. Tethering was occasionally practiced especially when herders were unavailable. Grazing time varied depending on season. In the dry season, when pasture were scarce (Figure 4.7), animals trekked long distances, in some cases, over 5 km, in search for alternative grazing lands as well as water. In the wet season, grazing was usually available nearby, as long as the plots weren't under crop cultivation. The grazing of dairy cattle in the wet season pasture is shown in Figure 4.8.

Management of semi-zero herds was very similar to the free-grazing herds except they were generally supplemented after grazing with cut and carry fodder (Thorpe *et al.*, 1993).

Water supply for dairy herds was usually from nearby piped or well water; however, animals had to travel to distant water sources such as permanent rivers and streams in the dry season and when the regular supply became scarce.

Figure 4.7 Scarcity of grazing pastures in the dry season in Kaloleni Division

Figure 4.8 Wet season grazing pastures in Kaloleni Division

At the time of the cattle census (mid 1989), supplementation with concentrates was practised by half of the exclusively dairy herds and a quarter of the mixed herds. Concentrates used were mainly maize bran and/or copra cake.

ii) Breeding

During the time of the research studies, breeding in most dairy herds was carried out by artificial insemination (AI). The AI service, using deep-frozen semen, was provided by the MOLD operating through the District Veterinary Office, Kilifi. The AI vehicle followed a designated route traversing the Division, and farmers whose cows required inseminations were advised to wait along the route. A selection of deep-frozen semen of dairy breeds, usually Ayrshire, Friesian, Jersey and Guernsey, was generally available for insemination. In most instances, farmers relied on the inseminators to advise them of the most suitable breed to use. The AI run, as it was usually referred to, was supposed to operate on a daily basis, but due to operational problems the service was erratic.

Due to the unreliability of the AI service, a number of farmers, particularly those managing their herds in the free-grazing system reverted to keeping breeding bulls. These bulls were either reared from calfhood on the farm or occasionally bought from other farms. Renting of bulls for service was not a common practice. There was a preference for cross-bred bulls by farmers possessing mixed herds where the majority of cattle in the herd were of the Zebu type.

iii) Animal husbandry and health practices

This section describes husbandry and recommended health practices generally carried out by farmers during the course of the epidemiological studies.

In the zero-grazing management system, female calves were bucket fed on whole milk twice a day until weaning at 10 to 12 weeks of age. At this time, the calves were introduced gradually to roughages and they adjusted easily to feeding on fodder or pasture. In most cases, male calves were disposed soon after birth, but occasionally they were reared for sale as young breeding bulls or fattened as steers for meat. There was a tendency to separate post-weaner males and females from their dams and they were quite often tethered outside the unit, particularly when there was insufficient space or other if cows showed hostile behaviour. Heifers, in most cases, were mated using AI when above two years of age.

In the free grazing herds, calves suckled their dams on milk which remained after the extraction of the morning and evening milk for human consumption. In general, calves were usually tethered nearby, but separated from the kraal (boma) where the adults were kept overnight. There was a tendency to raise bull calves for breeding purposes. The age at first breeding of heifers varied between two to three years depending on individual farm management practises.

In most circumstances free-grazing dairy herds were herded preferably as single herds so as to avoid contact with other dairy, and particularly the indigenous Zebu herds. However, segregation of the herds at watering points in the dry season and at the communal dips was impossible.

In the Division, the most common method of tick control was by the application of organophosphate-based acaricide. Cattle kept in the zero-grazing system were sprayed with acaricides using hand operated pumps. This practice was recommended by the Veterinary Department to be carried out once every week. With the help of the field veterinary staff, farmers practicing zero-grazing were shown the correct application technique. Acaricides were purchased mainly from chemists and agricultural stores from the urban town of

Mombasa, some 40 km from the Kaloleni trading centre, or when available from the local Veterinary Department drug store. The most widely used acaricide was 30% w/v quintiofos (Bacdip^{R}, Bayer, Germany) which was recommended to be diluted in water at 1:1400 before application.

In the case of free-grazing herds, the recommended tick control was mostly by weekly acaricidal application through the use of communal plunge dips. During the study a number of communal dips were non-functional, thereby forcing nearby patron farmers to switch to hand spraying. Several factors have contributed towards the failure of dips to function effectively. These include, inadequate supply of water, failure of the Veterinary Department to supply acaricides, mismanagement of acaricides and occasionally lack of supervision.

As a move towards privatisation of certain veterinary services, the responsibility and management of dips have been passed, since July 1991, to farmers, to be run by a committee under the guidance of the Veterinary Department.

Helminth control was more often practiced in zero-grazed dairy herds where regular anthelmintic treatments were recommended to be carried out every 2 months in young stock and every 6 months in adult cattle. In contrast, deworming practices in free-grazing herds varied. Some dairy herds were drenched with anthelmintic routinely, whereas in other herds, animals were treated upon clinical diagnosis of helminthiasis. Dewormers were purchased from the same sources as acaricides. The most widely used anthelmintic was 1.5% w/v levamisole, 3% w/v oxyclozanide with 0.382% cobalt sulphate (Nilzan Plus^{R}, Coopers, Kenya, Limited) at a recommended dosage of 5ml/20 kg bodyweight.

Control of trypanosomiasis in most areas of CL3 and CL4 was mainly by the use of therapeutic trypanocidal drugs. Occasionally a few farmers in

CL4 and CL5, used chemoprophylaxis on their dairy herds, particularly towards the start of the rainy season. Diminazene aceturate (Berenil^{R}, Hoechst, Germany), given mostly at 3.5 mg kg^{-1} and isometamidium chloride (Samorin^{R}, RMB, UK) at 0.5 mg kg^{-1} were used as curative and prophylactic drugs, respectively. According to the veterinary legislation, these drugs were only available through the Veterinary Department.

Annual vaccination against rinderpest was carried out by the Veterinary Department covering the whole Division. Although the Division was not a compulsory area for vaccinations against foot-and-mouth disease, the NDDP recommended that all zero-grazing herds should to be vaccinated annually. Zero-grazed herds were also vaccinated against blackquarter and anthrax. During the study period, an outbreak of lumpy skin disease occurred in the area and most dairy herds were immunised.

With the exception of hand spraying with acaricides and occasionally deworming of cattle done by the farmer themselves, most other animal health services were carried out by the veterinary personnel of the MOLD.

d) Animal health services.

This section describes the animal health services provided by the Divisional Veterinary Office at Kaloleni.

Disease control, meat inspection and clinical services formed the main components of animal health services which were coordinated and supervised by the divisional Veterinary Officer (VO). Most of the field activities were carried out by the Livestock Officer (LO) and the Animal Health Assistants (AHA), who had been trained in such disciplines as vaccination and chemoprophylaxis procedures, dip management, simple diagnostic and treatment methods, and meat inspection.

Beside the annual rinderpest vaccination campaign, the other main disease control activity were the supervision of the control of tick-borne disease by the use of acaricides. Widespread delivery of tick control depended on the regular dipping of cattle through the use of functional communal cattle plunge dips. However, of the 21 dips constructed in the early 1970's, with the help of European Commission (EC) grant to the Veterinary Department, only two thirds of them were operational at the time of the studies reported here. Figure 4.9 shows the distribution of the active and inactive communal dips.

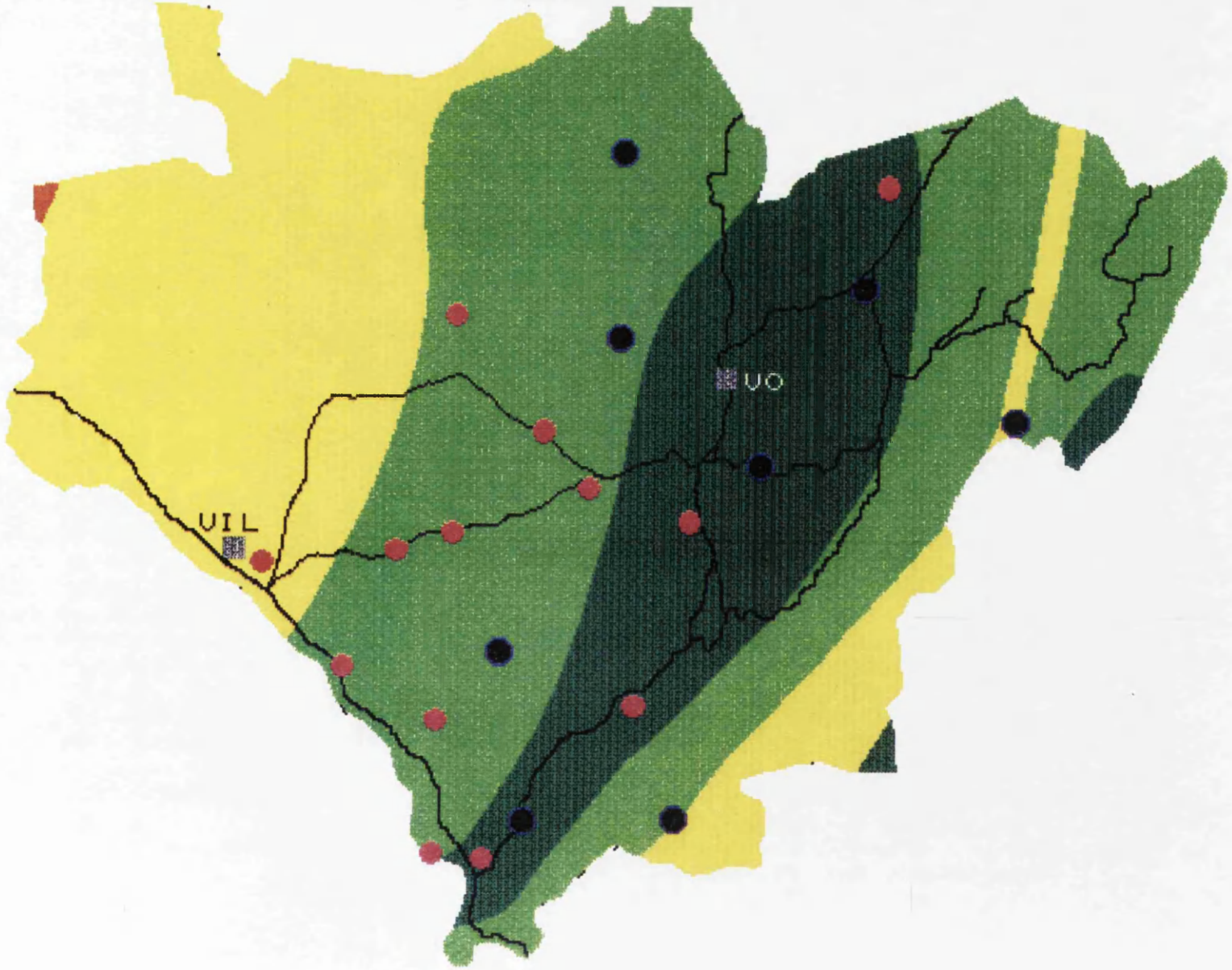
A nominal fee was charged for the clinical services provided by the Veterinary Department. During the study period, it ranged from 10 to 30 KShs. In addition, farmers had to pay for drugs used for each treatment. In areas where there was a high concentration of dairy herds, one LO and six AHAs provided the necessary animal health care. In the case of Zebu herds, most animal health practices, at individual or herd level, were delivered at places where animals congregated, such as the communal dips.

One of the major factors limiting effective delivery of health services was the lack of mobility of these field staff as a result of insufficient transport (Anon, 1991a). Occasionally, farmers had to call private veterinary practitioners from Mombasa, some 40 km away, to attend to clinical cases.

e) Diagnostic facilities

The Veterinary Investigation Laboratory (VIL), Mariakani, has the mandate to serve the Coast Province of Kenya. It provides facilities for the field diagnosis of most livestock diseases. From all over the Province, specimens are submitted by veterinary personnel, veterinary practitioners and by the farmers. As the laboratory was located in Kaloleni Division, it provided an excellent opportunity for the divisional field staff to carry out confirmatory diagnosis, particularly of major vector-borne protozoan diseases, and even submit

Figure 4.9 Location of functional and non-functional cattle dips in Kaloleni Division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carcasses for post-mortem examination. Beside confirmation of disease, the VIL staff can also be called upon to carry out epidemiological investigations in the event of serious outbreaks of disease at the farm or regional level. As well as testing biological samples, the laboratory was equipped to carry out chemical analysis of acaricide concentrations in the dip wash.

The VIL facilities were extensively used by the author and his team in performing parasitological diagnostic tests from samples collected from the study animals in Kaloleni Division.

4.3.2 KWALE

This was the second study area where small-holder dairy cattle raised in zero-grazing units were monitored for trypanosomiasis following chemoprophylaxis using 0.5 mg kg^{-1} isometamidium chloride (Samorin^{R},) given every 45 days.

Kwale District, one of the six administrative Districts of Coast Province, is located between latitudes 3^{O} 45' - 4^{O} 40'S and longitudes 38^{O} 50' - 39^{O} 40'E (Figure 4.2). The District has four Divisions, Kubo, Matuga, Msambweni and Kinango, which receive an annual rainfall averaging 1,000 - 1,200 mm near the coastline, decreasing to less than 900 mm towards the hinterland (Kinango), some 30 - 50 km inland from the coastline. Mean maximum and minimum temperatures are similar to those described for Kaloleni study area and ranged from 28^{O}C to 33^{O}C for the mean monthly maximum and 15^{O}C to 21^{O}C for the mean monthly minimum.

In the semi-humid areas (CL3), the main vegetation types are *Chlorophora/Sterculia* rain forest, interspersed with savanna mosaic grassland, *Brachystegia* woodland and *Acacia/Commiphora* thorn bushland which dominate in the semi-arid areas (Moomaw, 1960). Figure 4.10 shows the dense rain forested areas in the District.

Figure 4.10 Dense rain forested areas of Kwale District

The District supports a large and varied wildlife population, the most common being antelopes, wild pigs, bush pigs, warthhogs, monkeys, elands, buffaloes and elephants. The majority of them are concentrated in the national game reserve (Shimba Hills National Park), although presence of wild game is also common outside the park. Wild animals serve as reservoirs for both trypanosomiasis and tick-borne diseases (Aschroft, 1959; Grootenhuis and Young, 1981).

In the coastal strip, the Digo ethnic group form the majority of the indigenous inhabitants, whereas in the hinterland the Durumas are the dominant race. In the Settlement Schemes of the District, a number of different tribes have settled, the most common being the Waakambas.

Subsistence agriculture is the major activity in the semi-humid areas. Of the few commonly found tree-crops, coconut, cashew-nut, bixa and citrus fruits provide some income which, however, is declining due to poor market prices. Subsistence staple crops are mainly cassava and rice, though maize is grown in some areas. Damage by wild game, such as elephants, buffaloes and particularly suids, monkeys and baboons, inhibit farmers from cultivating larger agricultural plots. Until 1991, the only large-scale agricultural activity was a sugar-cane plantation at Ramisi, in Msambweni Division.

In general, the livestock sector, particularly dairy enterprise is under developed This has been attributed mainly to the risk of trypanosomiasis which has limited the establishment of bothcommercial beef and dairy enterprises. Of the cattle that are found, Zebu herds predominate. Majority of the herds belonging to the Digo villagers are sedentary and small in size (10-50), whereas relatively larger herds and cattle numbers owned by the Duruma people are found in the drier hinterland of Kinango. Livestock, and cattle in particular, are valued as assets which provide social security by forming an important

component of the risk aversive farming systems, particularly in the coconut-cassava (CL3) zone.

Rearing of dairy cattle is confined to a small number of commercial farms from which most of the milk is supplied to tourist hotels. In the villages, milk production is at subsistence level, but not all households keep cattle, particularly in the semi-humid zone. Therefore, there is an increasing demand for fresh milk, even at the village level.

In recent years, introduction of the zero-grazing package by NDDP has led to the adoption of dairy cattle by some small-holder farmers mainly the semi-humid areas of Kubo, Matuga and Msambweni Divisions. Management practices of the zero-grazing herds are similar to those described for the zero-grazed herd in Kaloleni Division.

During the time of the study, animal health services were provided by the Veterinary Department under the coordination and guidance of the District Veterinary Officer (DVO). In each Division, a VO was responsible for organising disease control activities, clinical services and meat inspection. Until 1989, a motorised AI service using deep frozen semen was operational. However, this was disbanded as the service required large Government subsidies. It was replaced by the use of room temperature semen at static insemination points, This system was found to be unpopular due to unreliability of the quality of semen as well as the increase in insemination fees from 10 Ksh to 40 Ksh (Anon, 1990a). Lately, some of the static points have started using deep frozen semen. The logistical problems of taking individual animals for insemination to static points and increased costs of the service have resulted in a number of dairy farmers rearing their own bulls for breeding.

At the divisional level, most of the field veterinary work is performed by the LOs and AHAs with diagnostic support provided by the Ukunda laboratory, a sub-centre of the regional VIL, Mariakani.

Bovine trypanosomiasis has been reported to be the most prevalent disease in the District (Anon,1990a) and its control depends on chemoprophylaxis using isometamidium chloride (Samorin^{R}). In most instances, due to operational limitations at the divisional as well as District level, widespread protection of cattle with regular Samorin inoculation was impractical. As a result, chemoprophylaxis was carried out on individual farm basis upon request from the farmer. From 1991 onwards, the District Veterinary Office, in collaboration with the NDDP, has embarked on a chemoprophylaxis programme for the small-holder dairy farmers in the District.

Although most parts of the District are thought to be under medium to high tsetse challenge, no large-scale programmes targeted at vector control have been initiated. However, in 1990, one commercial dairy farm started using flumethrin pour-on formulation with strategic chemoprophylaxis. This approach showed reasonable success in reducing the number of clinical cases of trypanosomiasis (Anon, 1991b).

4.3.3 MTWAPA

At this third study site (Table 4.2), an on-station experiment was carried out to monitor effect of trypanosomiasis on the health and production of dairy cattle under natural challenge and to assess the efficacy of trypanocidal drugs in the control of the disease. The experimental animals belonged to the Kenya Agricultural Research Institute's (KARI) Regional Research Centre (RRC), Mtwapa

Environment and herd management

The RRC, Mtwapa is located at longitude 39^{o} 44'E latitude 3^{o} 56'S at about 15 m above sea level (Figure 4.3) in the coconut-cassava agro-ecological zone (CL3) of the coastal strip (Jaetzold and Schmidt, 1983). The research centre lies 15 km north of Mombasa, along the main Mombasa-Malindi road.

Mean annual rainfall is about 1,100 mm with peak rainfall between April and June. Only January, February and March have on average less than 50 mm rain with wide variation recorded between years in the annual precipitation and its distribution. Daily temperatures are high with mean monthly maxima ranging between 27^{o}C and 32^{o}C and mean monthly minima between 20^{o}C and 24^{o}C. Relative humidity rarely falls below 70 per cent.

The herd studied was a Jersey herd established in the late 1950s. Its history, management and performance up to the late 1980s have been reported by Njubi, Rege, Thorpe, Lusweti and Nyambaka (1992). In summary, the breeding herd comprised of two groups, the lactating and the dry cows, which grazed day and night on natural pasture with shade trees or under the tree crops, cashewnut and coconut palm (Figure 4.11). In common with its management in recent years, the herd received little or no supplementary feed, except some legume forage during the experimental period. Cows were hand milked twice daily and the yield recorded of each milking. Calves were bucket reared. Mating was by artificial insemination. The animals were weighed monthly and heifers were introduced into the breeding herd at about 18 months of age.

Preventive veterinary practices carried out included annual vaccinations against rinderpest, foot-and-mouth disease, lumpy skin disease, anthrax and blackquarter. Acaricidal spraying using amitraz, 12.5% w/v diluted 1:500, (Triatex^{R}, Cooper Animal Health Ltd, UK) was performed weekly to control

Figure 4.11 Resident Jersey experimental dairy herd at the Regional Research Centre, Mtwapa.

tick-borne diseases. In 1987, the herd was immunised against East Coast fever using the infection and treatment method (Mutugi *et al.*, 1991a).

Young stock were drenched with an anthelmintic (Nilzan Plus) at a dose of 5ml/20kg. Treatment was carried out once every month in the wet season and once every two months in the dry season.

Trypanosomiasis in the dairy herd was controlled by the use drug isometamidium chloride (Samorin^{R}), at 0.5 mg kg^{-1} intramuscularly given one to three times a year depending on level of trypanosomiasis risk. The criteria for prophylactic treatment depended on clinical diagnosis of trypanosomiasis cases. Whenever, 10% of the herd was diagnosed clinically as suffering from trypanosomiasis, the whole herd was placed on isometamidium chloride. Clinical cases of trypanosomiasis were treated with 7.0 mg kg^{-1} diminazene aceturate (Berenil^{R},). Since mid-1980's there has been an increase in the number of curative treatments required, some even within a month of the chemoprophylaxis injection. It was against this background that the efficacy of trypanocidal drugs used on the dairy cattle was investigated.

4.4 SAMPLING AND DIAGNOSTIC METHODOLOGIES

This section describes the biological sampling and diagnostic techniques which were employed in this thesis. The design of the respective field studies and on-station experiments undertaken at the three study sites, Kaloleni, Kwale and Mtwapa are given in Chapters 5, 6 and 7.

4.4.1 Sample collection

Animals in zero-grazing units were usually restrained in the milking parlour, whilst those that were free-grazing were physically held against a convenient tree trunk aided by halters and tethering ropes. On certain occasions, animals had to be cast prior to sampling.

Blood samples were collected in two heparinised capillary tubes (75 mm x 1.5 mm) following prick puncture of the peripheral ear vein using sterile needles. Each capillary tube was filled to 3/4 of its length after which one end of the tube was sealed with Cristaseal (Hawksley and Sons,Ltd, UK). The filled tubes were then placed in an improvised cardboard capillary holder ensuring easy identification of samples. From every animal, 10-15 ml of blood from jugular puncture were also collected in plain vacutainers (Monoject, Sherwood, UK) for serum. Faecal samples were also taken from the rectum of these animals using polythene bags. The blood and faecal samples were stored in a cool box and analysed the same day at the regional VIL, Mariakani, situated some 20 km from the centre of the study area.

4.4.2. Estimation of anaemia by packed red cell volume

Whole blood in heparinised capillary tubes were centrifuged 12,000 g for 5 minutes using a microhaematocrit centrifuge (Hawksley and Sons Ltd, UK) After centrifugation, the packed red cell volume (PCV) was determined using a Hawksley micro-haematocrit reader and was recorded as a percentage of packed red blood cells to total volume of whole blood.

4.4.3 Parasitological techniques

a) Trypanosome detection

The darkground/phase contrast buffy coat (DG) technique (Murray, *et al.*, 1977) was used to detect and identify trypanosomes. Briefly, after centrifugation of the haematocrit capillary tube, it was broken at the buffy coat/plasma interface. Contents 1 mm below the interface, (to include the top layer of red cells), and 1 cm above, (to include plasma) were expressed on to a clean slide, mixed and covered with a 22 x 22 mm glass coverslip. The preparation was examined microscopically for trypanosomes by phase contrast

or darkground illumination using x 25 objective. Trypanosomes species were identified by their size and motility characteristic as described in Table 4.6. Thin blood smears were prepared from positive samples, stained with 10% Giemsa for morphological confirmation of the trypanosome species.

b) Detection of tick-borne haemoparasites

Approximately, 5 ul of blood from a second heparinised capillary tube was used to prepare thin blood films for haemoparasite examination. Following rapid air drying thin blood smears were fixed for 3 minutes in methanol and stained with 1/10 diluted Giemsa's stain for 20 minutes. After washing off the stain with tap water, the slides were allowed to dry. Microscopical examination for theilerial piroplasms, *Anaplasma* and *Babesia* parasites was done under oil immersion using x 100 oil objective.

c) Faecal egg counts

Faecal samples were analysed by the flotation technique (Hansen and Perry, 1990). Briefly, 2 gm of faeces were rubbed through a sieve into a beaker along with 30 ml of 1% formalin solution. Ten ml of the thoroughly mixed suspension were poured into a conical tube and centrifuged for 2 minutes at 1,200 g. The supernatant was discarded and the sediment resuspended in 10 ml of saturated salt (NaCl) solution (specific gravity 1.2). The contents in the tubes were mixed by inverting several times, then, using a pasteur pipette, both chambers of a McMaster worm counting slide (Hawksley and Sons Ltd, UK) were filled with the suspension. Helminths eggs were identified by their morphological appearance and grouped as either strongyles or strongyloides eggs. Both chambers were counted and the estimate of the number of eggs per gramme (e.p.g.) was obtained by multiplying the total count by 50.

Table 4.6 Morphological and motility characteristics used for the identification of trypanosome species

Species	Free flagellum	Kinetoplast	Undulating membrane	Size and motility in wet film and DG
T. vivax	Present	Large, terminal	Not prominent	Large, highly active, traverse the whole field rapidly, pausing rarely
T. brucei	Present in all but stumpy forms	Small, sub-terminal central	Prominent	Large with slender intermediate and stumpy forms, rapid movement in confined areas
T. congolense	Absent	Medium, sub-terminal marginal	Not prominent	Small, sluggish adheres to red blood cells by anterior end

4.4.4 Serology

a) Serum separation

Blood in vacutainers were kept either at room temperature for 2 to 3 hours or overnight at 4^{o}C to allow clot formation. After the removal of the clot, the serum was centrifuged at 1,200 g for 10 to 15 minutes. Using a fresh pasteur pipette for each sample, serum was aliquoted into three 1 ml serum storage tubes and stored at -20^{o}C. The serological analysis were carried out at the National Veterinary Research Centre (NVRC), Muguga, and the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi. All analysis at ILRAD were carried out by the author, while samples sent to NVRC were tested by the technical staff of the Protozoology Section of the Centre.

b) Detection of *Theileria parva* antibodies

Antibodies to *Theileria parva* schizont (TPS) antigen were detected using the indirect fluorescent antibody test (IFAT) as described by Burridge and Kimber (1972). Briefly, test and control (standard reference positive and negative) sera were diluted 1:40 in lymphocyte lysate diluting solution in tissue culture plates and incubated at room temperature for 30 minutes. *Theileria parva* schizont antigen, diluted in 0.1% bovine serum albumin solution, was applied to polytetrafluroethylene (PTFE) coated 10 well slides by dispensing 100 ul to each well. The slides were then allowed to dry at 37^{o}C.

Thereafter, 25 ul of diluted test serum were applied to each TPS well ensuring that every slide had both positive and negative controls. Slides were then placed in a moist chamber and incubated at room temperature for 30 minutes. After incubation, excess serum was shaken off, and the slides immersed in a container of Dulbecco's 0.01M phoshate buffer saline (PBS) pH 7.4 wash solution. Slides were allowed to soak for 30 minutes, with PBS pH 7.4 being discarded and refilled at 15 minutes interval.

After the final soak, each slide had its PTFE coated area around the wells carefully dried prior to adding 10 ul per well of 1:80 goat-antibovine fluorescein isothiocyanate conjugate (FITC). The conjugate was prepared with 1:100 Evans Blue stain, diluted in PBS pH 7.4. The slides were then placed in the moist chamber and allowed to incubate at room temperature for 30 minutes. Following incubation, excess conjugate was discarded and the slides were immersed in PBS pH 7.4 wash solution. Soaking of slides was carried out as described previously.

Thereafter, each slide was mounted with a cover slip ensuring no air bubbles got trapped and examined either on the same day or kept overnight at $4^{o}C$. Slides were read using a fluorescent microscope and the intensity of fluorescence recorded in relation to the positive and negative control in each slide. All positive samples were titrated at serum dilutions of 1:40, 1:160, 1:320 and 1:640, and the test repeated as described. Samples with antibody titres equal to or greater than 1:40 were considered positive.

c) Detection of *Babesia bigemina* antibodies

A sandwich antibody enzyme-linked immunosorbent assay (ELISA) using soluble extracts of *B. bigemina*, was used to test serum samples for the presence of antibody. Briefly, ELISA plates were coated with *B. bigemina* antigen by dispensing 100 ul of antigen at a concentration of 0.05 ug ml^{-1} diluted in PBS pH 7.4 into each well of the ELISA plates. The *B. bigemina* stock was used at a dilution of 1:1,000, which had been observed to be optimal after initial checkerboard titration against standard control sera. The plates were incubated at $37^{o}C$ for two hours and kept overnight at $4^{o}C$ or at $-20^{o}C$ for prolonged storage.

After overnight incubation or complete thawing of stored frozen plates, any unbound antigen was removed by washing the plates three times with PBS

pH 7.4 containing 0.1% Tween 20 (PBST). Thereafter, any remaining binding sites on the plates were blocked by adding 150 ul of PBST containing 5% normal sheep albumin to each well and incubated at 37^{o}C for 1 hour. Following incubation, the plates were washed three times by immersing in PBST.

One hundred microlitres of 1:100 dilution of test sera in PBST containing 0.5% sheep albumin, were dispensed into each well in duplicate. In addition, standard reference positive and negative control sera were added to each plate.

After 1 hour incubation at 37^{o}C, the plates were rinsed five times by immersing in PBST and then soaked with PBST for 15 minutes. The washing procedure was repeated once again. Plates were dried and 100 ul of 1:10,000 dilution in PBST with 0.05% sheep albumin of goat anti- bovine IgG horseradish peroxidase (HRPO)-labelled conjugate (Sigma, USA) were added to each well. Plates were incubated for 1 hour at 37^{o}C with continuous shaking on the Dynatech microtitre shaker. They were washed five times with PBST as previously described to remove any unbound conjugate.

Finally, 100 ul of substrate solution containing 125 ul of 2,2'-azino bis (3-ethyl)-benzthiazoline-6-sulfonic acid di-ammonium salt (ABTS, St Louis, USA) and 100 ul of 1% hydrogen peroxide in 25 ml of citrate buffer, pH 4.0, were added to each well. The plates were then covered with aluminium foil, placed in a drawer at room temperature for 30 minutes. Optical densities of the colour reaction were read at 414 nm wavelength using the ELISA autoreader. Optical readings of 0.100 and above were regarded as positive for the presence of antibody.

d) Detection of *Anaplasma marginale* antigens

Circulating *Anaplasma marginale* antigens were detected using a sandwich ELISA following a procedure similar to that described by Katende *et al.* (1990). Briefly, 96-well flat bottomed micro-ELISA plates (Nunc, Denmark) were coated with 100 ul/well of 75 kiloDaltons (kDa) *A. marginale* monoclonal antibody at a concentration of 10 ug/ml in PBS pH 7.4 and were incubated overnight at 4^{o}C. Thereafter, excess antibody was discarded, and plates subsequently blocked with 300 ul/well of 10% skimmed milk in PBS pH 7.4 for 1 hour at room temperature. The plates were emptied, rinsed three times with PBST, dried and stored at -20^{o}C until further use.

Sera were diluted 1:2 in 10% milk diluting buffer (10% skimmed milk in PBS pH 7.4 containing 0.1% Tween 20) before adding 75 ul of diluted test sera to each well of the monoclonal antibody coated plates. All test and control sera were tested in duplicate and each plate had a positive and negative control. Plates were incubated for 15 minutes at room temperature with continuous shaking using a Dynatech microtitre plate shaker. Thereafter, the contents were discarded, plates dried and blocked with 300 ul/well of 10% skimmed milk in PBS pH 7.4 for 1 hour at room temperature. After blocking, the plates were emptied, and 100 ul/well of 1:1000 dilution of HRPO labelled with *A. marginale* 75 kDa monoclonal antibody in 10% milk diluting buffer was added. Plates were incubated for 15 minutes at room temperature with continuous shaking. Thereafter, plates were emptied, and rinsed five times with PBST, soaked for 30 minutes followed by five more washes with a final soaking for a further 30 minutes.

Plates were dried and 100 ul of substrate solution consisting of 100 ul of 1% hydrogen peroxide and 125 ul of chromogen ABTS in 25 ml of substrate buffer (pH 4.0) were dispensed into every well. The plates were then incubated for 1 hour at room temperature. The optical densities, as a result of the colour

changes in the ELISA, were read at 414 nm wavelength using a Titertek Multiskan MCC/340 II (Flow UK) micro-ELISA autoreader. Absorbance values of 0.100 and above were considered positive for the presence of *A. marginale* antigens.

e) Detection of trypanosome antigens

Antigens were detected in sera by a sandwich ELISA following the procedure developed by Nantulya and Lindqvist (1989).

Briefly, flat-bottom micro-titre ELISA plates (Immunolon, USA) were coated with monoclonal antibodies for *Trypanosoma. vivax*, *T. congolense* and *T. brucei*, using 100 ul per well of a 1:100 (1 ug/well) dilution of appropriate monoclonal antibody in carbonate buffer pH 9.6 (coating buffer) and incubated overnight at $4^{o}C$. These monoclonal antibodies were raised against the three trypanosome species as described by Nantulya *et al.*, (1987). The excess antibody was drained off the plates and 100 ul washing buffer (0.15M PBS pH 7.4, containing 0.5% Tween 20) was dispensed to each well. Test and control sera were added to each well in duplicates, 10 ul for *T. congolense* and *T. vivax*, and 5 ul for the *T. brucei* The positive control sera used were known species-specific standard reference sera obtained from experimental infections, whilst the negative controls were from non-infected cattle kept at Kapiti Plains, a trypanosomiasis-free area of Kenya. The negative sera were confirmed as having no antibodies to trypanosomiasis on the IFAT (Katende *et al.*, 1987).

After incubation for 15 minutes at room temperature, the plates were emptied and rinsed with washing buffer. To each well, 100 ul of appropriate monoclonal antibody conjugated to HRPO diluted 1:100 in washing buffer containing 1% bovine albumin was added. The plates were incubated at room temperature for 15 minutes. Thereafter, plates were washed three times with

washing buffer, followed by a 10-minute soaking of the plates between each rinse to remove unbound conjugate.

One hundred microlitres of chromogen and substrate, consisting of 25 mg ml^{-1} of ABTS and 1% hydrogen peroxide in 50 mM citric acid buffer pH 4.0, were added to each well. The plates were incubated for 30 minutes at room temperature. Optical densities of the colour reaction were read using the ELISA autoreader. The predetermined cut-off optical density value for the test was 0.05 and absorbance values equal to or greater than 0.05 indicate presence of antigens.

f) Detection of trypanosome antibody

Presence of antibodies to trypanosome infection were tested using the ELISA test described by Luckins (1977).

A crude trypanosome lysate stock antigen was titrated for optimal concentration and 1:100 dilution in carbonate buffer pH 9.6 and used for coating plates for the assay. The plates were incubated for two hours at 37°C or kept overnight at 4°C before use. Antigen coated plates were washed three times with washing buffer (0.15M PBS pH 7.4, containing 0.5% Tween 20), shaken dry, and 100 ul of test and control sera diluted 1:50 in washing buffer containing 1% sheep albumin were dispensed in duplicates to each well. Plates were then incubated for 30 minutes at 37°C. The contents were emptied, and plates rinsed three times with washing buffer. To each well, 100 ul of goat anti-bovine HRPO conjugate diluted 1:1,000 in washing buffer containing 1% ovine albumin, was added. After incubation for 30 minutes at 37°C, the plates were washed five times and allowed to soak for 30 minutes. The washing and soaking steps were repeated to ensure removal of any unbound conjugate.

The rest of the procedure was similar to the one described for the antigen test. Optical densities readings more than twice that of the negative control in each ELISA plate were regarded as positive for antibody.

4.5 DATA MANAGEMENT AND ANALYSIS

All field and laboratory data of study animals were recorded on pre-designed data sheets for each herd. These were then transferred into computer files using the database management system, dBase III plus version 1.0 software programme (Ashton-Tate, 1986, USA).

For the epidemiological studies and the on-station experiment, categorical data with binary (0 or 1) responses were analysed using chi-squared tests or logistic regression models. Continuous quantitative data were analysed, where possible, by least squares analyses of variance models appropriate to the experimental design. Analyses were undertaken using generalised linear model routines provided in Statistical Analysis Systems (SAS) software. (SAS, Institute, Incorporation, Cary, North Carolina, USA). The details of the models used for the specific studies are described in the materials and methods section of their respective studies.

CHAPTER FIVE

VECTOR-BORNE DISEASES IN SMALL-HOLDER DAIRY CATTLE IN KALOLENI DIVISION, COASTAL KENYA

This chapter reports on two studies undertaken to investigate the epidemiological aspects of major diseases affecting small-holder dairy production in Kaloleni Division of Kilifi District in the coastal lowlands of Kenya. The first study was a cross-sectional study which gave a general overview of disease constraints, particularly in relation to tick-borne diseases and tsetse-transmitted trypanosomiasis, in the major AEZs and production systems. The results led to the design of the second study, a longitudinal cohort study to estimate the disease incidence and case-fatalities of the major diseases.

5.1 A CROSS-SECTIONAL STUDY OF DISEASE PREVALENCE IN SMALL-HOLDER DAIRY CATTLE IN KALOLENI DIVISION, COASTAL KENYA

5.1.1 INTRODUCTION

In the coastal lowlands of Kenya, diseases of cattle have long been recognised as major constraints to the productivity of indigenous and especially exogenous breeds and their crosses. Tick-borne diseases, particularly East Coast fever (ECF), and tsetse-transmitted trypanosomiasis, have probably been the principal biological factors limiting the productivity of existing small-holder cattle herds and restricting the adoption of specialised dairy cattle by small-holder farmers.

Reports of disease prevalence and the application of control measures in cattle herds in coastal Kenya and Tanzania have been restricted to studies of large-scale private and institutional units (Morzaria *et al.*, 1987; Paling *et al.*, 1987; Gaturaga *et al.*, 1989; Mutugi *et al.*, 1991a; Trail *et al.*, 1985). The efficacy of the control of trypanosomiasis through the use of chemoprophylactic drugs has been reported for indigenous Zebu herds in a

traditional village system by Maloo *et al.* (1988a), and for improved Boran cattle in a beef ranching system by Trail *et al.* (1985). However, there is a lack of systematic epidemiological studies examining disease prevalence in small-holder dairy cattle managed under the contrasting conditions present in the coastal region.

The study reported in this section was the first of a series of structured investigations carried out into the epidemiology of diseases affecting small-holder cattle and into the development of appropriate control strategies. A stratified cross-sectional study estimated disease prevalence rates and the influence on them of AEZ, cattle type (local Zebu and dairy) and age, and grazing system (zero and free grazing). The objectives of the study were to identify those diseases that limit dairy production in the small-holder sector and to quantify their occurrence by carrying out a parasitological and serological point-prevalence study.

5.1.2 MATERIALS AND METHODS

a) Study environment and cattle population

The cross-sectional study was carried out during 1989 in Kaloleni Division. A comprehensive description of the study area, cattle population and animal husbandry and health practices has been presented in Chapter 4.

b) Study design and methodology

The livestock census reported 157 dairy and mixed herds in the Division (Thorpe *et al.*,1993), and provided an indication of the AEZ and grazing systems in which these occurred. With the original objective of studying all herds with dairy cattle, a sample of 132 herds comprised of 734 dairy and 205 zebu cattle was obtained. Thus, 25 herds remained unsampled, mainly due to

difficulties of access or to the unwillingness of the owners to participate in the study.

Table 5.1.1 shows the number of herds and animals sampled by AEZ, grazing system, herd and cattle type. The study was carried out between July and September 1989 by two teams, each visiting on average two to three herds per day. Herds were identified by their AEZ location (CL3, CL4, and CL5), grazing system (zero- or free-grazing), herd type, (dairy or mixed), and by the name of the farmer. In every herd, cattle sampled were identified by their 'passport' data consisting of name, breed, sex and age. Whenever birth dates were unavailable, dentition was used to estimate age, according to Sisson and Grossman (1953). Categorisation of animals into their respective age groups by dentition is shown in Table 5.1.2.

Genetic composition of dairy cattle in most cases was difficult to determine as the majority of animals were cross-breds. However, cattle in zero-grazing units supported by the NDDP were mainly Ayrshire, Sahiwal and Brown Swiss crosses.

c) Sampling and diagnostic methodologies

Blood and faecal samples were collected from all cattle in dairy herds and, in the case of mixed herds, from both dairy and, whenever possible, an equal number of Zebu cattle, matched for age and sex. The procedures for obtaining biological samples have been described in Chapter 4. They were stored and transported in a cool box and analysed on the same day at the Veterinary Investigation Laboratory (VIL), Mariakani, some 20km from the centre of the study area.

Trypanosomes were detected in whole blood using the dark-ground/phase contrast DG buffy coat technique (Murray *et al.*, 1977). Tick-borne haemoparasites were identified by microscopical examination of Giemsa

Table 5.1.1 Number of herds and cattle classified by agro-ecological zones, grazing system, and herd type sampled to estimate disease prevalence in the cross-sectional study

Agro-ecological zone	Grazing system	Dairy herds		Mixed herds		
		No. herds	No. cattle	No. herds	No. of cattle	
					Dairy	Zebu
Coconut - cassava (CL3)	Zero	20	75	-	-	-
	Free	26	106	30	157	121
Cashew-nut - cassava (CL4)	Free	25	142	21	143	84
Livestock - millet (CL5)	Free	-	-	10	111	-
Total		71	323	61	411	205

Table 5.1.2 Categorisation of cattle into age-groups according to dentition

Age-group	Dentition
< 6 months	Milk teeth*
6-18 months	Milk teeth
>18-36 months	Eruption of 1st - 3rd permanent incisors
> 36 months	Presence of all permanent incisors

* Age was estimated by the farmer's recall of birth according to season, or other social events.

stained thin blood smears. This procedure was also used for differentiation of trypanosome species. Anaemia was estimated by measuring the packed red cell volume percent (PCV) of the heparinised whole blood samples after haemotocrit centrifugation. Faecal samples were analysed for nematode eggs, cestode eggs and segments, and coccidial oocysts using the flotation technique (Hansen and Perry, 1990). Strongyle and strongyloides egg counts of greater than 500 e.p.g. were considered significant and regarded as positive for helminth infection.

Serum samples were preserved in 1 ml aliquots at -20^{o}C. Sera from all age groups of animals were tested for antibodies to *Theileria parva*, *Babesia bigemina* and trypanosomes, and for antigens to *Anaplasma marginale* and trypanosomes. The procedures for serological assays using IFAT against *T. parva* schizont antigen (Burridge and Kimber, 1972) and ELISAs for other haemoparasites have been described in Chapter 4.

Serum dilutions of 1:40 and 1:160 were used for the *T. parva* schizont IFAT where fluorescence at titres equal to or greater than 1:40 were considered positive. Each individual ELISA test had predetermined positive and negative levels (Nantulya and Lindqvist, 1989; Deem *et al.*, 1993). Controls were included for every test and positive and negative results were established as follows: an optical density reading of >0.100 was considered positive for the *A. marginale* 75kDa antigen ELISA. For *T. congolense, T. vivax* and *T. brucei,* values of 0.050 and above were regarded as positive. An optical density of 0.200 was considered positive for the *Babesia* antibody, while optical readings twice that of the negative controls in each individual ELISA were recorded as positive for antibodies against trypanosomes. Antibody assays for *T. parva* and *B. bigemina* were performed at NVRC, Muguga, while ELISAs for detection of *A. marginale* and trypanosome antigens and trypanosome antibody were carried out by the author at ILRAD. Serum samples from 100 cows were

screened for antibodies to *Brucella abortus* using the Rose Bengal test at VIL Mariakani.

The number of samples tested for each serological assay varied. This arose due to logistical problems of carrying out these serological analyses in different institutes.

d) Questionnaire

In 92 of the 132 study herds, a questionnaire was completed. The farmers were asked about cattle health and management practices likely to influence the prevalence of diseases, particularly tick- and tsetse-borne diseases and helminthiasis. The questions included acaricide use, and grazing and watering management. Herds were omitted on the basis of a poor response to the questions or unavailabilty of the household head who was responsible for providing the relevant answers.

e) Statistical analyses

The results of the diagnostic tests were categorised as positive or negative on the basis of the criterion given above, and prevalence rates estimated. Overall differences in prevalence rates between AEZ, grazing system, cattle and herd type and animal age, were tested for statistical significance at the 5% level using chi-square tests. The combination of cattle and herd types and grazing systems in the three AEZs were referred to, in this context, as the farming systems. Data from the various farming systems were analysed using the linear logistic regression models (Collett, 1991). The variables analysed were the prevalence rates of antibodies to *T. parva* schizont antigen, *B. bigemina*, and *Trypanosoma* species, and of antigens to *A. marginale*, and to *T. congolense*, *T. vivax*, and *T. brucei*. The logistic regression models included the effects of farming system S_i, where i=1.....8, were the levels associated with the eight

combinations AEZ/cattle and herd type/grazing systems as shown in Table 5.1.3; age category of animal A_j, where j=1...4, with levels <6 months, 6-18 months, >18-36 months, >36 months;, sex of animal L_k, where k=1 or 2, with levels male of female. The interaction of farming system and age was also investigated. For the prevalence of *T. parva* antibodies and *A. marginale* antigens, only data from AEZ CL3 and CL4 were included in the model and so S_i was restricted to 1...7. The main effects logistic regression model explored was of the form:

$$\log \frac{(p_{ijk})}{(1-p_{ijk})} = \mu + S_i + A_j + L_k$$

where p_{ijk} is the probability of a positive diagnostic test for an animal in farming system i, of age j and sex k, and μ is an overall 'mean'. The model can be extended to include interaction terms.

The parameters of the model were estimated by maximum likelihood, using Genstat 5, Release 2.2 (Lawes Agricultural Trust, 1990). The statistical significance of effects was tested using deviances which had approximate chi-squared distributions. Predicted probabilities were estimated using the inverse of the logit transformation given by :-

$$p = \frac{\exp(\mu + S_i + A_j + L_k)}{1 + \exp(\mu + S_i + A_j + L_k)}$$

Approximate standard errors for the predicted probabilities were also obtained.

Table 5.1.3 Farming systems showing combinations of cattle and herd type, and grazing systems of small-holder dairy farmers in the three agro-ecological zones of coastal Kenya

Agro-ecological zones	Farming system	Cattle type	Herd[1] type	Grazing[2] system
Coconut-cassava (CL3)	1	Dairy	Dairy	Zero
	2	Dairy	Dairy	Free
	3	Dairy	Mixed	Free
	4	Zebu	Mixed	Free
Cashew-nut-cassava (CL4)	5	Dairy	Dairy	Free
	6	Dairy	Mixed	Free
	7	Zebu	Mixed	Free
Livestock-millet (CL5)	8	Dairy	Mixed	Free

1. Mixed herd: Herd comprising of both dairy and local Zebu cattle.
1. Dairy herd : Herd having only dairy animals.

2. Zero-grazing: Dairy cattle housed in stalls referred to as zero-grazing units where animals are fed on cut and carry pasture.
2. Free-grazing: Cattle managed by herding them to graze on natural pasture

The quantitative variable PCV was analysed by least squares analyses of variance using a fixed model in the general linear models procedure of SAS (Statistical Analysis Systems Institute, 1986). The model included the factors: farming system, age category of animal, sex of animal, the presence or absence of helminths, the presence or absence of theilerial piroplasms and the interaction of farming system and animal age. Linear contrasts estimated the significance of comparisons among farming systems and animal age classes. Tests were carried out at a 5% significance level.

5.1.3 RESULTS

a) Management practices

In the questionnaire survey, 95% of respondents said that they took measures to control tick infestations. All respondents keeping cattle in the zero-grazing system used their own hand-sprays for applying acaricide, with increasing numbers of those managing their herds in the free-grazing reverting to hand spraying rather than the use of communal plunge-dip tanks. Of those who had used the dip tanks, 27% had discontinued the practice because of poor dip management, the introduction of dipping fees and the non-functioning of dips in their area. Over 50% of the respondents said that they had not used any trypanocidal drugs to prevent or treat trypanosomiasis, while 28% said that they had treated their cattle for trypanosomiasis curatively. Only 16% of respondents had, at one time, protected their herds prophylactically. Intervention against helminthiasis was said to be carried out on their cattle by 80% of respondents. Anthelmintic drenching was performed as a preventive measure in most zero-grazed cattle, while in the majority of the free grazing herds, deworming was carried out after clinical diagnosis of helminthiasis.

b) Tick-borne diseases

Theilerial piroplasms were detected in 15% of the thin blood smears examined. The prevalence rate of piroplasms was twice as high in CL3 18% (95% confidence interval ci 14-22%) as in CL5 9% (c i 4-14%) ($p < 0.05$) with a significantly higher proportion detected in mixed (18%, c i 15-21%) compared to dairy herds (9%, c i 6-12%). No infections with *Anaplasma* or *Babesia* parasites were observed on blood smear examinations.

The antigen and antibody prevalence rates of the three tick-borne diseases (TBDs) in the three AEZs are presented in Table 5.1.4. Exposure to ECF, measured by the presence of antibodies to *T. parva* schizont antigen, gave an overall prevalence of 73%. Prevalence rate in CL5 (47%) was significantly lower than the rates in CL3 (76%) and CL4 (77%). Prevalence rates of over 70% and 90% for *B. bigemina* antibodies and *A. marginale* antigens, respectively, in the three AEZs were not significantly different.

Table 5.1.5 presents the prevalence rates of TBDs in dairy cattle kept in two management systems in the coconut-cassava (CL3) AEZ. Management of dairy animals in the zero-grazing system appeared to reduce the risk of exposure to tick-borne protozoan infections. Antibody prevalence to *T. parva* in zero-grazed cattle was 51% compared to significantly higher prevalence of 74% for cattle in free-grazing dairy herds. Similarly, prevalence rates of antibodies to *B. bigemina* in zero- and free-grazing dairy animals were significantly different at 42% and 68%, respectively. On the other hand, *A. marginale* antigen prevalence was over 90% in both grazing management systems, and did nor differ significantly.

In cattle managed in the free-grazing system, the prevalence rates of exposure to tick-borne infections according to type of cattle (dairy or local Zebu) and herd (dairy or mixed) are shown in Table 5.1.6. In general, antibody prevalences to *T. parva* (73%) and *B. bigemina* (76%) in dairy cattle were

Table 5.1.4 Antibody and antigen prevalence rates (%) (with 95% confidence interval) of major tick-borne diseases in small-holder cattle in the three agro-ecological (AEZ) zones of coastal Kenya

AEZ	*Theileria parva* Schizont IFAT			*Babesia bigemina* Antibody ELISA			*Anaplasma marginale* Antigen ELISA		
	n	%	c i	n	%	c i	n	%	c i
CL3	333	76	71-81	199	72	66-78	319	95	93-97
CL4	210	77	71-83	26	92	82-100	205	93	89-97
CL5	68	47*	35-59	50	74	62-86	NT	-	-
Overall	611	73	69-77	275	74	69-79	524	94	92-96

CL3 Coastal lowland 3 (Coconut-cassava)
CL4 Coastal lowland 4 (Cashew-nut- cassava)
CL5 Coastal lowland 5 (Livestock-millet)
n Number of animals tested
* Significantly different in the column ($p<0.05$)
c i Confidence interval
NT Not tested

Table 5.1.5 Antibody and antigen prevalence rates (%) (with 95% confidence interval) of major tick-borne diseases in small-holder dairy cattle managed in zero- and free-grazing systems in the coconut-cassava (CL3) AEZ of coastal Kenya

Grazing system	*Theileria parva* Schizont IFAT			*Babesia bigemina* Antibody ELISA			*Anaplasma marginale* Antigen ELISA		
	n	%	c i	n	%	c i	n	%	c i
Zero	49	51*	37-65	43	42*	27-57	64	91	84-98
Free	70	74	64-84	19	68	47-89	83	94	89-99

* Significantly different within the column ($p<0.05$)

n Number of animals tested

c i Confidence interval

Table 5.1.6 Antibody and antigen prevalence rates (%) (with 95% confidence interval) of major tick-borne diseases in small-holder cattle by cattle and herd type kept in the free-grazing system in coastal Kenya

	Theileria parva Schizont IFAT			*Babesia bigemina* Antibody ELISA			*Anaplasma marginale* Antigen ELISA		
Cattle type	n	%	c i	n	%	c i	n	%	c i
Dairy	436	73	69-77	157	76	69-83	334	93	90-96
Zebu	126	82*	75-89	75	88*	81-95	126	98	96-100
Herd type									
Dairy	138	75	68-82	22	73	54-92	150	93	89-97
Mixed	424	75	71-79	210	81	76-86	310	95	92-98

* Significantly different between dairy and Zebu cattle type ($p<0.05$)

c i Confidence interval

n Number of animals tested

relatively lower than the 80%, and more, estimated for exposure to the two parasite infections in local Zebu. Even though these differences were statistically significant, antibody prevalences to the two parasitic protozoa were high in both cattle types.

Managing cattle in dairy or mixed herds did not appear to have an influence on exposure to *T. parva* and *B. bigemina*, as antibody prevalences of 73% and above were estimated for both herd types. *Anaplasma marginale* antigen prevalence rates were estimated over 90% in both cattle as well as herd types and none of them were significantly different.

The overall age-specific antibody and antigen prevalence rates of the three TBDs are presented in Table 5.1.7. In general, there was an increase in the prevalence rates of exposure to these disease with animal age category. Antibody prevalences of *T. parva* increased from 64% in calves of less than 6 months to 79% in cattle over 36 months of age. A similar trend was observed with *B. bigemina*, where antibody prevalences increased from 58% to 81% for corresponding age cohorts. The pattern for *A. marginale* age-specific antigen prevalences was similar. Differences in the prevalences with animal age class appeared to be significant mainly due to the relatively lower prevalences in the young age cohorts (less than 19 months old). Despite these differences, it appeared that nearly 60% of the calf population experienced exposure to these tick-transmitted parasite infections by the time they were 6 months old.

The age-specific prevalence rates for *T. parva* antibodies in dairy cattle in CL3 and CL4 are shown in Figure 5.1.1. The prevalence for calves of less than 6 months of age in CL3 was 84% in free-grazing, and 56% in zero-grazing, whereas, the respective value for free-grazing in CL4 was 39%. In both zones, prevalences for animals over 18 months of age in the free-grazing

Table 5.1.7 Age-specifc antibody and antigen prevalence rates (%) (with 95% confidence interval) of major tick-borne diseases in small-holder cattle in coastal Kenya

Age (m)	*Theileria parva* Schizont IFAT			*Babesia bigemina* Antibody ELISA			*Anaplasma marginale* Antigen ELISA		
	n	%	c i	n	%	c i	n	%	c i
<6	74	64*	53-75	38	58*	42-74	59	81*	71-91
6-18	167	69	65-74	51	61	48-74	145	92	88-96
19-36	139	73	66-88	62	81	71-91	111	98	95-100
>36	231	79	74-83	124	81	74-88	209	97	95-99
Overall	611	73	69-77	275	74	69-79	524	94	92-96

(m) Age in months

* Significantly different from 19-36 and over 36 months age-classes in the column ($p<0.05$)

c i Confidence interval

n Number of animals tested

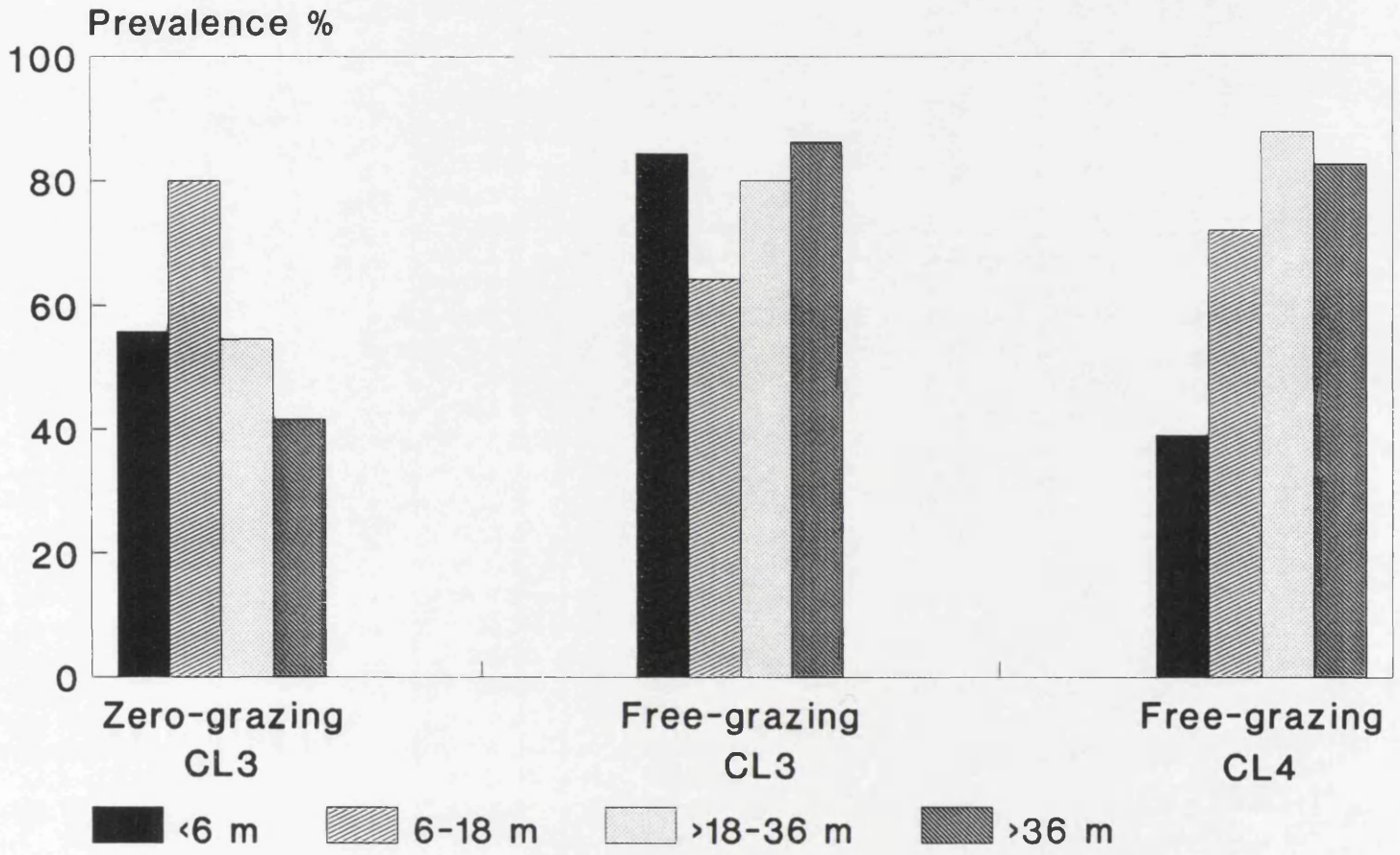


Figure 5.1.1 Age-specific prevalence rates of antibodies to *Theileria parva* schizont antigen for small-holder dairy cattle in coastal Kenya

system were 80% or higher. On the other hand, the prevalence for similar age cohorts in the zero-grazing system was 56% or less.

Using the logistic regression methods results of prevalence rates of antibodies and antigens to tick-borne diseases were similar (Table 5.1.8). The prevalences of *T. parva* antibodies in cattle in CL3 and CL4 differed significantly between farming systems (Appendix 1). This was due to the lower antibody prevalence estimated in zero-grazing system (57%) compared to over 70% for the rest of the cattle managed in their respective farming systems. Significant interaction of farming system and animal age category (Appendix 1) resulted from below average prevalence rates of three (less than 60%) of the four age categories of zero-grazed cattle. The exception was the 6 to 18 month old group, which had a prevalence of 80%, similar to their contemporaries in the other farming systems (Figure 5.1.1).

Antibody prevalences to *B. bigemina* were significantly different among farming systems and significantly among age class (Appendix 2). The differences in *B. bigemina* antibody prevalences among farming systems resulted mainly from the relatively lower prevalence of 42% estimated in zero-grazing dairy cattle compared to 67% to 100% estimated for cattle in other farming systems. Antibody prevalences of 61% or less for the 6-18 months and younger calves in comparison with 81% for above 18 months cattle was responsible for animal age significance. There were no differences in *A. marginale* antigen prevalences among farming systems as 90% and more prevalences were estimated in all types of farming systems, nor was their a significant interaction of farming system with age, the only exception being, animal age category (Appendix 3), where antigen prevalence in calves less than 6 months of 81% differed from over 90% observed in the rest of the age-cohorts.

Table 5.1.8 Antigen and antibody prevalence rates (%) of major tick-borne diseases in small-holder cattle kept under various farming systems in the three agro-ecological zones (AEZ) in coastal Kenya

AEZ	Cattle type	Herd type	Grazing system	*T. parva* Schizont IFAT			*B. bigemina* Antibody ELISA			*A. marginale* Antigen ELISA		
				n	%	s.e	n	%	s.e	n	%	s.e
CL3		Dairy	Zero	49	57[a]	7.4	43	42[b]	7.1	64	90	4.0
		Dairy	Free	70	74	5.3	19	67	10.1	83	93	2.7
	Dairy	Mixed	Free	125	83	3.1	66	74	5.0	95	96	1.7
	Zebu	Mixed	Free	89	84	3.6	71	88	3.9	77	96	2.6
CL4		Dairy	Free	68	73	5.6	3	100	1.7	67	93	3.1
	Dairy	Mixed	Free	105	79	3.9	19	94	5.5	89	94	2.0
	Zebu	Mixed	Free	37	71	5.9	4	86	12.9	49	99	1.3
CL5	Dairy	Mixed	Free	NI	-	-	26*	100	0.3	NT	-	-

a Differences between zero-grazing and free-grazing dairy cattle in CL3 approached significance (p=0.051)

b Differences beteween zero-grazing and free-grazing dairy cattle in CL3 were significant
($p<0.05$)

n Number of animals tested

s.e Standard error

NT Not tested

NI Data not included in the analysis

* Not all data records were included in the analysis

c) Trypanosomiasis

Only seven cases of trypanosome parasitaemias, all of them *T. vivax*, were detected by the buffy coat technique, giving a prevalence of less than 1%. By contrast, the overall prevalence of trypanosome antigens detected by ELISA was 34% (Table 5.1.9), with 40% of trypanosome antigens identified as *T. congolense*, 29% as *T. vivax*, and the remainder *T. brucei*. About 60% of antigenaemias were due to single species. The low antigen prevalence observed in CL5 (18%) differed significantly with the prevalences of 40% and 31% in CL3 and CL4, respectively. Generally, the prevalences of antibodies to *Trypanosoma* species were higher than antigen prevalences, ranging from 44% in CL5 to 62% and 60% in CL3 and CL4, respectively (Table 5.1.9).

In zero-grazing dairy cattle, trypanosome antigen prevalence was 11% compared to 48% estimated in dairy cattle kept in the free-grazing system in the same AEZ (Table 5.1.10). The four-fold difference was statistically significant. On the other hand, no apparent differences were observed in trypanosome antibody prevalences of dairy cattle managed in the two grazing systems, where prevalences estimated were 40% and 52% for zero- and free-grazing cattle, respectively (Table 5.1.10).

In general, both trypanosome antigen and antibody prevalences by cattle and herd type managed in the free-grazing system did not appear to be significantly different, but there was a tendency for antibody prevalence in Zebu cattle to be higher than dairy cattle. Likewise, the prevalence in mixed herds was higher than in dairy herds. (Table 5.1.11).

Results of trypanosome antigen prevalences estimated using the logistic regression analysis showed that farming systems significantly affected the prevalence of trypanosome antigens (Appendix.4). This resulted from dairy cattle managed in mixed, free grazing herds, where prevalences were almost twice as high in CL3 (37%) and in CL4 (43%) as in CL5 (22%) (Table 5.1.12).

Table 5.1.9 The prevalence rates (%) of trypanosome antigens and antibodies (with 95% confidence interval) in small-holder cattle in the three agro-ecological (AEZ) zones of coastal Kenya

AEZ	Trypanosome Antigen ELISA			Trypanosome Antibody ELISA		
	n	%	c i	n	%	c i
CL3	311	40	35-45	94	62	52-72
CL4	280	31	26-36	67	60	48-72
CL5	80	18*	10-26	16	44	20-68
Overall	671	34	30-38	177	59	52-66

* Significantly different in the column ($p<0.05$)

c i Confidence interval

n Number of animals tested

Table 5.1.10 The prevalence rates (%) of trypanosome antigens and antibodies (with 95% confidence interval) in small-holder dairy cattle managed in zero- and free-grazing systems in the coconut-cassava (CL3) AEZ of coastal Kenya

Grazing system	Trypanosome Antigen ELISA			Trypanosome Antibody ELISA		
	n	%	c i	n	%	c i
Zero	28	11**	0-24	5	40	0-83
Free	61	48	35-61	21	52	31-73

** Significantly different in the column ($p<0.01$)

c i Confidence interval

n Number of animals tested

Table 5.1.11 Trypanosome antigen and antibody prevalence rates (%) (with 95% confidence interval) in small-holder cattle by cattle and herd type kept in the free-grazing system in coastal Kenya

	Trypanosome Antigen ELISA			Trypanosome Antibody ELISA		
Cattle type	n	%	c i	n	%	c i
Dairy	481	33	29-37	123	55	46-64
Zebu	162	39	31-47	49	71	58-84
Herd type						
Dairy	170	31	24-38	42	48	33-63
Mixed	473	36	32-40	130	64	56-72

c i Confidence interval
n Number of animals tested

Table 5.1.12 Trypanosome antigen and antibody prevalence rates (%) in small-holder cattle kept under various farming systems in the three agro-ecological zones (AEZ) in coastal Kenya

AEZ	Cattle type	Herd type	Grazing system	Trypanosome Antigen ELISA			Trypanosome Antibody ELISA		
				n	%	s.e	n	%	s.e
CL3 Coconut-cassava		Dairy	Zero	28	10[a]	5.7	5	34	19.4
		Dairy	Free	61	47	4.7	21	46	10.2
	Dairy	Mixed	Free	122	37	4.4	36	70	7.2
	Zebu	Mixed	Free	100	45	5.0	32	65	8.0
CL4 Cashew-nut-cassava		Dairy	Free	109	22	3.9	21	47	10.7
	Dairy	Mixed	Free	109	43	4.8	29	61	8.5
	Zebu	Mixed	Free	62	29	5.8	17	73	11.8
CL 5 Livestock-millet	Dairy	Mixed	Free	52*	22	5.8	14*	43	13.1

a Significant difference between zero- and free-grazing dairy cattle in CL3 ($p<0.01$)

n Number of animals tested

s.e Standard error

* Not all data records were included in the analysis (the numbers of samples, therefore do not match for this zone with the overall data records presented elsewhere)

In addition, in CL3, free-grazing dairy cattle had an antigen prevalence rate nearly four to five times higher than that of zero-grazed dairy cattle, 47% and 10%, respectively.

Farming system did not appear to have a significant effect on the prevalence of trypanosome antibodies (Appendix 5), but there was a tendency for cattle managed in mixed herds to have a higher prevalence (over 60%) than those kept in dairy herds (less than 50%) in CL3 and CL4 zones (Table 5.1.12).

Trypanosome antigen prevalence did not differ among age categories and ranged from 32% to 37% in the four animal age categories (Table 5.1.13). On the other hand, significant differences in the trypanosome antibody prevalences were observed among animal age categories (Appendix 5). The prevalence of antibodies increased significantly with age from 32% and 40% in the two younger age groups, to 68% and 75%, respectively, for the two older age groups (Table 5.1.13).

d) Gastro-intestinal helminths

Prevalence of helminth infection measured by faecal nematode egg counts of >500 e.p.g was low for most cattle. Overall strongyle prevalence was 10%, with significant differences observed in the prevalence rates of 13% and 6% for CL3 and CL4, respectively, (Table 5.1.14).

Management of dairy cattle in free-and zero-grazing system, in CL3, had no influence the prevalence of strongyle infection, 9% and 7%, respectively (Table 5.1.15). In addition, the prevalences by cattle type, i.e., dairy or local Zebu, did not differ; however, two-fold increases in strongyle infection prevalences of 6% and 12%, were observed among dairy and mixed herd types, respectively (Table 5.1.16).

Table 5.1.13 Age-specifc prevalence rates (%) (with 95% confidence interval) of trypanosome antigens and antibodies in small-holder cattle in coastal Kenya

Age (months)	Trypanosome Antigen ELISA			Trypanosome Antibody ELISA		
	n	%	c i	n	%	c i
<6	73	37	26-48	19	32**	11-43
6-18	206	32	26-38	49	40	26-54
19-36	148	36	28-44	44	68	54-82
>36	244	34	28-40	65	75	64-86
Overall	671	34	30-38	177	59	52-66

** Significantly different from 19-36 and over 36 months age-class in the column ($p<0.01$)

c i Confidence interval

n Number of animals tested

Table 5.1.14 The prevalence rates (%) of helminth infections (with 95% confidence interval) in small-holder cattle in the three agro-ecological zones of coastal Kenya

Agro-ecological zones	Helminth infection (Faecal egg counts)		
	n	%	c i
Coconut- cassava (CL3)	451	13*	10-16
Cashew-nut-cassava (CL4)	322	6	3-9
Livestock-millet (CL5)	108	11	4-18
Overall	881	10	8-12

* Significantly different between CL3 and CL4 ($p<0.05$)

c i Confidence interval

n Number of animals tested

Table 5.1.15 The prevalence rates (%) of helminth infections (with 95% confidence interval) in small-holder dairy cattle managed in zero- and free-grazing systems in the coconut-cassava (CL3) AEZ of coastal Kenya

Grazing system	Helminth infection (Faecal egg counts)		
	n	%	c i
Zero	71	7	1-13
Free	106	9	4-14

** Significantly different in the column ($p<0.01$)
c i Confidence interval
n Number of animals tested

Table 5.1.16 The prevalence rates (%) of helminth infections (with 95% confidence interval) in small-holder cattle by cattle and herd type kept in the free-grazing system in coastal Kenya

	Helminth infection (Faecal egg counts)		
Cattle type	**n**	**%**	**c i**
Dairy	621	10	7-13
Zebu	189	11	6-16
Herd type			
Dairy	236	6*	3-9
Mixed	574	12	9-15

* Significantly different between dairy and mixed herds ($p<0.05$)

c i Confidence interval

n Number of animals tested

Age-specific prevalences for strongyle infection was significantly higher in calves (16%) than in cattle over 36 months (5%) (Table 5.1.17). There were very low prevalences of tapeworm segments (*Monezia* species) and coccidial oocysts.

e) Other diseases

Only one animal had a positive reaction to the Rose Bengal test for *Brucella abortus*.

f) Anaemia status

The overall mean PCV was 32.9%. It differed significantly among farming systems, and in the presence or absence of piroplasms. There was also a significant farming system X animal age category interaction (Appendix 6). Mean PCV differed among grazing systems and cattle types, but not between AEZ or herd types. In CL3, zero-grazing dairy cattle had a higher PCV than those in free-grazing, 33.7% and 32.1%, respectively, while in CL3 and CL4 the mean PCV of Zebu cattle was two to three percentage units lower than that of dairy cattle (Table 5.1.18). There was a tendency for PCV to increase with age. The maximum mean value, 32.5%, was observed in the 19 to 36 month category. The significant interaction of farming system and animal age resulted primarily because calves in zero- grazing units had high mean PCV (36.1%), while Zebu calves in the free-grazing system had mean PCV of 25.3% or lower. The presence of theilerial piroplasms significantly reduced mean PCV from 32.0% to 31.0%, though these biological differences were insignificant.

Table 5.1.17 Age-specifc prevalence rates (%) (with 95% confidence interval) of helminth infections in small-holder cattle in coastal Kenya

Age (months)	Helminth infection (Faecal egg counts)		
	n	%	c i
<6	99	16	9-23
6-18	248	14	10-18
19-36	201	11	7-15
>36	333	5**	3-7
Overall	881	10	8-12

** Significantly different in the column ($p<0.01$)

c i Confidence interval

n Number of animals tested

Table 5.1.18 Mean packed red cell volume percent (PCV) of small-holder cattle kept under various farming systems in the three agro-ecological zones (AEZ) in coastal Kenya

AEZ	Cattle type	Herd type	Grazing system	n	Mean PCV (%)	s.e
CL3 Coconut-cassava		Dairy	Zero	71	33.7	0.83
		Dairy	Free	106	32.1	0.70
	Dairy	Mixed	Free	153	32.0	0.57
	Zebu	Mixed	Free	119	29.8	0.73
CL4 Cashew-nut-cassava		Dairy	Free	130	33.1	0.69
	Dairy	Mixed	Free	123	32.1	0.68
	Zebu	Mixed	Free	69	29.1	1.00
CL5 Livestock-millet	Dairy	Mixed	Free	108	31.4	0.71

n Number of animals tested
s.e Standard error

5.1.4 DISCUSSION

This study provided an opportunity to undertake a quantitative assessment of prevalence rates of major vector-borne disease and other potential disease threats in order to assess the epidemiology of diseases in dairy and Zebu cattle kept under various farming systems in different agro-ecological zones in coastal Kenya.

In cattle belonging to small-holder dairy farmers in the three AEZs, there was evidence of exposure to ECF, babesiosis, anaplasmosis and trypanosomiasis as assessed by serological assays, with over 70% of the population testing positive for tick-borne haemoparasitic infections. On the other hand, helminth infection of 10%, at the time of sampling, did not appear to constitute a major problem.

Although theilerial piroplasms were detected in 15% of the population sampled, piroplasms of *T. parva* and *T. mutans*, the two most commonly observed infections of cattle in coastal Kenya, (Goddeeris *et al.*, 1982), cannot reliably be distinguished by examination of Giemsa stained blood smears (Young, 1981). Thus serodiagnosis was used to estimate the prevalence of *T. parva* infection.

Prevalence rates of *T. parva* antibodies of over 70% were significantly higher in the relatively wetter CL3 and CL4, then in drier CL5, where a prevalence rate of 47% was estimated. In CL3 and CL4, the high *T. parva* antibody prevalences, of over 70%, in dairy and Zebu cattle kept in the free-grazing system suggests widespread endemicity prevails with a large proportion of study population comprising of possible ECF survivors. These results were in agreement with other studies carried out in Zebu calves in the same study area, where prevalence rates of antibodies to *T. parva* schizont antigen of 85% and 59% in CL3 and CL4, respectively, were estimated (Deem *et al.*, 1993). In addition, these workers observed that calf population with high antibody

prevalences had higher mean tick infestation score of *R. appendiculatus*. Similar findings have been reported by Goddeeris *et al.*, (1982), where a high correlation was observed between presence of *R. appendiculatus* and antibodies to *T. parva* schizont antigen in bovine sera collected over larger areas of Kilifi District in coastal Kenya. In Kenya, *R. appendiculatus* normally requires a minimum of 500 mm of rainfall per annum and occurs in mostly savannah or woodland savannah habitats (Walker, 1974). Studies on the life cycle of *R. appendiculatus* at the Kenyan coast, in an area receiving on average 1200 mm of rainfall annually, showed activity of adult *R. appendiculatus* occurred throughout the year (Newson, 1978). In the CL3 agro-ecological zone of the study area, the climatic and environmental conditions were found to be suitable for *R. appendiculatus* activity to occur throughout much or all of the year.

On the other hand, the apparently low level of exposure in CL5 infers a reduced *T. parva* challenge, but the prevalence estimated was higher (47%) than that reported for Zebu calves in CL5 (22%), (Deem *et al.*, 1993). This was probably be due to the fact that most of the dairy cattle sampled in CL5 were found to be clustered in more or less a transitional area nearer to CL4. The relatively low antibody prevalence to *T. parva* reported in CL5 in this study, as well as in Zebu calves sampled from the same AEZ (Deem *et al.*, 1993) may be as a result of less favourable conditions for *R. appendiculatus* to be present for most parts of the year. Mean tick infestation scores of *R. appendiculatus* in Zebu calves in CL5 (0.52) were nearly four times lower than those recorded in CL3 (2.27) (Deem *et al.*, 1993). In the more arid regions of Kenya, conditions are known to be often marginal for the survival and development of the tick (Newson and Punya, 1978).

Generally, estimates of *T. parva* antibody prevalence rates in small-holder dairy cattle kept under various farming systems were higher than those

reported in large to medium scale institutional dairy herds found in similar environment of coastal Kenya. In CL3, antibody prevalence to *T. parva* was 20% in a pedigree Jersey herd kept at RRC, Mtwapa, whereas in CL5, a prevalence of 11.2% was estimated for Zebu-European crosses kept at the Animal Production Research Centre, Mariakani, (Mutugi *et al.*, 1991a). These low antibody prevalences were presumably the result of better application of tick control practices in these institutional herds.

On the other hand, the high level of *T. parva* antibodies in cattle belonging to small-holder farmers provides evidence that attempts to control ECF through application of acaricides did not prevent most cattle from being exposed to the parasite. Several reasons probably account for the failure of existing tick control practices. It may be related to the interval between acaricidal treatment, whereby the recommended practices of once a week application are not strictly adhered to, or it may be due to the increase in number of non-functional and poorly managed cattle dips in the area during the time of the study. In addition, the introduction of dipping fees probably resulted in the reduction in the number of cattle being dipped in the area. These factors must be instrumental in encouraging, particularly the dairy farmers, to revert to acaricidal application by hand spraying techniques; improper application of acaricides with hand pumps will not only render the method ineffective, but by being wasteful, incurs more costs.

By contrast, the prevalence of *T. parva* antibodies in dairy cattle managed in the zero-grazing system was lower (57%) than in their counterparts in the free-grazing system residing in the same AEZ. It was evident that the management approach for dairy animals in the zero-grazing system did appear, to a certain extent, to reduce the risk of exposure to the parasite. However, what could not be ascertained was whether it was confinement of cattle, particularly adult cattle, or application of acaricides or

combination of both, that was responsible for the relatively low antibody prevalence. Moreover, about half the number of cattle tested in this system had antibodies to *T. parva* implying that total prevention against exposure to the parasite was not achieved. This was the first study to report on *T. parva* antibody prevalences in small-holder dairy cattle kept under two different management systems.

In general, age-specific antibody prevalences increased from 64% in calves less than 6 months to 79% in adults over 36 months of age. These results suggest that under the small-holder management system, exposure to the parasite occurs in early life, possibly within the first 6 months of life, during which time it is thought that mortality may be occurring leaving behind a depleted, but generally immune population. However, in areas where endemic stability to theileriosis in Zebu calves existed, no mortalities due to ECF were reported, despite all calves developing theilerial infections and antibodies to *T. parva* schizont antigens within 6 months of age (Moll *et al.*, 1986). In the present cross-sectional study, antibody prevalences in free-grazing dairy calves under 6 months of age of 84% and 39% in CL3 and CL4 respectively, were similar to the 85% and 59% estimated in Zebu calves sampled in the two respective AEZs (Deem *et al.*, 1993). The relatively low antibody prevalence in the young age-cohort in CL4 is thought to be suggestive of delayed exposure to the parasite. However, in both AEZs, over 80% of the population over 18 months of age had developed antibodies to *T. parva* infection. In contrast, zero-grazed cattle showed a declining trend in antibody prevalence with increasing age, a reflection of reduced risk of exposure in this management system or possibly the result of introduction of recently purchased cows from ECF free farms.

Similarly, antibody and antigen prevalence rates of *B. bigemina* (over 70%) and *A. marginale* (over 90%) in free-grazing cattle in all AEZs were

comparable to the prevalences estimated in Zebu cattle across the three AEZs by Deem *et al.* (1993). Widespread endemicity to these diseases appeared to occur in the free-grazing system, whilst dairy cattle maintained in zero-grazing management system, had a relatively lower antibody prevalence to *B. bigemina* antibodies, but not to *A. marginale* antigens. The reason for the high prevalence rate of over 90% to *A. marginale* antigens in all farming systems across all AEZs remains unclear. In situations where cattle are constantly exposed to *Babesia* parasites, young animals become infected and develop immunity which protects them in adult life (Morrison, 1989). The increase in antibody prevalence to *B. bigemina* with age suggests such a scenario prevails in most small-holder free-grazing cattle in the area. Similar explanation may also apply to *A. marginale* infections.

Trypanosome infections diagnosed by parasite detection on DG gave a parasite prevalence of less than 1%. In addition, exposure to trypanosome infection as assessed by antigen and antibody prevalence indicated a relatively low risk to trypanosomiasis in comparison to the high risk to TBD as measured by their respective serological prevalences. Overall, trypanosome antigen prevalence of 34% was estimated compared to less than 1% parasite prevalence. Since the development of trypanosome species-specific antigen-ELISA for the detection of circulating antigens (Nantulya and Lindqvist, 1989), several laboratory and field based studies have described the increase in sensitivity in the diagnosis of trypanosome infections using this method (Masake and Nantulya, 1991; Nantulya *et al.*, 1992, Trail *et al.*, 1992).

In this study, trypanosome antigen prevalences declined from 40% in the relatively wetter, CL3 to less than 20% in the drier CL5, while antibody prevalence ranging from 44% to 66% across AEZs did not differ significantly. On the other hand, dairy cattle confined in zero-grazing units appeared to have

a lower antigen (10%), as well as antibody prevalence (34%), suggesting a relatively lower exposure to the disease.

The epidemiological significance of variation in trypanosome antigen prevalences among AEZs, in the face of few parasitologically detected cases, cannot be adequately explained based on a single testing of samples in a cross-sectional study. However, a combination of parasitological findings, antigenaemia and antibody status of the cattle in their respective AEZ may suggest Kaloleni Division to be an area of low or seasonal trypanosomiasis risk. This was supported by the fact that over 50% of the small-holder dairy farmers responded as not having used any trypanocidal drugs on their dairy herds, while 28% had treated their cattle curatively against trypanosomiasis. Moreover, only 16% of the respondents reported at some time as having protected their herds using chemoprophylaxis.

Of the antigenaemic cattle, nearly 60% of them had antigens to single trypanosome species. This was contrary to what has been observed in other parts of Kenya, such as at Nguruman, a trypanosomiasis endemic area, where 74.8% of bovine sera were positive for two or three trypanosome species (Nantulya *et al.*, 1992). However, in studies carried out in low challenge areas of Nguruman, antigen prevalence of 31% was estimated with 6% having mixed infections (Mwangi, 1993). It appears that antigens may be persisting for longer periods in circulation even in areas where trypanosomiasis challenge is thought to be low. However, in order to be able to interpret the significance of antigenaemias, longitudinal studies need to be planned using sentinel non-exposed herds in areas where the risk of the disease is preferably known to be low or seasonal.

In small-holder cattle, mean PCV of 32.9% was higher than average PCVs of below 30% reported in other areas on the Kenyan coast, where trypanosomiasis in sedentary Zebu and small-holder dairy cattle was the most

prevalent disease (Maloo *et al.*, 1988a). In this study, the mean PCV in Zebu cattle in CL3 of 29.8% was higher than mean weekly PCV of 24.2% in Boran and mean monthly PCV of 27.4% reported in non-parasitaemic Zebu cattle in areas of medium to high trypanosomiasis challenge (Maloo, *et al.*, 1988a; Munstermann *et al.*, 1992). Moreover, the mean PCV in Zebu cattle was similar to PCVs values obtained for Zebu dams in TBD endemically stable area in Kenya (Moll *et al.*, 1984). It therefore appears that in areas where trypanosomiasis and helminthiasis are not the major disease problems, and endemic stability to TBDs occur, anaemia, as measured by lowered PCV values, is not a major finding.

Generally, helminthiasis did not appear to be a an important problem. However, cross-sectional studies only indicate situations at one point in time. Therefore, periodic testing particularly in relation to rainfall pattern must be carried out to evaluate properly the prevalence of helminth infections.

In conclusion, the cross-sectional study highlighted the importance of vector-borne disease, particularly the TBDs, in different farming systems across the three AEZs as possible disease constraints limiting the adoption of dairying by small-holder farmers in coastal Kenya. However, the results of this study reported on serological prevalence of these diseases, in most cases indicating the proportion of population exposed to these infections. Information on exposure to these parasites does not describe the changing patterns of diseases caused by these parasites in a population, neither does it provide information on production losses due to disease. Therefore, in order to estimate incidence of diseases and case-fatalities, as well as factors such as season, age and grazing management practises influencing disease occurrences, longitudinal studies are required.

Consequently, these objectives formed the next study and are addressed in the second part of this chapter.

5.2 A LONGITUDINAL STUDY OF THE RISK OF MAJOR VECTOR-BORNE DISEASES IN SMALL-HOLDER DAIRY CATTLE.

5.2.1 INTRODUCTION

In general, cross-sectional studies, such as described in Section 5.1, are good indicators for providing an overview of the disease situation in an area, but they neither measure the changing pattern of disease over time, nor the losses resulting from these diseases. Disease incidence and case-fatality can only be determined by carrying out longitudinal studies. The measurement of these key parameters permits the assessment of the economic importance of disease and the introduction of appropriate control procedures. In Kenya, studies of Moll *et al.*(1984,1986) provide examples of this type of study in which they describe the epidemiology of theilerial infections in local Zebu cattle population in an endemic area. However, similar studies in small-holder dairy cattle do not exist.

Following the results of the cross-sectional study, where it was found that a large proportion (over 70%) of the sampled cattle population had been exposed to tick-borne infections, particularly in AEZs CL3 and CL4 (section 5.1), a longitudinal investigation was designed involving small-holder dairy cattle. The study was targeted at the dairy population in the coconut-cassava (CL3) AEZ, where dairying has been encouraged through the NDDP of the Ministry of Livestock Development. The objectives of the study were to estimate the prevalence and incidence of the major disease(s) and their case-fatalities in small-holder dairy cattle managed in the zero- and free-grazing systems.

5.2.2 MATERIALS AND METHODS

5.2.2.1 Study design

The longitudinal study was carried out from June 1990 to December 1991. A total of 30 dairy herds were selected, of which 16 were managed in the zero-grazing system. The remainder were free-grazing herds. These herds represented over 70% of the small-holder dairy herds in CL3 as reported in the livestock census (Section 4.3.1 b). The herds were composed of cattle of the *Bos taurus* dairy breeds, or their crosses with other taurine breeds or with *Bos indicus* (Sahiwal or Zebu) cattle. The geographical locations of these herds and their proximity to communal cattle dips are shown in Figure 5.2.1. In general, the study herds were within 1 to 3 km from the location of communal dips in CL3.

At the beginning of the longitudinal monitoring, 125 dairy cattle, representing 69% of the cattle population in dairy herds in CL3, were included in the study. Of the 125 animals, 56 were housed in zero-grazing units and the rest kept in the free-grazing system (Table 5.2.1). The cattle were identified by their passport data gathered in the same manner as described in the cross-sectional study (Section 5.1). An exception was age, which was derived from date of birth rather than dentition of the animal. Whenever birth dates of cows were unknown, their ages were approximated from their parity. The animals known to have had one or more calvings were grouped as three years and above.

5.2.2.2 Herd size and composition

The mean herd size of dairy cattle managed in the free-grazing system was 4.9 and ranged from 2 to 7 cattle per herd. In the zero-grazing system, the average herd size was 3.5 with a range of 1 to 7 dairy animals. The age structure of the

Figure 5.2.1 Location of small-holder dairy farms in the coconut-cassava (CL3) agro-ecological zone and the distribution of active and inactive cattle dips in Kaloleni Division.

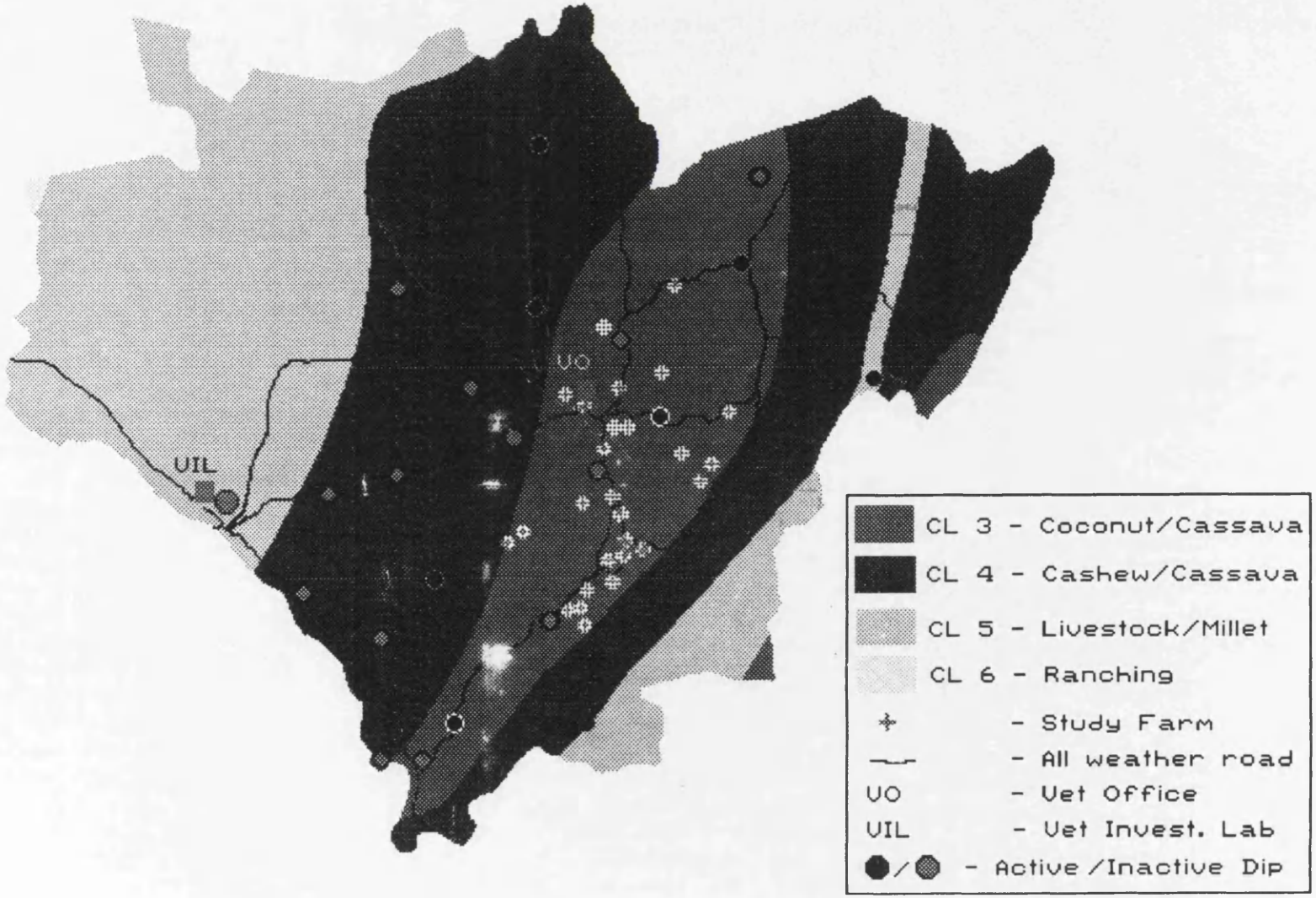

UO
UIL
CL 3 - Coconut/Cassava
CL 4 - Cashew/Cassava
CL 5 - Livestock/Millet
CL 6 - Ranching
- Study Farm
- All weather road
UO - Vet Office
UIL - Vet Invest. Lab
- Active /Inactive Dip

Table 5.2.1 Age-structure of small-holder dairy cattle in free- and zero-grazing systems at the begining of the longitudinal study .

Age (months)	Age-group	Grazing system		Total
		Free	Zero	
		n	n	n
<6	calves	10	5	15
6-12	post-weaners	12	10	22
12-24	replacement stock	7	11	18
24-36	heifers/young bulls	11	7	18
>36	adults	29	23	52
Overall		69	56	125

n : Number of animals

combined herds in their respective grazing management systems at the start of the study is given in Table 5.2.1.

Table 5.2.2 presents the initial number of females and males, categorized by age and their grazing system. Females accounted for 87% and 75% of the cattle kept in the zero-and free-grazing system, respectively. In the latter system, 37 females (71%) were of breeding age (>2 years), while a total of 30 heifers and cows (61%) were maintained in zero-grazing units.

All animals which entered the herds during the course of the study by virtue of birth, purchase or transfer were subsequently incorporated into the study. The management practices of small-holder dairy herds have been described in Chapter 4.

5.2.2.3 Rainfall data

During the longitudinal study, monthly rainfall was recorded from four locations in the coconut-cassava AEZ. The data presented are the means of the monthly rainfall recordings from these sites.

5.2.2.4 Herd monitoring and sample collection

Monitoring of the herds for diseases, particularly tick-borne diseases and trypanosomiasis, was carried out for a period of 19 months, starting June 1990. At the beginning, all animals were 'cleansed' of any trypanosome infection by treatment with 7.0 mg kg^{-1} of diminazene aceturate (Berenil^{R}, Hoechst). Young stock up to a year old, were dewormed at the start, and subsequently every alternate month, with 20ml/100kg of 1.5% w/v levamisole, 3% w/v oxyclozanide with 0.382% cobalt sulphate(Nilzan Plus^{R}, Coopers, Kenya Limited).

Herds were monitored and sampled once a month with the exception of three free-grazing herds which were left out during the dry months of January

Table 5.2.2 Number of male and female cattle categorised by age and grazing system at the start of the longitudinal study .

	Grazing system				
	Free		Zero		Total
Age (months)	Males	Females	Males	Females	
<12	12	10	6	9	37
12-24	2	5	1	10	18
>24	3	37	0	30	70
Overall	17	52	7	49	125

to March 1991 as they had been transferred temporarily to other farms in the same AEZ.

On each monthly visit, dairy cattle in all herds were clinically examined. In general, they were observed for demeanour, gait, body condition, hair coat, type of breathing, whether laboured or normal, pallor of conjunctival and/or vulval mucous membranes, texture of faeces, evidence of diarrhoea and ocular, nasal or vaginal discharges. Rectal temperatures were measured using a Centigrade thermometer. For every animal, the prescapular lymph nodes were palpated and the width diameter size (mm) of usually the right prescapular was measured with callipers (Arnold, UK). Whenever lymph nodes were enlarged, i.e., their sizes 50% larger than those observed the previous month, and or cattle had elevated temperatures of 39.5^{o}C or above, lymph node biopsies were taken using sterile 18G x 1.5 inches needles. The biopsy exudates were deposited on to glass slides and smears prepared which were air-dried and labelled before being stored in a slide box.

Blood and serum samples were obtained from all study cattle. Blood samples were collected from an ear-vein in a pair of heparinised capillary tubes, and from the jugular vein in plain vacutainers, as described in Section 4.4.1.

Faecal samples were collected from young stock to check for nematode egg burden and the efficacy of routine deworming.

5.2.2.5 Tick control

Information on tick control practices was gathered from farmers by questioning them on the frequency of acaricidal treatment, i.e., intervals in days between use, mode of acaricide application, i.e., hand spraying or dipping, and when these control measures were last implemented.

5.2.2.6 Breeding practices

The majority of the small-holder dairy farmers bred their animals using artificial insemination. Natural service was employed in two free-grazing herds. Those cattle reported pregnant by the farmer were examined by rectal palpation. In the event an animal failed to conceive following repeated inseminations, the owner was briefed on the probable causes, and whenever possible, assisted.

5.2.2.7 Laboratory analysis

Heparinised blood, lymph node biopsy smears and faecal samples were processed and analysed at the VIL Mariakani on the same day. The procedures have been described in Chapter 4. Serum samples were prepared and stored in 1 ml aliquots at -20^{o}C.

Giemsa stained thin blood smears were examined under 40 X 100 oil-immersion objectives for the presence of *Theileria* piroplasms, *Anaplasma* and *Babesia* infections. Similarly, lymph node smears stained with Giemsa were examined for theilerial macroschizonts. In addition, the status of anaemia was assessed by measuring the packed red cell volume percent (PCV) of uncoagulated blood using the micro-haematocrit centrifugation technique. Subsequently, the buffy coat preparations were examined for presence of trypanosomes by the DG technique (Murray *et al.*,1977). Identification of trypanosome species in parasitaemic cattle was based on their size and motility characteristics, and confirmed morphologically by examination of Giemsa stained thin blood smears.

5.2.2.8 Treatments

Animals exhibiting clinical signs of disease together with a febrile response (rectal temperatures above 39.5^{o}C), and detected positive for haemoparasitic

infections on microscopy were normally treated within 24 hours after laboratory diagnosis. Those in ill-health, but diagnosed negative for haemoparasitic diseases were also treated, based on clinical observations. There were few such cases.

East Coast fever cases, confirmed either by the presence of *T.parva* schizonts or both schizonts and piroplasms, were treated with parvaquone, (Clexon^R, Wellcome UK.) administered in two doses of 15 mg kg^{-1}, given intramuscularly (i.m.) 48 hours apart. Supportive therapy with intramuscular injections of multivitamins (Multivit, Intervet) and oxyteteracycline, 20 mg kg^{-1} (Terramycin LA^R, Pfizer,UK.) was given to animals in advanced stages of the disease. Not all cases of ECF were treated, as some cases were diagnosed at necropsy or had died before confirmatory diagnosis was made.

Treatment for anaplasmosis was only administered when the proportion of *Anaplasma marginale* parasitised erythrocytes exceeded 5% of the total red cells observed per microscopic field, and the corresponding animal's rectal temperature was above 39.5^{O}C. Oxytetracycline (Terramycin 100^R, Pfizer UK) at a dosage of 10 mg kg^{-1} given i.m. was used for treating clinical anaplasmosis.

Trypanosome parasitaemic cattle were treated with 7.0 mg kg^{-1} diminazene aceturate (Berenil^R, Hoechst) given i.m. The same treatment regime was used for cases of babesiosis.

In addition to routine deworming, young stock with strongyle and or strongyloides egg counts of greater than 500 e.p.g.of faeces were drenched with Nilzan Plus anthelmintic at a dose of 5ml/20kg bodyweight.

5.2.2.9 Mortalities

In the event of mortalities, post-mortem examinations were performed in the field and specimens submitted to the VIL for confirmatory diagnosis. Carrying out necropsies for all cases of mortalities proved to be difficult as farmers

sometimes failed to report the occurrence of deaths in their herds. In such situations, the cause of death was recorded as undiagnosed.

5.2.2.10 Additional information

Entries of all cattle into the study herds as a result of birth or purchases, and exits due to mortality, sale or slaughter were recorded. In addition to the monthly sampling, each herd was visited at least once more prior to the next sampling. These supplementary farm calls helped to provide information on births, sales and purchases of cattle within the herd. At the same time, it allowed a general check on health status of the herd. Animals observed to be unwell were clinically attended and relevant samples collected for confirmatory diagnosis.

Animals observed to be in ill-health in-between the monthly monitoring and farm visits were attended by the local animal health assistant (AHA) from the Veterinary Office. Under such circumstances, a tentative diagnosis was made from the description of clinical signs and treatment given. This was done only when the AHA's failed to submit biological samples to the laboratory for confirmatory diagnosis. If an animal died, a presumptive diagnosis was based on clinical history prior to death, nature of any treatment administered, and post-mortem findings if performed by the AHA. In cases where the cause of mortality was uncertain, it was recorded as undiagnosed.

5.2.2.11 Serology

a) Detection of *Theileria parva* antibodies

Serum samples were assayed for antibodies to *T.parva* schizont antigen using the IFAT (Goddeeris *et al.*, 1982). Every alternate month sera were screened for the presence of theilerial antibodies at 1:40 dilution. The procedure has been described in Chapter 4.

b) Detection of trypanosome antigens and antibodies

Serum samples were assayed for species-specific trypanosome antigens using the ELISA procedure described by Nantulya and Lindqvist (1989). The antigen-capture ELISA was performed on sera collected for the first 13 months (June 1990 to June 1991) of the study. Detection of antibodies to trypanosome infection was carried out in every alternate month serum samples using an ELISA as reported by Luckins (1977). Details of both techniques have been described in Chapter 4.

5.2.2.12 Data recording

All field and laboratory data of study cattle were recorded on pre-designed data sheets for each herd. Information containing bio-data of every animal present during the monitoring period was entered into a computer database management system, DBase III plus software package (Ashton and Tate, 1986).

5.2.2.13 Data presentation

a) Categorisation of cattle

Dairy herds involved in the study were grouped according to their grazing management practices as either free- or zero-grazing herds. Analyses at the herd level were impractical as herd sizes were small. Thus, comparative analyses of the data were based on individual animals grouped by their relevant grazing management practices. Under these circumstances, variations between and within the herds in either zero- or free-grazing systems could not be taken into consideration.

Categorisation of animals according to their respective grazing systems was carried out based on the following criteria.

1. All herds resided in the coconut-cassava AEZ throughout the study period.

2. All zero-grazing herds were managed similarly through attempting to adopt the nutritional requirements and health practice recommendations issued by NDDP of the Ministry of Livestock Development. These animal husbandry practices have been described in Chapter 4.

3. Grazing of the extensively managed free-grazing herds was entirely in the coconut-cassava AEZ. In the dry season, animals trekked longer distances, though never exceeding more than 5 kms, in search for pasture, but always grazed within the AEZ.

4. Tick control was practiced by all small-holder farmers in the study. Most of the free-grazing farmers controlled ticks by dipping their cattle in aqueous acaricide suspensions, while hand spraying of dairy animals with acaricides diluted to recommended concentration was the method of choice in the zero-grazing system. On average, the interval between acaricidal applications for controlling ticks was reported to be once every 10 days. This information was provided by farmers' response to questions pertaining to tick control practices.

5. Besides the veterinary care provided by the research team, all small-holder farmers called upon the local AHA or the veterinarian from the nearest Divisional Veterinary Office when requiring veterinary attention for their animals. None of these farmers sought the services of private veterinary practitioners.

b) East Coast fever immunisation

Towards the end of November 1990, 45 (24%) of the cattle in the study at that time were immunised against ECF using the infection and treatment (I&T) method (Radley, 1981). The breakdown of immunised and non-immunised animals is presented in Table 5.2.3. Therefore, in the analysis of data involving the calculation of incidence, prevalence and sero-prevalence of ECF, these cattle

Table 5.2.3 Number of study cattle that were immunised and not immunised against East Coast fever in zero- and free-grazing systems in the coconut-cassava agro-ecological zone of Kaloleni Division.

Grazing system	No. of dairy cattle		Total
	Immunised*	Non-immunised	
Zero	30	57	87
Free	15	93	108
Overall	45	150	195

* Immunisation by Infection and Treatment method

were excluded from the time they were immunised as they were considered not to be at risk.

c) East Coast fever

The prevalence, risk, incidence, cumulative incidence and antibody prevalence rates of ECF in dairy cattle in their respective grazing systems were estimated as described below.

i) Prevalence rate

The percent prevalence of ECF at each monthly sampling, was calculated as the number of cattle contracting the disease during the month, multiplied by 100, divided by the number of cattle tested.

ii) Risk rate

The risk rate of ECF during the monitoring period was calculated as follows.

$$\frac{\text{Number of animals contracting ECF}}{\text{Number of animals at risk}}$$

$$\text{Risk rate } p = \frac{r}{[n + \frac{a - (i + l)}{2}]}$$

where r = number of animals acquiring the disease under investigation (ECF),

n = initial number of animals at risk,

a = new entries of cattle during the study period,

i = number of immunised cattle present at the end of the study,

l = exits recorded due to other causes besides ECF.

This estimate was calculated on the assumption that infections were equally likely to occur over the monitoring period.

iii) Incidence

To calculate the incidence of ECF, two methods were used. These were incidences derived as function of animals at risk and animal months.

Incidence: animals at risk

In order to express the incidence rate of ECF within an age-specific group, all cattle were grouped into age-classes of 6 month intervals; 0-6 months, 6-12 months, 12-18 months, 18- 24 months, etc.

Age-specific incidence of ECF was calculated as:

$$\frac{\text{Number of cattle contracting the disease in an age-class}}{\text{Number of cattle at risk for 6 months in that age-class}}$$

An animal would be considered to be at risk in a particular age-class if present for all 6 months of that age-class (Figure 5.2.2). Example A illustrates a calf born at the start of the study and present for the entire period. This calf would be regarded as being at risk in three age-classes 0-6, 6-12 and 12-18 months. Similarly, if a calf was born during the study and completed 12 months before being sold (example B), it would be regarded as being at risk in both the 0-6 month and 6-12 month age-classes. An animal greater than 36 months of age at the start and present during the entire study would be considered to be at risk on three occasions for the three six-monthly periods.

In case an animal contracted ECF, and died before completing 6 months in its age-class (example C), it would be included as an animal at risk for that age-class as it acquired the event of interest under investigation. On

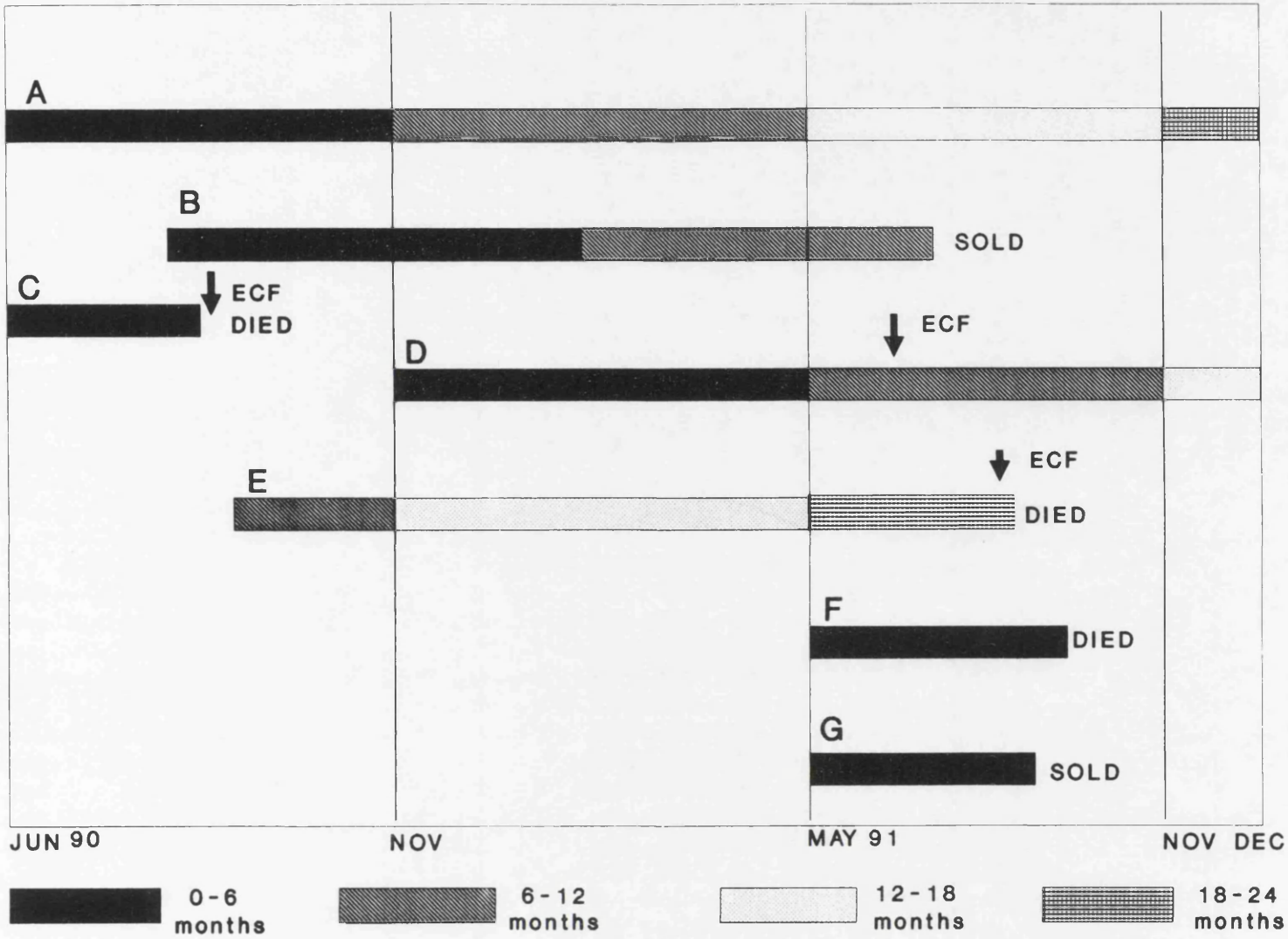


Fig5.2.2 A schematic diagram explaning the age-specific categorisation of animals at risk during the longitudinal study

the other hand, an animal recovering from ECF following treatment (example D), would be counted as being at risk up to the age-class it contracted the disease, but excluded from subsequent age-classes on the assumption that such an animal became immune and was no longer at risk.

In a situation where an animal entered the study in the middle of an age-class, for example, at 9 months of age, and died from ECF 12 months later, it would only be regarded at risk in 12-18 and 18-24 month age-classes (example E).

In the event an animal in any age-class left the study by virtue of being sold or dying from an unrelated cause, such a case would be excluded from being at risk for ECF for that age-class (example F and G).

The incidence and risk rate of ECF were calculated on the basis of an animal contracting ECF for the first time during the study period, irrespective of their previous *T. parva* schizont antibody status.

Incidence: animal months

Age-specific incidence of ECF expressed in animal months at risk was calculated as

$$\frac{\text{Number of cattle in a particular age-class acquiring ECF}}{\text{Total animal-months at risk contributed by animals in the age-class}}$$

Animal-months at risk in any age-class were calculated as sum of individual animal months contributed by animals present in that age-class. In the event an animal developed ECF, it would only contribute animal-months up to the time it contracted the disease.

The incidence was multiplied by six in order to correct for the observation period and to obtain the incidence rate over 6 months. Similarly, overall

incidence over the 19 month study period was calculated as the number of first occurrence of ECF cases, multiplied by 19 and divided by the total number of animal months contributed by the animals at risk.

iv) Cumulative incidence

Cumulative incidence can be calculated as an estimate of a susceptible animal contracting ECF under the small-holder dairy farming management systems by age of 6 months using

$$1 - (1-p_1)$$

where p_1 is the incidence estimated for 6 months of age. Similarly, cumulative incidence for a susceptible animal contracting ECF at ages 12 months, 18 months, 24 months, etc, will be

$$1 - (1 - p_1)(1 - p_2),$$

$$1 - (1 - p_1)(1 - p_2)(1 - p_3),$$

$$1 - (1 - p_1)(1 - p_2)(1 - p_3)(1 - p_4),$$

:

:

etc., where p_2, p_3, p_4 ... etc. were the observed incidences by 12, 18 and 24 months of age respectively.

v) Logistic regression models for occurrence of East Coast fever

Linear logistic regression models (Collett, 1991) were used for analysis of occurrence of ECF. The data set analysed excluded those animals which were immunised against ECF. The variable analysed was the occurrence of ECF. The logistic regression models included the effects of grazing system G_i, where $i = 0$ or 1 with levels zero or free-grazing; age category of animal A_j, where $j = 1..4$, with levels 0-6 months, 7-18 months, 19-36 months, >36 months; sex of

animal L_k, where k=1 or 2, with levels male or female. The logistic model explored was of the form:

$$\log \frac{(p_{ijk})}{(1-p_{ijk})} = \mu + G_i + A_j + L_k$$

where p_{ijk} is the probability of an animal contracting ECF in grazing system i, of age j and sex k, and μ is an overall 'mean'. The model can be extended to include interaction terms, but was not expanded as the two factor interactions were not significant.

The parameters of the model were estimated by maximum likelihood, using Genstat 5 (Lawes Agricultural Trust, 1990). The statistical significance of effects was tested using deviances which have approximate chi-squared distributions. Predicted probabilities were estimated using the inverse of the logit transformation given by :-

$$p = \frac{\exp(\mu + G_i + A_j + L_k)}{1 + \exp(\mu + G_i + A_j + L_k)}$$

Approximate standard errors for the probabilities were also obtained.

vi) Age-specific antibody prevalence

Age-specific antibody point prevalence of *T. parva* was estimated at 6 month intervals. This was calculated as the proportion of cattle with antibodies after completing 6 months in an age- class in relation to the number of cattle in that age cohort.

d) Trypanosomiasis

The percent monthly prevalence of cattle with trypanosome antigens was calculated as the number of antigen positive (for any trypanosome species)

cattle in any given month, multiplied by 100, and divided by the number of cattle tested in that month.

The percent prevalence of trypanosome antibodies, tested every second month, was calculated similarly.

e) Anaemia status

Anaemia status was assessed by measuring the packed red cell volume percent (PCV) of cattle. The mean monthly PCV of cattle kept in two management systems was calculated as the mean of PCV recordings during each sampling month.

In order to present the age-specific average PCVs, the mean PCV of each age-cohort was calculated for those cattle present for the entire 6 months period. For each animal in each age-cohort, average PCV was calculated from four or more individual PCV measurements. If the number of PCV recordings were less than four, the animal was excluded from the group. Differences in mean PCV between age-classes were tested using two sample t tests.

f) Mortality rate

The crude mortality rate was estimated using the formula below

$$\text{Mortality rate (MR)} = \frac{\text{Total deaths recorded over the study period}}{\text{Number of cattle at risk}}$$

$$= \frac{m}{n + \frac{(a-w)}{2}}$$

where m = number of mortalities of cattle during study period

n = initial number of animals at risk

a = new entries of cattle during the study

w= withdrawals due to sale or slaughter of animals

The denominator was calculated as the initial number of cattle at the start of the study, adjusted for subsequent additions and withdrawals over the course of the study as described in estimating the risk rates. Cattle immunised against ECF were not included in the withdrawals as immunisation does not prevent mortalities from other diseases.

g) Case-fatality rate

This was estimated as the number of animals dying from the disease under investigation divided by the total cases diagnosed as acquiring the disease of interest. In calculating the case-fatality of ECF, two cases having more than one episodes and three animals contracting the disease post ECF immunisation were included in the calculation.

h) Statistical analysis

Unless specified otherwise, comparison of differences in the occurrence of diseases, particularly the prevalence, risk, and incidence of ECF in small-holder dairy cattle kept under free-and zero-grazing systems were tested using chi square tests. Differences at the 5% significance level were considered statistically significant.

5.2.3 RESULTS

5.2.3.1 Rainfall

The mean monthly rainfall distribution from June 1990 to December 1991 is presented in Figure 5.2.3. A total of 1205 mm of rainfall was recorded during the study period with rains occurring in all but two months of the study. After the end of the long rains in June 1990, there was a short relatively dry spell of two months. Thereafter, a short rainy season of 3 months from September

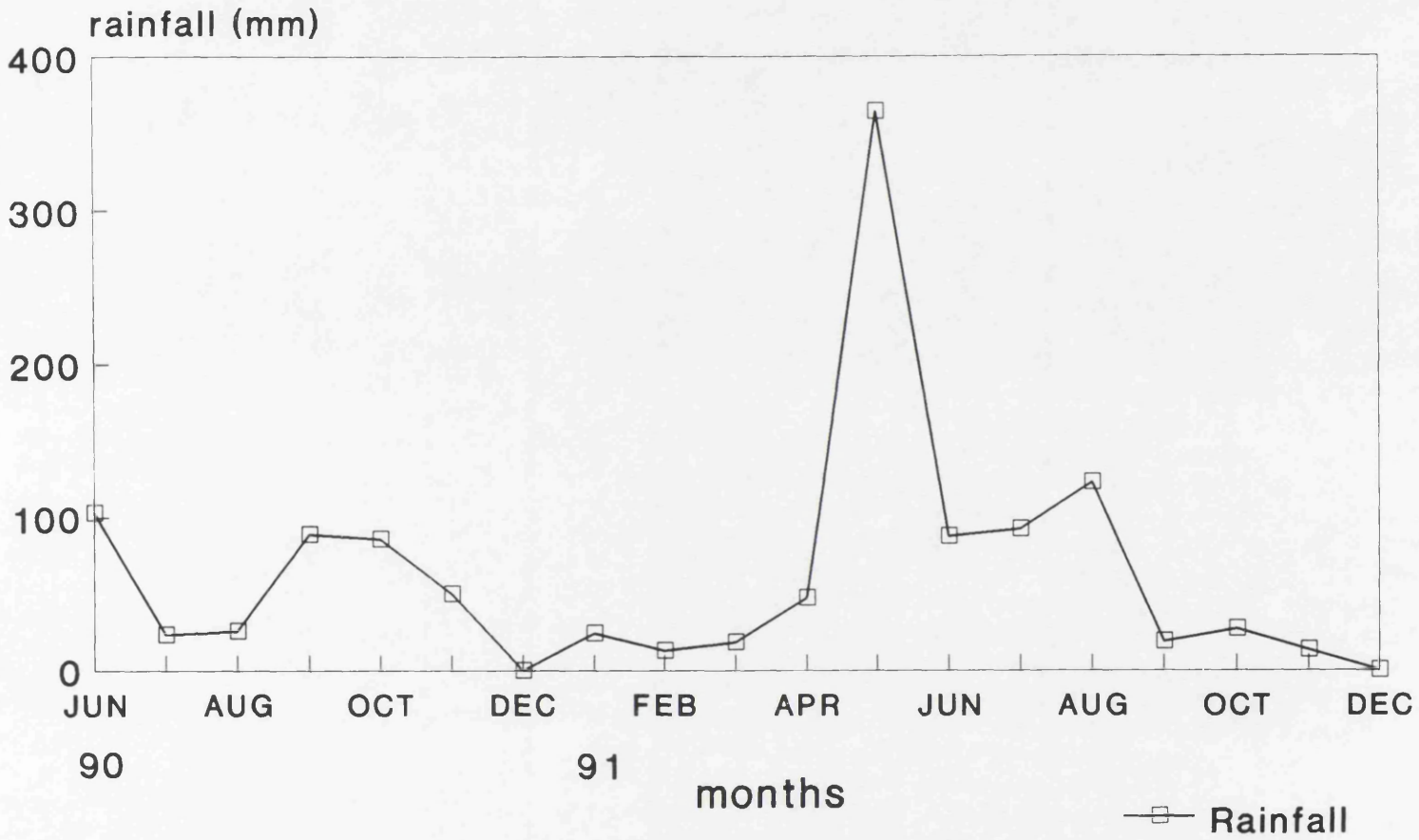


Fig.5.2.3 Mean monthly rainfall during the longitudinal study in the coconut-cassava agro-ecological zone of Kaloleni Division

1990 to November 1990 prevailed. This was followed by the long dry season of 4 months (December 1990 to March 1991) with only 55 mm of precipitation. The next rainy season lasted 5 months (April 1991 to August 1991) with May 1991 being the wettest month. A rainfall of 365mm in this single month accounted for 30% of the total precipitation recorded during the study period. The short rains failed and the last 3 months were relatively dry.

5.2.3.2 Population changes

The population changes of the small-holder dairy cattle maintained under free-and zero-grazing systems during the study period are presented in Table 5.2.4. In addition to 125 animals initially selected, 70 more entered the herds during the study period, of which 73% were calf births. On the other hand, there were 80 exits, with 56% resulting from death. In total, 195 animals contributed comprehensive records to the study. By the end of the longitudinal monitoring, four small- holder dairy farmers, two practicing zero-grazing and two free- grazing abandoned dairying due to losses from disease and labour constraints. Overall, a reduction of 8% in the study cattle population was observed.

5.2.3.3 Disease occurrence

During the 19 months of the monitoring period, a total of 78 out of 195 (40%) dairy cattle managed by the small-holder farmers suffered from one or more incidences of clinical disease. Clinical disease, in this context, was defined as those cases which required therapeutic interventions or those which resulted in mortalities. The diseases encountered were ECF, anaplasmosis, babesiosis, trypanosomiasis and helminthiasis together with some seven fatal cases which could not be conclusively diagnosed. Figure 5.2.4 displays the proportional occurrence of diseases in the small-holder dairy cattle observed during the

Table 5.2.4 The population changes of the study sample of small-holder dairy cattle during the longitudinal study in the coconut-cassava agro-ecoogical zone (AEZ) of Kaloleni Dvision, coastal Kenya (June 1990- December 1991).

Grazing system	No. of herds	N_i	Entries		Exits		N_e
			Births	Purchases	Deaths	Sales	
		bf					bf
Free	14	69 (40)	30	9	32	16	60 (36)
Zero	16	56 (30)	25	6	13	19	55 (34)
Overall	30	125 (70)	55	15	45	35	115 (70)

N_i Number of cattle at the start of the study

N_e Number of cattle at the end of the study

bf The figures in parenthesis represents number of breeding females

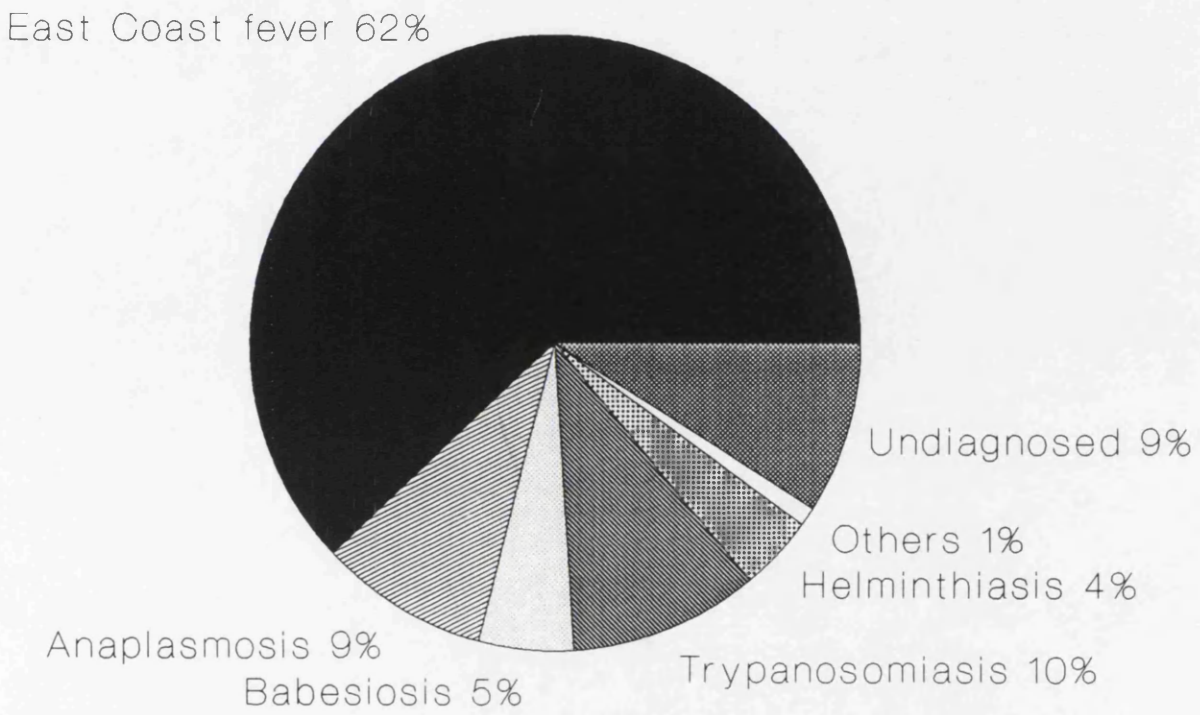


Fig. 5.2.4 Occurrence of diseases in small-holder dairy cattle in the coconut-cassava agro-ecological zone of Kaloleni Division

course of study. Tick-borne diseases (TBD) were the most common, accounting for more than 75% (59 out of 78) of all clinical cases.

a) East Coast Fever

East Coast fever was diagnosed as the disease of major importance, responsible for over 80% (48 out of 59 cases) of the TBD's and nearly two thirds (62%) of all disease occurrences. Of the 48 cases of ECF diagnosed in 46 cattle, two animals had a second episode of the disease and three cases occurred in immunised cattle.

In both free-and zero-grazing management systems, ECF was the most common disease (Figure 5.2.5) occurring in 21 out of the 30 study herds. More herds were affected by *T. parva* infections in the free-grazing system (12 out of 14), than in zero-grazing system (9 out of 16).

Although clinical cases of trypanosomiasis, anaplasmosis, babesiosis and helminthiasis were diagnosed in dairy cattle, in total, any single disease did not contribute to more than 15% of all detected clinical disease occurrences.

i) Prevalence of East Coast fever

East Coast fever was diagnosed throughout the course of the study, with the exception of the month of December 1991. These observations indicated that there were no marked seasonal differences in the prevalence of the disease between the relatively dry and wet rainfall months (Figure 5.2.6).

Overall, the mean monthly prevalence of ECF was 2.9% with the prevalence ranging from 0 to 7.7%. There was an increase in the prevalence of the disease one month after the beginning of the main rainy season in May 1991.

The overall monthly prevalences of ECF between zero- and free-grazing cattle were significantly different. East Coast fever was detected in

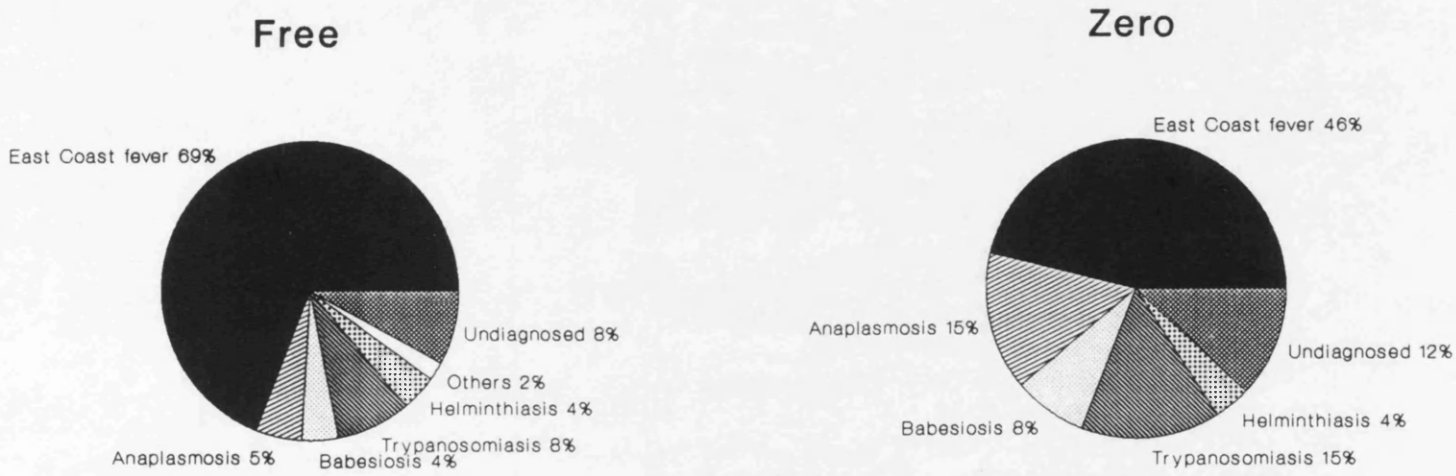


Fig.5.2.5 Occurrence of diseases in small-holder dairy cattle managed in two grazing systems in the coconut-cassava agro-ecological zone of Kaloleni Division

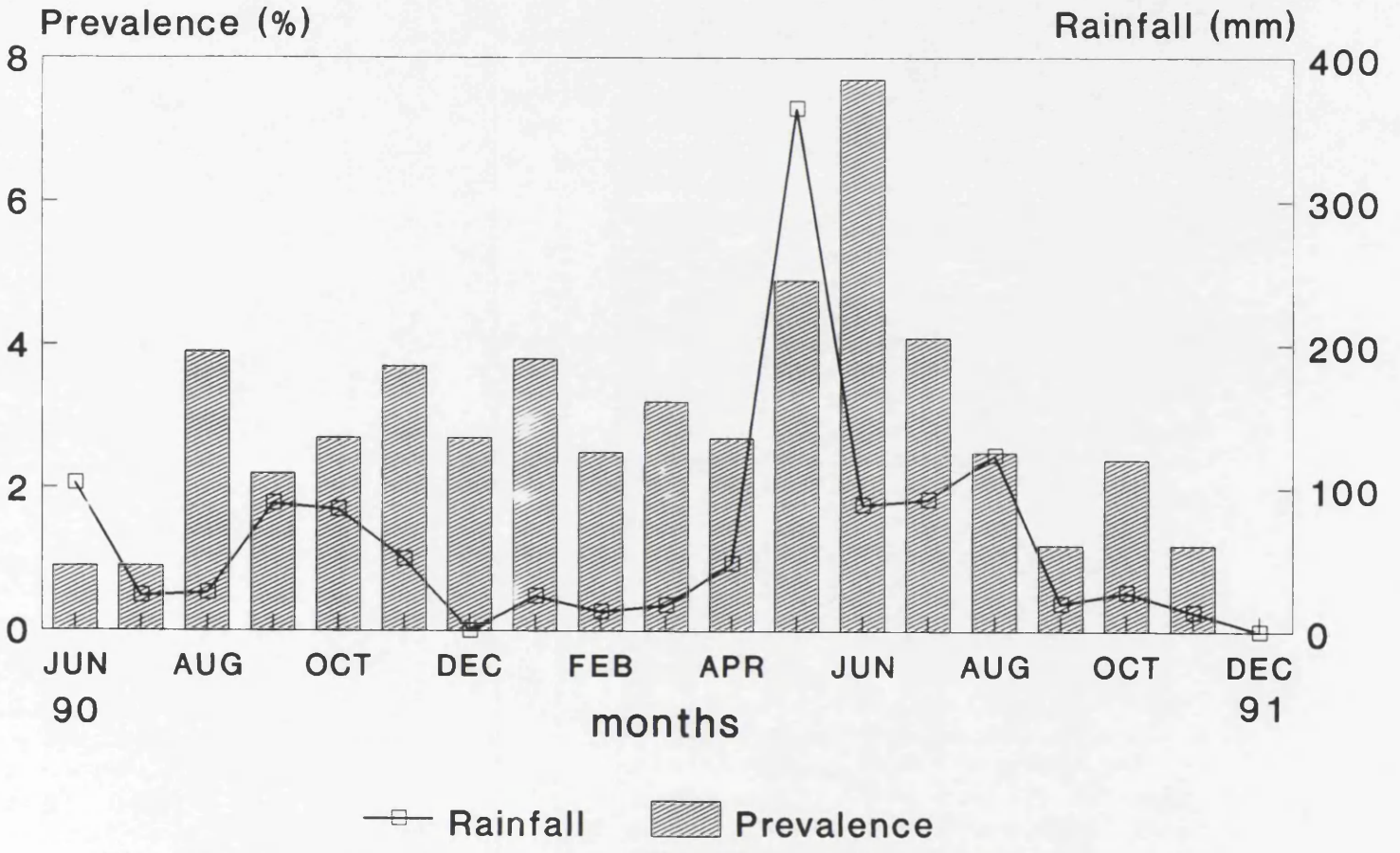


Fig 5.2.6 The prevalence of East Coast fever (ECF) during monthly testing of small-holder dairy cattle in relation to rainfall in Kaloleni Division

free-grazing dairy cattle in all, but four months of the study, where 34 ECF cases were diagnosed from a total of 953 observations. The mean monthly prevalence was 3.6% (95% confidence interval 2.5%-4.7%) with a range of 0 to 10% (Figure 5.2.7). On the other hand, in the zero-grazing system, episodes of ECF occurred in cattle in two periods; the first from June to October 1990, with the exception of July 1990, and the second from June to August 1991 (Figure 5.2.7). In both periods, the episodes of the disease coincided with the rainy seasons. A total of 11 ECF cases from 674 observations over 19 months gave a mean monthly prevalence of 1.6% (ci 0.7%-2.5%) with a range from 0 to 7.7%.

A two-fold or more difference in the *T. parva* antibody prevalence between animals in the zero and free-grazing management systems was significantly different. The bi-monthly antibody prevalences of cattle in the two management systems are presented in Figure 5.2.8. In free-grazing cattle, 356 of 461 samples tested were positive, giving an overall prevalence of 77%. The results showed a high exposure to ECF in free-grazing cattle with the antibody prevalence ranging from over 60% to 91%. On the other hand, the overall prevalence in zero-grazed cattle was low, 26% (87 positive out of 337 samples tested), and ranged from 12% to 39%.

ii) Incidence and risk rates of East Coast fever

The incidence and risk rates of ECF estimated over 19 months of the study are shown in Table 5.2.5. Incidence rate of ECF, expressed using animal-months at risk, in free-grazing cattle (0.58) was twice that of the cattle in the zero-grazing system (0.29). Expressing incidence rate by animal-months did not allow for statistical comparison as the animals-months reflect time contributed by the population at risk rather than individual number of animals at risk. Therefore, risk-rates were used. The estimated risk rate of ECF, over the same time

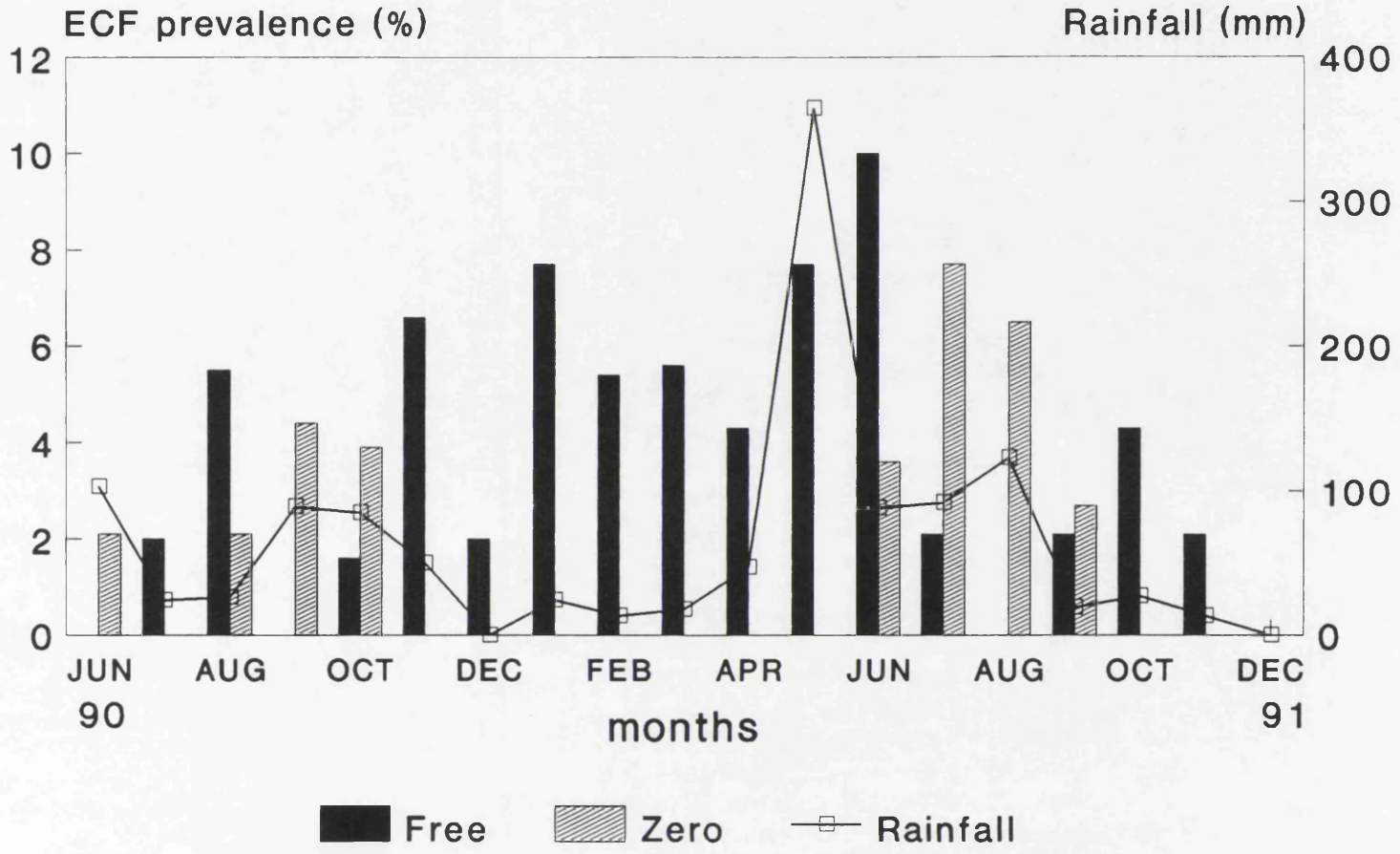


Fig.5.2.7 The prevalence of East Coast fever (ECF) during monthly testing of small-holder dairy cattle managed in free and zero-grazing systems in relation to rainfall at Kaloleni

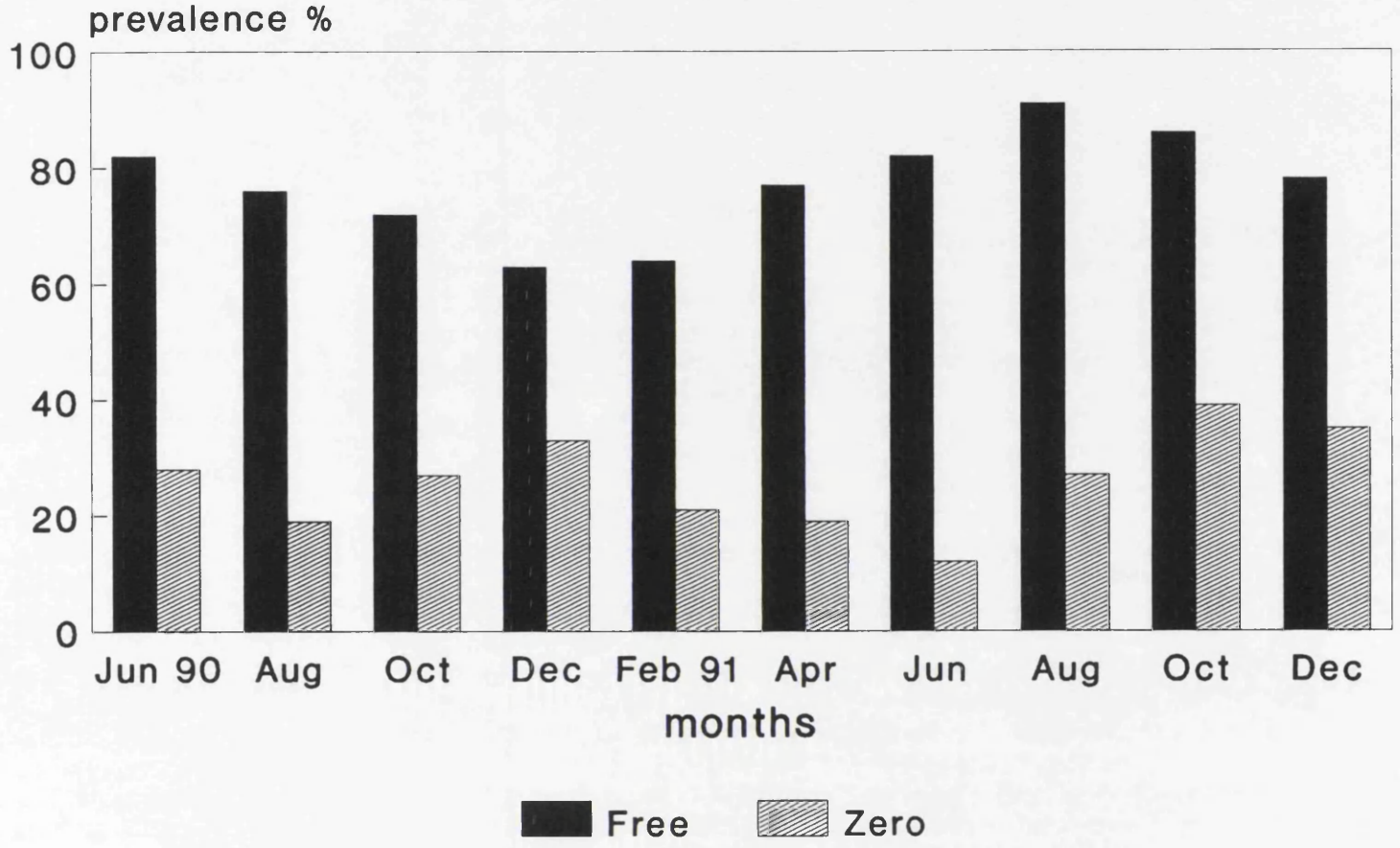


Fig.5.2.8 The prevalence of *Theileria parva* antibodies during bi-monthly testing of small-holder dairy cattle managed in free-and zero-grazing systems in Kaloleni Division

Table 5.2.5 The incidence and risk rates of East Coast fever (ECF) in small-holder dairy cattle during 19 months of the longitudinal study maintained in free-and zero-grazing systems

Grazing system	Animal months	Animals at risk	No. of ECF cases	Incidence [a] rate	Risk [b] rate
Free	1044	69	32	0.58	0.46*
Zero	729	41	11	0.29	0.27

* Significantly different in the column ($p < 0.05$)

a Incidence rate expressed as a function of animal-months over 19 months period (statistical comparision of incidence rates was not possible as the denominator used were animals-months rather than individual animals as experimental units)

b Risk rate estimated as a function of animals at risk derived from the risk rate formula

period, in free-grazing cattle was 0.46, whereas in those in the zero-grazing management system, the risk rate of 0.22 was significantly lower. Both forms of presentation showed that the incidence and risk rates of ECF was twice as high in free-grazing cattle than in cattle which were housed in the zero-grazing units.

iii) Age-specific incidence of East Coast fever

Age-specific incidence of ECF estimated for animals at risk over 6 months intervals is given in Table 5.2.6. The majority of the *T. parva* infections were diagnosed in animals less than 18 months of age; 81% and 73% occurring in cattle in the free-and zero- grazing systems, respectively. Incidence rates of ECF in these young age-cohorts were generally higher (0.40 to 0.57) in free-grazing cattle than in their counterparts in the zero-grazing system (0.20 to 0.40), the exception being the 6-12 month age-class, where they did not differ. On the other hand, ECF incidence in older (>18 months) age-classes did not exceed 0.2 in either of the management systems. Within the free-grazing system, the occurrence of ECF was significantly higher in 18 months or less age-cohorts than in older (above 18 months) age-groups. In contrast, differences in the occurrence of ECF between similar age cohorts in zero-grazing animals were not significant.

Age-specific incidence expressed as a function of animal months at risk is presented in Table 5.2.7. Estimates of ECF incidence for similar age-classes in the two management systems were comparable with the incidences presented as proportions of animals at risk. In the free-grazing system, age-specific ECF incidences ranged from 0 to 0.57, whereas in the zero-grazing system, the incidences never exceeded 0.25. In both forms of presentation, similar trends in the age-specific incidences of the disease were observed (Figure 5.2.9).

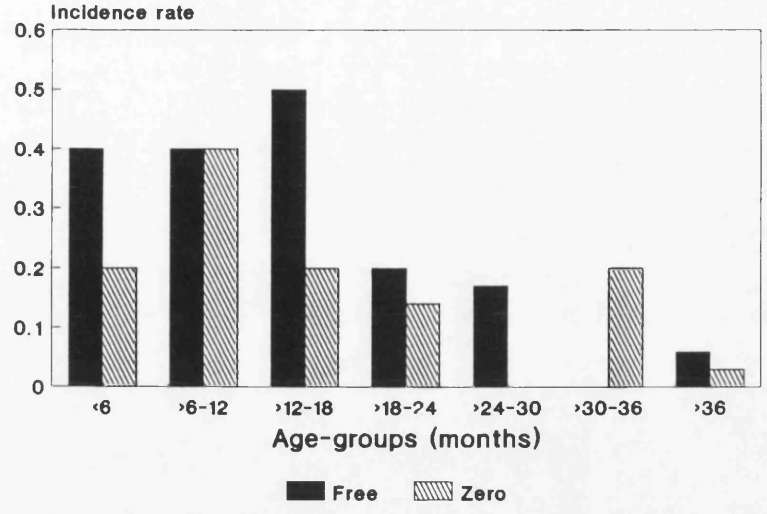
Table 5.2.6 Age-specific incidence rates of East Coast fever (ECF) expressed as a proportion of animals at risk in small-holder dairy cattle managed under the free- and zero-grazing systems in the coconut-cassava agro-ecological zone of Kaloleni Division

	Free-grazing			Zero-grazing		
Age-class (months)	Animals * at risk	No. of ECF cases	Incidence rate	Animals * at risk	No. of ECF cases	Incidence rate
<6	25	10	0.40	15	3	0.20
>6-12	20	8	0.40	10	4	0.40
>12-18	14	8	0.57	5	1	0.20
>18-24	4	1	0.25	7	1	0.14
>24-30	5	1	0.20	3	0	0.00
>30-36	7	0	0.00	5	1	0.20
>36	68	4	0.06	34	1	0.03

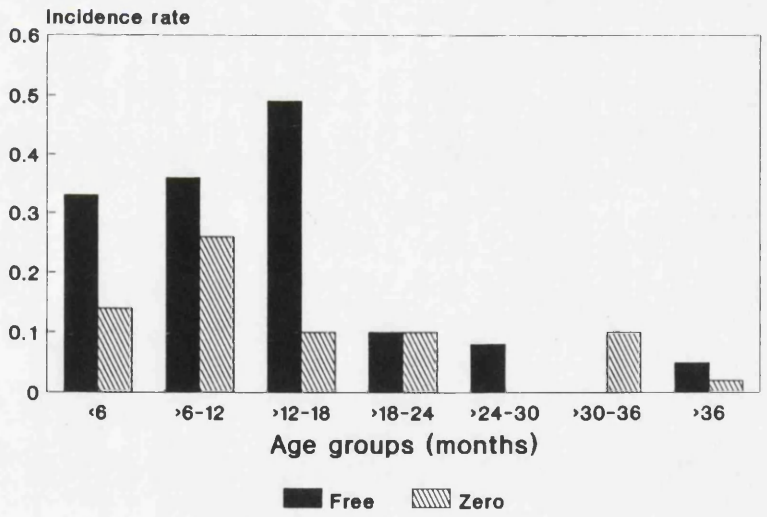
* Animals at risk were those that were present for the entire age-class period and included those that contracted ECF in that age-class

Table 5.2.7 Age-specific incidence rates of East Coast fever (ECF) expressed over six animal-months in small-holder dairy cattle managed under the free- and zero-grazing systems in the coconut-cassava agro-ecological zone of Kaloleni Division

	Free-grazing			Zero-grazing		
Age-class (months)	Animals months	No. of ECF cases	Incidence	Animals months	No. of ECF cases	Incidence
<6	180.2	10	0.33	136.6	3	0.13
>6-12	133.7	8	0.34	94.4	4	0.25
>12-18	84.9	8	0.57	58.7	1	0.10
>18-24	55.4	1	0.11	61.7	1	0.10
>24-30	69.7	1	0.09	44.4	0	0.00
>30-36	67.3	0	0.00	43.4	1	0.10
>36	452.4	4	0.05	289.5	1	0.02



Animals at risk



Animal months

Fig.5.2.9 Age-specific incidence rates of East Coast fever in small-holder dairy cattle expressed over six-monthly interval as functions of animals at risk and animal-months

iv) Cumulative incidence

The cumulative incidence of ECF in dairy cattle kept under the two management systems is shown in Figure 5.2.10. The results show that in this study the probability of free-grazing animal contracting ECF by 18 months of age was 0.83. Likewise, in the zero-grazing system, for the same age-cohort, the probability was 0.62.

In total, of the 43 first episodes of ECF cases observed in 150 cattle during the course of the study, 23 out of 50 (46%) were detected in males. The occurrence 20 ECF cases in 100 females (20%) was significantly lower than the number of cases observed in males. In addition, the number of ECF cases occurring in males (16 out of 31) and females (16 out of 60) in the free-grazing system were significantly different. The higher ECF incidence in younger age-groups in the zero-grazing system was mainly due to the majority of the infections, 7 out of 11 ECF cases occurring in males. As a result, the proportion of ECF cases in males kept in the zero-grazing system (7 out of 19) was significantly higher than the 4 out of 40 cases observed in females.

v) Logistic regression analyses

Analysis using the logistic regression models for probability of contracting ECF is shown in Table 5.2.8. The results were generally consistent with the previous analysis reported. Differences observed between grazing systems and between animal age-classes were statistically significant. Cattle in the free-grazing system had a two-fold higher probability (0.34) of occurrence of ECF than those kept in the zero-grazing system (0.16). In addition, the probability of age-related occurrence of ECF ranged from 0.07 to 0.44, with a higher probability of 0.40 and above occurring in less than 18 months old young stock. On the other hand, statistical differences between animal age-classes occurred due to a lower probability (0.07) of contracting ECF in over 36 months old adult

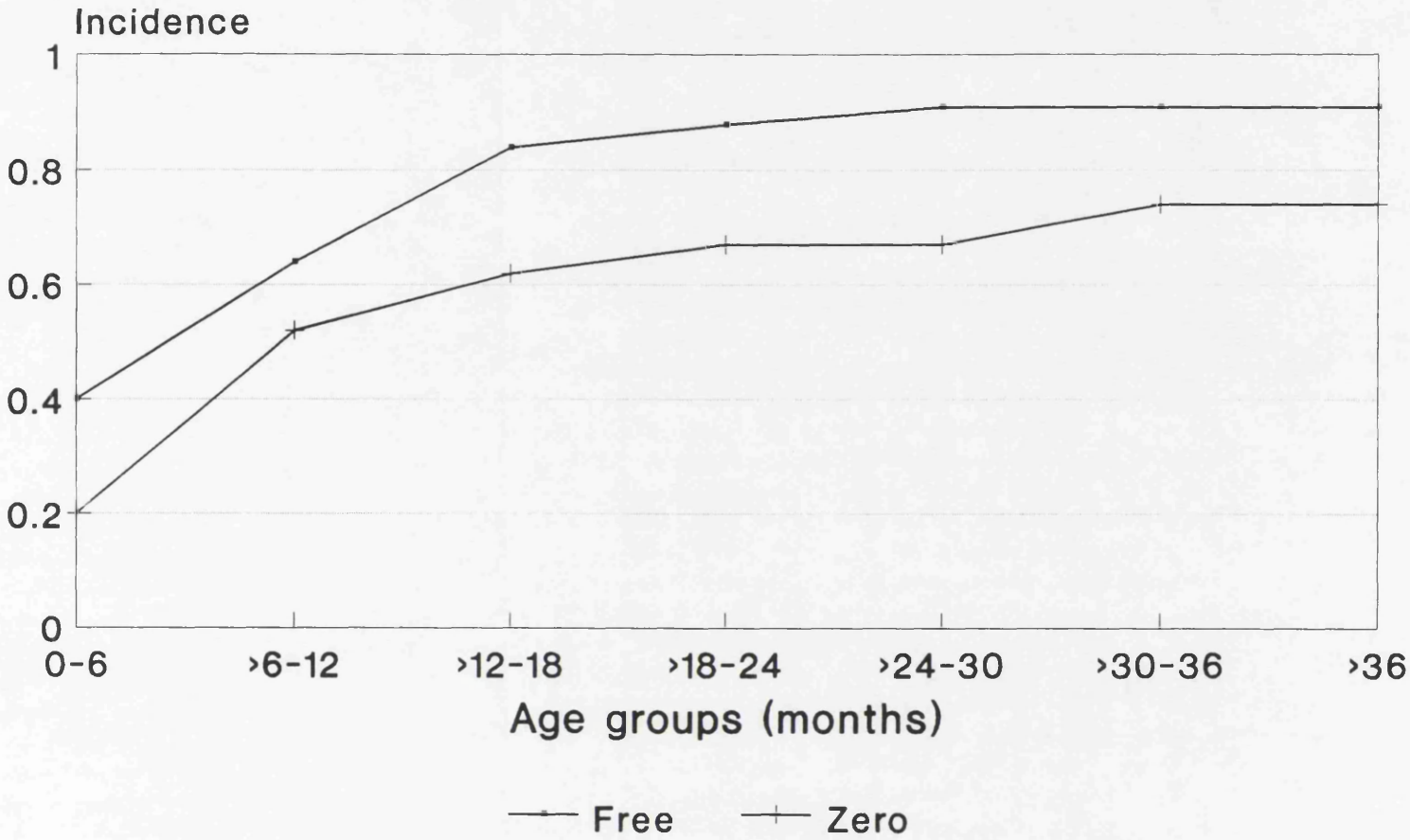


Fig 5.2.10 Cumulative incidence of East Coast fever in smallholder dairy cattle managed in free- and zero-grazing systems at Kaloleni

Table 5.2.8 The probablity of contracting East Coast fever estimated using logistic regression in small-holder dairy cattle during the longitudinal study in the coconut-cassava agro-ecological zone of Kaloleni Division

	n	probability	s.e	p <0.05
Grazing system				
Zero	57	0.16	0.05	*
Free	93	0.34	0.05	
Age-class (months)				
<6	27	0.40	0.09	
6-18	51	0.44	0.07	
18-36	18	0.22	0.10	
>36	54	0.07	0.04	*

Analysis of deviance using logistic regression

Analysis of deviance	Degrees of freedom	Deviance	p<0.05
Grazing system	1	6.5	*
Animal age-class	3	22.7	*
Residual	145	146.7	
Total	149	176	

* Significant at the 5% level

cattle. Sex of animal was excluded from the logistic regression model due to fewer number of males in the last two age-classes (less than four).

vi) Antibody prevalence

Figure 5.2.11 shows the age-specific antibody prevalence to *T. parva* schizont antigen in cattle at risk in the zero-and free- grazing management systems. By the time cattle attained 2 years of age, all of them in the free-grazing herds had developed antibodies to *T. parva*. On the contrary, cattle which were confined in stalls in the zero-grazing system had a lower antibody prevalence, never exceeding 40%.

b) Other tick-borne diseases

i) Anaplasmosis

There were a total of 27 *A. marginale* infections detected, but only seven showed clinical disease which warranted therapy. Of the seven cases, four were diagnosed in cattle raised in zero-grazing units. In contrast to ECF, no cases of the disease were detected in calves or young stock up to a year old. Anaplasmosis was observed in four cattle above 2 years old and in three between 1 to 2 years of age. All cattle treated fully recovered.

ii) Babesiosis

Babesia bigemina infections were observed in four cattle, two in free-grazing and the other two in a single zero-grazing herd. The disease was diagnosed in three cattle over 3 years of age, and in a nine month old weaner calf kept in a zero-grazing unit. All four *Babesia* infections were cured successfully by chemotherapeutic intervention.

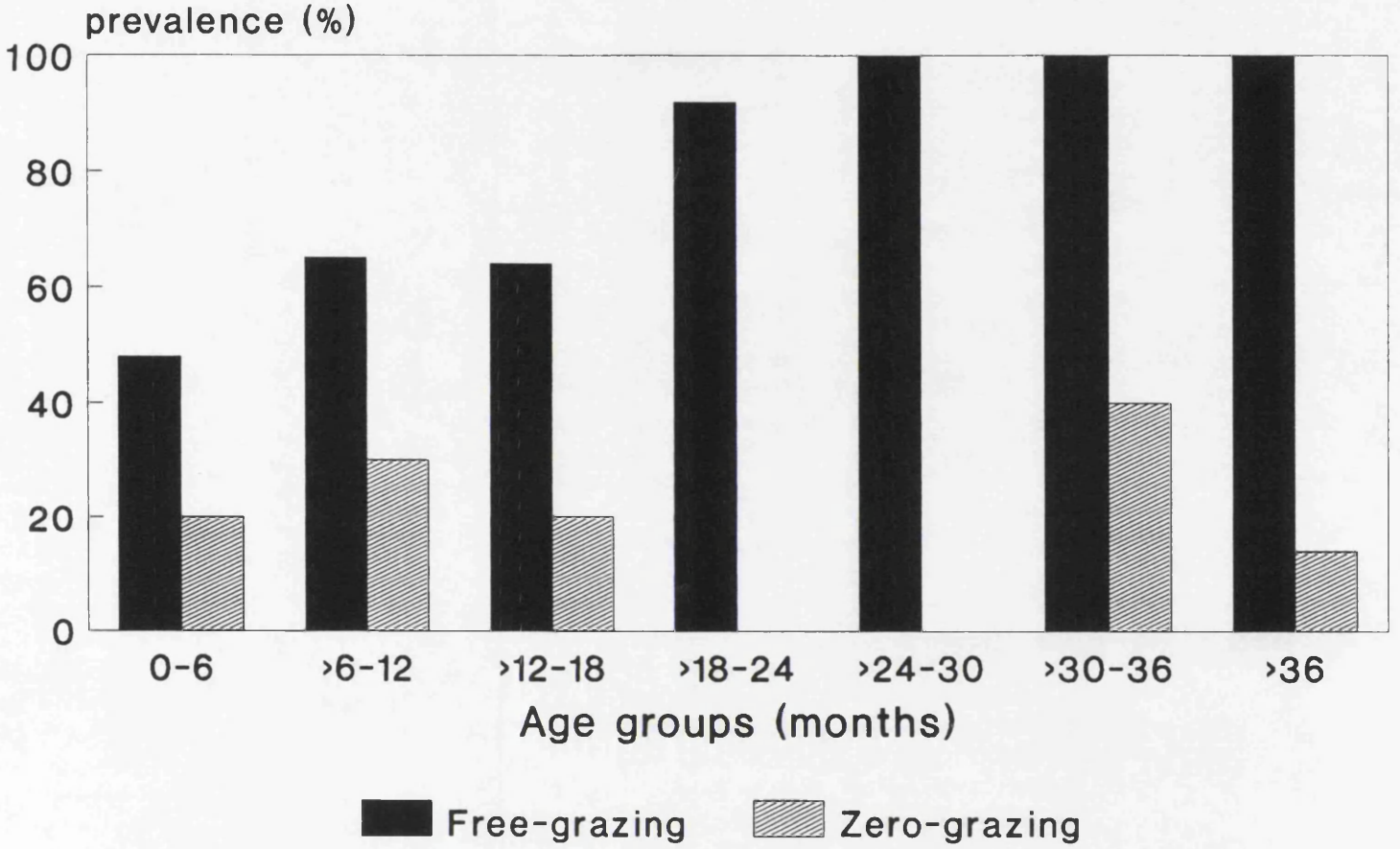


Fig.5.2.11 Age-specific *Theileria parva* schizont antibody prevalence in free- and zero-grazing small-holder dairy cattle at risk in the coconut-cassava agro-ecological zone of Kaloleni Division

iii) Cowdriosis

No cases of cowdriosis were observed.

c) Trypanosomiasis

Eight cases of trypanosome infections, seven *T. vivax* and one mixed infection of *T. vivax* and *T. congolense*, were diagnosed during the course of the study. The majority of the trypanosome parasitaemias were detected in cattle greater than 12 months of age; five in animals over 36 months of age and two in 12-24 months old. One case of trypanosomiasis was diagnosed in a 7.5 month old weaner calf.

Management of cattle in the zero-grazing systems did not prevent trypanosomiasis as out of the eight cases, three of them were diagnosed in cattle housed in the zero-grazed units. All parasitaemic cases were treated therapeutically. Six were successfully cured, but two died.

i) Trypanosome antigen and antibody prevalence rates

The monthly trypanosome antigen prevalence rates of cattle kept under zero- and free-grazing systems are shown in Figure 5.2.12. In general, the antigen prevalences were higher in free-grazing cattle than those kept in the zero-grazing system for most months. Overall, the antigen prevalence of 21% (156 antigen positive cases out of 729 samples tested) in cattle kept in the free-grazing system was significantly higher than the 9% (56 antigenaemic cases out of 636 tested) in zero-grazed animals (Table 5.2.9). Of the 212 antigenaemic animals, 48% of trypanosome antigens were identified as *T. vivax*, 48% as *T. congolense* and the remaining as *T. brucei*. About 78% of the antigenaemias were due to single species, with 76% and 84% of the antigenaemias attributed to single species in free- and zero grazing systems, respectively.

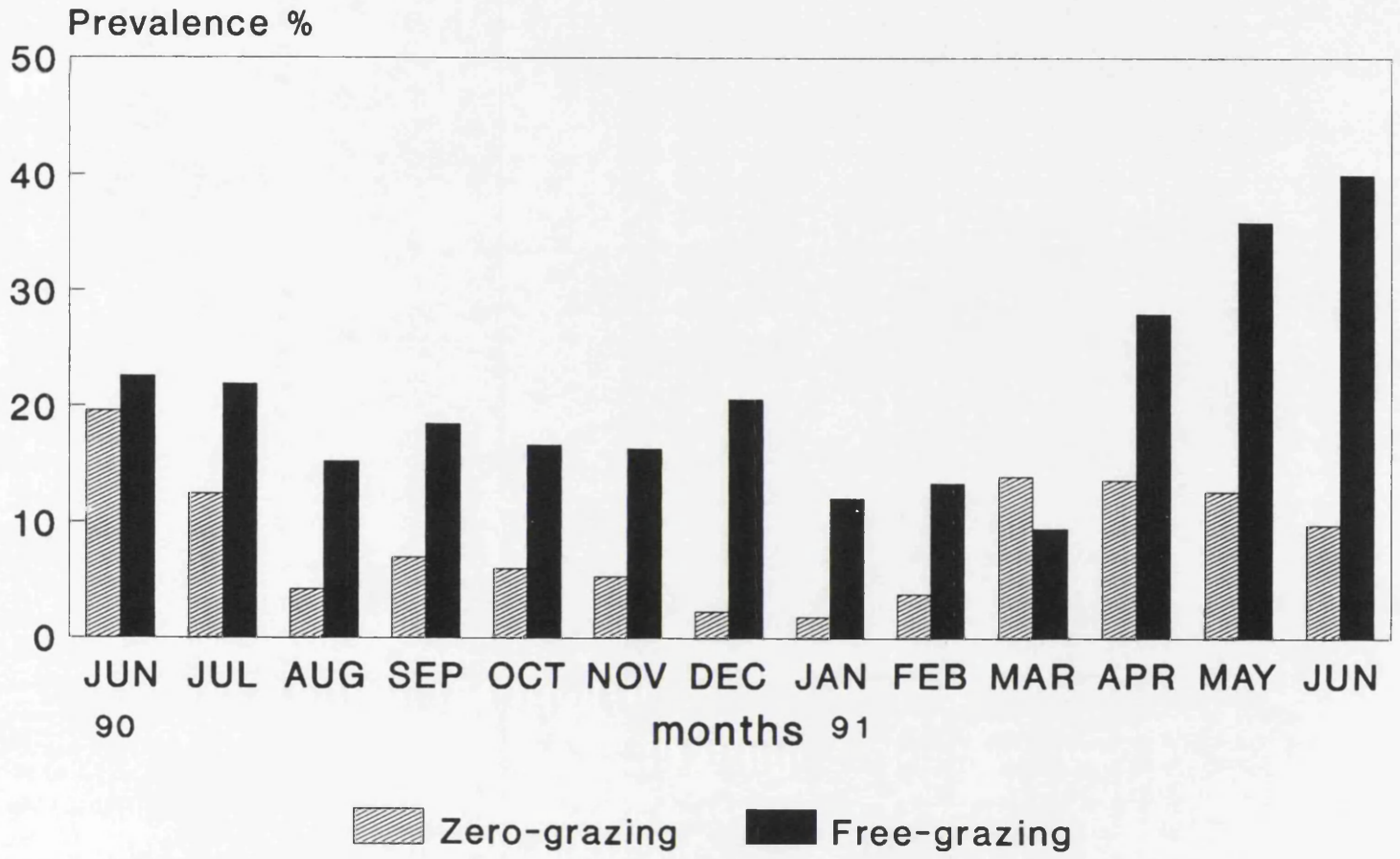


Fig.5.2.12 The prevalence of trypanosome antigens in small-holder dairy cattle kept in the two grazing systems in the coconut-cassava agro-ecological zone of Kaloleni Division

Table 5.2.9 Identification of single and mixed trypanosome infections using antigen-ELISA in small-holder dairy cattle managed in the two grazing systems in the coconut-cassava agro-ecological zone of Kaloleni Division

Grazing system	Free		Zero		Overall	
Species	n	%	n	%	n	%
Single infections						
Trypanosoma vivax (T.v)	55	35.2	26	46.4	81	38.2
T. congolense (T.c)	51	32.7	12	21.4	63	29.7
T.brucei (T.b.)	13	8.3	9	16.1	22	10.4
Mixed infections						
T.b ./ T. c	21	13.5	3	5.4	24	11.3
T. v. / T. c	8	5.1	4	7.1	12	5.7
T. b / T. v.	2	1.3	0	0	2	0.9
T. b / T. v. /T. c.	6	3.9	2	3.6	8	3.8
Total	156	100	56	100	212	100

n = Number of serum samples tested positive

The overall antibody prevalence was 18.8% and ranged from 11% to 32% with occasional large variations from one bimonthly analysis to the next. In the zero-grazing system, antibody prevalence of 19.8% (64 positive cases out of 323 samples tested) was not significantly different from the prevalence of 17.9% (72 positive samples out of 402 tested) observed in cattle managed in the free-grazing system.

d) Helminth infections

Despite the bi-monthly anthelmintic treatments, six young stock were detected with faecal nematode egg counts greater than 500 e.p.g. None of them showed clinical disease. Faecal egg counts ranged from 600 to 1700 e.p.g. with four of them having 600 e.p.g. All the six calves were successfully treated with Nilzan Plus. Apart from these six animals, three other calves which had never received anthelmintic treatments were diagnosed on necropsy as having died from helminthiasis.

e) Anaemia status

Figure 5.2.13 shows the mean PCV calculated from the monthly recordings from study cattle maintained in the two management systems. Mean PCVs were similar in the two grazing management systems. The overall mean PCV of dairy cattle raised in the zero-grazing system was 30.9% (se 0.15). The monthly means ranged from 28.5% to 33.2 %. In the free-grazing system, the overall mean was 30.1% (se 0.14) with the monthly means ranging from 28.3% to 33.1%.

The age-specific mean PCV of dairy cattle is presented in Table 5.2.10. Generally, mean PCV was higher in animals in zero-grazing than in free-grazing, though the differences were not significant, except for the 24-30 month age-class.

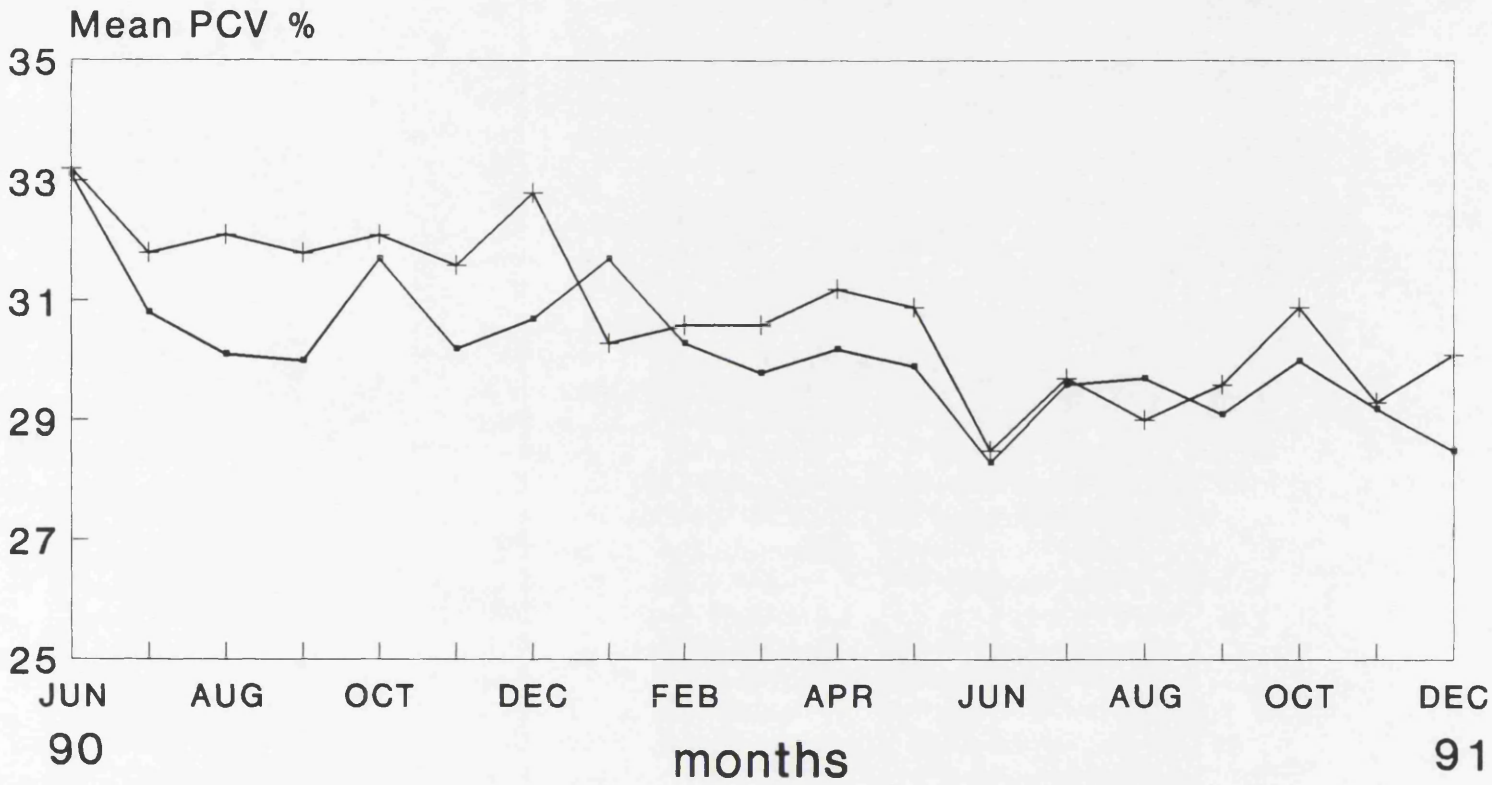


Fig.5.2.13 Mean monthly packed red cell volume percent (PCV) of smallholder dairy cattle managed in two grazing systems in the coconut-cassava agro-ecological zone of Kaloleni Division

Table 5.2.10 Mean packed red cell volume percent (PCV) of small-holder dairy cattle kept under free-and zero-grazing management systems in the coconut-cassava agro-ecological zone of Kaloleni Division

Grazing system	Free			Zero		
Age-class	n	Mean PCV	s.e	n	Mean PCV	s.e
< 6	15	32.1	0.68	13	32.7	0.7
6 - 12	15	29.5	0.5	15	29.0	0.52
12 - 18	10	29.5	1.3	13	29.5	0.71
18 - 24	8	30.0	1.0	12	31.5	0.51
24 - 30	12	29.0	0.54	12	31.8*	0.66
30 - 36	10	30.0	0.78	14	31.2	0.60
> 36	35	29.8	0.37	24	31.1	0.48

* Significantly different in the row ($p<0.05$)

n Number of animals

s.e standard error

5.2.3.4 Mortalities

During the course of the study, a total of 45 deaths were recorded, of which 32 (71%) and 13 (29%) occurred in the free- and zero-grazing systems, respectively. Table 5.2.11 gives the breakdown of the major causes of mortalities in dairy cattle managed in the two grazing systems. East Coast fever was responsible for nearly two thirds (62%) of the total losses, while none of the other individual diseases contributed to more than 20% of the losses. On several occasions (7 out of 45 cases), cause of death could not be established as animals were not presented for necropsy. Overall, the mortality rate in animals at risk was estimated as 0.32 (Table 5.2.12). Cattle in free-grazing herds had a significantly higher mortality rate (0.40) than those in zero-grazing units (0.19).

Table 5.2.13 gives the age-specific mortalities and those attributable to ECF in the two management systems. Overall, mortality rates ranged from 26% in calves less than 6 months to 32% in young stock up to 18 months, while in older cattle the mortality rate was estimated at 7%. Of these losses, ECF accounted for just above two-thirds of calf deaths, three-quarters of young stock mortalities and more than a third of the adult losses. Proportionally more deaths resulted from ECF in cattle in free- grazing herds than in the zero-grazing herds.

Contributing to the high losses resulting from ECF was the high case-fatality rate , the results of which are summarised in Table 5.2.14. During the study period, nearly 60% of occurrence of ECF caused fatality. There was a tendency for case-fatality rate to decrease as animal age increased. At the level of disease diagnosis and treatment prevailing during the study period, small-holder dairy cattle contracting ECF had only a 40% chance of surviving the disease.

Two cases of mortalities resulted from trypanosomiasis, one animal died from an acute case of haemorrhagic *T. vivax*, and another from a mixed

Table 5.2.11 Number and causes of mortalities in small-holder dairy cattle managed in two grazing systems in the coconut-cassava agro-ecological zone of Kaloleni Division

	Grazing system		
	Free	Zero	Overall
Cause of mortality	n	n	n
East Coast fever	21	7	28
Trypanosomiasis	1	1	2
Helminthiasis	2	1	3
Others (accidents)	4	1	5
Undiagnosed	4	3	7
Total	32	13	45

n Number of mortalities

Table 5.2.12 Estimation of mortality rate (MR) during the longitudinal study in small-holder dairy cattle kept in two grazing management system in the coconut-cassava agro-ecological zone of Kaloleni Division

Grazing system	Free	Zero	Overall
n	69	56	125
No. of Entries (e)	39	31	70
No.of Withdrawals (w)	16	19	35
No. of Mortalites (m)	32	13	45
Mortality rate !	0.40	0.19*	0.32

n = Nmber of cattle at the start of the study

* Significant at 95% level ($p<0.05$)

! Mortality rate calculated using the following formula

$$MR = \frac{m}{n + \frac{e-w}{2}}$$

Table 5.2.13 Age-specific mortalities and those attributable to East Coast fever in small-holder dairy cattle managed in two grazing systems in the cocout-cassava agro-ecological zone of Kaloleni Division, coastal Kenya.

Age-class (months)	Grazing system								
	Free			Zero			Overall		
	n	All (%)	ECF (%)	n	All (%)	ECF (%)	n	All (%)	ECF (%)
<6	30	11 (37)	8 (73)	21	2 (10)	1 (50)	51	13 (26)	9 (69)
6-12	24	7 (29)	5 (71)	24	4 (17)	3 (75)	48	11 (23)	8 (73)
12-18	17	6 (35)	5 (83)	8	2 (25)	1 (50)	25	8 (32)	6 (75)
<18*	110	8 (7)	3 (38)	76	5 (7)	2 (40)	186	13 (7)	5 (38)

n Number of animals at risk

* Number of animals contributing six-monthly periods in this age-class

Table 5.2.14 Age-specific case-fatality rate of East Coast fever in small-holder dairy cattle during 19 months of longitudinal study in the coconut-cassava agro-ecological zone of Kaloleni Division.

Age-classes (months)	Mortality / Morbidity	Case-fatality rate (%)
< 6	9 / 13	69
6 - 18	14 / 23	61
> 18	5 / 12	42
Overall	28 / 48	58

infection of *T. congolense/T. vivax*. In both cases, treatment was given at a fairly advanced stage of the disease.

Three calves (<6 months) which died from helminthiasis during the study period were diagnosed on necropsy. Of the three, two calves were raised in the free-grazing system and the other in a zero-grazing unit. Two male calves which died were neglected by the farmers as they were not reported for clinical attention. The other loss of a female calf occurred as it was introduced as a purchase and had not been presented for the routine deworming.

5.2.4 DISCUSSION

The results of the longitudinal study demonstrated the importance of disease as a major constraint to sustainable small-holder dairy production systems in the coconut-cassava AEZ of Kaloleni Division in coastal lowland, Kenya. This was reflected in the overall population changes, where an 8% reduction of the study population indicated the failure to propagate dairying under the small-holder sector. Moreover, four farmers abandoned dairying during the course of the study, with no new farmers venturing into the dairy industry. To a large extent, the majority of the study cattle losses occurred through disease-related mortalities.

In the study population, 40% of the dairy cattle developed clinical disease with 76% attributable to tick-borne diseases. East Coast fever was diagnosed as the predominant disease and was responsible for almost two-thirds (62%) of all clinical diseases cases encountered during the longitudinal monitoring. This inferred that the tick control practices implemented by the small-holder farmers failed to prevent occurrence of ECF. The disease was diagnosed throughout most of the study months and was the most important disease in cattle managed in both free- and zero-grazing systems. One month following the peak rainfall, 10% of the free-grazing cattle tested had clinical

ECF. Despite the surge in ECF cases during this month, no marked seasonal differences were evident. This was thought to be due to the ability of *R. appendiculatus* to persist throughout the year under the favourable coastal climatic conditions (Newson, 1978). Furthermore, it seems likely that in view of the high environmental temperatures, *T. parva* parasites can develop into infective sporozoite stage in the tick without the stimulus of prior feeding (Young, Leitch and Omwoyo, 1979, Young and Leitch, 1981). This means ticks can transmit sporozoites as soon as they start feeding. Studies carried out by Ochanda *et al.* (1988) have shown heat stimulated infected ticks can transmit fatal ECF within 24 hours of attachment to cows.

Besides the continuous presence of the vector, it is believed most resident cattle in free-grazing herds which had been previously exposed to the *T. parva* infection were carrier cattle and played a significant role in the epidemiology of the disease. This conclusion is supported by the fact that a large proportion of cattle in the free-grazing system had antibodies to *T. parva* indicating that they had been exposed to the parasite some time earlier and had survived either by spontaneous recovery or by therapeutic intervention. The latter seems to be more likely as cross-bred or European breed of dairy cattle are more susceptible to ECF than local Zebu in endemic areas (Barnett and Bailey, 1955; Guilbride and Opwata,1963). Moreover, cattle recovering following anti-theilerial treatment can become carriers (Dolan, 1986a). These observations were in agreement with those reported by Mutugi *et al.* (1991b), where clean nymphal ticks fed on resident dairy cattle, on a farm with previous history of often lethal theilerial challenge, were able to transmit fatal *T. parva* infection to all susceptible Friesian cattle when attached as resultant adults following moulting. Similar findings have been made by Kariuki (1990), where batches of *R. appendiculatus* ticks collected from previously exposed exotic dairy cattle in farms from various regions of Kenya, including one from the

coastal area, were applied to susceptible cattle under laboratory conditions. The majority of the tick batches, including the one collected from the Kenya coast, transmitted fatal ECF.

Although tick transmission studies were not carried out in this study, it appears that a similar situation occurs in Kaloleni. It is probable that ticks feeding on exposed cattle become infected and are brought into the homestead compound (kraal) where, after dropping off and moulting to either nymphs or adults, they attach on to calves transmitting *T. parva* infection over short feeding duration. This situation will arise when acaricidal application regimens are not rigidly implemented, a fault commonly noticed with the free-grazing farmers.

The prevalence of ECF in cattle managed in the zero-grazing system was two-fold lower than in the free-grazing system, and episodes of the disease occurred in two periods, both coinciding with the rainy seasons. The majority of the infections were detected in younger age cohorts (post-weaners) which tend to be tethered outside the unit, separated from their dams in order to prevent suckling by the offsprings. This usually occurs when the units lack cubicle space for the young stock. During the rainy seasons, calves kept outside the unit were at risk as tick numbers are known to increase with the onset of the rains (Newson, 1978), thereby increasing the possibility of transmitting infection. Infected ticks were thought to be introduced into zero-grazing homesteads by dropping off from herds of local Zebu cattle passing by. Zebu cattle population in the coconut-cassava AEZ had a high antibody prevalence to *T. parva* (Deem *et al.*, 1993) and were likely to be carriers.

Otherwise, the lower risk in the zero-grazed cattle is reflected in the relatively lower antibody prevalences, where the overall prevalence was 26% compared to the 77% antibody prevalence observed in the free-grazing cattle. The lower proportion of cattle exposed to *T. parva* infection indicated the

effectiveness of the management system, either by reducing tick contact of zero-grazed cattle as a result of confinement in stalls or by effective acaricidal application, or combination of both.

The changing population of the study herd by virtue of new entries and exits made estimation of population at risk over the entire monitoring period a difficult task. However, an attempt was made to estimate risk and incidence rates by expressing them as functions of animals considered to be at risk, and by animal months contributed by the study population at risk. Incidence rate expressed in animal-months allows results to be interpreted on a population basis, but does not estimate the probability of an individual contracting the disease. On the other hand, risk rate gives the probability of an animal becoming infected (Martin *et al* ., 1987).

In this study, both risk and incidence rates of ECF estimated for the duration of the study were two-fold higher in the free-grazing cattle compared to cattle managed in the zero-grazing system. Analysis of data using the logistic regression models predicted similar differences in the probability of cattle contracting ECF among the grazing management systems. The results clearly indicate the significance of managing cattle in zero-grazing unit in reducing the risk of contracting the disease. However, maintaining cattle in the zero-grazing system was labour intensive and during certain times of the year when planted fodder was scarce or insufficient, farmers depended on natural pasture brought in from nearby roadsides. These roadsides pastures were invariably tick-infested as roadside grazing by tethered cattle and small-ruminants was common. As a consequence, ticks were introduced into the unit. It was therefore concluded that the few cases of ECF in adult cattle might have arisen as a result of this practice and the failure to spray the cattle regularly with acaricides.

In the free-grazing system, a higher incidence rate of ECF was observed in animals less than 18 months of age compared to older age-cohorts. It appeared that most calves were exposed to *T. parva* infection early in life. In studies carried out by Moll *et al.* (1986) in an ECF endemic area, 44% of the local Zebu calves had developed patent *T. parva* macroschizonts by 6 to 7 months of age. Furthermore, in the free-grazing management system, the cumulative incidence gave a probability of an animal contracting ECF by 18 months of age as 0.83, inferring that eight or more out of 10 susceptible calves raised under these circumstances were likely to contract ECF before reaching 18 months of age. These calves would either succumb to the disease or recover following effective therapy. The age-specific *T. parva* antibody prevalence of cattle at risk showed that all animals by 24 months of age had been exposed to the parasite and were possibly immune. This possibly explains why there was a lower incidence of ECF in adult free-grazing cattle. The few cases of clinical ECF in exposed adult cattle was probably indicative of the existence of different strains of *T. parva* in the area. Moreover, the presence of antibodies does not always reflect immunity, nor will it prevent animals from developing clinical ECF. This was evident from the two exposed calves which developed a second episode of clinical ECF during the monitoring period. This is because the effector mechanism for an immune response has been shown to be cell-mediated (Emery, 1981; Emery, Morrison, Nelson and Murray, 1981).

In the zero-grazing system, the age-specific incidence of ECF was lower for most age-groups compared to the corresponding age-cohorts in the free-grazing system, but incidence of ECF among the age-groups in the zero-grazing system did not differ significantly. However, most cases were diagnosed in calves less than a year old, with six out of the seven clinical episodes occurring in males. Under this management system, the probability of an animal contracting ECF by 18 months of age as estimated by cumulative

incidence was 0.62. This high probability was because majority of the episodes of ECF occurred in pre- and post-weaner male calves. In the zero-grazing system, there was a tendency to keep males outside the units, and, due to their low economic value in the dairy enterprise which depended on AI service for breeding purposes, they were subjected to poor animal husbandry and health practises. Consequently, more males than females succumbed to infection with *T. parva*. The relatively low age-specific incidence of ECF in the zero-grazing system was reflected by the lower *T. parva* antibody prevalences (never exceeding 40%) detected in the various age-groups at risk. On the other hand, these animals must be considered to be highly susceptible in the event that infection be inadvertently be introduced into the units through ticks brought in with natural pastures or if, acaricidal application is relaxed. Moreover, of epidemiological significance was the fact that males undergoing clinical bouts of ECF might possibly become the source of infection for the rest of the susceptible cattle if tick control practices were not strictly adhered to.

Although none of the other diseases contributed more the 15% of all clinical disease cases, their economic importance, especially at an individual small-holder herd level should not be underestimated. Other diseases diagnosed were anaplasmosis, babesiosis, helminthiasis and trypanosomiasis. While in comparison to ECF, other tick-borne diseases were overshadowed, but their occurrence further reflects on the failure of tick control practices.

During the monitoring period only eight cases of trypanosomiasis were observed indicating the study area to be of low challenge. The results confirmed the earlier observations from the cross-sectional study suggesting Kaloleni Division to be an area of low to seasonal trypanosomiasis risk. Trypanosome antigen prevalences in free- and zero-grazing cattle of 21% and 9%, respectively, were lower than those observed for dairy cattle managed in the two grazing systems in the cross-sectional study (Section 5.1). The low

antigen prevalence was probably due to low tsetse challenge as observed by few numbers of trypanosome parasitaemic animals during the entire study period. The monthly antigen prevalences of free-grazing cattle never exceed 25%, except for the last 3 months. These results were comparable with those observed in the low tsetse challenge areas of Ngurumann, where monthly antigen prevalences did not exceed 15% (Mwangi, 1993). Of the antigenaemic cattle, 78% had antigens to single trypanosome species. The results confirmed the earlier findings where majority of the antigenaemic cases were due to single species. This was in contrast with observations from high trypanosomiasis challenge areas, where the reverse was true, with 78% of the antigenaemias due to two or more trypanosome species (Nantulya *et al.*, 1992). The relationship between the low trypanosome antigen prevalence in the face of few parasitological positive cases cannot be adequately explained from this study as cattle were already antigenaemic at the beginning of the study. Ideally, a sentinel herd from a trypanosomiasis-free area, tested to be antigenically negative should be closely monitored. Besides the routine screening for trypanosome infections on DG and antigen-ELISA, mice inoculation and more sensitive test involving the use of molecular techniques such as PCR need to be concurrently carried out on matching samples collected simultaneously. Presently, a decision for therapy against trypanosomiasis cannot be made on the basis of antigen ELISA alone. Ideally this test in combination with diagnosis using DG technique should be used. The use of antigen-ELISA in epidemiological studies will provide additional useful information, particularly in tsetse-eradicated areas where monitoring for trypanosomiasis is carried out routinely for surveillance of the disease.

The presence of antibodies indicates exposure to trypanosomes, but does not differentiate between current or past infections (Nantulya, 1991). In this study, the bimonthly trypanosome antibody prevalence of 18.8% indicated

a low level of exposure to trypanosome infection and further confirmed the low risk of trypanosomiasis in the study area, By contrast, in various parts of Kenya, where trypanosomiasis risk have been reported to be high, antibody prevalences in cattle measured by antibody-ELISA and IFAT were 78% and 80%, respectively (Mwangi, 1993; Zwart, *et al.*, 1973)

The low risk of trypanosomiasis in Kaloleni, is not likely to justify the need for a chemoprophylaxis programme, and control might best depend on prompt intervention with curative therapy in the face of clinical cases of trypanosomiasis

Moreover, the low prevalence of anaemia-causing haemoparasitic infections was reflected in the relatively high mean PCV of cattle managed in the two grazing systems. Overall, the mean PCV of 30.9% and 30.1% in zero- and free-grazing dairy cattle, respectively, showed that grazing management generally did not influence mean PCV, although there was a tendency for age-specific mean PCV of cattle in the zero-grazing system to be higher. The mean PCV results were similar to those observed in the cross-sectional study, where overall mean PCV of over 30% support the conclusion that the trypanosomiasis risk was low in the area. This was in contrast to a mean PCV below 26% observed in local Zebu and Boran cattle in known medium to high trypanosomiasis challenge areas aras of Kenya (Maloo, *et al.*, 1988a; Munstermann, *et al.*, 1992)

Although production parameters of dairy cattle were not recorded in this study due to the complexity of collecting these data, production losses due to mortalities appeared to be of significance. The mortality rate for the population in the study was estimated as 0.32, with those managed in the free-grazing system experiencing a two-fold higher rate than those in the zero-grazing system. In a similar environment, as on the island of Zanzibar, Tanzania, mortality rate in cross-bred calves at 10 months of age, kept under

free-grazing system, was estimated at 20% (Jacobsen, 1983). The low mortality rate in cattle kept in the zero-grazing units could be attributed to the reduced exposure to infections.

In both grazing systems, ECF was the major cause of death. Mortality attributed to ECF amounted to 62% with majority of the losses occurring in less than 18 months age-cohorts. In surveys carried out in Zanzibar, 31% of deaths were due to ECF in cross-bred cattle, 1 to 2 years old (Juma and Shambwana, 1985). This is likely to have been an underestimate as they did not report losses in calves. Moreover, they relied on farmers' opinion on the cause of death as post-mortem examinations were not performed. By contrast, a mortality rate of 25% in local Zebu calves in an ECF endemic area of Kenya was reported by Moll *et al.* (1984) with only 2.6% attributable to ECF. In studies carried out by Deem *et al.* (1993) mortality in local Zebu cattle, 6 to 18 months old, as reported by the farmers, was 14% in the coconut-cassava AEZ compared to 23% to 32% in similar age cohorts observed in dairy cattle in this study.

The results clearly indicated that under the present management systems of the small-holder dairy cattle, the majority of the losses due to ECF occurred in the younger age-groups. This is particularly reflected in the case-fatality rate where almost 60% of clinical cases of ECF died. However, not all animals were treated, particularly males calves as they were neglected by the farmers and not reported for clinical attention. Moreover, calling upon the local AHA, would mean incurring veterinary costs. In addition, anti-theilerial drugs are expensive and were not always readily available from the local Veterinary Office.

Besides ECF, mortalities occurred from trypanosomiasis and helminthiasis. Losses from trypanosomiasis in two cases were significant on an individual herd basis. This was important as both losses were cows in milk and

occurred on farms with mean herd size of three cattle. Deaths due to helminthiasis resulted from negligence on the part of the farmers. The result signifies the importance of control measures to prevent losses.

The predominant disease diagnosed and responsible for the majority of the losses in the study population was ECF. The study clearly highlighted its importance as the major disease limiting dairy development in the small-holder sector. Moreover, the longitudinal results confirmed those from the cross-sectional study (Section 5.1) which showed that the tick control practices implemented by the small-holder farmers failed to prevent infection. Managing cattle in the zero-grazing system reduced the risk of ECF, but did not prevent the occurrence of the disease in spite of the recommended acaricide applications for tick control. East Coast fever was observed particularly to effect the younger cattle population resulting in a high case-fatality rate.

In order to reduce the large economic losses resulting from ECF in these small-holder production systems, alternative control strategies are required which are economically sound and environmentally acceptable. In this respect, major consideration must be given to immunisation. Immunisation using the infection and treatment (I&T) method (Radley, 1981) offers the most appropriate alternative. This method has been successfully implemented in large institutional dairy herds in coastal Kenya (Morzaria, *et al.*, 1987; Mutugi *et al.*, 1991a).

To carry out effectively and economically the immunisation of small-holder cattle, it is necessary to identify the target population for the immunisation. The important factors to be considered are the age, the breed and the management system of the cattle at risk.

In order to develop recommendations for implementation of immunisation against ECF, specific experiments were carried out first to

determine the age-window for immunisation, and secondly to determine if it was necessary to protect local Zebu cattle. A third study describes a pilot immunisation trial of small-holder dairy cattle. These studies form the basis of the next chapter.

CHAPTER SIX

THE IDENTIFICATION OF A TARGET POPULATION SUITABLE FOR IMMUNISATION AGAINST EAST COAST FEVER

This chapter describes two experimental studies and a pilot immunisation trial against East Coast fever (ECF) carried out in small-holder dairy cattle in coastal Kenya. The experimental studies were aimed at identifying the target population for immunisation. The first study was conducted to determine the age-window for immunisation by assessing the age at occurrence of *Theileria parva* infection and case-fatality due to ECF in sentinel dairy calves exposed to natural tick challenge. Age-window refers to the suitable age range of calves for immunisation before they contract the disease. The second study was carried out to evaluate the immunity of naturally-infected local Zebu cattle and dairy calves to the designated immunisation stock of *T. parva*.

Subsequently, a pilot study for immunisation against ECF, using the infection and treatment (I&T) method (Radley, 1981) in small-holder dairy cattle was undertaken where the logistics of delivery of this method were addressed.

6.1 DETERMINATION OF AGE-WINDOW FOR IMMUNISATION BY ASSESSING AGE AT INFECTION WITH *THEILERIA PARVA* AND CASE-FATALITY DUE TO EAST COAST FEVER IN SUSCEPTIBLE CALVES EXPOSED TO NATURAL TICK CHALLENGE

6.1.1 INTRODUCTION

In the longitudinal study (Section 5.2), ECF was identified as the major disease constraint in small-holder dairy herds, with most of the cases occurring in young stock resulting in production losses through mortalities. The tick control practices of acaricidal application carried out by small-holder farmers appeared to be ineffective in preventing ECF. Moreover, the escalating costs of acaricides and anti-theilerial drugs under the present economic climate

prevailing in the country, as well as the increasing numbers of malfunctioning cattle dips, are bound to worsen the situation even further.

In order to reduce the risk of ECF and minimise its losses, the alternative method of control by I&T method of immunisation needs to be evaluated. The principle behind the I&T method is to mimic endemic stability by introduction of the immunising stock of *T. parva* Marikebuni, which is known to provide cross-protection against a wide range of coastal *T. parva* strains (Irvin *et al.*, 1983; Mutugi *et al.*, 1989).

From the results of the longitudinal study, it was evident that majority of ECF cases occurred in the younger age-cohorts. The target population for immunisation should cover all young stock and unexposed adult dairy cattle which are at high risk. The latter refers mainly to cattle kept in the zero-grazing system, where exposure to *T. parva* infection was low (Section 5.2).

In order to establish the age-window for immunisation of young animals, i.e., the age-range of calves which will allow protection by immunisation to be carried out before contracting ECF, a sentinel calf study was designed. The objective of the study was to determine age at detection of *T. parva* infection and case-fatality of ECF in susceptible calves exposed to natural tick challenge.

6.1.2 MATERIALS AND METHODS

a) Sentinel calves

Six groups of five cross-bred male calves, designated Groups I, II, III, IV, V and VI, were introduced on to a small-holder farm in the coconut-cassava AEZ of Kaloleni Division, starting in May 1990. Calves were released every two months, from May 1990, in order to ensure similar age cohorts were exposed during the wet and dry rainy seasons in the study year.

b) Source

All calves were purchased from Kilifi Plantations, a commercial dairy ranch located 60 km north of Mombasa in the Coast Province of Kenya. Due to routine use of acaricidal treatments for the control of ticks and tick-borne diseases (Paling *et al.*, 1987), the cattle on the ranch have been generally free of ECF.

The calves had a mean age of 26 days, with a range of 10-40 days. The majority of the calves were between 20-35 days old. Homogeneity in age of calves could not be controlled due to limited selection options available for calves offered for sale by the ranch.

c) Breed

The calves were crosses of the Brown Swiss, Ayrshire and Sahiwal breeds. Their breed composition varied from a small percent (<15%) of the *Bos indicus* Sahiwal breed up to a maximum of 50%, with the balance contributed by one or both of the *Bos taurus* breeds, Ayrshire and Brown Swiss. Generally, each group of five calves reflected this range of genetic composition which was representative, of the population of small-holder dairy cattle in coastal Kenya.

d) Adaptation phase

Prior to the study there was a period of 5-7 days when calves were gradually introduced to commercial milk replacer (Trilk, Kenya Cooperative Creameries). This phase of transition from one diet to another was carried out at the Regional Research Centre (RRC), Mtwapa.

e) Criteria for selection of the small-holder farm.

The selection of the small-holder farm was based on its history of high mortalities due to ECF, and the fact that all resident cattle had antibodies

against *T. parva* infection. In addition, the farm was chosen because of the willingness of the farmer to allow the study to be conducted on his farm.

f) Management of calves

Before the calves were exposed to natural tick challenge, they were ear-tagged for identification, dewormed with 1.5% w/v levamisole, 3% w/v oxyclozanide with 0.382% cobalt sulphate (Nilzan Plus, Coopers, Kenya Limited) at a dose of 5 ml/20kg, and treated topically with a flumethrin pour-on preparation (Bayticol, Bayer, Germany) at 1ml/10kg to ensure they were tick free. Prior to release, calves were bled and serum tested for the presence of antibodies to *T. parva*. All calves selected for the study were negative (<1:40 titre) for antibodies to *T. parva* schizont antigen on IFAT. Following release of calves on to the farm, tick control was stopped until they developed clinical ECF.

Once a calf contracted ECF, it was treated with parvaquone (Clexon^{R}, Wellcome) at a dose of 15mg kg^{-1} i.m, given twice, 48 hr apart. Upon diagnosis, the infected calf was treated with an acaricidal preparation to relieve it from the tick infestation. Thereafter, tick control in ECF recovered calves was carried out under the supervision of the farmer together with the rest of the cattle on the farm. Nine calves did not receive treatment in time and died before confirmatory diagnosis could be made. At necropsy, they were diagnosed as having ECF.

During the study, calves were fed on milk replacer, twice a day at 10% of their bodyweight, until weaned at 12 weeks of age. Thereafter, they were allowed to graze on pastures in the vicinity of the farm household and supplemented with maize bran and mineral lick.

g) Monitoring of calves

Calves were examined for demeanour and feed intake, and their rectal temperatures were recorded daily. At weekly intervals, calves were blood tested for the presence of tick-borne haemoparasites by microscopical examination of Giemsa stained thin blood smears. Whenever temperature of calves rose above 39.4^{O}C, considered to be a febrile response, and or peripheral lymph nodes were palpably enlarged, biopsy smears were prepared from the lymph nodes. Smears were stained with Giemsa and examined for the presence of theilerial schizonts.

Peripheral ear vein blood samples were collected in heparinised capillary tubes and tested for trypanosome infection using buffy coat DG technique (Murray *et al.*, 1977). Blood for serum was obtained by jugular puncture and separation of serum was done as described in Chapter 4. Control against helminthiasis was achieved by deworming calves with Nilzan every month.

During the exposure period, half body tick counts on all calves were recorded at weekly intervals. All tick species were identified, but no attempt was made to differentiate the number of ticks by species, except for *R. appendiculatus*, for which counts on the ears and head of individual calves were recorded. Tick counts were recorded for calves from the time of release until they contracted ECF.

Weekly monitoring of calves in all groups continued until the surviving calves attained 6 months of age. Thereafter, the calves were examined once a month until transferred to the Animal Production Research Centre, Mariakani or the RRC, Mtwapa.

In the event of mortalities, most carcasses were transported to the regional V.I.L. Mariakani for post-mortem examination. In some instances, calves were necropsied on the farm and after recording the gross pathological

findings, tissue samples of lymph nodes, spleen and lungs together with their impression smears were submitted to the V.I.L. for confirmatory diagnosis.

h) Serology

Serum samples collected weekly were stored at -20^{O}C until sent to the NVRC, Muguga for serological analysis. Sera were tested for antibodies to *T. parva* schizont antigen using the IFAT, as described by Burridge and Kimber (1972). All sera were tested using four-fold dilutions from 1:40. Fluorescence at dilutions of 1:40 or more was considered positive for antibodies.

i) Data analysis

Biological parameters recorded at weekly intervals were entered in data base files (DBase III Plus).

Age at detection of *T. parva* infection and days to infection post release on to the farm for each group of calves were analysed for significant differences using the one-way analysis of variance (ANOVA) test in Minitab Release 7.1, Minitab Incorporation (Appendix 7). Where significant differences were found, the Newman-Keuls Multiple Range Test was used to identify differences among groups. Differences were statistically tested at 5% level of significance.

Calves detected as having infections with *T. parva* and *B. bigemina* which resulted in death were recorded as dying primarily from the former infection.

6.1.3 RESULTS

a) Occurrence of East Coast fever

Table 6.1.1 presents the number of ECF cases, their case-fatalities and the proportion of infected calves developing antibodies to *T. parva* in each of the

Table 6.1.1 The occurrence and case-fatality due to East Coast fever (ECF) and seroconversion of calves contracting ECF to *Theileria parva* infection in the six groups of sentinel calves released on to a high ECF risk farm in the coconut-cassava agro-ecological zone of Kaloleni Division

Group	Month of release	Age at release (in days)		No. of ECF cases per group	Case-fatality of ECF	Sero-conversion of calves contracting ECF
		Mean	Range			
I	May 1990	17	10-26	5/5	3/5	2/5
II	July	30	22-40	3/5	2/3	2/3
III	September	23	18-25	4/5	2/4	4/4
IV	November	28	26-32	4/5	2/4	4/4
V	January 1991	32	27-35	5/5	2/5	4/5
VI	March	20	18-24	4/5	2/4	3/4
Overall		26	10-40	25/30	13/25	19/25

six groups of sentinel calves following natural tick challenge. East Coast fever, diagnosed by clinical assessment and detection of theilerial macroshizonts, occurred in all groups, irrespective of the month of release.

Of the total 30 calves released on to the high-risk farm, 25 (83%) contracted ECF, with 19 out of the 25 calves (76%) developing antibodies to *T. parva* schizont antigen. In total, there were 10 survivors at the end of the study and all were sero-positive. Overall, the case-fatality of ECF in calves was 52% (13/25).

b) Age at and days to detection of *Theileria parva* infection

The mean age (in days) at and days to detection of *T. parva* infection for all calves were 72 (SD $\pm$ 24.2) and 48 (SD $\pm$ 27.0) days, respectively (Table 6.1.2). The range for age at infection was 36-116 days, and 14-98 days for days to detection of infection.

Significant differences in age at detection of infection were observed between Group I and Groups III, IV and VI, where mean age of 102 days was higher than the 67 and less days of ages observed in the latter groups. In addition, the lowest mean age at infection of 46 days in Group III also differed from the mean age of 86 days observed in group II. The differences in age at infection among groups resulted in most cases from the variation in age of calves when released. This was reflected in the mean days to detection of *T. parva* infection where no differences were found among most groups, with the exception of Group I. In this group the mean days to infection of 88 days was significantly higher than the mean days to detection of 56 and less observed for the rest of the groups.

In general, almost two-thirds of the sentinel calf population developed ECF within 2 months after release, and by the third month nearly 90% of calves at risk were infected. The majority of the infections (80%) were

Table 6.1.2 Mean age (in days) at and mean number of days to detection of *Theileria parva* infection in sentinel calves belonging to six groups exposed to natural tick challenge

Group	Number of calves contracting ECF	Age (in days) at detection of infection			Days to detection of infection post release on to the farm		
		Mean	S.D	Range	Mean	S.D	Range
I	5	102[a]	11.3	87-116	88[c]	13.7	70-98
II	3	86	6.2	81-93	56	2.5	53-58
III	4	46[b]	7.6	36-53	22	7.5	15-28
IV	4	67	6.4	60-75	39	9.0	28-49
V	5	74	15.1	56-94	42	15.2	21-62
VI	4	58	33.2	38-107	37	33.2	14-86
Overall	25	72	24.2	36-116	48	27.0	14-98

ECF East Coast fever

a Significantly different from Groups III, IV. VI

b Significantly different from Groups I and II

c Significantly different from Groups II,III, IV,V,VI

SD standard deviation

detected by the time calves were 3 months of age, with nearly half of them occurring between the ages of 1 and 2 months.

Despite the tick control practices carried out under the supervision of the farmer, seven calves were diagnosed as having a second episode of ECF.

c) Other infections

Babesia bigemina infections were detected in four calves, with three of them having concurrent infections with *T. parva*. Four calves were diagnosed as having *A. marginale* infections and three occurred in calves which had clinical ECF earlier. Two other calves developed neonatal diarrhoea and another one suffered from navel ill (omphalophlebitis) within the first 2 weeks post release. No trypanosome infections were observed.

d) Treatment against East Coast Fever

Sixteen calves received treatment against ECF, of which 10 were successfully cured. These survivors developed antibodies to *T. parva* schizont antigen. Two out of six calves that died had mixed infections with *B. bigemina* where therapeutic intervention against both infections did not result in a successful cure. The other four had second episodes of ECF and succumbed to the disease as treatment was given too late.

e) Mortalities

The occurrence of mortalities in all groups of calves over the study period is shown in Figure 6.1.1. In total, 20 (66%) calves died, of which 13 (65%) were attributable to ECF, Of these 13 cases, two calves were diagnosed as having mixed infections of *T. parva* and *B. bigemina*. One mortality resulted from anaplasmosis, and another from babesiosis. Three calves, two suffering from

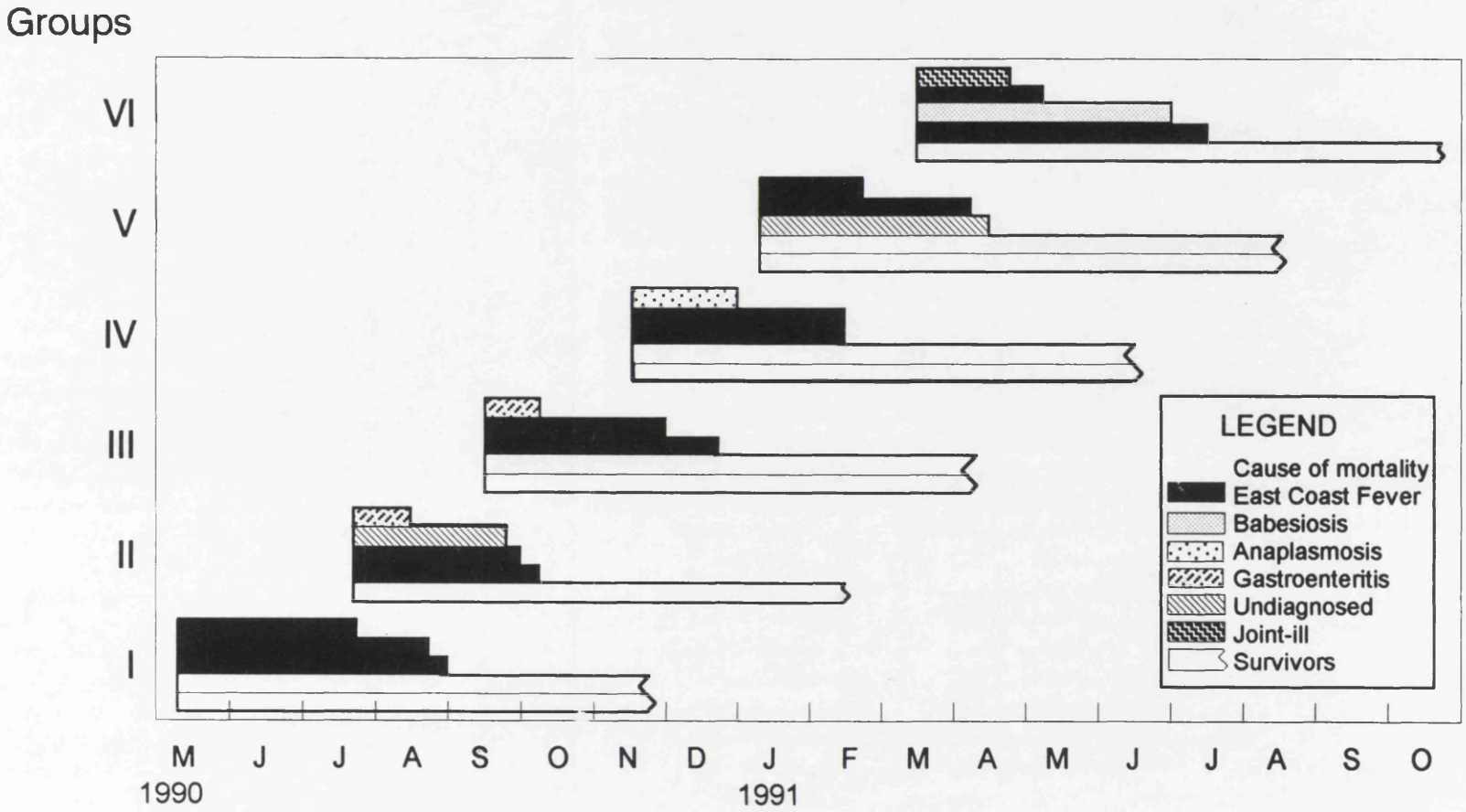


Fig. 6.1.1 Case-fatality due to East Coast fever (ECF) and other mortalities in the six groups of sentinel calves exposed to natural tick challenge

neonatal diarrhoea and one from navel ill (omphalophlebitis) died 2 weeks after release. In addition, two other calf deaths were undiagnosed.

f) Tick infestation

Tick species identified were *Rhipicephalus. appendiculatus, R. evertsi, R. pulcheus, Boophilus decolaratus, B. microplus* and *Amblyomma* species. During the exposure period, tick challenge increased progressively and reached a maximum of 141 (mean weekly half-body tick counts from all non-acaricidal treated calves) after 9 weeks (Figure 6.1.2). By this time, 19 out of 25 *T. parva* infections in calves had occurred.

Rhipicephalus appendiculatus ticks were observed on calves in all groups as early as the first week after release and reached a maximum half body count (mean 9.2 SD $\pm$ 3.4) by 9 weeks. There was considerable variation in the number of *R. appendiculatus* ticks infesting calves among the groups, but generally tick numbers increased over time. The mean weekly *R. appendiculatus* numbers for the first two groups of calves were low for the initial 4 weeks, never exceeding a mean count of 1.5, compared to the greater than 5 recorded in other groups for the same time period (Table 6.1.3).

6.1.4 DISCUSSION

The sentinel calf study clearly demonstrated the significance of ECF as a major calfhood disease in which 83% of the naive calves exposed to natural tick challenge became infected before they reached 4 months of age. The results also confirmed the earlier observations from the longitudinal study that the coconut-cassava AEZ was a high ECF risk area. In addition, no marked seasonal variation in the occurrence of the disease was observed as infection with *T. parva* was detected in all groups of calves released at bi-monthly intervals over 1 year. Moreover, the presence of the tick vector, *R.*

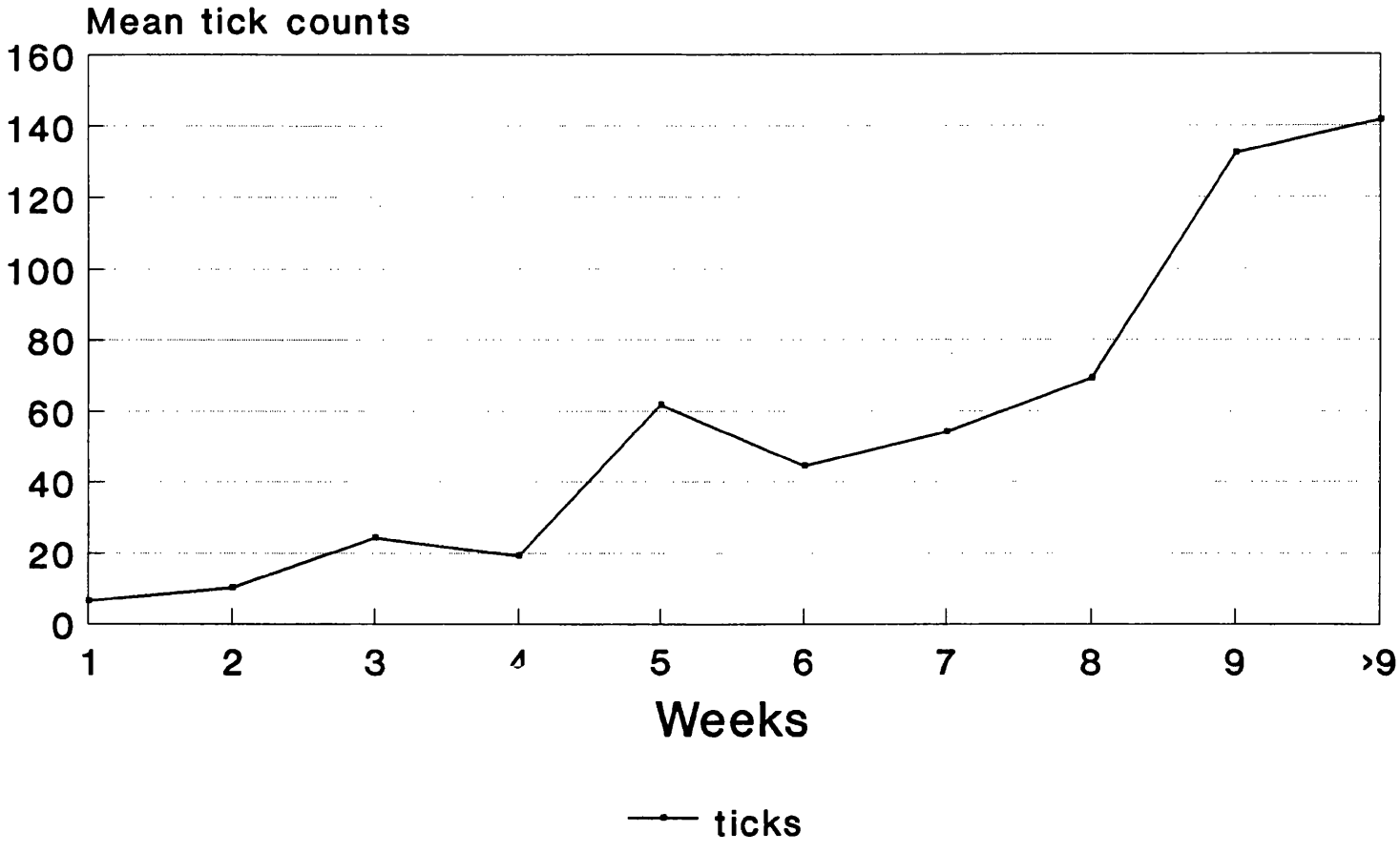


Fig.6.1.2 The mean weekly half body tick counts on sentinel calves in six groups kept under natural tick challenge

Table 6.1.3 Mean weekly (± SD) *Rhipicephalus appendiculatus* half body counts on sentinel calves in the six groups exposed to natural tick challenge

	Weeks								
	1	2	3	4	5	6	7	8	9
Groups									
I	0.3 ±0.5	0.8 ±0.8	1.2 ±0.8	0.4 ±0.6	1.4 ±1.1	2.0 ±0.7	2.8 ±1.1	4.8 ±2.5	8 6 ±3.4
II	0.4 ±0.6	1.2 ±2.2	1.0 ±1.4	1.5 ±0.6	4.0 ±2.7	9.0 ±3.0	5.5 ±0.7	15.5 ±3.5	NC
III	2.0 ±2.3	8.0 ±6.3	5.0 ±2.7	11.0 ±5.0	6.7 ±3.1	12.0 -	NC	NC	NC
IV	2.6 ±1.5	2.8 ±2.7	4.8 ±2.5	5.3 ±2.8	3.8 ±2.5	7.0 ±5.1	6.0 ±1.0	8.7 ±6.4	12.0 -
V	4.5 ±3.3	5.5 ±3.5	9.7 ±9.9	9.8 ±8.2	11.0 ±12.7	NR	NR	15.0 ±0.0	24.0 ±0.0
VI	11.2 ±5.2	12.8 ±4.8	13.8 ±6.8	NC	NC	NC	NC	NC	NC
Overall	3.5 ±4.9	4.9 ±5.5	5.0 ±5.4	4.8 ±5.8	4.9 ±4.8	6.6 ±5.7	5.1 ±4.1	8.1 ±5.6	9.2 ±3.4

NC Not counted as calves were treated with acaricides after contracting ECF
NR Not recorded
- Observation from only one calf
SD standard deviation

appendiculatus throughout the year, despite some variation, suggested continual challenge to calf population. These findings were in agreement with those observed by Newson, (1978) where more than one generation of *R. appendiculatus* were detected in a year on local Zebu cattle under similar environmental conditions.

In this study, age at detection of *T. parva* infection ranged from 36-116 days with 80% of the infections occurring by 3 months of age. Following release of groups of calves, days to diagnosis of ECF, on average, was 48 days and nearly two-thirds of the study calf population became infected within 2 months.

The longer time-period in mean days to infection for the first group of calves primarily occurred due to low *R. appendiculatus* challenge during the initial 1 month following release. This was thought to be as a result of restriction in the movement of calves on the homestead compound as a large proportion of the land, at the time of release of the group, was under maize cultivation. Nevertheless, all calves in this group contracted ECF.

The occurrence of more than one episode of clinical ECF in seven calves demonstrated the likelihood of the presence of antigenically diverse strains of *T. parva* in the area. Similar observations have been recorded in Zebu calves from an ECF endemic area of Kenya where some calves developed patent *T. parva* schizonts on more than one occasion (Moll *et al.*, 1986). In this respect, two calves were also diagnosed as having second bouts of clinical ECF in the longitudinal study (Section 5.2).

Besides ECF, anaplasmosis and babesiosis cases were also diagnosed in calves. Most of these other tick-borne parasitic infections occurred in calves which had either earlier or concurrently been exposed to *T. parva* infections. Prior or simultaneous infections with *T. parva* may have immunologically

compromised the calves rendering them vulnerable to these other parasitic infections (Irvin and Morrison, 1987).

Overall, 76% of the infected calves developed antibodies to *T. parva* schizont antigen, and those that did not seroconvert, died before the calves could mount an antibody response. Therefore, out of the 30 calves exposed, all the ten survivors became sero-positive. Case-fatality due to ECF of over 50% indicated a high mortality in spite of regular monitoring. Although ECF was the major cause of mortalities, deaths due to anaplasmosis and babesiosis, were also diagnosed. One calf which died from babesiosis appeared to be compromised by an earlier infection of *T. parva*. Deaths due to neonatal diarrhoea occurred as a result of poor adaptation to milk replacer.

Several workers have reported the higher susceptibility of exotic cattle breeds and their crosses with local Zebu or Sanga compared to the indigenous Zebu or Sanga to *T. parva* challenge (Cunningham, Joyner, Brown, Purnell, and Bailey, 1973; Radley, 1978; Paling, Mpangala, Luttikhuizen and Sibomana, 1991), and is generally accepted that mortality rates from ECF in untreated *Bos taurus* cattle can approach 100% (Irvin and Cunningham, 1981). The results from this study were generally in agreement with those reported by the earlier workers.

Under situations of high ECF risk prevailing in the coconut-cassava AEZ, this study was able to determine the likely age-window for immunisation against ECF using the I&T method. The range of 1 to 4 months of age at detection of infection in calves exposed to natural challenge defines the age for intervention by immunisation. The age-window for immunisation in the high ECF risk area should ideally be after calves are immunologically competent and before contracting ECF. Under practical situations, where tick control practices by small-holder farmers are likely to be inadequate, an age-window of 1 to 2 months is suggested because it will allow immunisation before the

majority of the infections occur. It has been shown that immunisation of calves on institutional farms in coastal Kenya has been successfully carried out in preweaned calves ranging from 4-6 weeks of age without any detrimental effects (Mutugi, *et al.*, 1991a).

In addition, calfhood immunisation has its advantages. Besides the ease of handling calves, it costs less in relation to the amount of drug used and most important of all, it reduces the risk of calf losses from the disease. Apart from preventing the occurrence of ECF and the resultant mortalities, calfhood immunisation will inhibit stunting, promote better growth and subsequently reduce the risk of contracting other diseases as a result of the immunosuppresive effects of *T. parva* infection (Irvin and Morrison, 1987). Production losses from theileriosis by stunting of calf development have been observed by Moll *et al.* (1986), where Zebu calves in an endemic area were found to have poor weight gains or weight losses during clinical infection. However, the major impact of this I&T technology will depend upon winning the confidence of more small-holder farmers to venture into sustainable and profitable dairy enterprise in the area.

In Kaloleni Division, the majority of the cattle population reported in the livestock census were indigenous Zebu (Thorpe *et al.*, 1993), and of the population tested for the presence of antibodies to *T. parva*, a large proportion of Zebu cattle (over 80%) in the coconut-cassava AEZ were positive (Deem *et al.*, 1993; Section 5.1). Similarly, the majority of the free-grazing dairy cattle (over 70%) had antibodies to *T. parva* schizont antigen (Sections 5.1 and 5.2). The question which therefore arises, is whether there is any justification in immunising local Zebu and the free-grazing exposed population in this AEZ.

An attempt to answer this question forms the basis of the next section in this chapter.

6.2 THE RELATIONSHIP BETWEEN PRESENCE OF ANTIBODIES TO *THEILERIA PARVA* SCHIZONT ANTIGEN IN ZEBU AND CROSS-BRED CATTLE AND IMMUNITY TO THE IMMUNISING STOCK OF *THEILERIA PARVA* MARIKEBUNI

6.2.1 INTRODUCTION

The principle of the I&T method of immunisation against ECF involves inoculation of cattle with stocks of *T. parva* exhibiting a broad-spectrum of immunogenicity together with simultaneous treatment with oxytetracycline (Radley, *et al.*, 1975a; Radley, 1981).

Under ideal circumstances, immunisation should cover the entire cattle population at risk, particularly in areas where endemic instability occurs due to seasonal variation in tick challenge, and total or partial tick control practices. However, implementation of blanket immunisation in all cattle types (local Zebu and dairy cattle) does not seem to be feasible mainly due to lack of financial and skilled manpower resources. Moreover, in high ECF risk areas, for example, in the coconut-cassava AEZ of Kaloleni Division, a large proportion of local Zebu and free-grazing dairy cattle already have antibodies to *T. parva* indicating the majority of them to be survivors (Section 5.1) and possibly immune to most of the local *T. parva* parasite stocks. In such situations, it is therefore, important to identify the target population for immunisation.

Cross-immunity trials have shown that the immunising parasite stock of *T. parva* Marikebuni cross-protected against several coastal and other parasite stocks isolated from various parts of Kenya (Irvin *et al.*, 1983, Mutugi *et al.*, 1989). However, what remains unknown is whether cattle populations exposed to natural infections with local *T. parva* strains are immune to the designated immunising stock of *T. parva* isolated from coastal Kenya.

In order to establish whether there was any justification in immunising the exposed local Zebu cattle and free-grazing dairy cattle, a challenge study was designed. The objective of the study was to determine whether presence of antibodies to *T. parva* in naturally exposed cattle of different ages was associated with immunity to the immunising stock of *T. parva* Marikebuni. Moreover, the study would retrospectively indicate whether partial immunisation of cattle herds, e.g., calves, would be a potential threat to non-immunised but exposed cattle within the herds, as it has been shown that I&T can lead to development of carriers of the immunising stock (Kariuki, 1991).

6.2.2 MATERIALS AND METHODS

a) Experimental animals

A total of 19 local Zebu cattle consisting of 10 young stock and nine adults were purchased from small-holder farmers in the coconut-cassava AEZ of Kaloleni Division. They were aged by their dentition. Ten indigenous Zebu whose permanent incisors had not yet erupted were considered as young cattle, aged between 6 to 18 months, while the remaining nine with first and second pairs of permanent incisors were regarded as adults between the age of 19-36 months.

Eight Ayrshire/Brown Swiss/Sahiwal cross-bred calves, survivors from the sentinel calf study (Section 6.1), which had previously had clinical ECF and been treated with parvaquone (Clexon) were also used in the challenge study. The cross-bred calves were 7 to 9 months old. Ideally exposed dairy cattle of similar age-cohorts as the Zebu cattle, belonging to small-holder farmers from the coconut-cassava AEZ, should have been used, but purchase of such a group was not possible.

Two cross-bred Ayrshire/Sahiwal dairy calves, less than 6 months old, obtained from the Animal Production Research Centre, Mariakani, which

were tested negative for antibodies (<1:40 titre) to *T. parva* schizont antigens on IFAT, were used as controls.

With the exception of five cross-bred calves from the sentinel calf study, all experimental cattle were kept at the Animal Production Research Centre, Mariakani, located some 40 km west of Mombasa on the main Mombasa-Nairobi road. Cattle were ear-tagged for identification and treated with an anthelmintic, 2.5% albendazole (Valbazen^{R}, Smith Kline Beecham) at 10 mg kg^{-1} and maintained under strict tick control by weekly application of flumethrin (Bayticol, Bayer, Germany) pour-on at 1 ml/10 kg bodyweight. The five cross-bred calves were managed similarly at the Regional Research Centre, Mtwapa, located 20 km north of Mombasa. All animals were supplemented with ranch cubes and mineral licks.

b) Pre-challenge serology

All cattle were bled for serum and screened for antibodies to *T. parva* schizont antigens using IFAT (Burridge and Kimber, 1972). Seventeen out of the 19 Zebu cattle were seropositive with antibodies titres greater than 1:160. Seven cross-bred calves had pre-challenge antibody titres of 1:160 or more, and one had a 1:40 antibody titre.

c) *Theileria parva* stabilate challenge

Each animal was challenged with 1 ml neat (undiluted) of cryopreserved *T. parva* Marikebuni sporozoite stabilate immediately after thawing. This dose was 10 x the dose used for immunisation (Mutugi *et al.*, 1989). It was inoculated subcutaneously in front of the left shoulder adjacent to the prescapular lymph node.

d) Monitoring

Following challenge, rectal temperatures of all animals were recorded daily, early in the day. Serum samples were collected every week from all cattle from the day of challenge. Monitoring continued for 35 days post challenge. Whenever rectal temperatures rose to 39.5^{o}C or above, considered to be a febrile response, biopsy smears from the prescapular lymph nodes were made. Smears were fixed with methanol and stained with Giemsa for microscopical examination. Blood in EDTA was also collected and tested for trypanosome infection using the buffy coat DG technique (Murray *et al.*, 1977).

Any animal eliciting a febrile response associated with presence of macroschizonts in the lymph node biopsies for 3 consecutive days was considered to be an ECF clinical reactor.

From the fifth day post challenge, the two control calves had their prescapular lymph node biopsies examined every day.

6.2.3 RESULTS

No clinical reactors were observed in the younger age group of the local Zebu cattle. In the adult Zebu group, one seronegative steer developed macroschizonts on day 15 post challenge. The macroschizonts persisted for 4 days, accompanied by pyrexia for 5 consecutive days, following which the steer recovered spontaneously (Table 6.2.1).

Of the eight cross-bred calves challenged, one calf that had a pre-challenge antibody titre of 1:40 developed a very severe clinical reaction and died from ECF 13 days after challenge. None of the other cross-bred calves, all of which had antibody titres of 1:160 or more, showed any clinical reactions.

Both control calves developed ECF. One died on day 18 while the other had a severe clinical reaction but recovered on day 20 post challenge without any treatment.

Table 6.2.1 Clinical reactions due to East Coast fever (ECF) in local Zebu and cross-bred dairy cattle following challenge with ten times the immunising dose of *Theileria parva* Marikebuni

Cattle type	n	Reactions due to ECF				Outcome
		Days to		Duration of		
		Pyrexia	Schizonts	Pyrexia	Schizonts	
Zebu						
Young stock (6-18m)	10	-	-	-	-	Survived
Adult (19-36m)	9	15(1)[a]	15	4	5	Recovered
Cross-breds						
Calves (>6m)	8*	11(1)	11	3	3	Died
Controls						
cross-bred	2	9(1)	7	14	11	Died
calves (<6 m)		11(1)	8	10	8	Recovered

n Number of cattle

- No reactions

a Figure in parenthesis represents number of reacting cattle

* Five cross-bred calves were challenged at Mtwapa Regional Research Centre

6.2.4 DISCUSSION

The presence of antibodies to *T. parva* reflects exposure to infection but is not necessarily indicative of immunity, as it has been shown that immunity to *T. parva* is cell-mediated (Emery, 1981; Emery *et al.*, 1981). Therefore, the immune status of cattle to particular stock *of T. parva* in a population can only be determined by challenge with the respective stock of the parasite.

In this study, the results showed that Zebu cattle and most cross-bred calves challenged with 10 x the immunising dose were immune to *T. parva* Marikebuni stock, whilst susceptible cross-bred control calves developed clinical ECF, with one resulting in mortality. This dose is known to cause fatal ECF in susceptible Friesian cattle (Mutugi *et al.*, 1989).

In addition, the study confirmed that the likelihood of cattle immunised with *T. parva* Marikebuni stock becoming carriers (Kariuki, 1990) and posing a threat to non-immunised cattle, is not necessarily important, as most cattle challenged were immune.

It therefore appears, that immunising local Zebu cattle above 6 months of age in an endemic area may not be justified. It is likely that the majority of local resident Zebus, which are in any case subjected to poor tick control, are exposed to *T. parva* parasites early in life, with some probably succumbing to the disease and dying, whilst others recover spontaneously and become immune. However, definitive studies are needed in order to investigate the outcome of early *T. parva* infections in local Zebu calves.

Ideally, Zebu calves between 1 to 6 months of age should have been included in the challenge study to ascertain their immune status. Although this was initially planned, the purchase of Zebu calves of less than 6 months of age proved difficult as farmers in the area were reluctant to sell their unweaned calves. This was because the act of suckling by calves stimulates milk 'let down' in dams allowing farmers to extract milk for human consumption.

Of the eight cross-bred calves challenged, one calf exhibited a clinical reaction and died. It was likely that the calf, which had a lower antibody titre (1:40), had not been exposed to the spectrum of *T. parva* strains to withstand challenge with *T. parva* Marikebuni stock. Even though most calves withstood the challenge, in general, free-grazing dairy cattle managed by small-holder farmers are subjected to a certain degree of tick control. It is probable that these control practices may prevent exposure of dairy cattle to a wider mosaic of antigenic types of *T. parva*. This was evident from the reoccurrence of ECF episodes in sentinel calves (Section 6.1) and in cattle in the longitudinal study (Section 5.2). Moreover, adult free-grazing dairy cattle in the longitudinal study, developed clinical ECF despite having antibodies to *T. parva* schizont antigen.

The high prevalence of antibodies against *T. parva* in local Zebu cattle reported in the cross-sectional study (Section 5.1) and by Deem *et al.* (1993), along with the results of the challenge experiment, clearly show that local Zebu above 6 months of age in an endemic area need not be immunised. Currently, it is not known whether immunisation of Zebu calves less than 6 months is essential, but it may well be that immunisation of this age-group would be advantageous in terms of their survival and performance, if carried out once the calves are immunologically competent. Furthermore, a reduction in calf losses from ECF will build the confidence of local Zebu farmers and provide them with an incentive to keep improved breeds particularly for dairy production.

The results in Sections 5.2 and 6.1 clearly demonstrated the susceptibility to ECF and the resultant losses through mortalities in dairy cattle managed in these small-holder production systems.

The aim of a vaccination strategy with I&T method must be firstly, to create an endemically stable immunised population and subsequently, to

immunise only animals that can upset this stability, namely, new animals introduced by birth or by purchase. In the present series of studies in endemically unstable population, it was clear that the target population was influenced by breed, age and management with the result that the following approach is recommended to create endemic stability to ECF (Table 6.2.2). Therefore, target population for immunisation must include dairy cattle of all age groups managed in both zero- and free-grazing systems, and all new entries into the area. Although majority of adult free-grazing dairy cattle were exposed and possibly immune to *T. parva* infection, immunising them would nevertheless be beneficial. As for local Zebu cattle, only calves less than 6 months and Zebu cattle introduced from non-ECF areas must be considered for immunisation.

In order to develop a strategic plan for the immunisation of the small-holder dairy cattle in Kaloleni Division, a pilot immunisation trial was carried out to define the logistics of the delivery of the infection and treatment method to small-holder dairy herds. The pilot study is described in the next section.

6.3 A PILOT STUDY OF THE IMMUNISATION OF SMALL-HOLDER DAIRY CATTLE AGAINST EAST COAST FEVER USING THE INFECTION AND TREATMENT METHOD

6.3.1 INTRODUCTION

Since 1986, successful trials of immunisation against East Coast fever (ECF) have been carried out on medium and large-scale institutional herds in Kilifi District, Coast Province of Kenya (Morzaria, *et al.*, 1987; Mutugi *et al.*, 1990, 1991a). The method of immunisation involves inoculation of host with a live

Table 6.2.2 Selecting target population for immunisation against East Coast fever (ECF) by artificially inducing endemic stability using infection and treatment (I&T) method based on cattle type (dairy or Zebu) and grazing management system (zero or free) in a high ECF risk area

	Zero-grazing	Free-grazing
Resident cattle population		
Dairy		
Calves <6 m	++	++
Young stock 6-18m	++	++
Adults >18m	++	+
Zebu		
Calves <6 m	N/A	++
Young stock 6-18m	N/A	-
Adults >18m	N/A	-
New cattle entries		
Dairy		
Calves (by birth)	++	++
Introduced (by purchase)	++	++
Zebu		
Calves (by birth)	N/A	++
Introduced (by purchase)	N/A	++*

++ Highly indicated

\+ May not be indicated, but beneficial

\- Not indicated

* Only those purchased from non-ECF endemic areas

N/A Not applicable as Zebu cattle are not managed in the zero-grazing system

m Age in months

sporozoite stabilate of *T. parva* Marikebuni parasite stock and simultaneous treatment with long-acting formulation of oxytetracycline (Radley, 1978; Mutugi, *et al.*, 1991a). Thereafter, animals are monitored for a period of 2 weeks, starting 14 days post-immunisation, for any clinical reactors caused by the method of immunisation.

The theilerial parasite stock selected for immunisation was isolated from Kilifi District (Morzaria, 1989a), characterised by monoclonal antibody profiles (Minami *et al.*, 1983) and cross-immunity studies (Irvin *et al.*, 1983). Cross-protection trials showed that *T.parva* Marikebuni conferred good protection when challenge with other stocks isolated from the Kenya coast (Irvin *et al.*, 1983; Morzaria *et al.*, 1987; Mutugi *et al.*, 1989).

Furthermore, in Kilifi District, the threat from buffalo- derived *T. parva* is virtually non-existent because the buffalo is absent in this area (Morzaria *et al.*, 1989a; Mutugi *et al.*, 1990). The Marikebuni stock, thus, was found to be ideal for intended large-scale immunisation to be undertaken in the District.

All successful immunisation studies using I&T method have been carried out in Government owned dairy herds (Mutugi *et al.*, 1991a). However, 85% of dairy herds in Kenya are small-holder (Mbogoh, 1984). Therefore there is a need to evaluate the use of I& T in this farming system.

There were several reasons why small-holder dairy farms in Kaloleni Division were selected for the ECF immunisation trial. In the Division, extensive in-depth studies have described the farming systems of the area, highlighting the importance of cattle diseases as one of the major constraints to dairy development (Thorpe *et al.*, 1993). Furthermore, a livestock census provided information on the number and distribution of dairy population within the Division (Thorpe *et al.*, 1993). In addition, a cross-sectional study of disease prevalence in small-holder dairy cattle, gave an antibody prevalence to *T. parva* schizont antigen of over 70% (Section 5.1) As was earlier discussed in

Section 5.1, this result indicated the failure of acaricide application for the prevention of tick-borne parasitic infections. Subsequently, the longitudinal investigation (Section 5.2) confirmed ECF to be the most important disease of small-holder dairy cattle in the area.

The importance of production losses resulting from ECF and the failure of recommended control methods, demonstrate the need to consider an alternative method for small-holder dairy cattle. The infection and treatment (I&T) method of immunisation (Radley, 1981) meets the requirements for a technology appropriate to the epidemiology of the disease, to the resources of the small-holders and to the current and likely future availability of veterinary services. Initially, all small-holder dairy cattle were identified as the target population for immunisation. The purpose of selecting all dairy cattle was to artificially create an endemically stable situation. Subsequently, immunisation would only be necessary in new-born calves and cattle introduced in the area.

The I&T method has only been tested in medium to large-scale dairy farms, where the procedure of immunisation and the post-immunisation 2-week monitoring were conveniently carried out on an individual farm basis (Morzaria *et al.*, 1987; Mutugi *et al.*, 1991a). A study was therefore required to test the logistical approach of delivery of the I&T method to small-holder dairy herds. Such a pilot study was designed and carried out in November 1990.

The study was undertaken in collaboration with National Veterinary Research Centre (NVRC), Muguga, Veterinary Investigation Laboratory (VIL), Mariakani, and the veterinary staff from the local Kaloleni Veterinary Office.

6.3.2 MATERIALS AND METHODS

a) Study herds

The pilot study involved 17 small-holder dairy herds with 64 cattle residing in the administrative locations of Kambe-Ribe and Ruruma of Kaloleni Division. Both locations lie in the coconut-cassava AEZ.

The selected herds had been sampled in the cross- sectional study, and 14 of them were included in the longitudinal study (Section 5.2). The herds were divided into two groups for the delivery of immunisation.

b) Briefing and reconnaissance

The Veterinary Officer responsible for field services in Kaloleni Division and two resident Animal Health Assistants (AHA) from the study area assigned to work with the immunisation team were briefed about the exercise. The researchers and AHAs made a reconnaissance visit to the small-holder dairy farms to explain the objective of the immunisation trial and the need for monitoring of the animals following immunisation. At the same time, distances between the farms in each group were noted in order to work out a schedule for the delivery of the immunisation and for the programme of post-immunisation monitoring.

c) Immunisation team

The immunisation team, also referred to as the task force, comprised of a research officer from NVRC, Muguga, a veterinarian from VIL, Mariakani, and the author. This team was supported by two laboratory technicians based at the VIL, Mariakani.

The two AHAs were paired with the two "experienced" stockmen from NVRC, Muguga, forming the field-based monitoring teams. The importance of monitoring was emphasised and the AHAs were trained at the VIL to carry

out lymph node biopsy smears. Details pertaining to monitoring, including the duration, frequency and timing of visiting immunised herds were also discussed. Each team was then provided with the work programme. During the monitoring period, members of the two teams were based in the two administrative locations ensuring close contact with the immunised herds and their owners.

d) Facilities

The VIL, Mariakani, 20 km from the study area, was well placed and equipped, and provided an ideal base for prompt processing and examination of samples.

e) Parasite stock for immunisation

The preparation of bulk sporozoite stabilate of *T.parva* Marikebuni stock (Stabilate IL 3014) has been described in several studies (Irvin *et al.,* 1983; Morzaria *et al.,* 1987; Mutugi *et al.*, 1989, 1991a). Briefly, a cryopreserved *T. parva* Marikebuni sporozoite stabilate was used to infect susceptible cattle, kept tick-free, at the International Laboratory for Research on Animal Diseases (ILRAD). During the theilerial reaction phase, a large number of clean *R. appendiculatus* nymphal ticks were applied to infected cattle to drop engorged during piroplasm parasitaemia. The resultant adult ticks were prepared into a *T. parva* sporozoite stabilate according to the methods described by Radley (1978). The stabilate was then distributed in 0.5 ml aliquots in pre-labelled colour-coded plastic straws and stored in liquid nitrogen. One ml of the stabilate was shown to cause fatal theileriosis when inoculated into susceptible cattle. The bulk stabilate was confirmed to have the same monoclonal antibody and the DNA profile as the original stabilate (Mutugi *et al.*, 1989).

f) Immunisation protocol

Immunisation of the dairy cattle was performed over 2 days, covering one group per day. The first group was in Ruruma with nine herds, while the second group in Kambe-Ribe, had eight herds. Of the 17 herds, 11 were managed in zero-grazing units, whilst the remainder were kept in the free-grazing system. The mean herd size of the selected farms was 3.7, ranging from 1 to 8 animals per herd, with the mode being 5. Cattle in each herd were recorded on pre-designed farm immunisation data sheets.

Prior to immunisation, cattle were weighed using a weighband,(Dalton Supplies Ltd U.K.) and ear-tagged for identification. They were also bled for serum. Rectal temperatures were recorded of all cattle, and five animals that were pyrexic (39.5^{o}C and above) were excluded from the immunisation programme. Three calves under a month old were noted, but not immunised. The majority of the dairy cattle were *Bos taurus* x *Bos indicus* cross-breds. As farmers could not accurately give the pedigree of their animals, no attempt was made to classify the study sample by their genetic composition.

i) Stabilate preparation

Plastic straws, each containing 0.5 ml of cryopreserved *T. parva* Marikebuni stabilate (IL 3014), were rapidly thawed by rubbing them between the palms of the hand. The contents of one straw were dispensed into a universal bottle containing 4.5 ml of Eagles Minimum Essential Medium with 3.5% w/v bovine plasma albumin and 7.5% w/v glycerol to give a stabilate dilution of 1:10. The mixture was allowed to equilibrate for 30 minutes and utilised whilst kept on ice through out the period of immunisation. The diluted stabilate was used within an hour of preparation.

ii) Stabilate inoculation

Out of the 64 dairy cattle in 17 herds, 56 were immunised using 1 ml of 1:10 diluted stabilate inoculated subcutaneously in front of the left shoulder, just adjacent to the prescapular lymph node.

iii) Drug administration

A long-acting formulation of oxytetracycline hydrochloric (Terramycin LA^{R} Pfizer Ltd, Sandwich, Kent, UK, injectable solution, 200mg/ml) was administered into the deep gluteal muscle just before stabilate inoculation. The dosage given was 20 mg kg^{-1} body weight plus 10% more as a precautionary measure because weighband weights are known not to be very accurate.

g) Monitoring

Monitoring started on day 14 post-immunisation for a period of 2 weeks and involved clinical examination and recording of rectal temperatures of all immunised cattle every second day. In the event, there was a febrile response with temperatures of 39.5^{o}C and above, prescapular or parotid lymph node biopsies smears were made for examination at the VIL.

To facilitate monitoring, herds belonging to each group were further divided into two sub-groups depending on the distance that was possible to cover on foot. On the basis of this arrangement, immunised herds were visited every alternate day and it took approximately 3 hr to carry out monitoring of one sub-group of herds. The two monitoring teams met with the task force daily at prearranged rendezvous points to collect samples for laboratory diagnosis and to check on any immunised animal showing a clinical reaction. Once an animal developed pyrexia, it was monitored daily by the task force. If a febrile response persisted for 3 successive days with lymph node biopsies

showing patent macroschizonts, such an animal was considered to be a clinical reactor. The reactors were treated with 20 mg kg^{-1} parvaquone (Clexon^R) given in two doses, 48 hr apart. Cattle were bled for serum on day 35 post immunisation, a week after end of the monitoring phase.

h) Serology

Pre and post-immunisation blood samples collected for serum were prepared and stored following the procedure described in Chapter 4. Sera were sent to NVRC, Muguga and assayed for antibodies to *T. parva* schizont antigen using the IFAT test (Burridge and Kimber, 1972).

6.3.3 RESULTS

All small-holder farmers were very cooperative and enthusiastic apart from one farmer who was excluded from the immunisation programme as he refused to allow his animals to be inoculated despite several attempts to convince him.

Analysis of the sera taken from the cattle prior to immunisation showed that 29 out of 56 cattle (51.8%) had antibodies to *T. parva* schizont antigen. Following the immunisation, seven animals (12.6%) developed clinical reactions which required treatment. Timely treatment with parvaquone (Clexon) successfully cured all clinical reactors. Of the seven reactors, five were managed in zero-grazing units with the remainder in free grazing herds. None of zero-grazing reactors had antibodies to *T. parva* before immunisation, whilst of the two free-grazing cattle, one was seropositive. Towards the end of the monitoring phase, five of the vaccinates were either sold or transferred to other farms and were therefore not bled for serum on day 35 post immunisation. Of the remaining 51 cattle, 44 (86.3%) had antibodies to *T. parva*.

6.3.4 DISCUSSION

The approach developed for the delivery of immunisation against ECF proved feasible for immunising small-holder dairy herds in the study area of Kaloleni Division.

This was the first attempt to immunise small-holder herds, and after almost two decades of research on the application of I&T method in Kenya, this exercise was clearly a historic event in the chronology of ECF control.

This pilot immunisation was also intended to ensure that resident members of the task force became familiar with the methodology and the potential logistical problems. Apart from the pilot study, the local task force personnel were being prepared for a wide-scale immunisation covering all dairy cattle in the Division.

The design of the immunisation schedule allowed inoculation and simultaneous treatment to be easily carried out. Moreover, involvement of the local AHAs during the reconnaissance stage of the exercise made the task of delivery further simpler as initial communication with farmers had helped to win their confidence and goodwill. This relationship was further strengthened by regular visits during the monitoring phase.

The importance of the monitoring phase was clearly demonstrated by the high numbers (12.6%) of clinical reactors detected. This was the crucial phase of the exercise. Failure to carry out effective monitoring could have lead to disastrous results and jeopardised the future implementation and acceptability of immunisation programme. In addition, effective monitoring prevented losses, and furthermore helped to enhance the trust built between farmers and those responsible for delivery of the technology.

The high number of clinical reactors will not be acceptable for a wide-scale delivery of immunisation to small-holder cattle. In immunisation trials of dairy herds on institutional farms on the Kenyan coast, Mutugi *et al.* (1991a)

attributed 14.7% clinical reactors in pedigree Jersey cattle to genetic factors. On the other hand, only 0.6% clinical reactors were seen when Zebu-European crosses were immunised on an institutional farm (Mutugi *et al.*, 1991a). In the present study, 12.6% reactors were observed in the cross-bred dairy cattle which had similar genetic composition as the Zebu-European crosses. However, the number immunised in this study were too few to draw any firm conclusions regarding the variation in the clinical reactor rates observed between the two studies.

Irrespective of the breed of cattle, the immunisation procedure should ideally have no clinical reactors. It is postulated that in order to avoid clinical reactors following immunisation, either lower doses of the stabilate or the use of an extended oxytetracycline cover, or both, should be used. Lowering the stabilate dose to produce mild immunisation reactions might present a problem as over-diluting of the stabilate could result in loss of some immunogenic components of the immunising stabilate (Mutugi *et al.*, 1988b). On the other hand, an additional treatment with oxytetracycline on the fourth day after inoculation with the stabilate will increase the cost of delivery of immunisation.

It would appear, therefore, that the most important factor in the safety of I&T method would be the determination of an optimal stabilate dose together with the evaluation of the most efficacious drug treatment for intended immunisation before wide-scale field immunisation is undertaken.

Apart from institutional herds and those herds involved in the pilot study in coastal Kenya, immunisation against ECF has been carried out on one of the islands of Zanzibar, off the coast of Tanzania. Using local isolates of *T. parva* stocks, about 500 European dairy cattle and their crosses with local Zebu in Government farms and belonging to small-holders were successfully immunised (Biwi, Rubia and Dolan, 1992). This is the only report of

immunisation against ECF in small-holder dairy cattle, although some 4,800 indigenous (Zebu and Sanga) calves between 2 to 12 months of age have been immunised in eastern Zambia (Berkvens, *et al.*, 1989).

The promising results from the pilot immunisation study paved way for a wide-scale implementation of immunisation in the rest of the dairy cattle belonging to small-holder farmers in Kaloleni Division. This venture, already completed, was carried out by researchers from NVRC, Muguga, VIL task force in association with the field veterinary staff. The group approach of technology delivery was used, and over 400 dairy cattle were successfully immunised (P.N.Ngumi, *pers comm.*)

Consequently, follow up immunisation of the new calf crop now needs to be undertaken. At present, the responsibility of follow up immunisation rests with the task force. The results from the sentinel calf study (Section 6.1) clearly demonstrated that in order to reduce the risk from ECF, calves must be immunised between one to two months of age. Calves greater than one month old have been successfully immunised in institutional herds in coastal Kenya (Morzaria *et al.*, 1989a; Mutugi *et al.*, 1991a) and under experimental conditions on a ranch in Kenya (Young, *et al.*, 1990b).

Regular visits to small-holder farms will be necessary to monitor the efficacy of immunisation, by observing the frequency of occurrence of ECF or other TBDs in the immunised population. This surveillance will contribute towards measuring the impact of immunisation in the small-holder sector. In this pilot study, out of the 56 animals immunised, 45 cattle were monitored during the longitudinal study from November 1990 to December 1991 (Section 5.2). Three cases of ECF were diagnosed in the immunised animals over the period, of which two occurred in cattle that had not seroconverted and resulted in mortality.

The success of this technology depends on the continual immunisation of the subsequent calves born in the area. Preventing calf losses from ECF will be a major contribution to the economic benefits of immunisation. Sustaining such a programme, is a large undertaking for the researchers and the task force and requires adequate funding. It is therefore recommended that immunisation in future is implemented on a full cost-recovery basis.

Controlling ECF by successful delivery of the I&T method immunisation will alleviate the risk of production losses from ECF, but not from other diseases. Although tsetse-transmitted trypanosomiasis was not observed to be a serious problem in the Kaloleni study area, that is not to say the disease is uncommon in other parts of coastal Kenya. In other study sites, less than 50 km from Kaloleni, trypanosomiasis appeared to be the major disease limiting the adoption of small-holder dairying. The significance of trypanosomiasis in this area highlights the complex epidemiology of vector-borne diseases in coastal Kenya. The importance of trypanosomiasis and an attempt to control the disease by chemoprophylaxis is addressed in the next chapter.

CHAPTER SEVEN

THE EFFICACY OF TRYPANOCIDAL DRUGS FOR THE CONTROL OF TRYPANOSOMIASIS IN DAIRY CATTLE EXPOSED TO NATURAL TSETSE CHALLENGE IN COASTAL KENYA

This chapter describes two studies carried out in areas of coastal Kenya where trypanosomiasis is known to occur and its control is by the use of trypanocidal drugs. The first study was an on-farm evaluation involving small-holder dairy cattle managed in the zero-grazing system in Kwale District (see earlier Figure 4.2). The second, an on-station experimental study was carried out at Regional Research Centre, (RRC) Mtwapa (see earlier Figure 4.3). Both studies evaluated the efficacy of trypanocidal drugs for the control of trypanosomiasis in dairy cattle exposed to natural challenge. In addition, the second study measured the effect of trypanosomiasis on production parameters such as milk yield and liveweight changes.

7.1 THE EFFICACY OF CHEMOPROPHYLAXIS FOR THE CONTROL OF TRYPANOSOMIASIS IN SMALL-HOLDER DAIRY CATTLE IN KWALE DISTRICT.

7.1.1 INTRODUCTION

Although tsetse-transmitted trypanosomiasis was not observed to be a major problem in Kaloleni Division, this did not reflect the situation in coastal Kenya as a whole. Several studies have described the importance of trypanosomiasis as one of the major constraints to livestock production in various parts of coastal Kenya (Maloo *et al.*, 1985; Paling *et al.*, 1987; Dowler *et al.*, 1989; Munstermann *et al.*, 1992; Gaturaga *et al.*, 1990).

In Kwale District, Coast Province, Kenya, tsetse-transmitted trypanosomiasis is widespread, and studies carried out in traditionally managed village Zebu herds and in a medium-scale Friesian dairy herd have identified trypanosomiasis as the major disease in the area (Maloo *et al.*, 1985; Gaturaga *et al.*, 1990). The major tsetse species in the sub-humid areas of the District are *Glossina pallidipes, G. austeni* and *G. brevipalpis* (Snow, 1979).

Glossina pallidipes is widespread, occurring is areas of ranging from bushland thicket to densely forested habitats, whereas *G. austeni* and *G. brevipalpis* are commonly found in areas of relatively undisturbed forests. In areas where habitats have been destroyed due to settlement and cultivation, tsetse densities have been affected (Snow, 1979).

In general, due to the high tsetse and trypanosomiasis risk the dairy enterprise in the District is underdeveloped and is mostly limited to a small number of medium-scale farms located along the coastline. These farms depend on regular use of the prophylactic trypanocide isometamidium chloride (Samorin^{R}), to protect their dairy herds against trypanosomiasis (Mwongela *et al.*, 1987; Gaturaga *et al.*, 1990). However, since 1989, several small-holder farmers in the District have ventured into dairying by adopting the zero-grazing package promoted by the National Dairy Development Project (NDDP).

Towards mid-1990, the NDDP approached the KARI/ILCA project to help in drawing up a trypanosomiasis control strategy for all small-holder zero-grazing dairy herds in the District. In early June 1990, a trypanosomiasis prevalence survey was carried out in randomly selected zero-grazing dairy herds within the District. Out of 49 cattle sampled, 13 had trypanosome infections, giving a prevalence of 26.5%. No other haemoparasitic infections were detected. In view of the high trypanosome prevalence, a chemoprophylaxis programme using isometamidium chloride was designed and initiated in collaboration with the District Veterinary Office (DVO) and the NDDP.

The objective of this study was to monitor the efficacy of the isometamidium chloride chemoprophylaxis for the control of trypanosomiasis in small-holder dairy herds in Kwale District.

7.1.2 MATERIALS AND METHODS

a) Study area

The study was carried out from June 1990 to December 1990, in Kubo, Matuga and Msambweni administrative Divisions of Kwale District. A comprehensive description of the study area is given in Chapter 4. In Kubo and Matuga Division, the majority of the zero-grazing units were located 10 to 20 km inland from the coastline in the vicinity of the Shimba Hills Game Reserve, while in Msambweni Division most zero-grazing units were nearer the coastline and in close proximity to a large sugar-cane plantation.

b) Study herds

A total of 71 dairy cattle managed in 25 small-holder zero-grazing units, representing all small-holder dairy farms in the District, were included at the beginning of the study. The distribution of the number of zero-grazing units and dairy cattle per Division at the beginning is presented in Table 7.1.1. Animals were identified according to their 'passport' information as described in the cross-sectional study (Section 5.1). The majority of the dairy cattle were Ayrshire/Brown Swiss/Sahiwal crosses purchased from Kilifi Plantations through the NDDP. Animal health and husbandry practices were generally similar to those described for dairy cattle managed in the zero-grazing system in Kaloleni Division (Chapter 4).

A chemoprophylaxis programme for the control of trypanosomiasis started in June 1990 with dairy cattle receiving isometamidium chloride (Samorin, RMB) at 0.5 mg kg^{-1} i.m, the manufacturer's recommended dosage, given approximately every 6 weeks. Calves less than 6 months old, were not inoculated with isometamidium chloride, but when detected parasitaemic, were treated curatively with diminazene aceturate (Berenil, Hoescht) at 7.0 mg kg^{-1}. The interval of 6 weeks between prophylactic treatment was selected on

Table7.1.1 Number of small- holder zero-grazing dairy herds and cattle at the begining of the study in the three Divisions of Kwale District, Coast Province, Kenya

Division	Number of herds	Number of cattle
Kubo	8	18
Matuga	11	25
Msambweni	6	28
Overall	25	71

the basis of what was generally recommended for a chemoprophylaxis programme in the sub-humid areas of Kwale District by the District Veterinary Office. Ideally the first chemoprophylactic treatment is timed to be given just before the start of the rainy season. In the present study, chemoprophylaxis was initiated on the basis of the high prevalence observed in the random survey and continued to be delivered at an interval of 6 weeks for the duration of the study.

The logistical approach of delivering the prophylaxis was worked out by preparing a treatment schedule for all herds in every Division. Prior to the chemoprophylactic treatment, weights of cattle were estimated using a weigh band (Daltons, UK). Isometamidium chloride inoculation of cattle was carried out in all Division over a period of 1 week. This exercise was implemented by the field veterinary personnel under the supervision of the research team. This was necessary as farmers were charged for the cost of prophylaxis and any subsequent treatments during the study period. Prior to the first prophylaxis, all animals were screened for trypanosome infection by microscopical examination of wet blood film preparations and those detected parasitaemic were treated with a diminazene aceturate at 7.0 mg kg^{-1}. The curatively treated animals were subsequently placed on isometamidium a week later. At the beginning, 56 out of the 71 (79%) cattle were prophylactically treated, while the remainder were calves under six months of age. During the course of the study, 19 newly purchased cows and eight newborn calves entered the study. In all, 98 cattle contributed comprehensive records to the study, with a total of 75 animals placed on isometamidium.

c) Monitoring

Monitoring for the efficacy of the chemoprophylaxis by checking for trypanosome infections in study cattle was carried out on a monthly basis,

starting July 1990. Study herds were visited for clinical examinations by the research team accompanied by the local animal health assistant (AHA). Following clinical examinations, rectal temperatures were recorded and blood and serum samples collected. Wet blood films prepared on the spot were examined by direct microscopy for any trypanosome infections. Peripheral ear vein blood samples were also collected in heparinised capillary tubes. Biological samples were processed the same day at the local veterinary diagnostic laboratory at Ukunda. Degree of anaemia was determined by measuring packed red cell volume (PCV) percent following haematocrit centrifugation of heparinised blood. Buffy coat preparations were examined for trypanosome infections using the DG technique (Murray *et al.*, 1977). Giemsa stained thin blood smears were screened for *Anaplasma*, *Babesia* and theilerial piroplasms. In addition, blood smears were used to identify trypanosome species from positive cases. Serum samples were stored at -20^{o}C. The sera were transported to ILRAD and assayed for trypanosome antigens and antibodies using ELISA. Details of the serological techniques have been described in Chapter 4.

Cattle detected positive for trypanosome infection during the course of the study were treated with diminazene aceturate (Berenil) at 7.0 mg kg^{-1}. Treatment was administered on the spot for those diagnosed positive on wet blood film examination, and within 24 hr for those detected on DG technique. All treatments were carried out by the local AHA accompanying the team (Figure 7.1.1).

In this study, the animals that died were not necropsied as they were not reported in time. However, presumptive diagnosis was made from previous observations and farmers' reports.

All field and laboratory data of study cattle were recorded on predesigned data sheets for each herd. Information containing the bio-data of

Figure 7.1.1 Treatment of a clinical case of trypanosomiasis by an animal health assistant (AHA).

every animal present during the study was entered into a computer data base management system, dBase III plus software package (Ashton Tate, 1986).

d)Data presentation and analysis

Cattle in each Division were grouped together on the basis of their zero-grazing management system and data were presented for each group rather than at individual herd level. This was because of small number of animals per zero-grazing unit which did not allow for individual herd comparisons. Trypanosome prevalence rates were calculated as the number of trypanosome parasitaemias divided by the number of cattle blood tested, and expressed as a percentage. Trypanosome prevalence rates were presented for only those cattle that were prophylactically treated with isometamidium chloride. Trypanosome antigen and antibody prevalence rates of cattle on prophylaxis were calculated in a similar manner.

Differences in trypanosome parasite, antigen and antibody prevalences among the Divisions were compared at 5% level of significance using chi-square test. Mean PCV differences were tested using one way analysis of variance (ANOVA) test in Minitab Release 7.1, (Appendix 8). Where significant differences in mean PCV were found, the Newman-Keuls Multiple Range Test was used to identify differences among the three Divisions.

7.1.3 RESULTS

Trypanosomiasis was the most common disease diagnosed in small-holder dairy cattle during the 6 months of the monitoring period. In total, 47 trypanosome parasitaemias were detected in 98 animals, of which 42 occurred in 75 prophylactically treated cattle. Five cases of trypanosomiasis were diagnosed in 23 unprotected calves. The majority of the parasitaemias were single infections, with *Trypanosoma vivax* and *T. congolense* contributing 70%

and 26% of the infections, respectively. Only two cases of mixed *T. vivax* and *T. congolense* infections were recorded. The only other haemoparasites detected were three cases of theilerial piroplasms, which showed clinical signs of East Coast fever (ECF) and were treated with parvaquone (Clexon^{R}) at 15mg kg^{-1} i.m, given twice, 48 hr apart.

a) Trypanosome prevalence rate and anaemia status

The overall trypanosome prevalence rate in prophylactically treated dairy cattle in Kwale District was 12.9% and varied from 16.5% and 18.1% in Kubo and Matuga Division, respectively, to 2.9% in Msambweni Division (Table 7.1.2). The five to six fold lower trypanosome prevalence in Msambweni was significantly different when compared with the prevalences observed in Kubo and Matuga. No significant differences were observed in the trypanosome prevalence among cattle in Kubo and Matuga. In addition, mean PCV of 27.6% and 28.1%, for cattle in Kubo and Matuga, respectively, were significantly lower than 31.1% recorded in cattle at Msambweni (Table 7.1.3).

In the Kubo and Matuga, trypanosome infections in cattle were detected in all months of the study, with the exception of one month in Kubo Division. The monthly trypanosome prevalence ranged from 0% to 33% and from 4% to 32% for cattle in Kubo and Matuga, respectively (Figure 7.1.2). Since implementation of the chemoprophylaxis regime, there appeared to be a decline in the monthly trypanosome prevalences in Kubo and Matuga, but total control was not achieved. By contrast, only one trypanosome infection was detected in each of the first 3 months of the study in cattle at Msambweni, giving a prevalence ranging from 0% to 6%. The effect of relatively low prevalence of trypanosome parasitaemia was reflected in the monthly mean PCV, where cattle in Msambweni had higher or equal mean PCV than their corresponding cohorts in Kubo and Matuga (Figure 7.1.2). Monthly mean PCV

Table 7.1.2 The prevalence of trypanosome infections in zero-grazing small-holder dairy cattle kept under isometamidium chloride (Samorin) prophylaxis in the three Divisions of Kwale District

Division	No.of samples tested	No. of trypanosome infections	Trypanosome prevalence (%)
Kubo	85	14	16.5
Matuga	138	25	18.1
Msambweni	103	3	2.9 *
Overall	326	42	12.9

* Significantly different in the column $p<0.05$

Table 7.1.3 Overall mean packed cell volume (PCV) of small-holder dairy cattle managed in the zero-grazing system in the three Divisions of Kwale District

Division	No.of PCV measurements	Mean PCV ± S.D
Kubo	83	26.9 ± 5.2
Matuga	137	28.0 ± 5.5
Msambweni	100	31.1* ± 5.3
Overall	320[a]	28.7 ± 5.3

SD standard deviation

* Significantly different in the column $p<0.05$

a PCV from six samples were not measured due to "run-off" during centrifugation

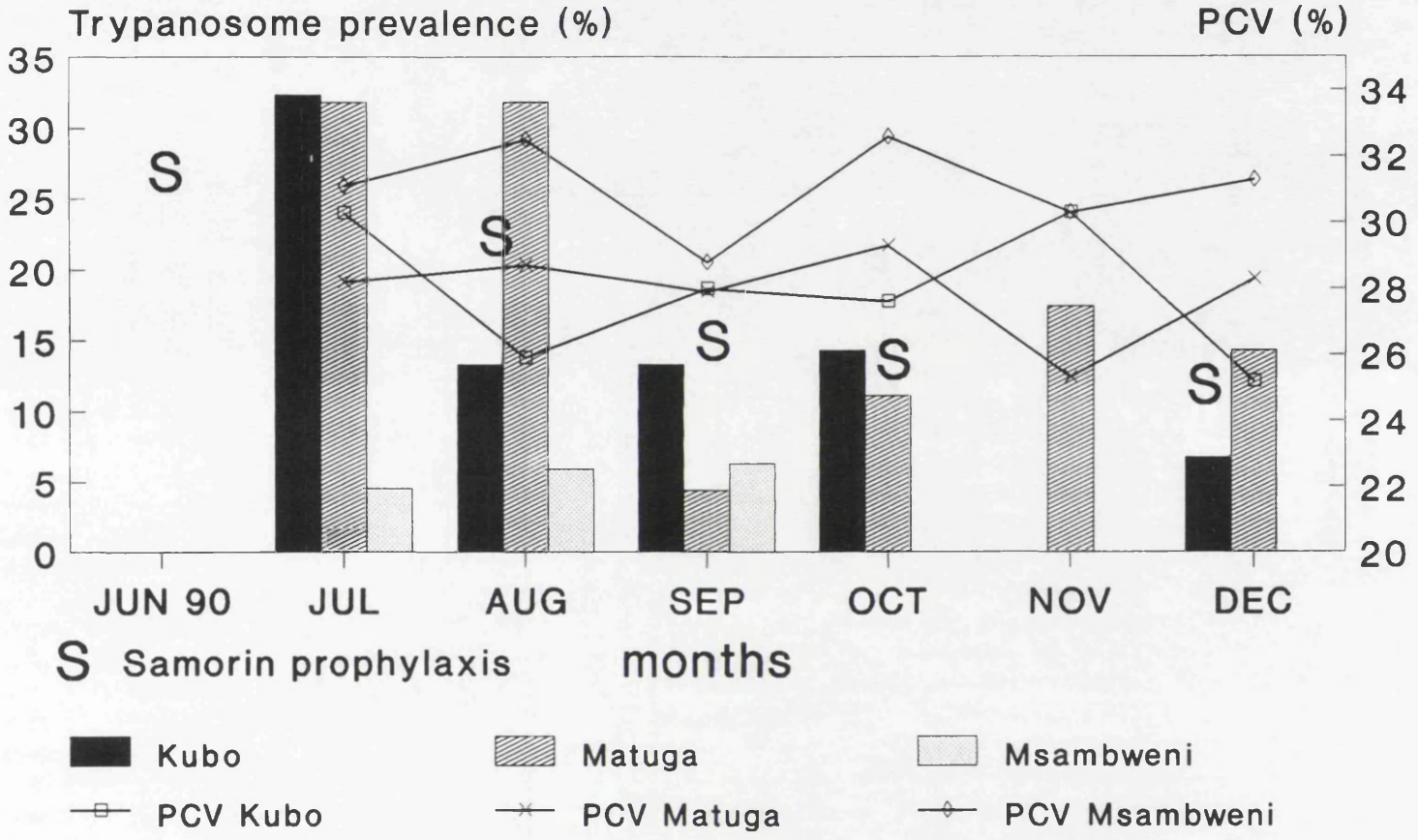


Fig.7.1.2 The monthly trypanosome prevalence and mean packed red cell volume (PCV) of chemoprophylactically treated cattle in the three Divisions of Kwale District

of cattle in Msambweni never dropped below 30% with, the exception of one month, while the monthly mean PCVs of cattle in the Kubo and Matuga were mostly lower than 30%.

b) Individual animal parasitaemias

Table 7.1.4 presents the frequency of occurrence of trypanosome parasitaemias in the dairy cattle. In Kubo and Matuga, 24 out of 52 (54%) cattle on the chemoprophylaxis regime became infected, while the majority of the cattle, 20 out of 23 (87%), remained aparasitaemic in Msambweni. Of the 24 parasitaemic cattle in Kubo and Matuga, 12 (50%) were detected trypanosome positive on more than one occasion with more than two-thirds of the parasitaemias, 27 out of 39 (69%) detected for the second or more times. One animal was diagnosed parasitaemic on four samplings. On the other hand, in Msambweni, three cattle were detected parasitaemic only once.

c) Days to detection of parasitaemia

There was a large variation in days to detection of trypanosome infection post chemoprophylaxis (Table 7.1.5). Parasitaemic animals were observed as early as 2 days post isometamidium chloride inoculation and ranged up to 45 days with a median of 21.5 days. Overall, 25 out of 42 parasitaemias (60%) occurred within 1 month after prophylaxis, with 11 cases detected by 15 days.

d)Trypanosome antigenaemia

The overall trypanosome antigen prevalences of 51%, 49% and 24% in cattle were observed in Kubo, Matuga and Msambweni Divisions, respectively. The two-fold lower prevalence of 24% in Msambweni was significantly different from the prevalences recorded in Kubo and Matuga. Out of the 213 sera tested

Table7.1.4 Frequency of occurrence of trypanosome infections in chemoprophylactically treated small-holder dairy cattle in the three Divisions of Kwale District

Frequency of parasitaemia	Kubo n=19	Matuga n=33	Msambweni n=23	Overall n=75
0	10	18	20	48
1	5	7	3	15
2	3	7	0	10
3	1	0	0	1
4	0	1	0	1

n Number of cattle

Table7.1.5 Range in days to detection of trypanosome parasitaemia in chemoprophylactically treated small-holder dairy cattle managed in the zero-grazing system in Kwale District

Days to detection of parasitaemia post prophylaxis	<15	15-30	31-45	Overall
Number of parasitaemic cattle	11	14	17	42
Range in days	2 - 13	15 - 29	31 - 45	2 - 45.
Median	7.0	17.5	32.0	21.5

from cattle in Kubo and Matuga, 106 were antigen positive, whereas in Msambweni only 24 out of 100 samples tested were detected positive.

By contrast, trypanosome antigenaemia prevalence in Kubo and Matuga were nearly three times the parasite prevalence of 16.5% and 18.1%, observed in cattle in the two Divisions, while in Msambweni, an eight-fold higher antigen prevalence (24%) compared to a parasite prevalence (3%) was found. Monthly antigen prevalences in Kubo and Matuga ranged from 46% to 57% and from 37% to 64%, respectively, and remained similar from July to December, while antigen prevalence in Msambweni declined from 42% to 13% (Figure 7.1.3).

Overall, 56% of the trypanosome antigens were identified as *T. congolense*, 25% as *T. vivax*, and the remainder as *T. brucei*. In Kubo and Matuga, 51% of the antigenaemias were due to single species, with the majority of them identified as *T. congolense* (Table 7.1.6). Most of *T. vivax* and *T. brucei* antigens were detected as mixed infections with *T. congolense*. Similarly, antigens of single trypanosome species contributed 75% of the antigenaemias detected in Msambweni, with most of them detected as *T. congolense*.

In general, out of the 42 parasitaemias detected on DG, nine were missed on antigen ELISA, while 97 samples which were negative on DG were tested antigen positive (Table 7.1.7).

e) Trypanosome antibody prevalence

Overall, antibodies to trypanosome infections were detected in 218 out of 298 (73%) sera tested during the study period with the monthly antibody prevalences ranging from 65% to 75%. Overall monthly prevalences of 78%, 74% and 68% in Kubo, Matuga and Msambweni Divisions, respectively, were not significantly different. Figure 7.1.4 shows the monthly antibody prevalences

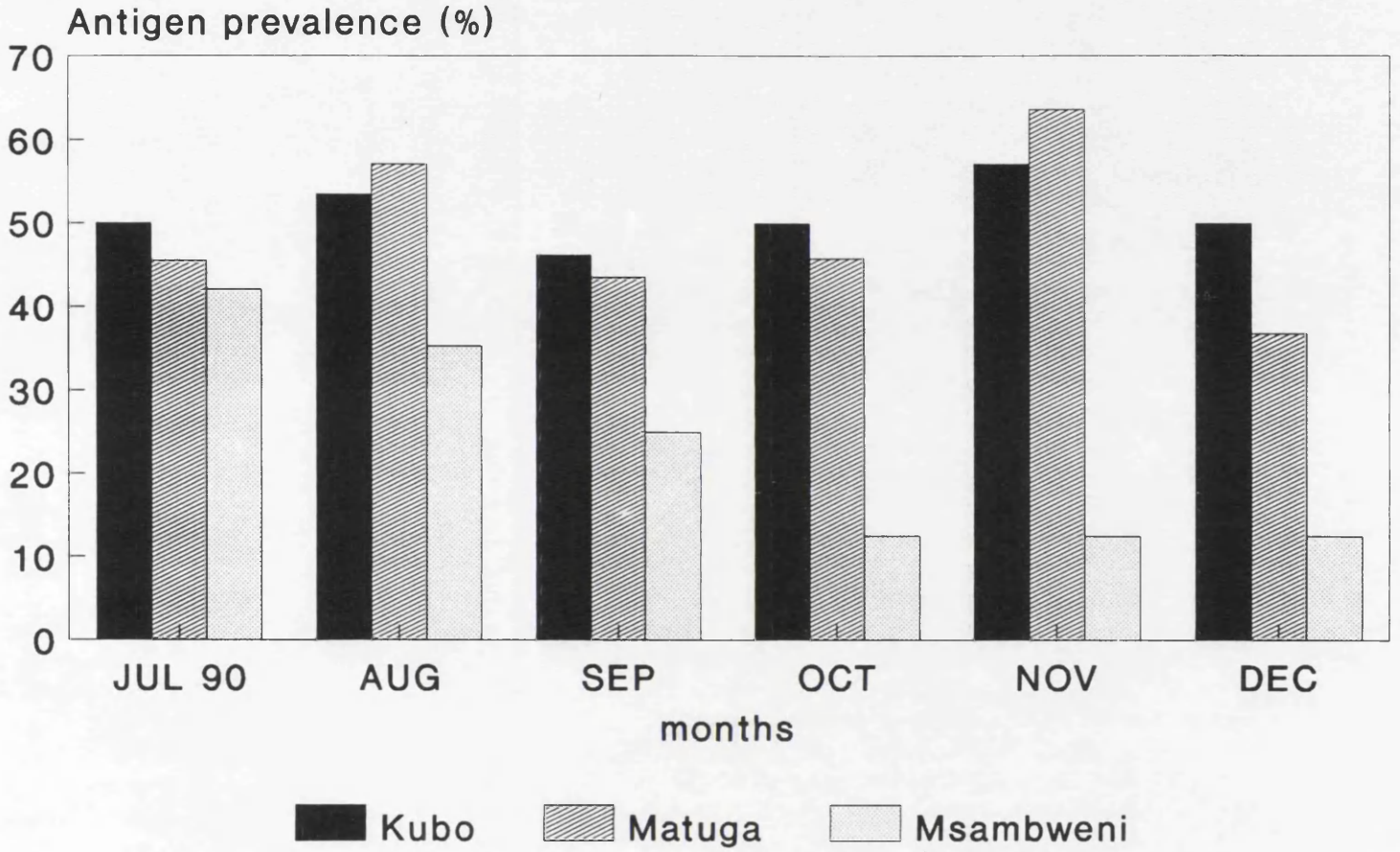


Fig 7.1.3. The monthly trypanosome antigen prevalence in chemoprophylactically treated small-holder dairy cattle in the three Divisions of Kwale District

Table 7.1.6 Identification of single and mixed trypanosome infections by antigen-ELISA in chemoprophylactically treated small-holder dairy cattle in the Kubo/Matuga and Msambweni Divisions of Kwale District

	Kubo/Matuga		Msambweni		Overall	
Species	n	%	n	%	n	%
Single infections						
Trypanosoma vivax (T.v)	4	3.8	6	25.0	10	7.7
T. congolense (T.c)	48	45.3	12	50.0	60	46.2
T.brucei (T.b.)	2	1.9	0	0.0	2	1.5
Mixed infections						
T.b ./ T. c	16	15.1	0	0.0	16	12.3
T. v. / T. c	16	15.1	5	21.0	21	16.2
T. b / T. v.	1	0.9	0	0.0	1	0.8
T. b / T. v. /T. c.	19	17.9	1	4.0	20	15.3
Total	106	100	24	100	130	100

n = Number of serum samples tested positive

Table 7.1.7 Comparision of antigen and antibody ELISA results with darkground (DG) technique in samples collected from cattle in Kwale District

	Antigen ELISA		Antibody ELISA	
	n = 313		n = 298	
	Positive	Negative	Positive	Negative
Buffy-coat (DG)				
Positive	33	9	36	6
Negative	97	174	182	74

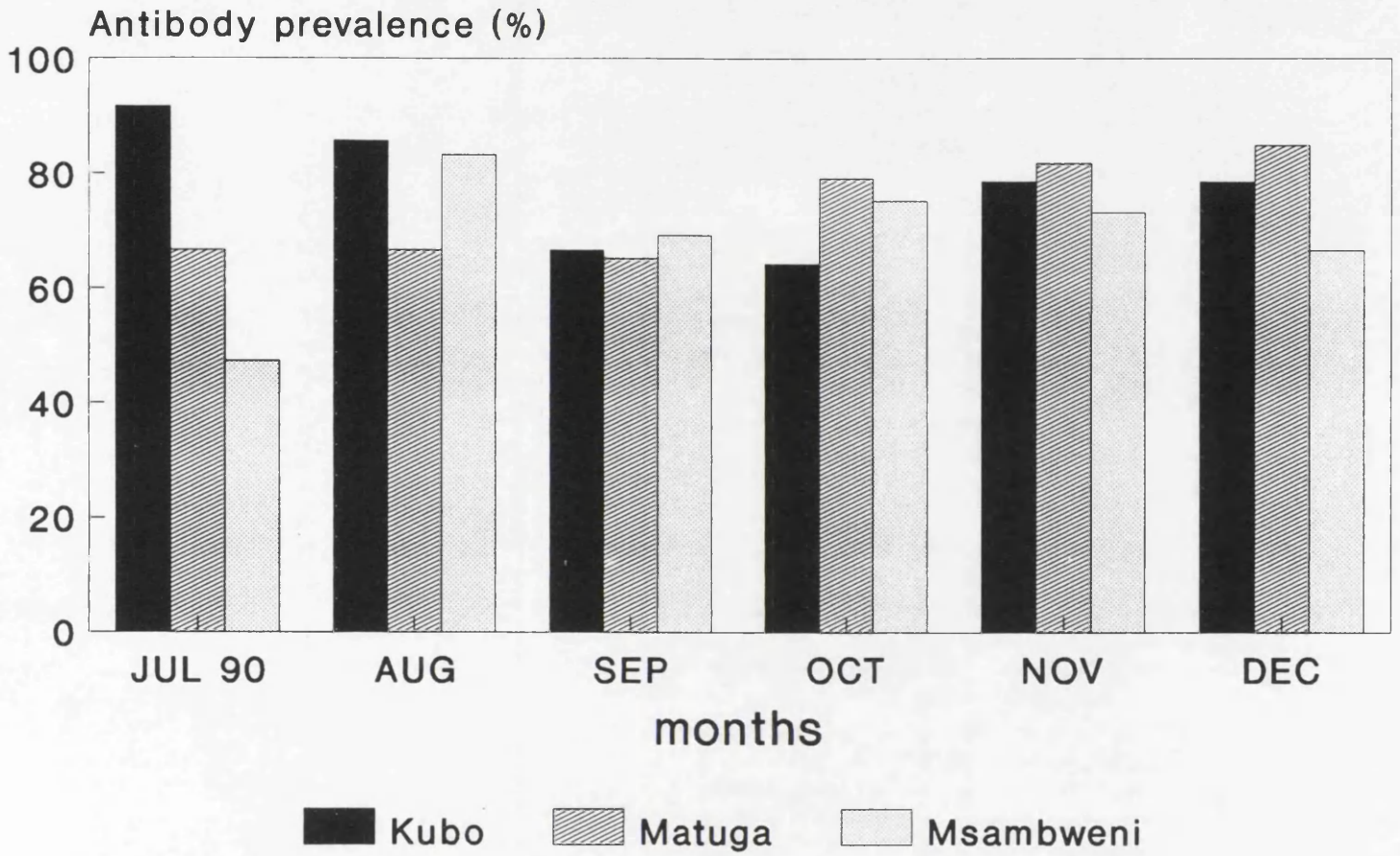


Fig.7.1.4 The monthly trypanosome antibody prevalence in chemoprophylactically treated small-holder dairy cattle in the three Divisions of Kwale District

in the three Divisions where antibody prevalences of above 60% persisted for most months in all the three areas.

Although a large proportion of the cattle in the study were antibody positive, six cattle which were detected parasitaemic on DG tested negative on antibody-ELISA (Table 7.1.7)

f) Mortalities

During the course of the study, four deaths occurred, three were in the prophylactically treated animals. The three cases were believed to have died from trypanosomiasis as they had recurrent trypanosome parasitaemias and were observed to be in ill-health for a long period. The fourth mortality occurred in a calf which died from a non-disease related cause (accident).

7.1.4 DISCUSSION

The observations made from this study confirmed the importance of trypanosomiasis as the major disease in the study population responsible for limiting the wide-scale adoption of dairying by the small-holder sector in Kwale District.

During the study period, apart from trypanosomiasis no other diseases were encountered in small-holder cattle managed in the zero-grazing system, with the exception of three cases of ECF which were successfully treated.

The study also demonstrated the variation in the efficacy of chemoprophylaxis using 0.5 mg kg^{-1} isometamidium chloride given every 6 weeks to small-holder dairy cattle in the area. The effectiveness of the chemoprophylaxis programme was assessed by monitoring for trypanosome parasitaemias in protected cattle. In areas of Kubo and Matuga, designated as the high trypanosomiasis risk area, the combined trypanosome prevalence was 17.5% compared to a significantly lower prevalence of 2.9% observed in

Msambweni, described as the low risk area. The definition of high and low trypanosomiasis risk areas, in this context, is based on the frequency of occurrence of trypanosome parasitaemias in small-holder cattle in a given area. The variation in the ability to control trypanosomiasis by chemoprophylaxis within a very small area emphasises the complexity in the epidemiology of the disease. On the other hand, treatment with diminazene aceturate appeared to cure most trypanosome parasitaemias.

In general, chemoprophylaxis failed to protect cattle against trypanosomiasis in the high risk area, where the majority of the zero-grazing units were located in the vicinity of the Shimba Hills National Game Reserve, an area known for its high tsetse population density (Snow, 1979). Recently, failure of isometamidium prophylaxis, even at 1.0 mg kg^{-1}, has been reported in several studies in the Coast Province, Kenya. On Galana ranch, in a trypanosomiasis endemic area, isometamidium afforded protection to beef cattle, for only 28.4 days, on average, with most breakthrough trypanosome populations detected as *T. vivax* (Dolan *et al.*, 1992). Likewise, on another beef ranch, located along the coastline in Lamu District, the prophylaxis regime provided total protection only for 1 week, with the majority of infections diagnosed as *T. congolense* (Munstermann *et al.*, 1992). These early infections were believed to be relapsing drug-resistant trypanosomes.

In the present study the high frequency of recurrence of trypanosome parasitaemias, where 12 out of 24 (50%) cattle became parasitaemic more than once during the study period, provided further evidence for the failure of chemoprophylaxis.

From this level of surveillance, it was difficult to assess whether trypanosome parasitaemias were new infections or relapses of parasites either from predilection sites in the animal where drug is inaccessible or due to drug-resistant trypanosomes. This was partly attributed to the study design where

monitoring intervals of 1 month were too long to determine conclusively the nature of frequent occurrence of trypanosome parasitaemias. In addition, lack of history of trypanosome infections and the frequency of trypanocidal treatments of cattle prior to the study made assessment of the nature of parasitaemias difficult. Moreover, parasite stocks were not isolated for drug-sensitivity evaluation.

In general, a large variation in the duration of prophylaxis ranging from 2 to 45 days was observed with half of the parasitaemic cases detected within 3 weeks. These observations were similar to those reported by Dolan *et al.* (1992) at Galana Ranch where the mean protection period was 4 weeks. In addition, more than 25% of the parasitaemias in the present study occurred by 15 days post prophylaxis suggesting an increasing likelihood of isometamidium drug-resistant trypanosomes. Under experimental conditions, the pre-patent period to detection of parasitaemia following experimental tsetse-transmitted trypanosome infections in cattle ranged from 10-16 days (Akol, Authie, Pinder, Moloo, Roelants and Murray 1986). Therefore, in the present study any parasitemias detected before 10-16 days post prophylaxis are likely to have been relapses due to drug-resistance.

Although trypanosome isolates were not tested for drug-resistance in this study, there have been increasing reports of isometamidium-resistant parasite stocks isolated from various parts of coastal Kenya (Githata,1979; Schonefield, *et al.*, 1987). Parasites stocks of *T. congolense* isolated from cattle in the same area as the present study, were found to be resistant up to 2.0 mg kg^{-1} of isometamidium chloride when tested in experimental drug-sensitivity cattle trials (Githata, 1979). More recently, the majority of the stocks of *T. vivax*, isolated from cattle in different areas along the Kenya coast were shown to be resistant to the prophylactic activity of 1.0 mg kg^{-1} isometamidium chloride (Schonefield *et al.*, 1987). Further, evidence of lowered sensitivity of

isolates of *T. vivax* from coastal region of Kenya to isometamidium was demonstrated in experimental studies where isometamidium treatment afforded protection for 1 month or less when cattle were experimentally fly-challenged with coastal stocks of *T. vivax* (Peregrine, *et al.*, 1991).

On the other hand, the situation in the low trypanosomiasis risk area of Msambweni was totally different, where within a short distance of less than 20 km, isometamidium prophylaxis appeared to protect cattle against trypanosomiasis. In this area, most zero-grazing dairy units were concentrated along the coastal belt in close proximity to a large sugar-cane plantation. It is possible therefore that the tsetse challenge was lower than in areas of Kubo and Matuga.

Apart from the low trypanosome prevalence in Msambweni, the mean PCV of cattle of 31.1% in this area was significantly higher than 26.9% and 28.0% observed for cattle in the high risk areas of Kubo and Matuga, respectively, where trypanosome parasitaemias commonly occurred. The higher mean PCV of 31.1% was comparable with PCV of 30.9% observed for zero-grazed dairy cattle in Kaloleni (Section 5.2), where trypanosomiasis risk was low. In contrast, the relatively low mean PCV in the high risk areas appeared to be similar to that reported in Zebu and Boran cattle in recognised medium to high tsetse challenge areas (Maloo *et al.*, 1988a; Munstermann *et al.*, 1992)

Immunological diagnosis by trypanosome antigen and antibody ELISA in the high risk area of Kubo and Matuga gave an overall antigen and antibody prevalence of 50% and 76%, respectively. This indicated that a relatively higher proportion of cattle were infected compared to an antigen and antibody prevalence of 9% and 20%, respectively, observed for dairy cattle managed in the zero-grazing system in Kaloleni Division (Section 5.2). The high antigen and antibody prevalences in the high risk area were comparable to those

reported in experimental cattle kept under ranching conditions in other areas of high tsetse challenge, as in Nguruman (Mwangi, 1993). In addition, the high proportion of mixed infections (49%) detected on antigen-ELISA, despite chemoprophylaxis, was similar to 74.8% and 73% reported in non-protected cattle in the high trypanosomiasis risk area of Nguruman (Nantulya *et al.*, 1992; Mwangi, 1993).

In the low risk area of Msambweni, trypanosome antigen prevalence was lower and appeared to be on the decline during the study period, whilst monthly antibody prevalence of 60% and higher persisted for most of the study period. The continual presence of antibodies even in the face of few parasitaemias is not unusual as trypanosomal antibodies are known to persist for several months even after anti-trypanosomal drug treatment (Luckins 1977; Nantulya, 1990). The explanation for the detection of relatively high numbers of antigenaemic cattle in the low risk area remains unclear.

The three-fold higher antigen levels in the high risk areas of Kubo and Matuga may be as a result of either low but undetectable levels of parasitaemias or chronic infections. Increased sensitivity of the antigen-ELISA in detection of trypanosome infection under laboratory based experimental conditions has been described by Masake and Nantulya, (1991). They reported a four-fold increase in sensitivity compared to the DG technique in detecting *T. congolense* infections. On the other hand, in this study antigen-ELISA missed nine cattle detected as parasitaemic by DG. Several studies have reported failure of antigen-ELISA to react positive to known parasitaemic cattle (Masake and Nantulya, 1991; Nantulya *et al.*, 1992; Trail *et al.*, 1992; Mwangi, 1993). It is postulated that in the initial phase of parasitaemia, sufficient parasite destruction by the host has not taken place to produce detectable antigen levels in the circulation (Nantulya and Lindqvist, 1989).

In view of the infections missed by antigen-ELISA, the use of DG technique will still remain an important field diagnostic test. As for the wider application of antigen-ELISA for field use, further studies need to be carried out to clarify the persistence of antigens, particularly for *T. congolense* in areas of low trypanosomiasis risk. At present, the combination of DG and antigen-ELISA provides an excellent tool for epidemiological studies. In addition, detection of antibodies provides essential information on the level of exposure to trypanosome infection in a given population.

The results of this study highlighted that one of the major concerns facing the small-holder dairy farmers in the high risk area of Kubo and Matuga Division was the continual detection of trypanosome infections despite efforts to control the problem with regular chemoprophylaxis using isometamidium chloride. To overcome this constraint, limited trypanocidal control options appear to be available. The initial intervention requires increasing the dose of isometamidium to 1.0 mg kg^{-1} given at the same interval. However, it is believed that effective control with this dosage may be questionable as unconfirmed reports by the local veterinary staff indicate that this dosage apparently does not afford sufficient protection.

On the assumption that chemoprophylaxis even at the higher dosage is likely to fail to prevent trypanosomiasis and with the likelihood of emergence of isometamidium drug-resistant trypanosome populations, the only alternative depends on the use of diminazene aceturate for curative therapy and as a sanative drug to treat against drug-resistant parasite stocks. Diminazene aceturate has been regarded as a sanative for treating isometamidium resistant parasite stocks (Whiteside, 1958). The term sanative refer to pairs of drugs that do not induce resistance to one another (Whiteside, 1958). Therefore, in order to carry out effective control, early detection and diagnosis of clinical trypanosomiasis will be of paramount importance. To supplement the efforts

of the veterinary staff, farmers will need to participate in the exercise and monitor temperatures of their animals on a daily basis. Farmer education on the importance of the disease together with training on taking, recording and reporting cases with elevated temperatures must be carried out. This can be done at the farm level with the assistance of field veterinary staff. Cases with elevated temperatures must be blood tested for parasites and once confirmed positive for trypanosome infection, prompt intervention with diminazene will help to prevent significant production losses.

The long-term control of trypanosomiasis aimed at reducing the tsetse challenge by the use of odour-baited traps and insecticidal impregnated odour-baited targets has successfully suppressed tsetse populations in many parts of Kenya (Dransfield *et al.*, 1991; Opiyo, *et al.*,1987;). In addition, the use of insecticides in dips or as pour-ons have been reported to reduce tsetse and trypanosomiasis challenge (Chizukya and Luguru, 1986; Thompson *et al.*, 1991; Lohr *et al.*, 1991). However, before embarking on tsetse control using trap and target technology, feasibility studies need to be carried out to determine the cost-effectiveness and economic justification of initiating such control methods. Under the present circumstances and with the small number of dairy herds that exist, large-scale tsetse control campaigns may not prove economical. On the other hand, the use of pour-ons may be beneficial, but studies on the effect of pour-on in small-holder dairy herds scattered over larger areas are lacking.

It therefore appears that the short-term solution depends on the importance of early detection and diagnosis of clinical cases followed by curative therapy. In the event such practices are inadequately implemented, significant production losses and mortality may result.

Production losses through trypanosomiasis in this area were of major concern to all small-holder farmers. However, due to the study design and difficulty in measuring production parameters, an on-station experimental

study to assess the effectiveness of a similar chemoprophylaxis programme on health and production of dairy cattle was carried out. This study forms the next section of this chapter.

7.2 EVALUATION OF THE EFFICACY OF PROPHYLACTIC AND THERAPEUTIC TRYPANOCIDAL DRUGS IN A DAIRY HERD EXPOSED TO NATURAL TRYPANOSOMIASIS RISK AT MTWAPA.

7.2.1 INTRODUCTION

In coastal Kenya and neighbouring regions, trypanosomiasis occurring in certain areas/locations, reduces cattle productivity directly through production losses in the current population, mainly Zebu herds managed in traditional small-holder systems (Maloo *et al.*, 1988a), and indirectly through limiting the use of breeds potentially more productive than the local Zebu. Studies in coastal Kenya and Tanzania have shown the importance of trypanosomiasis as a factor depressing the health and performance of Boran cattle in ranching systems (Trail *et al.*, 1985; Dowler *et al.*, 1989; Munstermann *et al.*, 1992), and of dairy cattle in extensive grazing systems (Paling *et al.*, 1987; Gaturaga *et al.*, 1990). Chemoprophylaxis with the trypanocidal drug isometamidium chloride (Samorin^{R},) was shown by Trail *et al.* (1985) and Maloo *et al.* (1988b) to reduce significantly the risk of trypanosomiasis and resulted in improved performance of the herds.

More recent field studies in coastal Kenya (Dolan *et al.*, 1992; Munstermann *et al.*, 1992), question the efficacy of trypanocidal prophylaxis in areas of high tsetse challenge. In addition, results from the previous study (Section 7.1) demonstrated the failure of isometamidium chloride to protect small-holder dairy cattle against trypanosomiasis under field conditions. However, due to the limited number of dairy cattle in small-holder herds

(Section 7.1), comparison of the efficacy of chemoprophylaxis with non-prophylactic controls was not possible, and neither was it practical to assess the effect of chemoprophylaxis on production parameters.

Therefore, a contemporaneous on-station experiment was designed to compare the efficacy of prophylactic and therapeutic trypanocidal drugs in dairy cattle exposed to natural trypanosomiasis risk in coastal Kenya. The study also attempted to determine the effect of trypanosomiasis on liveweight changes and milk yield of dairy cattle.

7.2.2 MATERIALS AND METHODS

a) Environment and herd management

The experiment was carried out from July 1990 to December 1991 on a dairy herd of Jersey cattle belonging to Kenya Agricultural Research Institute's Regional Research Centre (RRC), Mtwapa, Coast Province. Detailed description of the experimental site and herd management practices are given in Chapter 4.

Mean annual rainfall over the last 20 years at the Centre, was about 1,100 mm with peak rainfall between April and June. During the period of the study, total rainfall was close to the long-term mean, but exceptionally heavy rain fell in May to July 1991 which was followed by a period of below monthly average rainfall (Figure 7.2.1).

The Jersey herd was established in the late 1950s. Its history, management and performance up to the late 1980s have been reported by Njubi *et al.* (1992). In brief, the breeding herd comprised of two groups, the lactating and the dry cows, which grazed day and night on natural pastures with shade trees or under the tree crops, cashew-nut and coconut palm, receiving little or no supplementary feed. Cows were hand milked twice daily and the yield of each milking recorded. Breeding practices depended on AI services

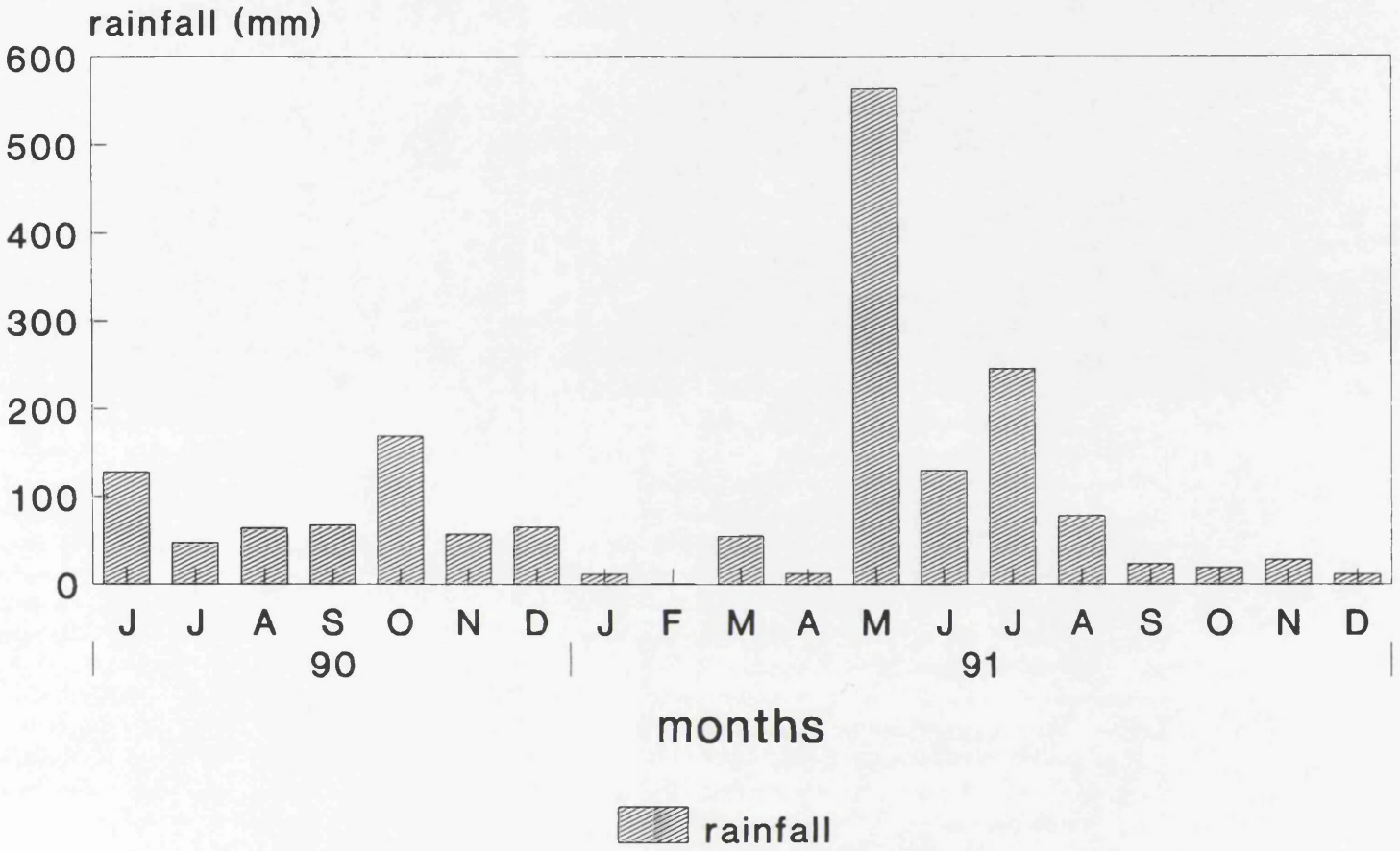


Fig.7.2.1 Monthly rainfall distribution during the experimental period at the Regional Research Centre, Mtwapa.

provided by the Veterinary Department. Heifers were introduced into the breeding herd at about 18 months of age. Preventive veterinary practices carried out were vaccinations against rinderpest, foot-and-mouth disease, lumpy skin disease, anthrax and blackquarter, and weekly acaricidal spraying to control against tick-borne diseases. In 1987, the herd was immunised against East Coast fever using the infection and treatment method (Mutugi *et al.*, 1991a).

The tsetse challenge at Mtwapa has generally been considered to be low and tsetse surveys carried out by the Veterinary Department in the area have indicated the presence of *Glossina pallidipes* and *G. austeni* as the main tsetse species (Anon, 1991a). In nearby areas, e.g., at Vipingo, an area 20 km north from the study site, *G. pallidipes* was the only tsetse species caught (Dowler *et al.*, 1989), whereas at Kilifi Plantations, 40 km from Mtwapa, *G. austeni* was the predominate tsetse trapped (Paling *et al.*, 1987). In recent years, the low tsetse challenge at Mtwapa has been thought to be due to the increasing pressure from human settlement leading to destruction of tsetse habitats. Furthermore, tsetse surveys at Mtwapa caught proportionally more biting flies (*Stomoxys* and *Tabanids* species) than tsetse (Anon 1991a).

The herd had a history of trypanocidal prophylaxis, in which isometamidium chloride at 0.5 mg kg^{-1} was given one to four times a year, depending on the level of trypanosomiasis risk. The criterion for prophylactic treatment was based on clinical diagnosis of trypanosomiasis cases. Whenever, 10% of the herd was diagnosed clinically as suffering from trypanosomiasis, the whole herd was placed on isometamidium chloride. This system was not very efficiently implemented and mortalities due to trypanosomiasis were common. Prior to the start of the experiment, 10 cows had died from trypanosomiasis in the previous 3 months.

b) Experimental design and procedures

Towards the end of June 1990, all breeding females in the Jersey herd were treated with diminazene aceturate (Berenil^{R}) at 7.0 mg kg^{-1} to eliminate any current trypanosome infection. In July 1990, 69 cattle categorised by age into <5 years, and 5 years and above age-class, and by lactation status (lactating or dry), were assigned at random to two treatment groups.

The first group was given the prophylactic drug, isometamidium chloride, at 0.5 mg kg^{-1} bodyweight, the dose rate recommended by the manufacturer. The prophylaxis was repeated every 3 months, given in the months of October 1990, January 1991, April, July and the last administration in October 1991. The 3 months interval was worked out based on the number of prophylactic treatments given per year for the last 6 years. Apart from the routine prophylaxis, animals diagnosed as having trypanosome infections in this group were treated with diminazene aceturate at 7.0 mg kg^{-1}.

The second group of animals received no prophylactic drug, but was therapeutically treated with 7.0 mg kg^{-1} diminazene aceturate when detected parasitaemic.

Heifers which entered the breeding herd during the course of the experiment, were placed into respective treatment groups at random. For the six 3-monthly prophylactic periods, an average of 39 out of a total of 78 animals, received the trypanocidal prophylaxis.

c) Monitoring of experimental herd

Cattle in both the groups were observed at least once daily and blood sampled once every week. Peripheral ear vein blood collected in heparinised capillary tubes from all animals were examined for trypanosomes using the dark-ground buffy coat technique (Murray *et al.*, 1977). All parasitaemic cattle were treated with diminazene aceturate at 7.0 mg kg^{-1}. In addition, clinical cases observed

between the weekly samplings in both groups were tested for trypanosomes, and if positive were treated curatively and recorded accordingly. At the weekly sampling, whole blood was measured for packed red cell volume (PCV) percent using the haematocrit centrifugation technique. Trypanosome species were identified by their motility on buffy coat examination and by preparing Giemsa stained thin blood smears. The animals were weighed monthly using a suspension cattle weigh-bridge.

When diseases other than trypanosomiasis were diagnosed, they were treated and recorded. Mortalities occurring during the study period were recorded and post-mortem examinations were performed to determine the cause of death.

d)Data analysis

The data collected over the 18 month experimental period were grouped into six periods, the periods being the three months intervals between prophylactic treatments. The periods were designated I, II, III, IV, V and VI. The variables submitted to statistical analyses within each period were occurrence of trypanosome parasitaemia, days to first trypanosome parasitaemia, mean PCV, liveweight change and daily milk yield.

i) Logistic regression models

Linear logistic regression models (Collett, 1991) were used separately for each period for the analyses of occurrence of trypanosome parasitaemia. Each animal was categorised as either having no infection in the period or as having one or more infections. Effects of prophylactic treatment (S_i, $i=1,2$), cow age (A_j, $j=1,2$) and cow lactation status (L_k, $k=1,2$) were fitted. The logistic model was of the form:

$$\log \frac{(p_{ijk})}{(1-p_{ijk})} = \mu + S_i + A_j + L_k$$

where p_{ijk} is the predicted probability of infection for an animal with prophylactic treatment i, of age j and lactation status k and μ is an overall 'mean'. The model can be extended to include interaction terms.

The parameters of the model were estimated by maximum likelihood, using Genstat 5 (Lawes Agricultural Trust, 1990). The statistical significance of effects was tested using deviances which have approximate chi-squared distributions. Predicted probabilities were estimated using the inverse of the logit transformation given by :-

$$p = \frac{\exp(\mu + S_i + A_j + L_k)}{1 + \exp(\mu + S_i + A_j + L_k)}$$

Approximate standard errors for the predicted probabilities were also obtained.

The two-factor interaction terms were not significant and none was included in the final models. In period I, where a considerable number of animals had two infections, a log-linear model was used to analyse three categories of infection (0, 1 or 2 parasitaemias). However, this analysis did not alter the conclusions of the logistic model described above. In addition, observed means for the occurrence of trypanosome parasitaemias were calculated as arithmetic means.

ii) Cox's proportional hazards model

Cox's proportional hazards model (Cox and Oakes, 1984) was used to analyse in periods I and III the dependent variable, days to first detection of trypanosome parasitaemia. The other periods had zero or low trypanosome occurrences (Table 7.2.1). Effects included in the model were prophylactic treatment, cow age and cow lactation status and their two-factor interactions. The proportional hazards model (without interactions) has the form:

$$h(t) = h_0(t)\exp(\mu + S_i + A_j + T_k)$$

where h(t) is the hazard function which defines "instantaneous" probability of infection at time t, given that the animal is not already infected $h_0(t)$ is an unspecified baseline hazard function. The parameters S_i, A_j and L_k are defined as before and now represent relative risk factors. Estimation was by maximum likelihood using the programme EGRET (Statistics and Epidemiology Research Corporation, 1991).

iii) Analyses of variance

The continuous (quantitative) variables were analysed by least squares analyses of variance using fixed models in the general linear models procedure of SAS (Statistical Analysis Systems Institute, 1986). The model for the analyses of the mean PCV in a period included the independent factors, treatment groups (receiving or not receiving isometamidium), trypanosome parasitaemias (0, 1 or more), cow age (<5 years, 5 years or more), lactation status (lactating or dry) and their two-factor interactions.

The effect of trypanosomiasis on liveweight change was estimated for cows of known pregnancy status in periods I and III, the periods when number of trypanosome cases observed were high (Table 7.2.1). Cows calving during a

period were excluded. The model included the independent factors, treatment group, trypanosome parasitaemia (0, 1 or more), cow physiological status at the end of the period (empty, 1-6 months pregnant or 7-9 months pregnant) and their two-factor interactions. Liveweight at the beginning of the period was fitted as a covariate.

The effect of trypanosomiasis on daily milk yield was estimated by first identifying those lactating cows with detected trypanosome parasitaemia. For these cows and for their non- parasitaemic contemporaries, mean daily milk yield (DMY) was calculated for the week at the end of which parasitaemia was detected (week 0), for the preceding week (week -1), and for the 4 weeks following detection of parasitaemia (weeks 1 to 4). Any cow in which another parasitaemia was detected in weeks -1 or 1 to 4, was excluded. The model for DMY included the independent factors, prophylactic group (PG), cow nested within prophylactic group, trypanosome parasitaemia (TP), and the interaction of PG and TP. Mean DMY in week -1 was fitted as a covariate in the analyses of DMY in weeks 0 to 4.

Differences between the occurrence of and days to trypanosome parasitaemias in prophylactic and non-prophylactic groups were tested at 5% level of significance. Similarly, continuous (quantitative) variables of mean PCV, liveweight changes and mean DMY were also tested at 5% level of significance.

7.2.3 RESULTS

a) Herd health

In this research herd in which routine acaricide application and other preventive medicine practises were implemented, trypanosomiasis was by far the most frequent cause of ill-health. Over 130 trypanosome parasitaemias were detected during the 18 month experiment. In periods I and III, clinical

cases of trypanosomiasis associated with *Trypanosoma vivax* infections developed into an acute form of the disease exhibiting a haemorrhagic syndrome. Apart from being parasitaemic, animals were pyrexic, developed bloody diarrhoea and had severe petechiations of the conjunctival and vulval mucosae. Occasionally, discharges of frank blood were observed from the nostrils and the ears of the infected cattle.

By comparison there were only a total of 58 reported cases of other diseases or ill-health, of which 33 (57%) were mastitis diagnosed in 20 cows. There were eight cases (14%) of foot-rot and lameness and a total of six (10%) cases of dystocia, retained placenta and metritis. Eleven cases (19%) had minor problems with abscesses, and kerato-conjunctivitis (pink-eye).

During the study there were five mortalities. Three were attributed directly to haemorrhagic *T. vivax* infection, while East Coast fever was diagnosed in a cow which died after trypanosomiasis was detected and treated, and another cow had trypanosomiasis complicated by unidentified causes. Post mortem examinations of cattle dying from the haemorrhagic syndrome were characterised by marked ecchymotic and petechial haemorrhages of the gastrointestinal mucosa, "blood splashed" appearance of the skeletal muscles, petechiations of the epicardium, and enlarged, oedematous and haemorrhagic lymph nodes. All mortalities attributed to trypanosomiasis were in the group not receiving isometamidium. With the exception of the ECF case associated with trypanosomiasis, no other of tick-borne infections were detected.

b) Trypanosome parasitaemia

Trypanosomes in all detected parasitaemias were identified as *T. vivax*. During the 18 month experiment, trypanosome parasitaemias observed 60 times in the 242 animal/periods in the isometamidium chloride prophylactic group, compared to 74 parasitaemias in 227 animal/periods in the non-prophylactic

group were significantly not different. There were only 10 animal/periods in each of the prophylactic and non-prophylactic groups which had two detected parasitaemias. The occurrence of one or more trypanosome parasitaemias varied widely between the six 3-monthly periods, ranging from 0% to 58.1% and 0% to 72.5% in the prophylactic and non-prophylactic treatment groups, respectively (Table 7.2.1). Whether trypanosomiasis challenge was high, as in July to October (period I) and in January to April (period III), or low as in periods II, IV and VI, the proportion of parasitaemic animals in all periods was similar in the prophylactic and non-prophylactic groups. Consequently, there was no significant effect of the prophylactic treatment on detection of trypanosome infection in any single period.

The detection of trypansome parasitaemias in a period was not significantly affected by lactation status or age, except in period IV, when the older (>5 years) group of lactating cows had a significantly higher occurrence of trypanosome parasitaemia, 36.7% (se 11.73), than the other lactating and dry age groups (5.6% to 10.0%). During the experiment, there was no tendency for one lactation status or age class to have a consistently different parasitaemia detection rate.

Diminazene aceturate was effective as a therapeutic agent. Animals detected with trypanosome parasites and which were treated the same or the next day with diminazene aceturate, became aparasitaemic when tested a week later, with two exceptions, which remained parasitaemic at the next sampling. After further treatments with diminazene aceturate, both animals were successfully cured as neither were detected parasitaemic the following week.

Just as prophylactic treatment did not prevent occurrence of trypanosome infections (Table 7.2.1), time (in days) to detection of first trypanosome parasitaemia (DTP) was not affected by prophylactic treatment in periods I or III. For those cows with a detected parasitaemia in period I, the

Table 7.2.1 Observed and estimated* mean percentages of dairy cattle detected with one or more trypanosome parasitaemia during each of the six 3-monthly periods in prophylatic and non- prophylactic treated groups

	Prophylactic				Non-prophylactic			
		Parasitaemic cattle				Parasitaemic cattle		
Period	Number of cattle	Observed mean (%)	Estimated mean (%)	s.e	Number of cattle	Observed mean (%)	Estimated mean (%)	s.e
I	33	54.5	55.9	8.34	36	58.3	57.1	8.05
II	36	2.7	2.8	2.79	38	13.2	13.0	5.38
III	43	58.1	58.9	7.40	40	72.5	71.8	7.09
IV	43	9.3	9.5	4.29	37	18.9	18.6	6.00
V	44	0.0	0.0	-	39	0.0	0.0	-
VI	43	4.7	5.0	3.42	37	5.4	5.0	3.38

* Estimated mean was calculated by logistic regression methods

s.e Standard error

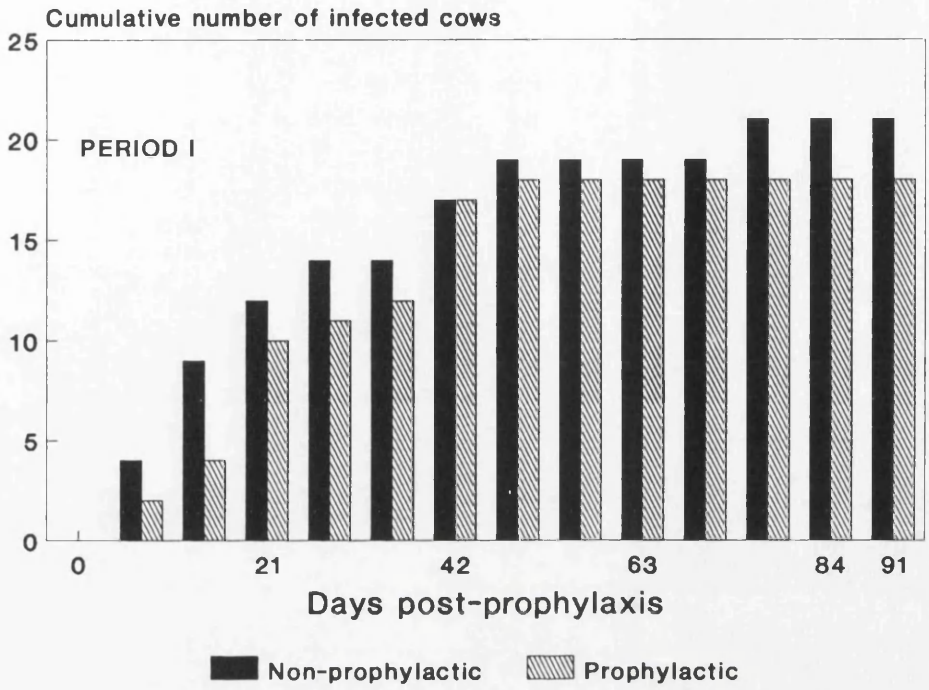
mean DTP for cows not receiving and those receiving isometamidium chloride were 27.4 (se 4.70) and 26.8 (se 3.23) days, respectively. In period III, the respective figures were 70.3 (se 3.48) and 69.5 (se 2.94) days.

Figure 7.2.2 shows the weekly distribution of detected parasitaemias in prophylactic and non-prophylactic groups in periods I and III, confirming that in both periods parasitaemias were detected as early and with similar frequency in the prophylactic as in the non-prophylactic groups. In period I, trypanosomiasis risk was high from the beginning of the period (Figure 7.2.2). By the end of the fourth week (28th day), trypanosomes had been detected in 67% of the animals in the non-prophylactic group and 61% in the prophylactic group. Within one week of receiving the isometamidium chloride, two animals were parasitaemic, and by 21 days post-administration 10 parasitaemias were detected, an infection rate very similar to that of the group not receiving isometamidium chloride (Figure 7.2.2). In period III, most infections were detected after day 63, and infection rates were similar in both groups (Figure 7.2.2).

Cow age and cow lactation status were not significant factors affecting days to detection of first parasitaemia. However, the effect of lactation status approached significance ($p<0.07$) in period I, in which the hazard ratio (lactating versus dry) was 1.82 with a 95% confidence interval of 0.97 to 3.42, implying that lactating cows were at higher risk from trypanosomiasis than dry cows.

c) Anaemia status

In periods I and III, when the majority of trypanosome infections were observed, mean PCV was significantly depressed by one or more parasitaemias (Table 7.2.2). In no period did prophylactic treatment (PT) affect mean PCV, and neither was there any significant interaction of PT x trypanosome



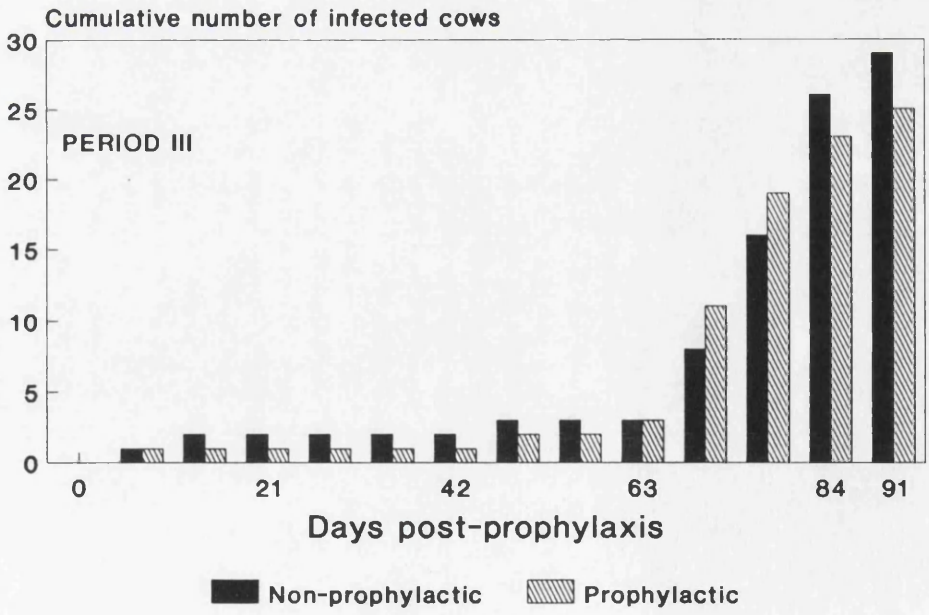


Fig.7.2.2 The cumulative number of cows with detected trypanosome parasitaemia in the prophylactic and non-prophylactic groups in the first and third periods of the study

Table 7.2.2 Mean least square estimates of packed red cell volume (PCV) percent in the 3-monthly periods for parasitaemic and non-parasitaemic dairy cattle

	Parasitaemic			Non-parasitaemic			
Period	Number of cattle	Estimated mean PCV (%)	s.e	Number of cattle	Estimated mean PCV (%)	s.e	Significance
I	38	29.4	0.41	28	31.0	0.54	$p<0.05$
II	6	32.4	2.09	68	30.6	0.41	NS
III	53	27.4	0.34	29	28.6	0.39	$p<0.05$
IV	10	28.1	0.82	67	29.9	0.31	$p<0.06$
V*	0	-		80	31.0	0.35	-
VI	4	30.2	1.89	76	30.6	0.30	NS

s.e Standard error

NS Not significant

* Period V had no parasitaemic animals.

parasitaemia. In all periods, mean PCV of lactating cows was significantly lower by some two to three percentage units than that of dry cows (Table 7.2.3). Age of cow significantly affected mean PCV in periods I, and III where older cows had a lower mean PCV than the relatively younger cattle (<5 years), whilst the reverse was true for period V (Table 7.2.3). Overall, the effect of age was inconsistent in the different periods.

e) Liveweight change

In the two periods, I and III, where most trypanosome parasitaemias were detected, liveweight change was not significantly affected by trypanosome infection or by trypanocidal prophylaxis, and neither was there any significant or apparently important interaction of trypanosome parasitaemia and prophylactic treatment. In period I, animals were, on average, gaining weight, while in period III they were losing weight. Although there was no significant effect, there was a tendency for parasitaemic animals to have poor liveweight changes. In period I, animals with one or more detected trypanosome parasitaemias gained 13.3 kg (se 2.94) compared to 17.7 kg (se 4.03) for those with no parasitaemia (Table 7.2.4). In period III, those with detected parasitaemia lost 17.1 kg (se 5.55) compared to a gain of 2.8 kg (se 10.47) for those with no parasitaemia (Table 7.2.4).

f) Daily milk yield

Daily milk yield was not significantly affected by trypanocidal prophylaxis in any of the weeks -1 to 4, nor was there any significant interaction of trypanosome infection and prophylactic treatment, nor any indications of an important interaction.

Cows in which trypanosome parasitaemia had been detected had significantly lower daily milk yield (DMY) in the week following the detection

Table 7.2.3 Mean least squared estimate of packed red cell volume (PCV) of dairy cattle presented by lactation status and age during the six 3-monthly periods of the study

Period	Lactation status				Age			
	Milk		Dry		<5 years		>5 years	
	n	PCV[a]	n	PCV	n	PCV	n	PCV
I	27	29.3	39	31.0	36	30.6	30	29.7[b]
II	23	29.4	49	33.7	40	31.1	32	31.9
III	22	26.7	57	29.3	47	28.7	32	27.8[b]
IV	27	28.0	49	30.0	43	28.8	33	29.2
V	37	29.3	43	32.6	47	30.3	33	31.5[b]
VI	38	28.8	41	32.0	43	30.6	36	30.2

n Number of cattle

a All mean PCV values were significantly different from mean PCV estimated for the dry herd ($p<0.05$)

b Mean PCV significantly different between age-classes ($p<0.05$)

Table 7.2.4 Estimated liveweight changes ± s.e (kg) of parasitaemic and non-parasitaemic dairy cattle exposed to natural trypanosomiasis risk in coastal Kenya during periods I and III

	Parasitaemic			Non-parasitaemic			
Period	Number of cattle	Estimated liveweight changes (kg)	s.e	Number of cattle	Estimated liveweight changes (kg)	s.e	Significance
I	37	+13.3	2.94	25	+17.7	4.03	NS
III	45	- 17.1	5.55	22	+2.8	10.47	NS

s.e Standard error

NS Not significant

of parasitaemia than non-parasitaemic cows. Their respective DMY were 3.14 (se 0.132) and 3.40 (se 0.106) kg, a depression of 10% (Table 7.2.5). In the following week (week 2,), the depression in DMY was similar, 9%, and significant, but in the subsequent two weeks, DMY of the parasitaemic and non-parasitaemic cows did not differ (Table 7.2.5).

7.2.4 DISCUSSION

From this study, it was evident that the prophylactic strategy of isometamidium chloride given at 0.5 mg kg^{-1} every 3 months failed to control trypanosomiasis. Chemoprophylaxis appeared to have no significant effect on occurrence of trypanosome parasitaemia, days to detection of infection, mean PCV, liveweight changes and milk yield. On the other hand, treatment with diminazene aceturate successfully cured most trypanosome infections.

All parasitaemias were diagnosed as *T. vivax* infections, with a marked variation in the detection of infections occurring during the course of the study. In the first and third periods, most of the *T. vivax* clinical cases developed an acute disease followed by a haemorrhagic syndrome. Outbreaks of haemorrhagic *T. vivax* in dairy farms in coastal Kenya have been reported by Mwongela *et al.* (1981) where acuteness of the disease in the field often results in mortalities of adult cattle and in abortions before diagnosis or treatment can be made. In the present study, in spite of close monitoring, haemorrhagic *T. vivax* was responsible for the three mortalities during the study.

Isometamidium chloride prophylaxis did not protect cattle against the outbreaks of haemorrhagic *T. vivax* infections. Similar observations were made in beef cattle placed on isometamidium chloride, where breakthroughs of trypanosome infections were again caused by *T. vivax* frequently exhibiting the haemorrhagic syndrome (Dolan *et al.*, 1992). Dolan *et al.* (1992) attributed the

Table 7.2.5 Mean least square estimate of daily milk yield (DMY, kg) of dairy cows in the week prior to detection of trypanosome parasitaemia (week 0), in the previous week (week -1) and in each of the 4 weeks following parasite detection (weeks 1-4) and the DMY of the contemporary non-parasitaemic cows

	Parasitaemic			Non-parasitaemic			
Week	Number of milk records	Estimated mean DMY (kg)	s.e	Number of milk records	Estimated mean DMY (kg)	s.e	Significance
-1	36	3.62	0.238	283	3.60	0.109	NS
0	36	3.40	0.106	283	3.54	0.048	NS
1	36	3.14	0.132	280	3.48	0.061	$p<0.01$
2	34	3.10	0.148	268	3.42	0.067	$p<0.05$
3	30	3.31	0.186	259	3.37	0.080	NS
4	29	3.33	0.191	258	3.29	0.081	NS

s.e Standard error

NS Not significant

breakthrough trypanosome infections to heavy tsetse challenge. However, isolates of *T. vivax* from the same area and from several others parts of the Kenya coast, including two haemorrhagic isolates, were found to be resistant to the prophylactic activity of isometamidium chloride at up to 2 mg kg^{-1} (Rottcher and Schillinger, 1985; Schonefield, *et al.*, 1987). On the other hand, the same isolates were found to be sensitive to diminazene aceturate (Berenil) at 7.0 mg kg^{-1}. Further, experimental studies have shown that protection afforded by isometamidium chloride when cattle were fly-challenged with two cloned derivatives of haemorrhagic *T. vivax* isolates from coastal Kenya was for only one month or less (Peregrine, *et al.*, 1991).

The detection of trypanosome parasitaemia in the first and third month of periods I and III, respectively, suggests infections occurred as outbreaks. No differences were observed in mean days to detection of parasitaemias in either the prophylactic and non-prophylactic groups for the two periods, indicating that no protection was afforded by isometamidium chloride.

Apart from failure to protect cattle against trypanosomiasis, isometamidium chloride prophylaxis had no significant effect on mean PCV values in any period of the study. Irrespective of the treatment group, parasitaemic animals had significantly lower mean PCV in periods I and III. This was reflected in the greater number of infections detected during these periods in comparison with the low infection rates in other periods. Lower PCVs in parasitaemic Zebu cattle placed under isometamidium chloride prophylaxis in areas of medium and high tsetse challenge have been observed in various parts of coastal Kenya (Maloo *et al.*, 1988b; Munstermann *et al.*, 1992). In this study, mean PCVs of non-parasitaemic cattle was 29.9% and above for all periods, with the exception of period III. The relatively lower mean PCV in period III was attributed to nutritional stress as grazing pastures were scarce due to the dry season from January to April 1991. Depression of

PCV from nutritional stress in non-parasitaemic N'Dama cattle has been reported by Agyemang, *et al.* (1990) when cattle maintained on pastures during the dry season dropped their mean PCV by four percentage points within 6 months.

Apart from the short period of nutritional stress, lactating cows had significantly lower mean PCV values (below 30%) than the corresponding dry animals in the herd in all study periods. In addition, stress of lactation during period I, appeared to predispose cattle to trypanosomiasis as milking cows were observed to be at a higher risk from the disease than dry cows. Interaction between physiological status and trypanosome infections, and its effect on health and productivity have been reported in N'Dama cows by Agyemang, *et al.* (1992), where significantly higher trypanosome prevalences were observed in lactating cows compared to the dry animals. Furthermore, average PCVs of lactating N'Dama cows, whether infected or not, were lower than the corresponding dry animals (Agyemang, *et al.*, 1992).

Neither chemoprophylaxis nor presence of trypanosome infection had any significant effect on liveweight changes during periods I and III. In period I, weight gains observed were attributed to better quality and abundance of grazing pastures, whilst the contrary was true for period III, where the effects on dry season on weight changes were pronounced. Although, no significant differences in liveweight changes were observed in both periods, there was a tendency for parasitaemic animals to either gain less (Period I) or lose more (Period III) than the non-parasitaemic cattle. Moreover, all parasitaemic animals were treated with diminazene aceturate immediately upon diagnosis, thereby preventing substantial weight losses.

Liveweight recordings at monthly intervals, failed to detect any obvious effect of trypanosome infection. At the same time, the effect of parasitaemia on daily milk yield was assessed. In general, the mean lactation yield of the

herd of 1,500 kg in the 1980s was below the average milk yield of 2,200 kg observed when the herd received supplementary feeding in the mid-1970's (Njubi *et al.*, 1992). This was attributed to managemental factors rather than the suitability of genotype for coastal climatic conditions (Njubi *et al.*, 1992). The present study showed that the milk yields of cows which became parasitaemic were significantly lower for 2 weeks following the detection of trypanosome infection than those of non-parasitaemic cows. This loss of production occurred in spite of prompt therapeutic intervention against infection. This has been the first study to report on quantified milk production losses from clinical trypanosomiasis under natural field challenge and signify the importance of effects of trypanosomiasis on milk production. Extrapolating these results to the small-holder situation, the losses might be expected to be even higher as facilities for diagnosis and early intervention with trypanocides are not readily available.

In this study, chemoprophylaxis was not effective possibly because of drug resistance of the parasites at this dosage of isometamidium. Previous studies have reported the successful use of isometamidium chloride as a prophylactic drug (Trail *et al.* 1985); it was found that Boran cattle kept on prophylaxis in a high tsetse challenge area in coastal Tanzania, performed at 80% of the productivity achieved by improved Kenyan Boran cattle kept in tsetse-free areas. In addition, isometamidium chloride chemoprophylaxis increased productivity by 20% in Zebu cattle kept as sedentary village herds in areas of medium tsetse challenge on the Kenyan coast (Maloo *et al.*, 1988b).

Nevertheless, in the present study it appeared that the use of isometamidium chloride at 0.5 mg kg^{-1} was not effective in controlling trypanosomiasis and neither did it prevent production losses arising from clinical trypanosomiasis. However, treatment with diminazene appeared to cure most infections and prevented significant mortalities. This approach was

only made possible by intensive monitoring of the dairy herd, whereby infections were detected early followed by immediate curative therapy. On a large-scale beef ranch, regular monitoring for trypanosomaisis and subsequent treatments of parasitaemic animals have been shown to prevent mortalities and have improved productivity of the herd (Dowler *et al.*, 1989). Lately, several dairy farms in Kilifi District have started implementing this method of blood-testing and curative treatments, as isometamidium chloride prophylaxis does not appear to afford adequate protection (C. Wilson, *pers. comm.*; M. Jauss, *pers. comm*). This is most likely to be the result of the increasing emergence of drug-resistant strains of trypanosomes.

In general, chemoprophylaxis with isometamidium chloride is the recommended method for controlling trypanosomiasis in medium to high tsetse challenge areas, and there have been several reports on its successful use (Trail *et al.*, 1985; Maloo *et al.*, 1988b). However, these studies (Section 7.1 and 7.2) and other recent studies have shown that there is increasing evidence of its failure to protect against trypanosomiasis in the coastal region of Kenya (Dolan *et al.*, 1992; Munstermann *et al.*, 1992), probably due to emergence of drug-resistant strains. In such situations, diagnosis and chemotherapeutic treatment offers the only drug-control alternative. The successful implementation of this control strategy depends on good management requiring diagnostic facilities, skilled personnel and the commitment to continue regular monitoring even in the face of low or undetectable tsetse challenge.

In the small-holder sector carrying out this form of control will require active farmer participation with increasing dependence on the private sector to provide effective and sustainable animal health services.

CHAPTER EIGHT

THE FUTURE ROLE OF EPIDEMIOLOGICAL STUDIES AND PREVENTIVE MEDICINE PROGRAMMES FOR SMALL-HOLDER DAIRY FARMERS IN COASTAL KENYA

8.1 A systematic approach to epidemiological studies and implementation of appropriate control programmes for small-holder dairy production systems.

The series of epidemiological studies reported in this thesis culminated in developing systematic methods for assessing the importance of disease, and in particular, of major vector-borne disease, as constraints to small-holder cattle production in coastal Kenya. In addition, the results from these studies influenced the adoption and delivery of appropriate preventive medicine programmes for improved herd productivity.

The step-wise approach towards identification and quantification of disease risk with consequent development of suitable control programmes was based on the following studies:

a) Cross-sectional study

The aim of the cross-sectional study was to estimate the prevalence of major diseases occurring in cattle population in Kaloleni Division (Section 5.1). In the design of the study, factors likely to influence disease prevalence were taken into consideration. Thus, the study population was stratified by agro-ecological zones (AEZ), herd and cattle type and grazing management system. In this study, the sampling strategy was planned to include all small-holder dairy cattle. In total, a sample size of over 900 head of cattle was selected to obtain sufficient numbers in each stratum.

The results from the Kaloleni study showed that tick-borne diseases and trypanosomiasis were the most important. Over 70% of the small-holder dairy cattle sampled had antibodies or antigens to tick-borne diseases (TBD), while a relatively lower trypanosome antigen (33%) and antibody prevalence (55%) were observed. With regard to East Coast fever (ECF), *Theileria parva* antibody prevalences decreased from the wetter AEZs, CL3 and CL4, to the drier CL5 AEZ. In addition, dairy cattle kept in the zero-grazing system had a

lower *T. parva* antibody prevalence compared to dairy cattle in the free-grazing system, indicating the influence of grazing management in relation to exposure to infection with *T. parva*.

One of the limitations of selecting a large sample size, is the cost-factor and the capacity of laboratories to handle biological samples for analysis. Although the samples collected during the cross-sectional study were adequately handled, a few logistical problems arose with serological analysis.

Thus, future cross-sectional studies should aim at determining a representative sample size for a given cattle population in an area/region. If the study population is to be stratified, then sample size should be representative of each stratum, e.g., AEZ, cattle type, or grazing system. For a stratified random sample, assuming a 10% tolerable error, the desired number of cattle required to achieve 95% confidence in the estimate of the prevalence of disease under investigation can be calculated as follows:

$$n = 1.96^2 \times \frac{[p\,(1-p)]}{L^2}$$

where, n = sample size, p = expected prevalence of the disease under investigation, and L is the tolerable error (Martin *et al.*, 1987). The expected prevalence (p) can be determined by carrying out a pilot study involving samples from all strata. In the event the population is grouped into herd clusters, the desired sample size should be inflated with a factor, $1 + (m\text{-}1)\,v$, where m is the mean herd size, and v is the intra-herd correlation coefficient.

In general, cross-sectional studies provide an overview of disease situation in an area, but they neither measure the changing pattern of disease over time, nor the losses resulting from these diseases.

b) Longitudinal study

The purpose of the longitudinal study in this thesis (Section 5.2) was to determine disease incidence, mortality rates and case-fatality rates, as well as to observe cattle population changes in an area. In Kaloleni Division, ECF was the most important disease diagnosed. Incidence rates of 0.58 and 0.29 were estimated over the 19 months study period for dairy cattle kept in the free-and zero-grazing system, respectively. The two-fold lower incidence of ECF detected in the zero-grazing system was a reflection of the influence of grazing management in reducing tick contact and the risk of contracting the disease. The majority of *T. parva* infections were detected in age-cohorts of less than 18 months, managed in both zero- and free-grazing systems. In addition, a 32% overall mortality rate was estimated, with ECF accounting for two-thirds of the calf deaths, three-quarters of the young stock mortalities and more than a third of all adult losses. Case-fatality due to ECF approached 60%.

In Kwale District, another area of coastal Kenya, the disease situation was totally different with tsetse-transmitted trypanosomiasis identified as the major disease. In this study, no attempt was made to estimate the incidence of trypanosomiasis as it was difficult to ascertain whether trypanosome parasitaemias detected were new infections or relapses due to drug-resistant strains. Nevertheless, an overall prevalence of 12.9% in chemoprophylactically treated small-holder dairy cattle was recorded, with the majority of the trypanosomiasis cases detected in cattle found in areas of known high tsetse challenge in the District.

Apart from estimating incidence of disease and their case-fatalities, the results also indicated that existing control measures applied by the farmers failed to protect dairy cattle from contracting vector-borne diseases. This was particularly so for ECF in Kaloleni Division and for trypanosomiasis in most parts of Kwale District.

Longitudinal studies can be time-consuming, and ill-defined investigations can fail to provide relevant answers required to meet the objectives of a study. Moreover, the population changes occurring during the course of the study, as reported in the thesis (Section 5.2), can cause difficulty in defining the population at risk over the entire study period.

Therefore, in designing such studies, consideration must be given in making allowances for the changing population over the duration of study. In addition, depending on the disease(s) under investigation, in this case, vector-borne diseases, the time-period of the study should incorporate both the wet and dry seasons.

Another impo ·ant factor when considering the design of a longitudinal study is the frequency of monitoring of the study population. In areas, where trypanosomiasis risk is high, monitoring should preferably be carried out once every 2 weeks or, if practical, even once a week. From experiences gathered from the studies reported in this thesis (Section 7.1), once a month monitoring did not appear to be ideal in areas of high trypanosomiasis risk. On the other hand, in Kaloleni Division, an area of low tsetse challenge, but high ECF risk, monthly monitoring, with in-between supplementary visits, was found to be adequate.

Working with small-holder farmer cattle, the success of the longitudinal study depends on ability of the research team to establish a good working relationship with the farmers, by providing regular feedback to the farmer on the health status of their herds. In addition, successful therapeutic intervention in the face of clinical disease, not only provides necessary epidemiological data, but further strengthens goodwill and confidence between the farmer and the researcher.

In the event of mortalities, identifying their cause is essential for completeness of the epidemiological data. In many instances, farmers fail to

report mortalities. Experience from working with small-holder farmers show that cattle owners do not understand the need for necropsies. Moreover, social customs and taboos aggravate the situation further. Therefore, farmer education becomes very important, and the significance of post-mortem examinations has to be fully explained to the farmer. In the study reported in the thesis (Section 5.2), establishing a sound working relationship with the farmers was fruitful as some of them did make an effort to contact the team when mortalities occurred in their herds. Furthermore, this 'dialogue' motivated the farmer to provide adequate history, information pertaining to health practices carried out, expressed their satisfaction or dissatisfaction on the available veterinary services, and allowed for appreciation of the nature of work researchers undertook towards identifying and resolving disease constraints for small-holder dairy production.

Results from studies reported in this thesis clearly showed that under the small-holder situation in Kaloleni Division, tick control practices failed to prevent the occurrence of TBDs, particularly ECF (Chapter 5), and with the escalating cost of acaricides it is likely that control activities in future will deteriorate even further. In addition, chemotherapy against ECF using anti-theilerial drugs is expensive and cannot be afforded by most small-holder dairy farmers. Moreover, availability of drugs can be unreliable. Therefore, under the present circumstances, it is unlikely that tick control programmes will have a major impact in reducing the incidence of ECF and other TBDs in the cattle population in the region. Furthermore, the likelihood of ticks developing resistance to the acaricides makes this control method less sustainable in the long term. In order to reduce dependence on expensive acaricides and chemotherapy, there was a need to identify and implement alternative measures for the control of ECF and other TBDs. In this thesis, the alternative approach used for the control of ECF was immunisation by using the infection

and treatment (I&T) method (Radley, 1981). However, before carrying out immunisation it was important to identify the target population for immunisation.

In other situations, such as at Kwale and Mtwapa (Chapter 7), where trypanosomiasis was the major disease problem, chemoprophylaxis using isometamidium chloride failed to prevent the occurrence of the disease. On the other hand, curative treatment with diminazene aceturate was successful. With no new trypanocides being developed in the foreseeable future, and the prospects of a vaccine against trypanosomiasis unlikely (Murray *et al.*, 1991), control of the disease depends on use of existing trypanocidal drugs. Therefore, in the short term there is a need to develop strategies for the more effective use of drugs for the control of trypanosomiasis.

c) Identification of target population for immunisation against East Coast fever

Results from the cross-sectional and longitudinal studies in Kaloleni Division, showed that young small-holder dairy cattle, irrespective of their grazing management system were at high risk from contracting ECF. At the same time, the low incidence of clinical disease as well as the low *T. parva* antibody prevalence in zero-grazed adult dairy cattle classified them to be a high ECF risk group. On the other hand, the high prevalence of antibodies in local Zebu cattle and the free-grazing adult dairy cattle indicated they had been exposed to *T. parva* infection, but what was not known was whether this population was immune to the immunising stock of *T. parva.*

The principle behind immunisation using I&T method is to create an artificially induced endemic stable cattle population in an area. Therefore immunisation has to be carried out in two phases; first to immunise the entire susceptible population to mimic endemic stability, and second to maintain

stability by vaccinating calves subsequently born and newly introduced cattle. To achieve endemicity in the first phase, small-holder dairy cattle of all ages, irrespective of their grazing system, were selected as target population for immunisation. As this method provides life-long immunity, further immunisation will only be necessary in new-born calves and newly purchased dairy cattle in the area. Thus, a specific study to determine the age-window for immunisation of susceptible dairy calves was carried out (Section 6.1). The results showed that dairy calves exposed to natural tick challenge contracted ECF by 1 to 4 months of age and should ideally be immunised as soon as possible within this age range.

With regard to Zebu cattle residing in the coconut-cassava AEZ, they were not considered for immunisation as they were believed to be immune. To test this hypothesis, a specific study was undertaken to assess the immune status of local Zebu cattle over 6 months of age (Section 6.2). The results showed that Zebu cattle were immune as they withstood challenge with the immunising stock of *T. parva* Marikebuni. The same could possibly be said for adult free-grazing dairy cattle, but a challenge study with this population was not carried out. On the other hand, although the immune status of less than 6 month old Zebu calves was not established, immunisating them would be beneficial, as ECF is known to cause high morbidity and stunting of Zebu calves in an endemic area (Moll *et al.*, 1986).

In the pilot trial carried out in Kaloleni Division, all small-holder dairy cattle were selected for initial immunisation using I&T method. Zebu calf population was not included in the immunisation as it was only targeted at the high investment cost dairy cattle, with all dairy calves and adult dairy cattle particularly in the zero-grazing units being priority target groups. As the immune status of exposed adult dairy cattle in the free-grazing system was not

established, they were nevertheless considered for immunisation even though it was likely that most of the exposed cattle were immune.

d) Control of East Coast fever by immunisation using infection and treatment method: An alternative approach

The pilot study for immunisation of small-holder dairy cattle against ECF in Kaloleni Division using the I&T method led to the development of an approach for the delivery of immunisation to groups of small-holder dairy farms (Section 6.3). Using this approach, over 95% of the dairy cattle in Kaloleni Division have since been immunised (P.N. Ngumi, *pers. comm.*). The success of the control method depends on the ability to carry out follow-up immunisation of new-born calves. At present, this remains the responsibility of the task force based at VIL, Mariakani. However, under the declining operational funding allocated to government institutions, the sustainability of the exercise will require total cost-recovery from small-holder farmers. During the pilot immunisation trial, due to the small number of animals immunised realistic estimates for the cost of immunisation could not be made, but studies carried out elsewhere in Kenya estimated the cost of delivery of immunisation on a ranch to be US$4.29 per animal (Young, *et al.*, 1992). On a larger scale involving cattle population at risk in a region or a country, where immunisation is planned over a long-term period, the estimated cost of immunisation for a national programme involving 100,000 cattle per annum, would be in the region of US$ 2.37 per head of animal with a range of US$ 2.14-2.97 (Mukhebi, Morzaria, Perry, Dolan and Norval, 1990).

Since the successful delivery of immunisation to small-holder farms in Kaloleni Division, the veterinary authorities have permitted immunisation to be carried out on a wider scale for the control of ECF. This policy will also allow veterinarians in the private sector to carry out such procedures in liaison

with the task force which will act as a "backstopping" team. With the increasing emphasis on privatisation of veterinary services, such opportunities will act as a stimulus for veterinarians to venture into establishing clinical practices in rural and peri-urban areas of coastal Kenya.

In this respect, the next group of target dairy cattle population for immunisation against ECF by I&T will be small-holder dairy cattle in Bahari Division, Kilifi District. This study is to be used as a model example for delivery of immunisation by the private sector. In the Division there are over 100 small-holder dairy farmers and the majority of them belong to a farmers' association called the Bahari Dairy Club which acts as a milk collecting and cooling centre for most of the milk. As well as marketing the milk, the club provides facilities for purchasing concentrates, mineral and salt licks and dairy equipment.

In the highlands of Kenya, dairy cooperative societies managed by small-holder farmers, also provide veterinary services such as AI, and run on a cost-recovery basis with farmers paying their clinical fees through milk sales. At present, such a service, is not provided by the Bahari Dairy Club and most members depend on government-run clinical services which are deteriorating due to insufficient operational funds. Therefore, the dairy club plans to employ a veterinarian and animal health assistants (AHA) either on a full-time basis or on contract terms to provide essential clinical services. With the freeze on employing more veterinarians and AHAs in the public sector, veterinary graduates and AHAs are seeking jobs in the private sector. In addition to providing the regular clinical services, the animal health staff will be trained to carry out I&T method of immunisation against ECF.

Initial training of the team on the procedure of immunisation against ECF will be provided by the task force based at the VIL. Upon successful completion of training, a request for purchase of the *T. parva* sporozoite

stabilate by the dairy club, endorsed by the VIL task force certifying competency to handle the stabilate and carry out immunisation, will be made to NVRC, Muguga. Preparation, quality control and testing of the stabilate remains the responsibility of this research institute. Moreover, selling of sporozoite stabilate will boost the commercial sector of the research institute, meeting a new directive given by the Director of KARI to generate income for further research activities.

Immunisation will be carried out in small groups of dairy herds to facilitate delivery and post immunisation monitoring. Members of the dairy club will pay for the service through their milk sales. During the first phase of immunisation, aimed at blanket cover of all small-holder dairy herds, additional AHAs, will be employed on short-term contracts for monitoring and sampling. Prior to immunisation, a delivery and monitoring schedule of the targeted cattle herds will be prepared. A copy of the schedule will be sent to the task force team for reference in the event the team needs to intervene. Farmers will be briefed on the immunisation method and be made aware about the likelihood of clinical reactors developing.

On the first day of delivery of immunisation, a member of the task force from VIL will accompany the team to supervise the method of delivery, and upon satisfactory implementation authorise immunisation of the rest of the herds. Monitoring will be carried out by AHAs, with farmers involved in temperature recording on a daily basis for a period of 2 weeks. Animals with elevated temperature will be reported to AHAs for taking lymph node biopsies and blood smears for 3 consecutive days, and in the event of an animal being considered a clinical reactor, anti-theilerial therapeutic intervention will be carried out by the veterinarian. Spot checks by a task force team during the monitoring phase may be advisable. This will ensure monitoring is effectively carried out.

After the blanket immunisation of all dairy cattle, follow-up vaccinations of new-born calves and purchased animals can be done by the veterinarian employed by the club. The success of the exercise will depend on the sustainability of the immunisation programme by continual vaccination of new additions to the immunised cattle population. Ideally animals purchased by small-holder farmers should be immunised at the source, e.g., at Kilifi Plantations. The advantage of immunising on one large dairy farm is that it is carried out at a single site, making monitoring a much easier task.

While effectively protecting cattle against the disease, the I&T method has some limitations. It relies on use of live parasites which need to be cryopreserved to remain infective. The immunised animals remain carriers of the parasite, and thus act as a reservoir for infection of ticks and non-immunised cattle. At present, these drawbacks do not outweigh the advantages over conventional methods. However, further research is being carried out to identify broad-spectrum immunogenic antigens of *T. parva* parasite. Scientists at ILRAD have identified a sporozoite protein with a molecular mass of 67 kDa as a promising candidate for a novel vaccine against ECF (ILRAD, 1992). Before such a vaccine is adequately tested and appropriate antigen delivery systems identified for commercial marketing, I&T method remains the only method of vaccination available.

Although, successful immunisation against ECF will prevent major losses due to this disease, it does not justify cessation of tick control due to the risk of contracting other tick-borne diseases. Dairy cattle which have been subjected to intensive tick control or have had minimal tick contact as in the case with zero-grazing adult cattle, are likely to be highly susceptible to the other TBDs. Vaccination against anaplasmosis and babesiosis is commonly practised in South Africa using live parasite vaccines (FAO, 1984), but such practices are not carried out in Kenya and clinical cases are treated using

drugs. Therefore, studies need to be designed to develop strategic and effective tick control programmes against other TBDs in ECF immunised herds. It is envisaged that following successful immunisation against ECF, it will be possible to reduce the frequency of acaridical application resulting in significant economic benefits for the small-holder dairy farmer.

e) Control of trypanosomiasis: a short-term and long-term solution.

In areas of high tsetse challenge along the coastal region of eastern Africa, trypanosomiasis control has usually depended on chemoprophylaxis with isometamidium chloride (Trail *et al.*, 1985), whereas in areas of low tsetse challenge, such as at Kilifi Plantations, curative therapy has been being practised (Paling, *et al.*, 1987) Successful use of chemoprophylaxis has been achieved in beef cattle on a ranch at Mkwaja, Tanzania, and in traditionally managed village Zebu herds at Muhaka, Kenya (Trail *et al.*,1985; Maloo *et al.*, 1988). However, the results from studies carried out in small-holder dairy cattle in serious tsetse risk areas of Kwale District and in a Jersey herd at Mtwapa showed that chemoprophylaxis with isometamidium chloride was ineffective, but curative treatment with diminazene aceturate worked offering a short-term solution. In the two study areas, failure of chemoprophylaxis was thought to be due to drug-resistant trypanosomes strains or reduced sensitivity of *Trypanosoma vivax* strains to isometamidium when given chemoprophylactically (Peregrine *et al.*, 1992). Therefore in such situations, the control strategy must depend on early diagnosis and prompt curative therapy.

For the future short-term control strategy, the high tsetse challenge area of Kwale District will be used as a model for describing the delivery and implementation of trypanosomiasis control programme aimed at blood-testing and curative treatment of parasitaemic cattle, with the possible role of the

private sector. In Kwale District, there are less than 30 small-holder dairy farmers and they are widely distributed within the District. Unlike, farmers in Kilifi District, these farmers do not have a dairy club and milk is sold by individual farmers locally. Production losses from trypanosomiasis appear to the one of the most important constraints. Therefore, early diagnosis of clinical cases is of paramount importance to prevent substantial losses and even mortalities. Although the cause of the problem is well known, the early diagnosis and delivery of clinical services are poor. Therefore, to carry out an effective trypanosomiasis monitoring and curative treatment programme, a cost-effective approach needs to be planned. Initiation of the control method should start at farm level requiring active farmer participation. The farmers' role would involve daily recording of temperature of all cattle in their herds, and animals with elevated temperatures being reported for clinical attention. In addition, blood-testing of the animals should be carried out at least once every 2 weeks, either by staff from the local government-run veterinary office, or by engaging the services of the increasing number of available AHAs. The latter seems to be the best option as under the present circumstances, the DVO's office cannot accommodate the routine blood-testing exercise due to lack of sufficient funds.

Thus, the routine monitoring can be effectively carried out by AHAs for a negotiated fee. Farmers can also call upon the services of the AHAs in the event that animals are febrile between the blood-testing visits. Moreover, these activities will stimulate the growth of the private sector in providing animal health services for the rural small-holder dairy farmers. The AHAs will be responsible for blood sampling, and submission of samples to the nearest diagnostic centre and carrying out necessary treatments. Similar services could also be provided by veterinarians planning to establish private practices in the District.

Depending on the tsetse challenge, the frequency of curative treatments given should indicate as to whether dairying in areas of high challenge would be viable or not in the long term. Furthermore, evidence of drug-resistance to curative drugs can be detected if animals become parasitaemic within 12 to 16 days after the last treatment. Thus, not only would these activities control trypanosomiasis, they would also monitor for development of drug-resistance. Emergence of drug-resistance is likely to appear if trypanocides are not efficiently and correctly utilised. The most common cause of drug-resistance is thought to be underdosing (Leach and Roberts, 1981), as under field conditions reliable methods for liveweight measurement are rarely available for calculating dosage when treating cattle.

With the threat of drug-resistance, it is necessary to consider alternative methods of control with a long-term solution. One such approach relies on an integrated control strategy aimed at reducing tsetse population and keeping cattle free from trypanosome infection by regular monitoring and treatment. Suppression of tsetse population has been achieved by use of odour-baited traps (Dransfield, *et al.*, 1992) and odour-baited insecticide impregnated targets, (Opiyo, *et al.*, 1987). In both study areas, *Glossina pallidipes* was the predominate tsetse species. Controlling tsetse by use of targets and traps would appear to be the method of choice for reducing tsetse challenge, but at present, studies evaluating the effectiveness of traps and targets against the three major coastal tsetse species, *G. pallidipes*, *G. austeni* and *G. brevipalpis*, are lacking. Research has currently been undertaken to develop an effective trap for the control of *G. brevipalpis* and initial results show promise (C.Kyorku, *pers. comm.*).

The use of synthetic pyrethroids as pour-on formulations or in dip wash appeared to have reduced tsetse and trypanosomaisis risk in similar environments along the coastal belt of eastern Africa (Lohr, *et al.*, 1992;

Thompson, *et al.*, 1992; Fox, *pers. comm.*). However, these studies were carried out on ranching conditions involving large cattle numbers. Whether similar results can be reproduced in the small-holder situation, is yet to be determined.

Therefore, the need to develop cost-effective, environmentally acceptable and sustainable tsetse control methods suited for coastal conditions requires the urgent attention of scientists working in the field of tsetse control.

Implementation of tsetse control programmes must be economically viable. Under the present economic circumstances, the small number of dairy herds would not appear to justify tsetse control programmes. One might argue that the reason why more farmers are not venturing into dairying is because of the high tsetse and trypanosomiasis risk. Therefore, before embarking on control programmes, consideration should be given on the economic feasibility and long-term sustainability of such activities especially by the public sector where, dwindling operational resources barely allow functioning of existing programmes, such as vaccination against infectious diseases. Involving the private sector requires the end-users to fund this exercise. The end-users in this case, are the small-holder farmers who under present circumstances, cannot support such an investment. Therefore, unless social and economic justification allow for tsetse control to be carried out at the regional or national level through international donor funding, the optimal use of trypanocidal drugs will continue to be the best option for dairy farming in the small-holder sector.

f) Animal health package

In this thesis, although major emphasis was on vector-borne diseases with consequent development of appropriate methods for controlling these diseases, preventive medicine programmes need to be delivered as a complete animal health package, incorporating control of epidemic infectious diseases,

vector-borne heamoparasitic diseases, helminthiasis, and diseases arising as a result of intensified dairy production.

In coastal Kenya, epidemic infectious diseases such as rinderpest, foot-and-mouth disease and lumpy skin disease are controlled by vaccination programmes coordinated through the District Veterinary Offices. Vaccination against rinderpest is carried out annually in all yearling cattle and the delivery of this vaccine has been boosted by the launching of a regional Pan African Rinderpest Campaign programme. Control against foot-and-mouth disease and lumpy skin disease is carried out only in the face of an outbreak. However, since the initiation of the National Dairy Development Programme (NDDP), small-holder dairy herds are vaccinated against foot-and-mouth disease twice a year upon farmers' request.

Helminthiasis can cause significant losses, particularly in calves. Currently, helminth control depends on strategic use of anthelmintics as advocated by the veterinary authorities. For effective control, deworming is recommended for calves at an interval of once a month in the wet season and once every 2 months in the dry season, and for yearlings and adults, once every 6 months. The majority of the small-holder dairy farmers abide by the recommendations, but with the recent price increases in anthelmintics and other veterinary pharmaceutical products, there is an increasing tendency to relax from the regular worm control.

Small-holder dairy production in Kenya is unique compared to many parts of Africa. Therefore, diseases of intensification, usually unheard off in most sub-Saharan African countries, become important in the small-holder sector. Of the diseases directly attributed to management, mastitis is probably one of the most important diseases effecting dairy production. Although mastitis did not appear to cause serious problems in small-holder dairy herds in the study areas reported in this thesis, the risk of severe production losses

should not be forgotten. Control can be achieved by improving hygiene and using appropriate antibiotic therapy in the face of clinical cases. In addition, bacterial diseases such as brucellosis, anthrax and blackquarter occur sporadically and can be effectively controlled by use of vaccines.

Preventive medicine programmes delivered as a complete animal health package must incorporate control strategies to be delivered by government-run services, such as rinderpest control with constant surveillance, as well as those that can be carried out by the private sector. Some of the major components for herd health tailored for small-holder dairy farmers in the subhumid agro-ecological zone include control and treatment of epidemic infectious viral diseases, bacterial diseases, heamoparastic diseases, gastrointestinal parasitism and nutritional deficiencies (Table 8.1). With the move towards privatisation of the veterinary services, the private sector can play a significant role in the effective implementation of preventive medicine programmes for sustainable and profitable small-holder dairy production in coastal Kenya.

8.2 Way forward

The step-wise approach employed in the research reported in this thesis forms a blueprint for the type of epidemiological studies needed for identification and quantification of the main diseases and allows the assessment of existing control programmes in small-holder dairy production systems. In addition, the studies highlighted the need to implement alternative, but epidemiologically, economically and environmentally acceptable and sustainable methods for improving cattle productivity in the small-holder sector.

While developing a systematic approach for identification and quantification of the major diseases, and carrying out immunisation against ECF and chemotherapeutic programmes for tsetse-transmitted

Table 8.1 Animal health packages for small-holder dairy production systems in coastal Kenya

Vaccinations	Treatment	Management
Viral diseases	**Haemoparasitic diseases**	Provision of salt and
Rinderpest	Trypanosomiasis, anaplasmosis,	minerals for mineral and
Lumpy skin disase	babesiosis and heartwater	vitamin deficiency
Foot-and-mouth disease		conditions
Bacterial diseases	**Gastrointestinal parasitism**	Strategic tick control
Anthrax	Helminthiasis	Strategic worm control
Blackquarter	Coccidiosis (for calves)	
Brucellosis		Milking hygiene
Parasitic disease	**Bacterial infections**	Tsetse control
East Coast fever	Mastitis	(pour-on ?)
(I & T) method	Kerato-conjunctivitis (pink eye)	
	Foot -rot	

I & T : Infection and treatment method of immunisation.

trypanosomiasis in coastal Kenya, more studies at the local level are required to address the following issues:

1. The epidemiological and economical significance of other tick-borne diseases in ECF immunised dairy cattle and the implication and evaluation of the need for strategic tick control.
2. Studying the socio-economic impact of immunisation against ECF in small-holder dairy production system in Kaloleni Division.
3. The development, testing and implementation of environmentally and economically acceptable tsetse control methods effective against the three major tsetse species, *G. pallidipes*, *G. austeni* and *G. brevipalpis* commonly found along the eastern coast of Africa.

In most parts of the sub-humid zones of the sub-Saharan Africa, vector-borne diseases limit the expansion of cattle production systems. Thus, application of such a blueprint of epidemiological methods at national and even regional level will help to identify and quantify major biological constraints limiting the development and expansion of rural and peri-urban small-holder livestock production systems.

Apart from collecting site-specific epidemiological data, this information could further be used in developing epidemiological models to predict disease situation in similar environments, particularly for the small-holder sector. Under these circumstances, epidemiological models should take into consideration a combination of factors likely to influence the epidemiology of disease. Some of these factors include AEZ, cattle and herd type, grazing management systems, vector densities, infection rates in the vectors and the hosts, carrier status and reservior of infection in hosts and existing disease control programmes. Epidemiological models developed for the small-holder sector would not only be useful in predicting disease, but also in assessing and implementing appropriate disease control strategies.

Epidemiological data can be further improved with the use of advanced molecular techniques for detecting the causative organisms of the important livestock diseases in the hosts and their vectors. Apart from evaluating the feasibility and cost-effectiveness of carrying out these diagnostic tests under field conditions, employing these methods will help in upgrading the knowledge-base of the epidemiology of major diseases.

Similarly, the molecular technology can be exploited in improving current methods for disease control. This may be achieved by developing appropriate cost-effective genetically engineered subunit vaccines against the main animal diseases effecting livestock production. Besides being efficacious, these vaccines must be environmemtally, economically and socially acceptable for small-holder production systems in sub-Saharan Africa.

As a result of this work in coastal Kenya, the blueprint developed has been used to assess disease situations not only in other dairy potential areas of coastal Kenya, but is also being implemented in several other countries in East, West, Central and Southern Africa. By adopting this systematic approach, activities in various African countries can be coordinated into a network. At the same time, the spatial data modelling can be used in developing sensitive epidemiological methods to predict the significance of major animal diseases affecting livestock production and assist in decision-making for effective disease control strategies in sub-Saharan Africa.

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APPENDIX

Appendix 1. Analysis of deviance for the presence of *Theileria parva* antibodies using logistic regression

	d.f	deviance	p value
Sex	1	0.91	
Age	3	4.22	
Farming system 7!	6	21.4	**
Farming system x age	18	43.9	**
Residual	514	522.7	
Total	542	593.1	

** Significant at 1% ($p < 0.01$)

! Only seven combinations of the farming systems were included in the analysis due to missing cells in an age class in farming system 8

Appendix 2. Analysis of deviance for the presence of *Babesia bigemina* antibodies using logistic regression

	d.f	deviance	p value
Sex	1	0.39	
Age	3	13.4	**
Farming systems!	7	53.2	**
Residual	239	204.4	
Total	250	271.4	

** Significant at 1% ($p < 0.01$)

!These analysis were carried out without interaction due to missing cells.

Appendix 3 . Analysis of deviance for the presence of *Anaplasma marginale* antigens using logistic regression

	d.f	deviance	p value
Sex	1	0.28	
Age	3	19.7	**
Farming system7!	6	5.0	
Farming system x age	18	21.0	
Residual	495	190.0	
Total	523	235.4	

** Significant at 1% ($p < 0.01$)

! Only seven combinations of the farming systems were included in the analysis due to missing data from farming system 8

Appendix 4. Analysis of deviance for presence of trypanosome antigens using logistic regression methods

Source	degrees of freedom (df)	deviance
Sex	1	0.004
Age	3	1.47
Farming system	7	34.39**
Residual	631	792.91
Total	642	828.8

** Significant at 1% ($p < 0.01$)

Appendix 5. Analysis of deviance for the presence of trypanosome antibody using logistic regression methods

Source	degrees of freedom (df)	deviance
Sex	1	6.3
Farming system	7	10.2
Age	3	15.9**
Residual	163	204.6
Total	174	237.1

** Significant at 1% ($p < 0.01$)

Appendix 6 Least squared analysis of variance for mean packed cell volume (PCV) using a general linear model

Source	degrees of freedom (df)	Mean squares	F Value	Pr >F
Farming system	7	138.2	4.29	0.0001 **
Age	3	77.8	2.42	0.07
Farming system x age	21	59.9	1.86	0.011 *
Sex	1	18.7	0.58	0.45
Theilerial pioplasms	1	154.2	4.79	0.029 *
Strongyles	1	42.3	1.31	0.25
Error	844	32.2		

** Significant at 1%

* Significant at 5%

Appendix 7 One-way analysis of variance for age at infection with *Theileria parva* in sentinel calves in six groups exposed to natural tick challenge

Source	DF	SS	MS	F	P value
Age at infection	5	9032	1806	6.7	0.001*
Error	19	5105	269		
Total	24	14137			

* Significant at 5% level

One-way analysis of variance for days to infection with *Theileria parva* in sentinel calves in six groups exposed to natural tick challenge

Source	DF	SS	MS	F	P value
Days to infection	5	13891	2778	9.99	0.00
Error	19	5286	278		
Total	24	19177			

* Significant at 5% level

DF Degrees of freedom
SS Sum of Squares
MS Mean sum of squares
F F value
P value probability

Appendix 8 One-way analysis of variance on mean packed red cell volume (PCV) of cattle in three Divisions of Kwale District

Source	DF	SS	MS	F	P value
Divisions	2	930.7	465.4	16.05	0.000*
Error	317	9189.1	29.0		
Total					

* Significant at 5% level

DF Degrees of freedom

SS Sum of Squares

MS Mean sum of squares

F F value

P value probability