THE EPIDEMIOLOGY OF EAST COAST FEVER IN MALAWI ZEBU CATTLE AND THE ECONOMICS OF TICK-BORNE DISEASE CONTROL IN MALAWI

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DECLARATION

The data presented in this thesis was collected as part of a study carried out by myself and Ms T L Norman. Data collection was shared but the study planning, data processing and interpretations presented in this thesis are my work except where stated otherwise. Paper 5 was jointly authored by the stated authors.

This thesis was composed by myself and it has not been submitted in candidature for any other degree, diploma or professional qualification.

Andrew Soldan
1 November 2007

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LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 8

LIST OF PHOTOGRAPHS

CONTENTS

Page

Page

Page

Page

ABSTRACT

Morbidity, mortality and seroconversion to *Theileria parva* were studied in Malawi zebu cattle in six areas in the same ecological zone. A total of 3,257 animals were intensively monitored over a period of three years. Strategic tick control was carried out in four areas and no tick control was performed in a further two areas. Strenuous efforts were made to diagnose illness and deaths in the cattle.

The seasonal fluctuations in numbers of ticks on the cattle were observed at four-weekly intervals for three-and-a half years. Productivity of the cattle belonging to 143 farmers in the six areas was also monitored. Seroconversion to *Cowdria ruminantium* was monitored for the first year of the study.

Strategic dipping using nine immersions, at two-week intervals, from December to March gave almost complete control of *R. appendiculatus* but the numbers of *B. microplus* and *A. variegatum* were similar in dipped and undipped animals.

One undipped area was in an epidemiologically unstable state with respect to East Coast fever (ECF) due to prior dipping. East Coast fever mortality and morbidity were low in the first year after the cessation of dipping but rose over the second and third year until 46% of calves died of ECF before reaching one year of age. In the other undipped area ECF mortality and morbidity were low for all three years, despite high *T. parva* seroconversion rates. Dipping had ceased three years before the study began and it was concluded that this area was in a stable state with respect to ECF.

Strategic dipping in the other four areas caused very low ECF morbidity and mortality, as determined by comparison with the undipped control cattle. ECF mortality in strategically dipped calves was zero in most areas for most years.

Adult *R. appendiculatus* were responsible for most of the *T. parva* transmission causing clinical disease with nymphs responsible for a significant amount of sub-clinical infection. The existence of enzootic stability to ECF in an undipped area without continuous adult *R. appendiculatus* activity was demonstrated and the significance of nymphal transmission to the maintenance of this stability is discussed.

The costs and benefits of various tick-borne disease control strategies were calculated. Policies of vaccination or strategic dipping where tank construction was necessary were significantly less cost effective than policies involving stopping dipping or the continuation of strategic dipping at an existing tank. The most cost-effective option would be to stop dipping and accept mortalities while endemic stability becomes established. This could however have a large social cost due to mortality in the early years.

CHAPTER 1

INTRODUCTION

The work described and discussed in this thesis concerns the control of East Coast fever in Malawi zebu cattle in the Central Region of Malawi. The history of tick and tick-borne disease control is riven with debate on the most appropriate control strategies. This debate still rages today and impacts on policy and policy makers across East and Central Africa. The following correspondence was published in the Veterinary Record in 1991 and provides an introduction to the debate and this thesis.

Mares R.G. (1991) East Coast fever in the days of the Empire (Book Review) Veterinary Record, 128, 482.

> THE dust cover of this interesting book states simply 'Paul F. Cranefield is Professor at the Rockefeller University, New York.' It gives no information as to what he professes. He admits in chapter 11 that he has '... no special expert knowledge' so it must be concluded that he is not a veterinary surgeon, but he obviously admires our profession and has a love of history. A few years ago he gave a lecture on East Coast fever as part of a symposium at the Wellcome Institute. This was so admired by those members of the Veterinary History Society present that they have been trying ever since to get him to address them.
>
> The book requires some concentration, as the events are not recounted in strictly chronological order. But, particularly to those closely acquainted with the struggles of the veterinary profession in one time Colonial Africa, the story it tells is fascinating. It is lit by periodic flashes of dry humour. The epilogue states that '...Great Britain was the place where most of the veterinarians who coped with East Coast fever had been trained'; and a footnote adds, 'All of them were British; some even English'!
>
> The book shows once again that the only thing we learn from history is nothing. Koch tried vainly to immunise cattle with massive injections of infected blood, thus delaying the discovery that seven-day dipping was the right answer. Yet, in spite of the fact this has eradicated the disease from vast areas of Africa, internationally funded projects are still trying to develop a vaccine.

When the story starts in 1902 the Cape of Good Hope, the Orange Free State, the Transvaal, Natal and Rhodesia were all separate colonies of this country with varying degrees of independence. Each colony had its own agricultural service including one or more veterinary officers. These men are little known today except for Sir Arnold Theiler. He, of course, gave his name to the parasite involved in the disease.

Theiler also founded the world renowned Onderstepoort Veterinary Research Institute which Cranefield rightly says, was, in the 1920s, '... in terms of veterinary research ... far ahead of the United Kingdom.' Other notable characters, Gray, Hutcheon, Lounsbury and Watkins-Pitchford are less well known than they deserve. The German expert, Robert Koch, hired for the job of investigating the disease by the British South Africa Company, got too much credit, and, worse, led others to defer to his fame and go astray.

It was cattle from Natal sent to Rhodesia by Watkins-Pitchford that gave an early clue to the fact that the disease was new and not as Koch first insisted, identical with the 'Texas fever' he had studied in Tanganyika. It was also Watkins-Pitchford who pioneered dipping, in spite of the early reluctance of Arnold Theiler and Stewart Stockman. Gray at first found it so hard to be recognised as a veterinary surgeon in Rhodesia that for some years he was forced to earn his living as a telegraphist. Now a handsome framed testimonial to his later work in the Transvaal is on the walls of the Centre for Tropical Veterinary Medicine in Edinburgh. It was the American born entomologist, Lounsbury, working with Hutcheon in the Cape, who discovered that the vector was not the blue tick (then known as *Rhipicephalus decolouratus*) but the brown tick.

The penultimate chapter, 'What is East Coast fever?', does perhaps digress from the book's title 'Science and Empire' as it deals with the interesting link between the multiplication of infected lymphocytes in East Coast fever and the lymphoid invasion of leukaemia. A study of this chapter indicates what an ornament to our profession Cranefield might have been.

There are a few very minor slips in the proof reading. Watkins-Pitchford's death is recorded once as 1950 and later as 1951. The latter is correct. On page 93 Koch is stated as arriving in Rhodesia in February 1903, and on the next page that he submitted his first report in March 1902: pretty good even for the great Koch! When the blue tick is mentioned on page 47 it is called Rhipicephalus. This was its name at the time: but a note that it is now Boophilus might help.

The sources used are extensive and impressive. It is good to note that proper reverence is accorded to Henning's masterly 'Diseases of animals in South Africa'. Among the many other names of those who have helped are Miss Horder in the Wellcome Library, Professor Brocklesbury of the CTVM, Professor Lawrence of Zimbabwe and P.J. Posthumus,

regional veterinary officer for Natal, who provided an index of veterinarians in South Africa.

Creek M.J. (1991) Eradication of East Coast fever. Veterinary Record, 128, 575.

SIR, - In his review of 'Science and Empire: East Coast Fever in Rhodesia and the Transvaal', R.D. Mares asserts that seven-day dipping is the 'right' answer to eradicate East Cost fever (ECF); and that Koch's vain use of infected blood as a method of immunisation should have taught us not to waste time in developing a vaccine (*VR*, May 18, p482).

I submit that there is an alternative view, and I would make three points. The original and successful dipping campaign was contemporaneous with land settlement and wholesale slaughter of wildlife. Does Mr Mares think that these policies are currently practical in countries affected by ECF?

Secondly, the native zebu cattle are remarkably resistant to ECF, except in situations where the disease moves into a new area or where seven-day dipping has been practised but then has broken down. The limited cost benefit studies done so far all indicate that there is no economic justification for intensively dipping these cattle.

Thirdly, the modern approach to immunisation with precisely targeted vaccines is so vastly different from injecting infected blood that the comparison has little relevance.

For a variety of reasons national dipping programmes do not work well in modern Africa, so it is difficult to see how they can be 'right'. By default, there is therefore a prima facie case for the use of a vaccine, especially to protect valuable crossbred dairy cattle. Arguably the approach to dealing with ECF will vary to cope with various circumstances. Intensive dipping, alternative acaricide regimes, choice of cattle breed as well as vaccination will each have a role, either alone or in combination. As far as the vaccines are concerned they need to be both reliable and cost effective which will require efficient manufacture and also improvement of the delivery system.

Mares R. G. (1991) Eradication of East Coast fever. Veterinary Record, 129, 59.

SIR, - it is satisfying to fly a kite and have someone notice; so perhaps you will permit further discussion on the points raised by Dr Creek (*VR*, June 15, p575) on my review of Professor Cranefield's book (*VR*, May 18, p482) with reference to the merits of cattle dipping. I do not altogether grasp Dr Creek's first point. To which 'original and successful

dipping campaign' does he refer? To the best of my knowledge the 'original' was that in Natal in the first years of the Union of South Africa. The publisher's preface to a small book 'An illustrated pamphlet on tick-destruction and the eradication of East Coast fever' by H. Watkins-Pitchford (published by P. Davis & Sons of Maritzburg and Durban about 1912) says '... the work in Natal must remain ... the pioneer and standard work upon which all future South African efforts at tick eradication must be based.' At this time it is probably true to say that this was 'contemporaneous with land settlement and wholesale slaughter of wildlife'. The later, also successful, eradication of East Coast fever in Transkei was the subject of an excellent film I saw first during my diploma in tropical veterinary medicine course in 1958.

The South African methods were draconian but worked. East Coast fever was also eradicated from the southern region of Malawi and, I think (subject to correction), in the Kikuyu rural dairy scheme and by the Iringa dipping scheme in Tanganyika. I do not think land settlement and wholesale slaughter of wildlife had any impact in these cases.

On the second point it is quite true that native cattle are more resistant to tropical disease. But surely the object of Third World development projects was, and I suppose still is, to up-grade, or even replace these cattle with exotic breeds that are more productive. The successful commercial dairy herds in Malawi during the 1960s could not have survived without seven day dipping. Because there was no East Coast fever there was no need for vaccine or curative drug.

On the third point, perhaps I am out of date, but until very recently there were no 'precisely targeted vaccines' for heart water, anaplasmosis, piroplasmosis, streptothricosis or just plain 'tick worry': all tick-borne or tick-associated diseases. Has science advanced so much since I retired that we can ignore the vectors when combating vector-borne disease? And have any cost benefit analyses been done to confirm the economic justification for a 'precisely targeted vaccine' against East Coast fever?

National dipping programmes are not the only things that do not work well in modern Africa. Has Dr Creek had the experience of trying to mass vaccinate a wild herd of zebu cattle with a wilder herd of cattle guards and veterinary assistants using intravenous heart water vaccine? Rinderpest campaigns, too, can fail if paraffin refrigerators are not maintained. As Dr Creek ways, we 'require efficient manufacture and improvement of the delivery system'.

The big point in favour of dipping, as I see it, is that the infrastructure is still in place. Not all dipping tanks in Africa are broken, not all field staff have forgotten how to check and run a tank. Any cost benefit analysis must take into account the fact that this capital still exists to pay dividends.

Soldan A.W., Norman T.L., Edelsten M. and Chinombo D. (1991) Eradication of East Coast fever. Veterinary Record, 129, 179.

SIR, - As a unit involved in a large scale cost benefit study into the dipping of zebus in Malawi we write in support of the excellent letter by M.J. Creek (*VR*, June 15, p575).

R.G. Mares in his book review (*VR*, May 18, p482) and subsequent letter (*VR*, July 20, p59) intimates that the 'right' approach to tick-borne disease control, in exotic and zebu cattle, is seven-day dipping to eradicate the ticks and therefore the diseases. As Dr Creek points out, this is not possible, and we submit not desirable, in modern Africa.

The intensive dipping versus increased interval dipping versus non-dipping debate has more than animal health implications, it strikes at the very heart of an 'old style' veterinary service whose main role was the dipping of cattle. Is this the chief worry of Mr Mares?

Malawi is having to re-examine its policy of dipping every seven days in arsenic trioxide. There is resistance to arsenic and it is a dangerous environmental pollutant. A change to a modern, highly efficacious (and expensive) acaricide at a similar seven-day interval (as advocated by Mr Mares) would destroy the excellent calfhood-acquired immunity of our indigenous herds. This would lead to severe problems when dipping is interrupted; as it will be from time to time, due to shortage of dip or distribution problems. Only 20,000 (2.5 per cent) of Malawi's 800,000 head of cattle are exotic or improved breeds. It is not legitimate to impose this risk on the village farmer for the sake of the very few with improved cattle. For the latter, vaccination and, or, the use of a modern pour-on acaricide should be offered as alternatives.

Mr Mares comments that in the past development projects wished to upgrade or replace indigenous cattle with exotic breeds. After years of severe problems with both disease and environmental adaptation of exotics in the small farmer/village situation, the qualities of indigenous breeds are being appreciated. Their disease resistance, tick resistance and environmental adaptation are considered important traits to preserve in breeding programmes to provide more productive animals. The aim of whole scale replacement of indigenous breeds with exotics in the rural areas is hopefully a thing of the past.

Finally, we would ask Mr Mares why he would want to vaccinate zebus against heartwater (*VR*, July 20, p59). Malawi zebus do not suffer from heartwater (or babesiosis or anaplasmosis for that matter) unless they are under an intensive dipping regime that breaks down. The cost benefit analysis of such a situation would keep several economists happy for a long time! We believe that the sensible answer is to dip indigenous animals less frequently, or not at all, thereby maintaining enzootic stability and animal health, reducing environmental pollution and expenditure of foreign currency.

Yeoman G.H. (1991) East Coast fever: Africa's stubborn problem. Veterinary Record, 129, 414-415.

NEARLY 100 years have elapsed since Robert Koch confusingly misinterpreted what was to become the 'new' disease, East Coast fever. In The Veterinary Record of May 18 (p482), R.G. Mares reviewed the book, 'Science and Empire: East Coast Fever' ... by Professor Cranefield. This thrillingly authoritative retrospective and the subsequent exchanges in The Veterinary Record remind us that East Coast fever still provides veterinarians in Africa with the same obdurate challenge that it did those of us in the colonial era.

This bovine *Theileria parva* infection is a unique and commonly fatal condition in which the piroplasm hijacks T-lymphocyte mitosis, causing a catastrophic lymphoblastosis that destroys vital organs and impairs the immune system. There are philosophical connotations with human leukaemia and AIDS. East Coast fever is arguably the most economically important disease of cattle in eastern Africa. While there have been laboratory advances in immunisation, a practical field method has yet to be developed, and control measures have so far been based on the use of acaricides. Since the introduction of cattle dipping at the turn of the century, there has been a debate, once more raised by Creek (*VR*, June 15, p575) and Soldan and others (August 24, p179), as to the respective merits of attempting the eradication of the tick vector, *Rhipicephalus appendiculatus*, or merely aiming at a moderation of its numbers.

In a letter (*VR*, July 20, p59) Mr R.G. Mares mentions the Iringa (Tanzania) dipping scheme as an example of the successful large-scale eradication of East Coast fever. I was the veterinarian responsible for the planning and execution of this scheme up to 1955. I regret that an analysis of the reasons for its success and ultimate termination has never been published, but some comments may be helpful to present-day veterinarians faced with this disease.

Over the period 1950-57 East Coast fever was eradicated from an enzootic area is south west Tanzania about half the size of Northern Ireland, containing a zebu cattle population that increased from 160,000 to 250,000 over that period. Neither land settlement nor wildlife were relevant factors. Political action closed the scheme in 1957, but my prediction that the disease would recur within months proved wrong: it took many years.

Among the reasons for success should be noted (i) that the enzootic zone was a plateau salient, on three sides of which the ecology was unsuitable for the vector tick; (ii) adequate funding enabled the provision of sufficient dipping tanks (over 100) to obviate onerous trekking; (iii) the then new availability of a gammexane acaricide relieved us of the problems of arsenic and meant that a seven-day regime was efficacious;

(iv) by building small tanks (final optimum capacity only about 10,000 litres) with a continuing top-up system, regular sludge removal and frequent strength testing, a rapid turn over of the gamma isomer was attained, and the problems of selective stripping and biodegradation (and so under-strength dipping) obviated; (v) registration and recording were meticulous and ensured virtual 100 per cent compliance.

There were equally cogent reasons for the termination of the operation. The overnight refusal to dip in 1957 was an act of political protest in the run-up to independence: but underlying this were errors in basic planning. First, no prior tick survey was carried out: the area was selected on the basis of the recorded occurrence of East Coast fever, without differentiation between enzooticity and epizooticity, filled out to cover the whole salient on the grounds of casually observed 'brown ear ticks'. A survey would have revealed that in the marginal areas such ticks could be, according to altitude, *Rhipicephalus hurti/jeanelli* group, *R sanguineus*, *R tricuspis*, *R punctatus*, *R pravus* or *R neavei*. None of these Rhipicephalids, which to the non-expert are indistinguishable from *R appendiculatus*, are effective field vectors of *T parva*. Enforcing dipping in these areas, with the plausibly laudable aim of 'making assurance doubly sure', in fact meant that a significant proportion of owners were required to dip without good reason. Further, no methodical process of *quantitative* vector monitoring (that is, by means of sentinel immune cattle) was built in to the plan. If it had been, we would have realised after perhaps only two or three years that eradication had been achieved, and so we would have been able to start moderating the regime, thus pre-empting political opposition.

Subsequent quantitative studies on the population dynamics of *R appendiculatus* (Yeoman 1966a, b, 1967, Yeoman and Walker 1967, Tatchell and Easton 1986) showed that there is a quantifiable graded tick burden relationship between enzootic, epizootic and East Coast fever free zones, but that this is only meaningful at low infestation rates. Thus an average of as few as five ticks per head (that is, two to three per ear – detectable only by trained staff) will sustain enzooticity; one to four per head will invite epizooticity; while an average of less than one can allow sporadic outbreaks.

In planning eradication by dipping, epizootic areas should at first be omitted and the true enzootic focus precisely delineated by quantifiable methods based on the accurate identification of ticks taken from repetitive transect stations. This study cannot take less than a year, for reasons of seasonal cyclicity. Then, if it is decided to introduce dipping (and the various important arguments adduced by the correspondents referred to above must first be carefully balanced), the initial target should be this enzootic zone. Once under control, the epizootic zone may be found to have disappeared, but continuing study will be the guide as to whether to extend or relax the operation.

For such tick survey work, it is essential to have on-site taxonomic expertise competent in the differentiation of *R appendiculatus* from its congeners. This is more difficult than it sounds: every specimen must be critically examined under the stereomicroscope by operators who are so experienced that they can correctly determine problem cases almost on the basis of the 'jizz' of ornithologists.

Irrespective of the above, however, the first question that must be asked in contemplating East Coast fever control in enzootic areas must be whether there is a justification for introducing a cumbersome and elaborate form of Western intervention that, if it is successful, must almost inevitably add to the complex process of environmental denudation that is destroying the continent. By our success we vets, like our medical colleagues, have caused enormous damage in Africa. We should not persist in the error of introducing western technology just because it exists. This is a subject that I have addressed elsewhere (Yeoman 1989).

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The following is extracted from Radostits O.M., Blood D.C. and Gray C.C. (1994)

Veterinary Medicine. Baillière Tindall, London, Edition 8, pp 1210

Diseases caused by Protozoa – East Coast fever

Control

Until now, the main method of control of ECF is to break the transmission cycle between cattle and ticks. This is achieved through widespread and strict application of acaricides at short intervals, adherence to legislation on cattle movements and quarantine, and good livestock and pasture management. With the ever rising costs of acaricides, their effect on the environment, the development of acaricide resistance and frequent political problems in the affected regions, this

strategy to control ECF and other tick-borne diseases in Africa warrants urgent review (Dolan, 1987, Tatchell, 1987, Yeoman, 1991, and Soldan et al, 1991). An integrated approach is advocated involving the use of genetically resistant breeds, a judicious and selective application of acaricides, and the use of vaccines.

Cattle that recover from the disease have a solid immunity to homologous challenge. In endemic areas, premunity is established early and this provides lifelong immunity if reinfection continues and the cattle are not moved to a different location where they may be exposed to a different strain of the parasite. However, there is some cross-immunity between antigenically different strains. Nutritional or climatic stress may seriously reduce the animals' premunity. In enzootic areas, reasonable control is maintained by avoiding nutritional stress and controlling the tick population. Improved or exotic breeds will require regular dipping or the use of pour-on acaricides but it has been suggested that indigenous animals should be dipped less frequently, or not at all, thereby maintaining enzootic stability (Soldan et al, 1991). For example, in countries like Malawi, indigenous breeds presently constitute up to 97% of the cattle population and it has been argued that it is not legitimate to impose the risk of losing enzootic stability on the village farmer for the sake of the few improved cattle. It may also not be advisable to replace indigenous, tick-resistant stocks with exotic breeds, at least in the rural areas.

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Tatchell, R.J. (1987) Parasitol. Today, 3, 7.
Yeoman, G.H. (1991) Vet. Rec., 129, 414.
Soldan, A.W. et al (1991) Vet. Rec., 129, 179.

The above correspondence and book section give an introduction to some of the history and controversy that surrounds the issue of dipping to control East Coast fever in Africa. In Malawi, as in several other countries, the field structure of the Government Veterinary Department was organised around the dip tank. The Government had been telling farmers that dipping was necessary, and even enforcing dipping by statute, for many years. Politicians keen to see improvements in local services would request the construction of a human health centre and a dip tank.

In the late 1980s Malawi still had compulsory weekly dipping of all cattle in the ECF endemic Central and Northern regions. There was an awareness in the scientific community that other countries had, by accident or design, moved away from this policy for traditionally managed indigenous cattle. However, such a momentous change in policy would be difficult to defend without good data on its impact.

This thesis presents the results of a prospective study of the tick population dynamics, ECF epidemiology and the economics of tick and ECF control carried out between December 1990 and May 1994 in the area around Lilongwe Malawi. The thesis is presented as a literature review followed by a series of five papers. Further data was collected on diseases other than ECF and on production levels. This will be presented separately by T.L. Norman.

STUDY OBJECTIVES

A. To investigate the epidemiology of East Coast fever in traditionally managed Malawi zebu cattle in the area around Lilongwe.

B. To evaluate a reduced/strategic dipping regime for its ability to control ticks and tick-borne disease.

C. To examine herd immunity outcomes under dipping and non-dipping management strategies.

D. To investigate the advantages and disadvantages of using chlorfenvinphos and total replacement amitraz acaricides.

E. To evaluate the economics of various tick control strategies compared to the cessation of dipping.

F. To generate enough data to be able to advise the Chief Veterinary Officer on future policy decisions relating to dipping.

Chapter 2 of this thesis provides a literature review concentrating on *Rhipicephalus appendiculatus* and *Theileria parva*, but with mention of other tick-borne diseases present in the study area.

Chapter 3 considers the seasonality of ticks in the study area and the effect of strategic dipping. These findings help to meet objectives B and D above.

Chapter 4 reports on the epidemiology of ECF in the study area and helps to meet objectives A, B and C of the study.

Chapter 5 gives details of an experiment to evaluate the immunity to challenge of animals monitored under different tick control regimes. This helped to meet

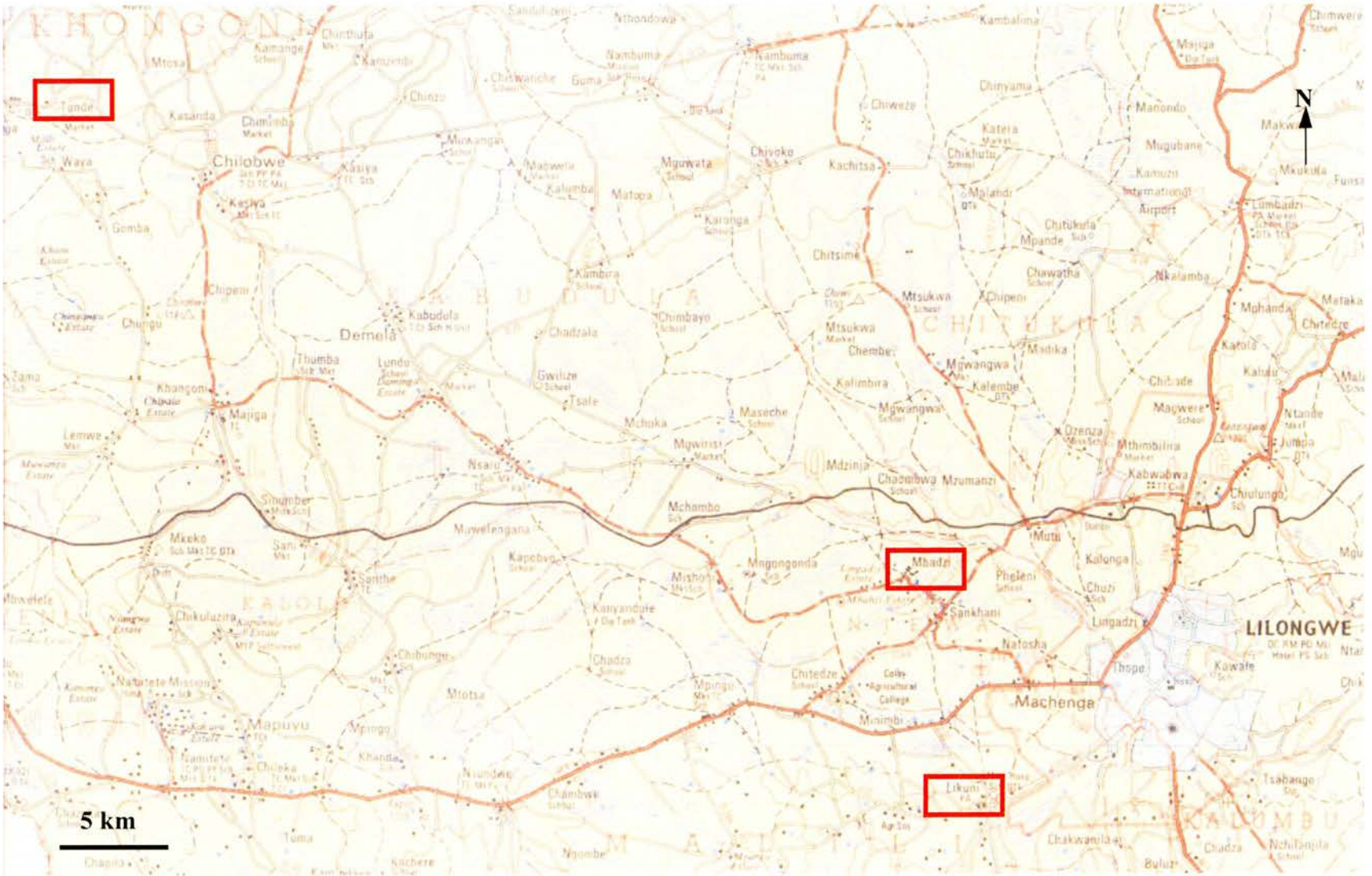
objectives B and C of the study.

Chapter 6 looks at herd productivity levels and the economic aspects of tick-borne disease control. This helped to meet objective E of the study. All results, but especially those from chapter 4 and 6 enabled objective, evidence based advice to be given to the Chief Veterinary Officer on future tick and tick-borne disease control policy, thus meeting objective F of the study.

Chapter 7 reports the results of a study into seroconversion to *Cowdria ruminantium* in animals monitored under different tick control strategies. Even with strategic dipping to control ECF there is a danger that endemic stability to other tick-borne diseases will be upset. This chapter shows that in the study reported this was not the case. This helped meet objective C and therefore also advice to the Chief Veterinary Officer (objective F).

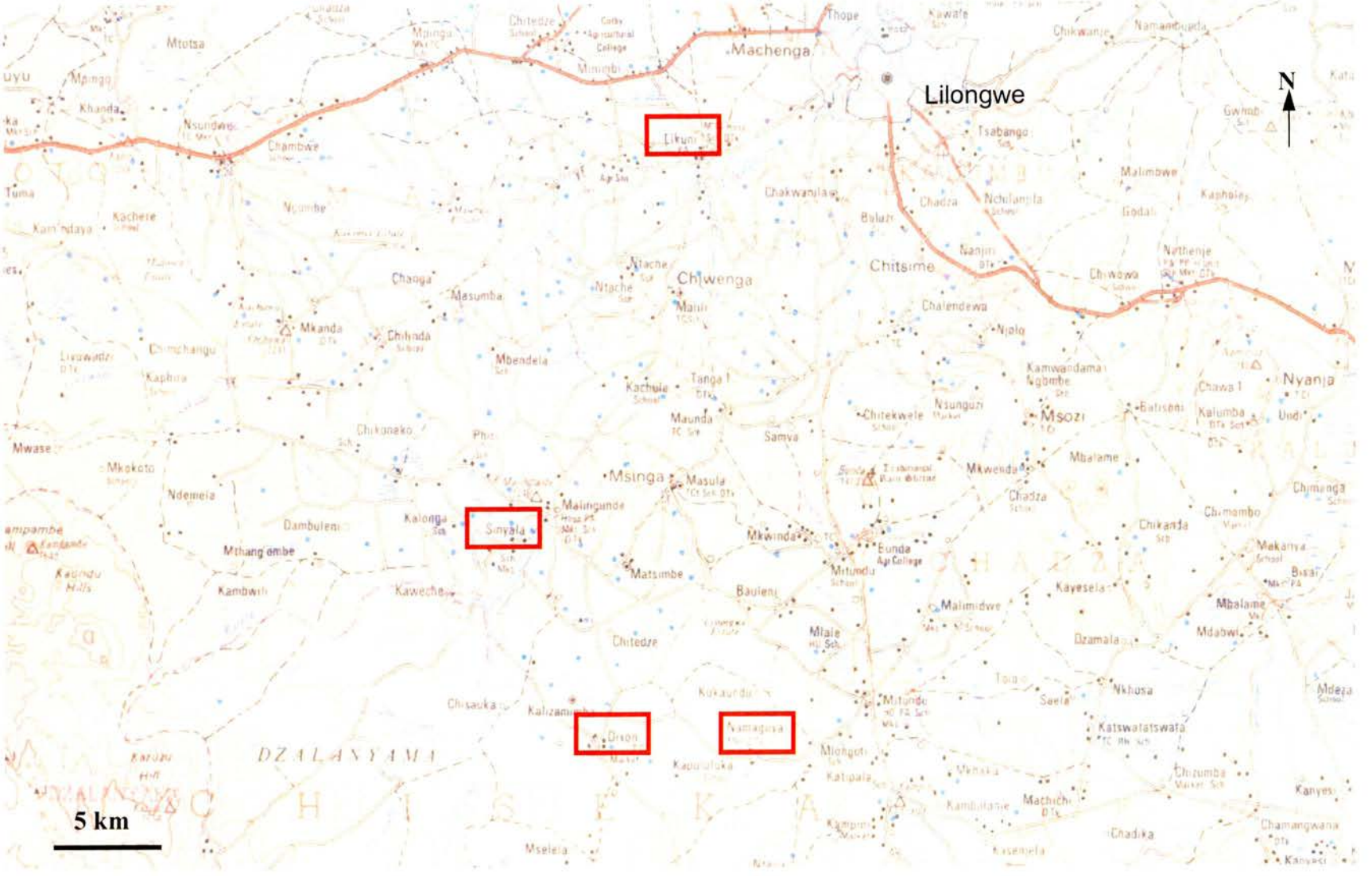
Figure 1.1 Map of Malawi

Figure 1.2. Location of study areas – North West of Lilongwe. 1 cm = 2.8 km

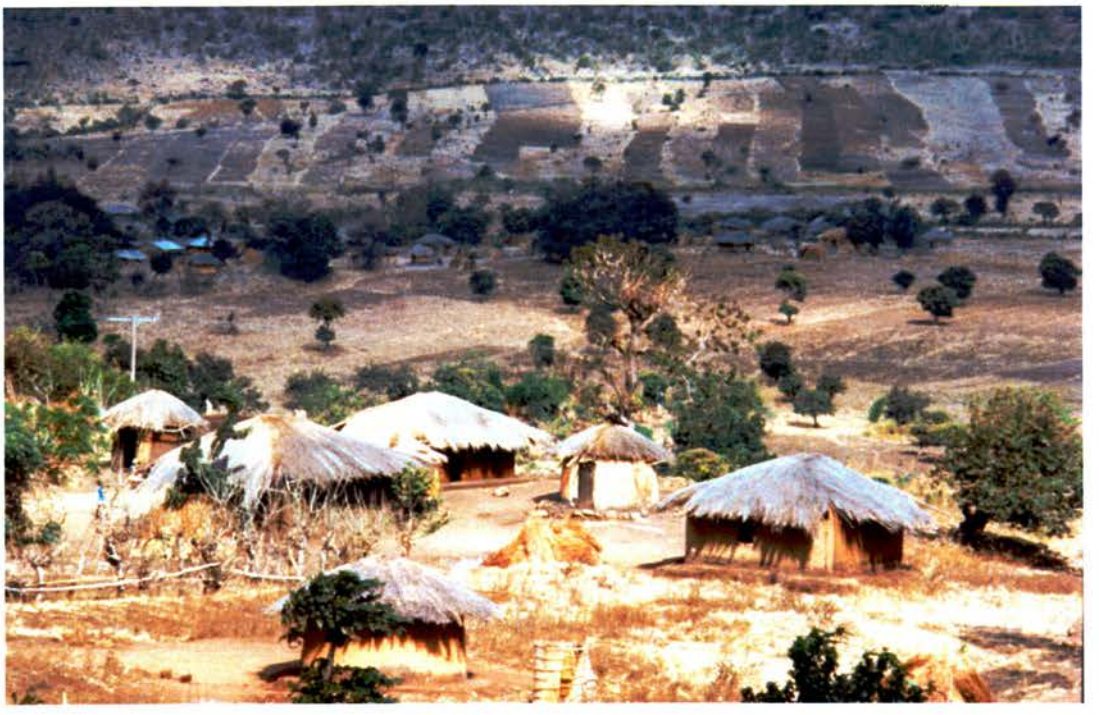


Taken from: The National Atlas of Malawi

Figure 1.3. Location of study areas – South West of Lilongwe. 1 cm = 2.8 km



Taken from: The National Atlas of Malawi



Photograph 1.1 View of village and surrounding heavily-cultivated farmland. This photograph was taken in mid-dry season.

Photograph 1.2 Typical khola (cattle pen) with "living fence" used to contain cattle overnight. Small boy seen on the left herds the cattle during the day.

Photograph 1.3 Smaller khola with poor fence. Photograph taken in dry season, the ground within khola can become deep mud in the rainy season.

Photograph 1.4 Malawi zebu cattle. Study ear tags can be seen in the right ears. Body condition varies between individuals but the animals in this picture are fairly typical.

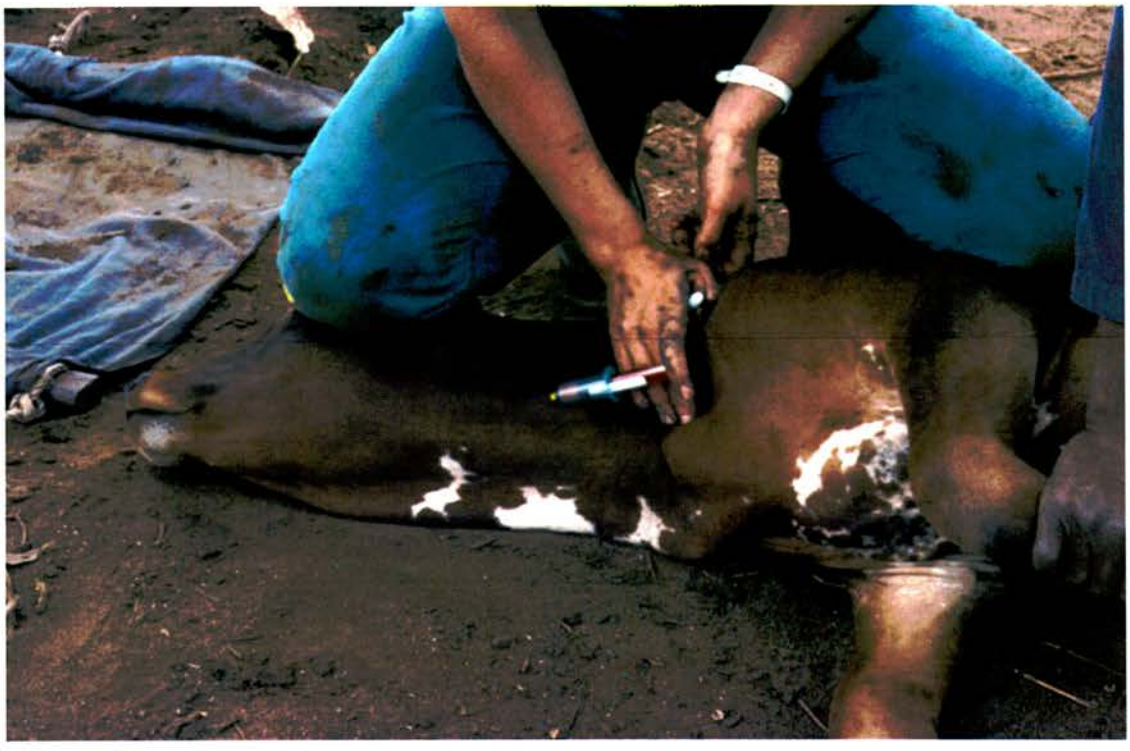
Photograph 1.5 Malawi zebu cattle. Note body condition, hump and short horns. Malawi zebu have a large range of colours. Animals are used to human presence and relatively docile.

Photograph 1.6 Dixon dip tank and collecting pens. This tank was well constructed. Roofing to keep rainwater out of the tank can be seen. Erosion around the tank due to repeated gathering of cattle is also visible.

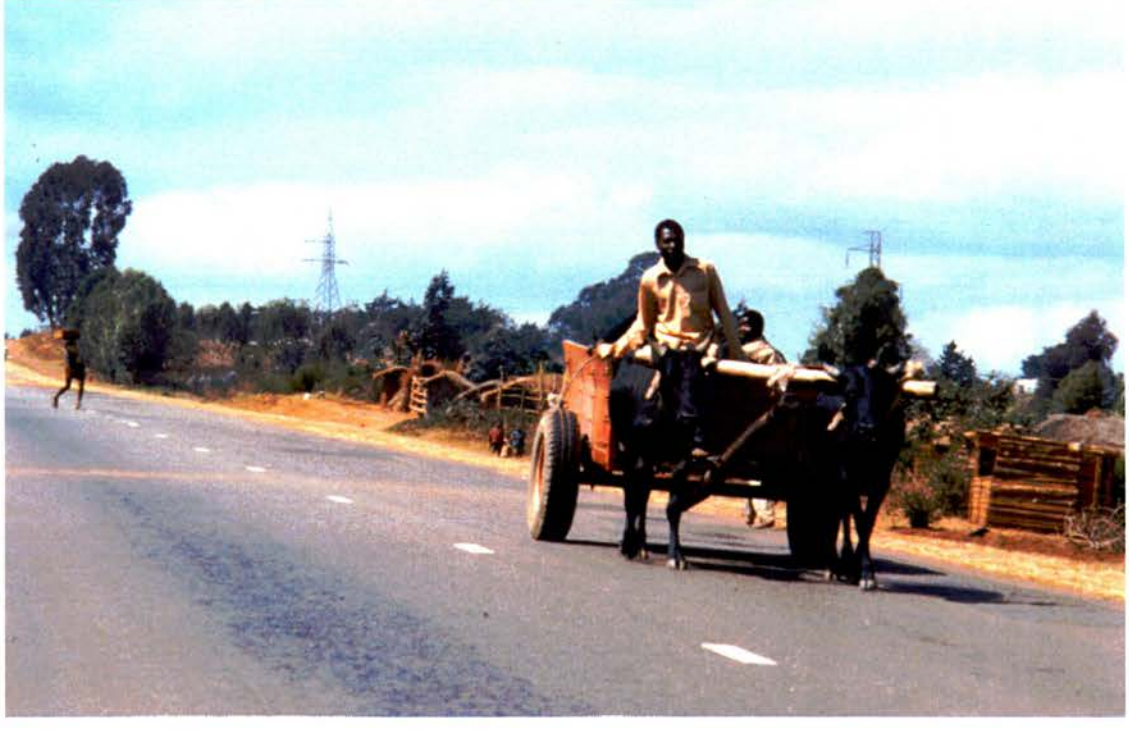
Photograph 1.7 Dip tank. Cattle exit tank in the foreground of the picture. The chemical store is situated near the entrance to the tank on the right of the picture.

Photograph 1.8 Catching calves for tick counting at Tonde. Erosion and mud within this large khola can be clearly seen. The calf being caught is approximately 6 months old.

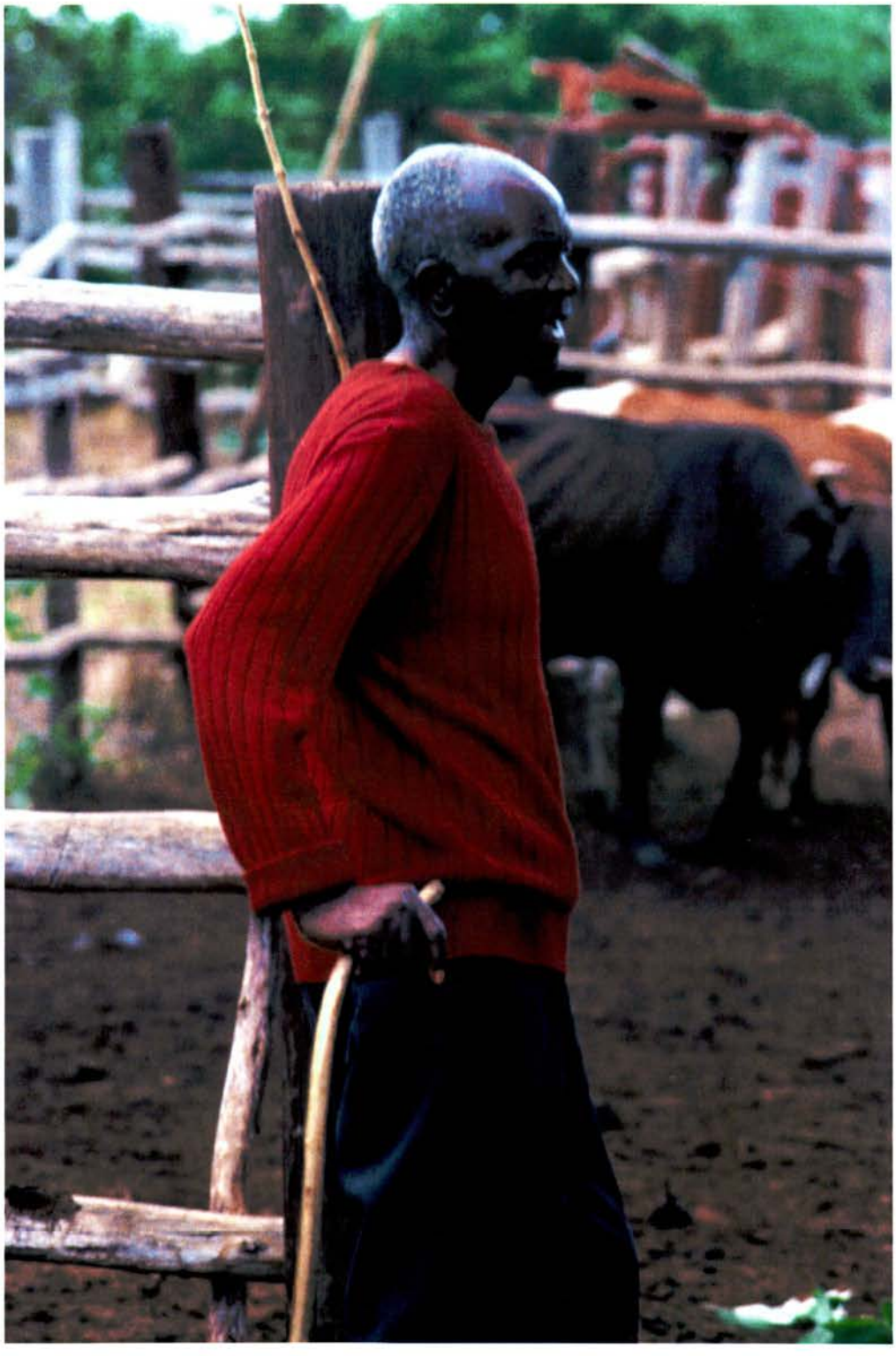
Photograph 1.9 Study cattle pen at Tonde.



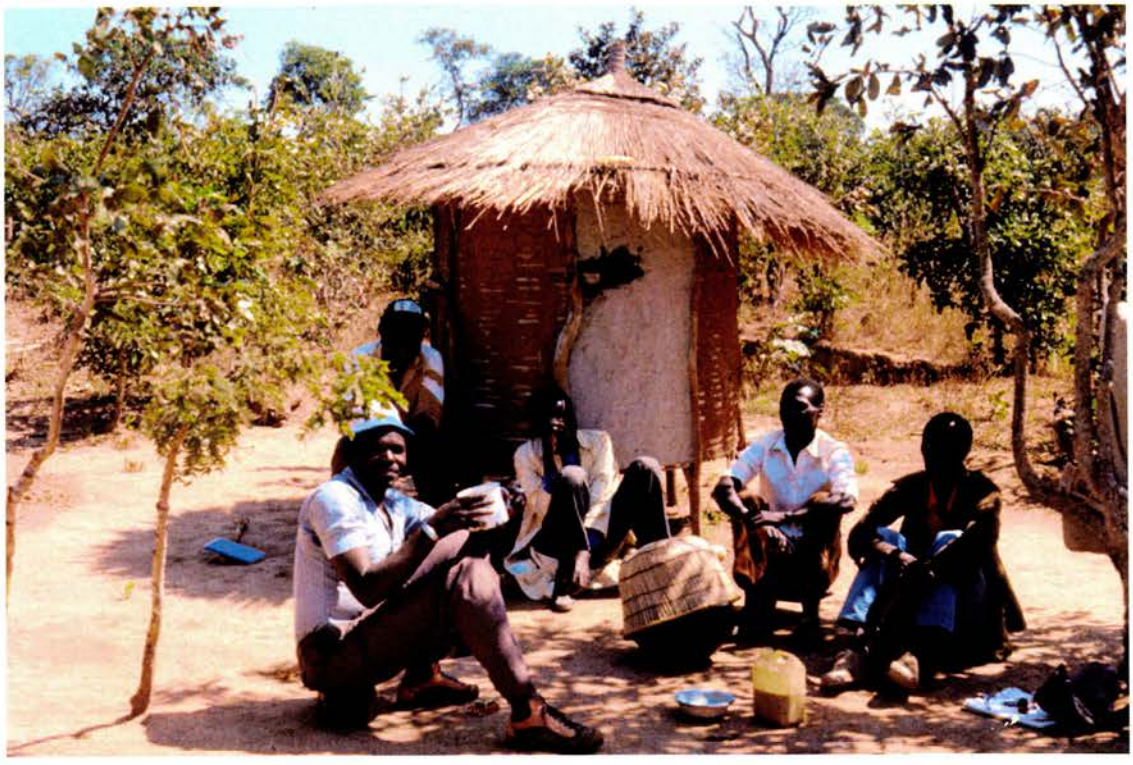
Photograph 1.10 Collecting a blood sample from a calf.



Photograph 1.11 Draft oxen at work.



Photograph 1.12 Let the young men catch the calves.



Photograph 1.13 Relaxing drinking maize beer during the heat of the day.

CHAPTER 2

EPIDEMIOLOGY AND CONTROL OF EAST COAST FEVER WITH PARTICULAR REFERENCE TO MALAWI

1.0 *THEILERIA PARVA*

1.1 CLASSIFICATION

Theileria parva is a tick transmitted intracellular protozoon and its classification and features were described by Irvin (1987).

SUB KINGDOM	Protozoa;	single cell eukaryotes.
PHYLUM	Apicomplexa;	apical complex present at least in some stages; reproduce sexually by syngamy.
CLASS	Sporozoea;	sporogonic stage producing sporozites.
SUB CLASS	Piroplasmia;	piroform, rod shaped or amoeboid; parasites in erythrocytes and some other cells.
ORDER	Piroplasmida;	asexual and sexual reproduction; ticks are vectors.
FAMILY	Theileriidae;	schizont stages in lymphocytes.
GENUS	*Theileria*;	piroplasm stage in erythrocyte lacks pigment.

The *Theileria* species of domestic animals are shown in table 2.1.

Table 2.1 The main Theileria species found in domestic animals

Parasite	Principal host	Principal vector	Pathogenicity in cattle	Geographical range
Theileria parva	Cape Buffalo, Cattle	*R. appendiculatus*	+++	Parts of East and Central Africa
Theileria mutans	Cape Buffalo, Cattle	Amblyomma spp.	+	East, Central and Southern Africa
Theileria taurotrogi	Eland, cattle	*R. appendiculatus*	-	East, Central and Southern Africa
Theileria annulata	Cattle, Asiatic Buffalo	Hyalomma spp.	++	Asia, Middle East, North Africa

1.2 HISTORY

East Coast fever, the disease caused by *T. parva,* was first recognised in 1901/1902 in Southern Rhodesia (now Zimbabwe). Although initially thought to be an acute form of babesiosis (Grey and Robertson, 1902) the disease seemed to be spreading along the transport routes and killing almost all cattle on the way. Because of the severe mortality and consequent disruption to transport, the British South Africa Company employed Robert Koch to investigate. He concluded that the disease was distinct from babesiosis and that it had been introduced from the Portuguese East African Coast and proposed the name African Coast fever (Koch, 1903). This name was adapted in the Southern African colonies to East Coast fever reflecting its presumed origin and the fact that it did not occur on the South African coast. Although first identified as a separate disease in Southern Africa, it had probably been endemic in Eastern Africa for a long time (Perry and Young, 1993).

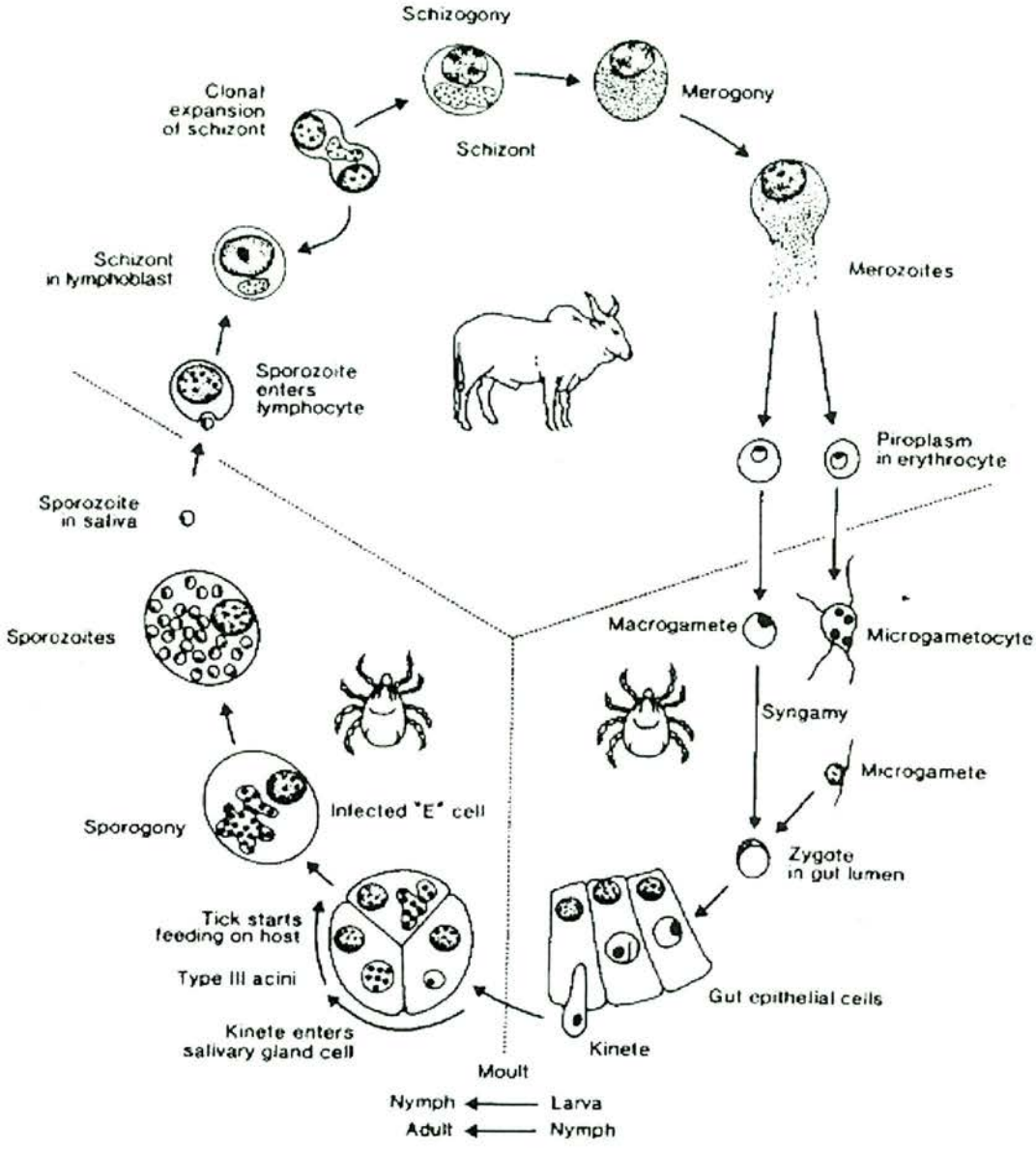
1.3 VECTORS

Three species of Rhipicephalus ticks are considered to be responsible for field transmission of *T. parva*. The most important of these is *Rhipicephalus appendiculatus*, with *R. zambeziensis* and *R. duttoni* playing a role in some areas (Lessard, L'Eplattenier, Norval, Kundert, Dolan, Croze, Walker, Irvin and Perry, 1990). R. duttoni only occurs in Angola and Zaire in the semi-arid belt along the Atlantic coast (da Graca and Serrano, 1971). R. zambeziensis replaces R. appendiculatus in some of the hotter, drier river valleys (e.g. Luangwa, Zambezi and Limpopo) and in some of the drier parts of Zambia, Mozambique, Zimbabwe and South Africa as well as the non-desert areas of Botswana and Namibia (Perry et al 1992). In the light of this, it may be expected to be found in the rift valley and lake shore area of Malawi but has not been recorded.

1.4 LIFE CYCLE

Figure 2.1 shows the life cycle of *T. parva* in cattle and in its host tick *Rhipicephalus appendiculatus* as drawn by A.S. Young (Norval et al, 1992).

Figure 2.1 The life cycle of *T. parva*



As drawn by A.S. Young in Norval et al (1992)

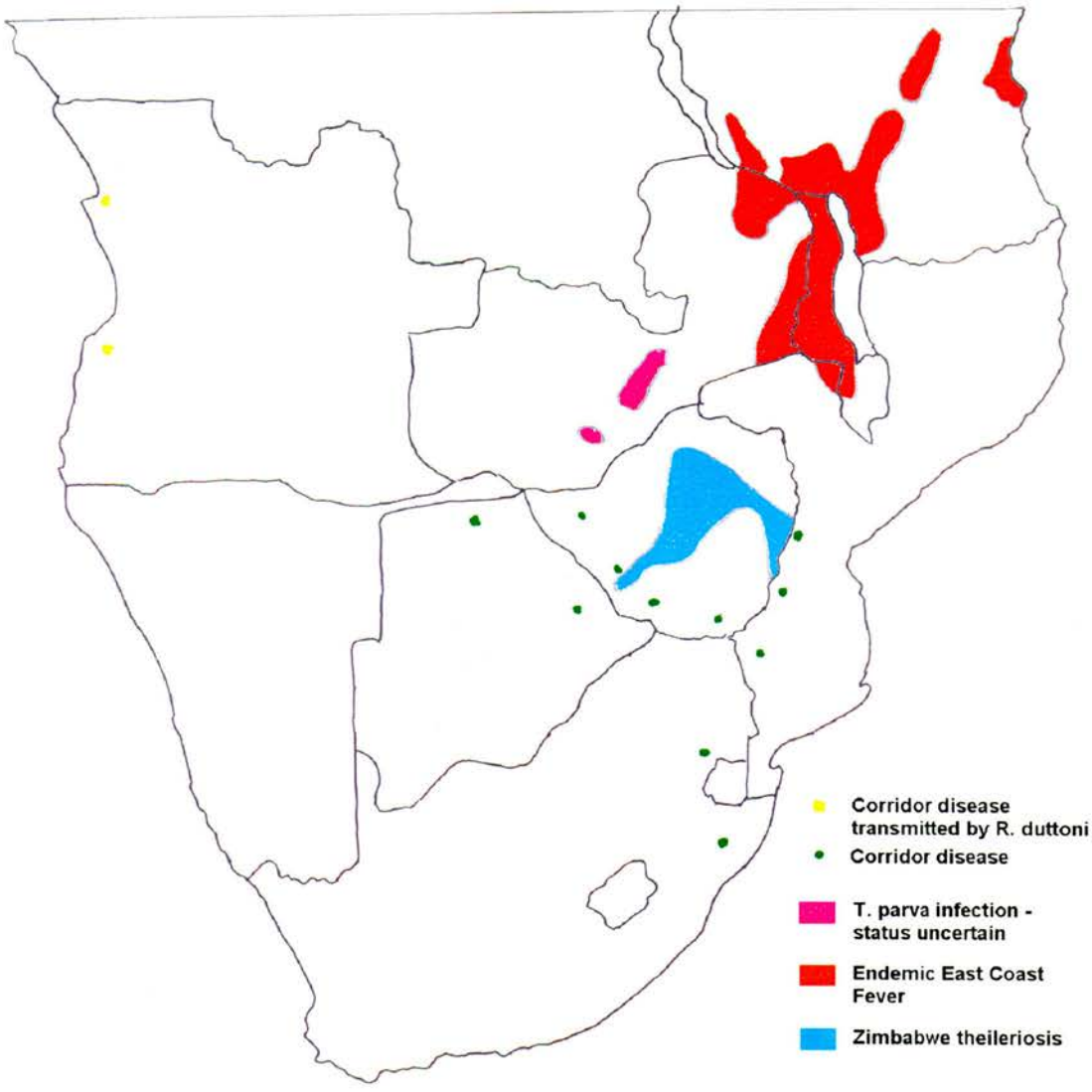
After a tick ingests infected erythrocytes, piroplasms differentiate into macro and microgametes. The sexual stage of the life cycle then occurs when these come together to form a zygote in the gut lumen of the tick. The zygote invades the gut epithelial cells and transforms into a motile kinete. When the tick moults the kinete undergoes sporogony in the salivary gland. Tick feeding stimulates maturation of the sporozoites and after three to four days sporozoites are released into the saliva. These are transferred to the mammalian host with the tick saliva and enter circulating lymphocytes were they form a schizont. The presence of a schizont transforms the lymphocyte and it starts colonal expansion. Usually within 14 days these infected cells infiltrate all lymphoid and other body tissues. The damage to infected tissue and to the lymphoid system underlies most of the pathogenesis of East Coast fever. Schizonts develop into merozoites which destroy host cells allowing the release of the merozoites, which infect erythrocytes (Norval et al, 1992). When in the erythrocytes, these merozoites are called piroplasms. It is not known if piroplasmosis of *T. parva* can infect new cells as happens with other Theileria species (Norval et al, 1992).

1.5 RANGE*

Figure 2.2 shows the distribution of the diseases that can be collectively described as East Coast fever in Central and Southern Africa.

* See chapter 8, section 1.1 for selected recent publications

Figure 2.2 Distribution of East Coast Fever in Central and Southern Africa



Redrawn from Lawrence, de Vos & Irvin, in Coetzer, Thompson and Tustin (eds) 1994. Infectious diseases of livestock with special reference to Southern Africa. Oxford University Press, Vol 1, pp310

1.6 DISEASE AND DIAGNOSIS*

East Coast fever was first described in Southern Rhodesia by Gray and Robertson (1902), although it was confused by concurrent *Babesia bovis* infections to the extent that it was classified as a severe form of redwater. Shortly afterwards, Koch (1903) reported that it was a new disease, similar to cases he had seen in Tanzania in 1897.

Bruce, Hamerton, Baterman and Mackie (1910) working in Uganda provided the first description of the endemic form of East Coast fever which was predominantly a disease of calves with a lower case fatality rate than the epidemic disease described in Southern Africa.

Clinical East Coast fever is characterised by pyrexia, malaise, anorexia, lachrymation, digestive disturbances, emaciation, dyspnoea and swelling of superficial and internal lymph nodes due to the lympho-proliferative nature of the disease. The incubation period varies from 8 to 25 days with a mean of 13 days (Losos, 1986). At the onset of pyrexia there is a lympho-destructive phase leading to a severe leucopenia in fatal cases. In severe cases, pulmonary oedema leads to dyspnoea and nasal discharge. Subcutaneous oedema is also sometimes seen. Different stocks of *T. parva* seem to damage different organs (Norval et al, 1992). Diarrhoea is often present and can contain blood or even be black and tarry. Lachrymation and corneal opacity are sometimes present. In some cases the brain is

* See Chapter 8 section 2.1 for selected recent publications

infected and a disease sometimes called Turning Sickness is seen.

The host reaction following *T. parva* infection ranges from no apparent reaction to severe reaction and death. A useful classification system for the severity of clinical disease is shown below (Anon, 1989).

- *No apparent reaction*: No parasites detected and no clinical signs apparent.
- *Mild reaction*: Few schizonts are detected, no fever occurs, or fever persists for less than 4 days. The animal is otherwise clinically normal and recovers.
- *Moderate reaction*: Schizonts are detected, fever persists for longer than 4 but less than 9 days. The animal shows mild and transient clinical signs and recovers.
- *Severe reaction*: Schizonts are detected, fever persists for 8 days or longer and the animal has obvious clinical signs of theileriosis. The animal may recover, but usually dies.

Diagnosis of clinical cases in the field relies on clinical signs and light microscopic examination of stained lymph node and blood smears. Yeoman, (1966a) used a set of criteria for his studies in the lake Victoria region which have become generally accepted. Cases showing clinical signs of East Coast fever and in which schizonts were detected were accepted as *T. parva* infection. Cases showing clinical signs of ECF but in which no schizonts were detected, irrespective of the presence of piroplasms in the blood, were classified as suspect.

Confusion between *T. parva* and *T. taurotragi* can occur when diagnosing mild clinical disease on the basis of light microscopy as the schizonts of these parasites are difficult to differentiate on morphological grounds (Perry et al, 1992). The schizonts of *T. mutans* are also too similar to reliably distinguish, although the main clinical sign with this normally benign parasite is anaemia (Young et al, 1978). *T. taurotragi* infections in cattle are usually mild, but De Vos (1982) has attributed cerebral theileriosis of cattle in South Africa to this parasite.

The piroplasms, like the schizonts, of *T. parva*, *T. mutans* and *T. taurotragi* cannot be reliably distinguished on morphological grounds (Perry et al, 1992). Therefore the only reasonably reliable method of confirming a diagnosis of ECF is the presence of schizonts in an animal displaying typical clinical signs. The presence of schizonts and/or piroplasms in an animal showing no clinical signs provides evidence of theilerial infection but not the species of parasite involved.

1.7 SEROLOGY*

Although several serological tests have been developed, the indirect fluorescent antibody test (IFAT) described by Burridge and Kimber (1972) has become the most widely used. This uses cell culture schizont *T. parva* antigen fixed onto glass microscope slides. Diluted test sera are added to the slides and then after washing, an anti-bovine fluorescein isothiocyanate conjugate is added. After another wash and

* See chapter 8 section 1.3 for selected recent publications

drying, the slide is examined under ultra violet light for the presence of specific fluorescence. Although there is no cross-reaction with *T. mutans* at a dilution of 1/40 (Burridge and Kimber 1972) there are cross-reactions with *T. taurotragi* and *T. annulata* (Burridge, Brown, Crawford, Kirimi, Morzaria, Payne and Newson, 1974a, and Burridge, Brown and Kimber, 1974b). In experimental *T. parva* infections, in the absence of rechallenge, antibody continues to be detected at a dilution of 1/40 for a mean of 7 months, with a range of 3 to 17 months (Burridge and Kimber, 1973). An ELISA for *T. mutans* has been described by Katende, Goddeeris, Morzaria, Nkonge and Musoke (1990).

1.8 PARASITE DYNAMICS

1.8.1 Cattle to tick transmission of *T. parva*[*]

Barnett (1957) found experimental evidence that recovered cattle were able to transmit *T. parva* for many months after recovery. Despite this it was generally believed for many years that the maintenance of *T. parva* in the field relied on infected ticks. However Young, Leitch, Newson and Cunningham (1986) showed conclusively that recovered cattle were still infective to ticks. Eighteen of 23 batches of *R. appendiculatus* applied to 1½ year old naturally infected and clinically normal zebu cattle over a 13 month period transmitted *T. parva* as adults to naive cattle (100 ticks applied to each cow).

[*] See chapter 8 section 1.4.1 for selected recent publications

As high parasitaemias associated with clinical cases are relatively short-lived, it is likely that most ticks that become infected with *T. parva* do so by feeding on carrier animals (infected, recovered animals able to infect other animals via ticks, Young et al, 1986). The total amount of infection in the tick population is dependent on the number of ticks in the population and host resistance (Fivaz, Norval and Lawrence, 1989). The susceptibility of *R. appendiculatus* to infection with *T. parva* from infected cattle was studied by Purnell, Ledger, Omwoyo, Payne and Peirce (1974). They fed nymphs on infected cattle and then assessed the infection rates in the ticks. All the cattle used for feeding the nymphs were in the acute phase of experimentally induced ECF. Parasitaemias ranged from 0.1 to 50% of red blood cells. Although animals with very high parasitaemias did result in significantly higher tick infection rates the difference was relatively small and very variable. No clear correlation between the piroplasm parasitaemia in the cattle on which the ticks engorged as nymphs and the infection rates in the adult ticks could be found. An interesting calculation made by these authors was that a nymph feeding on a parasitaemic animal may ingest 10 million to 100 million piroplasms. Their final conclusion was that the adult tick infection rate probably depends on a factor not measured. They suggest, but present no evidence, that this could be the juxtaposition of infected gut epithelial cells and developing salivary glands during the nymphal moult.

It is possible that, regardless of the parasitaemia, carrier animals are much less infective to ticks because of the well-established immune reaction. This is alluded to by Purnell et al (1974) when discussing stages in the acute phase of infection. It is

also known that treatment of infected animals with corticosteroids makes them more infective to ticks (T.T. Dolan and F. N. Mwakima, unpublished results, 1990 quoted in Norval et al 1992). It is possible that by reducing the efficiency of the host's immune system and causing an increase in parasitaemia, corticosteroids may also increase the infectivity of the piroplasms.

1.8.2 *T. parva* strains

Strain differences between different stocks of T. parva were noted by Young, Brown, Burridge, Cunningham, Kirime and Irvin (1973), Young, Radley, Cunningham, Musisi, Payne and Purnell (1977), Radley, Brown, Burridge, Cunningham, Kirimi, Purnell and Young (1975), Radley, Young, Brown, Burridge, Cunningham, Musisi and Purnell (1975) and Pinder and Hewett (1980). Buffalo derived *T. parva* appears to be significantly different to cattle derived strains with incomplete cross protection being provided by immunity to cattle derived strains.

Despite considerable evidence of strain differences of *T. parva* and the less than solid cross protection between some of the strains, there is also evidence for homogeneity of the stocks across Africa. *T. parva* Boleni from Zimbabwe protects against a range of Kenyan stocks (Irvin, Morzaria, Munatswa and Norval, 1989). Part of the reason for this may be the ability of heterologous strains to recombine during the sexual cycle of *T. parva* in the ticks (Morzaria, Young and Batavia, 1990).

1.8.3 Tick to cattle transmission of *T. parva*.

Purnell, Brown, Cunningham, Burridge, Kirime and Ledger (1973) showed that ticks only transmit *T. parva* after 4 days of attachment.

The severity of a *T. parva* infection is dose-dependent. Barnett and Bailey (1955) found that when 4-month-old calves from immune dams were infected with *T. parva* by one tick, none died (although 54% of calves from susceptible dams died) and that there was a graduation of mortality up to 60% when 50 infected ticks were applied. No data was presented to substantiate the infectiveness of all ticks and some of the survivors may not have received an infected tick. However, even after allowing for this, there appears to be a dose-dependent effect on the development of ECF disease. Barnett (1957), probably commenting on the same work, reported graduated survival rates in 3 to 4-month-old calves born to dams reared in an enzootic area from 100% with one infected tick to 36% with 50 ticks. He presented data to support the infectivity of the ticks. He states

"The results provide experimental proof for the belief that in the enzootic areas limitation of tick numbers by means of long interval or irregular dipping periods could be effective in reducing mortality from ECF and at the same time maintain the immunity of the cattle population".

Barnett & Brocklesby (1961) reported in a letter to the Veterinary Record that they had isolated a mild strain of *T. parva* which caused graduated mortality rates in naïve cattle depending on how many infected ticks were used for transmission. Other workers (Radley, Brown, Burridge, Cunningham, Peirce and Purnell, 1974, and Cunningham, Brown, Burridge, Musoke, Purnell, Radley and Sempebwa, 1974)

provided conclusive evidence to substantiate this dose-related effect. The latter group inoculated susceptible cattle with titrated suspensions of *T. parva* derived from ground-up ticks. The 3 dilutions used gave a graduated response. The highest dose killed 7 of 10 animals while the lowest dose resulted in one death, 7 had mild reactions that resisted re-challenge and 2 animals that failed to seroconvert and died on re-challenge. Similar results were obtained by Dolan, Young, Losos, McMilian, Minder and Soulsby (1984). Eight of 10 animals given undiluted stabilate became infected and died. Nine animals were given a dilution of the stabilate, 8 became infected and only 2 died. Four further dilutions were used each resulting in less animals becoming infected and less dying. The experiments showed that reducing the dose of infective material can result in a significantly higher survival rate even in the animals that become infected.

Short and Norval (1981a) found that the nymph to adult ratio on the vegetation was approximately 10:1. However Yeoman (1966b) found the nymph:adult ratio of *R. appendiculatus* on adult cattle to be 1:6.2 but 1:1.3 on calves. Yeoman went to considerable trouble to count nymphs accurately and although his team undoubtedly missed a proportion of them, his figures are probably reliable. The fact that nymphal *R. appendiculatus* are known to feed on a wide range of alternative hosts (Yeoman, 1966 and Branagan, 1974) is probably the reason why the population on cattle differs from the pasture population. MacLeod and Colbo (1976), working in the Southern Province of Zambia, considered that alternative hosts for *R. appendiculatus* larvae were very important for the maintenance of the tick population. This will obviously

have implications for *T. parva* epidemiology in this and similar regions.

Purnell, Boarer and Peirce (1971) studied the comparative infection rates of adult and nymphal *R. appendiculatus* when their previous instars fed together on an experimentally infected calf. They found that there were approximately 3 times as many infected acini in the resultant adults as there were in the nymphs. Adults are therefore the most potent stage of *R. appendiculatus* in transmitting *T. parva* to cattle (they carry a higher parasite burden than nymphs). However it also follows that an individual nymph will transmit a smaller dose of parasite which, as discussed earlier, may result in less severe disease than that caused by an adult tick. Purnell, Young, Brown, Burridge and Payne (1974) had similar findings when working with a strain of *Theileria lawrencei* previously passaged in cattle.

A field survey by Barnett (1957) indicated that mortality was directly attributable to ECF among calves in enzootic areas was low, and carried out an experiment to find out why. He obtained two zebu herds, one from an enzootic area and the other from a government farm where they had been kept tick-free for 3 generations. He ran both herds separately under tick-free conditions and subjected the calves to infestations with a single *T. parva* infected tick. His results are given below.

Table 2.2 Survival of *T. parva* challenged calves of different ages

Age of calf	Survival of calves from immune dams	Survival of calves from susceptible dams	Controls
1 month	100% (20)	20% (20)	0% (9)
4 months	100% (20)	46% (24)	9% (11)
14 months	80% (20)	37% (7)	0% (3)
27 months	64% (11)		0% (2)

The controls were 1 to 2-year-old high-grade steers.

This work shows that calves from enzootic area have considerable resistance to ECF and that this appears to decrease with age. Age related immunity is central for the concept of endemic stability and it is surprising that this is the only published experimental data that appears to support the case for it existing in ECF.

The high recovery rate at 4 months suggests that some factor other than colostral immunity is the major determinant of survival. This is supported by the findings of Muhammed, Lauerman and Johnson (1975) who found that transfer of immune serum to naïve cattle did not protect them from ECF. Cunningham, Brown, Burridge, Morzaria and Urquhart (1989) showed that *Bos taurus* calves from dams hyper immunised with *T. parva* were not protected when challenged at one to two months old. Emery (1981) showed that immunity could be transferred to susceptible animals by transfer of thoracic duct leucocytes from *T. parva* vaccinated animals and concluded that cell mediated immune responses played a major part in resistance. McKeever, Taracha, Innes, Machugh, Awino, Goddeeris and Morrison (1994) showed that cytotoxic T lymphocytes (CTL) produced in the lymph node draining the

infection site are probably responsible for elimination of the parasite. They obtained evidence for this by selectively transferring CTLs from immune calves to their naïve monozygotic twins and showing that this gave protection in the face of an otherwise lethal challenge.

2.0 *RHIPICEPHALUS APPENDICULATUS*

2.1 HISTORY

In November 1902 *R. appendiculatus* collected from a sick ox in Southern Rhodesia were sent to Charles Lounsbury in Cape Town who conducted several experiments with them. Theiler and Stockman in the eastern Transvaal carried out further work. The work of these 3 workers has been summarised by Lawrence (Norval et al 1992) as follows:

1. That *R. appendiculatus* was the principle vector of *T. parva*, although other *Rhipicephalus* species could transmit the parasite experimentally.
2. That it required only a single tick to transmit infection.
3. That transmission occurred only transstadially and not transovarially.
4. That infected ticks on a pasture died out within a period of 15 months after which time cattle could be grazed with safety.
5. That recovered cattle were not infective for ticks*
6. That the periods of attachment of the ticks ranged from 3 days for larvae to 9 days for adults.
7. That the time from detachment of one stage to the reattachment of the next was on average 18 (larva-nymph) to 21 (nymph-adult) days.
8. That the incubation period for East Coast fever from the time of attachment of ticks was 10-20 days.

* It has since been discovered that this is not the case. See section 1.8.1.

2.2 LIFE CYCLE

After feeding, engorged female ticks fall to the ground and lay eggs approximately 1 week later. Larvae hatch from these eggs in approximately 1½ to 2 months and seek a host. Larvae complete their feeding after about 5 days, fall to the pasture and moult

to nymphs within 2-3 weeks. These seek a second host and feed for about 5 days before again falling to the pasture and moulting. Adults emerge in approximately 2-3 months and again seek a host before feeding for approximately 10 days. Timings for this 3 host tick life cycle are taken from Wilson (1946b).

2.3 SEASONAL ACTIVITY

Short and Norval (1981b) analysed published data on the seasonal occurrence of *R. appendiculatus* in eight localities in East and Central Africa. They showed that the pattern of seasonal occurrence is largely dependant on the timing of the activity period of the adult stage (see also Short and Norval, 1981a). They identified the regulatory factors as rainfall, temperature and day length. Sutherst and Maywald (1985) described a computerised system for matching climates in ecology and showed that it was useful for predicting the distribution of *R. appendiculatus*.

2.3.1 Rainfall

It is not rainfall that is important to the actual development of the tick but rather the humidity in the tick's microclimate. Short and Norval (1981b) concluded that, as a generalisation, mean monthly rainfall of 10mm or more was the minimum for adult activity. Pegram and Banda (1990) considered that microclimate humidity has little effect on development rates but a large influence on duration of survival of each stage. Rechav (1982) concluded that tick population sizes are generally governed by humidity. Neither Yeoman (1966b) nor McCulloch, Suda, Tungaraza and Kalaye

(1968) could detect any evidence of high humidity accelerating development rates.

The egg is the stage most vulnerable to desiccation (Branagan, 1973, Kaiser, Sutherst, Bourne, Gorissen and Floyd, 1988, Norval et al, 1992, Short, Floyd, Norval and Sutherst, 1989) and in areas of seasonal rainfall the tick has adapted to ensure that this stage occurs in the latter half of the rainy season when the microclimate is most favourable. As humidity is influenced by temperature (through evaporation) it is important to note that the effect of rainfall on activity can be substantially modified by temperature (Short and Norval, 1981b).

2.3.2 Temperature

Wilson (1946a) and Yeoman (1966b) suggested that low temperatures in the cooler months of the year may be at least part of the reason for little adult activity during these times in Malawi and Tanzania. Short and Norval (1981a) observed a significant correlation between temperature and adult activity in the highveld of Zimbabwe. The main useful measurable parameter was daily minimum temperature, with a rapid decline in adult activity below 15°C.

Temperature also affects development periods for the various life cycle stages (Branagan, 1973). Short and Norval (1981a) noted that unusually cold winters delayed nymphal activity for several months. Short et al (1989) studying ticks in soil cages found that development of all stages was most rapid during warm conditions

and slowest during the cool season although they also found that the preovipositional period was lengthened by very hot conditions.

Randolph (1993) analysed data from five locations in southern Africa and concluded that the most important environmental factor for *R. appendiculatus* was the night time minimum temperature as this determined condensation and saturation deficit and thus the tick's ability to replenish moisture lost during the day time and so survive while questing for hosts. Larvae numbers were correlated most closely with these factors, which the author considers to be consistent with earlier experimental results showing that larvae are the most susceptible stage to desiccation.

2.3.3 Day length

Several workers (Rechav, 1982, Short and Norval, 1981a, and Short and Norval, 1981b) have implicated day length as a possible controlling factor in the seasonal occurrence of *R. appendiculatus* in Southern Africa. Short and Norval (1981b) concluded that as 11 hours (light) is the shortest day length at which adult activity has been recorded over the entire distribution range of *R. appendiculatus*, this must be the minimum day length necessary for adult activity.

2.3.4 Field studies – East Africa

In Eastern Africa there is almost constant day length throughout the year and in many areas there is no prolonged dry period. Kaiser et al (1988) reported continuous

activity of larvae, nymphs and adults through out the year in some regions of Burundi. They also recorded extremely high infestation rates with mean adult *R. appendiculatus* of over 300 per animal for most of the two years of their study. Kaiser, Sutherst and Bourne (1982) counted *R. appendiculatus* on cattle at Entebbe in Uganda over an 18-month period. Three hundred or more adult *R. appendiculatus* per animal were recorded during 19 of the 22 months of the study, with no obvious pattern through the year. In both of these studies (Kaiser et al, 1982 and Kaiser et al, 1988) nymphs and adults were present in large numbers at the same time. Kaiser et al (1988) also demonstrated the presence of larvae, albeit at much lower infestation rates, throughout the year.

Tatchell and Easton (1986) found no evidence of any marked seasonal variation in infestation rates near Lake Victoria, Tanzania. All three instars were present at the same time for almost the whole two years of their study. The almost constantly favourable climate for the development of the various tick stages means that in certain areas of East Africa *R. appendiculatus* can have at least two (Branagan, 1973) and possibly three (King, Gettinby and Newson, 1988) generations per year with all stages on the host at the same time.

2.3.5 Field studies – Southern Africa

There have been a number of well-designed studies of the seasonal variations of *R. appendiculatus* in Southern Africa. Wilson (1946a) in the Northern region of

Nyasaland (now Malawi) was struck by the marked seasonal periodicity of *R. appendiculatus* . He found adults were most abundant during the months of December to March. Larvae and nymphs first appeared in his collections in April. The larvae remained common in May, were fewer in June and had disappeared entirely after July. Nymphs were common throughout the dry season from April to November but disappeared during the wet months of December to March. Yeoman (1966b) considered that the seasonality of *R. appendiculatus* in the lake region of Tanzania to be very similar to that seen by Wilson in Malawi.

Jooste (1966a) studied a herd of Red Poll cows 30 miles North of Salisbury, Rhodesia (Harare, Zimbabwe) and found adult *R. appendiculatus* occurred almost exclusively during the wet months of November to March. Subsequent studies by the same author (Jooste, 1966b) showed that larvae could be found on the pasture between January and October with two marked peaks in April and October. Nymphs were found on the pasture between May and October with the main peak in August and September. The data was only collected over a single 12 month period and from a single 50 yard drag area. Potentially this may have led to bias in the data due to uneven distribution of ticks, especially larvae, on the pasture. The two peaks of larval abundance do not match the nymphal or adult pattern seen by the same author. She states that the two larval peaks may be due to slow development of eggs during the cooler months of May to July. This is not well borne out by the graphs presented and more data would be needed to check if the two peaks really occurred of if they were a consequence of the sampling methodology.

The work done by B. A. Matson in a similar Highveld location to Jooste (1966a) and later published by Matson and Norval (1977) also documented peak infestations of *R. appendiculatus* adults occurring in the middle half of the rainy season. This led Matson and Norval (1977) to conclude that there was only one generation per annum.

Rechav (1982) in South Africa saw a very similar pattern with adult numbers increasing sharply in November, peaking in January/February, then decreasing and finally disappearing in May. Larvae were present mainly during April to June and nymphs were active mainly during July/August. Pegram, Perry, Musisi and Mwanaumo (1986) found a very similar seasonal pattern in Zambia. Adults were present from December to April, larvae mainly from March to April and nymphs from May to September.

Working in Eastern Zambia, Berkvens (1990) found the same basic seasonal pattern but in one location saw a second wave of adults in May/June and a second wave of nymphs in August and September after a first nymphal wave starting in March. His general impression was that at least part of the tick population cycled through two generations per annum.

2.3.6 Diapause

Tick diapause is a special category of dormancy, which is important in the seasonal

regulation of the life cycle. The advantage of diapause is that it is a pre-adaptive behaviour that precedes the actual onset of unfavourable environmental conditions and thus ensures tick survival. Diapause is regulated by inherent mechanisms and is not a direct response to unfavourable conditions. (Belozerov, 1982)

Short and Norval (1981a) concluded from their work in the Highveld of Zimbabwe that seasonal regulation of the life cycle of *R. appendiculatus* occurs through the regulation of adult activity. It is through this regulation that a fairly constant pattern of larval and nymphal occurrence is maintained from year to year. They developed this argument to explain how, by using a combination of factors for regulation of adult activity (day length, rainfall and temperature), *R. appendiculatus* has evolved an extremely adaptable seasonal cycle that enables the species to survive in a wide range of climates. The same authors, in another publication, further develop this theory by considering the seasonal occurrence of *R. appendiculatus* in eight locations in East, Central and Southern Africa (Short and Norval, 1981b). The significance of this phenomenon is that it ensures that the desiccation sensitive egg and larvae stages occur at the wettest time of the year and that the highly resistant adult stage can live through the most unfavourable time of the year.

The basic process of diapause is that adult ticks, after moulting from nymphs have a resting phase and only commence seeking a host when some environmental trigger is reached. Pegram et al (1986), Pegram and Banda (1990) and Short et al (1989) demonstrated such a diapause under field conditions in Zambia and Zimbabwe.

On the basis of published data and his findings in the Eastern Province of Zambia, Berkvens (1990) formulated a general model of diapause for *R. appendiculatus* by dividing the behaviour into three groups by latitude.

1. Close to the equator (latitudes below 7°) the minimum day length never drops below the threshold value of 11h45m and the newly moulted adult ticks are unable to enter diapause. *R. appendiculatus* is therefore restricted to areas where it can survive possible adverse climatic conditions without having to enter a diapause. It is excluded from areas where the climatic conditions during the dry season are so adverse that adults would have to enter diapause to survive them.

2. At latitudes above approximately 7°, day lengths are below the threshold photoperiod for increasing periods of time with increasing latitude and newly moulted adults can enter diapause. However at latitudes lower than 16-30° (actual figure not known), the maximum day length is not long enough to terminate the diapause by means of a long day signal. Between 7° and 16-30° the diapause is terminated by a weakening of its photoperiodic maintenance because of ageing of the adults. *R. appendiculatus* is able to survive in areas where a diapause is required to survive the viscitudes of the dry season.

3. At latitude above 16-30°, the minimum day lengths are shorter than the threshold required to induce diapause and maximum day lengths are longer than the minimum required to terminate it. *R. appendiculatus* is able to take full advantage of a proper diapause, entirely governed by day length.

This explanation fits well with another of Berkvens' findings (Berkvens, Pegram and Brandt, 1995) that ticks from East Africa entered diapause when exposed to Zambian conditions. The logical conclusion from his first category (the equatorial population) is that in East Africa the tick can not enter diapause when it needs to survive adverse conditions due to day lengths being too long for the induction of diapause. Norval et al (1989) found that unfed adults from Kenya did not diapause when exposed to natural day lengths of 12 hours light 12 hours dark, whereas ticks from Zambia and

Zimbabwe did enter diapause and did not attach readily to hosts. Berkvens (1991) does qualify his claim that east African stocks diapause by pointing out that the tick he used had been maintained under laboratory conditions for many years and this may have affected its behaviour although it is difficult to imagine how laboratory conditions would select for diapausing behaviour. There is no doubt that *R. appendiculatus* at the equator does not enter diapause even when it would be beneficial to do so and that in Southern Africa diapausing behaviour is vital to the ticks survival. What is still unclear is whether all *R. appendiculatus* stocks have the ability to diapause when day lengths are short enough for its induction or if East African ticks are genetically different and do not have the ability to diapause.

At present there is no supporting evidence to show that physiological ageing of *R. appendiculatus* allows the breaking of diapause without the day length stimuli (Berkvens, 1991 and Berkvens et al, 1995) although this was suggested as a possibility by Belozerov (1982).

2.4 HOSTS

While cattle are the economically important host of *R. appendiculatus* the tick is known to feed on other species. The main laboratory species found to be useful for feeding all stages is the rabbit. Branagan (1974) found that Cattle, Buffalo, Eland, Waterbuck and Oryx were at least as good as rabbits as hosts but Thomson's Gazelle, Wildebeest, Sheep and Goats were less so. He also found that immature stages successfully engorged on Mongoose, Cane Rat, Genet, Domestic Fowl and Spur Fowl.

Yeoman (1966b) found that sheep were reasonable hosts for *R. appendiculatus* adults but only seemed to carry significant burdens when closely grazed with cattle. Even in suitable areas, sheep did not carry many ticks when there were no cattle. He found goats to be poor hosts and failed to find adult *R. appendiculatus* on dogs, cats, donkeys and horses. On small mammals (e.g. hares) he found no adults but some nymphs.

Macleod, Colbo, Madbouly and Mwanaumo (1977) found adult *R. appendiculatus* on a wide range of wild animals with one exceptional kudu having 548 adult ticks. These workers also determined that 89 % of *R. appendiculatus* adults fed on the head of their host (85 % in the ear).

2.5 HOST RESISTANCE

De Castro and Newson (1993) in a review paper outline the effects of host resistance on ticks.

- Hypersensitivity reactions at the site of the bite that provoke licking, rubbing and scratching which dislodges or squashes the ticks.
- Decreased percentage of ticks surviving after attachment due to defensive cellular reaction in the bite lesion.
- Reduced engorged weight of larvae and nymphs that do complete feeding. This produces undersized nymphs and adults with lower capacity at the next feed. For the adult female a reduced blood meal yields fewer eggs. The stunted ticks also have a lower chance of survival off the host.
- The combination of these host resistance effects can greatly reduce the overall tick population. This was seen by Chiera, Newson and Cunningham (1985) who found that when all 3 stages of *R. appendiculatus* fed on resistant hosts the egg production of the population was reduced by 98% compared with non resistant hosts.

Wikel and Allen (1982) and Wikel and Whelen (1986) reviewing the immunological basis of host resistance to ticks concluded that antibody, cell mediated and complement dependent immune effector mechanisms are important for host resistance.

Repeated infestations of *R. appendiculatus* on cattle were shown to result in a decline in engorgement weights and recovery rates of ticks (Chiera et al, 1985). Fivaz et al (1989) conducted an experiment where tick resistant and tick naive Friesland cross Hereford oxen were exposed to ticks infected with *T. parva*. The difference in tick resistance was demonstrated by significant reductions in mean tick weights and recovery of detaching females compared to the naive controls. The resistant cattle all developed a severe exudative dermatitis of the ear pinnae within 24 hours of tick

attachment that trapped the feeding ticks when it dried. By contrast there was little clinical evidence of host reaction in the tick-naive group. All the oxen developed febrile responses and evidence of *T. parva* infection. Fascinatingly the four tick-resistant animals suffered from mild self limiting disease whereas the three tick-naive animals became seriously ill and two died. The authors concluded that the difference in severity of the clinical signs in the two groups was likely to be due to qualitative differences in the dose of infective sporozoites which was related to host response to tick infection. Francis and Little (1964) reported similar findings in infections due to *Babesia* species, with transmission to tick-resistant cattle being greatly reduced in comparison to non-resistant cattle. The authors associate this with the lower number (by a factor of 10) of engorging ticks on the tick-resistant animals. Similar results were reported by Fivaz and Norval (1990).

The degree of acquired resistance in cattle varies between breeds. Norval, Sutherst, Kurki, Gibson and Kerr (1988) found that Hereford x Friesian cattle allowed the engorgement of at least 4 times as many adult and nymphal *R. appendiculatus* as Sanga cattle. All cattle had been tick-naive at the start of the experiment and had been grazed on pastures heavily seeded with *R. appendiculatus* for four months before the start of tick counting.

Utech, Wharton and Kerr (1978) found a range of resistance to *Boophilus microplus* in different breeds of cattle. *Bos indicus* cattle were highly resistant while *Bos taurus* cattle had very low resistance to tick maturation. Cross breeds were of intermediate

resistance and 20% of these cattle carried 50% of the mean tick population. They also reported that resistance to tick may be passed on to the next generation of cattle. Fivaz, De Waal and Lander (1992) also reported that cross-bred oxen (Afrikander x Sussex) carried significantly more *Boophilus decoloratus* ticks than indigenous (Mashona) oxen.

3.0 THE EPIDEMIOLOGY OF EAST COAST FEVER

East Coast fever is estimated to cause the deaths of 1.1 million cattle annually from among the 24 million at risk in 11 countries (Mukhebi, Perry and Kruska, 1992). Despite these losses well designed studies to evaluate a possible link between infection pressure and production losses are not common in the published literature.

3.1 FIELD STUDIES OF EAST COAST FEVER EPIDEMIOLOGY – EAST AFRICA*

Barnett and Bailey (1955) in their annual report and Barnett (1957) note an ECF mortality rate, in zebu cattle in an enzootic area of Kenya, of approximately 8% during the first year of life. This was before dipping was introduced to the area. They believed that the infection rate in the ticks was low and that this combined with adult *R. appendiculatus* infestations of 10-30 ticks per calf maintained endemicity without heavy calf losses. These authors considered that there was a link between relatively low *R. appendiculatus* infestation rates and high rates of recovery from ECF in zebu cattle. Barnett (1957) noticed a dramatic fall in total mortality (29% to 7%) and ECF mortality in calves (8% to 1%) when dipping was started every 2 weeks in benzine hexachloride (BHC). This was despite no movement control and the fact that not all the animals in the area were dipped. Barnett and Bailey (1955) summarised as follows;

* See chapter 8 section 2.1 for selected recent publications

> "In general it can be said that reductions of tick numbers has an advantageous effect on the recovery rate from ECF and that a 100% recovery rate can be expected from infections with a single adult infected tick. Even with 5 infected ticks the recovery rate should be about 90%".

In 1956, the year after Barnett and Baileys annual report was published, there was a severe epizootic ECF in the Sukumaland area of the Lake Region in Tanzania. G. H. Yeoman was sent to investigate and studied the epidemiology of ECF from 1957 to 1960 in the area. The first of his 3 papers on this study (Yeoman 1966a) dealt with the quantitative relationship between *R. appendiculatus* and the epizooicity of ECF. He found that at any one time up to 5 ECF zones could be identified.

1. Permanently enzootic, characterised by disease in calves only. Average adult *R. appendiculatus* infestation rates were approximately 40-60 per animal.
2. Recently enzootic, having passed through an epizootic stage and mortality having now shifted from adults to calves. Average adult *R. appendiculatus* infestation rates were approximately 5-20 per animal.
3. Epizootic, characterised by continuing and spreading heavy losses, particularly but not exclusively in adults. Adult *R. appendiculatus* infestations in this area averaged 1-4 per animal.
4. Sporadic, characterised by localised, limited losses often explainable by movement of stock from infected area. Adult *R. appendiculatus* infestations were only 0.3 - 0.8 ticks/animal.
5. ECF free zones. *R. appendiculatus* infestations were below 0.2 ticks per animal.

While Yeoman was not able to study tick infection rates his work confirmed that of Barnett and Bailey (1955) that when cattle were exposed to high levels of parasite challenge a situation of enzootic stability could develop through the means of herd immunity, disease only being seen in calves. He showed that the highest mortality from ECF was seen in the intermediate zone where there was not enough parasite

circulating from vector to host to generate solid herd immunity. He also demonstrated the instability of these intermediate areas in that changes in the vector population or the introduction of infected cattle could spark serious mortality. This intermediate zone was not static geographically and the state in any particular location could change with time. The author did not however produce any evidence that reducing tick numbers reduced the ECF mortality while maintaining endemic stability. Yeoman established the principle, still widely held today, that the higher the challenge the greater the endemic stability.

Five years after Yeoman made his study, McCulloch et al (1968) conducted a livestock survey in the same area. They estimated annual losses of adults and calves from ECF in the enzootic zone to be 9% and 46%, in the epizootic zone to be 15% and 47% and in the clean areas to be 5% and 4% respectively. These figures have to be interpreted with caution as they were based on retrospective owner reports with no diagnostic backup.

Moll, Lohding and Young (1984) noted that if the epidemiology of ECF is to be understood fully, intensive studies of limited epidemic and epidemic areas were essential. They chose an endemic area in the Trans-Mara division of Kenya for their work. They studied 116 zebu calves born over a 14-month period. Nineteen percent died before 6 months of age and 25% by 18 months of age. They recorded 2.6% mortality due to theileriosis. One problem however is that the authors do not clearly state their criteria for diagnosis. They comment that other studies (notably Barnett

and Bailey, 1955 and Barnett, 1968) may have over estimated the mortality from ECF by considering the presence of macroschizonts in post-mortem smears to be evidence of death from theileriosis. Moll et al (1984) found that up to 25% of calves had patent macroschizont parasitosis at any one time. Despite this criticism the paper was a landmark in the study of ECF epidemiology under field conditions. They comment in their discussion that "It is evident that the disease caused by *Theileria* species are complex and cannot be studied in a vacuum separated from other disease problems within the cattle populations". Their main epidemiological finding was that while theileriosis morbidity was 100% (detected parasitologically) calf mortality from the disease was very low. They also considered it likely that *Theileria* infections stunted calf growth. However they do not make any mention of this as a possible contributory factor in the rest of the calf mortalities.

Moll et al (1984) felt that their study was hampered by the monthly sampling interval. Therefore Moll, Lohding, Young and Leitch (1986) went on the following year to study 31 zebu calves born during one month in the same area. These animals were examined every day for 6 months. While all calves developed *Theileria* infections as judged by slide examination and serology, none died. Again they noticed the effect of infection on calf growth rates. With a cohort size of 31 this study should not be used as evidence that calf mortality can be zero in an endemic area. There is a 25 % probability that they would have failed to detect a mortality rate as high as 5% (Cannon and Roe, 1982).

In both of the Trans-Mara studies the demarcation between *T. parva* and *T. mutans* infection was not always clear. It was evident that both were common and the authors resorted to using the term theileriosis to describe the disease complex. Despite this, the most important finding of these studies was that in an endemic area the vast majority of calves could resist *T. parva* challenge but that theileriosis was only one of the challenges that calves had to survive.

Latif, Rowlands, Punyua, Hassan and Capstick (1995) conducted a study on Rusinga Island, Lake Victoria, in which farmers only kept zebu stock and practised no tick control. A total of 162 calves born on 10 farms during 1986 and 1987 were monitored until 12 months old. Calves were examined weekly and a diagnosis of death due to ECF was based on a post-mortem examination and backed up by examination of smears. Thirty three percent of calves died before they reached 12 months. Twenty-three of the 42 deaths were attributed to general loss of condition. The authors acknowledged the reality of the field situation where a number of factors could have been involved in these cases and that it was impossible to separate one factor from another as the primary cause of death. Nine deaths were positively attributable to ECF, which means that 7% of calves died before 12 months old from ECF. These deaths were not evenly spread, with all deaths occurring on 2 of the 10 farms and in one year. Approximately 20% of calves showed clinical signs of ECF in each of the 2 years of study. Unfortunately no serology for *T. parva*, monitoring of older cattle or tick counts were carried out. This makes interpretation of the prevailing epidemiological state on the island difficult. In their discussion the

authors appear to assume that there is a degree of endemic stability but do not state this.

The last 3 studies discussed (Moll et al, 1984, Moll et al, 1986 and Latif et al, 1995) show a general pattern of relatively low calf mortality due to ECF (up to about 10%) in endemic areas. They also show that this mortality is unpredictable from year to year and farm to farm. There is an obvious need for larger scale studies over longer time periods. However the papers also demonstrate the difficulties of collecting reliable data from the field situation and analysing this in a meaningful way.

3.2 FIELD STUDIES OF EAST COAST FEVER EPIDEMIOLOGY – CENTRAL AND SOUTHERN AFRICA*

Central and southern Africa has enormous potential for studying the epidemiology of ECF due to the fact that for most of the region the 3 instars of *R. appendiculatus* show a strict seasonal pattern. However, no prospective studies have been carried out and published (up to 1995).

* See chapter 8 section 2.2 for selected recent publications

4.0 *COWDRIA RUMINANTIUM* AND ITS VECTORS

4.1 INTRODUCTION

Heartwater is a disease of cattle, sheep, goats, buffalo and several species of wildlife caused by the rickettsia *Cowdria ruminantium* and is transmitted by Amblyomma ticks (Uilenberg 1983). A review of the historical background and global importance of Heartwater is given by Provost & Bezuidenhout (1987). The first record of what was probably heartwater was in 1838 in South Africa. In 1900 Lounsbury confirmed that the bont tick (*Amblyomma hebraeum*) was the vector in South Africa. In 1925 E.V. Cowdry confirmed the suspicion of Sir Arnold Theiler that the disease was caused by a rickettsia. He named this *Rickettsia ruminantium* which was later changed to *Cowdria ruminantium* (Moshkovski 1947).

Heartwater occurs in most African countries south of the Sahara, in Madagascar, the Mascarene Islands and some islands in the Eastern Caribbean (Provost & Bezuidenhout 1987)

4.2 VECTORS

Following Lounsbury's confirmation of Amblyomma hebraeum as a vector in 1900 many other species of 3 host Amblyomma ticks have been shown to transmit Heartwater. The most important of these is Ambyoma variegatum (Doubney 1930). This species is well adapted to domestic livestock, is an efficient vector and has a wide distribution including most of tropical sub-Saharan Africa, Madagascar, the

southern part of the Arabian peninsula and several Caribbean islands.

Transmission is transstadial and not transovarial (Ilemobade & Leeflang, 1978). Ticks infected as larvae transmit disease as nymphs and adults. However, larvae do not usually feed on hosts susceptible to Heartwater and therefore are rarely infected. Ticks infected as nymphs transmit in the adult stage (Lounsbury 1990, Doubney 1930). Male ticks, although capable of transmitting Heartwater (Alexander 1931) appear to be poor vectors (Ilemobade & Leeflang 1977).

Unlike the transmission of babesiosis and theileriosis where feeding occurs for several days before transmission, Heartwater is transmitted within 24 hours of tick attachment (Neitz 1968). This has practical implications for the frequency of acaricidal treatment necessary to stop disease transmission.

4.3 DISEASE AND DIAGNOSIS

Fever is the first clinical sign and develops approximately 18 days after tick infestation (Neitz, 1968). The course of infection varies from subclinical to peracute. Subclinical and mild forms are frequently seen in indigenous cattle and in young calves of all breeds (Uilenberg, 1981). Peracute cases are usually seen in exotic breeds. Animals can suddenly collapse and die in convulsions. However, Heartwater in susceptible cattle is usually an acute febrile disease accompanied by nervous signs (staggering, drunken gait, circling movements, twitching of eyelids,

etc). Profuse, sometimes haemorrhagic diarrhoea is common (Uilenberg, 1981).

On post mortem the most obvious lesion is oedema of the lungs and the presence of froth in the trachea and bronchii. Hydropericardium, hydrothorax and ascites are common but not always seen (Uilenberg 1981).

Diagnosis is confirmed by the microscopic examination of brain capillaries for the organism. This is usually accomplished by staining a brain squash smear with Giemsa (Purchase, 1945) and examination under high power.

4.4 SEROLOGY*

Du Plessis (1981) has described an indirect fluorescent antibody test (IFAT) for detecting *Cowdria ruminantium* antibodies. IFAT antibodies first appeared two weeks after infection and peaked 8-10 days after the febrile reaction had subsided. Significant levels were still detectable 18 months later.

4.5 EPIDEMIOLOGY⊥

Local cattle in endemic regions usually show low mortality due to high levels of resistance. It is likely that this resistance is due to natural selection within a

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* See chapter 8 section 3.1 for selected recent publications
⊥ See chapter 8 section 3.2 for selected recent publications

population rather than any particular resistance linked to breed (Uilenberg 1981 and 1983).

Calves, even of susceptible stock, are relatively resistant to disease for a 3 week period after birth. Lambs are resistant to disease within the first week of life (Neitz and Alexander 1941, Uilenberg 1971).

Susceptible cattle can survive for years in an endemic region (Neitz and Alexander 1945, Uilenberg 1971) which is evidence for a low infection rate in the Amblyomma ticks in these areas. This combined with the short duration of age resistance led Uilenberg (1983) to conclude that an endemically stable condition is probably not possible except in cattle populations with a high level of genetic resistance.

Du Plessis, Loock and Ludemann (1992) demonstrated endemic stability in a herd of Hereford cattle that had been maintained for 50 years on an endemic farm. With *Amblyoma hebraeum* cowdria infection rates of 3-5%, endemic stability was maintained by only dipping the herd when an average of 10 adult ticks were seen per animal. When the average was increased to 15 during the calving period 97% of calves acquired immunity by 6 months of age. Interestingly the authors record 3 distinct isolates of *C. ruminantium* from the farm. One of these isolates was almost non-pathogenic to cattle.

5.0 *BABESIA BOVIS, BABESIA BIGEMINA* AND OTHER VECTORS

5.1 HISTORY AND CLASSIFICATION AND VECTORS

Babesia parasites were first described by Babes (1888) as a cause of redwater (haemoglobinuria) in South African cattle.

The genus Babesia is a member of the order Prioplasmia and the family Babesidae and the bovine species include *Babesia bigemina*, *B. bovis* (synonymous with *B. argentina*), *B. divergens* and *B. major*. *Babesia bigemina* is a large piroplasm measuring 4-5μm by 2-3μm. *B. bovis* is a small piroplasm measuring 2.5μm by 1.5μm. Both occur in Europe, Africa, Australia and South America. Babesiosis (called Texas fever) was eradicated from the United States by intensive tick eradication Ristic (1981). *B. bigemina* is principally transmitted by the one host ticks *Boophilus microplus* and *Boophilus decoloratus* whereas *B. bovis* is only transmitted by *B. microplus*.

5.2 LIFE CYCLE

After inoculation by the vector tick Babesia merozoites enter the bloodstream and multiply asexually in red blood cells (Hoyte 1961). Multiplication within the red cell is by binary fission, eventually causing the disruption of the cell and release of parasites to invade new cells. Following ingestion of parasites by ticks, sexual reproduction occurs in the gut. Following detachment, the infected engorged adult

female Boophilus tick passes the Babesia parasite to the larvae of the next generation through the eggs. Following attachment of *Boophilus microplus* to a new host infection is transmitted by the larval stages (*B. bovis*) or nymph and adult stages (*B. bigemina and B. bovis*) (Friedhoff and Smith 1981)

5.3 DISEASE

Mahoney (1979) has given a description of the clinical presentations of babesiosis. Clinical signs can range from hyper-acute to subclinical depending on the parasite species, the infecting dose, the breed of the host, the age of the host and the immune status of the host. Body temperature rises in parallel with the increased parasitaemia, the animal becomes listless, anorexic and has a roughened coat. Haemoglobinaemia, haemoglobinuria and jaundice follow. The faeces is dry and dehydration causes the eyes to sink in their sockets. Infections with *B. bovis* can lead to sludging of red cells in the capillaries and consequent cerebral damage (Leeflang 1972).

The post mortem changes associated with acute babesiosis are congestion of internal organs (especially the spleen), jaundice, dark urine, thick granular bile and myocardial haemorrhages. (Mahoney 1979)

5.4 DIAGNOSIS

Diagnosis in clinical cases is usually easily obtained by examination of Giemsa stained thin blood films (Todorovic and Carson 1981). In subclinical infections the

percentage of parasitized red cells is lower and more sensitive techniques may be needed. Examination of thick smears has been described by Mahoney and Saal (1961) and the use of acradine orange stain by Winter (1967). Brain crush smears stained with Giemsa are particularly useful in the diagnosis of fatal *B. bovis* (Callow and Johnston 1963) and have the added advantage that they can be made up to 28 hours after death.

Serological diagnosis is most commonly made using the Indirect Fluorescent Antibody Test although complement fixation and indirect haemagglutination have also been used (Mahoney 1979).

5.5 EPIDEMIOLOGY

In enzootic areas the disease is usually only seen in cattle newly introduced into the area. Indigenous cattle are rarely affected because natural resistance of the very young and passive colostral immunity is present during initial challenge (Hall 1963). Following challenge calves are immune.

As with other tick borne diseases heaviest losses occur in areas marginal for the vector tick where herd immunity (especially in older animals) is low. Similar conditions can be caused by an inefficient dipping programme. (Joyner and Donnelly 1979)

6.0 TICK-BORNE DISEASE CONTROL

There are four main methods of tick-borne disease control. These can be used singularly or, more commonly, in combination:

- Control of the vector ticks.
- Movement control of the host.
- Immunological protection of the host.
- Using or breeding naturally resistant stock. This has been discussed in section 2.3.2.

Discussions of tick control methods have been hampered by a plethora of ill-defined terms. The following terms have been defined by Pegram, Hargreaves and Berkvens (1995) and are used throughout the text.

- *Intensive tick control* - Acaricide application aimed at keeping animals totally free of ticks to prevent transmission of pathogens causing tick-borne disease; this usually involves frequent application of acaricide throughout the year.
- *Strategic tick control* - Acaricide application aimed at (substantial) reduction of tick populations and consequent reduction in the transmission of pathogens causing tick-borne disease; the timing of acaricide application is based on ecological information on the seasonal numbers of ticks and the frequency of application will vary during the year.

A full review of the use of acaricides for tick control has been provided by FAO (FAO, 1984) and will not be repeated here. The following is a list, roughly in order of importance, of the acaricide application methods that have been used on any scale in Africa.

Plunge dipping.

Spray race.

Pour-on preparations.

Hand spraying.

Tick grease.

Impregnated ear tags.

The first acaricide of importance in Africa was arsenic oxide which widely used from the early part of the century until the 1960's and 1970's (Norval et al, 1992) Subsequently there have been organochlorines in the 1960's and 1970's; organophosphates and carbamates in the 1980's and amidines and synthetic pyrethroids in the 1980's and 1990's.

During the 1950's and 1960's work intensified on the development of an ECF vaccine (Neitz, 1953, Brocklesby and Bailey, 1965). Radley (1981) reviewed the development of a practical field vaccination that has come to be known as the "infection and treatment" method. This involves infecting an animal with virulent *T. parva* and treating with long acting oxytetracycline to control the disease but allowing the development of immunity.

6.1 INTEGRATED TICK AND TICK-BORNE DISEASE CONTROL

In Zimbabwe compulsory dipping of cattle started in 1914 for the control of ECF (Lawrence, Fogin and Norval, 1980). The policy was very successful in controlling disease, allowing a thriving commercial cattle industry and resulted in over grazing of the Tribal Trust lands (Norval, 1977). Disruption to the dipping service and increased cattle movements during the 7 years of conflict in Rhodesia (Zimbabwe) resulted in an increase in tick numbers and an estimated 1 million cattle deaths in the Tribal Trust lands from tick-borne disease. Lawrence et al (1980) attributes this to the lack of immunity in the cattle population due to the historically effective dipping program. The authors request that, the lessons learnt be remembered and that a new approach to the control of tick-borne diseases which results in a stable situation, not prone to disaster, be evolved. One of the authors of this paper makes a personal, almost philosophical point, in another paper (Lawrence, 1990).

> "To conclude, in my early years I believed that parasites were put into the world to plague cattle and that it was the veterinarians task to kill them at every opportunity. Now I am sure that this approach is wrong, and that it is the veterinarians task to evaluate the damage that the parasite causes, and find ways of reducing that damage and increase production in an economic way."

The experiences of Zimbabwe, the cost of dipping and worries about environmental contamination and residues have lead to the concept of integrated control of ticks and tick-borne diseases. Norval (1983) puts forward the arguments against intensive dipping. He suggests that intensive tick control was introduced in a crisis to control

ECF at a time when there were no effective alternatives and this in turn led to significant problems with instability to babesiosis, anaplasmosis and heartwater. He claims that total tick control is generally uneconomic and proposes a future based on integrated systems employing host immunity (presumably by vaccination in exotic breeds and making use of natural immunity in zebu cattle).

There have been several reviews published on integrated tick control (Young, Groocock and Kariuke, 1988, Perry, Mukhebi, Norval and Barrett, 1990, Anon, 1990). The concept of integrated control strategies has gained widespread theoretical acceptance and most of the published work on it involves immunisation of susceptible cattle against ECF and subsequent relaxation of intensive dipping.
De Castro, Young, Dransfield, Cunningham and Dolan (1985) found that, at the expense of some loss of productivity, zebu cattle immunised against ECF could be kept in the Trans-mara area of Kenya despite tick infestations. However their group size was small (15 dipped and 15 undipped) and the study only lasted for 30 weeks.
Morzaria, Irvin, Wathanga, D'Sousa, Katende, Young, Scott and Gettinby (1988) exposed naive groups of zebu cattle to naturally transmitted ECF. Eighty animals were immunised and divided into four groups of 20. Each group had a different acaricidal application regime ranging from spraying twice per week to no tick control. Eighty similar cattle were not immunised and divided up into the same tick control groups. The authors concluded that in the coastal province of Kenya, ECF immunised beef cattle can be maintained with limited tick control without significant loss of productivity.

Berkvens, Geysen and Lynen (1989) undertook mass vaccination of traditionally managed zebu calves in the Eastern Province of Zambia. They believed that although ECF had only been introduced to the area relatively recently some form of endemic stability had been reached. Despite this, the authors estimated that ECF killed about 50% of the calves. A total of 5,900 calves were immunised over a 7-month period. By the authors own admission the monitoring of the campaigns results was not very efficient. However they estimated approximately an 80% reduction in mortality in immunised calves. This study is the only published record of large-scale vaccination of traditionally managed zebu cattle and raises issues not discussed in the on-farm trial publications. Farmer suspicion of new technologies and novel interventions, financing of such vaccination programs and the huge logistical problem of monitoring their success or failure are all mentioned.

The practical problems and cost of ECF vaccination raises the question of whether a similar level of tick-borne disease control can be achieved using the established technology of acaricide application but in a more targeted and cost-effective way than intensive dipping. There have been two large-scale studies published. The original was by Barnett (1957) who closely observed zebu calves for disease in an endemic area of Kenya. He estimated ECF losses to be approximately 8 to 10% of all stock born. In one area a program of dipping every 2 weeks was introduced and there was a dramatic fall in ECF mortality rates to just over 1%. However, there were no

animal groups to act as controls and the program ran into difficulties in subsequent years when the dip became under-strength and mortality rose again.

Moran and Nigarura (1990) took the reduction in acaricide use a stage further and evaluated strategic tick control in Burundi. They limited acaricide treatment to a 4-month period that was the main season of adult *R. appendiculatus* activity. After two seasons there was a 44% reduction in adult female ticks and no significant correlation between the lack of tick control for 8 months of the year and a higher incidence of disease. The authors claimed that this strategic control had not disrupted endemic stability and based this on the proportion of 7 to 12 month old calves that were serologically positive to the indirect fluorescent antibody test at 1/160 to *T. parva* before the start of the program and after 2 years. This study attempted to evaluate a strategic dipping policy on a national scale and as such should not be viewed as a well controlled experiment. The scientific data generated suffers greatly from low recording intensity and lack of controls. Presumably cattle were dipped or sprayed before the start of the project and comparisons of tick numbers and mortality rates between years is unsatisfactory. Economic losses due to tick damage can be large in Burundi (Kaiser et al, 1988) with counts of over 500 adult *R. appendiculatus* being common. Because of this, the main reason for the strategic dipping program was not to control tick-borne diseases but to reduce tick damage and increase productivity without upsetting enzootic stability.

7.0 THE ECONOMICS OF TICK AND TICK-BORNE DISEASE CONTROL

Perry and Young (1995) reviewed the role of economics in the control of tick-borne diseases. Identification of the target client is vital if the correct methods are to be chosen. They define four principal client groups and their needs.

Primary client	**Product**	**Epidemiological tool**
Farmer	Decision support, based on production targets	Longitudinal studies of production profiles: effect of infection on productivity: economic models
Veterinary services	Disease distribution occurrence, relative importance and trends, by admin. Boundary: cost and benefits of intervention	Sample-based active reporting: georeferenced data; distribution and dynamic models; expert systems
Development planners/donors	Information and decision support on relative importance of TBDs by production system; returns on investment and control	Impact assessment models; investment analysis
Animal health research organisations	Information and decision support on validity, efficacy and impact of new technologies	Experimental epidemiology, clinical trials, model development

As tick-borne diseases in Africa do not affect trade in livestock it is their effect on production that has the most economic impact. The authors note that in some countries the economic responsibility for control is moving from government veterinary departments to the farmers and that there is thus a growing need for data on the economic effect of tick-borne infections at the farm, production system and national levels. They note that there have been few studies to determine the relationship between tick-borne infections and productivity loss and most current estimates rely largely on assumptions.

A general estimate of the economics of theileriosis control in Africa was made by Mukhebi et al (1992). They estimated that the losses due to theileriosis in 1989 were US$ 168 million, which includes mortality of 1.1 million cattle. They also did cost benefit analysis for vaccination and found a positive ratio when vaccination cost was US$5-7 per head. However, the main conclusion of their paper was that while these calculations were useful as illustrations, they were severely hampered by the fact that the data sources used were inadequate in content and quality. Like Perry et al (1995) they call for more accurate study based data to enable more accurate economic analysis.

Authors considering the situation in Zimbabwe have lead the way in considering the economic (and epidemiological) implications of intensive dipping. Perry et al (1990) proposed alternative strategies to replace intensive dipping first started in 1914 and which failed so dramatically in the 1970s. The proposed strategies were a combination of reduced dipping, strategic dipping, minimal dipping and tick-borne disease vaccination. Working from a model, rather than field trials, they found that all alternative options were more cost effective than the present intensive dipping. Amongst their recommendations for the future they suggest location specific studies to assess the validity of the assumptions made in the model. The same authors provide a very similar assessment for the whole of Southern Africa in Norval, Barrett, Perry and Mukhebi (1990).

7.1 EXPERIMENTAL STUDIES ASSESSING THE ECONOMICS OF TICK AND TICK-BORNE DISEASE CONTROL

There are surprisingly few experimental studies that analyse the economics of ticks and tick-borne disease control. A study was carried out using adult, ECF naïve, Boran cattle in an ECF endemic area of Kenya (Morzaria et al, 1988, and Mukhebi, Wathanga, Perry, Irvin and Morzaria, 1989). The cattle were divided into 8 groups of 20. Four groups were vaccinated against *T. parva*. One vaccinated group and one unvaccinated group were maintained under each of four tick control regimes ranging from twice weekly spraying to no tick control. Financial analysis revealed that the immunised groups were more profitable due to lower mortality and higher weight gains than the unimmunised groups. Of the unimmunised groups the most profitable was the group sprayed twice weekly and this was mostly due to a lower mortality. While the results of this study are interesting, the financial analysis is not very surprising. It is well known that adult naïve cattle moved into an ECF endemic area experience heavy mortality. The finding that a vaccine, which protects against the disease, is cost effective under these circumstances is hardly surprising. The study conditions were appropriate for a beef-fattening unit but bore little resemblance to most farm or traditional management systems. No calves or young stock were included, the effect on reproduction was not examined and the study only lasted 9 months. The last factor did not allow for seasonal factors to be taken into account. These have a significant effect on the epidemiology of tick-borne diseases and the shortness of the trial prevented the effect of the establishment of endemic stability from being measured. The authors acknowledge these factors and call for further

trials under different production circumstances and over a longer period of time span before any recommendations can be made for widespread adoption.

Pegram, James, Oosterwijk, Killorn, Lemche, Ghirotti, Tekel, Chizyuka, Mwase and Chizhuka (1991) studied two herds of 60 female Sanga cattle for 3 years in Central Zambia. One herd was kept tick free by regular acaricide treatment while the other was given no tick control. The study tried to simulate an on-farm situation and measured a wide range of productivity factors including mortality, calf growth rate, reproductive performance and milk production. The productivity data was analysed using a computer program that allowed the calculation of accurate rates and for the interaction of the productivity parameters. The tick free herd performed significantly better than the tick infested herd but the additional production was much less than the acaricide cost. The authors concluded that there was no economic justification for intensive tick control under the study conditions. They did however calculate that strategic tick control would be justified if the quantity of acaricide used could be reduced by 50% without any major reduction in benefits. Mortality rates were low in adults (approximately 4.5% in both herds) and moderately high in calves (but similar in both herds). No calves died after weaning. However, the authors make no mention of tick-borne diseases and it has to be assumed that the study area was ECF free. A potential anomaly in the authors' calculations was that they based their tick control costs on acaricide cost only, assuming that the cost of using a backpack sprayer for a reasonable number of cattle was negligible. However the use of a backpack sprayer is generally considered to be impractical for large numbers of cattle

and not efficient enough to control ECF in an endemic area. De Castro, James, Minjauw, Giulio, Permin, Pegram, Chizyuka and Sinyangwe (1997) followed the same cattle for a further 3 years. They divided the non-sprayed and intensively sprayed groups to make a third, strategically sprayed, group. They confirmed that there was no ECF in the area until the last days of their study. The three groups were grazed on the same pasture, and it is interesting to note that the tick numbers were in steep decline on the untreated group. The *R. appendiculatus* numbers were 10-20% of their former levels, and the authors speculate that this was due to two-thirds of the cattle being sprayed and the associated reduction in the pasture tick population. Their economic analysis concludes that herds under tick control gave better returns than the unsprayed herd. This is surprising, as it differs from the findings of Pegram et al (1994) for the previous 3 years of the study, and was at a time when tick numbers were significantly reduced. The main difference between the groups came about by a difference in mortality rates both in calves and adults. Unfortunately the causes of death were not monitored, and the authors pass no comment as to the reason for the differences except to say that there was no evidence of any tick-borne diseases.

8.0 TRADITIONAL CATTLE KEEPING IN MALAWI AND THE IMPACT OF EAST COAST FEVER

Malawi represents the most southerly habitat of the short-horned East African zebu cattle which are locally known as Malawi zebu (Faulkner & Epstein, 1957). These thoracic-humped cattle were introduced to Africa in the 4th century AD but did not increase significantly until after the Arab invasion of 669 AD (Norval et al 1992). They differ from the Sanga cattle of the rest of central and southern Africa in having short horns and a thoracic hump rather than the longer horns and cervico-thoracic hump of the Sanga.

In northern Malawi there is an intermediate breed usually called Angoni after the people who own them. These have longer horns than Malawi zebu and a hump that can be cervico-thoracic or thoracic. Angoni are slightly larger than Malawi zebu and the predominant coat colour is red (Faulkner & Epstein, 1957).

Hugh Stannus, a medical officer in Nyasaland, described an outbreak of ECF that occurred during the 1908/09 rainy season in Mombera district, near Mzimba, Northern Region (Stannus, 1910). According to Aspinall (1973), G. Garden, the first veterinary surgeon in Nyasaland, accurately described ECF in 1911 without naming it. He impressed on the Government the importance of the control of tick-borne diseases by dipping (Mares, 1973). In 1914 two spray machines came into use (Aspinall, 1973) and the cattle population was estimated at 63,000 (Mares, 1973). In 1917 the first dip tanks were built in the Southern Province and according to Aspinall

(1973) Nyasaland was one of the first colonial territories to start tick control. In 1925, seven-day dipping became compulsory in certain areas (De Meza, 1925).

In 1929 the Veterinary Department was formed and employed 56 dip-tank labourers and 26 veterinary assistants (Aspinall, 1973). At this point the cattle population had grown to 166,000 (a ratio of 1:10 with the human population – Mares, 1973). By 1950 there were 72 dip tanks and by 1964 this had grown to 128 servicing a cattle population of 450,000, still 1:10 with the human population (Aspinall, 1973 and Mares, 1973). By 1973 there were 200 dip tanks and by 1991 there were 371 tanks serving 800,000 cattle (Edelsten, personal communication). This was still approximately 1:10 with the human population. Mares (1973) states that "ECF eradication is being attempted by intensive tick control, area by area but that there is little sign as yet of any reduction of incidence". Arsenic trioxide acaricide was used in almost all tanks until 1994 (Aspinall, 1973, Edelsten, personal communication). Malawi was probably the last country to still be using short interval arsenic dipping. Most other countries had moved on to newer acaricides often due to worries, confirmed or otherwise, about resistance. Resistance of *Boophilus microplus* to arsenic had undoubtedly occurred, for example in Australia after 50 years of use (Wharton and Roulston, 1977) and in Rhodesia (Hammant, 1977, and Tomson and Bryson, 1971). However resistance to newer chemicals appeared much faster (Whitehead, 1973) and some countries have considered a move back to using arsenic. (Arteche, Laranja and Arregui, 1979). Resistance in *R. appendiculatus* is less of a problem than for the shorter life cycle Boophilus ticks. It is not known to what

extent, if any, *R. appendiculatus* in Malawi had become resistant to arsenic.

Wilson (1946a and 1946b) was the first person to publish systematic survey data illustrating the seasonal occurrence of ticks in Nyasaland. He found that *R. appendiculatus* adults engorge and oviposit from December to November. Wilson was struck by the marked seasonality and noted that *R. appendiculatus* completed only one life cycle per annum. Berggren (1978) carried out a tick survey of Malawi in 1974/5. He found that *R. appendiculatus* was widespread and common in the Northern and Central Regions but rare in the Southern Region. He found a few *R. appendiculatus* in the Shire Highlands (Southern Region).

Wilson was also the first to study the incidence of ECF through the seasons and estimate the mortality it caused (Wilson, 1944). By examination of large numbers of spleen smears he showed that the incidence of ECF was strictly seasonal with most deaths occurring during the 3 hot rainy months of January, February and March. Wilson (1945) estimated that ECF caused 2% of the total annual cattle mortality in the Northern Province, in which spleen smears were examined in all cases of death. During 1944, schizonts were detected in 342 out of 16,000 smears examined. The same author studied the cattle population around Likuni dip tank near Lilongwe in 1943/44 (Wilson 1944). Here he had data on the whole population and extrapolated from the number of schizont containing spleen smears, the total number of smears submitted and the knowledge of the overall actual mortality rate, to estimate that ECF annual mortality was approximately 5% of the population. Unfortunately Wilson

made no comment on the age profile of the animals dying from ECF. He concluded that the mortality at Likuni and in the Northern Province were lower than those reported in South Africa and Tanganyika. He acknowledged that dipping may be playing a part but attributed most of the difference to a genetic resistance that has built up over many years of contact with the *Theileria* parasite. He also concluded that *R. appendiculatus* was the sole vector and that only a low proportion of the ticks were infected. However he had no methods by which to verify this.

In a review and analysis of historical Veterinary Department and laboratory data, Grindle (1979) estimated the total annual mortality rate of cattle in Malawi to be 5% of the adult population and 30% of the calves. He estimated that ECF caused 10% mortality in calves and 1% in adults. This estimate is very similar to that of Wilson when the likely age profile of the cattle population is considered. Moodie (1984) performed a year-long study in Mzimba district in the Northern Region and estimated an ECF mortality rate of 38% in calves (less than 2 years old) and 18% in adults. Unfortunately very little information was given regarding the methods used.

Grindle (1979) states that the veterinary services fee at that time (50 tambala, UK£0.30) was almost sufficient to cover the acaricide and labour costs of dipping. He concludes that the overall benefit of dipping zebu cattle was marginal unless large numbers of cattle used the tank.

9.0 CONCLUSION

This literature review has looked at the published information relevant to the study reported in this thesis. Most of the published studies on ECF epidemiology have been carried out in East Africa and there is a need to investigate the situation in Malawi. There is also a need to study the effect of strategic dipping on ticks and tick-borne diseases in the particular climatic, land use and cattle husbandry situation in Malawi. An economic analysis of these factors is needed to allow evidence based decision making by the Malawian veterinary authorities. The research work carried out from 1990 to 1994 and reported in this thesis was designed to answer these questions.

CHAPTER 3

THE SEASONAL PATTERN OF TICK INFESTATIONS ON MALAWI ZEBU CATTLE AND THE EFFECT OF STRATEGIC DIPPING; WITH SPECIAL REFERENCE TO *RHIPICEPHALUS APPENDICULATUS*

ABSTRACT

The seasonal fluctuations in numbers of ticks on Malawi zebu cattle were recorded at six sites within 60 km of Lilongwe, Malawi, to provide data for the improved control of East Coast fever (*Theileria parva* infection) by strategic dipping. Half body tick counts were performed on 5 calves, every 4 weeks at each site. At two sites there was no dipping, at two sites the study cattle and other herds in the area were dipped strategically in the amidine acaricide amitraz and at a further two sites in the organophosphate acaricide chlorfenvinphos. Tick and meteorological data was collected from January 1991 to May 1994. Rainfall and temperature were uni-modal with the highest rainfall being between December and March and the highest temperatures being between September and January.

Rhipicephalus appendiculatus adults had a seasonal distribution with the highest numbers in the rainy season (December to March). *Boophilus microplus* adults had a complex distribution, with the main peak between March and May, and smaller peaks in August to September and November to December, presumed to indicate

three generations per year. *Amblyomma variegatum* had one generation per year with peak adult numbers in November to January. *Hyalomma truncatum* adults were found in very small numbers in March and April. Strategic dipping using nine immersions, at two-week intervals, from December to March gave almost complete control of *R. appendiculatus* but the numbers of *B. microplus* and *A. variegatum* were similar in dipped and non-dipped animals. It is concluded that strategic dipping at this timing is effective for the control of *R. appendiculatus* numbers on zebu cattle in the study area.

INTRODUCTION

Malawi has just over 800,000 cattle (Anon, 1991a), the vast majority (approximately 97%) being traditionally managed, communally grazed, Malawi zebu. There are 371 dip tanks (Anon, 1991b) scattered over the country and for many years Malawi has had a compulsory programme of weekly dipping in arsenic trioxide. A small number of cattle, mainly dairy crossbreds, are dipped in chlorfenvinphos. Seven day dipping was first made compulsory in certain areas in the early 1920s (De Meza, 1925). In 1929 the Veterinary Department was set up as a separate department (Mares, 1973) and in 1930 government dip tank construction began. Forty seven tanks were built in Central and Northern Region (Wilson 1946b) and over the years the number of dip tanks has increased to the present 371. The objective of tick control prior to 1969 was to control tick-borne disease (Aspinall, 1960). But in 1969 new legislation was introduced ("The Eradication of East Coast Fever Rules 1969") which gave the Chief Veterinary Officer special extra powers of cattle movement control and tick control with the aim of eradicating ECF. Mares (1973) stated that "An attempt is being made to eradicate ECF by intensive tick control, area by area, but there is little sign as yet of any reduction in the incidence."

In more recent years, although weekly dipping has remained compulsory, the law has not been strictly enforced. Coupled to this were periods of non-dipping when there was shortage of acaricide or water or tank breakdown. It is estimated that only 20-40% of cattle are dipped regularly (Edelsten, 1990).

Like other countries in Central and Southern Africa, Malawi has been reconsidering its policy of intensive compulsory dipping for zebu cattle. Several workers have concluded that intensive tick control for these animals is both economically and technically unjustifiable (Norval, 1983, Pegram and Chizyuka, 1990, Anon, 1990a, Kaiser, Sutherst, Bourne, Gorissen and Floyd, 1988, and Perry, Mukhebi, Norval and Barrett, 1990).

Four tick genera are commonly found on cattle in Malawi: *Boophilus, Amblyomma, Rhipicephalus*, and *Hyalomma*. *Boophilus* is the most prevalent tick, found throughout Malawi (Berggren, 1976, Mfitilodze, 1991). *Amblyomma* are common throughout Malawi except in the Lower Shire valley and around Mangochi where low rainfall may limit their occurrence (Berggren, 1976). *Rhipicephalus* is found throughout Central and Northern regions (Berggren, 1976), and in low numbers in Southern region (Mfitilodze, 1991). The Southern region has no ECF. *Hyalomma* species are found, but in low numbers. The highest population of cattle is found in the Central region.

A study was started in late 1990 to examine the epidemiology of tick-borne diseases, particularly East Coast fever, in Malawi and their control by strategic dipping as defined by Pegram et al (1995). This paper reports the results of an investigation into tick dynamics on undipped and strategically dipped traditionally managed Malawi zebu cattle.

MATERIALS AND METHODS

The study was carried out in six areas around Lilongwe (33 °E, 14 °S) on the central African plateau at an elevation of approximately 1000 metres. The dip tank serving each area had been using arsenic trioxide acaricide prior to the start of the study, with the exception of Tonde where dipping had been suspended nearly three years before, in early 1988. The areas were selected because they were all in the same ecological zone within a 60 km radius of the Central Veterinary Laboratory, Lilongwe, and had enthusiastic resident veterinary assistants. The area is typical of the mango savannah of Central Africa, and is heavily cultivated.

During the wet season, when crops are cultivated, livestock graze in dambos (low lying areas prone to flooding) and along rivers. During the dry season cattle forage in the fields after harvesting but still use the dambos for green grass and water. Cattle management in the study area is similar to that found elsewhere in Malawi. Cattle are kept in small herds utilising communal grazing. They are enclosed at night in a khola (small pen) and are herded during the day by young boys.

In the six areas a total of 1,800 Malawi zebu cattle belonging to 143 farmers were tagged in December 1990 with easily readable ear tags. Only farmers who were willing to allow their cattle to be monitored and sampled were included in the study. The tagged animals comprised between 10 % and 16 % of the total cattle population in each area. All animals belonging to an individual farmer were tagged and all calves born to

tagged cows during the study were also tagged. A total of 3,257 animals were tagged and monitored over a three-year period.

During the study no dipping or topical acaricide application was performed in two areas, namely Likuni and Tonde. In November 1990 the other four tanks were emptied, cleaned, calibrated and re-filled. Some minor repairs were made to collecting pens and crushes. At the beginning of December 1990 dipping started in four tanks. The organophosphate acaricide chlorfenvinphos at a concentration of 0.05 % m/v (Supona 30, Shell Chemicals) was used at Dickson and Namaguya. Measurement of dip concentration had proved impractical in the past and a total replacement acaricide that could be added fresh just before each dipping had obvious advantages. Therefore the amidine acaricide amitraz at a concentration of 0.005 % m/v (Triatix TR, Coopers Animal Health) was used at Sinyala and Mbabzi. Dipping was carried out at two-weekly intervals from December 1990 to March 1991 and four-weekly intervals from April 1991 to November 1991. This strategy was designed to reduce ECF incidence (based on occurrence of ECF in the central region; Edelsten, 1990, Wilson, 1946, Moodie, 1981, Central Veterinary Laboratory data, 1989/90) while minimising acaricide costs. First year study results indicated that although 17 immersions per year gave excellent control of ECF, this was too intensive and endemic stability to other tick-borne diseases was likely to be upset. The study protocol was changed to 9 immersions per year, dipping strategically (fortnightly from December to March with no dipping in the dry season) for the rainy seasons of 1991/92, 1992/93 and 1993/94. The author (or Theresa Norman MRCVS) plus one or two veterinary assistant staff

attended every dipping. A person was employed as a counter at each tank to record the numbers of tagged and non-tagged cattle dipped.

To estimate the tick population tagged cattle were exposed to, half body tick counts were performed every four weeks on five calves in each area. Each calf selected belonged to a different farmer and had not been sampled four weeks before. Every four weeks, in each area, the five youngest tagged calves over the age of 6 months were selected for tick counting. This sampling protocol ensured that there was no confounding effect due to calf age. The use of sentinel animals (tick naïve cattle grazed with the study cattle) for tick counting was not possible under the field conditions of this study. Dipped cattle were dipped at two-weekly intervals and counts were made just before dipping. As *R. appendiculatus* adults usually remain on their host for less than two weeks (Branagan, 1974), counts made on dipped calves were a measure of the tick population dipped cattle were exposed to, and therefore comparisons with counts in undipped cattle were valid. Ticks were identified on the cattle as *Amblyomma, Boophilus, Hyalomma* or *Rhipicephalus* species. The rhipicephalids were recorded as coming from the head or body. With the exception of *Amblyomma* nymphs, no attempt was made to count larvae or nymphs due to the difficulty of doing this accurately. Counts and identifications were performed on the calves without removing the ticks. All adult ticks were counted as infestations were low, with very few standard ticks. At various times in the study sample collections were made (100-200 ticks) and taken back to the laboratory for identification. For statistical analysis of *R. appendiculatus* counts, the logarithmically transformed

individual tick counts (log n+1) were pooled over the four main months of adult activity (December to March), checked for similarity of variance and then compared using a single factor analysis of variance. Where a significant difference was seen, individual groups were compared using a two-tailed 't' test.

In May 1994 ten adult *R. appendiculatus* were collected from calves in the Likuni area from which all adult ticks had been manually removed 24 hours earlier. These were dissected immediately and the salivary glands age graded according to the method of Walker and Fletcher (1985).

By farmer and veterinary assistant questionnaire and field investigation the main rainy season grazing areas of dipped cattle in the study were identified and mapped. It was then established which areas were used by farmers with tagged cattle and then which farmers with non tagged cattle used the same areas. The dipping records for the 1992/93 rainy season were then examined to establish a mean dipping percentage for all cattle that grazed these areas. The purpose was to estimate the dipping pressure on the *R. appendiculatus* population that the tagged cattle were exposed to.

Meteorological data from five recording stations in the study area was collected and analysed. Rainfall data from 1957 to 1994 for Chitedze Research Station (10 km from Likuni dip tank), Bunda College of Agriculture (10 km from Namaguya dip tank), Kasiya (10 km from Tonde dip tank) and Sinyala (1 km from the tank) was supplied by the Chief Meteorological Officer, Blantyre (personal communication). Rainfall data

from 1967 to 1994 at Mbabzi (1 km from tank) was supplied by Mbabzi Estates Ltd (personal communication).

RESULTS

WEATHER

Figure 3.1 shows the mean rainfall for five reporting stations in the study area plus the mean monthly maximum and minimum temperatures. It can be seen that there is a single rainy season from November to April followed by a cold dry season with mean monthly minimum temperatures below 15 ^{O}C from May to September. There is then a short hot dry season before the next rains.

Figure 3.2 shows mean annual rainfall for five recording stations in the study area over the previous 37 years. The 1990/91 and 1991/92 rainy seasons were the driest for 33 years but the 1992/93 season was only just below the 37-year median value. Therefore after two very dry years the 1992/93 rainy season could be said to be typical for the study area. There was again very low rainfall in the 1993/94 season.

Figure 3.3 illustrates annual rainfall at the two undipped tanks for the last 14 years. The 37-year median for the two tanks is similar and it can be seen that the 1989/90 season had almost normal rainfall for both areas. Tonde then had four consecutive rainy seasons with low rainfall. Likuni had two rainy seasons with low rainfall followed by normal amounts in 1992/93 and then another dry year in 1993/94.

Figure 3.1

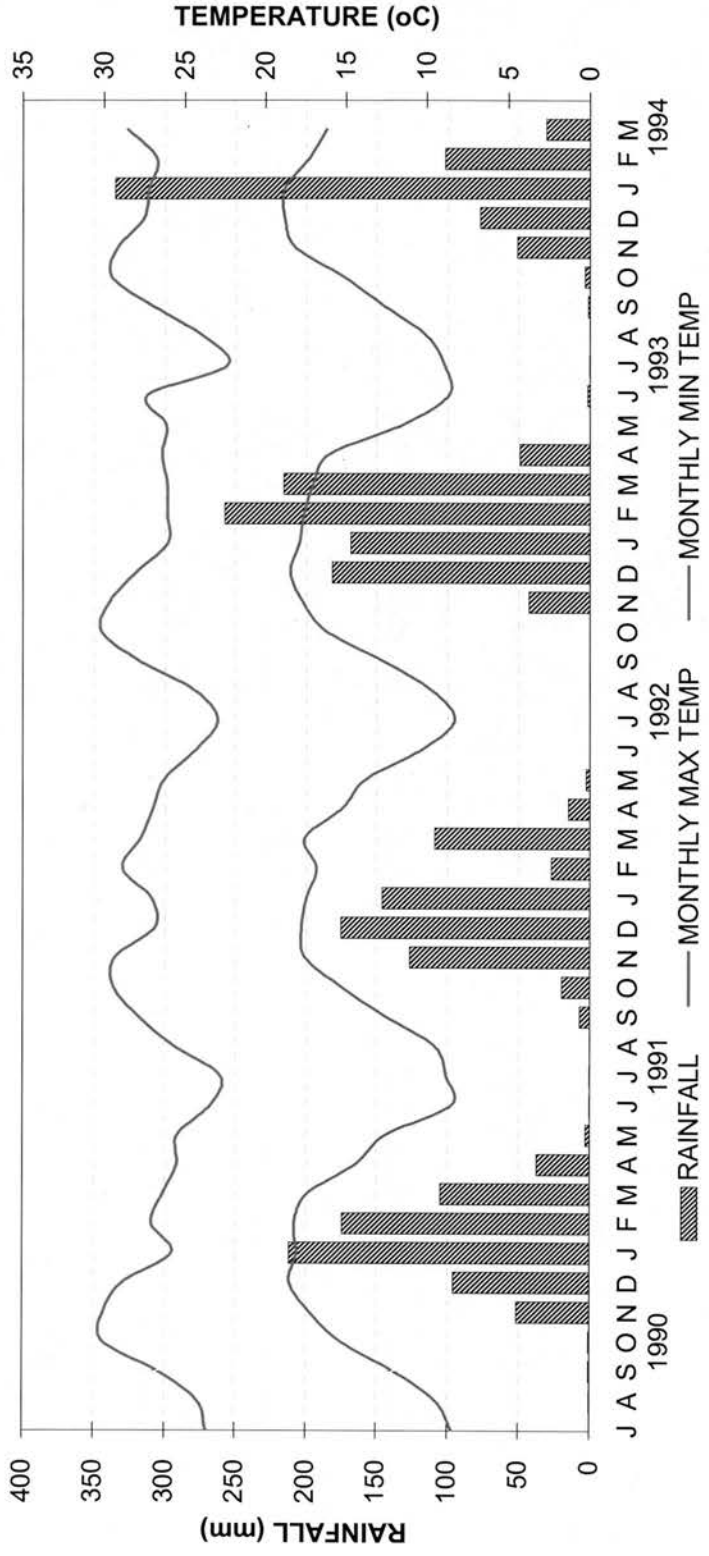


Figure 3.1 Rainfall and mean monthly maximum and minimum temperatures in the study area

Figure 3.2

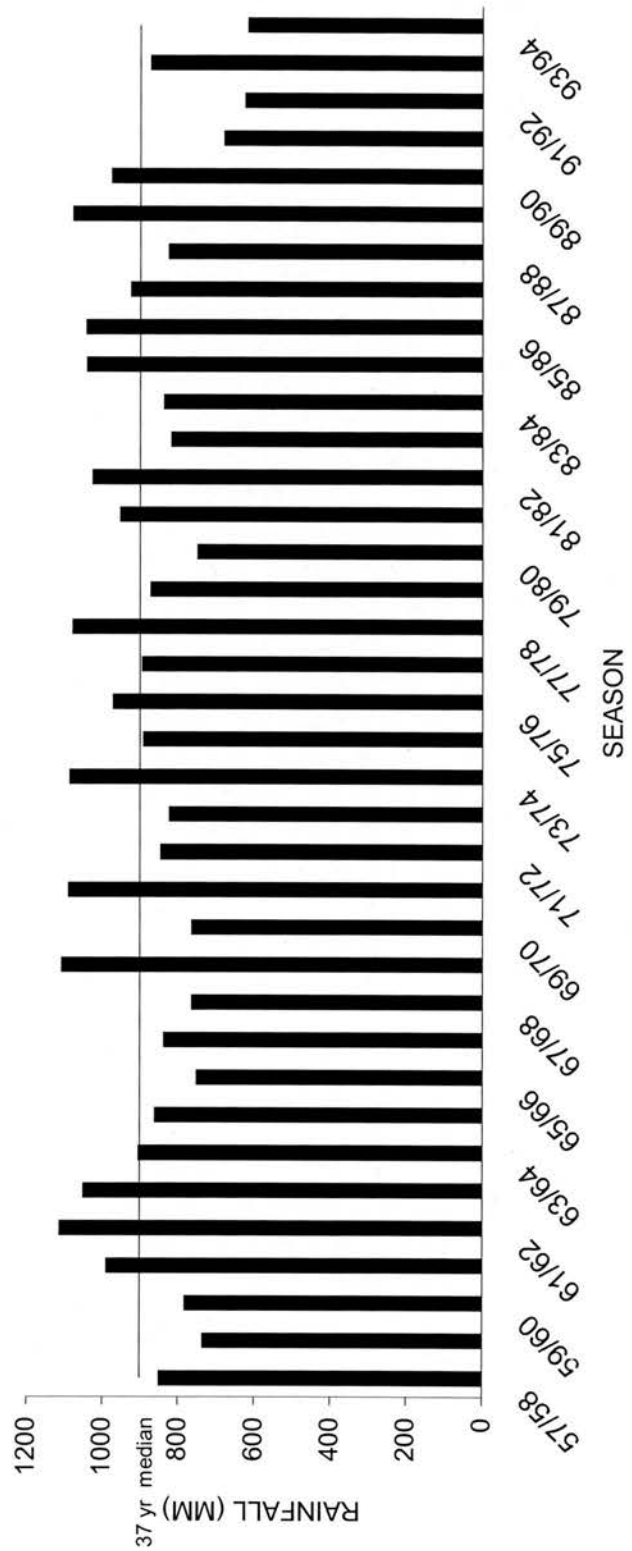


Figure 3.2 Annual rainfall in the study area. Mean of five reporting stations.

Figure 3.3

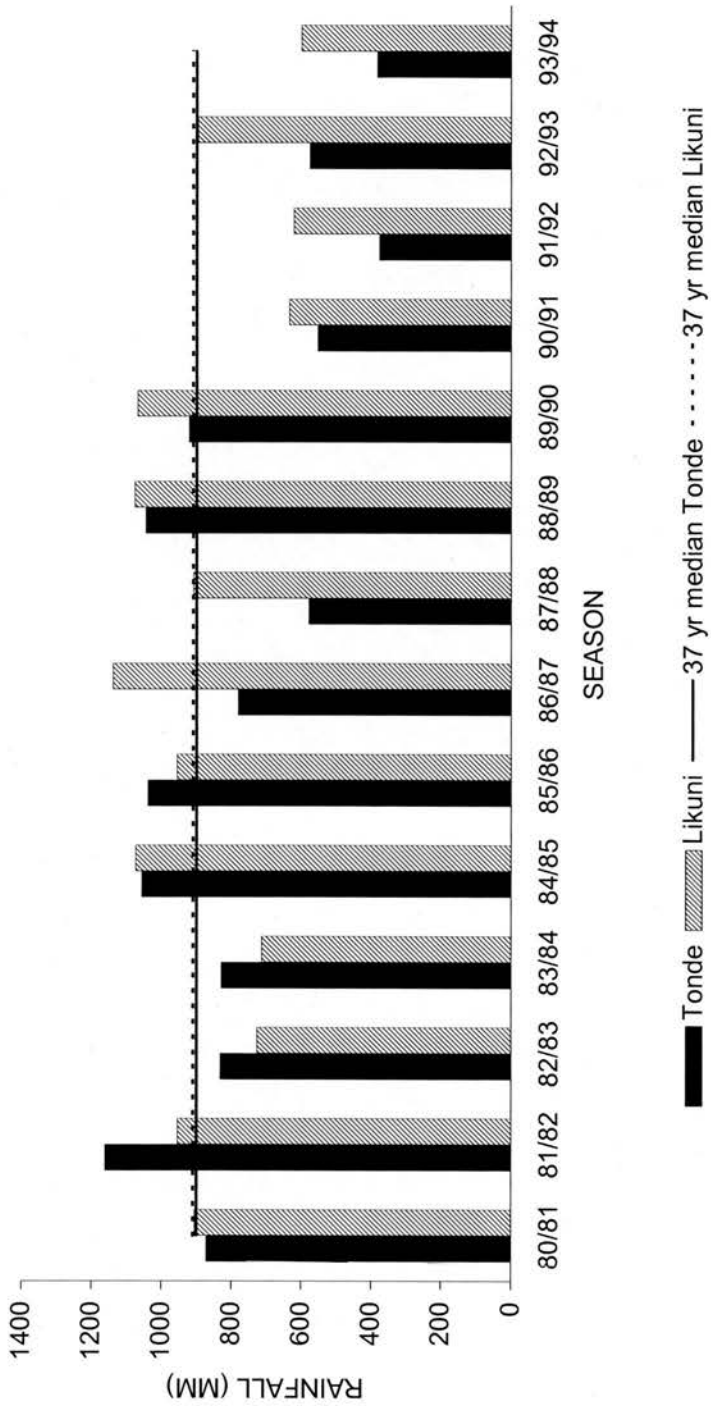


Figure 3.3 Annual rainfall in the undipped areas of Tonde and Likuni.

In the 1990/91 rainy season Tonde had only 61%, in 1991/92 only 42%, in 1992/93 only 64% and in 1993/94 only 42% of its 37-year median rainfall. Therefore although 1992/93 had normal rainfall for the area, Tonde was unusual in again receiving greatly below median rainfall.

TICKS

Mean dip attendance was 74 to 95 % for tagged cattle and 16 to 49 % for untagged cattle that lived within five kilometres of the dip tank. The owners and herders of cattle within the areas provided information on the grazing areas (Dambos) used by their cattle. Results of this retrospective study for grazing areas used by tagged cattle are shown in Table 3.1 along with the dipping percentage of all cattle (tagged and non-tagged) that grazed these areas. These cattle had a dipping attendance of 42 to 69 %. These figures give a better indication of the dipping pressure on the tick population that tagged cattle were exposed to, than gross figures for the whole tank area.

Tick counts from dipped cattle represent maximum tick numbers, as counts were carried out just prior to dipping. In the 1991 dry season cattle were dipped every four weeks, whereas in 1992, 1993 and 1994 the protocol was changed and there was no dipping in the dry season. Figures 3.4, 3.5, 3.6 and 3.7 show geometric mean half body tick counts on dipped and undipped cattle for *Rhipicephalus* adults on the head, *Boophilus* adults, *Amblyomma* adults and *Amblyomma* nymphs respectively over three and a half years (January 1991 to May 1994). Small (100-200) samples of adult ticks

showed that almost 100 % of *Rhipicephalus* ticks found on the head were *R. appendiculatus*, that 100% of the *Amblyomma* were *Amblyomma variegatum* and that 100% of the *Boophilus* ticks were *Boophilus microplus*.

Table 3.1. Rainy season grazing patterns of dipped cattle.

	DICKSON	NAMAGUYA	SINYALA	MBABZI
Number grazing areas used by tagged cattle	6	7	3	5
Number of cattle using above areas	1649	1193	1758	603
Dipping percentage for these cattle in 1992	42%	57%	53%	69%

Figure 3.4 *Rhipicephalus* adults. Geometric mean half head counts on dipped and undipped calves.

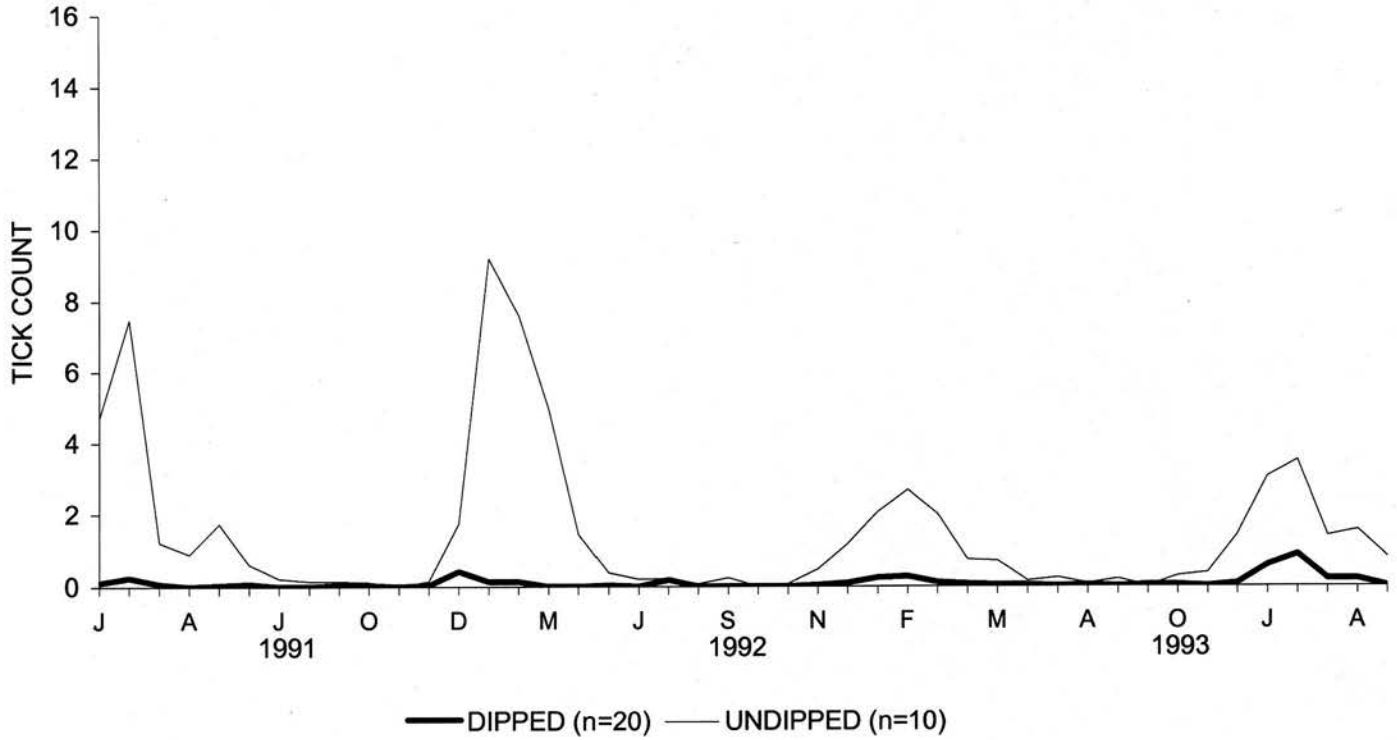


Figure 3.5

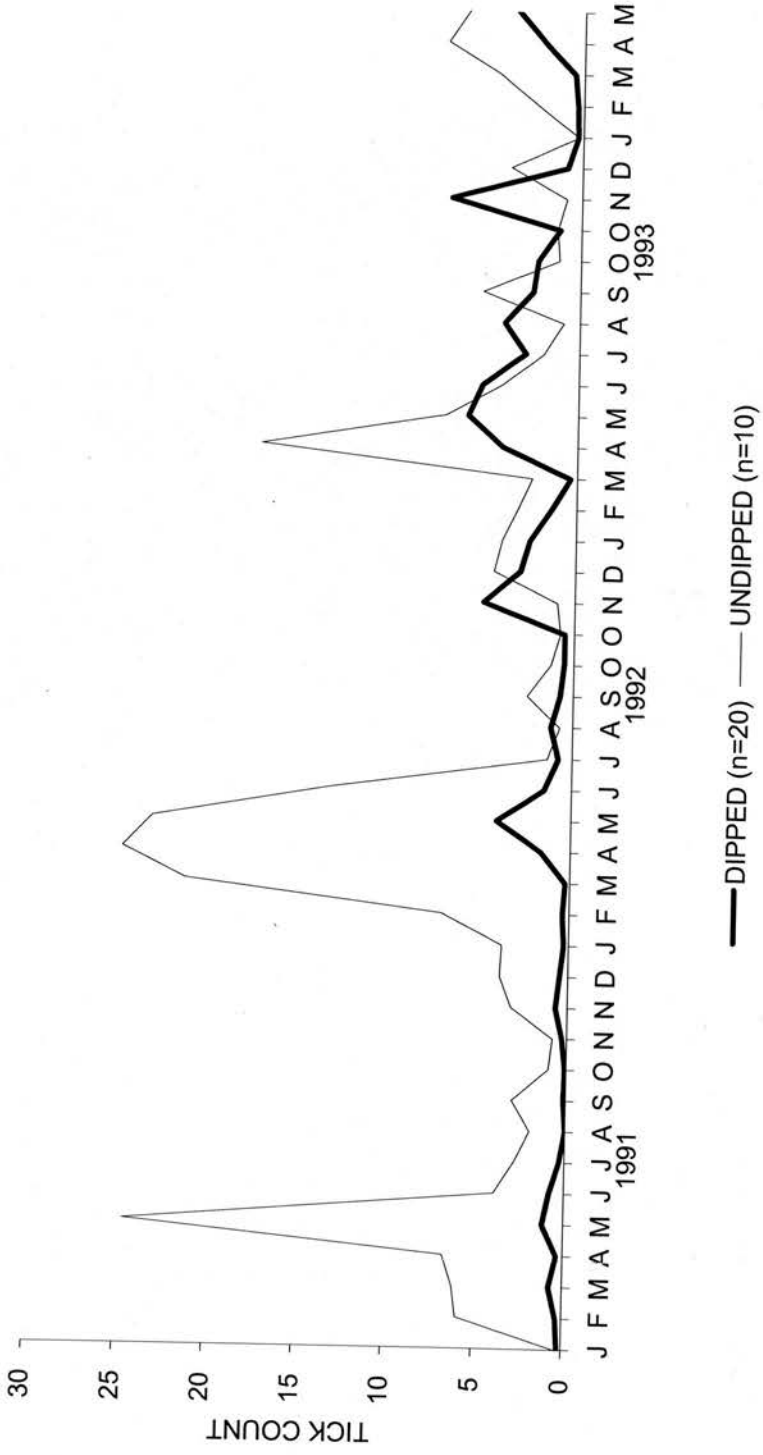


Figure 3.5 *Boophilus* adults. Geometric mean half body counts on dipped and undipped calves.

Figure 3.6 ***Amblyomma*** **adults. Geometric mean half body counts on dipped and undipped calves.**

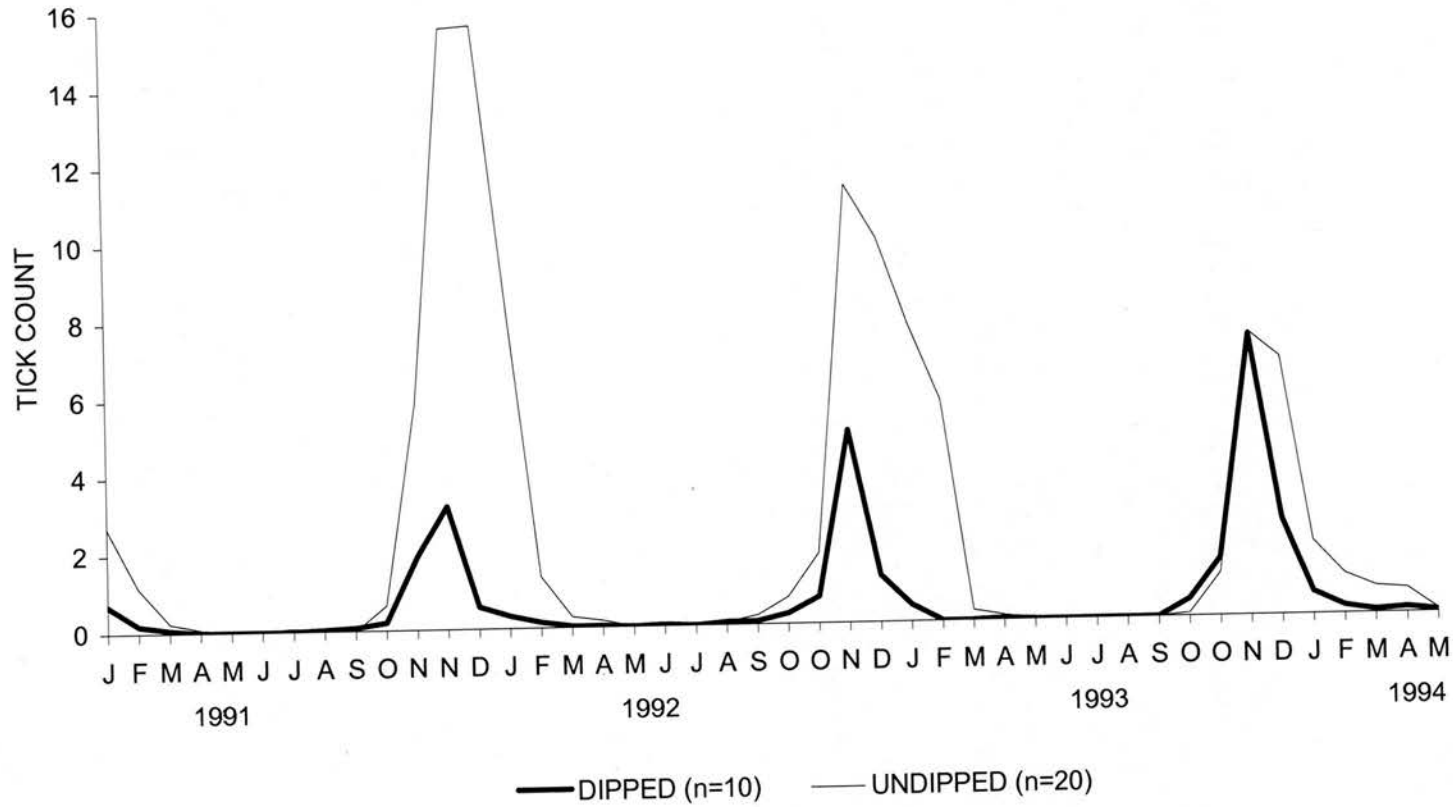
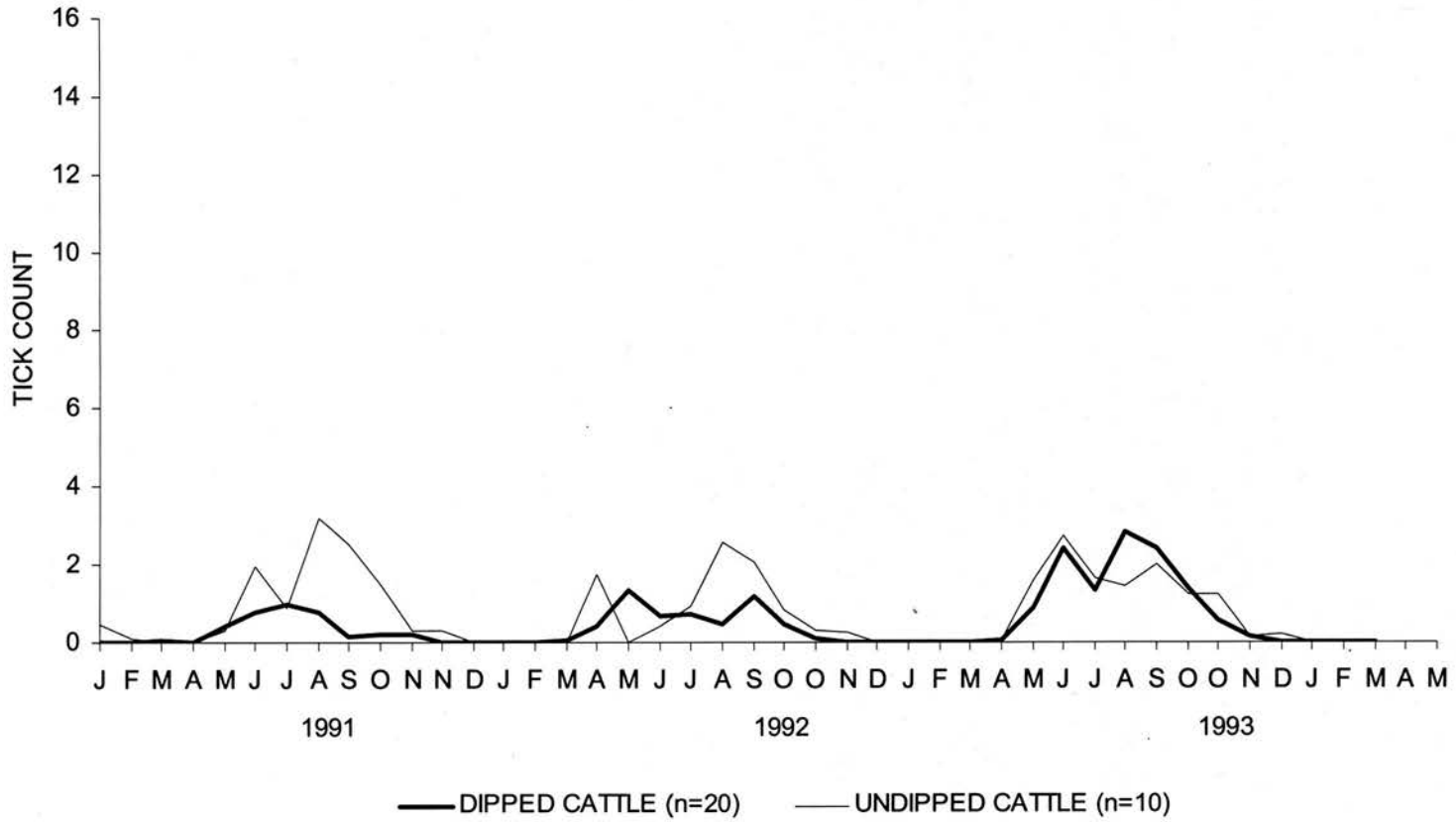


Figure 3.7 *Amblyomma* nymphs. Geometric mean half body counts on dipped and undipped calves.



Amblyomma ticks showed a strict seasonal pattern with a single generation per year. *Amblyomma* nymphs were common through the dry season (figure 3.7) and adults during the late dry season and rainy season (figure 3.6). The numbers of nymphs counted was considerably lower than adults, suggesting that the majority of *Amblyomma* nymphs fed on alternative hosts or that adults remain attached for longer. *Rhipicephalus appendiculatus* adults also had a seasonal pattern being most common during the rains and peaking in January/February (figure 3.4). Dipping every two weeks in the rainy season (nine immersions) gave almost total control of *R. appendiculatus*. *Boophilus* adults occurred throughout the year with peak numbers in April (figure 3.5). Undipped cattle had fewer *Boophilus* in 1993 and 1994 than in the two previous years. Compared to 1991 dipped cattle had more *Boophilus* and *Amblyomma* (both adults and nymphs) ticks during 1992 to 1994 due to the change of dipping protocol with no dry season dipping during these years.

Figures 3.8 and 3.9 show *R. appendiculatus* numbers on undipped animals at Tonde and Likuni respectively. They also show the percentage of median rainfall that occurred in each rainy season at each location. Analysis of variance of the rainy season *R. appendiculatus* numbers at Tonde and Likuni showed that there was a significant difference ($P < 0.01$) between areas and years. For Likuni there was no significant difference ($P > 0.05$) in *R. appendiculatus* tick numbers for any of the four seasons of the study, whereas at Tonde there was a significant difference ($P < 0.01$). At Tonde 1992/93 and 1993/94 saw a large drop in *R. appendiculatus* numbers ($P < 0.001$) compared with the previous two seasons.

Figure 3.8 *Rhipicephalus* adults. Geometric mean half head counts on calves at the undipped area of Tonde and percentage of median rainfall occurring each season over the study period.

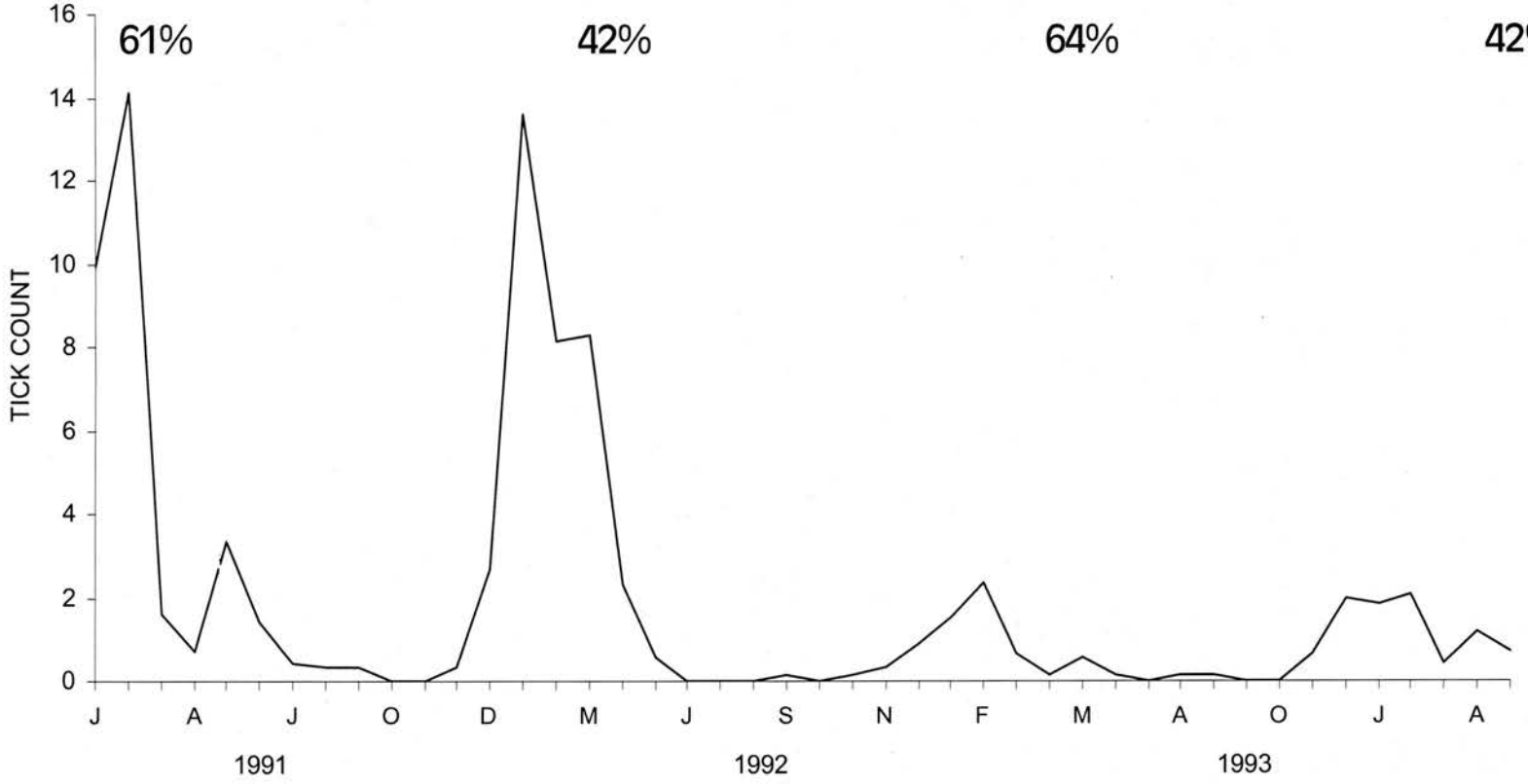
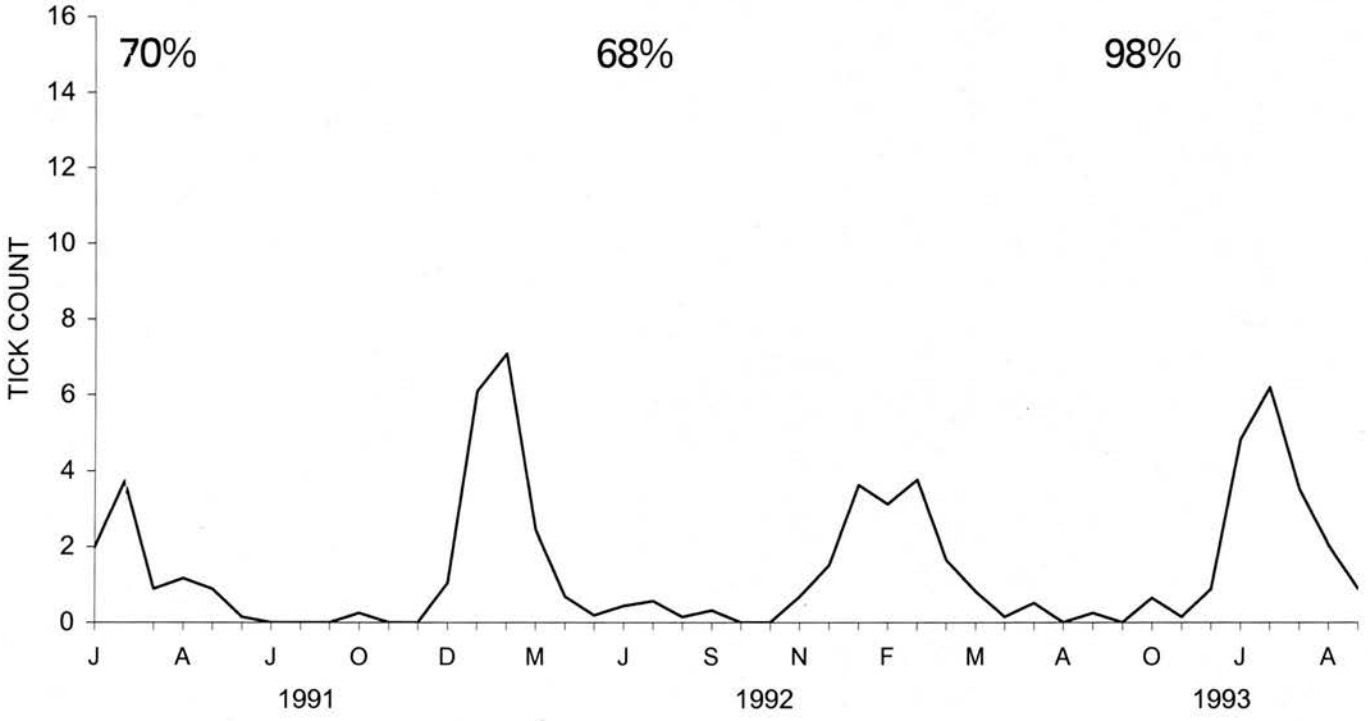


Figure 3.8

Figure 3.9 *Rhipicephalus* adults. Geometric mean half head counts on calves in the undipped area of Likuni and percentage of median rainfall occurring each season over the study period.

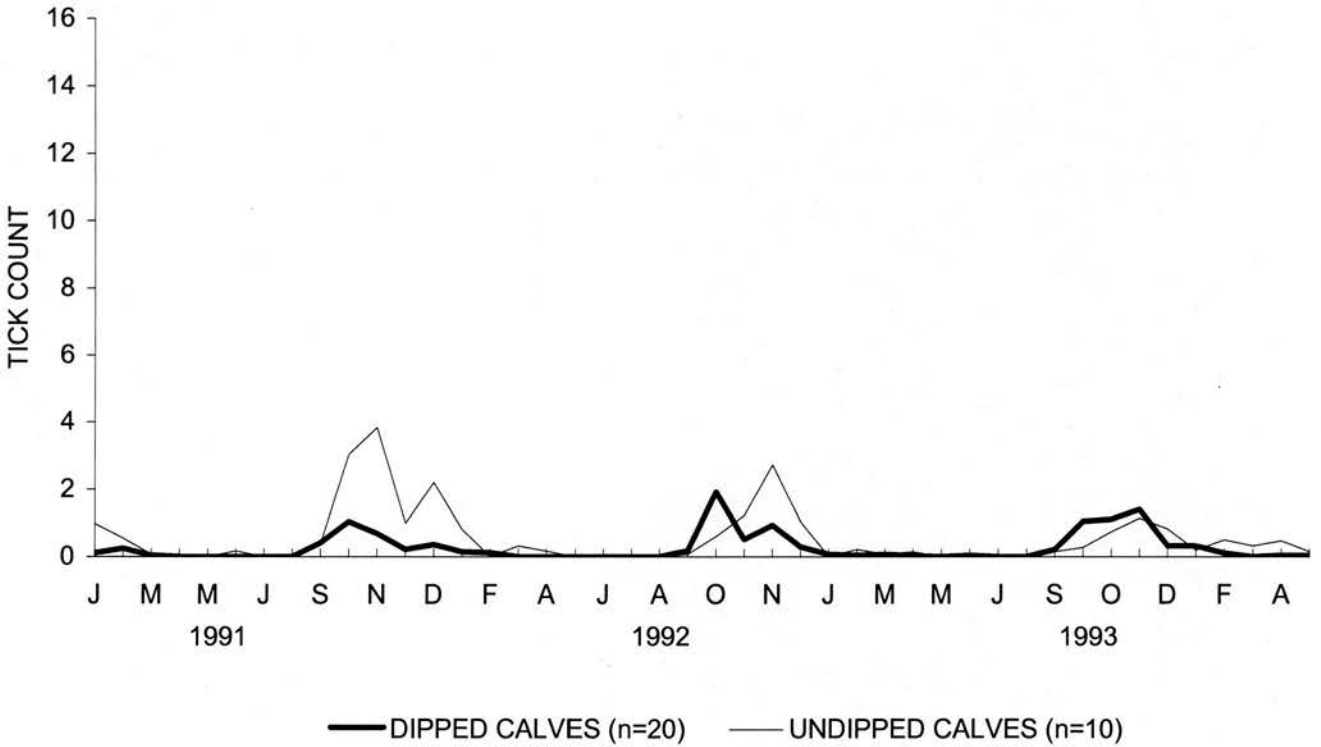


During the first wet season of the study (1990/91) *R. appendiculatus* numbers at Tonde were significantly higher ($P < 0.05$) than at Likuni, in the second season there was no difference ($P > 0.05$) and in the third and fourth season the numbers at Likuni were significantly higher ($P<0.05$) than at Tonde. An outbreak of ECF in a single village in May 1992 (see Chapter 4) was associated with a geometric mean half head count of 12.2 adult *R. appendiculatus* per affected animal. This late flush of ticks is not seen in Figure 3.9 because of its local nature. The 10 adult *R. appendiculatus* collected from Likuni in May 1994 were age graded as young and therefore had moulted within the last six months.

Figure 3.10 shows the number of *Rhipicephalus* adults found on the body of calves during the study. It can be seen that there were many fewer than on the head and that they occurred earlier, being most common at the end of the dry season. Seventy-one per cent of these ticks were *Rhipicephalus compositus* and 29 % *R. appendiculatus*. Most of the *R. appendiculatus* found off the head were in the tail brush.

Hyalomma truncatum were found in very low numbers, mostly in March and April and usually in the tail brush or groin. It was rare to find single ticks of this species and usually they occurred in groups of four to eight.

Figure 3.10 *Rhipicephalus* adults. Geometric mean half body counts, excluding the head, in dipped and undipped calves.



DISCUSSION

Rhipicephalus appendiculatus adults tick numbers on calves showed a seasonal pattern similar to that reported by Wilson (1946) for Lilongwe. There was however evidence of an extended period of adult activity and possibly a second peak on cattle in May or June that was not reported by Wilson. A similar finding has been reported in Eastern Zambia by Berkvens et al (1989) and Berkvens (1990) associated with a second peak of ECF. Lawrence (1991) and Norval et al (1991) commenting on the seasonal pattern of ECF in Zimbabwe before 1954 suggest that their findings may have been caused by a non-diapausing tick population that later died out in Zimbabwe under the pressure of tick control and adverse climatic conditions. The disease pattern seen in this study at the undipped tanks was very similar to that seen in Zimbabwe before 1954 (Chapter 4).

The adult *R. appendiculatus* seen on cattle in May and June in this study may have been due to a separate non-diapausing tick population as proposed by Lawrence (1991) and Norval et al (1991) for Zimbabwe. However a simpler, and more likely explanation, is that a new generation of adults emerge before the environmental trigger for the induction of diapause has been reached in late May or early June. Adult ticks that engorge in November or December may produce the next generation of adults before some threshold (for example day length) causing the onset of diapause is reached. December to April is 150 days and considering the data of Wilson (1950) and Branagan (1973) this is sufficient time to complete a generation through the wet warm months in Lilongwe (mean minimum temperature of 17.1^{O}C, mean maximum

temperature of 26.8^{O}C). Supporting evidence that these ticks are the start of a second generation is that the small number of adult *R. appendiculatus* age graded in May 1994 were all young and therefore recently moulted. In the Eastern Province of Zambia Berkvens (1990) noted the presence of a second wave of adults in May and two waves of nymphs in May/June and August/September. Wilson (1946 and 1950) considered there to be only one generation per year. He noted that the few adults present on cattle outside of the period from November to March did not lead to viable larvae. This was due to non-engorgement, failure of oviposition or failure of larval hatching. Newson (1978) working at the Kenya coast saw a bi-modal annual cycle of adults. He suggested that the second peak of adults occurred because the temperature was sufficient to drive the life cycle fast enough. However the humidity after the second peak was too low to allow survival of the larvae. Although no nymphal counts were made, the second wave of nymphs seen by Berkvens (1990) was evidenced in this study by the number of clinical cases of ECF and seroconversions to *T. parva* that occurred in September when very few adult ticks were present (Chapter 4, figures 4.5, 4.6 and 4.7). It is doubtful that this second peak of nymphs is a second generation following the second generation of adults in May/June. It is more likely that the second generation of adults is a dead end and that the second nymphal peak is caused by nymphs becoming active again after the cold months of June, July and August. Nymphal survival during this period may be facilitated by the permanently humid micro-climate to be found in the dambos that are such a prominent feature of the study area.

The few adult *R. appendiculatus* seen on cattle during July to October are probably ticks that broke diapause the year before and failed to find a host until later in the year. Survival of these ticks for long periods while seeking a host would also be aided by the micro-climate available in dambos (Punyua, 1985, and Newson, Chiera, Young, Dolan, Cunningham and Radley, 1984).

After the two driest years for 36 years the 1992/93 rainy season had only very slightly below the 37-year median rainfall for the area. However Tonde had another year of abnormally low rainfall. It would appear that the three consecutive dry years at Tonde had a severe effect on the *R. appendiculatus* populations. There is potential for higher tick numbers following normal or wet years. The effect of rainfall on *R. appendiculatus* tick numbers at Likuni is harder to determine. The variation in rainfall was not so great as at Tonde and the effect on tick numbers not so dramatic. Unlike cattle at Tonde that had not been dipped since 1988, cattle at Likuni were dipped up to the start of the study in late 1990. These factors make correlation of rainfall and adult tick numbers at Likuni problematical.

Strategic dipping had a dramatic effect on the *R. appendiculatus* population. Undipped cattle carried adult ticks from December to May but strategic dipping almost completely controlled these burdens. The survey of rainy season grazing patterns showed that the dipping percentage for all cattle grazing in areas that tagged cattle grazed was approximately 50%. This, along with reasonable *R. appendiculatus* control that existed before the study started (no adult *R. appendiculatus* tick rise occurred in

early 1991) was sufficient pressure on the tick population to keep numbers very low. The fact that weekly dipping in arsenic trioxide during 1989/90 controlled *R. appendiculatus* and *A. variegatum* adults in 1990/91 is contrary to the widely held belief that arsenic dipping had been of very low efficacy. However since the four tanks included in the study were in good physical condition and had efficient veterinary assistants they may not be a true representation of the general situation in Malawi.

Cessation of dipping during the dry season in 1992, 1993 and 1994 allowed *B. microplus* and *A. variegatum* tick numbers in dipped animals to return to approximately those seen in undipped animals. As expected this change took longer for three-host *Amblyomma* ticks (late 1993) with only one generation per year than for the more rapidly multiplying one-host *Boophilus* ticks (late 1992) which appear to have three generations per year.

Nine acaricide applications during the rainy season gave almost total control of *R. appendiculatus* but allowed similar *Boophilus* and *Amblyomma* numbers to the undipped areas. These results show that in the study area strategic dipping is technically feasible for the control of *R. appendiculatus* numbers. Whether this is desirable for disease control or economically justifiable is the subject of Chapter 6.

CHAPTER 4

THE EPIDEMIOLOGY OF EAST COAST FEVER IN UNDIPPED MALAWI ZEBU CATTLE AND THE EFFECT OF STRATEGIC DIPPING

ABSTRACT

Morbidity, mortality and seroconversion rates due to *Theileria parva* infection were studied in Malawi zebu cattle in six areas in the same ecological zone. A total of 3,257 animals were intensively monitored over a period of three years. Strategic tick control was carried out in four areas and no tick control was performed in a further two areas.

One undipped area was in an epidemiologically unstable state with respect to East Coast fever (ECF) due to prior dipping. East Coast fever mortality and morbidity were low in the first year after the cessation of dipping but rose over the second and third year until 46% of calves died of ECF before reaching one year of age. In the other undipped area ECF mortality and morbidity were low for all three years, despite high *T. parva* seroconversion rates. Dipping had ceased three years before the study began and it was concluded that this area was in a stable state with respect to ECF.

Strategic dipping in the other four areas resulted in a very low ECF morbidity and mortality. ECF mortality in calves was zero in most areas for most years.

East Coast fever case mortality in the undipped areas was 57% overall and there were no significant differences between areas or ages. In strategically dipped areas the ECF case fatality proportion in calves and young-stock was considerably lower at 28%. This is attributed to lower infective doses of *T. parva* in these areas. Adult *R. appendiculatus* were responsible for most of the *T. parva* transmission causing clinical disease with nymphs responsible for a significant amount of sub-clinical infection. The existence of enzootic stability to ECF in an undipped area without continuous adult *R appendiculatus* activity was demonstrated and the significance of nymphal transmission to the maintenance of this stability is discussed.

INTRODUCTION

East Coast fever (ECF) is caused by the protozoan parasite *Theileria parva* which is transmitted primarily by the tick *Rhipicephalus appendiculatus*. The disease occurs in Eastern, Central and Southern Africa and is estimated to cause the deaths of 1.1 million cattle annually from among the 24 million at risk in 11 countries (Mukhebi, Perry and Kruska, 1992). Despite these losses, well designed field studies of the epidemiology of ECF are surprisingly rare in the published literature (Perry and Young, 1995). Much of the present understanding of its epidemiology stems from the work of Barnett and Bailey in Kenya (Barnett and Bailey, 1955 and Barnett, 1957) and Yeoman in the south-east Lake Victoria area of Tanzania (Yeoman, 1966a and 1966b).

Barnett and Bailey (1955) observed calf mortality in inefficiently dipped cattle in Kenya over a six year period. Moll, Lohding and Young (1984) and Moll, Lohding, Young and Leitch (1986) made detailed studies of ECF epidemiology in zebu calves from an endemically stable area of Kenya. Latif, Rowlands, Punyua, Hassan and Capstick (1995) reported on the ECF incidence in monitored calves on Rusinga Island, Lake Victoria, Kenya. These studies were carried out in East Africa where all the instars of the vector tick exist on cattle at the same time. This is not the case in Central and Southern Africa where tick activity, and therefore disease challenge, is seasonal. The only published work on ECF epidemiology in Malawi was by Wilson (1944b and 1945) who obtained excellent data on the seasonal incidence of the disease from

routinely submitted spleen smears. He was also able to relate this to the seasonality of *R. appendiculatus* (Wilson, 1945).

Like other countries in Central and Southern Africa, Malawi has been reconsidering its policy of intensive compulsory dipping for zebu cattle. The need for more information led to the setting up of a study to evaluate strategic dipping as a form of tick-borne disease control. An account of the tick population dynamics seen in this study is given in Chapter 3. The epidemiology of ECF over a three-year period in the two control areas, where there was no acaricide application, and in the four strategically dipped areas is reported in this paper.

MATERIALS AND METHODS

The study was carried out at six dip tank areas around Lilongwe (33 ^{O}E, 14 ^{O}S) on the central African plateau at an elevation of approximately 1000 metres. The areas were selected because they were all in the same ecological zone and within 60 km of the Central Veterinary Laboratory. The area is typical of the mango savannah of Central Africa and is heavily cultivated. During the wet season when crops are cultivated, livestock graze in dambos (low lying areas prone to flooding) and along rivers. During the dry season cattle forage in the fields after harvesting but still use the dambos for green grass and water. Cattle management in the study area is similar to that found elsewhere in Malawi. Cattle are kept in small herds utilising communal grazing. They are enclosed at night in a khola (small pen) and are herded during the day by young boys.

Cattle were recruited into the study by farmer agreement, all farmers within 5 km of the tank were asked if they wished to join. Herd sizes ranged from one to 54 with a median of 11, which is similar to previous reports (Grindle 1979, Aspinal 1960).

UNDIPPED AREAS – TONDE AND LIKUNI

The dip tank at Likuni had been using arsenic trioxide acaricide up to the start of the study. Dipping in arsenic trioxide at Tonde had been suspended nearly three years before in 1988. During the study no dipping was carried out in either area. In the two areas a total of 629 Malawi zebu cattle belonging to 53 farmers were tagged in

December 1990 with easily readable ear tags. These comprised approximately 12% of the total population in each area. All animals belonging to an individual farmer were tagged and all calves born to tagged cows during the study were also tagged. A total of 1,213 undipped animals were tagged and monitored during the three years.

STRATEGICALLY DIPPED AREAS – DICKSON, NAMAGUYA, SINYALA AND MBABZI

The four dip tanks serving these areas had been using arsenic trioxide prior to the start of the study. In November 1990 the four tanks were emptied, cleaned, calibrated and re-filled. Some minor repairs were made to collecting pens and crushes. At the beginning of December 1990 dipping was started in these four tanks. The organophosphate acaricide chlorfenvinphos at a concentration of 0.05% m/v (Supona 30, Shell Chemicals) was used at Dickson and Namaguya. The amidine acaricide amitraz at a concentration of 0.005% m/v (Triatix TR, Coopers Animal Health) was used at Sinyala and Mbabzi. Dipping was carried out at two-weekly intervals from December 1990 to March 1991 and four-weekly intervals from April 1991 to November 1991. This strategy was designed to reduce ECF incidence (based on occurrence of ECF in the central region; Edelsten, 1990, Wilson, 1946, Moodie, 1981, Central Veterinary Laboratory data 1989/90) while minimising acaricide costs. First year study results indicated that although 17 immersions per year gave excellent control of ECF, this was too intensive and endemic stability to other tick-borne diseases was likely to be upset (Chapter 3, figures 3.5 and 3.6). The study protocol was changed to 9 immersions per year, dipping strategically (fortnightly from December to March with no dipping in the

dry season) for the rainy seasons of 1991/92, 1992/93 and 1993/94. In the four areas 1171 Malawi zebu cattle belonging to 90 farmers were tagged in December 1990 with easily readable ear tags. These comprised approximately 12% of the total population in each area. All animals belonging to an individual farmer were tagged and all calves born to tagged cows during the study were also tagged. A total of 2044 dipped animals were tagged and monitored during the three years.

DIPPED AND UNDIPPED AREAS

Animals under one year were classified as calves, from one to two years as young-stock and those over two as adults. In return for agreeing to their cattle being included in the study, farmers received free veterinary advice and treatment. An active disease surveillance system was set up in an attempt to diagnose all causes of death and clinical disease in tagged animals. The veterinary assistants who resided in each area were provided with the equipment and drugs necessary. Each area was visited fortnightly by a member of the study team, sick animals were examined and samples collected. The veterinary assistant or his tank messenger visited each khola at least once per week and sampled sick animals as presented by the farmers. Several farmers' meetings were held to explain the purpose of the study and to emphasise the need to report sicknesses.

Samples were examined at the Central Veterinary Laboratory, Lilongwe. All smears were examined by the protozology section and by the author or T.L. Norman. Post-mortem material was examined by the author or T.L. Norman. ECF morbidity in this

paper refers to clinically detectable disease (including enlarged lymph nodes, rectal temperature over 39.5 °C, lacrimation), confirmed by the presence of schizonts in lymph node or spleen smears. Cases of ECF were treated with a single dose (20 mg/kg body weight) of long acting oxytetracycline (Terramycin LA, Pfizer Ltd.). Treatments always occurred well into the period of clinical disease. Buparvaquone and Halifuginone were not used because of their unavailability in Malawi and the lack of any justification for their use on economic grounds. Full diagnoses were made where possible. In the cases where insufficient diagnostic samples were obtained to test for tick-borne disease or establish any other cause of death, a classification of "died no sample" was made. A calculation was made, based on the confirmed ECF mortality rate for each age group in each area each year, to estimate the proportion of animals that died without being sampled that were likely to have died from ECF.

To estimate the *R. appendiculatus* tick population tagged cattle were exposed to, half body tick counts were performed every four weeks on five calves in each area. Each calf selected belonged to a different farmer and had not been sampled four weeks before. Every 4 weeks, in each area, the five youngest tagged calves over 6 months of age were selected for tick counting. This sampling protocol ensured that there was no confounding effect due to calf age. The use of sentinel animals for tick counting was not possible under the field conditions of this study. No attempt was made to count larvae or nymphs. Counts and identifications were performed on the calves without removing the ticks. Sample collections showed that all the adult Rhipicephalus ticks found on the head were *R. appendiculatus* (Chapter 3). All adult ticks were

counted as infestations were low and there were therefore very few standard ticks (ticks within 24 hours of full engorgement).

Seventy-five calves born during 1991 in the undipped areas (approximately four calves born each month during 1991 in each of the two areas) were monitored for seroconversion to *T. parva.* A 10 ml blood sample was collected from the jugular vein of each calf every eight weeks until twelve months of age. This was designed to give an indication of seasonal variations in seroconversion without the confounding effect of calf age. In a separate exercise, cohorts of approximately 15 calves born in each of the 6 areas during May/June 1992 were serologically monitored until 12 months of age. Each calf had a 10ml jugular blood sample collected every four weeks. All blood samples were allowed to clot overnight at 4 ^{o}C before the sera was removed and stored at minus 20 ^{o}C. Sera was tested for *T. parva* schizont antibody using the indirect fluorescent antibody test at a dilution of 1:640 by the FAO East Coast fever vaccine production and quality control project, Central Veterinary Laboratory, Malawi. To avoid age bias (due to high non-ECF mortality) data on calves that died before nine or twelve months was not used in the seroconversion calculations for the May/June 1992 cohort.

Individual animal and disease data were stored and analysed using the Panacea and Monitor computer programs produced by Reading University, UK. This allowed animal-days to be calculated for any analysis period and therefore the calculation of true rates. True risk rates quantify the risk, proportional to the period an animal is at

risk. However true rates are meaningless to a farmer with only a few animals, not least because rates of over 100% are possible. To quantify the risk to individual calves the cumulative mortality probability (over a one-year period) was calculated from the formula :

$$\text{Cumulative mortality probability} = 1 - \exp(-\text{mortality rate})$$

(Clayton and Hills, 1993)

This gives the probability that an individual calf (being representative of the population) will die before one year old.

The chi-squared test was used to compare rates from an incidence table. The standard error (SE) of each rate was calculated from the formula:-

$$SE_R = (\text{Rate/animal years})^{0.5}$$

(James, 1995)

The chi-squared test was used to compare proportions from a contingency table (see Appendix 30). Tick counts were logarithmically transformed (log n+1) to allow geometric means to be calculated.

RESULTS

UNDIPPED AREAS – TONDE AND LIKUNI

During the three years of the study 106 undipped animals were confirmed as having clinical ECF. Of these 60 died giving a case fatality proportion of 57%. The ECF case fatality proportion was similar in both areas for all ages (59% [34/58] at Likuni and 54% [26/48] at Tonde) and for calves (62% [23/37] at Likuni and 62% [18/29] at Tonde). Figure 4.1 shows ECF mortality in calves relative to other causes of death. East Coast fever accounted for between 0 and 63% of all calf deaths at Likuni and between 7 and 47% at Tonde. Figure 4.2 shows the age distribution of the 87 ECF cases that occurred (82% of the total number of cases) in animals 24-months or under from both areas. The denominator for each age group was the number of animals, in which clinical ECF had not been confirmed, reaching that age during the three years of the study. Five-hundred-and-twenty-eight animals were recorded for the 0-month-old group reducing to 321 for the 24-month-old group. Most cases (66/106) occurred in the first 12 months of life with significantly fewer cases ($P<0.001$) in young-stock (19/106) and adults (21/106). The peak time for calving was April to August when 70% of calves were born.

Table 4.1 gives total mortality, ECF specific mortality and ECF specific morbidity data by age group and area. The adjusted figures account for the proportion of deaths classified as "died no sample" that were likely to have died from ECF. For the three years in both areas 29% (71/244) of deaths were classified as "died no sample". The

Figure 4.1

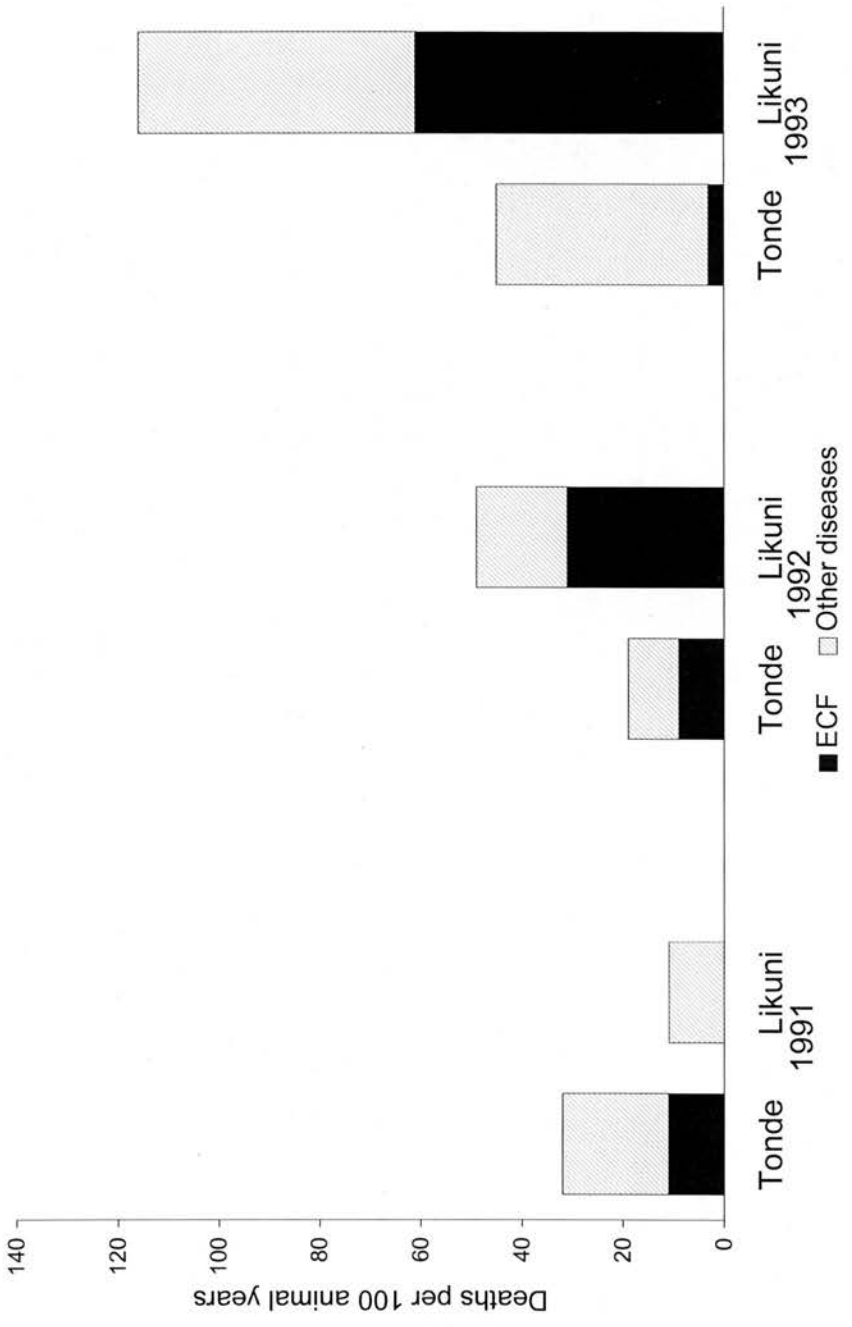


Figure 4.1 Calf mortality due to ECF and other causes in undipped cattle for 1991 to 1993.

Figure 4.2

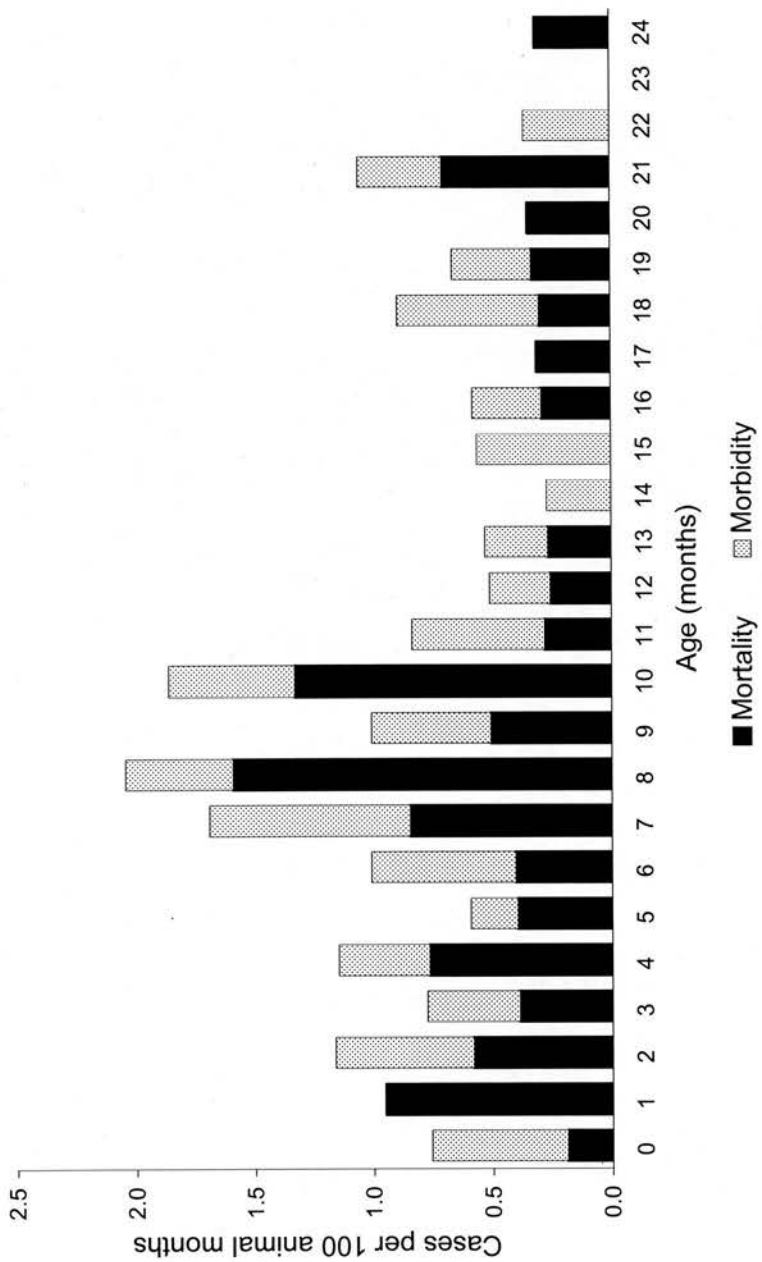


Figure 4.2 Age of ECF confirmation in animals of 24 months old or less, in both undipped areas (Tonde and Likuni).

Table 4.1 Total mortality, ECF mortality and ECF morbidity by age from 1991 to 1993

	CALVES			YOUNGSTOCK			ADULTS		
LIKUNI	**1991**	**1992**	**1993**	**1991**	**1992**	**1993**	**1991**	**1992**	**1993**
Animal years	54	43	31	26	46	54	176	111	85
Total deaths	6	21	36	2	7	7	6	11	16
Total mortality rate (SE)	0.11 (0.05)	0.49 (0.11)	1.16 (0.19)	0.08 (0.05)	0.15 (0.06)	0.13 (0.05)	0.03 (0.01)	0.10 (0.03)	0.19 (0.05)
Confirmed ECF deaths	0	12	11	1	2	2	3	3	0
Adjusted ECF deaths	0	13.3	18.9	1	7	3.5	3	4.7	0
Adjusted ECF mortality rate (SE)	0.00 (0.00)	0.31 (0.08)	0.61 (0.14)	0.04 (0.04)	0.15 (0.06)	0.06 (0.03)	0.02 (0.01)	0.04 (0.02)	0.00 (0.00)
Confirmed ECF cases	2	19	16	2	5	2	4	6	2
Adjusted ECF cases	2	20.3	23.9	2	9	3.5	4	7.7	2
Adjusted ECF morbidity rate (SE)	0.04 (0.03)	0.47 (0.10)	0.77 (0.16)	0.08 (0.05)	0.20 (0.07)	0.06 (0.03)	0.02 (0.01)	0.07 (0.02)	0.02 (0.02)
TONDE	**1991**	**1992**	**1993**	**1991**	**1992**	**1993**	**1991**	**1992**	**1993**
Animal years	76	104	117	92	127	147	220	203	202
Total deaths	24	19	53	5	4	5	6	4	12
Total mortality rate (SE)	0.32 (0.06)	0.18 (0.04)	0.45 (0.06)	0.05 (0.02)	0.03 (0.02)	0.03 (0.02)	0.03 (0.01)	0.02 (0.01)	0.06 (0.02)
Confirmed ECF deaths	6	9	3	1	3	1	0	1	2
Adjusted ECF deaths	8.5	9	3.8	1.7	3	1.7	0	1	2
Adjusted ECF mortality rate (SE)	0.11 (0.04)	0.09 (0.03)	0.03 (0.02)	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	0.00 (0.00)	0.00 (0.00)	0.01 (0.01)
Confirmed ECF cases	10	14	5	4	4	2	0	2	7
Adjusted ECF cases	12.5	14	5.8	4.7	4	2.7	0	2	7
Adjusted ECF morbidity rate (SE)	0.16 (0.05)	0.13 (0.04)	0.05 (0.02)	0.05 (0.02)	0.03 (0.02)	0.02 (0.01)	0.00 (0.00)	0.01 (0.01)	0.03 (0.01)

SE = standard error of the rate

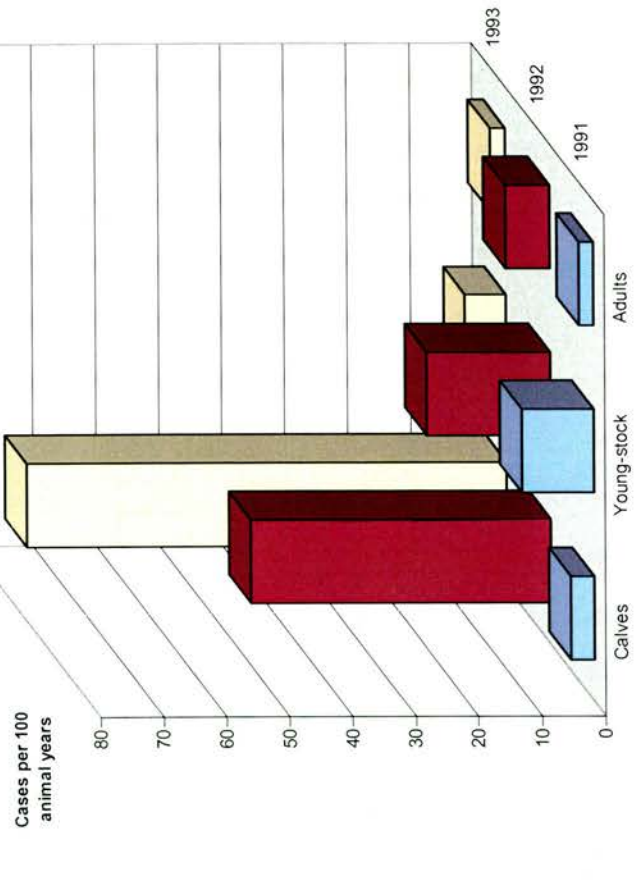
ECF morbidity pattern for the two areas is illustrated in Figures 4.3 and 4.4. At Likuni the ECF specific calf morbidity was low in 1991 but rose dramatically for each of the subsequent two years to reach 77% in 1993 ($P < 0.001$ for 1991 v 1992 but $P > 0.05$ for 1992 v 1993). ECF specific morbidity in young-stock at Likuni reached a peak in 1992 and declined in 1993. ECF morbidity at Tonde was comparatively low with the highest morbidity rate of 16% being seen in calves in 1991. The ECF calf morbidity rate at Tonde fell with each subsequent year to only 5% in 1993 ($P < 0.05$ 1993 v 1991).

Cumulative ECF calf mortality and morbidity probabilities up to one year of age are shown in Table 4.2. At Likuni in 1993, 54% of calves had clinical ECF and 46% had died of ECF by one year of age. In contrast, for the same year at Tonde, 5% of calves had clinical ECF and 3% had died of ECF by one year of age.

Figures 4.5 and 4.6 show monthly ECF mortality and morbidity rates for all ages along with monthly counts of *R. appendiculatus* adults for the two undipped areas. Cases occurred in every month of the year with the exception of November. The main clusters of cases were in January to March coinciding with the main peak of adult *R. appendiculatus* feeding. There was a rise in the number of cases in May/June in some years. During the period of very low adult *R. appendiculatus* feeding in August/September there was another definite cluster of cases in some years. The mortality and morbidity data in Figures 4.5 and 4.6 represent confirmed ECF cases only.

Figure 4.3

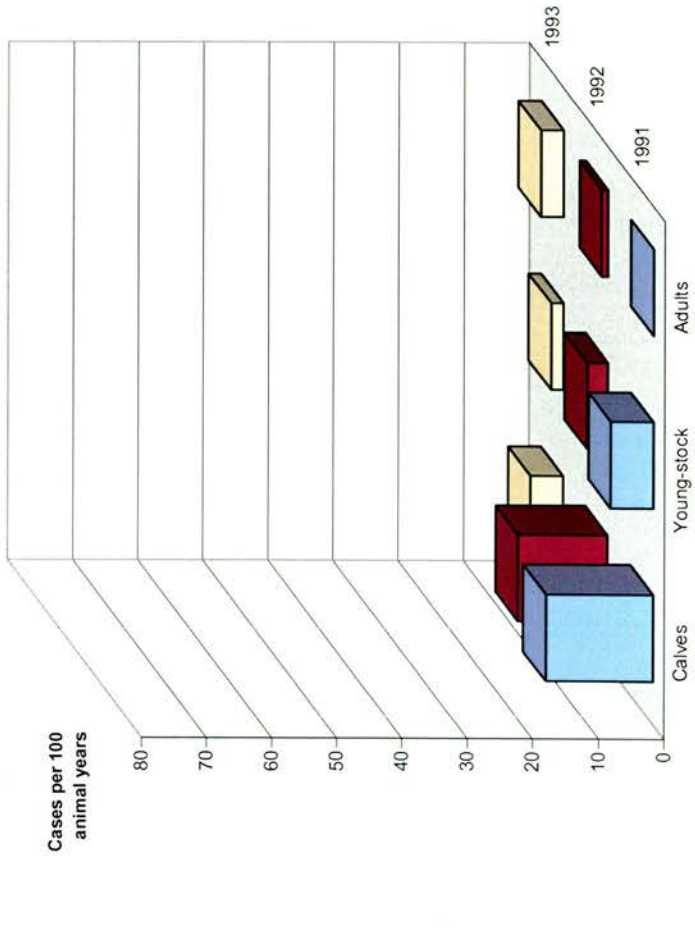
Figure 4.3 ECF morbidity in calves, young-stock and adults from 1991 to 1993 at Likuni (undipped).



See appendix 10 for data

Figure 4.4

Figure 4.4 ECF morbidity in calves, young-stock, and adults from 1991 to 1993 at Tonde (undipped).



See appendix 10 for data

Table 4.2 Cumulative ECF calf morbidity and mortality probability in calves up to one year of age in the undipped areas (Likuni & Tonde) between 1991 and 1993

LIKUNI	1991	1992	1993
ECF cumulative calf morbidity probability	4%	37%	54%
ECF cumulative calf mortality probability	0%	27%	46%
TONDE			
ECF cumulative calf morbidity probability	15%	13%	5%
ECF cumulative calf mortality probability	10%	7%	3%

Figure 4.5

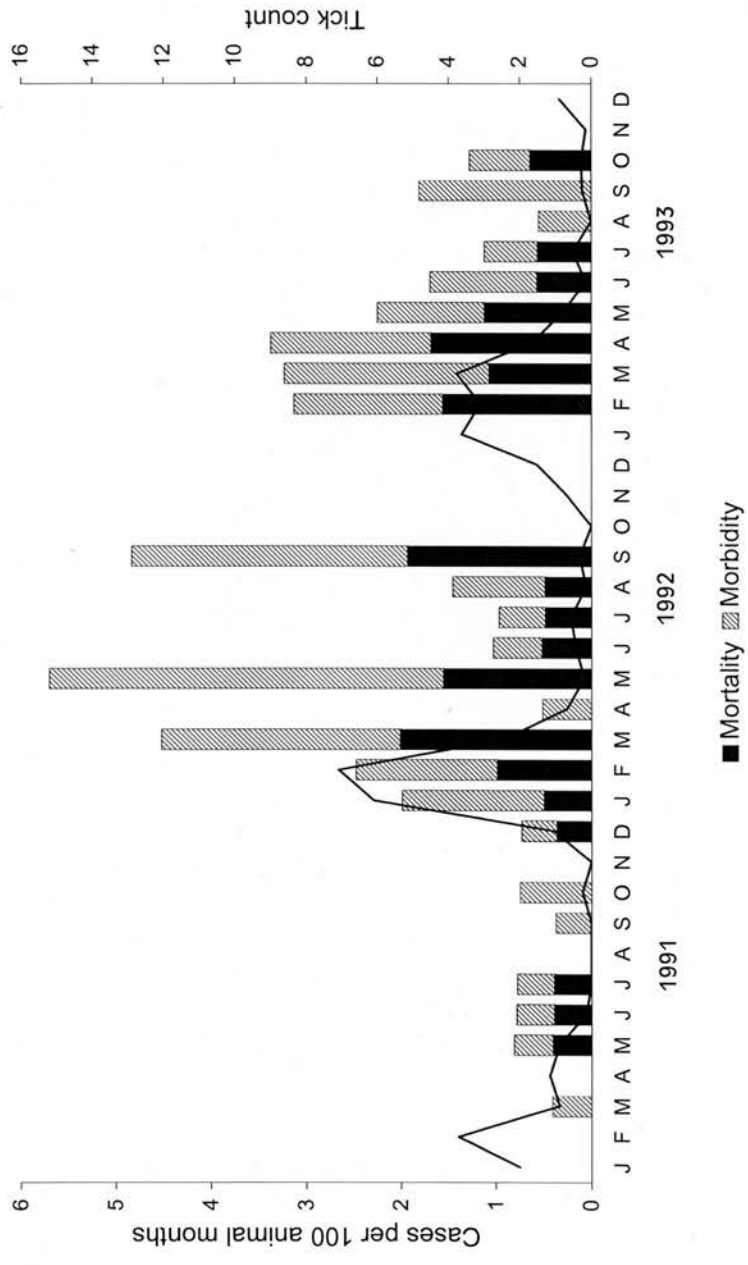


Figure 4.5 Monthly ECF morbidity and mortality for all ages, and monthly geometric mean half head counts of adult *R. appendiculatus* at Likuni (undipped) over 3 years.

Figure 4.6

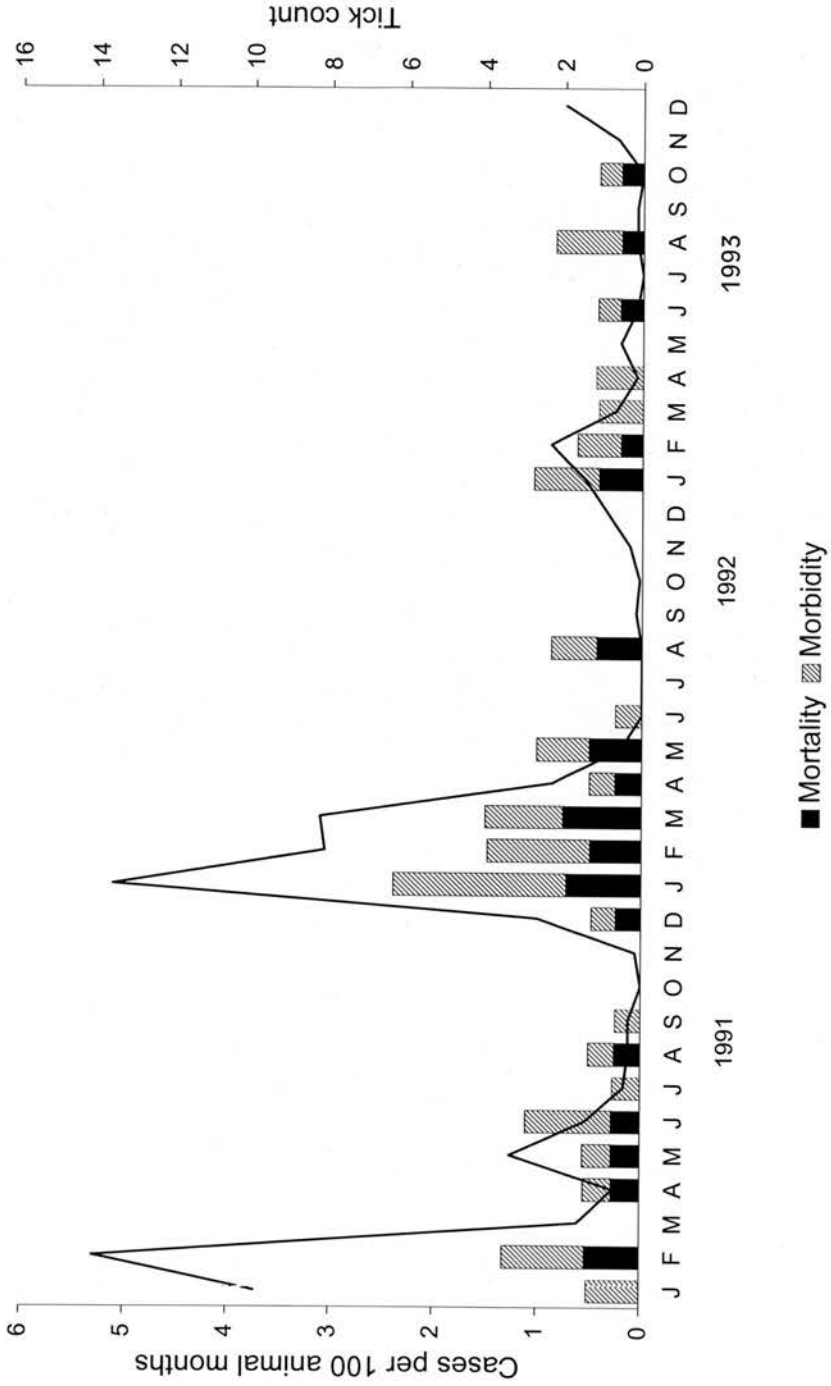


Figure 4.6 Monthly ECF morbidity and mortality for all ages, and monthly geometric mean half head counts of adult *R. appendiculatus* at Tonde (undipped) over 3 years.

As the proportion of deaths classified as "died no sample" was not constant over the three years of the study this data shows seasonal trends but does not accurately represent the relative levels of disease between years.

In 1991 and 1992 at Tonde 9/36 (25%) of calves born during the main period of adult *R. appendiculatus* activity (December to March) developed clinical ECF. Most cases occurred soon after birth. This compares with 17/218 (8%) of calves born between April and November that developed clinical ECF. Most cases occurred in the following season of main adult tick activity.

Figure 4.7 shows the number of seroconversions to *T. parva* at four-week intervals in 75 calves born in 1991. January was the peak month for seroconversion with slowly decreasing numbers until June. There was a second smaller peak of seroconversion in September and October. Because of the four-week sampling period the figure shows two bars for October. One was taken early in the month, the other at the end. The sampling protocol ensured that the age distribution was equal for each month.

Table 4.3 shows cumulative seroconversion rates to *T. parva* at twelve-months-of-age in cohorts of calves born in May/June 1991 and May/June 1992. The results represent cumulative incidence and not point prevalence. Titres of over 1/640 were often only maintained for two to three months. Point prevalence figures at twelve months old were considerably lower. Cumulative seroconversion at 12 months of age was 67% or over in all of the undipped cohorts.

Figure 4.7

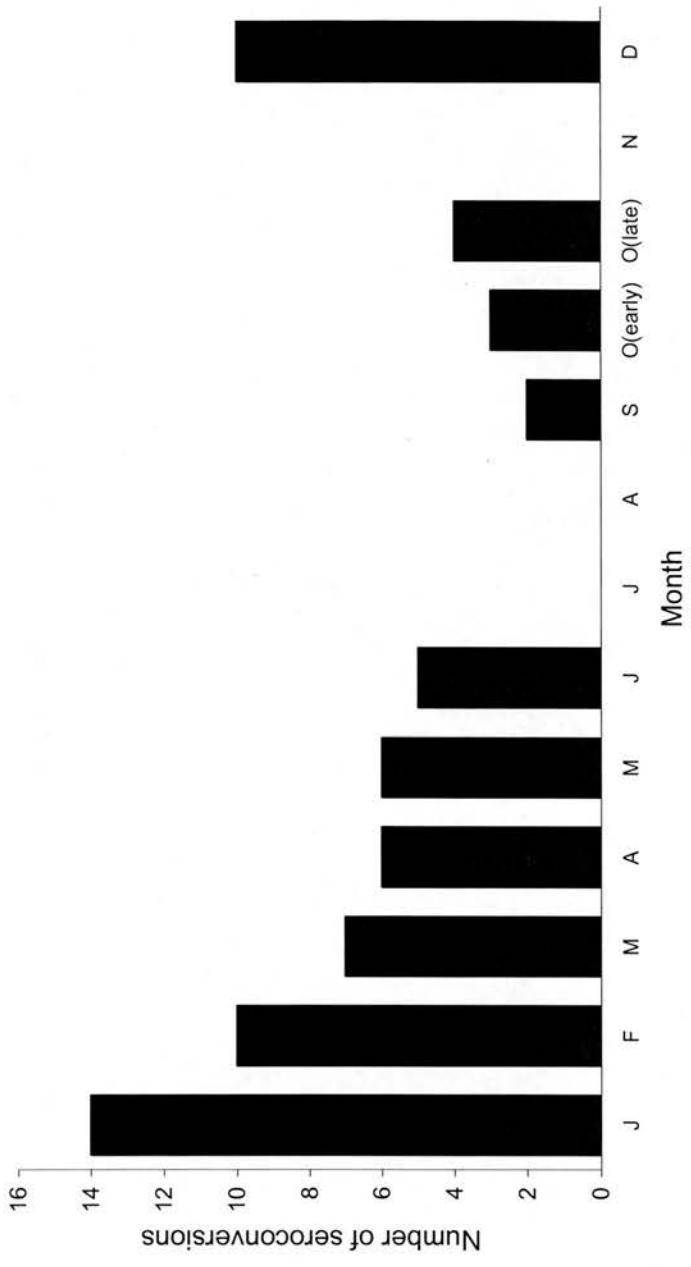


Figure 4.7 Number of seroconversions to *T. parva* per month in a group of 75 calves, with a constant age distribution, born in 1991 and monitored until 12 months old.

Table 4.3

Table 4.3 Cumulative *T. parva* seroconversion at 12-months of age in cohorts of calves born in May/June 1991 and May/June 1992.

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	Dickson	Namaguya	Sinyala	Mbabzi	Tonde	Likuni
1991					9/11 (82%)	9/10 (90%)
1992	0/16 (0%)	2/9 (22%)	4/11 (36%)	4/10 (40%)	6/9 (67%)	5/7 (71%)

STRATEGICALLY DIPPED AREAS – DICKSON, NAMAGUYA, SINYALA AND MBABZI

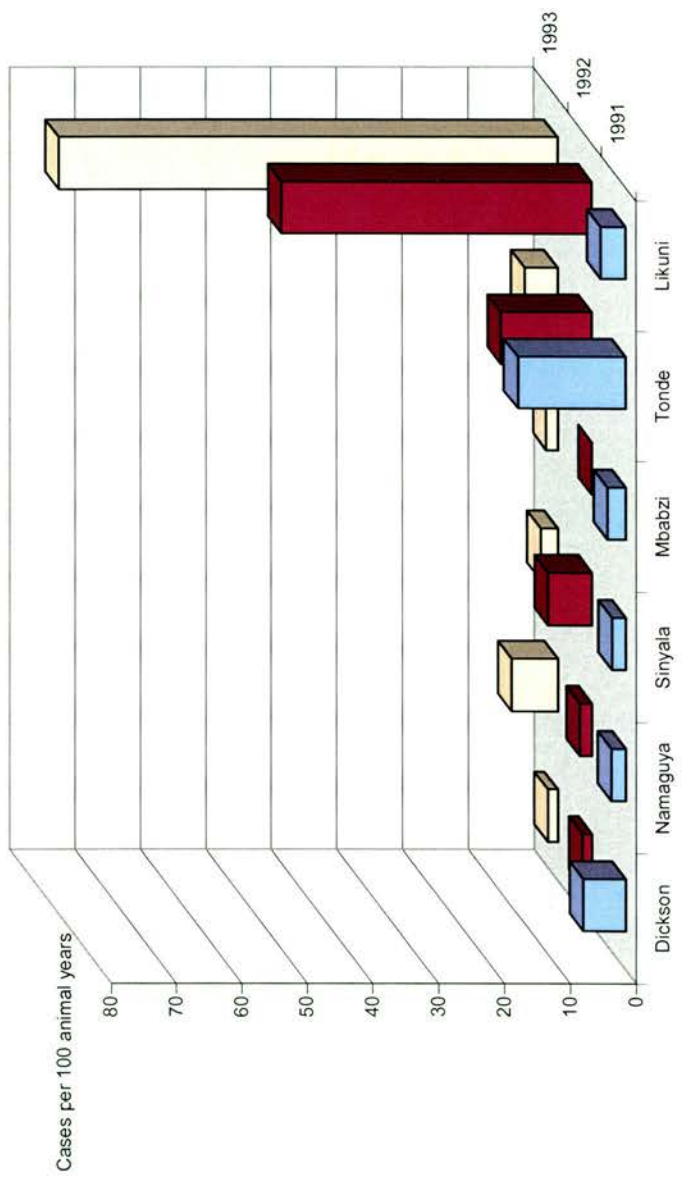
During the three years of the trial 58 strategically dipped animals were confirmed as having clinical ECF. Of these 27 died giving a case fatality proportion of 47%. This was not statistically significantly different ($p>0.05$) from the 57% case fatality proportion seen in the undipped animals. However there was a difference between the age groups. For strategically dipped calves the case fatality proportion was 29% (5/17 – confirmed cases only), for young-stock it was 27% (3/11) and for adults it was 63% (19/30). One hundred and thirty-eight strategically dipped calves died, 8 (including 3 that died without being sampled, that are statistically likely to have died from ECF) of these due to ECF (6%). This is considerably lower than the 34% (54/159) of calf deaths in the undipped areas which were caused by ECF.

Figure 4.8 shows the annual calf ECF morbidity rates for the three years of the study. It can be seen that the ECF morbidity rate in the dipped calves ranged from 0% in Mbabzi in 1992 to 7% in Namaguya in 1993. Overall the East Coast fever morbidity in strategically dipped calves was 3% (20/706). ECF morbidity rates for the undipped areas of Tonde and Likuni are also given in Figure 8 for comparison.

Figure 4.9 shows the annual ECF morbidity rates for young-stock and adults. For the strategically dipped areas this varied from 0.4% (1/269) to 3.7% (9/241) and was

Figure 4.8

Figure 4.8 ECF morbidity rates in calves from 1991 to 1993 for the six study areas.



See appendix 10 for data

Figure 4.9

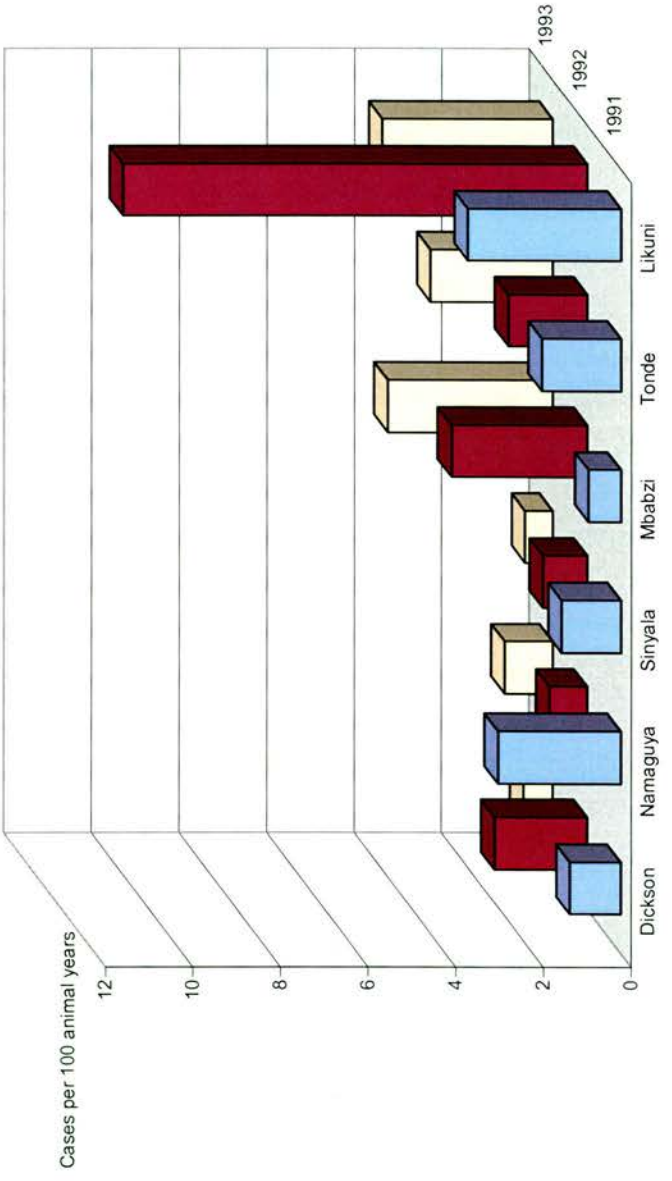


Figure 4.9 ECF morbidity rates in young-stock and adults from 1991 to 1993 for the six study areas.

See appendix 10 for data

1.6% (49/3005) overall. The ECF annual morbidity rates for the undipped areas of Tonde and Likuni are given for comparison.

Figure 4.10 shows the annual ECF mortality rates for calves. In the strategically dipped areas this was zero in most years but was 7% (4/55) in Namaguya in 1993. Overall the calf ECF mortality rate was 1% (8/706) in strategically dipped calves. Rates for the undipped areas of Tonde and Likuni are given for comparison.

Figure 4.11 shows the annual ECF mortality rates for young-stock and adults. In strategically dipped areas this ranged from zero to 3% (7/241) with an overall rate of 1% (30/3000). Rates for the undipped areas of Tonde and Likuni are given for comparison.

Table 4.3 shows cumulative seroconversion up to 1 year old in calves born in May/June 1992. Serologically monitored calves that died of any cause before 1 year old were excluded from the analysis. Between 0% and 40% of dipped animals had seroconverted by 12 months of age. This compares with 67 and 71% of undipped animals at Tonde and Likuni in the same time period.

Figure 4.10

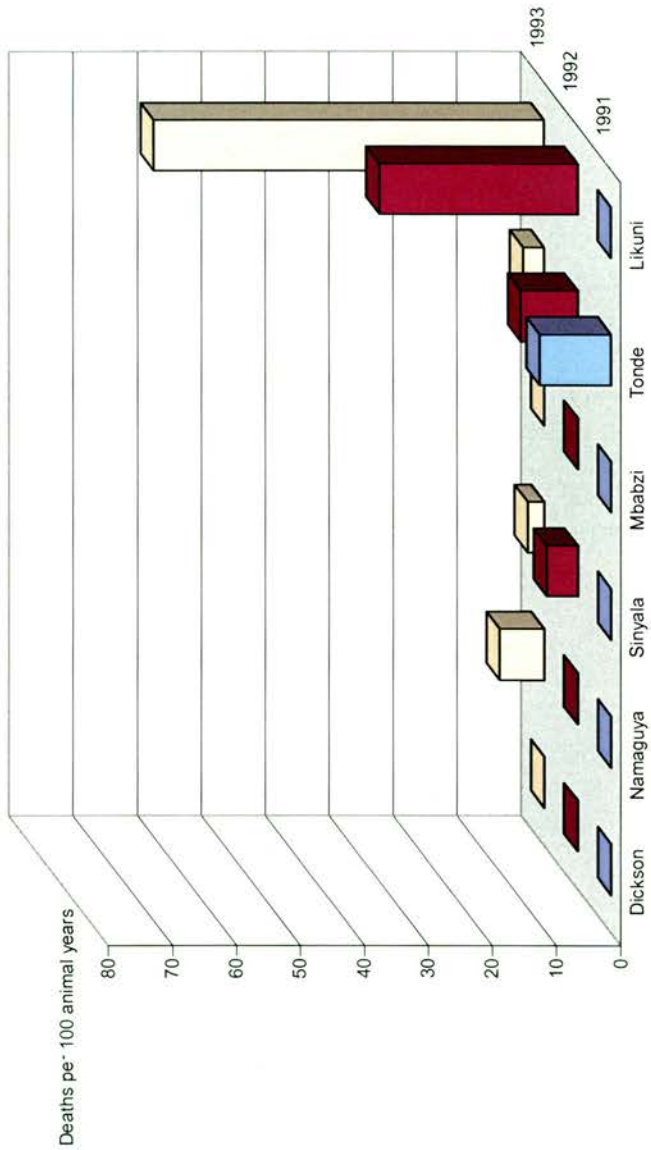
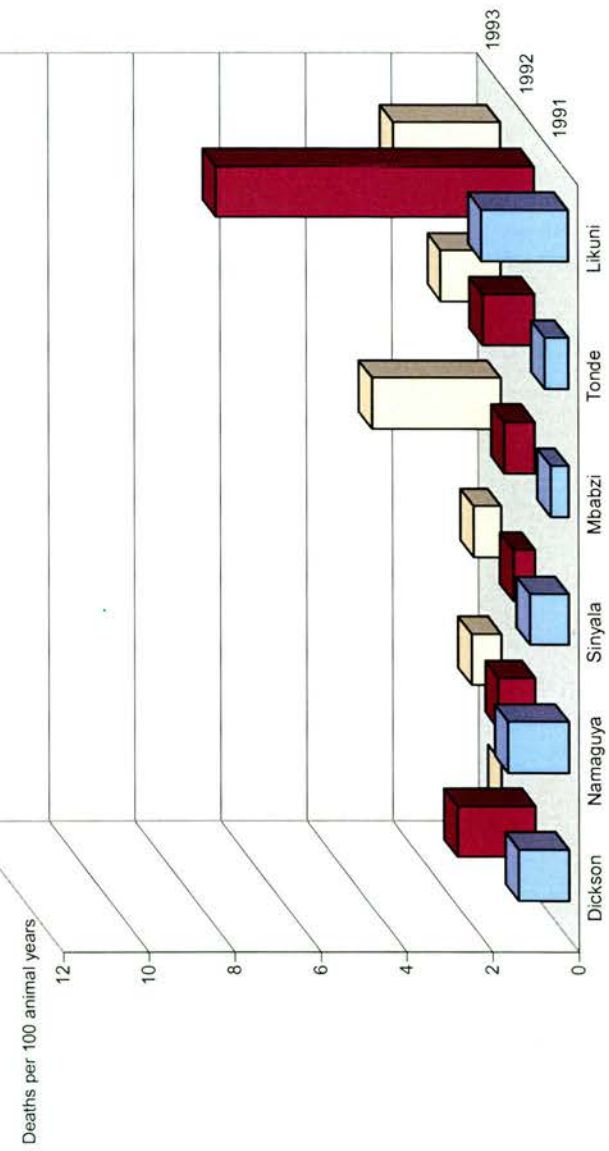


Figure 4.10 ECF mortality rates in calves from 1991 to 1993 for the six study areas.

See appendix 9 for data

Figure 4.11

Figure 4.11 ECF mortality rates in young-stock and adults from 1991 to 1993 for the six study areas.



See appendix 9 for data

DISCUSSION

It was not thought that the oxytetracycline given to clinical cases of ECF in this study had any effect on the course of the disease as treatments always occurred well into the period of clinical disease (Brocklesby and Bailey, 1962).

Norval, Perry and Young (1992) defined four epidemiological states for cattle derived *T. parva*. These were:

"Very unstable" - low antibody prevalence with low to high disease incidence in all ages.
"Unstable" - low to medium antibody prevalence with medium to high disease incidence mostly in immature animals.
"Stable" - medium to high antibody prevalence with low disease incidence mostly in calves.
"Very stable" - high antibody prevalence with no disease or very low incidence in young calves only.

UNDIPPED AREAS

The two undipped areas included in this study appear to illustrate two of these states. At the start of the study in 1991 Likuni was unstable but had low ECF mortality, presumably due to prior dipping. However the cessation of dipping, with a lag period of one year, resulted in a severe outbreak of ECF with a cause specific mortality of nearly 50% in calves before one year old in 1993. The factor that differentiated Likuni from a very unstable area was the fact that adult mortality remained low. It is presumed that the relatively inefficient dipping carried out before 1991 allowed sufficient parasite transmission to ensure an immune adult population. By contrast Tonde, where dipping had been stopped in 1987, was stable by 1991 with subsequent

ECF cumulative mortality probability between 3% and 10% in calves before one year old. ECF mortality in older animals was also very low. One point of difference from the definitions given by Norval et al (1992) was that in this study there were no differences in case fatality proportions between the stable and unstable areas.

In both areas the peaks of ECF incidence coincided with the main peaks of *R. appendiculatus* adult activity (Figures 4.5 and 4.6) but cases occurred in every month except November. In several years a higher than expected number of cases occurred in May/June. These were probably caused by the second generation of adult *R. appendiculatus* (Chapter 3) that had fed and become infected as nymphs during the early part of the year when there were many clinical cases. After the induction of diapause in the newly moulted adult ticks, probably in July, transmission was continued by nymphs and a second peak of disease and seroconversion (Figure 4.7) occurred in August/September when, it is presumed, nymphs became active again as the mean monthly temperature rose and infected the newly born crop of calves (70% of calves born in the period from April to August).

The importance of nymphal transmission in the development of immunity rather than disease is shown by the difference in the proportion of calves that show clinical ECF depending on when they were born. Calves born during the period of adult tick activity (December to April) were three times more likely to show clinical disease than calves born during the rest of the year. Yeoman (1966) and Kaiser, Sutherst and

Bourne (1982) found the ratio of *R. appendiculatus* on calves to be one nymph to 1.3 adults. Purnell, Boarer and Peirce (1971) showed that there are approximately three times as many infected acini in adult ticks as there are in nymphs resultant from the synchronous engorgement of their previous instars. However, it also may be the case that individual nymphs can carry a lighter parasite load than even an adult with a single infected salivary acini (Purnell, Young, Brown, Burridge and Payne, 1974, Ochanda, Young, Wells, Medley and Perry, 1966). As the severity of a *T. parva* infection is dose dependent (Wilde, Brown, Hulliger, Gall and MacLeod, 1968, Jarrett, Crighton and Pirie, 1969, Cunningham, Brown, Burridge, Musoke, Purnell, Radley and Sempebwa, 1974, Radley, Brown, Burridge, Cunningham, Peirce and Purnell, 1974, Dolan, Young, Losos, McMilian, Minder and Soulsby, 1984) then nymphal challenge would be more likely to produce sub-clinical disease followed by immunity rather than clinical disease or mortality. The field evidence from this study confirms the experimental findings of Ochanda et al (1996). There is no doubt that the majority of clinical ECF in this study was due to adult tick transmission (Figures 4.5 and 4.6) and it would seen likely that the majority of sub-clinical reactions leading to immunity were due to nymphal challenge. The seroconversion distribution (Figure 4.7) does not appear to support this hypothesis, but this data is based on a cohort that had a uniform age distribution. The calving pattern resulted in the majority of calves being exposed to nymphal challenge for 4 to 6 months before the main period of adult challenge. Also screening at a dilution of 1/640 may have failed to detect some seroconversions resulting from sub-clinical disease. It was the significant contribution to the overall parasite transmission by nymphs that allowed

the generation of a stable state in a region without continuous adult *R. appendiculatus* activity. This finding is different from the conclusion of Latif, Hove, Kanhai, Masaka and Pegram (2001) who did not consider nymphal challenge sufficient to maintain stability on three commercial farms in Zimbabwe. There were many factors which may have altered the epidemiology of ECF in comparison with the field situation in Malawi. However, they did conclude that nymphs are a significant factor in determining the epidemiology of ECF in Southern Africa.

At Tonde for the three years of the study there was relatively little disease but medium seroconversion rates (Table 4.3). However, at Likuni in 1991 when the tick population was released from the pressure of dipping, the severity of *T. parva* challenge increased and this resulted in a considerable number of clinical cases in calves and young-stock in 1992. The clinical cases in early 1992 would have lead to even higher tick infection rates in mid 1992 and early 1993 respectively. Extremely high mortality in the calves followed in 1993.

The question arises as to what the future situation will be in the two undipped areas. It is likely that Tonde will remain, and Likuni will become, stable with an ECF cumulative mortality probability of 5% to 10% in calves up to one year of age and little ECF in older animals. Provided tick numbers remain high enough, the major transmission to calves will be from nymphs (due to the seasonality of calving and nymphal activity) that have fed as larvae on infective carrier animals, and will result in high levels of herd immunity rather than clinical disease. However tick numbers

fell in 1993 due to severe drought (Chapter 3) and subsequent observations by Norman, Claes and Banda (1995) showed that at Tonde very low rainfall in 1994 (40% of normal) resulted in even lower *R. appendiculatus* numbers and low ECF incidence followed. Significantly higher ECF incidence was then seen in the early 1994/95 rainy season when tick numbers were higher. Transmission rates apparently fell sufficiently for population immunity to fall with subsequent increase in disease (as described by Yeoman, 1966 in an area marginal for *R. appendiculatus*). The possibility of this happening periodically should be taken into account when a policy of non-dipping is being considered on economic grounds. At Likuni Norman et al (1995) saw low levels of ECF in 1994 (under 10% in calves, very low in other age groups) but did comment that areas for cattle grazing were becoming more restricted and that overgrazing was a growing problem. Likuni is close to Lilongwe and more houses were being built and bush cleared. They conclude that this was creating a more hostile environment for tick survival which may restrict tick numbers even in years with good rainfall. The definitions of the epidemiological states given by Norval et al (1992) makes no mention of time-scale. In marginal areas "stability" can be fragile and can be converted to instability by a variety of changes including reductions in rainfall, grazing habits or land use.

It has been suggested (Barnett and Bailey, 1955, Norval et al, 1992) that calves under six months of age are more resistant to *T. parva* challenge than older animals and that this forms part of the basis for the generation of endemic stability. However, the data presented by Barrett and Bailey (1955) is the only convincing published data to

support this hypothesis. In the study reported here, clinical ECF and mortality was seen in calves of all ages. Figure 4.2 illustrates this and also shows an increase in cases in calves 7 to 10 months old. However as 70% of calves were born from April to August this finding is probably the result of the age distribution of calves during the main period of adult *R. appendiculatus* feeding. Another crucial factor is that although clinical ECF cases were removed from the denominator in the rate calculations for Figure 4.2 no allowance was made for the increasing proportion of immune animals in older age groups. By comparing the ECF morbidity and seroconversion rates it can be seen that most challenged calves, especially in the stable area of Tonde, seroconverted without showing clinical signs severe enough to be detected by the monitoring system in place. Therefore, as seroconversion rates were high, the true risk rate in older animals would have been much higher than that shown in Figure 4.2. It is therefore not possible to draw conclusions as to the influence of age on the susceptibility to *T. parva* challenge from these data. However, the use of the term 'stable' for a situation where up to 10% of calves die of ECF can be misleading.

This study has demonstrated that ECF has the potential to cause severe calf mortality, in indigenous cattle, when even relatively inefficient dipping is stopped. There was however a lag period of one year between the end of dipping and the severe mortality. It has also shown that after three to four years this mortality can decrease to a low level and that an area in Central Southern Africa can become endemically stable for ECF as defined by Norval et al (1992). However, this stability can be

fragile and still involve significant calf mortality. The role of age related immunity to ECF in calves appears to be significantly less important than the contribution to this stability from nymphal transmission to calves before their first exposure to adult ticks, which has been demonstrated by this study.

STRATEGICALLY DIPPED AREAS

Over the three years of the study strategically dipped calves and young-stock had significantly ($p < 0.01$) lower ECF mortality rates compared with the two undipped areas. Strategic dipping dramatically reduced adult *R. appendiculatus* numbers (Chapter 3) and as a consequence significantly reduced ECF mortality in animals under two years old. This was especially true for calves. Interestingly the ECF case fatality proportion was lower in strategically dipped calves and young-stock (27-29%) than in all other age groups or in the undipped animals (around 55-60%). This was likely to be due to lower adult *R. appendiculatus T. parva* infection rates in dipped areas because of the relatively fewer numbers of clinical cases. Another contributory reason could have been that a greater proportion of the transmission was being carried out by nymphs.

ECF mortality in adult cattle showed a different pattern. Strategically dipped adults had similar low ECF mortality rates to the undipped areas. The ECF case fatality proportion was similar for all adults (approximately 60%). Strategic dipping did not appear to affect the ECF situation in adult animals. The reasons for this are not clear from the results of this study which only ran for three years. This is too short a

period to see the full effect of a strategic tick control policy on adult animals. Over a longer time period falling herd immunity would be expected in the strategically dipped areas which could lead to high adult ECF mortality rates if dipping were to stop. It is possible that a proportion of adult ECF cases in the dipped areas were due to re-activation of latent infection due to metabolic or other stress and not due to tick transmission. The evidence for this is circumstantial, but there is a marked difference between the calf (and to a lesser extent the young-stock) mortality rates and the adult rates (c.f. Figure 4.10 and 4.11 taking account of the differences in scale). All animals grazed the same pasture and it is unlikely that tick transmission alone could result in this difference in disease rates. A similar phenomenon has been seen with *Cowdria ruminantium* infections (Pullen 1980).

There can be no doubt that the strategic dipping regime implemented in this study reduced disease caused by *T. parva* to a very low level. In areas containing only Malawi zebu animals there could be no justification for a more intensive regime if control, and not eradication, was the aim. The level of control achieved may appear desirable but it is at the expense of herd immunity. This creates an unstable situation that is vulnerable to interruptions in acaricide supply or tank break down. Cessation of dipping would lead to severe mortality in a situation similar to that seen at Likuni in this study or to that in Zimbabwe described by Lawrence, Foggin and Norval (1980).

CHAPTER 5

EXPERIMENTAL CHALLENGE OF GROUPS OF 18-MONTH-OLD MALAWI ZEBU CATTLE, PREVIOUSLY MAINTAINED UNDER DIFFERENT TICK CONTROL REGIMES, WITH *THEILERIA PARVA*

ABSTRACT

As part of a larger study, eighteen-month-old Malawi zebu cattle previously maintained under different tick control regimes were challenged with *Theileria parva*. Two of the 7 previously undipped animals had severe reactions but none died or needed anti-theilerial treatment. Nine of the 12 previously strategically dipped animals had severe reactions, of which two died of East Coast fever and one was treated to prevent its death. The conclusion is drawn that undipped animals had significantly higher resistance to *T. parva* challenge.

Of the 11 animals that had severe reactions, 5 needed treatment for, or died of, babesiosis 3 to 4 weeks after *T. parva* challenge. Immuno-suppression caused by the *T. parva* infections was thought to be the cause.

INTRODUCTION

Like other countries in Central and Southern Africa, Malawi has been reconsidering its policy of intensive compulsory dipping for zebu cattle. Several workers have concluded that intensive tick control for these animals is both economically and technically unjustifiable (Norval 1983, Pegram and Chizyuka 1990, Kaiser, Sutherst, Bourne, Gorissen and Floyd 1988, and Perry, Mukhebi, Norval and Barrett 1990).

A study was carried out to evaluate economically and technically feasible options for tick-borne disease control in Malawi zebu cattle in the future. An objective of the study was to investigate whether dipping could economically control East Coast fever (ECF) while maintaining clinical freedom from other tick-borne diseases (i.e. maintain assumed endemic stability). Results of tick studies are given in Chapter 3, entitled "The seasonal pattern of tick infestations on Malawi zebu cattle and the effect of strategic dipping with special reference to *Rhipicephalus appendiculatus*". ECF epidemiology is reported and discussed in Chapter 4, entitled "The epidemiology of East Coast fever in undipped Malawi zebu cattle and the effect of strategic dipping". This paper gives the results of an experiment challenging groups of cattle with *T. parva*. The cattle that had been maintained under different tick control regimes and serologically monitored since birth for *T. parva* antibodies.

MATERIALS AND METHODS

The main study was carried out in six dip tank areas around Lilongwe (33 °E, 14 °S) on the central African plateau at an elevation of approximately 1000 metres. The dip tank serving each area had been using arsenic trioxide acaricide prior to the start of the study, with the exception of Tonde where dipping had been suspended nearly three years before in early 1988.

In the six areas a total of 1,800 Malawi zebu cattle belonging to 143 farmers were tagged in December 1990 with easily readable ear tags. These comprised between 10 % and 16 % of the total population in each area. During the study no dipping was carried out or topical acaricide used in two areas (Likuni and Tonde). In November 1990 the other four tanks were emptied, cleaned, calibrated and re-filled. At the beginning of December 1990 dipping started in four tanks. The organophosphate acaricide chlorfenvinphos at a concentration of 0.05 % m/v (Supona 30, Shell Chemicals) was used at Dickson and Namaguya. The amidine acaricide amitraz at a concentration of 0.005 % m/v (Triatix TR, Coopers Animal Health) was used at Sinyala and Mbabzi. Dipping was carried out at two-weekly intervals from December to March (9 immersions per year) with no dipping in the dry season. No difference in TBD mortality rates were seen between the areas using the two different acaricides (see Chapter 4) and these two groups (4 dip tank areas) are considered together as "strategically dipped" cattle.

In November 1993 three animals from each area were randomly selected from the 17 to 18 month old age group to take part in a challenge experiment. These animals had been serologically monitored every 2 months for *T. parva* from birth until May 1993 when they were 12 months old. For the challenge experiment they were housed in specially constructed pens at two locations near to the Central Veterinary Laboratory. Four control animals originated from Southern Region, Malawi, which is free of *T. parva* infection, and were housed at the Central Veterinary Laboratory during the experiment. The animal details are given in Table 5.1. All animals were treated with the acaricide Drastic Deadline (1% Flumethrin, Bayer) 5 days before being challenged and two-weekly thereafter to keep them tick free.

The 23 animals were challenged with 1 ml of *T. parva* (Muguga) stabilate number 73 subcutaneously below the right ear. All animals were blood sampled on the day of challenge (Day 0) and weekly thereafter. Day 0 sera were tested for antibodies to *B. bovis, B. bigemina, T. parva* and *T. mutans*. Day 7, 14 and 28 sera were tested for *T. parva* antibodies. The proportions of dipped and undipped animals showing a severe reaction were compared using chi-squared test from a contingency table (see Appendix 30).

All blood samples were allowed to clot overnight at 4 ^{o}C before the sera was removed and stored at minus 20 ^{o}C. Sera were tested for *T. parva* schizont antibody using the indirect fluorescent antibody test (IFAT) at dilution of 1:640 and for *T. mutans* at a

Table 5.1

Table 5.1 Details of animals used in challenge experiment.

Tag No.	Origin	Sex	Age	Breed
1563	S. Region	M	14 m	FrxMz
1568	S. Region	M	14 m	FrxMz
1569	S. Region	M	14 m	FrxMz
1570	S. Region	M	11 m	FrxMz
D443	Dickson	F	18 m	Mz
D438	Dickson	F	18 m	Mz
D441	Dickson	F	18 m	Mz
S357	Sinyala	M	18 m	Mz
S362	Sinyala	M	18 m	Mz
S356	Sinyala	F	18 m	Mz
N357	Namaguya	M	18 m	Mz
N363	Namaguya	F	18 m	Mz
N359	Namaguya	M	18 m	Mz
M439	Mbabzi	F	18 m	Mz
M448	Mbabzi	M	18 m	Mz
M455	Mbabzi	F	18 m	Mz
L337	Likuni	M	18 m	Mz
L333	Likuni	M	18 m	Mz
L340	Likuni	M	18 m	Mz
T523	Tonde	F	17 m	Mz
T533	Tonde	F	17 m	Mz
T527	Tonde	F	18 m	Mz
T516	Tonde	F	18 m	Mz

dilution of 1:160, also by IFAT (by the FAO East Coast fever vaccine production and quality control project, CVL, Malawi). Sera were tested for *B. bovis* and *B. bigemina* antibodies by IFAT, both at dilution of 1:90 (by the FAO Regional Babesia, Anaplasma and Heartwater Vaccine Production Project, CVL, Malawi).

All animals were examined daily for temperature and clinical signs. Enlarged parotid and prescapular lymph nodes were biopsied daily and giemsa stained smears examined. Blood smears were similarly examined on a periodic basis from day 13 onwards.

The severity of reaction was classified as follows (Anon 1989):-

- No reaction or no apparent reaction: No parasites are detected and no clinical signs are apparent.
- Mild reaction: few schizonts are detected, no fever occurs or fever persists for less than 4 days. The animal is otherwise clinically normal and recovers.
- Moderate reaction: schizonts detected, fever persists for longer than four but less than nine days. The animal shows mild and transient clinical signs and recovers.
- Severe reaction and recovery or death: schizonts are detected, fever persists for 8 days or longer and the animal has obvious clinical signs of theileriosis.

Severe reactions to *T. parva* were treated with buparvaquone (Butalex, Pitman Moore) and babesiosis cases were treated with imidocarb (Imizol, Pitman Moore).

RESULTS

Tables 5.2 and 5.3 give the results of the experiment. Nine out of twelve (75%) calves that had been dipped strategically through the last rainy season had a severe reaction whereas only two out of seven (29%) undipped calves had a severe reaction. Five of the eleven calves that had a severe reaction recovered without treatment, three recovered after treatment and three died despite treatment. Five of the calves that were treated were given imidocarb due to recurrence of fever and very high *B. bigemina* parasitaemias on day 24-26, two of these died.

All four of the ECF naïve control animals had a severe reaction and needed treatment.

Four of the 7 undipped animals and one of the 12 dipped animals had titres of greater than or equal to 1/640 to *T. parva* at some point during the first 12 months of life. Only one calf was seropositive to *T. parva* on day 0 and this showed no reaction when challenged. All calves (except the three that died and could not be tested), including the controls, were seropositive to *T. parva* on day 28.

The Friesian cross control calves had a prepatent period to schizonts of 7.5 days compared to 9.4 days for Malawi zebu calves that had a severe reaction. Control calves also had a shorter time to onset of fever of 8.3 days compared to 9.6 days for non-immune Malawi zebu calves.

Table 5.2 Results of experiment challenging 18 month old calves with *T. parva*. November 1993.

	Day 0 *B. bovis* serology	Day 0 *B. bigemina* serology	Day 0 *T. mutans* serology	Day 0 *T. parva* serology	0-12 months *T.parva* serology	P.P.P. schizonts	P.P.P. fever	Day 28 *T.parva* serology	ECF reaction	Final outcome
Controls										
1563				-VE		7	8	+VE	Severe	Recovered (treated B)
1568				-VE		7	8	+VE	Severe	Recovered (treated B)
1579				-VE		8	9	+VE	Severe	Recovered (treated B)
1570				-VE		8	8	+VE	Severe	Recovered (treated B)
Study calves										
D443	+VE	+VE	+VE	-VE	-VE	10	11	Died	Severe	Died ECF
D438	+VE	-VE	+VE	-VE	-VE	7	8	+VE	Severe	Recovered (no treatment)
D441	+VE	+VE	+VE	-VE	-VE	13	11	+VE	Mild	Recovered (no treatment)
N357	+VE	-VE	-VE	-VE	+VE	11	10	+VE	Severe	Recovered (no treatment)
N363	-VE	+VE	+VE	-VE	-VE	13	11	+VE	Mild	Recovered (no treatment)
N358	-VE	+VE	-VE	-VE	?	11	10	+VE	Moderate	Recovered (no treatment)
S357	+VE	-VE	+VE	-VE	-VE	8	10	Died	Severe	Died ECF/Babesiosis
S362	+VE	+VE	+VE	-VE	?	10	11	+VE	Severe	Recovered (treated B+I)
S356	+VE	-VE	+VE	-VE	-VE	9	9	+VE	Severe	Recovered (no treatment)
M439	+VE	-VE	+VE	-VE	-VE	10	9	+VE	Severe	Recovered (treated I)
M448	+VE	-VE	+VE	-VE	-VE	10	11	+VE	Severe	Recovered (no treatment)
M455	-VE	+VE	+VE	-VE	-VE	10	9	+VE	Severe	Recovered (treated I)
T523	+VE	+VE	+VE	-VE	?	10	11	+VE	Moderate	Recovered (no treatment)
T533	+VE	+VE	-VE	-VE	+VE	9	9	+VE	Severe	Recovered (no treatment)
T527	+VE	+VE	+VE	-VE	-VE		7	+VE	No reaction	
T516	+VE	+VE	+VE	-VE	+VE	9	9	Died	Severe	Died Babesiosis
L337	-VE	-VE	+VE	-VE	+VE	10	10	+VE	Moderate	Recovered (no treatment)
L333	+VE	+VE	+VE	+VE	+VE			+VE	No reaction	
L340	+VE	+VE	-VE	-VE	-VE	7	10	+VE	Mild	Recovered (no treatment)

Key: I=Imidocarb, B=Buparvaquone, P.P.P = Pre-Patent Period

Table 5.3 Summary of reactions to experimental *T. parva* challenge.

Number of reactions/Number challenged

Area	No Reaction	Mild Reaction	Moderate Reaction	Severe Reaction
Controls	0/4	0/4	0/4	4/4
Dickson (SD)	0/3	1/3	0/3	2/3
Namaguya (SD)	0/3	1/3	1/3	1/3
Sinyala (SD)	0/3	0/3	0/3	3/3
Mbabzi (SD)	0/3	0/3	0/3	3/3
Tonde (UD)	1/4	0/4	1/4	2/4
Likuni (UD)	1/3	1/3	1/3	0/3

SD = Strategically dipped
UD = Undipped

DISCUSSION

Chapter 4 concluded that the strategic dipping carried out in this trial was preventing the development of endemic stability whereas the undipped areas were, by 1993, in some form of endemic stability to East Coast fever. The animals used in this experiment had survived one rainy season and their immune status could reasonably be expected to be representative of their group. The low number of animals limits the conclusions that can be drawn from the experiment, however 9 out of 12 dipped animal showed a severe reaction compared to 2 out of 7 undipped animals ($p < 0.05$). Likuni experienced very high East Coast fever mortality in the 92/93 rainy season (Chapter 4) and any calf that survived could be expected to be immune. This was the case, with none of the Likuni animals showing a severe reaction. Animals from all groups demonstrated the ability to withstand challenge, however the previously undipped animals showed significantly higher resistance to challenge.

Although there appeared to be variations in resistance to the experimental challenge it is interesting that of the 19 Malawi zebu animals taken from the trial area only two died of East Coast fever and one other was treated to prevent its death. Most animals, even the 11 that had severe reactions, recovered without anti-theilerial drugs. This demonstrates a remarkable degree of resistance compared to the control animals. Either they were partly immune or this was another facet of the natural resistance of zebu cattle to *T. parva* challenge seen by other workers (Barnett and Bailey, 1955).

The use of *T. parva* (Maguga) in this experiment was not ideal but there were no well characterised local strains available for use. The serological history of the animals was of no value in predicting the outcome of the challenge. One explanation would be that there was little cross protection between the local *T. parva* strains and the challenge strain (*T. parva* Maguga). However it is known that cell mediated immunity is the basis of acquired resistance to *T. parva* (Emery 1981) and it is possible that in the field situation in Malawi zebu cattle serological status is a poor indicator of the cell mediated immunity status. Also the screening dilution at which the *T. parva* IFAT was performed was fairly high (1/640). This is the usual dilution to check for seroconversion after experimental infection but may not have been appropriate for detecting responses to field challenge in zebu cattle. When Moll, Lohding, Young and Leitch (1986) sampled zebu calves weekly in an endemic area of Kenya they found that 84% of animals had peak titres of over 640 by the age of 6 months. In the same area the year before, Moll, Lohding and Young (1984) had found that only 43% of calves had peak titres over 1/640 by 6 months of age when they were sampled monthly. In both studies it was concluded, from other data, that the challenge was high and that most calves were immune to *T. parva* by 6 months of age. In the Malawi experiment the sampling interval was 8 weeks and it could be expected that an even lower proportion of seroconversions would be detected.

In contrast to the situation with East Coast fever 5 of the 11 animals that had a severe reaction to *T. parva* challenge died of, or needed treatment for, *Babesia bigemina*.

This was probably caused by immuno-suppression resulting from the *T. parva* infection. While immuno-suppression in severe cases of ECF could be expected due to the marked leukopenia induced by parasite multiplication (Wilde 1967) there are few published reports of it actually occurring. Wagner, Jessett, Brown and Rudley (1975) showed that there was a reduced antibody response to rinderpest vaccine in cattle with severe ECF. Immuno-suppression may have been the indirect cause of the death of some animals in the field study whose deaths were not attributed to ECF.

CHAPTER 6

HERD PRODUCTIVITY IN MALAWI ZEBU CATTLE AND THE ECONOMICS OF TICK-BORNE DISEASE CONTROL

ABSTRACT

The productivity of Malawi zebu cattle belonging to 143 farmers in six areas was monitored for three-and-a-half years. The cattle in four of the areas were strategically dipped to control tick-borne disease, principally East Coast fever. No tick control was carried out in the other two areas. The output per carrying capacity unit (approximately 2.9 animals) ranged from 400 Malawi Kwacha per year to 170 Malawi Kwacha per year (US$1 = MK6.5). The costs and benefits of various tick-borne disease control strategies were calculated. Policies of vaccination or strategic dipping where tank construction was necessary were significantly less cost effective than policies that stopped dipping or that continued strategic dipping at an existing tank. The most cost-effective option would be to stop dipping and accept mortalities while endemic stability becomes established. This would however have a large social cost due to mortality in the early years and extension services would need to provide relevant information to allow farmers to make informed decisions.

INTRODUCTION

Malawi has just over eight hundred thousand cattle with approximately 97% being Malawi Zebu. There are 371 dip tanks scattered over the country and for many years there has been a compulsory programme of weekly dipping in arsenic trioxide. The dipping service is the largest single expenditure for the Department of Animal Health and Industry. It is estimated that 5 million Malawi Kwacha (MK) is spent annually, of which MK 1 million is for purchase of acaricides. This amounts to approximately half the Department's recurrent budget. The dipping service is essentially free to farmers except for an annual fee levied per head of cattle owned. In 1993 this was MK 0.5 per head per year (equivalent to US$ 0.08) and has remained at this level for 20 years.

Like other countries in Central and Southern Africa, Malawi has been reconsidering its policy of intensive compulsory dipping for zebu cattle. Several workers have concluded that intensive tick control for these animals is both economically and technically unjustifiable (Norval 1983, Pegram and Chizyuka 1990, Anon 1990a, Kaiser, Sutherst, Bourne, Gorissen and Floyd 1988, and Perry, Mukhebi, Norval and Barrett 1990).

In view of the large proportion of the veterinary budget devoted to dipping, a study was carried out to evaluate economically and technically feasible options for tick-borne disease control in Malawi zebu cattle in the future. An objective of the study was to investigate whether dipping could economically control East Coast fever (ECF) while

maintaining clinical freedom from other tick-borne diseases (i.e. maintain assumed endemic stability). If responsibility for funding tick-borne disease (TBD) control is to move from central government to cattle owners, then the costs must be compared with benefits and the overall output of the farming system. This paper gives the economic results of a three-year study into herd productivity and tick-borne disease control in Malawi zebu cattle. Results of the tick studies are given in Chapter 3 and the ECF epidemiology in Chapter 4.

MATERIALS AND METHODS

The study was carried out in six dip tank areas around Lilongwe (33 °E, 14 °S) on the central African plateau at an elevation of approximately 1000 metres. The dip tank serving each area had been using arsenic trioxide acaricide prior to the start of the study, with the exception of Tonde where dipping had been suspended nearly three years before in early 1988. The areas were selected because they were all in the same ecological zone within 60 km of the Central Veterinary Laboratory, and had enthusiastic resident veterinary assistants. The area is typical of the mango savannah of Central Africa, and is heavily cultivated. The study area was free from trypanosomiasis, anthrax, foot and mouth disease and rinderpest. It was considered endemic for East Coast fever (*Theileria parva*), babesiosis (*Babesia bovis* and *B. bigemina*), heartwater (*Cowdria ruminantium*) and anaplasmosis (*Anaplasma marginale)*. During the wet season, when crops are cultivated, livestock graze in dambos (low lying areas prone to flooding) and along rivers. During the dry season cattle forage in the fields after harvesting but still use the dambos for green grass and water. Cattle management in the study area is similar to that found elsewhere in Malawi. Cattle are kept in small herds utilising communal grazing. They are enclosed at night in a khola (small pen) and are herded during the day by young boys.

In the six areas a total of 1,800 Malawi zebu cattle belonging to 143 farmers were tagged in December 1990 with easily readable ear tags. These comprised between 10% and 16% of the total population in each area. Herd sizes ranged from one to 54 with a

median of 11 which is similar to previous reports (Grindle, 1979, Aspinal, 1960). All calves born to tagged cows during the study were also tagged. A total of 3,257 animals were tagged and monitored during the three years of the study. Animals under one-year were classified as calves, from one-to-two years as young stock and those over two years as adults. In return for agreeing to their cattle being included in the study, farmers received free veterinary advice and treatment. Calvings, sales, slaughters and other entries or exits from the monitored herds were recorded.

During the study no dipping was carried out or topical acaricide used in two areas, namely Likuni and Tonde. In November 1990 the other four tanks were emptied, cleaned, calibrated and re-filled. Some minor repairs were made to collecting pens and crushes. At the beginning of December 1990 dipping started in four tanks. The organophosphate acaricide chlorfenvinphos at a concentration of 0.05 % m/v (Supona 30, Shell Chemicals) was used at Dickson and Namaguya. The amidine acaricide amitraz at a concentration of 0.005 % m/v (Triatix TR, Coopers Animal Health) was used at Sinyala and Mbabzi. Dipping was carried out at two-weekly intervals from December 1990 to March 1991 and then four-weekly intervals from April 1991 to November 1991. This strategy was designed to reduce ECF incidence (based on occurrence of ECF in the central region; Edelsten 1990, Wilson 1946a, Moodie 1981, Central Veterinary Laboratory, Lilongwe, data 1989/90) while minimising acaricide costs. First year study results indicated that although 17 immersions per year gave excellent control of ECF, this was too intensive and endemic stability to other tick-borne diseases was likely to be upset. The study protocol was changed to nine

immersions per year, dipping strategically (fortnightly from December to March with no dipping in the dry season) for the rainy seasons of 1991/92, 1992/93 and 1993/94. The author (or Theresa Norman MRCVS) plus one or two veterinary assistant staff attended every dipping. No difference in TBD mortality rates were seen between the areas using the two different acaricides (Paper 2) and in the calculations they are considered together as "dipped" cattle.

An active disease surveillance system was set up in an attempt to diagnose all causes of death and clinical disease in tagged animals. The veterinary assistants who resided in each area were provided with the equipment and drugs necessary. Each area was visited fortnightly by a member of the study team; sick animals were examined and samples collected. The veterinary assistant or his tank messenger visited each khola at least once per week and sampled sick animals as presented by the farmers. Several farmers' meetings were held to explain the purpose of the study and to emphasise the need to report sicknesses. Full diagnoses were made where possible. In the cases where insufficient samples were obtained to confirm tick-borne disease or establish any other cause of death, a classification of "died no sample" was made. A calculation was made, based on the confirmed TBD mortality rate for each age group in each area each year, to estimate the proportion of animals that died without being sampled that were likely to have died from TBD.

Cohorts of 15-17 calves born during May/June 1991 in each of the six areas were monitored every four weeks until 10-months-old for growth rate and half body adult

female tick count. The ticks were differentiated in the field as *Amblyomma, Boophilus, Hyalomma* or *Rhipicephalus* species. Weights were measured using a 200 kg spring balance (Salter Ltd, UK) attached to a sling and pulley system suspended from a tripod. The growth rate of similar cohorts born during May/June 1992 was calculated by measuring calf weights in July, November and May.

In November 1992 a survey was carried out to identify the number of tagged cows milked for human consumption, the average milk yield, and the number of tagged oxen used for pulling carts.

Individual animal and disease data were stored and analysed using the Panacea and Monitor computer programs produced by Reading University. This allowed animal-days to be calculated for any analysis period and therefore the calculation of true rates.

Comparison of production levels between dipped and undipped cattle was made using a computer based "Livestock Production Efficiency Calculator" (Anon, 1990b). This program has been used by other authors in similar work to estimate the value of an intervention (Pegram, James, Oosterwijk, Killorn, Lemche, Ghirotti, Tekle, Chizyuka, Mwase & Chizhuka, 1991 and de Castro, James, Minjauw, Guilio, Permin, Pegram, Chizyuka and Sinyangwe, 1997). The net result of the LPEC calculation is an estimate of the value of production that can be obtained from a fixed feed resource. This productivity is expressed in terms of the value of output per unit of feed intake (the unit is called a "carrying capacity unit" or CCU, and is equal to a feed supply providing

100MJ of Metabolisable energy (ME) per day throughout the year).

LPEC requires the input of several rates which are defined as follows:

$$\text{Annual percentage mortality rate} = \frac{\text{No. deaths}}{\text{No. animal Days}} \times 365 \times 100\%$$

$$\text{Annual percentage parturition rate} = \frac{\text{No. parturitions}}{\text{No. breeding female animal days}} \times 365 \times 100\%$$

$$\text{Annual percentage culling rate} = \frac{\text{Net sales}}{\text{No. animal days}} \times 365 \times 100\%$$

Breeding females are defined as females after their first calving. Therefore first parturitions are counted in the numerator, but immature replacement females (heifers before first calving) are not included in the denominator. Net sales are the number of animals sold or transferred out of the herd for any reason, less the number of animals purchased or transferred in.

A financial analysis of tick and tick-borne disease control strategies was made comparing the costs of strategic dipping and alternative methods of tick and tick-borne disease control over a 10 year period. Due to the large variation in parturition rates and non-TBD mortality rates between years, overall rates were used as input data to LPEC for the analysis. All cattle in the study over the three years were used to calculate the parturition rate. The non-TBD mortality rate for all animals in each of the three age groups was calculated and added to the TBD mortality rate for each age group and analysis group.

The analysis is based on 1000 animals in an area with access to a central dip tank or vaccination centre. The major costs incurred by stopping dipping in an endemically unstable area are due to the mortality experienced until endemic stability is established. The calculation assumes that the cessation of dipping and the immunisation of susceptible cattle would lead to the same TBD mortality rate as that seen in this study in the strategically dipped cattle (Minjauw, Otte, James, de Castro and Sinyangwe, 1998). The vaccination model assumes that all cattle are vaccinated in year one, and that in subsequent years only calves are vaccinated. At the end of 1993, one US dollar was approximately equal to 6.5 Malawi Kwacha (MK).

RESULTS

REPRODUCTION

Figure 6.1 shows the annual parturition rates over the three years of the study for the six areas studied. The variation in rates between years was as great as the variation between areas. The cow parturition rates (excluding heifer calvings) followed the same pattern ranging from 44% to 70% in 1992 and 38% to 55% in 1993. Most calvings occurred in the middle of the year and Figure 6.2 shows the number of calvings each month for all the monitored cattle. The median age at first calving during 1992 and 1993 was 42 months, with a range between tanks and years of 38 to 48 months.

The number of breeding females per breeding male in late 1992 ranged from 17.7 at Tonde to 5.4 at Namaguya with an overall mean of 8.7.

CALF GROWTH

Figure 6.3 shows the growth rate of two cohorts of calves (n=99 in 1991/92 and n=93 in 1992/93). Ten-month-old calves in April 1993 were not as heavy as those in April 1992. The fastest-growing calves were at Tonde with mean monthly weight gains (MMWG) of 8.4 kg and 6.9 kg for 1991/92 and 1992/93 respectively. The slowest growing calves were at Likuni in 1991/92 (MMWG of 4.4 kg) and Mbabzi in 1992/93 (MMWG of 3.9 kg).

Figure 6.1

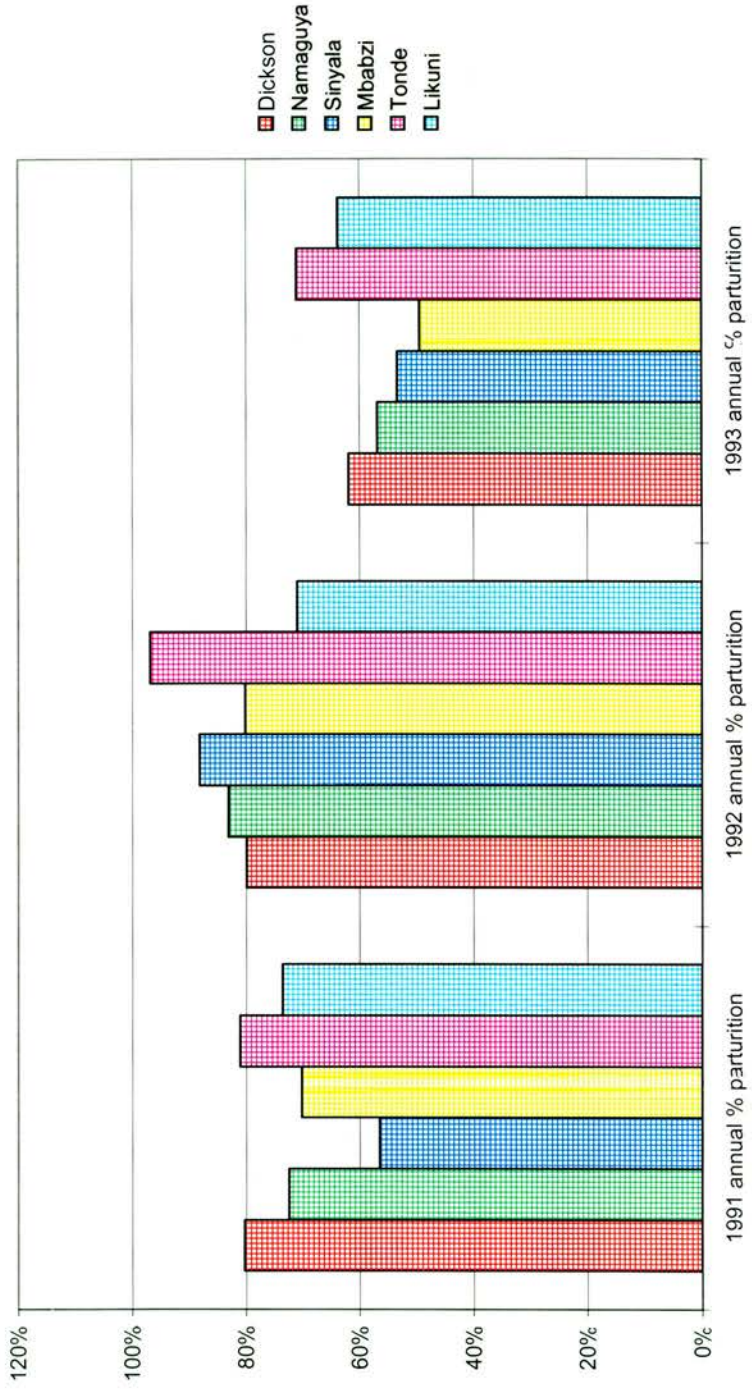


Figure 6.1 Annual percentage parturition rate for each monitored area for 1991 to 1993

Figure 6.2

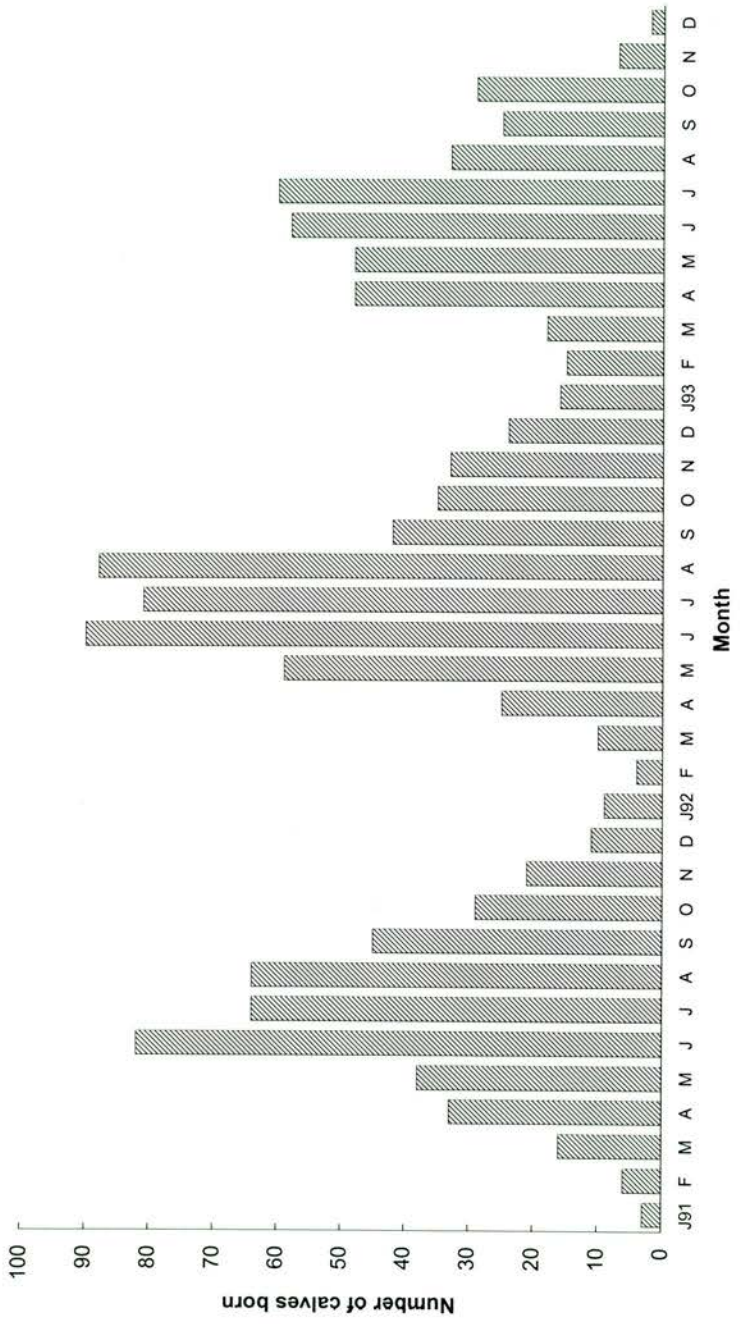


Figure 6.2 Calving pattern of all monitored cattle for 1991 to 1993.

Figure 6.3

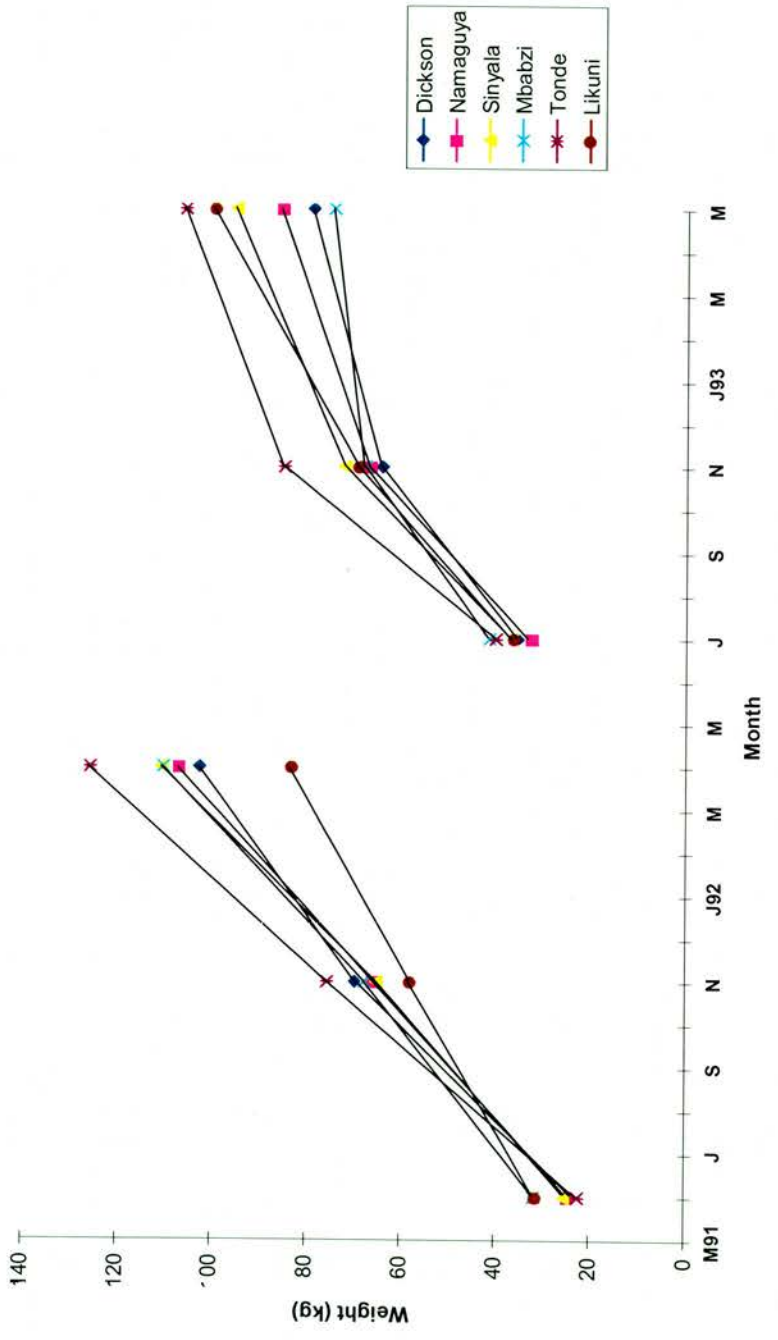


Figure 6.3 Growth curves for cohorts of calves born in 1991 and 1992.

In 1993 the 57 calves whose dams were not milked for human consumption grew faster (MMWG of 5.4 kg) than the 36 whose dams were milked (MMWG of 4.8 kg) but the difference was not significant ("t" test, $p>0.05$). Mean monthly weight gain per undipped calf in 1991 showed no significant correlation between weight gain and *Boophilus* species or *R. appendiculatus* numbers. There was however a significant negative correlation (Pearson correlation, -0.482, $p<0.01$) between weight gain and adult female *Amblyomma* numbers.

MORTALITY RATES

Mortality and morbidity rates seen in this study are given in Tables 6.1, 6.2 and 6.3. Calf mortality rates were below 20 % in dipped cattle during 1991 and 1992 but rose to between 30 and 50 % in 1993. Calf mortality at Tonde was 30 % in 1991, 20 % in 1992 and 40 % in 1993. At Likuni the calf mortality rate climbed steadily from 10 % in 1991 to 115% in 1993. Adult and young stock mortality rates were less than 10 % in all tanks for the three years of the study except for Mbabzi in 1993 and Likuni in 1992 and 1993 where it rose to 12-18 %. Deaths due to tick-borne disease were rare in dipped cattle of all ages. The TBD mortality rate was never greater than 6 % in dipped cattle, and in most dipped areas in most years there were no deaths attributed to TBD.

Table 6.1 Total mortality by age 1991

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
CONFIRMED	5	4	8	9	24	6
ANIMAL YRS	47	46	47	70	76	54
RATES	10.8%	8.6%	17.0%	12.8%	31.4%	11.0%
YOUNG STOCK						
CONFIRMED	4	1	1	0	5	2
ANIMAL YRS	75	51	59	68	88	26
RATES	5.4%	2.0%	1.7%	0.0%	5.7%	7.6%
ADULTS						
CONFIRMED	7	3	8	5	6	6
ANIMAL YRS	218	165	163	201	223	176
RATES	3.2%	1.8%	4.9%	2.5%	2.7%	3.4%

Table 6.2 Total mortality by age 1992

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
DEATHS	5	3	4	3	19	21
ANIMAL YRS	71	55	56	77	102	43
RATES	7.0%	5.5%	7.1%	3.9%	18.6%	49.2%
YOUNG STOCK						
DEATHS	0	2	4	2	4	7
ANIMAL YRS	105	86	83	120	125	46
RATES	0.0%	2.3%	4.8%	1.7%	3.2%	15.2%
ADULTS						
DEATHS	12	4	8	11	4	11
ANIMAL YRS	200	147	127	170	202	111
RATES	6.0%	2.7%	6.3%	6.5%	2.0%	9.9%

Figures are adjusted for Butalex use in 8 cases at Likuni

Table 6.3 Total mortality by age 1993

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
DEATHS	24	23	18	31	53	36
ANIMAL YRS	69	55	54	59	117	31
RATES	34.9%	42.2%	33.6%	52.6%	45.2%	114.6%
YOUNG STOCK						
DEATHS	3	4	4	19	5	7
ANIMAL YRS	130	100	97	118	147	55
RATES	2.3%	4.0%	4.1%	16.0%	3.4%	12.8%
ADULTS						
DEATHS	5	9	10	18	12	16
ANIMAL YRS	169	124	106	123	202	85
RATES	3.0%	7.3%	9.5%	14.6%	6.0%	18.9%

In the ECF-stable area of Tonde the TBD mortality rate in calves varied from 11.2 % (1991) to 7.5 % (1993) with the adult and young stock TBD mortality rate between 0 and 2 %. At the ECF unstable area of Likuni the TBD calf mortality rate rose from 0 % in 1991 to 61 % in 1993. The young stock TBD mortality rate peaked in 1992 at 15.2 % and adult TBD mortality rates were low for all three years (maximum 4.2 %). Of the 99 TBD deaths recorded during the 3 years of the study, 86 were caused by ECF.

OXEN AND MILK FOR HUMAN CONSUMPTION

Figure 6.4 shows the herd structure for all monitored animals in November 1992. Approximately 30 % of the 641 cows in the study were milked for human consumption with an average daily yield of 600 ml. Approximately 50 % of the 568 male animals over one year were used as work oxen.

OFFTAKE RATES

The annual adult male culling rate for all animals in the study was 25 % in 1991, 41 % in 1992 and 103 % in 1993. The annual adult female culling rate was 10 % in 1991, 13 % in 1992 and 36 % in 1993. The mean sale price of animals sold or bought during 1993 was: breeding males MK754 (n=16), breeding females MK506 (n=100), replacement females MK435 (n=39), mature surplus males MK671 (n=130) and calves MK260 (n=3).

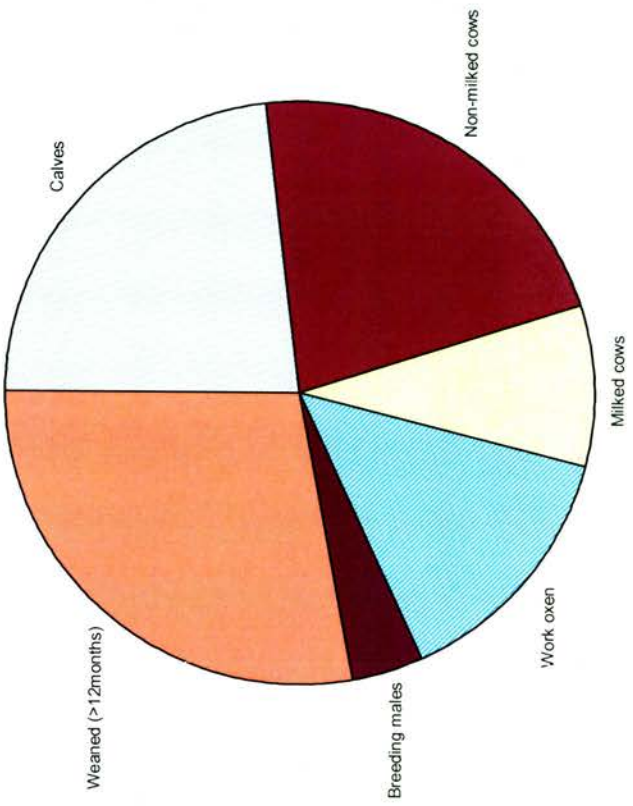


Figure 6.4 Herd structure of all monitored cattle in November 1992.

OUTPUT

The economic output in each area for each year is shown in Figure 6.5 and a breakdown of the output for Dickson is shown in Table 6.4. In 1991 all the areas had similar outputs of approximately MK350/CCU (US $53.8). In 1991 Namaguya had the highest number of animals per CCU (2.96) and Mbabzi had the lowest (2.83). In 1992 the output at Likuni fell to MK250/CCU and fell again in 1993 to MK160/CCU. In 1993 the output in the other five areas dropped slightly compared with 1991 and 1992 to a median of MK304/CCU. The number of animals per CCU remained stable for all areas in 1992 and 1993 at just over 2.9.

FINANCIAL ANALYSIS OF TICK-BORNE DISEASE CONTROL STRATEGIES

Table 6.5 shows the output adjusted for a common annual parturition rate of 73 % (the rate for all animals in the study) and an underlying non-TBD mortality rate of 19.7 % in calves, 3 % in young stock and 4 % in adults. The parameters used in the financial analysis spread sheet are shown in Table 6.6 and the results of the analysis are illustrated in Figure 6.6.

The calculated cost of vaccination against ECF was MK 34 for a calf and MK 65 for an adult. This rises to MK 42 and MK 73 respectively if there is only a 25 % up-take within an area. The lowest periodic average cost per head per year after 10 years

Figure 6.5

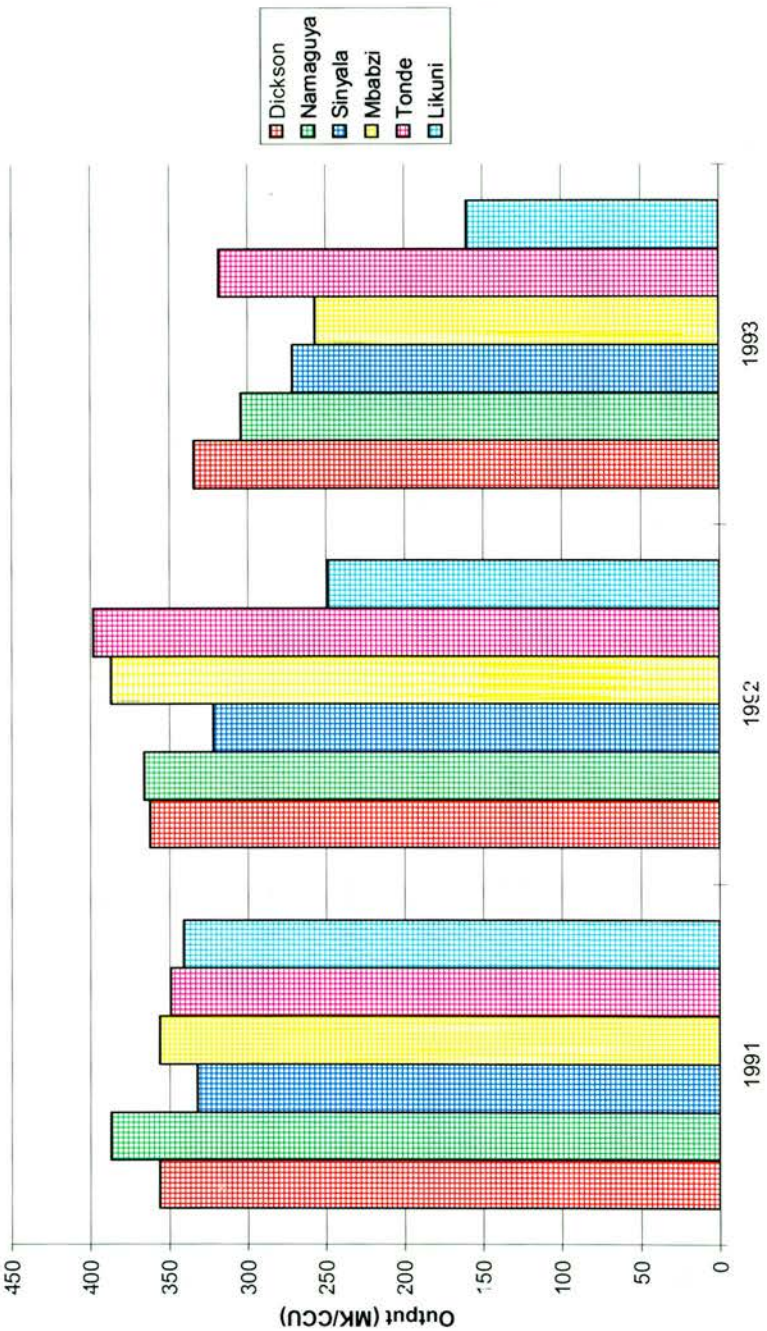


Figure 6.5 Economic output of each monitored area for 1991 to 1993.

Table 6.4 Output for Dickson

Values in Malawi Kwacha per carrying capacity unit (C.C.U.)

	1991	1992	1993
Breeding female culls	41.90	29.40	216.40
Breeding male culls	11.80	17.80	117.80
Mature surplus animals	259.20	280.30	85.20
Milk	20.00	18.90	19.70
Salvage value of deaths	22.80	15.60	30.10
Purchase of replacement females	0.00	0.00	-103.20
Purchase of replacement males	0.00	0.00	-31.70
Net total	355.70	362.00	334.30
Number of animals per C.C.U.	2.94	2.93	2.94
Net total per animal	120.99	123.55	113.71

Table 6.5 Value of output adjusted for common annual parturition rate and non-TBD mortality rate

	Output (MK/CCU)	Animals per CCU
Dipped areas 1991-1993	334	2.94
Tonde 1991-1993	326	2.93
Likuni 1991	326	2.95
Likuni 1992	262	2.93
Likuni 1993	272	2.88

Table 6.6 Parameters used in financial analysis of tick-borne disease control strategies

GENERAL		**CESSATION OF DIPPING**	
		Losses compared with endemically stable area:	
Total herd size	1000	Year 1	0.80 *d*
Breeding cows (%)	31	Year 2	34.20 *d*
Calving rate (%)	73	Year 3	16.90 *d*
Discount rate (%)	10	Year 4	10.00 *c*
DIPPING		**ECF IMMUNIZATION**	
Dip capacity (litre)	15000	Stabilate Cost Per Dose (MK)	11.9 *e*
Maximum period between rechargings (years)	1	Tetracycline cost per 100ml (MK)	135 *f*
No. Head dipped between rechargings	25000	Tetracycline dosage (ml/10 kg)	1
Labour cost / recharging	80		
		Mean liveweight of cows (kg)	300
Name of acaricide product	Supona 30	Mean liveweight of followers (kg)	180
Concentration (units product/1000 l water)	1.67	Mean liveweight of calves immunized (kg)	80
Replenishment (units product/1000 l water)	2.86		
Cost of product / unit (MK)	68 *a*	Professional fees: visit charge (MK)	150
Dip construction cost (MK)	50000 *b*	Professional fees: per animal immunized (MK)	2
Year number for reconstruction	1		
Maintenance overhead cost / year (MK)	4000 *b*	Number calf vaccination visits per year	2
		Number follow-up visits per year	2
Labour cost per 1000 head dipped (MK)	8		
Management cost per dipping day (MK)	20	Percentage adverse reactors (vet. treatment)	3 *c*
Water loss (litre/dipping)	1.5 *c*	Average treatment cost for reactor (MK)	230 *g*

NOTES

a = Actual cost of 30,000 purchase by Malawi government in late 1993, corrected for currency fluctuations.

b = Estimate based on Lilongwe ADD figures for 1990 to 1993.

c = Estimate

d = Table 1.

e = F.A.O. tick borne disease vaccine unit quote Feb 94.

f = Malawi pharmacy quote Feb 94.

g = Estimate based on Malawi Pharmacy quote for Butalex Feb 94.

Figure 6.6

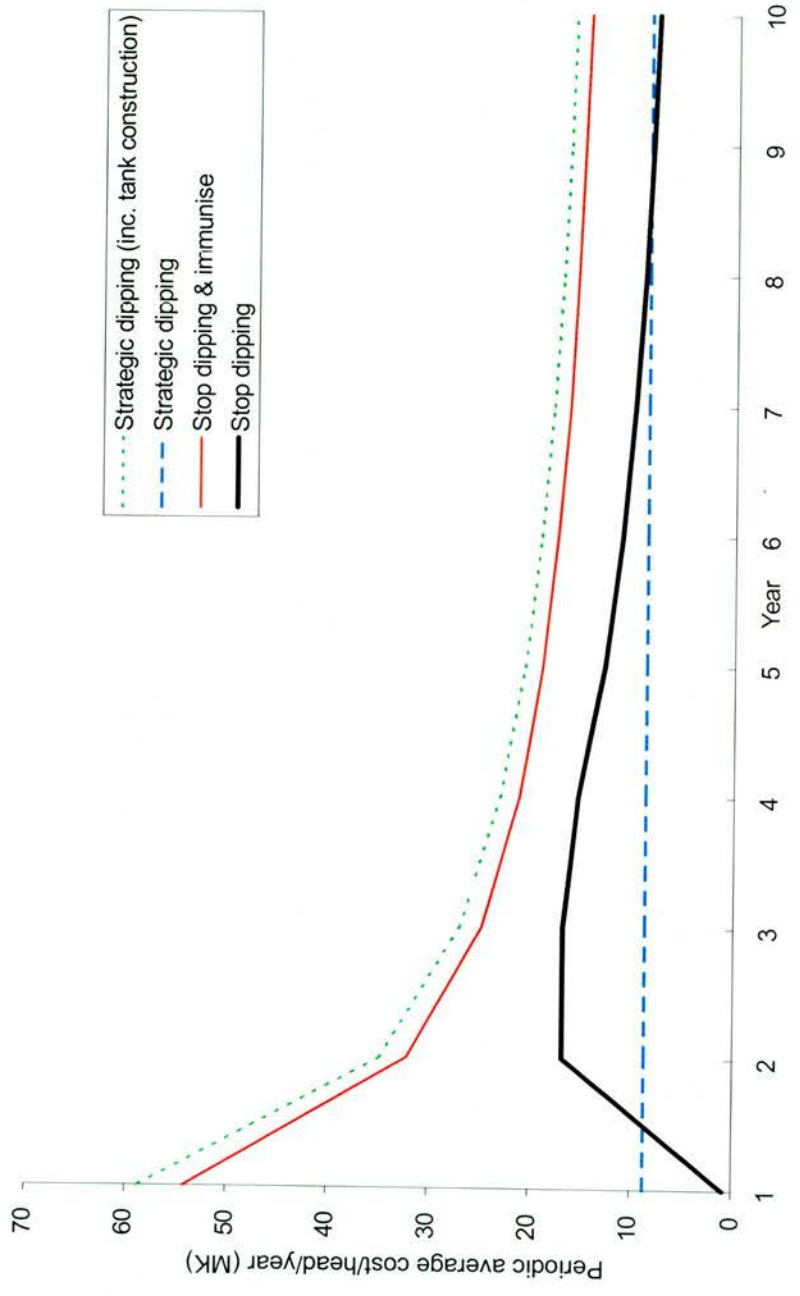


Figure 6.6 Relative costs of ECF control

would be achieved by a cessation of dipping and allowing endemic stability to establish naturally. The cost of this option would be MK7.9/head/year (an average of MK7.9/head for each of the 10 years). Over the same period strategic dipping where no tank construction was needed would cost MK8.66/head/year and stopping dipping coupled with vaccination against ECF would cost MK14.64/head/year. The most expensive option at MK16.06/head/year would be strategic dipping where tank construction was necessary.

DISCUSSION

HERD PRODUCTIVITY

The calving rates seen in this study were similar to those seen in extensively herded and grazed, traditionally managed zebu cattle in Zambia by Perry, Mwanaumo, Schels, Eicher and Zaman (1984) although the breeding female to breeding male ratio was considerably lower than that seen in Zambia. Calf growth rates were slightly lower than for Malawi zebu reared at livestock improvement centres (MMWG of 9.7 kg - Faulkner and Epstein, 1957) but considerably higher than for zebu calves on Rusinga island, Kenya (MMWG of 3.7 kg - Latif, Rowlands, Punyua, Hassan and Capstick, 1995). Depression in growth rates due to milking of dams and the feeding of *A. variegatum* was not unexpected, and has been seen in similar situations (Stobbs, 1967, Pegram, Lemche, Chizyuka, Sutherst, Floyd, Kerr and McCosker, 1989, Pegram and Oosterwijk, 1990). However the significance of the *Amblyomma* effect should not be overstated. The strategic dipping used in this study was designed to control *R. appendiculatus* and ECF only. In years two and three, when there was no dry season dipping, the *A. variegatum* infestation rates were similar in all groups (Chapter 3).

Draft power and manure contribute a significant proportion of the total economic output from cattle in Malawi. In this study no differences between the dipped and undipped groups were likely. LPEC bases its calculations on a fixed feed resource and this results in a fixed manure output. This would only fall if the number of

animals in an area decreased and not all the feed was utilised. This is an unlikely situation in Malawi where one of the most important limiting factors on cattle production is food availability. TBD morbidity and mortality rates in dipped and undipped adults were very similar, and therefore draft production lost due to TBD would have been similar in both groups. However the study only ran for 3 years which is unlikely to have been long enough to see the full effect on the disease situation in adult animals.

Milk production for human consumption in this study (30% of cows milked with an average yield of 660 ml) was very similar to that seen by Perry, Carter, Hill and Milne (1987) where 37% of traditionally managed Mashonaland type cows were milked with an average yield of one litre per day.

TBD CONTROL STRATEGIES

Following the conclusion of this study in 1994, government policy for areas of Malawi that have endemic ECF was changed to nine immersions per year, every two weeks, during the rainy season. No new dip tanks are to be constructed, partly because this study has shown them to be too expensive. Dipping will cease when the tanks come to the end of their serviceable life, or before if government decides to stop funding them. Responsibility for tick and tick-borne disease control will then presumably move to the cattle owners. The effectiveness of strategic tick control depends on a high proportion of cattle in an area being dipped, and is therefore

unlikely to work under a cost-recovery system.

Vaccination against ECF would prevent serious losses occurring following the cessation of dipping but as yet lacks a robust delivery service and is a novel technology to most farmers. The very high initial cost in the first year, due to the need to vaccinate all animals, would be a deterrent to use by farmers accustomed to a low level of ECF mortality while dipping. It may be that demand for vaccine would come two to three years after the cessation of dipping, when high mortality would be seen. However by this time the establishment of endemic stability would be well advanced, and some farmers might choose to do nothing and allow stability to develop naturally. Vaccination of calves may be attractive in marginal areas where endemic stability was prone to failure due to annual variations in weather patterns and consequent changes in tick populations (Chapter 3).

The estimated cost of ECF vaccination in this study was in excess of 10 % of the animals' market value. It is difficult to envisage farmers operating a low input system paying so much for vaccination. If there is to be vaccine use, it is likely to be in conjunction with the upgrading of cattle and a general increase in the value of inputs and outputs.

The most cost-effective option (MK 7.9/head/year over 10 years) of stopping dipping and accepting mortalities while endemic stability becomes established is also the least technically demanding. Although this is financially the most cost-effective

option the social and political ramifications should not be ignored.

The most likely scenario is that individual farmers will make choices based on their own situation and various control strategies will be practised. The extension services will need to provide relevant information to allow informed decision making.

CHAPTER 7

SEROCONVERSION TO *COWDRIA RUMINANTIUM* OF MALAWI ZEBU CALVES, REARED UNDER DIFFERENT TICK CONTROL STRATEGIES

A.W. Soldan, T.L. Norman, S. Masaka, E.A. Paxton, R.M. Edelsten, and K.J. Sumption (1993) Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux, **46**, (1-2), 171-177

ABSTRACT

The seroconversion by indirect ELISA to *Cowdria ruminantium* over the first year of life of sixty-six Malawi zebu calves born into groups which were dipped 17 times per year was compared to seroconversion of 32 calves born into non-dipped groups. *Amblyomma variegatum* tick counts and clinical disease in each group of cattle were monitored throughout the study period. No cases of heartwater were seen in either group of calves over the first 22 months of life. Only one case of heartwater was observed, in an eight-year-old cow, in the 1,800 intensively monitored cattle over the same period. By 12 months of age almost all undipped calves had seroconverted and 50% of seroconversions were attributed to nymphal challenge. In contrast, only 41% of calves had become seropositive by 12 months of age in the dipped groups. The dipping regime used therefore significantly decreased seroconversion rates to

C. ruminantium in these calves. 73% of calves had detectable levels of maternal antibodies to *C. ruminantium* in the first 4 weeks of life. Antibody levels in each of the calves in dipped groups had waned to below the cut off point for the ELISA by eight to 12 weeks. Seroconversion did not occur in the first eight to 12 weeks of life in dipped herds. The indirect ELISA test results were not significantly different in the proportion positive in single tests at 12 months of age, or by cumulative test results of the previous nine months, and therefore the test may be of value as a test of herd immunity. It is concluded that a state of enzootic stability exists to *C. ruminantium* in undipped Malawi zebu cattle in the study area, which is characterised by a high innate resistance to the infection and seroconversion of the majority of the calves born in May-June to the agent between three and nine months of age.

INTRODUCTION

The vector of *Cowdria ruminantium* in Malawi is considered to be *Amblyomma variegatum,* which has a widespread distribution in the country, except for lowland areas of southern Malawi such as the Lower Shire Valley (Du Plessis et al, 1984). Heartwater has been frequently reported in unprotected *Bos taurus* cattle, but the disease is seldom reported in indigenous Malawi zebu cattle *(Bos indicus).* The number of taurine cattle in Malawi is very low (less than 20,000) in comparison with numbers of Malawi zebu in the national herd of 800,000 (Anon, 1991). The status of traditionally managed Malawi zebu to *Cowdria ruminantium* is not known, although a state of enzootic stability and/or genetic resistance in indigenous ruminants to the infection, is considered to be present in *Amblyomma variegatum* infested areas. The occurrence and timing of seroconversion to *C. ruminantium* in relation to *Amblyomma* infestations in cattle is important to understanding the nature of infection in both undipped and dipped cattle populations.

Dipping to control tick-borne disease was first made compulsory in certain areas of Malawi in the early 1920's (De Meza, 1925, cited in Mares, 1973). Although current legislation provides for weekly dipping of cattle in arsenic trioxide it is estimated that only 20-40% are dipped regularly. A survey of cases presented to veterinary assistants at dip tanks in north and central regions showed that ECF morbidity varied between 0.5 and 1.8% (all ages); clinical cases of babesiosis and anaplasmosis were rare and heartwater was not recorded. The dipping attendance over the year was only

50% and therefore the low ECF morbidity was not attributed to the suppression of ticks through dipping (Edelsten, 1990).

A three-year trial commenced in 1990, which undertook to investigate the effect of reduced intensity dipping and non-dipping in traditionally managed cattle, upon morbidity, mortality, productivity and economic indicators. Seroconversion to *C. ruminantium* in cohorts of calves born during this trial into study herds at six dip tanks is reported here.

An indirect ELISA test was used to test serum samples for antibodies to detergent soluble antigens extracted from the purified elementary body of *C. ruminantium* (Sumption, Masaka and Paxton, unpublished results). The significance of positive test results in immunofluorescent antibody (IFA) tests upon sera from animals from areas where *Amblyomma* ticks are present is unclear, because positive reactions have been observed in IFA tests with sera from some *Amblyomma* free areas (Du Plessis et al, 1987). The latter results are presumed to be the result of cross-reactions caused by antibodies to *Ehrlichia* species, because serological cross-reactions in IFA tests have been observed between antisera raised to various *Ehrlichia* species and *C. ruminantium* antigens in infected mouse macrophages (Du Plessis et al, 1987) or neutrophils (Jongejan et al, 1989 and Logan et al, 1986). The indirect ELISA used to test sera from Malawi utilised detergent soluble elementary body antigens, because a number of cross-reactive antigens to *E. phagocytophila* and *E. ondiri* were removed

during the detergent extraction process; the ELISA test consequently has a low level of detection of antibodies to these pathogens in comparison with IFA using *Cowdria* infected goat neutrophils or infected endothelial cells (Sumption and Paxton, unpublished). The presence of *E. ondiri* or *E. bovis* has not been demonstrated in Malawi, and therefore antibody reactions in this study are assumed to be to *C. ruminantium.*

MATERIALS AND METHODS

LOCATION OF STUDY AREA

Malawi is located between 9°-17° South and 33°-36° East in Central Africa. As part of a larger study, six dip-tanks in the same ecological zone were chosen in the Lilongwe area. This area is on the Central African Plateau with an undulating, almost flat topography about 1000m above sea level. Four of the tanks chosen were in good repair and had been using arsenic trioxide up to the start of the study in November 1991. Cattle at two dip tanks which were to act as non-dipped controls had effectively not been regularly dipped as a result of tank disrepair, or because there was a large group of farmers who did not dip their cattle.

ORGANISATION OF STUDY

Approximately 300 animals at each of the six tanks were tagged in November 1990. Each of the cattle were Malawi zebu and belonged to small holders, and were communally grazed. No alteration in management was instituted and no prophylactic treatments were given during the trial. Dipping was carried out in four tanks at two-weekly intervals in the rainy season (December 1990 to March 1991 and December 1991 to March 1992) and at four-weekly intervals through the dry season (April 1991 to November 1991). Dipping at two tanks was in chlorfenvinphos (Supona 30, Shell Chemicals Ltd.) and at the other two in amitraz (Triatix TR, Coopers Animal Health).

Acaricide concentrations and replenishment were as recommended by the manufacturers, and the total replacement method was used for amitraz. Dipping of cattle was not carried out at the two control tanks. An active disease monitoring system was set up with the aim of identifying the specific cause of each case of death or disease in cattle at each dip tank in the trial. The routine samples collected from dead animals were faeces, blood, lymph node, spleen, and brain crush smears. These were examined by staff of the protozoology section of the Central Veterinary Laboratory and a project veterinary officer. The veterinary assistant associated with each dip tank visited owners with tagged cattle every week, and project staff visited every two weeks throughout the study period.

CALF COHORT STUDY

The peak calving season in the Lilongwe area occurs between May and July each year. As part of a productivity study 15 calves born in May/June 1991 had serum samples collected at eight-week intervals with the first sample being collected at the first visit after birth. Samples were frozen at -20°C and aliquots for *Cowdria* serology were forwarded on ice by airfreight to the Centre for Tropical Veterinary Medicine, Edinburgh, where they were tested by indirect ELISA. Chi-squared tests were used to compare proportions.

INDIRECT ELISA FOR THE DETECTION OF ANTIBODIES TO *C. RUMINANTIUM*

An indirect ELISA developed at the CTVM was used to test sera at a dilution of 1 in 50. The ELISA uses soluble antigens extracted from the elementary body (EB) stage of *C. ruminantium* (Welgevonden stock) following release from cell cultures, and has an extremely low reactivity to antibodies present in antisera raised to *Ehrlichia phagocytophila,* in comparison with immunofluorescent antibody tests (IFA) using Welgevonden infected neutrophils or infected endothelial cells. It also has an excellent sensitivity in the detection of experimentally infected animals (Sumption and Paxton, unpublished results*). Cross-reactions with *Ehrlichia* spp. present considerable difficulties in the interpretation of IFA tests for heartwater (Du Plessis et al, 1987 and Du Plessis et al, 1992). The indirect ELISA was developed using detergent soluble fractions of the *Cowdria* elementary body because these fractions have a reduced number of antigens with cross-reactivity to *E. phagocytophila* and *E. ondiri* sera, than is found in whole EB or infected cell preparations (Sumption and Paxton, manuscript in preparation). Soluble antigen is prepared from EB's semipurified from culture medium by centrifugation for 20 min at 1,000 g for the pelleting of endothelial cells, followed by centrifugation at 10,000 g for the pelleting of EB's. Pellets were washed in sterile phosphate buffered saline (PBS, pH 7.4), and re-centrifuged at 10,000 g for 20 min. The procedure was repeated two times,

* Sumption K.J., Paxton E.A., and Bell-Sakyi L (2003) Development of a polyclonal competitive Enzyme Linked Immunosorbent Assay for the detection of antibodies to *Ehrlichia ruminantium*. Clinical and Diagnostic Laboratory Immunology. **10** (5), 910-916

followed by detergent lysis of the elementary body in 0.5% nonidet NP40 and 0.5% sodium deoxycholate in 50 mM Tris-HCL (pH 8.0), 2 mM EDTA, 150 mM sodium chloride and 1 mM phenylmethyl-sulfonylfluoride for 2 min at room temperature followed by rapid passage through a 26 g needle to disaggregate elementary bodies, and incubation at 37°C for 30 min. After a further round of needle passage, insoluble antigen was removed by centrifugation at 4°C for 30 min at 16,000 g. Soluble antigen extracts were then stored at -20°C until used in ELISA. Antigen was diluted in 0.05M carbonate-bicarbonate buffer (pH 9.6) and 100 μl added to each well of 9 well immunoplates (Immulon II, Dynatech Laboratories) and incubated overnight at 4°C. Plates were then washed 5 times in PBS diluted 1:4 in distilled, de-ionised water which contained 0.05% Tween 20. Serum samples were diluted to 1 in 50 in PBS containing 0.05% Tween 20 (PBST) and 100 μl added to duplicate wells and incubated for 60 minutes at 37°C in a shaking incubator. Plates were then washed as before and 100 μl of rabbit anti-bovine IgG horse radish peroxide conjugate (Sigma Chemical Company) added to each well and incubated for 60 min at 37°C. Plates were then washed and 100 µl orthophenylene diamine (OPD, 0.4 mg/ml) and hydrogen peroxide (0.015%) added to each well and incubated for 6 min at 20°C. 100 μl of IM sulphuric acid was then added to each well to stop the reaction and the optical densities recorded at 492 nm in a Titertek Multiscan Spectrophotometer. Four positive controls, two negative controls and two blank wells (no serum) were used per plate.

TICK COUNTS

Half body counts of *Amblyomma variegatum* adults and nymphs were conducted every 4 weeks at each dip tank on 5 animals between the ages of 6 and 12 months. Individual animals were not counted more than once in the study period and each animal, on any one date, came from different farmers. In dipped cattle, tick counts represent maximal burdens as they were carried out immediately prior to dipping.

RESULTS

DISEASE

A laboratory confirmation of diagnosis was reached in over 80% of deaths occurring in tagged cattle during this study. East Coast Fever was the only tick-borne disease observed in both dipped and undipped cattle. No cases of heartwater were observed between May 1991 and June 1992. A significant difference was not observed in disease mortality and tick numbers between cattle in herds in which a regime of chlorfenvinphos or amitraz was used. Dipped cattle are therefore compared to non-dipped cattle populations for the comparison of seroconversion.

DIPPING PERCENTAGES OF CATTLE

For tagged cattle the average dipping percentage was 83% and for untagged cattle attending the same tanks it was 41%. Tagged cattle made up between 10 and 22% of the cattle population for each tank.

TICK COUNTS

Figure 7.1 shows the relationship between *A. variegatum* adults and nymphs in undipped cattle. Infestations of cattle showed a highly seasonal distribution, with peak numbers of nymphs in August and September, followed by peak numbers of adults in November and December. Nymphal activity was from March to November

Figure 7.1

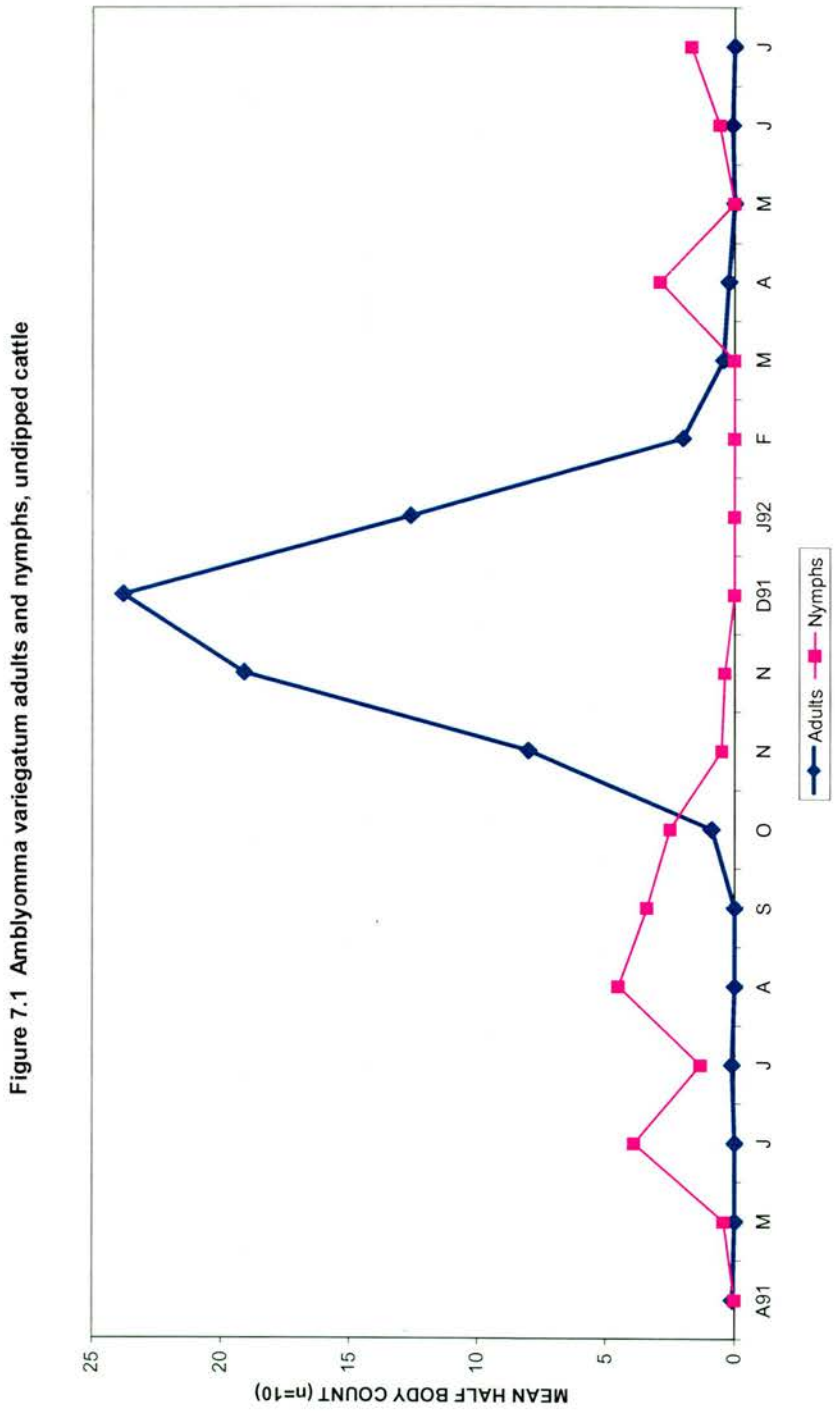


Figure 7.1 Amblyomma variegatum adults and nymphs, undipped cattle

with adults present from October to February. Table 7.1 shows mean adult and nymph *A. variegatum* half body counts for various periods of the study.

DETERMINATION OF CUT-OFF VALUES FOR ELISA

Sera collected from calves in October-November 1991 and May-June 1992, from dipped and non-dipped groups, were tested in ELISA at a dilution of 1/50 and the frequency distributions of OD values was plotted (figures 7.2, 7.3, 7.4 and 7.5). The dipped calves in October-November had OD values which were characterised by a bimodal frequency distribution with a group of values less than 0.25 OD units, and a small number of sera with values greater than this value. A similar distribution was also observed for the same calves in May-June 1992 (figure 7.4), whereas non-dipped calves in October-November (figure 7.3) had a comparatively high proportion of values (18/29) greater than 0.25 OD and a single peak of values for sera collected in May-June (figure 7.5) of which a high proportion (15/16) were greater than 0.25 OD units. The low values (less than 0.25 OD units) were assumed to represent a population of calves which had not seroconverted to heartwater, because dipped calves had very low tick counts from birth to the time of sampling in October-November. The presumed negative population had an expected skewed distribution which was characterised by a tail of high OD values; the latter was also observed for sera from heartwater free areas of Europe and the Caribbean (unpublished results). The chosen cut-off value of 0.25 was similar to that determined for sera from other parts of the world, and was chosen on the basis of OD values which separated the

Table 7.1 *Amblyomma variegatum* counts on 6 to 12-month old calves for three periods of the year.

	May-October 1991		November 1991 - February 1992		March - June 1992	
	non-dipped	dipped	non-dipped	dipped	non-dipped	dipped
Adults	0.2	0.1	13.1	3.3	0.2	0.0
Nymphs	2.7	1.0	0.2	0.1	0.9	0.9

Figures are mean half body tick counts for the three periods indicated, for 20 calves in the four dipped groups and 10 calves in the two non-dipped groups.

Figure 7.2

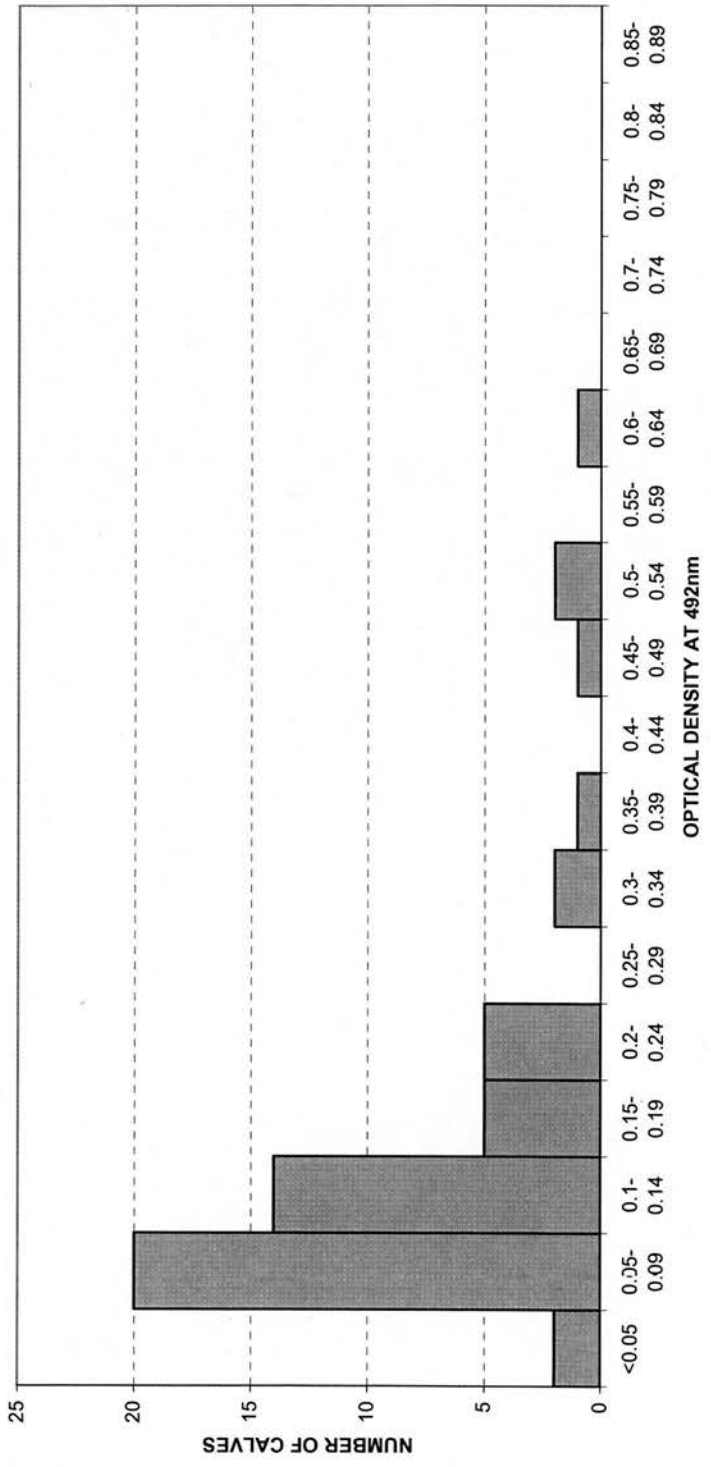


Figure 7.2 Indirect ELISA for antibodies to Cowdria. OD values frequency distribution for dipped calves sampled in October or November 1991

Figure 7.3

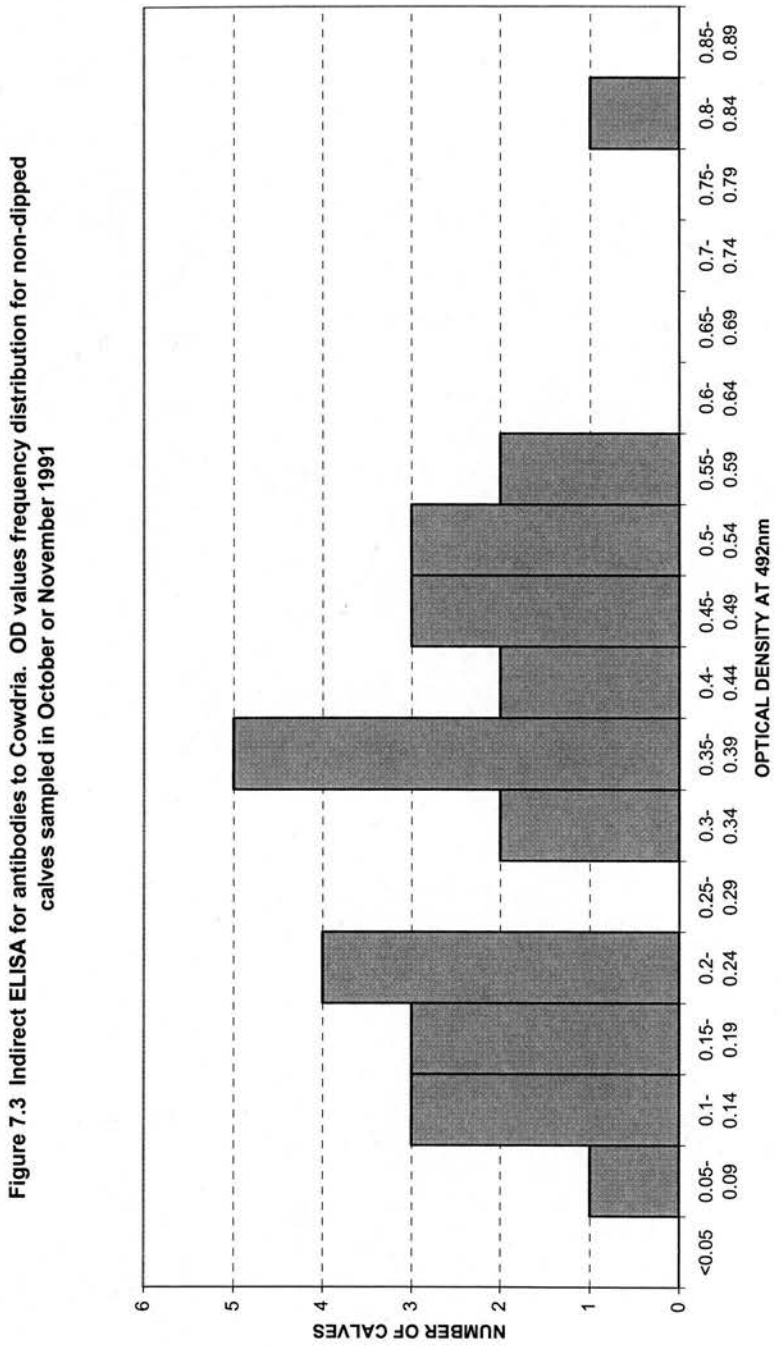


Figure 7.3 Indirect ELISA for antibodies to Cowdria. OD values frequency distribution for non-dipped calves sampled in October or November 1991

Figure 7.4

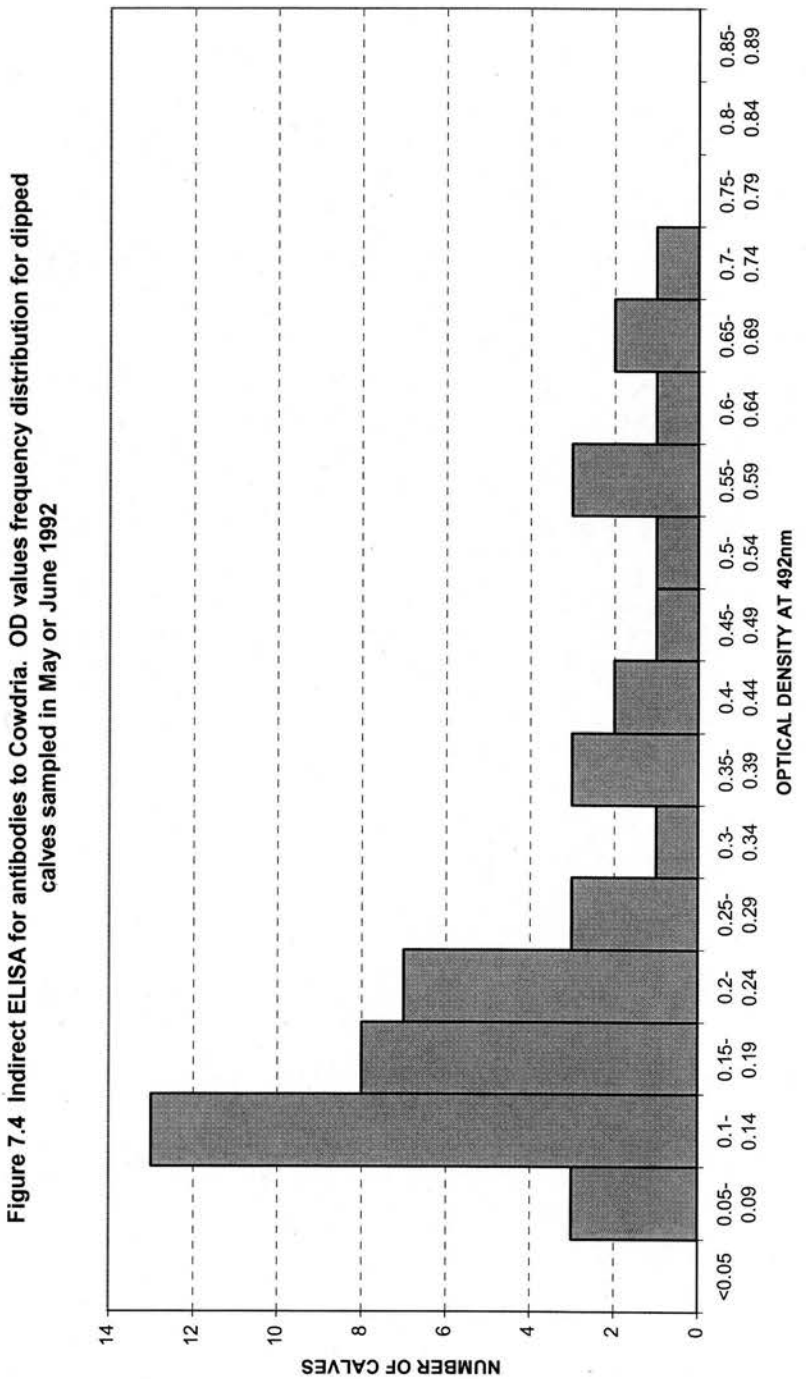


Figure 7.4 Indirect ELISA for antibodies to Cowdria. OD values frequency distribution for dipped calves sampled in May or June 1992
NUMBER OF CALVES
0
2
4
6
8
10
12
14
OPTICAL DENSITY AT 492nm
<0.05
0.05-0.09
0.1-0.14
0.15-0.19
0.2-0.24
0.25-0.29
0.3-0.34
0.35-0.39
0.4-0.44
0.45-0.49
0.5-0.54
0.55-0.59
0.6-0.64
0.65-0.69
0.7-0.74
0.75-0.79
0.8-0.84
0.85-0.89

Figure 7.5

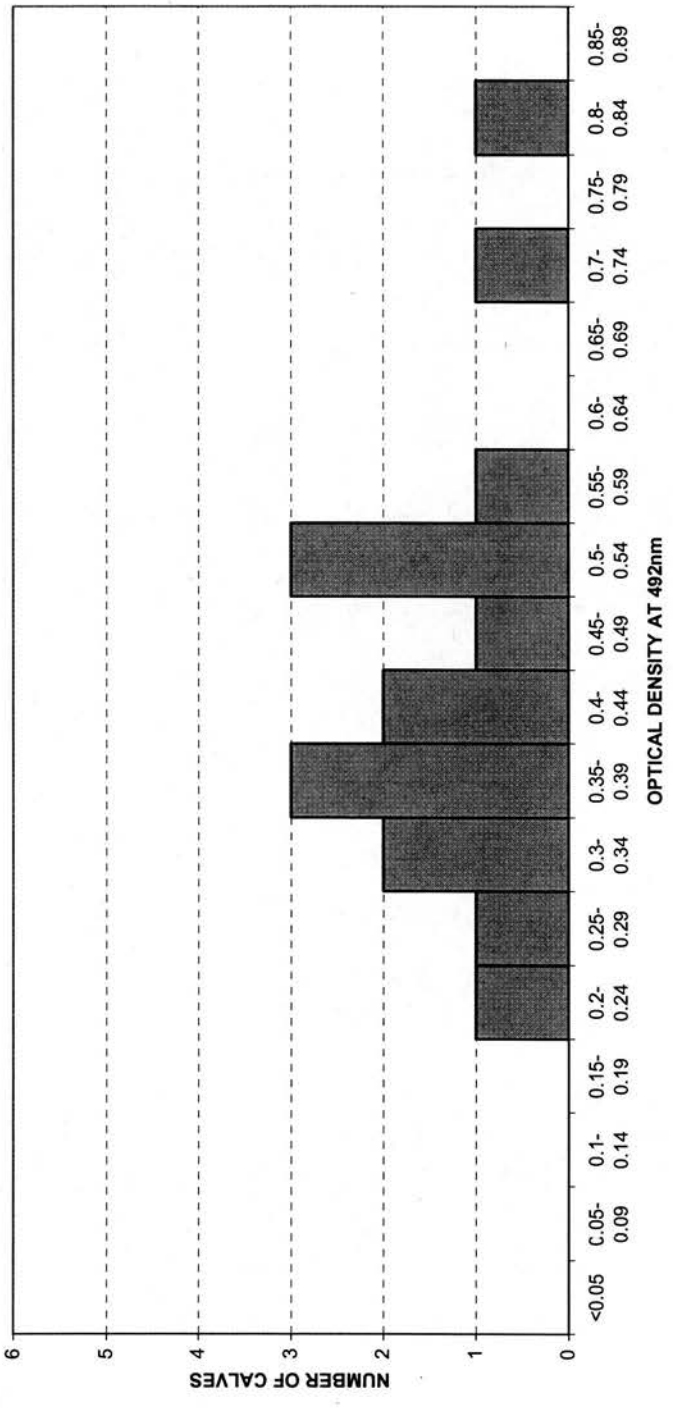


Figure 7.5 Indirect ELISA for antibodies to Cowdria. OD values frequency distribution for non-dipped calves sampled in May or June 1992

high and low OD value distributions, and from the baseline values observed following the decline in maternal derived antibody in dip trial calves.

MATERNALLY DERIVED ANTIBODY LEVELS IN CALVES

Twenty-nine of the forty calves (72.5%) which were sampled in the first four weeks of life were seropositive (table 7.2). This was considered to be the result of maternal antibody. The number of calves with detectable maternal antibody was slightly higher in the undipped group (10/12, 80%) than in the dipped group (19/30, 63%) but the difference was not significant ($P > 0.05$). No calves in dipped herds had a positive result in samples taken when they were aged between eight and 12 weeks. The chosen cut-off value of 0.25 was higher than the maximum value (0.247) observed (median = 0.113, n = 42) in calves eight to 12 weeks of age in dipped groups. Evidence of seroconversion was therefore considered to be the finding of an OD value greater than 0.25 in calves over 12 weeks of age.

SEROCONVERSION TO *C. RUMINANTIUM* IN COHORT CALVES

The proportion of calves considered seropositive by ELISA in samples collected in October 1991, and February and June 1992 (or in samples collected in the preceding month if no sample was taken in that month) is shown in table 7.3. There was a significant difference ($P < 0.01$) between seroconversion in dipped and undipped calves at all three periods of the year. After 12 months of the trial 96% (23/24) of

Table 7.2 Proportion of seropositive serum samples from cohort calves less than 16 weeks of age at the six diptanks in the trial.

Diptank	Regime	0 to 4 weeks	4 to 8 weeks	8 to 12 weeks	12 to 16 weeks
Likuni	non-dipped	1/2	3/5	2/10	3/4
Tonde	non-dipped	9/10	6/6	0/1	3/6
	Total non-dipped	10/12	9/11	2/11	6/10
Namaguya	dipped	2/3	1/13	0/8	0/11
Dickson	dipped	8/11	0/7	0/11	1/14
Mbabzi	dipped	6/9	2/15	0/5	1/10
Sinyala	dipped	3/5	2/12	0/3	1/12
	Total dipped	19/28	5/47	0/27	3/47
	Total all tanks	29/40 (73%)	14/58 (24%)	2/38 (5%)	*9/57 (16%)

Serum considered seropositive if the OD value exceeded 0.25 OD units relative to a positive control (Nyaga 2) at 1.053 OD units.

** 8 of the positives were from probable seroconversions.*

Table 7.3 Seroconversion to *Cowdria ruminantium* in calves born May/June 1991.

	October 1991		February 1992		June 1992	
	Spot	Cumulative	Spot	Cumulative	Spot	Cumulative
Dipped	3/55 (5%)	5/56 (9%)	17/49 (35%)	19/51 (37%)	15/42 (36%)	18/44 (41%)
Non-dipped	15/29 (52%)	15/29 (52%)	21/26 (81%)	23/26 (88%)	17/18 (94%)	23/24 (96%)

Results are given of single sample tests (spot tests) and cumulative seroconversion of the groups (including samples collected in the month of which results are stated).

undipped calves had seroconverted compared to only 41% (18/44) of dipped calves. Tagged cattle at these dip tanks were monitored for a further year but clinical cases of heartwater were not observed in cohort calves or other tagged cattle at the dip tank except for a single confirmed case in an 8-year-old cow.

The seroconversion of cohort calves was also determined from the results of the serial collection of serum samples, and the results are given as a cumulative seroconversion for the cohort (table 7.3). The proportion of calves considered seropositive was very similar by both methods ($P > 0.05$).

The highest OD value observed for serum samples from calves which seroconverted varied from 0.261 to 0.993 OD units, with a median value for 58 calves of 0.501. Optical density values for serum samples from ten of the 58 calves declined to below the cut-off value, between eight and 24 weeks after seroconversion. However, a decline to below the cut-off value was observed in only 3 of the 31 calves which seroconverted in the non-dipped groups which had received a higher tick challenge.

DISCUSSION

The undipped calves in this study had an almost continuous *Amblyomma* challenge through the first year of life (figure 7.1). For the first 5 months this was almost entirely of nymphae (table 7.1), and approximately 50% of the calves seroconverted to *C. ruminantium* in this period (table 7.2). The remaining calves seroconverted during the months of adult *Amblyomma* activity. At the end of one year of life almost 100% of undipped calves had seroconverted to *C. ruminantium.* In contrast the dipped calves had a significantly reduced level of seroconversion throughout the study period, and therefore the reduced intensity dipping had significantly affected exposure of calves to the agent of heartwater. Dipped cattle carried significantly lower tick burdens than undipped cattle and this was reflected in significantly lower seroconversion rates in dipped calves, which reached only 41% at 12 months of age. In the subsequent 10 months (June 1992- April 1993), heartwater was not observed in any of the dipped calves which had not seroconverted by June 1992, despite a suspension in dipping from April 1992 to November 1992. Seroconversion in the absence of clinical cases of heartwater was observed among the calves aged between 5 and 12 months in the non-dipped groups, between October 1991 and June 1992. Approximately 50% of the non-dipped calves seroconverted in this period. The results therefore suggest that Malawi Zebu are resistant to heartwater until at least one year of age. The observation of a case of heartwater in an 8 year old cow is surprising, because of the high seroconversion in non-dipped herds and the low dip attendance prior to the onset of the dip trial which would be expected to result in a

very high herd immunity of animals older than 2 or 3 years. Cases of heartwater in this age group have been attributed to relapse as a result of stress (Pullan, 1980). In addition, an increased susceptibility to immunisation with the Ball3 vaccine stock in animals older than 8 years has been observed (Van Der Merwe, 1979). The apparently high innate resistance of Malawi Zebu calves to the development of clinical heartwater suggests that a combination of factors may have been associated with clinical heartwater on this occasion.

The suppression of *Babesia bovis* seroconversion in the same calves by dipping during the dry season was considered to create an enzootically unstable situation and therefore at the end of the rains in March 1992, dipping was discontinued in favour of a strategy of non-dipping in the dry season. This strategy may be expected to increase nymphal *Amblyomma* infestations and seroconversion to *C. ruminantium* at 6 months of age in calves born in May-June 1992.

The finding that there was no significant difference between the proportion of dipped and undipped calves which had maternal antibodies is probably the result of similar exposure of cattle in these groups to *Amblyomma* ticks despite the statutory requirements which existed for dipping. This suggests that the dipping in arsenic trioxide which occurred before the trial in 4 of the groups resulted in a similar proportion of seropositive cows to the non-dipped groups; this may have occurred because of insufficient dip attendance or acaricide activity. The observation that

maternal antibody to *C. ruminantium* was not detected after 8-12 weeks of age is similar to that of Du Plessis et al (1992) who found that in 18 out of 21 calves born to naturally exposed dams, maternal derived antibody was not detected after 12 weeks of age, in IFA tests. Comparison of spot seroconversion rates and cumulative seroconversion rates (table 7.2) showed that there was no difference between the two methods. This suggests that this ELISA test may be of value in herd studies for the investigation of comparative seroconversion rates in the field, with serum samples collected at a single point in time. In this study, seropositive status continued for at least 8-10 months after seroconversion in the majority of calves which seroconverted, in the presence of intermittent or continuous tick challenge, and only 3 out of 31 calves exposed to high tick numbers in the non-dipped groups underwent a temporary reversion to a seronegative status.

CONCLUSION

In undipped Malawi Zebu cattle in the study area, *C. ruminantium* appears to be in a state of enzootic stability with high seroconversion rates in cattle and the absence of clinical disease. Malawi Zebu seem to have some degree of innate resistance to heartwater because calves not challenged in their first year showed no clinical disease under tick challenge in their second. The ELISA proved useful in determining comparative seroconversion rates in herds kept under different management regimes, and results of single sampling were not significantly different from serial sampling in the determination of seroconversion rates at a given point in time.

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CHAPTER 8

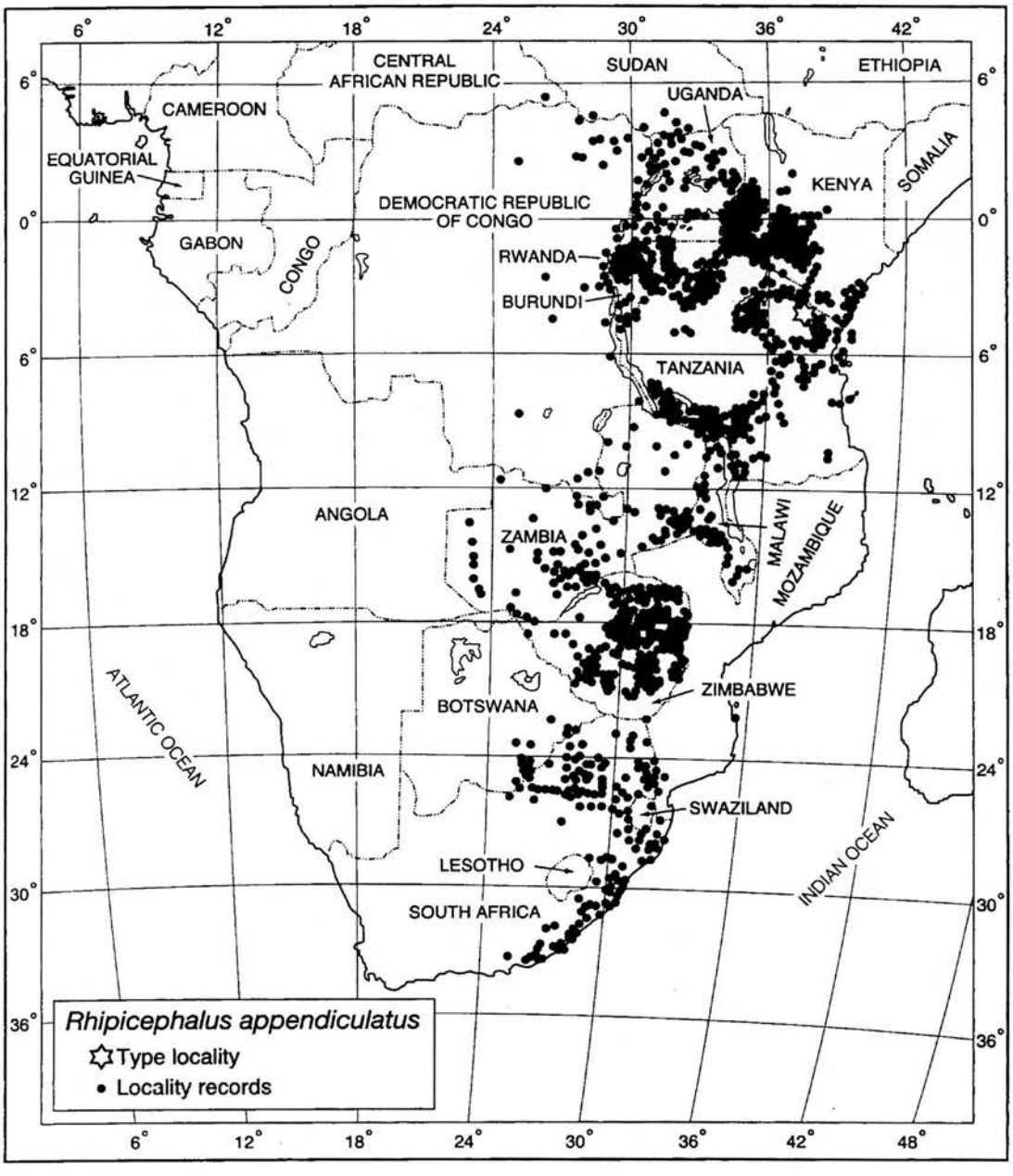
KEY LITERATURE PUBLISHED SINCE 1995

1.0 *THEILERIA PARVA*

1.1 RANGE

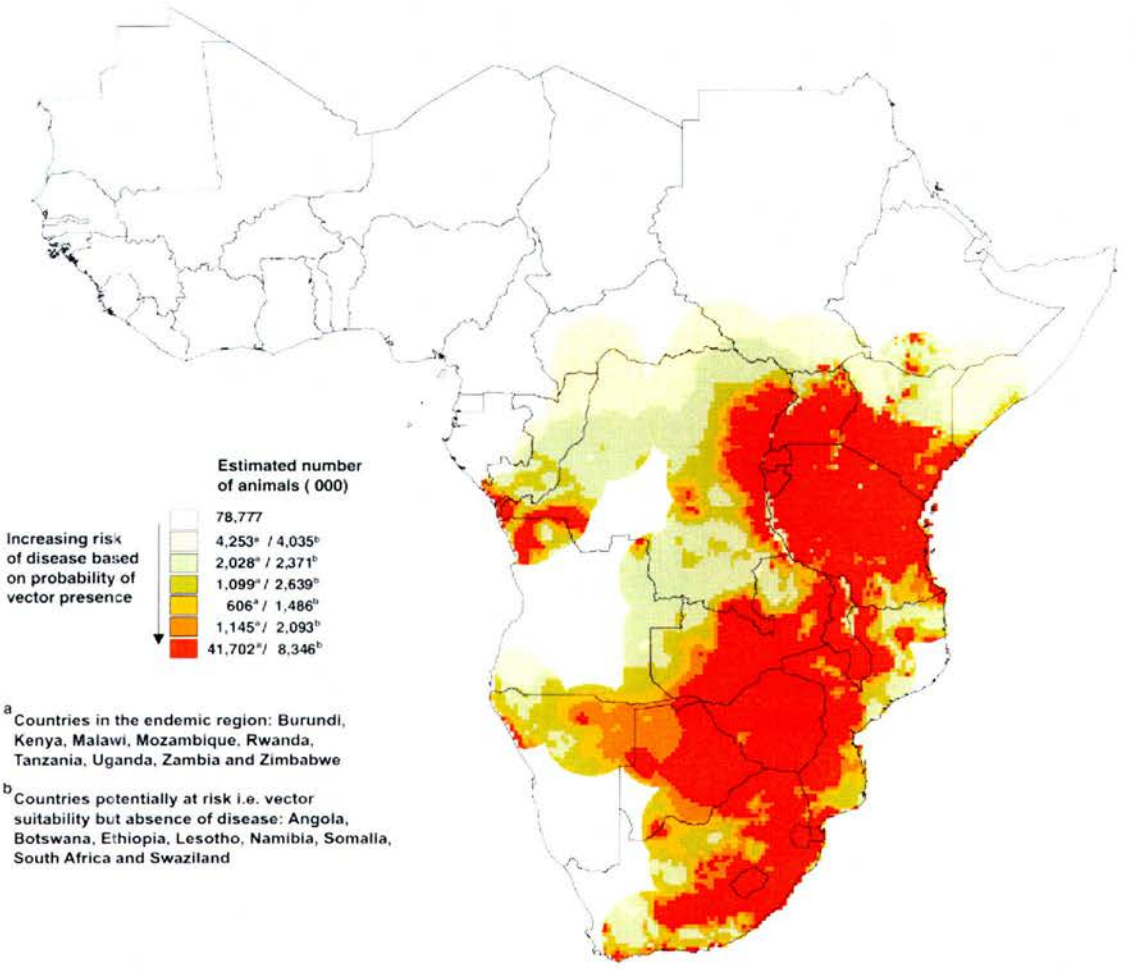
Figure 8.1, from Walker, Keirans and Horak (2000), shows the known range of *R. appendiculatus* in Africa. Figure 8.2, taken from Minjauw (2001), gives an indication of the potential distribution of East Coast fever based on the distribution of the known vector *R. appendiculatus* and implicated vectors *R. duttoni* and *R. zambeziensis*. These distributions are derived from tick records plus extensions into areas having potentially suitable habitats as defined mathematically.

Figure 8.1 Known range of *R. appendiculatus*



From Walker, Keirans and Horak (2000).

Figure 8.2 Potential distribution of East Coast fever (*T. parva*) vectors (*R. appendiculatus*, *R. duttoni* and *R. zambeziensis*)



From Minjauw B. (2001) published by Animal Health Programme, Department for International Development, Centre for Tropical Veterinary Medicine, University of Edinburgh, Easter Bush, Midlothian, Scotland.

1.2 DISEASE AND DIAGNOSIS

1.2.1 Molecular tests

Morzaria, Katende, Musoke, Nene, Skilton and Bishop (1999) review the molecular methods available for the diagnosis and study of *T. parva*. These methods can be used as sensitive diagnostic tools and to discriminate between strains of the parasite. Ogden, Gwakisa, Sawaï, French, Fitzpatrick, Kambaroge and Bryant (2003) describe the use of the polymerase chain reaction (PCR) to detect *T. parva* in Tansanian cattle and ticks.

1.3 SEROLOGY*

An ELISA for *T. parva* has been described by Katende, Morzaria, Toye, Skilton, Nene, Nkonge and Musoke (1998). The latter test showed a sensitivity of over 99% and a specificity of between 94 and 98%. It is likely that the publication by Muraguri, Gitau, Murangi, Mbago and Karinki (1999) is further validation of this test. The authors actually refer to the paper by Katende et al (1990) as a reference for the test being validated, however Katende (1990) refers to a test for *T. mutans*. They show that for the *T. parva* ELISA there is very poor agreement between the ELISA and IFAT (with a 1:40 cut-off point). They show a similar sensitivity between the two tests and a better specificity for the ELISA of 90% compared with 80% for the IFAT.

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* Cross reference chapter 2 section 1.7

1.4 PARASITE DYNAMICS*

1.4.1 Tick to cattle transmission of *T. parva*

Latif, Hove, Kanhai, Masaka and Pegram (2001), working with naïve 7-month old tracer Hereford animals on commercial farms in Zimbabwe with significant wildlife populations, found 10-16 times as many adult *R. appendiculatus* as nymphs on the cattle. These authors concluded that most nymphs must have fed on alternate hosts. Nineteen of 24 (76%) of the cattle showed patent theileria schizonts during the period of nymphal activity, but it is not stated whether these were *T. parva* alone or in combination with *T. taurotragi*. There was a range of clinical manifestations with mostly mild or inapparent disease but also with some severe and fatal reactions. The disease transmitted by adult ticks was very pathogenic with 30 of 35 (86%) showing severe reactions. Surprisingly, cattle which survived *T. parva* challenge transmitted by nymphs and seroconverted, showed a variable degree of resistance to subsequent challenge transmitted by adult ticks. Thirteen of 18 (72%) of these cattle had a second disease episode with a case fatality of 46%. The authors suggest that these results indicate that endemic stability to *T. parva* could not be maintained by natural exposure of the calves during the season of nymphal activity. However they acknowledge that the presence of *T. taurotragi* may be a complicating factor and that their work may not agree with results from other studies. Land management on the farms restricted cattle and nymphal tick contact and the authors note this may have had the effect of partly breaking the transmission cycle. It is possible that the

* Cross reference chapter 2 section 1.7

situation with naïve *Bos taurus* cattle moved onto a commercial farm is very different to that found in a communal grazing situation with highly resistant *Bos indicus* cattle. However, one factor that this publication does make clear is that nymphs can be very significant in the epidemiology of theileriosis. This fact may not have been fully appreciated in other East African studies due to the presence of all stages of *R. appendiculatus* at one time. Dolan (1999) takes this further and is of the opinion that the epidemiological studies carried out in East Africa have biased interpretation of the disease. He states that:

"There is a real danger that the subtleties of the epidemiological variation in these environments, and the graduations in between, will be missed under the weight of data and opinion emanating from the eastern African experience."

Ochanda, Young, Wells, Medley and Perry (1996) compared the infection levels and salivary gland morphology in nymphal and adult *R. appendiculatus* fed concurrently on groups of *T. parva* infected cattle. By using more sensitive detection methods for assessing *T. parva* infection in nymphs than had been used previously, they were able to show that larval/nymphal transmission is not as efficient as nymphal/adult transmission. They also estimated that there were fewer sporozoites in an infected nymphal acini than an adult acini. The implication is that nymphal challenge would tend to cause milder infections.

Marcotty, Brandt, Billiouw, Chaka, Losson and Birkvens (2002) showed that both adults and nymphs were all able to transmit *T. parva* from carrier animals which

carried clinical and lethal ECF. However, they found that the prevalence of infection in nymphs was much lower than in adults. Their study was not designed to establish differences in virulence of *T. parva* transmitted by nymphs and adults.

2.0 THE EPIDEMIOLOGY OF EAST COAST FEVER

2.1 FIELD STUDIES OF EAST COAST FEVER EPIDEMIOLOGY – EAST AFRICA*

Gitau and colleagues (Gitau, Perry and McDermott, 1999 and Gitau, McDermott, Katende, O'Callaghan, Brown and Perry, 2000) carried out a prospective study of ECF epidemiology in small holder dairy farms in Murang'a district, Kenya, by monitoring a total of 225 female calves from birth to 6 months old in five agro-ecological zones. ECF morbidity, mortality and seroconversion varied greatly between zones and grazing practices, being highest with unrestricted grazing at the lower elevations. In these areas, 10-12% of calves were reported to have died of ECF before 6 months old, based on clinical signs only. Tick data was collected but was not presented in a way that allows detailed analysis. In the most heavily infested zone approximately half of examinations revealed adult *R. appendiculatus* with maximal counts being 39 per calf. The specific fatality rate due to ECF was approximately 35%. The authors acknowledge that the study was hampered by the low number of calves in each group, but conclude that none of the areas studied were endemically stable with respect to ECF. They contrast their results with those of

* Cross reference chapter 2 section 3.1

Moll et al (1986) but do not discuss the major differences in cattle breeds between the two studies.

2.2 FIELD STUDIES OF EAST COAST FEVER EPIDEMIOLOGY – CENTRAL AND SOUTHERN AFRICA*

Central and southern Africa has enormous potential for studying the epidemiology of ECF due to the fact that for most of the region the 3 instars of *R. appendiculatus* show a strict seasonal pattern. However, very few prospective studies had been published prior to 1995. Since then several studies have appeared in the published literature.

Minjauw, Otte, James, de Castro and Sinyangwe (1998) studied 5 groups of Sanga cattle (approximately 45 animals per group of which 7 to 9 were calves) kept under different tick and tick-borne disease control regimes in central Zambia. They reported that there were approximately 5 adult *R. appendiculatus* per animal during the rainy season but no data was given on tick seasonality. ECF mortality was highest in the non-immunised, non-treated control group. The study was conducted during an ECF epidemic following the disease's introduction into the area, and in this group adult mortality was 26% and calf mortality was 90% (rates based on animal years of risk). All the adult mortality was due to ECF. However, there was a dramatic reduction in ECF incidence (even in the control group) once a large proportion of the cattle in the area had been immunized. It would appear that the

* Cross reference chapter 2 section 3.2

transition to endemic stability had been accelerated by widespread ECF vaccination.

Minjauw, Rushton, James and Upton (1999) conducted a financial analysis of the Minjauw et al (1998) study. They conclude that immunization and strategic spraying gave the highest net present value and that the unsprayed, non-immunized regime gave the lowest. While this analysis may have been valid for the 2.5 years following the new introduction of ECF into such an area, the authors did not discuss the possibility that the generation of endemic stability may have radically altered the calculations in subsequent years. However, the finding that immunization in the face of an outbreak in this type of production system can be economically justified was important.

Billiouw, Vercruysse, Marcotty, Speybroeck, Chaka and Berkvens (2002) working in Eastern Zambia, also report a study conducted during an ECF epidemic. Forty percent of calves showed clinical ECF and 29% died. They noted that adult tick transmission was most important for causing disease and suggest that the fact that the nymphal wave in September was never responsible for disease outbreaks indicated that nymphal transmission played a minor part in the transmission dynamics. However, as 79% of calves, and presumably a higher percentage of adults, survived the outbreak and there was no serological monitoring of calves, this suggestion is difficult to substantiate. While nymphs were obviously not responsible for most disease transmission, it was a large jump to claim that they play a minor part in the overall transmission dynamics.

Latif, Hove, Kanhai, Masaka and Pegram (2001) conducted a study in Zimbabwe where Hereford cattle (>7 months old) with no previous exposure to ECF were moved in groups onto 3 commercial, ECF endemic, farms to act as sentinels. They demonstrated *T. parva* transmission by adults between January and April and by nymphs between June and December. Transmission by adults was consistently followed by severe ECF, whereas the severity of disease during the nymphal season was low. They postulate that grazing practices usually protected cattle from exposure to nymphs and conclude that endemic stability could not be maintained by natural exposure of calves during the season of nymphal activity. However, their data showed that 7 of the 18 cattle moved during the nymphal season died of ECF (6 of them died in the subsequent adult tick season) whereas 25/30 cattle moved during the period of adult activity died or had severe reactions to ECF and required treatment. This evidence, along with the fact that the study animals were all naïve *Bos taurus* animals over 7 months old, contradicts the authors' conclusion that endemic stability could not be maintained by natural exposure of calves to nymphal transmission.

Kivaria, Heuer, Jongejon, Okello-onen, Rutagwenda, Unger and Boehle (2004) in a study lasting 21 months observed 931 traditionally grazed Ankole calves born over a 12-month period. The study has some serious flaws and the authors make some unsubstantiated and erroneous assumptions, e.g. the statement in the introduction – "As with most tick-borne diseases, young (up to 4 months of age) calves are protected against ECF by colostrum derived maternal immunity."

The abstract claims that a relationship between rainfall pattern and whole body *R. appendiculatus* counts was determined, however, the paper provides no data or analysis of either of these. The abstract also states that the *T. parva* related calf mortality rate ranged from 0 to 67% with an average of 5.4%. Even this figure is open to question as there were no laboratory or veterinary diagnoses made and all results are based on farmer opinion. Due to logistical problems some of these were historical (up to 3 months) rather than contemporary. Total calf mortality up to one year of age was 10%. The authors suggest that a small peak of mortality at 3 months of age was due to the fact that antibody levels were lowest at this stage (maternal antibodies having waned and endogenous antibodies yet to be produced). However in the sentence before making this conclusion they point out that at 3 months calves start to accompany their mothers to graze. This is a much more likely explanation of the increased mortality at 3 months.

3.0 *COWDRIA RUMINANTIUM*

3.1 SEROLOGY*

Sumption, Paxton and Bell-Sakyi (2003) describe the validation of the competitive ELISA for measuring antibodies to *Ehrlichia ruminantium* used in the study described in chapter 7. Sera from experimentally infected calves in South Africa (10), The Netherlands (1) and sera from 19 heartwater vaccinated calves from South

* Cross reference chapter 2 section 4.4

Africa, were used to calculate a sensitivity of 79% at 70 per cent inhibition (PI). The specificity using a cut-off of 70 PI was estimated to be 100% following testing 10 sera from cattle in Scotland (heartwater free), six sera from Scottish cattle experimentally infected with *E. ondiri* and two Namibian cattle suspected to be infected with other Ehrlichia species. The accurate calculation of specificity could have been improved in this work had sera from African cattle naturally exposed to tick challenge, but heartwater free, been available.

3.2 EPIDEMIOLOGY*

Tice, Bryson, Stewart, du Plessis and Waal (1998) conducted a two-year field trial in four communal grazing areas of South Africa during a period when farmers were changing from an intensive dipping programme. They found cessation of tick control in communally grazed *Bos indicus* cattle resulted in no outbreaks of babesiosis, anaplasmosis or heartwater despite the parasites being present in the indigenous tick vectors. Herd antibody levels for the parasites were low at stages in the study but still no outbreaks were observed. The authors suggest various possible reasons for the lack of observed disease but the innate resistance of *Bos indicus* cattle, which made up the bulk of the population, is the most likely explanation.

* Cross reference chapter 2 section 4.5

CHAPTER 9

GENERAL DISCUSSION

Since the completion of this study there have been major advances in the testing technology for *T. parva*. The ELISA for detection of antibodies (Katende et al, 1998) shows improved sensitivity over the IFAT used in this study. The ELISA is almost certainly a cheaper and more robust test to run. Of greater significance is the development of molecular tests (Morzaria et al, 1999). Their use in this study would have greatly aided the understanding of the epidemiology of theileriosis in the study area. An initial prevalence study of the Theileria species and strains present in the area would have been very helpful. Also, a study estimating the proportion of the adult cattle population that were carriers for *T. parva* would have been very interesting. Finally, a specific *T. parva* PCR would have provided useful additional data when diagnosing clinical disease.

The findings of more recent publications that examine the role of nymphal transmission of *T. parva* are highly relevant to the situation in Malawi seen in this study. There is no clear consensus about the ability of nymphal transmission to maintain endemic stability, but there is strong evidence that nymphal infections cause milder disease (although still capable of killing a naïve *Bos taurus* calf) and that the true role of nymphal transmission has been overlooked in East African studies. This study has shown that Malawi zebu calves exposed to nymphal transmission before their first exposure to adult *R. appendiculatus* are significantly less likely to die from

ECF than those whose first *T. parva* challenge is transmitted by adult ticks.

Perry and Young (1995) pointed out that much information has been gathered on the epidemiology of *T. parva* by studies into extreme cases. Moll et al (1986) documented a situation with very high transmission rates and zero mortality. Many authors have documented situations of very severe mortality when ECF moves into a new area where there is no herd immunity to the disease. This study presents an intermediate case with significant transmission rates, low mortality in adults and low-moderate mortality in calves maintained with no tick control. Heavy calf mortality was seen in the two years after dipping ceased.

It is concluded that the interaction of the seasonality of *R. appendiculatus* lifecycle stages and the calving season determine epidemiology of ECF in the region studied. The timings of larval, nymphal and adult feeding are all significant.

This study has shown that in the area around Lilongwe:

- Strategic dipping nine times per year at 2-weekly intervals with chlorfenvinphos or amitraz during the rainy season gave almost total control of *R. appendiculatus* and allowed the numbers of other tick species to remain similar to undipped controls.
- Controlling *R. appendiculatus* by strategic dipping gave almost total control of ECF but did not allow the development of endemic stability to the disease.

- Cessation of dipping in arsenic trioxide could lead to very heavy mortality from ECF in calves and young stock in years 2 and 3.
- Following the cessation of dipping and heavy mortality, endemic stability to ECF could be established.
- One of the major contributors to endemic stability for ECF was the combination of the calving pattern and the period of *R. appendiculatus* nymphal activity. The majority of calves were exposed to low dose *T. parva* infections from nymphs before the high dose challenge from adults. Nymphal challenge produced mostly subclinical infections and immunity.
- Economically the most cost effective option would be to stop dipping and allow the generation of endemic stability. However this could possibly result in major social and political problems caused by high cattle mortality in years 2 and 3.
- The second best policy, in economic terms, would be to continue using serviceable tanks with a strategic dipping policy (nine immersions per year). The main disadvantage would be the lack of endemic stability and vulnerability to interruptions in dipping. However, in areas where endemic stability does not exist due to prior dipping, it is an attractive option.
- The cost of strategic dipping, where tank construction was necessary, was more than twice the cost of the cheapest option.

The tick-borne disease control policy in Malawi has evolved through the twentieth century. The policy in the early 1990's was based on assumptions and livestock management practices that were no longer realities. The intensive dipping of local

cattle was designed to eradicate ECF and to allow the widespread introduction of high grade cattle. By the 1990's the aim of ECF eradication had been long forgotten and high grade cattle were not being introduced into the communally grazed rural herds. For economic, political, scientific and environmental reasons the policy needed to be changed. This study had the full support of the Chief Veterinary Officer from its conception and results were presented in 1994. Consideration of the results and consultation with a group of experts in tick-borne disease control in Malawi lead to a change in Government policy. A decision was made to build no new tanks for the dipping of communally grazed Malawi zebu cattle and that existing tanks would move to strategic dipping nine times per year.

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LIST OF APPENDICES

APPENDIX 1. MONTHLY MORTALITY RATE IN CALVES 1991-1993

DEATHS

	D	N	S	M	T	L	TOT
J91	0	0	1	0	4	0	5
F	0	0	0	2	2	1	5
M	0	1	5	1	0	0	7
A	0	0	0	1	7	1	9
M	1	1	1	0	1	1	5
J	0	1	0	1	2	1	5
J	0	0	0	1	0	1	2
A	2	1	1	1	2	1	8
S	2	0	0	1	2	0	5
O	0	0	0	0	1	0	1
N	0	0	0	0	0	0	0
D	0	0	0	1	3	0	4
J92	0	0	0	0	4	1	5
F	0	0	0	0	3	2	5
M	0	0	0	0	3	3	6
A	1	0	1	0	0	1	3
M	0	0	0	0	1	1	2
J	0	1	0	1	2	1	5
J	2	2	0	0	0	2	6
A	0	0	0	0	1	3	4
S	0	0	1	0	1	5	7
O	1	0	0	0	2	0	3
N	0	0	1	1	0	0	2
D	2	0	1	1	2	0	6
J93	1	1	1	2	3	1	9
F	4	4	0	4	11	7	30
M	2	2	5	8	23	11	51
A	4	6	3	3	3	5	24
M	4	2	1	3	0	4	14
J	2	5	3	2	3	1	16
J	3	0	0	2	3	2	10
A	2	1	0	1	1	1	6
S	0	1	1	2	1	0	5
O	0	0	0	2	2	1	5
N	1	1	4	2	1	3	12
D	1	0	0	0	2	0	3

NO AT START OF MONTH

	D	N	S	M	T	L	TOT
J91	50	40	53	71	86	35	335
F	42	40	53	77	78	38	328
M	34	37	50	79	77	39	316
A	32	37	47	73	68	44	301
M	26	38	43	73	62	52	294
J	20	35	37	60	54	59	265
J	34	41	41	66	59	61	302
A	43	44	46	59	65	60	317
S	57	49	43	61	74	58	342
O	61	56	48	64	78	57	364
N	66	58	47	65	88	57	381
D	66	56	49	68	91	61	391
J92	66	55	48	69	90	46	374
F	67	55	47	70	84	47	370
M	67	55	46	66	81	44	359
A	67	54	46	67	76	39	349
M	69	55	48	70	75	35	352
J	74	54	51	78	81	40	378
J	72	46	52	74	103	51	398
A	63	46	51	79	121	49	409
S	74	56	65	82	128	48	453
O	72	58	66	86	126	39	447
N	74	59	68	90	131	40	462
D	77	61	68	86	135	43	470
J93	79	66	69	90	138	43	485
F	77	66	67	86	142	43	481
M	74	64	66	81	135	38	458
A	70	62	67	76	114	29	415
M	74	55	65	68	123	30	415
J	74	58	63	61	126	25	407
J	72	58	57	59	111	29	386
A	75	59	51	52	111	35	342
S	62	49	42	49	107	33	342
O	62	49	40	42	106	30	329
N	61	47	42	37	106	28	321
D	59	44	36	31	104	24	298

MONTHLY DEATH RATE

	D	N	S	M	T	L	Overall
J91	0.0	0.0	1.9	0.0	4.7	0.0	1.5
F	0.0	0.0	0.0	2.6	2.6	2.6	1.5
M	0.0	2.7	10.0	1.3	0.0	0.0	2.2
A	0.0	0.0	0.0	1.4	10.3	2.3	3.0
M	3.8	2.6	2.3	0.0	1.6	1.9	1.7
J	0.0	2.9	0.0	1.7	3.7	1.7	1.9
J	0.0	0.0	0.0	1.5	0.0	1.6	0.7
A	4.7	2.3	2.2	1.7	3.1	1.7	2.5
S	3.5	0.0	0.0	1.6	2.7	0.0	1.5
O	0.0	0.0	0.0	0.0	1.3	0.0	0.3
N	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D	0.0	0.0	0.0	1.5	3.3	0.0	1.0
J92	0.0	0.0	0.0	0.0	4.4	2.2	1.3
F	0.0	0.0	0.0	0.0	3.6	4.3	1.4
M	0.0	0.0	0.0	0.0	3.7	6.8	1.7
A	1.5	0.0	2.2	0.0	0.0	2.6	0.9
M	0.0	0.0	0.0	0.0	1.3	2.9	0.6
J	0.0	1.9	0.0	1.3	2.5	2.5	1.3
J	2.8	4.3	0.0	0.0	0.0	3.9	1.5
A	0.0	0.0	0.0	0.0	0.8	6.1	1.0
S	0.0	0.0	1.5	0.0	0.8	10.4	1.5
O	1.4	0.0	0.0	0.0	1.6	0.0	0.7
N	0.0	0.0	1.5	1.1	0.0	0.0	0.4
D	2.6	0.0	1.5	1.2	1.5	0.0	1.3
J93	1.3	1.5	1.4	2.2	2.2	2.3	1.9
F	5.2	6.1	0.0	4.7	7.7	16.3	6.2
M	2.7	3.1	7.6	9.9	17.0	28.9	11.1
A	5.7	9.7	4.5	3.9	2.6	17.2	5.8
M	5.4	3.6	1.5	4.4	0.0	13.3	3.4
J	2.7	8.6	4.8	3.3	2.4	4.0	3.9
J	4.2	0.0	0.0	3.4	2.7	6.9	2.6
A	2.7	1.7	0.0	1.9	0.9	2.9	1.8
S	0.0	2.0	2.4	4.1	0.9	0.0	1.5
O	0.0	0.0	0.0	4.8	1.9	3.3	1.5
N	1.6	2.1	9.5	5.4	0.9	10.7	3.7
D	1.7	0.0	0.0	0.0	1.9	0.0	1.0

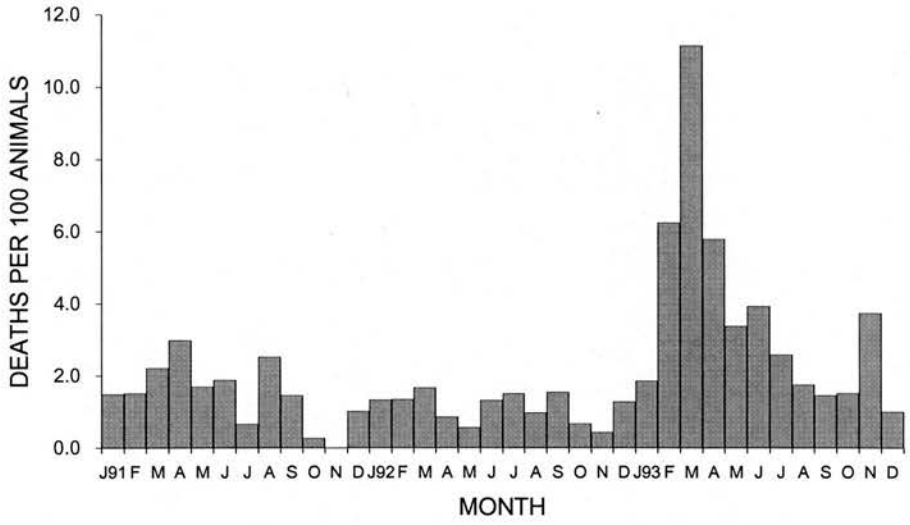
APPENDIX 2. MONTHLY ANIMAL NUMBERS AND MORTALITY RATES IN CALVES

	NO. ANIMALS AT START OF MONTH							TOTAL	ECF	OTHER	NO	NO	OTHER	DEATH	ECF	OTHER TBD	NO DIAGNOSIS	NO SAMPLE	OTHER
	D	N	S	M	T	L	TOT	DEATHS	DEATHS	TBD	DIAGNOSIS	SAMPLE		RATE	RATE	RATE	RATE	RATE	RATE
J91	50	40	53	71	86	35	335	5	0	0	0	1	4	1.5	0.0	0.0	0.0	0.3	1.2
F	42	40	53	77	78	38	328	5	2	0	0	1	2	1.5	0.6	0.0	0.0	0.3	0.6
M	34	37	50	79	77	39	316	7	0	0	0	2	5	2.2	0.0	0.0	0.0	0.6	1.6
A	32	37	47	73	68	44	301	9	1	0	1	4	3	3.0	0.3	0.0	0.3	1.3	1.0
M	26	38	43	73	62	52	294	5	1	0	0	2	2	1.7	0.3	0.0	0.0	0.7	0.7
J	20	35	37	60	54	59	265	5	1	0	2	1	1	1.9	0.4	0.0	0.8	0.4	0.4
J	34	41	41	66	59	61	302	2	0	0	1	1	0	0.7	0.0	0.0	0.3	0.3	0.0
A	43	44	46	59	65	60	317	8	0	0	1	2	5	2.5	0.0	0.0	0.3	0.6	1.6
S	57	49	43	61	74	58	342	5	0	0	4	0	1	1.5	0.0	0.0	1.2	0.0	0.3
O	61	56	48	64	78	57	364	1	0	0	0	1	0	0.3	0.0	0.0	0.0	0.3	0.0
N	66	58	47	65	88	57	381	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
D	66	56	49	68	91	61	391	4	1	0	1	0	2	1.0	0.3	0.0	0.3	0.0	0.5
J92	66	55	48	69	90	46	374	5	4	1	0	0	0	1.3	1.1	0.3	0.0	0.0	0.0
F	67	55	47	70	84	47	370	5	3	0	1	1	0	1.4	0.8	0.0	0.3	0.3	0.0
M	67	55	46	66	81	44	359	6	5	0	1	0	0	1.7	1.4	0.0	0.3	0.0	0.0
A	67	54	46	67	76	39	349	3	0	0	2	0	1	0.9	0.0	0.0	0.6	0.0	0.3
M	69	55	48	70	75	35	352	2	1	0	1	0	0	0.6	0.3	0.0	0.3	0.0	0.0
J	74	54	51	78	81	40	378	5	0	0	2	0	3	1.3	0.0	0.0	0.5	0.0	0.8
J	72	46	52	74	103	51	398	6	0	0	4	0	2	1.5	0.0	0.0	1.0	0.0	0.5
A	63	46	51	79	121	49	409	4	2	0	0	1	1	1.0	0.5	0.0	0.0	0.2	0.2
S	74	56	65	82	128	48	453	7	5	0	1	0	1	1.5	1.1	0.0	0.2	0.0	0.2
O	72	58	66	86	126	39	447	3	0	0	1	0	2	0.7	0.0	0.0	0.2	0.0	0.4
N	74	59	68	90	131	40	462	2	0	0	1	1	0	0.4	0.0	0.0	0.2	0.2	0.0
D	77	61	68	86	135	43	470	6	1	0	1	2	2	1.3	0.2	0.0	0.2	0.4	0.4
J93	79	66	69	90	138	43	485	9	2	0	1	4	2	1.9	0.4	0.0	0.2	0.8	0.4
F	77	66	67	86	142	43	481	30	4	1	8	16	1	6.2	0.8	0.2	1.7	3.3	0.2
M	74	64	66	81	135	38	458	51	4	5	20	19	3	11.1	0.9	1.1	4.4	4.1	0.7
A	70	62	67	76	114	29	415	24	4	0	9	7	4	5.8	1.0	0.0	2.2	1.7	1.0
M	74	55	65	68	123	30	415	14	1	0	4	5	4	3.4	0.2	0.0	1.0	1.2	1.0
J	74	58	63	61	126	25	407	16	0	1	6	5	4	3.9	0.0	0.2	1.5	1.2	1.0
J	72	58	57	59	111	29	386	10	1	0	2	3	4	2.6	0.3	0.0	0.5	0.8	1.0
A	75	59	51	52	111	35	342	6	0	0	2	1	3	1.8	0.0	0.0	0.6	0.3	0.9
S	62	49	42	49	107	33	342	5	0	0	0	4	1	1.5	0.0	0.0	0.0	1.2	0.3
O	62	49	40	42	106	30	329	5	1	0	2	1	1	1.5	0.3	0.0	0.6	0.3	0.3
N	61	47	42	37	106	28	321	12	0	0	3	3	6	3.7	0.0	0.0	0.9	0.9	1.9
D	59	44	36	31	104	24	298	3	0	0	0	0	3	1.0	0.0	0.0	0.0	0.0	1.0

NOTE:- NO DIAGNOSIS = SUFFICIENT SAMPLES WERE COLLECTED TO RULE OUT EAST COAST FEVER BUT NOT TO MAKE A FULL DIAGNOSIS OF THE CAUSE OF DEATH.

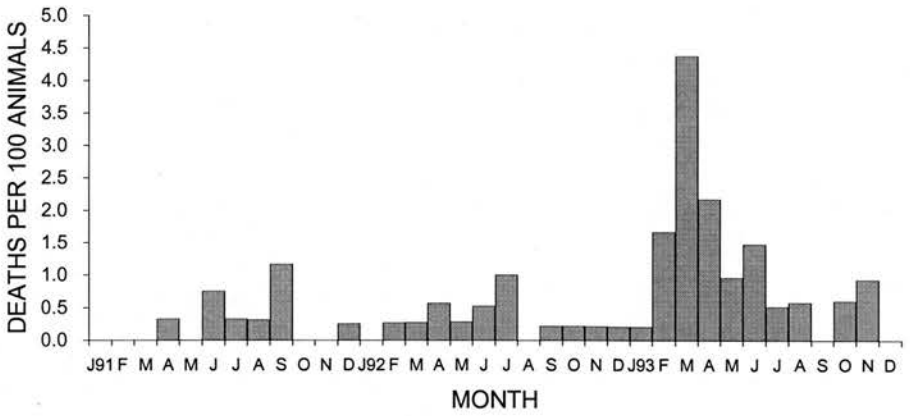
APPENDIX 3. MONTHLY DEATH RATE IN CALVES. ALL AREAS 1991-1993.

Appendix 3a. Monthly death rate in calves - all causes. All tanks 1991-1993.



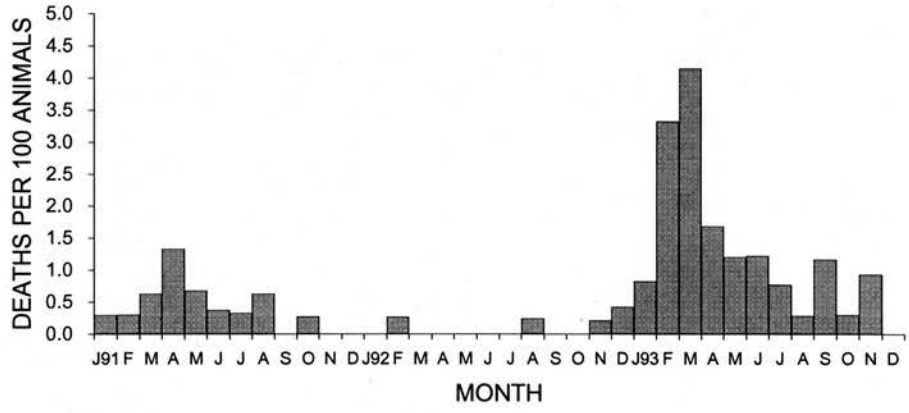
Data in appendix 2

Appendix 3b. Death no diagnosis rate in calves. All tanks 1991-1993.



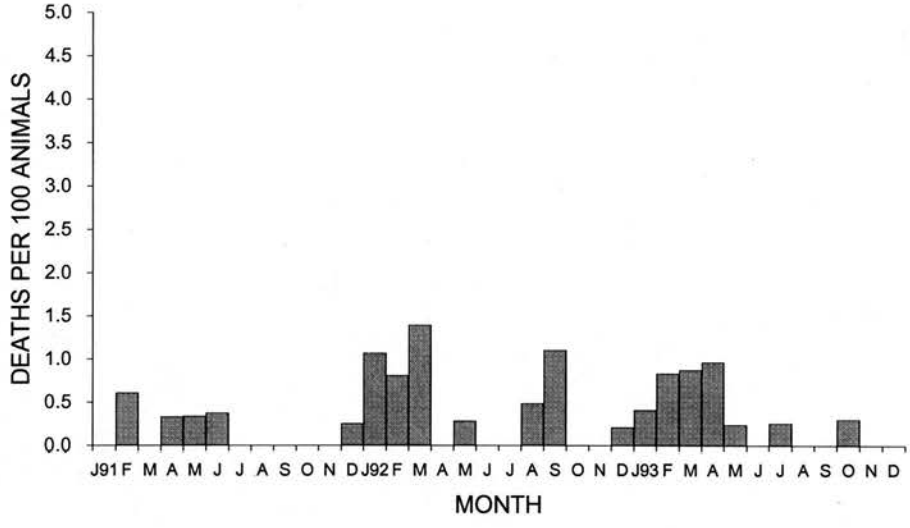
Data in appendix 2

Appendix 3c. Death no sample rate in calves. All tanks 1991-1993.



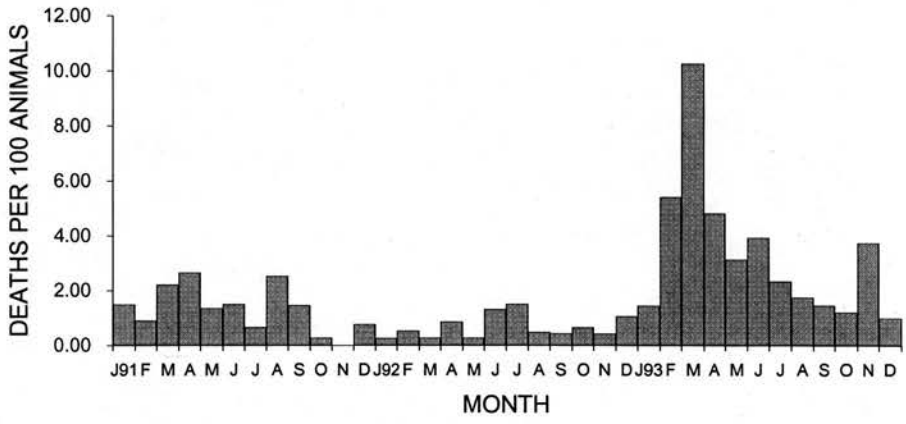
Data in appendix 2

Appendix 3d. Monthly ECF mortality rate in calves. All tanks 1991-1993



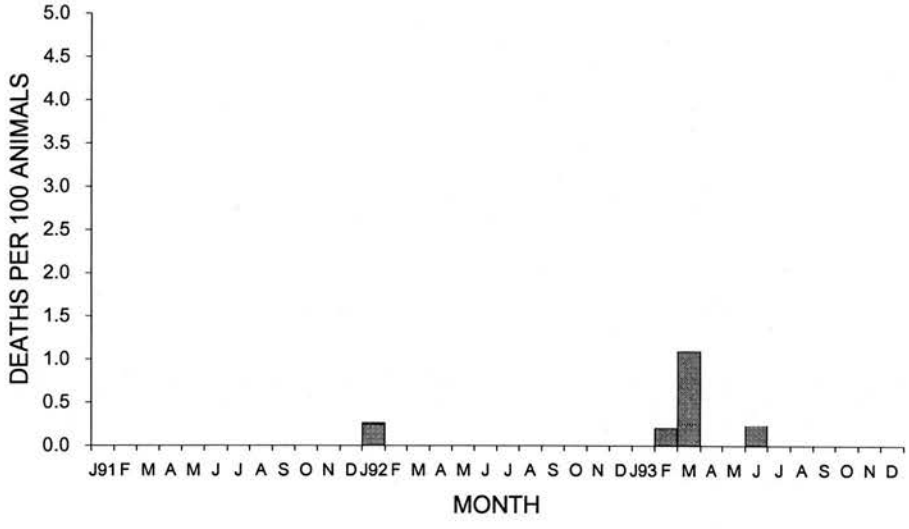
Data in appendix 2

Appendix 3e. Non ECF deaths. All tanks.



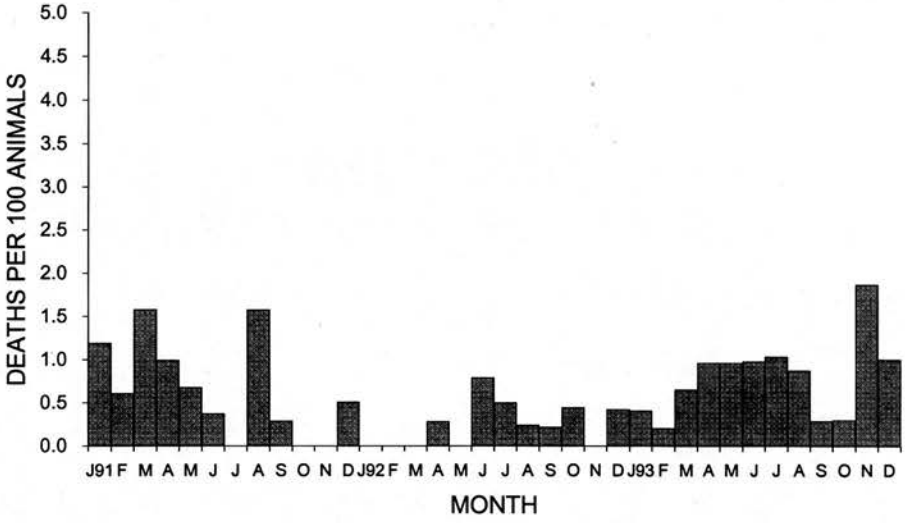
Data in appendix 2

Appendix 3f. Monthly other TBD death rate in calves. All tanks 1991-1993.



Data in appendix 2

Appendix 3g. Monthly miscellaneous death rate in calves. All tanks 1991-1993.



Data in appendix 2

APPENDIX 4. MONTHLY ANIMAL NUMBERS AND MORTALTY RATES IN ALL AGES

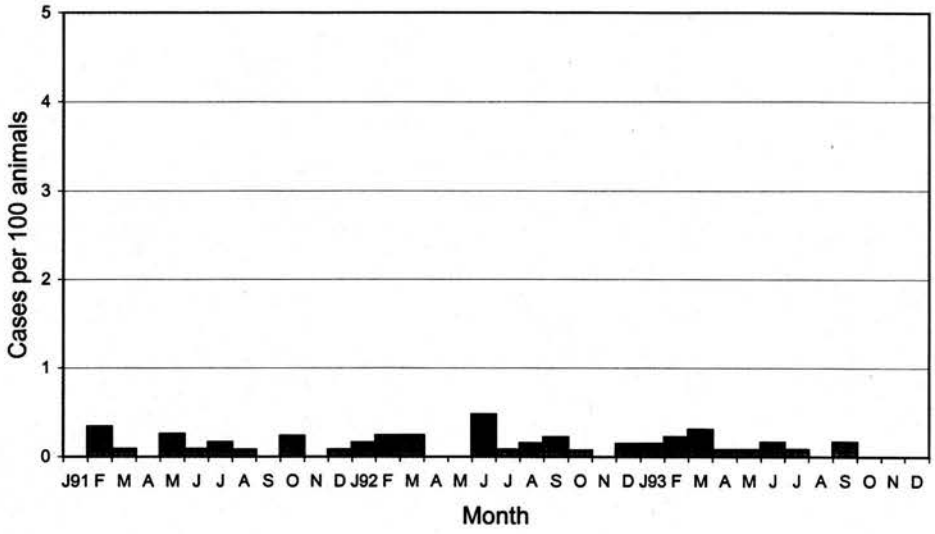
	NO. ANIMALS AT START OF MONTH							TOTAL	ECF	OTHER	NO	NO	OTHER	DEATH	ECF	OTHER TBD	NO DIAGNOSIS	NO SAMPLE	OTHER
	D	N	S	M	T	L	TOT	DEATHS	DEATHS	TBD	DIAGNOSIS	SAMPLE		RATE	RATE	RATE	RATE	RATE	RATE
J91	325	263	277	312	389	240	1806	7	0	0	0	1	6	0.4	0.0	0.0	0.0	0.1	0.3
F	325	262	276	316	378	241	1798	8	3	0	0	3	2	0.4	0.2	0.0	0.0	0.2	0.1
M	320	254	277	320	372	243	1786	11	1	0	2	2	6	0.6	0.1	0.0	0.1	0.1	0.3
A	321	250	275	323	366	243	1778	15	1	0	3	6	5	0.8	0.1	0.0	0.2	0.3	0.3
M	322	245	274	329	361	247	1778	13	5	0	0	2	6	0.7	0.3	0.0	0.0	0.1	0.3
J	322	249	271	335	362	255	1794	12	2	0	3	5	2	0.7	0.1	0.0	0.2	0.3	0.1
J	338	263	278	347	372	257	1855	5	2	0	1	1	1	0.3	0.1	0.0	0.1	0.1	0.1
A	350	264	283	348	397	262	1904	12	1	0	1	3	7	0.6	0.1	0.0	0.1	0.2	0.4
S	361	274	279	352	410	265	1941	10	0	0	4	1	5	0.5	0.0	0.0	0.2	0.1	0.3
O	365	278	277	356	409	267	1952	3	0	0	0	2	1	0.2	0.0	0.0	0.0	0.1	0.1
N	367	277	273	352	418	270	1957	1	0	0	0	1	0	0.1	0.0	0.0	0.0	0.1	0.0
D	367	277	272	354	418	274	1962	7	2	0	2	1	2	0.4	0.1	0.0	0.1	0.1	0.1
J92	360	276	260	355	418	201	1870	8	5	1	1	0	1	0.4	0.3	0.1	0.1	0.0	0.1
F	359	273	261	356	404	202	1855	10	5	0	1	3	1	0.5	0.3	0.0	0.1	0.2	0.1
M	355	272	258	353	399	199	1836	12	9	0	1	1	1	0.7	0.5	0.0	0.1	0.1	0.1
A	356	272	257	355	396	195	1831	8	1	0	2	2	3	0.4	0.1	0.0	0.1	0.1	0.2
M	357	273	257	360	395	193	1835	6	2	0	1	1	2	0.3	0.1	0.0	0.1	0.1	0.1
J	366	276	254	366	395	194	1851	13	4	0	2	2	5	0.7	0.2	0.0	0.1	0.1	0.3
J	375	279	263	367	425	207	1916	10	1	0	4	2	3	0.5	0.1	0.0	0.2	0.1	0.2
A	382	285	267	378	457	206	1975	12	4	0	1	3	4	0.6	0.2	0.0	0.1	0.2	0.2
S	401	305	276	384	471	207	2044	10	5	1	1	0	3	0.5	0.2	0.0	0.0	0.0	0.1
O	398	310	287	389	474	200	2058	11	0	0	2	0	9	0.5	0.0	0.0	0.1	0.0	0.4
N	397	309	290	379	472	197	2044	9	0	1	4	1	3	0.4	0.0	0.0	0.2	0.0	0.1
D	397	313	288	372	479	199	2048	12	2	1	1	4	4	0.6	0.1	0.0	0.0	0.2	0.2
J93	393	312	286	371	477	197	2036	18	3	1	2	6	6	0.9	0.1	0.0	0.1	0.3	0.3
F	388	308	280	362	479	192	2009	38	5	2	10	18	3	1.9	0.2	0.1	0.5	0.9	0.1
M	370	303	275	350	470	186	1954	66	4	6	23	24	9	3.4	0.2	0.3	1.2	1.2	0.5
A	364	294	268	335	441	178	1880	37	4	0	14	14	5	2.0	0.2	0.0	0.7	0.7	0.3
M	370	286	265	310	450	178	1859	22	3	0	7	8	4	1.2	0.2	0.0	0.4	0.4	0.2
J	368	289	261	308	461	177	1864	27	2	1	12	8	4	1.4	0.1	0.1	0.6	0.4	0.2
J	376	287	259	301	469	178	1870	17	2	0	2	5	8	0.9	0.1	0.0	0.1	0.3	0.4
A	378	291	253	290	476	182	1870	15	1	0	5	3	6	0.8	0.1	0.0	0.3	0.2	0.3
S	371	286	252	291	481	166	1847	17	2	0	2	5	8	0.9	0.1	0.0	0.1	0.3	0.4
O	373	251	250	280	475	156	1785	15	2	0	2	2	9	0.8	0.1	0.0	0.1	0.1	0.5
N	354	250	248	253	473	146	1724	17	0	0	3	8	6	1.0	0.0	0.0	0.2	0.5	0.3
D	344	242	238	229	463	139	1655	8	0	1	1	1	5	0.5	0.0	0.1	0.1	0.1	0.3

NOTE: NO DIAGNOSIS = SUFFICIENT SAMPLES WERE COLLECTED TO RULE OUT EAST COAST FEVER BUT NOT TO MAKE A FULL DIAGNOSIS OF THE CAUSE OF DEATH.

APPENDIX 5. EAST COAST FEVER CONFIRMED CASES BY MONTH 1991-1993

REGIME	CHLORFENVINPHOS				AMITRAZ				UNDIPPED			
DIPTANK	DICKSON		NAMAGUYA		SINYALA		MBABZI		TONDE		LIKUNI	
	CASES	DIED	CASES	DIED	CASES	DIED	CASES	DIED	CASES	DIED	CASES	DIED
JANUARY 91	0	0	0	0	0	0	0	0	2	0	0	0
FEBRUARY	0	0	2	1	1	0	1	0	3	2	0	0
MARCH	0	0	1	1	0	0	0	0	0	0	1	0
APRIL	0	0	0	0	0	0	0	0	1	1	0	0
MAY	2	2	0	0	1	1	0	0	1	1	1	1
JUNE	1	0	0	0	0	0	0	0	3	1	1	1
JULY	0	0	0	0	1	0	1	1	1	0	1	1
AUGUST	0	0	1	0	0	0	0	0	1	1	0	0
SEPTEMBER	0	0	0	0	0	0	0	0	1	0	1	0
OCTOBER	0	0	2	0	0	0	1	0	0	0	2	0
NOVEMBER	0	0	0	0	0	0	0	0	0	0	0	0
DECEMBER	1	0	0	0	0	0	0	0	1	1	1	1
JANUARY 92	0	0	1	1	1	0	0	0	7	3	3	1
FEBRUARY	1	0	0	0	0	0	2	1	4	2	3	2
MARCH	1	1	0	0	0	0	2	1	3	3	5	4
APRIL	0	0	0	0	0	0	0	0	1	1	1	0
MAY	0	0	0	0	0	0	0	0	2	2	8	3
JUNE	3	3	2	1	0	0	1	0	1	0	1	1
JULY	0	0	0	0	0	0	1	0	0	0	1	1
AUGUST	0	0	0	0	2	1	0	0	2	2	2	1
SEPTEMBER	0	0	0	0	1	1	2	0	0	0	6	4
OCTOBER	1	0	0	0	0	0	0	0	0	0	0	0
NOVEMBER	0	0	0	0	0	0	0	0	0	0	0	0
DECEMBER	1	1	0	0	1	1	0	0	0	0	0	0
JANUARY 93	1	0	1	1	0	0	0	0	3	2	0	0
FEBRUARY	1	0	1	0	0	0	1	1	2	1	3	3
MARCH	1	0	1	1	1	1	1	0	2	0	4	2
APRIL	0	0	1	1	0	0	0	0	2	0	3	3
MAY	0	0	0	0	0	0	1	1	0	0	2	2
JUNE	0	0	0	0	0	0	2	0	1	1	2	1
JULY	0	0	0	0	1	1	0	0	0	0	1	1
AUGUST	0	0	0	0	0	0	0	0	3	1	1	0
SEPTEMBER	0	0	0	0	0	0	2	2	0	0	3	0
OCTOBER	0	0	0	0	0	0	0	0	1	1	1	1
NOVEMBER	0	0	0	0	0	0	0	0	0	0	0	0
DECEMBER	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 6. ECF MORBIDITY IN DIPPED ANIMALS BY MONTH



Data in appendix 5

APPENDIX 7. ANIMALS SUSPECTED TO HAVE DIED FROM ECF BY AGE, AREA AND YEAR

Appendix 7a. Animals suspected to have died from ECF in 1991

CALVES

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	5	2	3	0	0.0
NAMAGUYA	4	1	3	0	0.0
SINYALA	8	2	6	0	0.0
MBABZI	9	0	9	0	0.0
TONDE	24	7	17	6	2.5
LIKUNI	6	3	3	0	0.0

YOUNG STOCK

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	4	2	2	1	1.0
NAMAGUYA	1	0	1	0	0.0
SINYALA	1	1	0	0	0.0
MBABZI	0	0	0	0	0.0
TONDE	5	2	3	1	0.7
LIKUNI	2	0	2	1	0.0

ADULTS

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	7	2	5	1	0.4
NAMAGUYA	3	1	2	2	1.0
SINYALA	8	4	4	1	1.0
MBABZI	5	0	5	1	0.0
TONDE	6	1	5	0	0.0
LIKUNI	6	0	6	3	0.0

NOTE:- "SUSPECT ECF" AS USED HERE IS REFERRING TO A STATISTICAL CALCULATION OF THE PROPORTION OF ANIMALS THAT DIED WITHOUT BEING SAMPLED, AND NOT SUSPECTED CLINICAL DISEASE SEEN IN THE FIELD.

Appendix 7b. Animals suspected to have died from ECF in 1992

CALVES

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	5	1	4	0	0.0
NAMAGUYA	3	0	3	0	0.0
SINYALA	4	1	3	2	0.7
MBABZI	3	1	2	0	0.0
TONDE	19	0	19	9	0.0
LIKUNI	21	2	19	12	1.3

YOUNG STOCK

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	0	0	0	0	0.0
NAMAGUYA	2	2	0	0	0.0
SINYALA	4	1	3	0	0.0
MBABZI	2	0	2	0	0.0
TONDE	4	0	4	3	0.0
LIKUNI	7	5	2	2	5.0

ADULTS

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	12	1	11	5	0.5
NAMAGUYA	4	0	4	2	0.0
SINYALA	8	1	7	1	0.1
MBABZI	11	0	11	2	0.0
TONDE	4	0	4	1	0.0
LIKUNI	11	4	7	3	1.7

MORTALITIES ARE ADJUSTED FOR BUTALEX USE IN 8 CASES AT LIKUNI.
(ASSUMING A CASE MORTALITY RATE OF 58% IN NON BUTALEX TREATED CASES).
NOTE:- "SUSPECT ECF" AS USED HERE IS REFERRING TO A STATISTICAL CALCULATION
OF THE PROPORTION OF ANIMALS THAT DIED WITHOUT BEING SAMPLED, AND NOT
SUSPECTED CLINICAL DISEASE SEEN IN THE FIELD.

Appendix 7c. Animals suspected to have died from ECF in 1993

CALVES

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	24	7	17	0	0.0
NAMAGUYA	23	11	12	2	1.8
SINYALA	18	5	13	1	0.4
MBABZI	31	18	13	0	0.0
TONDE	53	11	42	3	0.8
LIKUNI	36	15	21	11	7.9

YOUNG STOCK

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	3	1	2	0	0.0
NAMAGUYA	4	2	2	0	0.0
SINYALA	4	0	4	0	0.0
MBABZI	19	9	10	2	1.8
TONDE	5	2	3	1	0.7
LIKUNI	7	3	4	2	1.5

ADULTS

TANK	TOTAL DEATHS	DIED NO SAMPLE	DIED KNOWN CAUSE	DIED ECF	SUSPECT DIED ECF
DICKSON	5	0	5	0	0.0
NAMAGUYA	9	3	6	1	0.5
SINYALA	10	2	8	1	0.3
MBABZI	18	7	11	2	1.3
TONDE	12	0	12	2	0.0
LIKUNI	16	6	10	0	0.0

NOTE:- "SUSPECT ECF" AS USED HERE IS REFERRING TO A STATISTICAL CALCULATION OF THE PROPORTION OF ANIMALS THAT DIED WITHOUT BEING SAMPLED, AND NOT SUSPECTED CLINICAL DISEASE SEEN IN THE FIELD.

APPENDIX 8. TOTAL MORTALITY BY AGE, AREA AND YEAR

Appendix 8a. Total mortality by age 1991

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
DEATHS	5	4	8	9	24	6
ANIMAL YRS	47	46	47	70	76	54
RATES	10.8%	8.6%	17.0%	12.8%	31.4%	11.0%
YOUNG STOCK						
DEATHS	4	1	1	0	5	2
ANIMAL YRS	75	51	59	68	88	26
RATES	5.4%	2.0%	1.7%	0.0%	5.7%	7.6%
ADULTS						
DEATHS	7	3	8	5	6	6
ANIMAL YRS	218	165	163	201	223	176
RATES	3.2%	1.8%	4.9%	2.5%	2.7%	3.4%

Appendix 8b. Total mortality by age 1992

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
DEATHS	5	3	4	3	19	21
ANIMAL YRS	71	55	56	77	102	43
RATES	7.0%	5.5%	7.1%	3.9%	18.6%	49.2%
YOUNG STOCK						
DEATHS	0	2	4	2	4	7
ANIMAL YRS	105	86	83	120	125	46
RATES	0.0%	2.3%	4.8%	1.7%	3.2%	15.2%
ADULTS						
DEATHS	12	4	8	11	4	11
ANIMAL YRS	200	147	127	170	202	111
RATES	6.0%	2.7%	6.3%	6.5%	2.0%	9.9%

FIGURES ARE ADJUSTED FOR BUTALEX USE IN 8 CASES AT LIKUNI.

Appendix 8c. Total mortality by age 1993

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
DEATHS	24	23	18	31	53	36
ANIMAL YRS	69	55	54	59	117	31
RATES	34.9%	42.2%	33.6%	52.6%	45.2%	114.6%
YOUNG STOCK						
DEATHS	3	4	4	19	5	7
ANIMAL YRS	130	100	97	118	147	55
RATES	2.3%	4.0%	4.1%	16.0%	3.4%	12.8%
ADULTS						
DEATHS	5	9	10	18	12	16
ANIMAL YRS	169	124	106	123	202	85
RATES	3.0%	7.3%	9.5%	14.6%	6.0%	18.9%

APPENDIX 9. EAST COAST FEVER MORTALITY BY AGE, AREA AND YEAR

Appendix 9a. East Coast fever mortality by age 1991

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
CONFIRMED	0	0	0	0	6	0
SUSPECTED	0	0	0	0	2.5	0
TOTAL	0	0	0	0	8.5	0
ANIMAL YRS	47	46	47	70	76	54
RATES	0.0%	0.0%	0.0%	0.0%	11.1%	0.0%
YOUNG STOCK						
CONFIRMED	1	0	0	0	1	1
SUSPECTED	1	0	0	0	0.7	0
TOTAL	2	0	0	0	1.7	1
ANIMAL YRS	75	51	59	68	88	26
RATES	2.7%	0.0%	0.0%	0.0%	1.9%	3.8%
ADULTS						
CONFIRMED	1	2	1	1	0	3
SUSPECTED	0.4	1	1	0	0	0
TOTAL	1.4	3	2	1	0	3
ANIMAL YRS	218	165	163	201	223	176
RATES	0.6%	1.8%	1.2%	0.5%	0.0%	1.7%

SUSPECT CASES ARE THOSE THAT "DIED NO SAMPLE" BUT WERE STATISTICALLY LIKELY TO HAVE DIED FROM ECF. SEE APPENDIX 7a.

Appendix 9b East Coast fever mortality by age 1992

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
CONFIRMED	0	0	2	0	9	12
SUSPECTED	0	0	0.7	0	0	1.3
TOTAL	0	0	2.7	0	9	13.3
ANIMAL YRS	71	55	56	77	102	43
RATES	0.0%	0.0%	4.8%	0.0%	8.8%	31.1%
YOUNG STOCK						
CONFIRMED	0	0	0	0	3	2
SUSPECTED	0	0	0	0	0	5
TOTAL	0	0	0	0	3	7
ANIMAL YRS	105	86	83	120	125	46
RATES	0.0%	0.0%	0.0%	0.0%	2.4%	15.2%
ADULTS						
CONFIRMED	5	2	1	2	1	3
SUSPECTED	0.5	0	0.1	0	0	1.7
TOTAL	5.5	2	1.1	2	1	4.7
ANIMAL YRS	200	147	127	170	202	111
RATES	2.7%	1.4%	0.9%	1.2%	0.5%	4.2%

FIGURES ARE ADJUSTED FOR BUTALEX USE IN 8 CASES AT LIKUNI.

SUSPECT CASES ARE THOSE THAT "DIED NO SAMPLE" BUT WERE STATISTICALLY LIKELY TO HAVE DIED FROM ECF. SEE APPENDIX 7b.

Appendix 9c. East Coast fever mortality by age 1993

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
CONFIRMED	0	2	1	0	3	11
SUSPECTED	0	1.8	0.4	0	0.8	7.9
TOTAL	0	3.8	1.4	0	3.8	18.9
ANIMAL YRS	69	55	54	59	117	31
RATES	0.0%	7.0%	2.6%	0.0%	3.2%	60.2%
YOUNG STOCK						
CONFIRMED	0	0	0	2	1	2
SUSPECTED	0	0	0	1.8	0.7	1.5
TOTAL	0	0	0	3.8	1.7	3.5
ANIMAL YRS	130	100	97	118	147	55
RATES	0.0%	0.0%	0.0%	3.2%	1.2%	6.4%
ADULTS						
CONFIRMED	0	1	1	2	2	0
SUSPECTED	0	0.5	0.3	1.3	0	0
TOTAL	0	1.5	1.3	3.3	2	0
ANIMAL YRS	169	124	106	123	202	85
RATES	0.0%	1.2%	1.2%	2.7%	1.0%	0.0%

SUSPECT CASES ARE THOSE THAT "DIED NO SAMPLE" BUT WERE STATISTICALLY LIKELY TO HAVE DIED FROM ECF. SEE APPENDIX 7c

APPENDIX 10. EAST COAST FEVER MORBIDITY BY AGE, AREA AND YEAR

Appendix 10a. East Coast fever morbidity by age 1991

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
CONFIRMED	3	1	1	2	10	2
SUSPECTED	0	0	0	0	2.5	0
TOTAL	3	1	1	2	12.5	2
ANIMAL YRS	47	46	47	70	76	54
RATES	6.5%	2.2%	2.1%	2.8%	16.4%	3.7%
YOUNG STOCK						
CONFIRMED	1	0	0	1	5	3
SUSPECTED	1	0	0	0	0.7	0
TOTAL	2	0	0	1	5.7	3
ANIMAL YRS	75	51	59	68	88	26
RATES	2.7%	0.0%	0.0%	1.5%	6.5%	11.4%
ADULTS						
CONFIRMED	1	5	2	1	0	4
SUSPECTED	0.4	1	1	0	0	0
TOTAL	1.4	6	3	1	0	4
ANIMAL YRS	218	165	163	201	223	176
RATES	0.6%	3.6%	1.8%	0.5%	0.0%	2.3%

SUSPECT CASES ARE THOSE THAT "DIED NO SAMPLE" BUT WERE STATISTICALLY LIKELY TO HAVE DIED FROM ECF. SEE APPENDIX 7a

Appendix 10b. East Coast fever morbidity by age 1992

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
CONFIRMED	1	1	3	0	14	19
SUSPECTED	0	0	0.7	0	0	1.3
TOTAL	1	1	3.7	0	14	20.3
ANIMAL YRS	71	55	56	77	102	43
RATES	1.4%	1.8%	6.6%	0.0%	13.7%	47.5%
YOUNG STOCK						
CONFIRMED	0	0	1	4	4	5
SUSPECTED	0	0	0	0	0	4
TOTAL	0	0	1	4	4	9
ANIMAL YRS	105	86	83	120	125	46
RATES	0.0%	0.0%	1.2%	3.3%	3.2%	19.6%
ADULTS						
CONFIRMED	6	2	1	5	2	6
SUSPECTED	0.5	0	0.1	0	0	1.7
TOTAL	6.5	2	1.1	5	2	7.7
ANIMAL YRS	200	147	127	170	202	111
RATES	3.3%	1.4%	0.9%	2.9%	1.0%	6.9%

SUSPECT CASES ARE THOSE THAT "DIED NO SAMPLE" BUT WERE STATISTICALLY LIKELY TO HAVE DIED FROM ECF. SEE APPENDIX 7b

Appendix 10c. East Coast fever morbidity by age 1993

	CHLORFENVINPHOS		AMITRAZ		UNDIPPED	
	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES						
CONFIRMED	1	2	1	1	5	16
SUSPECTED	0	1.8	0.4	0	0.8	7.9
TOTAL	1	3.8	1.4	1	5.8	23.9
ANIMAL YRS	69	55	54	59	117	31
RATES	1.5%	7.0%	2.6%	1.7%	5.0%	76.1%
YOUNG STOCK						
CONFIRMED	1	0	0	3	2	2
SUSPECTED	0	0	0	1.8	0.7	1.5
TOTAL	1	0	0	4.8	2.7	3.5
ANIMAL YRS	130	100	97	118	147	55
RATES	0.8%	0.0%	0.0%	4.1%	1.8%	6.4%
ADULTS						
CONFIRMED	1	2	1	3	7	2
SUSPECTED	0	0.5	0.3	1.3	0	0
TOTAL	1	2.5	1.3	4.3	7	2
ANIMAL YRS	169	124	106	123	202	85
RATES	0.6%	2.0%	1.2%	3.5%	3.5%	2.4%

SUSPECT CASES ARE THOSE THAT "DIED NO SAMPLE" BUT WERE STATISTICALLY LIKELY TO HAVE DIED OF ECF. SEE APPENDIX 7c.

APPENDIX 11. 1992/93 COHORT CALVES *THEILERIA PARVA* SEROLOGY RESULTS

INDIRECT FLUORESCENT ANTIBODY TEST AT A DILUTION OF 1/640

	TAG No.	BIRTH MONTH	APR	MAY	JUN	JUL	AUG	AUG SEP	SEP OCT	OCT NOV	NOV DEC	DEC	JAN	FEB	MAR	APR	MAY	JUN
DICKSON	438	-17		N		N	N		N	N		N	N	N	N	N	N	N
	440	-17		N		N		N	N	N	N	N	N	N	N		N	N
	441	-17				N			N	N		N	N	N	N	N	N	N
	442	-17				N			N	N		N	N	N	N	N	N	N
	443	-17				N	N	N	N	N	N	N	N		N	N	W	N
	444	-17				N	N	N	N	N	N	N	N		N	N	N	N
	445	-17				N			N	N	N	N	W\N	N	N	N	N	N
	446	-17				P		P\N	N	N	N	N	N	N	N	N	N	
	448	-17			N	N		N	N	N		N	W	N	N	N	DIED N.S.	
	449	-18			P	N	N		N	N	N	N	N	N	N	N	N	N
	450	-18			N	N			N	N	N	N	N	N	N	N	CONE	
	451	-18				N	N		N	N		N	N	N	N	N	N	N
	452	-18			N	N		N	N		N		N	N	N	N	W\N	W
	453	-18				N			N	N		N	N	N	N	N	N	N
	455	-18				P		N	P\N	N	N	N	N	N	N	N	N	N
	456	-18				N		N	N	N	N	P\N	N	N	N	N	N	N
	467	-18				N		N	N	N	N	N	N	N	N	N	N	N
NAMAGUYA	357	-17				N		N		N	N	N	N	N	N		W	W
	370	-17				N		N	N	N	P\N	N\N	P\N	N			EMAC	
	359	-18				N	N	P	N	N	N	P\N	N	N	N	DIED N.S.		
	361	-18				N		N	N	N	N	N		N	N	TRAUM		
	364	-18				N			N		N		N		N	EMAC		
	365	-18				N		N	N	N	N	N	N	N	N	DIED N.S.		
	369	-18				N		N	N	N	N	N	N		N	N	N	N
	363	-19				N		N	N	P\N	N	N	W	N	N	N	N	N
	366	-19				N		N	N	N	N	N	N	N	N		N	N
	367	-19				N		N	N	N	N	N	N	N	N		N	N
	368	-19				P		N	P	N\N	P	N\N	N	N	N	N	W	P
	371	-19				N			N	N	N	N	N	N	N		N	N
	372	-19				N		N	N		N	P	W	W	N	DIED N.S.		
	373	-19				P		N	P	N	P	P				N	N	
	375	-19				N		N	P\N	N	N		N	N	N	N	N	N
SINYALA	350	-16				N			N	N	N	N	N		DIED N.S.			
	352	-16		P		N			N	N	N	N	N	N	N		N	N
	354	-17		N	N	P	N	P	P	N		N	P	P	P	P	P	P
	355	-17		N	N	N		N	P\N		N		N	N	N	W\N	SOLD	
	356	-17				N			P\N	N	P\N	N		N	N		N	N
	357	-17				N		N	N	P\N		N	N	N	N	N	N	N
	358	-18						N	N	N		N	N		N	N	N	N
	359	-18				N		N	N	N		N	N		N	N	N	N
	360	-18				N	N	N	N		N	N	P	W		N	N	N
	361	-18				P		N	N		N	P	W		W	W	W	W
	364	-18				N		N	N	N	N	N	N	N	N	N	N	
	365	-18				N		N	N	N	N	P	N\N	W	W	MALN		
	367	-18				P			N	N	N	N		P	P\N	P\N	W	W

	TAG No.	BIRTH MONTH	APR	MAY	JUN	JUL	AUG	AUG SEP	SEP OCT	OCT NOV	NOV DEC	DEC	JAN	FEB	MAR	APR	MAY	JUN
MBABZI	437	-17		W		N		P\N	N	N	N	P	W	W	W	N	N	N
	438	-17				N		N	N	P	N\P	P	W	W	N	N	N	N
	439	-17		N		N		N	N	N	N	N		N	N	N	N	N
	440	-17			N	N		N	P\N	N\N	P	N\N	N	N	N	N	N	N
	446	-17				P		N	N	N	N	N		N	N	N	N	N
	447	-17				N		N	N	N	N	N			CDNE			
	448	-17				N		N	N	P\N		N	N	N	N	N	N	W
	449	-17				N	N	N	N		N	N	W	W	W	N	N	DIED
	452	-17				N		N	N	N	N	P\N	N	N	N	N	N	N
	453	-17				P		N	N	N	N		N	N	W\P	CDNE		
	450	-18			P	P	N	N	N	N	N	P	W	N	N	DIED		
	451	-18			N	N		N	N	N	N	N	W\N	N	N	N	N	N
	454	-18				N		N	N	N		SLAUGH						
	455	-18				P		N	N	N	N	N	N	N	N	N	N	N
TONDE	515	-17			N	N	N	N	N	N	N	N	P	P	TRAUM			
	516	-17				N			N	N	N	P	W	N	N	N	N	N
	518	-17				N	N			N	N	N	N	N		N	N	N
	521	-17				N		N		N	P	P	W	N	B.BOVIS			
	522	-17				N		N	N	N	N		N	N	CDNE			
	524	-17				P		P	P	N	P	N	N	N	N		N	N
	525	-17				N		N		N	N	N	N	N	N	B.BOVIS		
	527	-17				N		N	N			N	N	N	N	N	N	N
	533	-17				N	N	N	N	N	N		N	W	P	P	P	
	534	-18				P		N	N	N	N	N	N	N		N	N	N
	519	-18				P			N	N	N		W	P	CDNE			
	520	-18				N			N	N	N	N	N	N	CDNE			
	523	-18				N		N	N	N	N	P	N	W	W	P	P	P
	526	-18				N		N	N	P\N	N	N	N	N	W	W	W	W
	532	-18				N		N	N	N	N	N	W\N	W\N	N	N	N	N
LIKUNI	328	-17		N		N		N	N	N	N	N	N	N	DIED N.S.			
	333	-17		P	N	P	P	P	P\P	P\P	P	P	P	P	P	P	P	P
	337	-17				N		N	N	N	N	N	N	N	N	N	W	P
	338	-17				N		N	N\N	P	N	N	W	P	P	P	W	P
	339	-17				N		P\N	N	N		P	W	P	P	P	P	P
	340	-17				N		N	N	N	N	N	N	N	N	N	N	N
	348	-17				N	N	P\P	P	P		P	DIED N.S.					
	341	-18				P		N	P\N	N	N	N	N	N	N	W	P	P
	342	-18				P		P\N	N	N	N	N	W	P	P	P	P	P
	343	-18				P		N	N	N		N	W	P	ECF			
	344	-18				N		N	N	N	P\N	N	N	N	DIED N.S.			
	345	-18				N		N	N	N	N	N	N	N	SLAUGH SICK			
	346	-18				N		N	N	N	N	N	N	N	DIED N.S.			
	350	-18				P		N	N	N	N	N	N	N	N	ECF		

NOT ALIVE

NOT SAMPLED

P POSITIVE

N NEGATIVE

W WEAK POSITIVE

P\N POSITIVE ON FIRST TEST, NEGATIVE ON RETEST

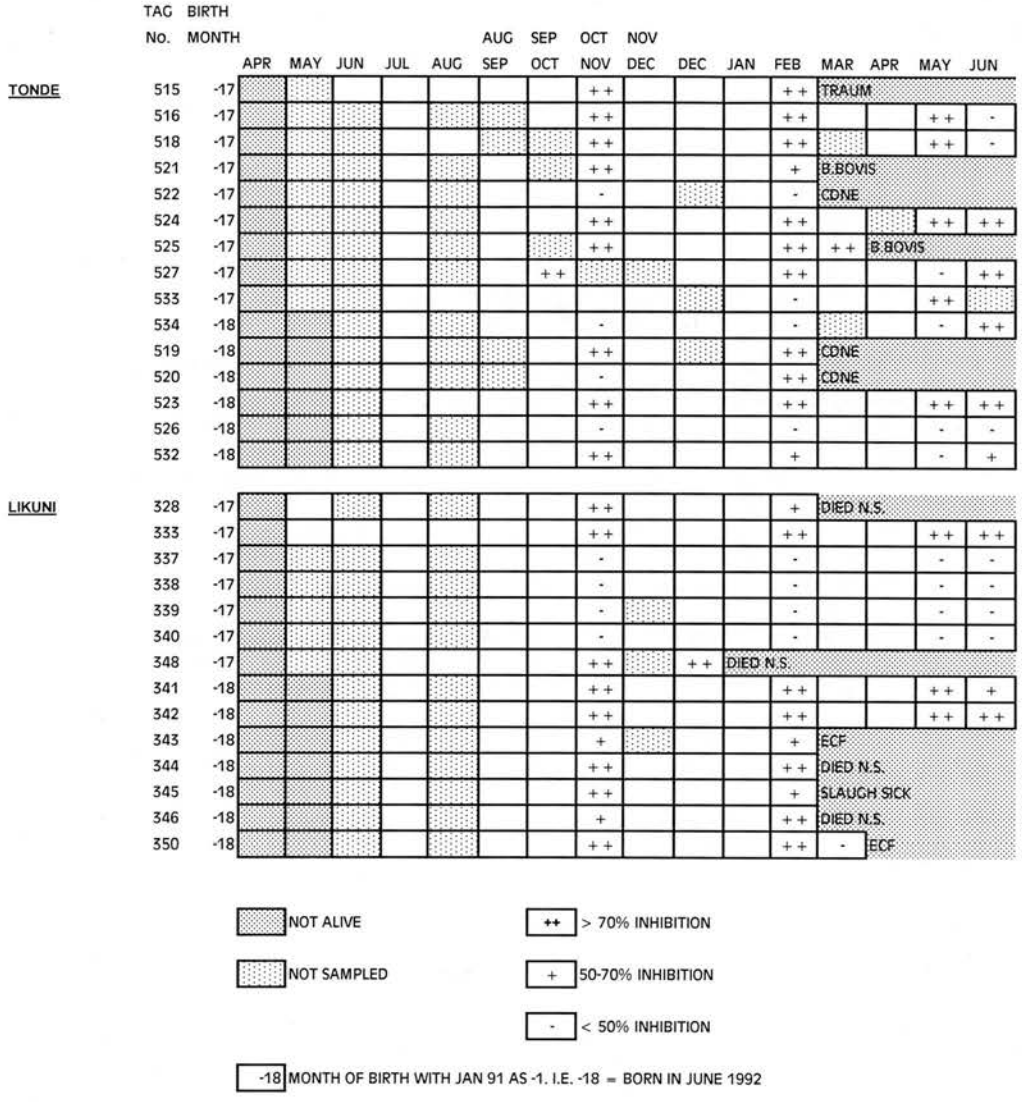
-18 MONTH OF BIRTH WITH JAN 91 AS -1. I.E. -18 = BORN IN JUNE 1992

APPENDIX 12. 1992/93 COHORT CALVES *C. RUMINANTIUM* SEROLOGY RESULTS

COMPETITIVE ELISA RESULTS

	TAG No.	BIRTH MONTH	APR	MAY	JUN	JUL	AUG	AUG SEP	SEP OCT	OCT NOV	NOV DEC	DEC	JAN	FEB	MAR	APR	MAY	JUN
DICKSON	438	-17							-					++			+	-
	440	-17							-					+			+	++
	441	-17							++					++			++	++
	442	-17							+					++			+	-
	443	-17							++				++				++	++
	444	-17							+				-				-	++
	445	-17							-					++			++	++
	446	-17							-					-			+	
	448	-17							++					++		+	DIED N.S.	
	449	-18							++					-			-	-
	450	-18							++					-		-	CDNE	
	451	-18							-					+			++	++
	452	-18							++					++			+	+
	453	-18							++					+			++	++
	455	-18							++					++			++	++
	456	-18							-					++			++	++
	467	-18							++					-			-	-
NAMAGUYA	357	-17						-						-	-		-	-
	370	-17							-					-			EMAC	
	359	-18							-					-	-	DIED N.S.		
	361	-18							-					-	-	TRAUM		
	364	-18							-				++		++	EMAC		
	365	-18							-					-	-	DIED N.S.		
	369	-18							-				-				-	-
	363	-19							-					+			-	-
	366	-19							-					-			-	-
	367	-19							-					-			-	-
	368	-19							-					-			-	-
	371	-19							-					-			+	-
	372	-19							-					++	++		DIED N.S.	
	373	-19							-			-					-	
	375	-19							-					-			-	-

	TAG No.	BIRTH MONTH	APR	MAY	JUN	JUL	AUG	AUG SEP	SEP OCT	OCT NOV	NOV DEC	DEC	JAN	FEB	MAR	APR	MAY	JUN
SINYALA	350	-16							+				-		DIED N.S.			
	352	-16							++					++			++	++
	354	-17							++					++			++	++
	355	-17						++						++		++	SOLD	
	356	-17							++					+			++	++
	357	-17							++					++			++	-
	358	-18							-				-				+	++
	359	-18							++				++				++	++
	360	-18							++					++			++	++
	361	-18							-				++				+	++
	364	-18							++					++			+	
	365	-18							-					++	++	MALN		
	367	-18							++					++			+	++


TAG No.
BIRTH MONTH
APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN
AUG SEP OCT NOV DEC
TONDE
515 -17 ++ ++ TRAUM
516 -17 ++ ++ + -
518 -17 ++ ++ + -
521 -17 + B.BOVIS
522 -17 - - CDNE
524 -17 ++ ++ ++ ++
525 -17 ++ ++ ++ B.BOVIS
527 -17 ++ ++ - ++
533 -17 - ++
534 -18 - - - ++
519 -18 ++ ++ CDNE
520 -18 - ++ CDNE
523 -18 ++ ++ ++ ++
526 -18 - - - -
532 -18 ++ + - +
LIKUNI
328 -17 ++ + DIED N.S.
333 -17 ++ ++ ++ ++
337 -17 - - - -
338 -17 - - - -
339 -17 - - - -
340 -17 - - - -
348 -17 ++ ++ DIED N.S.
341 -18 ++ ++ ++ +
342 -18 ++ ++ ++ ++
343 -18 + + ECF
344 -18 ++ ++ DIED N.S.
345 -18 ++ + SLAUGH SICK
346 -18 + ++ DIED N.S.
350 -18 ++ ++ - ECF
NOT ALIVE
NOT SAMPLED
++ > 70% INHIBITION
+ 50-70% INHIBITION
- < 50% INHIBITION
-18 MONTH OF BIRTH WITH JAN 91 AS -1. I.E. -18 = BORN IN JUNE 1992

APPENDIX 13. GEOMETRIC MEAN HALF BODY TICK COUNTS (n=5)

RHIPICEPHALUS ADULTS ON HEAD

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
1991 J	0.1	0.0		0.1	6.4	1.5
F	0.0	0.4	0.0	0.5	8.6	2.6
M	0.2	0.0	0.1	0.0	1.2	0.7
A	0.0	0.0	0.0	0.0	0.7	0.9
M	0.2	0.0	0.0	0.0	1.7	0.6
J	0.0	0.3	0.0	0.0	0.8	0.1
J	0.0	0.0	0.0	0.0	0.4	0.0
A	0.0	0.0	0.0	0.0	0.3	0.0
S	0.0	0.0	0.0	0.1	0.3	0.0
O	0.0	0.0	0.1	0.0	0.0	0.2
N	0.0	0.0	0.0	0.0	0.0	0.0
N	0.0	0.0	0.0	0.1	0.3	0.0
D	1.3	0.5	0.0	0.0	2.0	0.8
1992 J	0.3	0.0	0.0	0.2	8.3	4.1
F	0.2	0.2	0.0	0.1	5.3	4.8
M	0.0	0.0	0.0	0.0	5.5	1.3
A	0.0	0.0	0.0	0.0	1.7	0.4
M	0.0	0.0	0.0	0.1	0.4	0.1
J	0.0	0.0	0.0	0.0	0.0	0.4
J	0.3	0.1	0.0	0.3	0.0	0.4
A	0.0	0.0	0.0	0.0	0.0	0.1
S	0.0	0.0	0.0	0.0	0.1	0.3
O	0.0	0.0	0.0	0.0	0.0	0.0
O	0.0	0.0	0.0	0.0	0.1	0.0
N	0.0	0.0	0.0	0.1	0.3	0.4
D	0.0	0.0	0.0	0.3	0.7	1.1
1993 J	0.7	0.1	0.0	0.2	1.5	1.1
F	0.0	0.1	0.1	0.8	2.3	2.2
M	0.0	0.1	0.1	0.1	0.4	2.7
A	0.0	0.1	0.0	0.1	0.1	1.2
M	0.0	0.1	0.0	0.0	0.4	0.6
J	0.2	0.0	0.0	0.0	0.1	0.1
J	0.0	0.0	0.0	0.0	0.0	0.4
A	0.2	0.0	0.0	0.0	0.1	0.0
S	0.0	0.0	0.0	0.0	0.1	0.2
O	0.0	0.0	0.1	0.0	0.0	0.0
O	0.2	0.0	0.0	0.0	0.0	0.5
N	0.0	0.0	0.0	0.0	0.5	0.1
D	0.3	0.0	0.0	0.0	2.0	0.7
1994 J	1.0	0.6	0.1	0.5	1.4	3.4
F	0.8	0.4	0.4	1.6	2.1	4.1
M	0.1	0.0	0.0	0.6	0.4	2.5

RHIPICEPHALUS ADULTS ON BODY

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
1991 J	0.4	0.0		0.0	1.0	0.5
F	0.5	0.0	0.3	0.0	0.8	0.1
M	0.0	0.0	0.0	0.1	0.0	0.1
A	0.0	0.0	0.0	0.0	0.0	0.0
M	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.0	0.0	0.0	0.3	0.0
J	0.0	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	0.0	0.0	0.1	0.0
S	0.6	0.1	0.8	0.0	0.7	0.0
O	0.1	0.6	2.1	0.7	5.5	0.6
N	0.0	0.0	2.4	0.5	3.2	2.3
N	0.5	0.1	0.0	0.1	1.5	0.3
D	0.0	0.4	0.9	0.0	1.7	1.5
1992 J	0.1	0.1	0.3	0.0	1.0	0.3
F	0.0	0.1	0.1	0.1	0.0	0.0
M	0.0	0.0	0.0	0.0	0.3	0.1
A	0.0	0.0	0.0	0.0	0.1	0.1
M	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	0.0	0.0	0.0	0.0
S	0.3	0.1	0.0	0.1	0.1	0.0
O	1.0	0.4	2.2	3.0	0.5	0.4
O	0.3	0.0	0.7	0.7	0.9	1.0
N	0.7	0.0	1.9	0.8	2.2	1.2
D	0.1	0.0	0.6	0.3	0.7	1.0
1993 J	0.0	0.0	0.3	0.0	0.0	0.0
F	0.0	0.0	0.0	0.1	0.0	0.4
M	0.0	0.0	0.1	0.1	0.0	0.1
A	0.0	0.0	0.0	0.1	0.0	0.3
M	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.1	0.0	0.0	0.0	0.0
J	0.0	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	0.0	0.0	0.0	0.0
S	0.1	0.3	0.0	0.3	0.3	0.0
O	0.3	0.3	1.2	1.7	0.5	0.0
O	0.5	0.8	0.7	1.6	0.6	0.6
N	0.6	0.3	2.0	2.1	0.2	1.9
D	0.6	0.0	0.1	0.5	1.2	0.4
1994 J	0.3	0.1	0.9	0.0	0.3	0.0
F	0.0	0.0	0.4	0.0	0.1	0.9
M	0.0	0.0	0.0	0.0	0.5	0.1

AMBLYOMMA ADULTS

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
1991 J	1.8	0.3		0.0	2.0	2.0
F	0.5	0.2	0.0	0.0	1.0	0.7
M	0.0	0.0	0.0	0.0	0.1	0.3
A	0.0	0.0	0.0	0.0	0.1	0.0
M	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.0	0.0	0.0	0.0	0.1
A	0.0	0.0	0.1	0.0	0.0	0.0
S	0.1	0.0	0.1	0.0	0.0	0.0
O	0.3	0.3	0.0	0.1	0.9	0.2
N	3.8	0.0	1.8	1.8	5.0	3.2
N	2.2	0.2	3.5	7.1	7.9	11.0
D	2.9	0.2	0.1	0.0	14.5	5.9
1992 J	0.8	0.1	0.3	0.0	7.7	3.7
F	0.1	0.0	0.3	0.0	0.6	1.6
M	0.0	0.0	0.1	0.0	0.4	0.0
A	0.1	0.0	0.0	0.0	0.3	0.0
M	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.0	0.1	0.0	0.1	0.0
J	0.0	0.0	0.0	0.0	0.0	0.0
A	0.1	0.0	0.1	0.0	0.1	0.0
S	0.1	0.0	0.0	0.1	0.4	0.0
O	0.0	0.0	0.3	0.6	0.7	0.4
O	1.3	0.0	0.6	0.8	1.5	1.3
N	7.7	1.9	5.9	2.2	9.2	3.3
D	0.5	0.2	4.1	0.4	3.6	10.7
1993 J	0.0	0.0	0.7	0.9	9.2	4.1
F	0.0	0.0	0.0	0.0	6.1	3.7
M	0.0	0.0	0.0	0.0	0.0	0.4
A	0.0	0.0	0.1	0.0	0.2	0.0
M	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.0	0.0	0.0	0.0	0.0
J	0.0	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	0.0	0.0	0.0	0.0
S	0.0	0.0	0.0	0.0	0.0	0.0
O	0.9	0.0	0.4	0.4	0.0	0.1
O	0.5	0.3	1.2	1.1	0.9	0.9
N	2.3	2.7	7.5	12.5	5.8	4.1
D	2.5	0.2	4.6	3.1	6.4	4.7
1994 J	1.0	0.1	1.5	0.0	1.4	1.5
F	0.3	0.0	0.5	0.0	0.2	2.1
M	0.1	0.0	0.1	0.0	0.5	0.8

AMBLYOMMA NYMPHS

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
1991 J	0.0	0.0		0.0	0.9	0.0
F	0.0	0.0	0.0	0.0	0.0	0.2
M	0.1	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	0.0	0.0	0.0	0.0
M	1.4	0.3	0.0	0.0	0.1	0.3
J	1.6	0.3	0.3	0.5	2.8	0.3
J	1.2	0.3	0.3	1.6	0.3	0.9
A	0.5	0.1	1.0	0.9	1.8	2.8
S	0.0	0.0	0.7	0.0	1.6	2.1
O	0.1	0.0	0.1	0.3	0.5	2.1
N	0.5	0.0	0.1	0.0	0.0	0.6
N	0.0	0.0	0.0	0.0	0.4	0.1
D	0.0	0.0	0.0	0.0	0.0	0.0
1992 J	0.0	0.0	0.0	0.0	0.0	0.0
F	0.0	0.0	0.0	0.0	0.0	0.0
M	0.0	0.0	0.0	0.2	0.0	0.0
A	0.5	0.3	0.3	0.3	1.6	0.7
M	2.0	0.3	0.3	2.4	0.0	0.0
J	0.3	0.9	0.6	0.3	0.5	0.2
J	0.5	0.2	0.9	0.9	0.0	1.7
A	0.4	0.0	1.2	0.3	3.9	1.1
S	0.9	1.3	1.7	0.1	1.1	2.0
O	0.3	0.3	0.6	0.3	0.6	0.7
O	0.0	0.1	0.3	0.0	0.3	0.2
N	0.0	0.0	0.0	0.0	0.3	0.1
D	0.0	0.0	0.0	0.0	0.0	0.0
1993 J	0.0	0.0	0.0	0.0	0.0	0.0
F	0.0	0.0	0.0	0.0	0.0	0.0
M	0.0	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	0.1	0.0	0.0	0.1
M	0.4	0.8	0.1	2.8	0.7	1.4
J	3.2	1.0	0.1	4.0	2.9	1.3
J	1.1	0.1	0.4	4.0	2.0	0.7
A	1.5	0.7	2.6	6.2	0.8	1.5
S	1.4	0.5	3.3	2.8	1.1	1.9
O	0.3	0.3	1.0	4.1	1.2	0.7
O	0.3	0.0	1.3	0.6	1.1	0.9
N	0.0	0.0	0.3	0.3	0.1	0.1
D	0.0	0.0	0.0	0.0	0.1	0.3
1994 J	0.0	0.0	0.0	0.0	0.0	0.0
F	0.0	0.0	0.0	0.0	0.0	0.0
M	0.0	0.0	0.0	0.0	0.0	0.0

BOOPHILUS ADULTS

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
1991 J	0.4	0.1		0.0	0.0	0.7
F	1.5	0.0	0.1	0.0	6.2	2.4
M	0.9	0.9	0.7	0.1	4.3	4.0
A	0.7	0.4	0.1	0.1	7.3	4.1
M	1.3	2.0	0.7	0.3	7.5	14.1
J	5.9	0.0	0.3	0.1	2.2	2.4
J	0.1	0.2	0.4	0.2	3.8	0.6
A	0.0	0.0	0.0	0.0	1.4	1.6
S	0.0	0.0	0.5	0.0	2.3	2.0
O	0.3	0.0	0.0	0.0	0.3	1.4
N	0.3	0.0	0.5	0.1	0.7	0.6
N	1.0	0.7	0.1	0.6	1.0	4.4
D	1.1	0.3	0.3	0.1	4.4	1.5
1992 J	0.8	0.2	0.1	0.0	3.4	1.9
F	2.3	0.0	0.0	0.0	2.7	7.9
M	0.6	0.4	0.0	0.0	13.1	6.4
A	6.4	0.4	0.0	2.9	16.8	6.4
M	7.7	1.1	9.0	0.8	13.4	7.3
J	3.4	1.0	1.4	0.3	5.2	12.8
J	1.3	0.5	0.3	0.9	1.4	0.5
A	0.1	0.3	1.5	3.4	0.3	1.0
S	0.1	0.4	2.0	0.3	1.2	2.8
O	0.7	0.7	0.3	0.3	1.0	0.9
O	0.8	0.1	0.5	0.6	0.3	0.9
N	6.8	4.2	2.9	2.6	0.5	0.9
D	2.6	0.5	2.5	4.5	1.2	6.8
1993 J	1.7	0.0	4.1	5.0	1.6	4.8
F	1.5	0.0	1.8	2.1	1.1	5.8
M	0.1	0.1	0.0	1.3	0.8	2.6
A	10.0	0.8	0.1	19.9	9.0	11.9
M	2.8	3.4	7.9	10.0	2.0	6.9
J	6.2	0.5	6.8	4.5	2.9	3.1
J	5.3	0.6	1.6	4.1	2.0	1.0
A	2.5	2.0	11.0	1.9	1.0	0.4
S	0.1	2.5	2.2	4.6	3.5	4.0
O	0.7	0.7	2.6	4.7	0.8	1.1
O	0.8	0.5	3.6	0.2	1.1	0.9
N	3.4	5.3	6.1	6.2	0.1	1.5
D	1.3	0.1	0.5	1.3	3.3	3.4
1994 J	0.3	1.0	0.0	0.0	0.1	0.3
F	0.4	0.4	0.0	0.1	1.9	2.5
M	0.7	0.1	0.0	1.2	2.2	6.6

APPENDIX 14. MONTHLY RAINFALL IN STUDY AREA

CHITEDZE	JUL	AUG	SEPT	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN
81/82	0	0	0	31	105	112	253	293	66	61	33	0
82/83	0	2	0	38	82	156	145	185	67	43	3	6
83/84	12	0	0	5	14	185	206	165	110	13	4	0
84/85	6	0	0	0	116	253	192	208	245	54	0	0
85/86	3	0	0	15	144	177	188	200	192	36	1	0
86/87	0	0	0	19	28	349	289	179	224	50	0	0
87/88	0	0	1	22	44	140	203	172	272	53	2	0
88/89	0	0	0	43	7	102	359	271	268	12	11	4
89/90	0	0	0	2	153	217	327	149	88	53	80	0
90/91	0	1	0	1	82	59	229	149	80	30	5	1
91/92	1	0	23	1	109	177	132	21	147	11	1	0
92/93	0	0	0	0	45	185	208	267	187	5	0	0
93/94	1	0	5	1	60	42						
MBABZI												
81/82												
82/83							88	174	95	16	2	1
83/84	17	0	0	0	14	248	118	229	135	11	5	0
84/85	14	0	0	0	109	303	241	239	238	24	0	0
85/86	0	0	0	28	102	213	201	192	130	29	0	0
86/87	0	0	0	19	89	270	287	106	204	89	0	0
87/88	0	0	0	6	16	232	139	188	151	27	4	0
88/89	0	0	0	50	53	162	409	249	288	13	9	9
89/90	0	0	0	7	100	45	311	83	8	124	74	0
90/91	0	0	0	0	45	178	230	145	99	13	0	0
91/92	0	0	8	0	125	190	170	65	97	5	2	0
92/93	0	0	0	0	27	220	177	172	232	9	0	0
93/94	0	0	0	0	47	61	370					
BUNDA												
81/82	0	0	0	30	119	128	395	236	74	52	30	0
82/83	0	0	0	52	85	224	119	229	188	39	4	9
83/84	1	0	1	11	110	175	182	230	153	37	27	5
84/85	3	1	3	0	113	355	71	245	168	0	0	0
85/86	0	1	0	22	103	128	276	263	276	181	6	0
86/87	0	0	0	40	33	225	219	170	139	6	13	0
87/88	0	0	1	5	36	152	225	253	181	102	4	0
88/89	0	0	0	70	72	121	249	257	242	37	12	0
89/90	0	0	0	19	207	287	251	87	99	78	86	0
90/91	0	0	0	3	84	82	254	182	119	34	11	1
91/92	2	0	5	25	260	195	178	23	120	7	5	0
92/93	0	0	0	0	36	141	173	388	212	89	0	7
93/94	0	0	0	0	56	104						
KASIYA												
81/82	0	0	0	7	94	402	292	167	10	130	59	0
82/83	0	1	0	100	63	145	165	223	60	73	2	0
83/84	0	0	0	0	58	243	107	208	197	17	0	0
84/85	0	0	0	6	119	407	49	220	196	22	37	0
85/86	0	0	7	29	179	299	153	205	109	58	0	0
86/87	0	0	0	17	28	152	167	238	181	0	0	0
87/88	0	0	0	7	25	97	121	181	137	9	2	0
88/89	0	0	0	57	56	80	455	236	147	15	0	0
89/90	0	0	0	0	114	263	301	104	54	34	54	0
90/91	0	0	0	0	30	82	80	147	125	89	0	0
91/92	0	0	0	1	86	109	86	15	70	6	2	0
92/93	0	0	0	0	45	185	190	111	34	13	0	0
93/94	0	0		7	50	61						

SINYALA

81/82	0	0	0	13	91	111	367	246	33	186	35	0
82/83	0	0	0	26	120	92	287	209	92	57	4	16
83/84	12	0	0	22	50	183	287	160	181	46	3	0
84/85	0	4	17	5	111	324	132	102	151	113	0	0
85/86	0	0	0	1	117	202	260	240	170	87	0	0
86/87	0	0	0	44	53	212	237	152	100	7	0	0
87/88	0	0	0	15	37	87	305	248	157	78	1	0
88/89	0	0	0	72	54	116	317	203	189	27	1	0
89/90	0	0	0	3	200	194	316	115	107	96	0	0
90/91	0	0	5	0	16	79	265	250	100	21	0	0
91/92	0	0	0	70	55	205	165	10	111	31	0	0
92/93	0	0	0	0	62	180	95	352	199	131	0	0
93/94	0	0	0	9	42	117						

APPENDIX 15. ANNUAL RAINFALL IN STUDY AREA

	CHITEDZE	MBABZI	BUNDA	KASIYA	SINYALA	MEAN
57/58	846.6		788.7	1015.0	753.6	851.0
58/59	784.4		708.7	756.4	697.5	736.7
59/60	771.9		859.3	751.1	751.1	783.3
60/61	1105.2		852.4	1120.9	882.1	990.2
61/62	1045.0		922.3	1215.4	1275.1	1114.4
62/63	833.9		853.7	1191.3	1326.1	1051.2
63/64	913.9		774.7	877.1	1055.1	905.2
64/65	807.5		904.5	1001.5	739.4	863.2
65/66	559.1		928.9	691.6	832.4	753.0
66/67	810.8		923.5	705.6	919.0	839.7
67/68	646.7	707.9	724.9	873.3	873.3	765.2
68/69	916.7	1095.0	1258.6	1136.9	1136.9	1108.8
69/70	947.4	780.0	575.1	763.5	763.5	765.9
70/71	1118.1	1056.1	1195.3	1043.4	1043.4	1091.3
71/72	926.1	682.2	936.0			848.1
72/73	726.9	621.0	715.8	1030.2	1030.2	824.8
73/74	1226.1	1163.3	1114.0	968.2	968.2	1088.0
74/75	994.2	834.1	784.6	926.1	926.1	893.0
75/76	1014.2	875.0	848.1	1065.5	1065.5	973.7
76/77	987.8	914.9	846.1	865.9	865.9	896.1
77/78	1106.7	1039.9	932.4	1330.7	993.1	1080.6
78/79	764.3	856.5	843.0	987.5	917.2	873.7
79/80	707.6	760.0	921.0	613.2		750.4
80/81	905.7	1001.8	1065.5	872.3	929.2	954.9
81/82	954.7	876.0	1063.9	1160.6	1083.7	1027.8
82/83	727.2	690.0	948.3	832.4	901.2	819.8
83/84	714.6	777.0	930.9	829.8	944.1	839.3
84/85	1074.1	1168.0	958.2	1056.8	958.1	1043.0
85/86	956.1	895.0	1256.9	1038.8	1077.3	1044.8
86/87	1139.2	1064.0	845.2	781.9	805.3	927.1
87/88	908.6	763.0	959.8	578.2	925.5	827.0
88/89	1076.7	1242.0	1059.0	1045.7	978.7	1080.4
89/90	1069.0	752.0	1113.9	922.3	1030.6	977.6
90/91	634.7	710.0	770.3	552.5	736.4	680.8
91/92	622.4	662.0	819.9	376.1	646.8	625.4
92/93	896.1	837.0	1044.0	576.0	1017.0	874.0

OVERALL MEAN FOR 30 YEARS, 5 STATIONS = 907.3 MM

APPENDIX 16. MONTHLY MEAN MINIMUM & MAXIMUM TEMPERATURES IN STUDY AREA

CHITEDZE (MAXIMUM)

	J	F	M	A	M	J	J	A	S	O	N	D
1989	26.9	26.9	27.0	26.8	25.7	24.0	24.0	25.6	28.5	29.8	29.9	27.5
1990	26.0	26.9	28.1	27.5	25.3	24.9	24.3	24.6	27.2	30.5	30.5	29.2
1991	26.1	27.3	26.8	26.1	26.3	24.1	23.5	26.4	28.6	30.0	29.7	27.0
1992	27.5	29.4	28.4	27.4	26.8	24.8	23.5	25.0	28.5	30.6	30.0	28.2
1993	26.3	26.5	27.1	26.9	26.5	32.2	23.0	24.3	27.4	30.0	29.5	29.5

KAMUZU INTERNATIONAL AIRPORT (MAXIMUM)

1989	24.7	25.6	24.9	24.8	23.9	22.7	22.4	23.5	26.5	28.4	27.9	26.1
1990	25.8	26.6	27.1	27.1	24.7	24.1	23.0	23.8	26.1	29.6	29.5	28.4
1991	25.5	26.8	25.9	24.9	24.6	22.7	22.1	24.9	27.1	28.8	28.9	26.7
1992	26.6	28.3	27.0	26.5	25.7	23.8	22.4	23.8	27.1	29.7	29.3	27.8
1993	25.8	25.7	25.1	25.9	25.8	22.4	21.8	23.4	26.4	29.0	28.8	25.5
MEAN MAXIMUM	26.1	27.0	26.7	26.4	25.5	24.6	23.0	24.5	27.3	29.6	29.4	27.6

CHITEDZE (MINIMUM)

	J	F	M	A	M	J	J	A	S	O	N	D
1989	17.5	17.3	16.7	14.7	11.4	8.6	8.5	9.5	12.1	15.0	16.6	17.5
1990	17.7	17.4	15.9	15.8	13.5	10.3	8.7	9.7	12.0	14.8	17.2	18.5
1991	18.2	18.4	17.5	14.8	12.8	8.9	9.1	9.7	12.3	15.3	17.5	17.7
1992	17.6	16.7	17.6	15.1	13.7	10.7	8.8	10.1	12.6	15.5	17.6	18.5
1993	18.0	17.9	17.3	15.8	11.7	9.2	9.1	10.1	12.5	14.9	18.1	18.3

KAMUZU INTERNATIONAL AIRPORT (MINIMUM)

1989	17.5	17.6	16.7	14.9	12.1	9.5	8.3	10.4	12.8	15.9	17.7	17.5
1990	17.5	17.2	15.4	15.5	13.0	10.1	8.2	9.2	12.1	15.9	17.3	18.5
1991	17.9	18.0	17.3	14.0	12.3	8.1	8.5	9.3	12.8	15.3	17.6	17.8
1992	17.3	17.0	17.5	15.4	13.8	9.9	7.8	9.3	12.9	17.0	17.8	18.4
1993	17.8	17.3	16.8	16.3	11.3	8.1	8.7	9.9	12.7	15.4	18.2	19.3
MEAN MINIMUM	17.7	17.5	16.9	15.2	12.6	9.3	8.6	9.7	12.5	15.5	17.6	18.2

APPENDIX 17. NUMBER OF CATTLE DIPPED AT STUDY DIP TANKS IN 1992.

	10/1/92	24/1/92	2/2/92	21/2/92	6/3/92	20/3/92	27/11/92	11/12/92	25/12/92	MEAN	% AGE
DICKSON											
TAGGED	232	264	250	333	234	320	257	251	365	278	82%
UNTAGGED	1543	827	1100	1740	1255	889	538	591	337	980	31%
TOTAL	1775	1091	1350	2073	1489	1209	795	842	702	1258	36%
MBABZI											
TAGGED	258	310	329	287	321	NR	299	364	316	311	85%
UNTAGGED	651	881	939	1108	847	NR	1403	1330	548	963	39%
TOTAL	909	1191	1268	1395	1168	NR	1702	1694	864	1274	44%
NAMAGUYA											
TAGGED	234	221	205	244	219	247	253	283	256	240	84%
UNTAGGED	829	887	814	961	841	1464	1303	1273	1102	1053	48%
TOTAL	1063	1108	1019	1205	1060	1711	1556	1556	1358	1293	52%
SINYALA											
TAGGED	88	NR	NR	259	NR	NR	NR	NR	247	198	74%
UNTAGGED	1264	NR	NR	865	NR	NR	NR	NR	613	914	42%
TOTAL	1352	NR	NR	1124	NR	NR	NR	NR	860	1112	45%

NR = NOT RECORDED

1992 CATTLE POPULATIONS (A + B REGISTERS)

DICKSON =	3493
MBABZI =	2868
NAMAGUYA =	2479
SINYALA =	2448

NUMBER OF TAGGED CATTLE 1992 (ANIMAL YEARS)

DICKSON =	337
MBABZI =	367
NAMAGUYA =	287
SINYALA =	266

NUMBER OF FARMERS WITH TAGGED CATTLE 1992

DICKSON =	22
MBABZI =	21
NAMAGUYA =	22
SINYALA =	22

APPENDIX 18. NUMBER OF CATTLE DIPPED AT STUDY DIP TANKS IN 1993.

	8/1/93	22/1/93	5/2/93	19/2/93	5/3/93	19/3/93	3/12/93	17/12/93	31/12/93	MEAN	% AGE
DICKSON											
TAGGED	250	332	341	317	290	242	239	214	234	273	74%
UNTAGGED	546	547	665	678	646	528	511	446	475	560	16%
TOTAL	796	879	1006	995	936	770	750	660	709	833	22%
MBABZI											
TAGGED	332	271	317	349	302	327	113	229	175	268	96%
UNTAGGED	787	811	630	666	654	451	356	432	385	575	27%
TOTAL	1119	1082	947	1015	956	778	469	661	560	843	35%
NAMAGUYA											
TAGGED	288	284	279	271	228	248	160	184	199	238	95%
UNTAGGED	1144	798	818	282	880	1107	1416	1707	1502	1073	49%
TOTAL	1432	1082	1097	553	1108	1355	1576	1891	1701	1311	54%
SINYALA											
TAGGED	261	253	270	251	262	242	208	229	216	244	81%
UNTAGGED	451	709	709	808	511	266	863	694	641	628	41%
TOTAL	712	962	979	1059	773	508	1071	923	857	872	47%

NR = NOT RECORDED

1993 CATTLE POPULATIONS (A + B REGISTERS)

DICKSON =	3788
MBABZI =	2409
NAMAGUYA =	2428
SINYALA =	1849

NUMBER OF TAGGED CATTLE 1993 (ANIMAL YEARS)

DICKSON =	368
MBABZI =	279
NAMAGUYA =	250
SINYALA =	300

NUMBER OF FARMERS WITH TAGGED CATTLE 1993

DICKSON =	23
MBABZI =	21
NAMAGUYA =	21
SINYALA =	22

ACARICIDE USED FOR EACH TANK IN 1993

DICKSON =	76.40 LITRES
NAMAGUYA =	56.40 LITRES
MBABZI =	27.00 KGS
SINYALA =	27.00 KGS

APPENDIX 19. ACTUAL ACARICIDE USAGE 1992

REGIME	CHLORFENVINPHOS		AMITRAZ	
DIPTANK	DICKSON	NAMACUYA	SINYALA	MBABZI
NUMBER OF IMMERSIONS	9	9	9	9
QUANTITY OF TRIATIX TR USED (KG'S)			27	27
QUANTITY OF SUPONA 30 USED (L'S)	60	62.6		
COST AT 1992 PRICES (MALAWI KWACHA)	1419	1480	2788	2788
AVERAGE COST PER TANK FOR 1992 (MK)		1449.5		2788

COSTINGS ARE BASED ON:-

TRIATIX TR AT MK 103.25/KG AND SUPONA 30 AT MK 23.65/KG

APPENDIX 20. BREEDING MALES AND FEMALES (NOVEMBER 1992).

REGIME	CHLORFENVINPHOS		AMITRAZ		UNDIPPED		TOTAL
DIPTANK	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI	
NUMBER BREEDING FEMALES	109	86	95	118	159	74	641
NUMBER BREEDING MALES	15	16	17	7	9	10	74
BREEDING FEMALES PER BREEDING MALE	7.3	5.4	5.6	16.9	17.7	7.4	8.7

NOTE:- BREEDING FEMALE = FEMALE AFTER DAY OF FIRST CALVING
BREEDING MALE = ENTIRE MALE OVER 2 YEARS OLD

APPENDIX 21. PERCENTAGE WORK OXEN AND MILK COWS NOVEMBER 1992.

REGIME	CHLORFENVINPHOS		AMITRAZ		UNDIPPED		TOTAL
DIPTANK	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI	
NO. MILKED	34	30	32	52	28	13	189
NO. OXEN	73	61	36	32	65	20	287
NO. COWS	109	86	95	118	159	74	641
NO.MALES >1 YR	131	101	69	83	135	49	568
COWS MILKED %	31.2%	34.9%	33.7%	44.1%	17.6%	17.6%	29.5%
WORK OXEN %	55.7%	60.4%	52.2%	38.6%	48.1%	40.8%	50.5%

COWS YIELD APPROX 600mls DAILY.

APPENDIX 22. SALE PRICES 1992

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI	TOTAL
BREEDING MALES							
NO. SOLD	0	0	0	1	3	0	4
MEAN PRICE	0	0	0	920	865	0	879
BREEDING FEMALES							
NO. SOLD	3	3	3	10	7	0	26
MEAN PRICE	547	413	425	496	591	0	510
REPLACEMENT FEMALES							
NO. SOLD	3	0	3	2	8	0	16
MEAN PRICE	432	0	380	490	400	0	414
MATURE SURPLUS MALES							
NO. SOLD	3	5	6	13	21	0	48
MEAN PRICE	632	620	591	568	698	0	637
CALVES							
NO. SOLD	0	0	0	0	1	0	1
MEAN PRICE	0	0	0	0	150	0	150

APPENDIX 23. SALE PRICES 1993

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI	TOTAL
BREEDING MALES							
NO. SOLD	0	5	3	3	3	2	16
MEAN PRICE	0	602	567	1057	865	790	754
BREEDING FEMALES							
NO. SOLD	13	13	17	36	19	2	100
MEAN PRICE	539	423	458	493	609	500	506
REPLACEMENT FEMALES							
NO. SOLD	6	1	4	18	10	0	39
MEAN PRICE	406	550	435	450	415	0	435
MATURE SURPLUS MALES							
NO. SOLD	33	13	16	31	36	1	130
MEAN PRICE	782	677	661	539	694	400	671
CALVES							
NO. SOLD	0	0	1	1	1	0	3
MEAN PRICE	0	0	280	350	150	0	260

APPENDIX 24. OFFTAKE RATES BY AREA AND YEAR

Appendix 24a Offtake rates 1991.

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
ADULT MALE SALES	6	6	3	5	10	0
ADULT MALE OTHER	2	7	1	4	6	12
ADULT MALE PURCHASES	1	1	0	0	4	0
NET ADULT MALE SALES	7	12	4	9	12	12
ADULT MALE YEARS	56	41	21	27	43	35
ANNUAL ADULT	12.6%	29.0%	19.4%	33.0%	27.8%	34.1%
MALE				22.1%		30.6%
CULLING RATE						25.1%

ADULT FEMALE SALES	4	5	4	2	10	0
ADULT FEMALE OTHER	6	11	13	4	11	11
ADULT FEMALE PURCHASES	0	0	0	2	11	2
NET ADULT FEMALE SALES	10	16	17	4	10	9
ADULT FEMALE YEARS	109	86	95	134	151	116
ANNUAL ADULT	9.1%	18.6%	18.0%	3.0%	6.6%	7.8%
FEMALE				11.1%		7.1%
CULLING RATE						9.6%

Appendix 24b Offtake rates 1992

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
ADULT MALE SALES	3	6	9	11	18	1
ADULT MALE OTHER	7	6	1	6	2	5
ADULT MALE PURCHASES	0	2	0	3	0	0
NET ADULT MALE SALES	10	10	10	14	20	6
ADULT MALE YEARS	52	34	18	17	31	17
ANNUAL ADULT	19.3%	29.3%	56.8%	82.8%	64.9%	34.5%
MALE				36.6%		53.9%
CULLING RATE						41.5%

ADULT FEMALE SALES	6	5	8	15	13	0
ADULT FEMALE OTHER	7	3	9	16	10	8
ADULT FEMALE PURCHASES	6	1	10	2	2	0
NET ADULT FEMALE SALES	7	7	7	29	21	8
ADULT FEMALE YEARS	108	82	82	121	146	80
ANNUAL ADULT	6.5%	8.5%	8.5%	23.9%	14.4%	10.0%
FEMALE				12.7%		12.8%
CULLING RATE						12.7%

Appendix 24c. Offtake rates 1993

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
ADULT MALE SALES	29	18	9	13	9	5
ADULT MALE OTHER	8	9	7	6	6	3
ADULT MALE PURCHASES	1	0	0	0	0	0
NET ADULT MALE SALES	36	27	16	19	15	8
ADULT MALE YEARS	38	27	13	10	20	9
ANNUAL ADULT	94.7%	101.5%	123.1%	191.9%	74.6%	85.1%
MALE				112.0%		78.0%
CULLING RATE						103.4%

ADULT FEMALE SALES	22	13	15	33	13	5
ADULT FEMALE OTHER	18	32	13	31	6	17
ADULT FEMALE PURCHASES	2	0	2	0	2	2
NET ADULT FEMALE SALES	38	45	26	64	17	20
ADULT FEMALE YEARS	107	78	81	94	157	68
ANNUAL ADULT	35.5%	57.6%	32.3%	68.2%	10.8%	29.2%
FEMALE				48.1%		16.4%
CULLING RATE						35.9%

NOTE:- "Adult" means 3 years and over.

APPENDIX 25. MEDIAN AGE AT FIRST CALVING 1992 & 1993

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
1992	41	42	43	48	39	43
MEDIAN AGE	41		45		41	
AT FIRST	43				41	
CALVING (MONTHS)	43					

1993	42	38	40	44	40	48
MEDIAN AGE	41		43		41	
AT FIRST	41				41	
CALVING (MONTHS)	41					

NOTE:- AGES OF ANIMALS OVER 2 YEARS OLD IN 1992 AND 3 YEARS OLD IN 1993 ARE ESTIMATES AND ARE ONLY ACCURATE +/- 3 MONTHS.

APPENDIX 26. COW PARTURITION RATES 1992

	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES BORN TO COWS	49	48	44	62	99	42
COW YEARS	104	81	82	114	141	75
COW PARTURITION RATE	47%	59%	54%	54%	70%	56%
	53%				65%	
	58%					

APPENDIX 27. CALVING RATES

1991	DICKSON	NAMAGUYA	SINYALA	MBABZI	TONDE	LIKUNI
CALVES IN PERIOD	71	59	54	79	105	68
COW YEARS	85.5	66.4	79.4	102.7	121.4	86.2
ANNUAL PERCENTAGE	83.0%	88.9%	68.0%	76.9%	86.5%	78.9%
CALVING				78.7%		83.3%
RATE						80.5%

1992						
CALVES IN PERIOD	83	68	72	88	135	51
COW YEARS	104	81	82	114	141	75
ANNUAL PERCENTAGE	79.8%	84.0%	87.8%	77.2%	95.7%	68.0%
CALVING				81.6%		86.1%
RATE						83.2%

1993						
CALVES IN PERIOD	67	45	44	47	116	40
COW YEARS	108	79	82	95	163	65.8
ANNUAL PERCENTAGE	61.8%	56.9%	53.5%	49.5%	71.3%	60.8%
CALVING				55.6%		68.3%
RATE						60.5%

NOTE:- First parturitions are included in the numerator but heifers before first calving are not included in the der

APPENDIX 28. PERIODIC AVERAGE COST/HEAD/YEAR OF VARIOUS CONTROL OPTIONS

Strategic dipping:

No. dippings per year 9

SUPONA 30

		Cost (MK)										
ITEM	Year	1	2	3	4	5	6	7	8	9	10	25
Dip capital		50000	0	0	0	0	0	0	0	0	0	0
Dip maintenance		4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000
Recharging labour/mach.		80	80	80	80	80	80	80	80	80	80	80
Labour/management dipping		252	252	252	252	252	252	252	252	252	252	252
Acaricide		4329	4329	4329	4329	4329	4329	4329	4329	4329	4329	4329
Water		0	0	0	0	0	0	0	0	0	0	0
TOTAL		58661	8661	8661	8661	8661	8661	8661	8661	8661	8661	8661
COST PER HEAD PER YEAR		58.66	8.66	8.66	8.66	8.66	8.66	8.66	8.66	8.66	8.66	8.66
PERIOD AVE. COST/HEAD/YEAR		58.66	34.85	26.94	23.00	20.65	19.10	18.00	17.18	16.55	16.06	13.67

TRIATIX TR

		Cost (MK)										
ITEM	Year	1	2	3	4	5	6	7	8	9	10	25
Dip capital		50000	0	0	0	0	0	0	0	0	0	0
Dip maintenance		4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000
Recharging labour/mach.		80	80	80	80	80	80	80	80	80	80	80
Labour/management dipping		252	252	252	252	252	252	252	252	252	252	252
Acaricide		5832	5832	5832	5832	5832	5832	5832	5832	5832	5832	5832
Water		0	0	0	0	0	0	0	0	0	0	0
TOTAL		60164	10164	10164	10164	10164	10164	10164	10164	10164	10164	10164
COST PER HEAD PER YEAR		60.16	10.16	10.16	10.16	10.16	10.16	10.16	10.16	10.16	10.16	10.16
PERIOD AVE. COST/HEAD/YEAR		60.16	36.35	28.44	24.50	22.15	20.60	19.50	18.68	18.06	17.56	15.17

Strategic spraying:

No. sprays per year 9

SUPONA 30

		Cost (MK)									
ITEM	Year	1	2	3	4	5	6	7	8	9	10
Sprayer capital		700	0	0	0	0	0	0	0	0	0
Labour/management		252	252	252	252	252	252	252	252	252	252
Acaricide		5110.2	5110.2	5110.2	5110.2	5110.2	5110.2	5110.2	5110.2	5110.2	5110.2
TOTAL		6062.2	5362.2	5362.2	5362.2	5362.2	5362.2	5362.2	5362.2	5362.2	5362.2
COST PER HEAD PER YEAR		6.06	5.36	5.36	5.36	5.36	5.36	5.36	5.36	5.36	5.36
PERIOD AVE. COST/HEAD/YEAR		6.06	5.73	5.62	5.56	5.53	5.51	5.49	5.48	5.47	5.47

Strategic pour-on

No. applications per year 6

		Cost (MK)										
ITEM	Year	1	2	3	4	5	6	7	8	9	10	25
Labour/management		168	168	168	168	168	168	168	168	168	168	168
Pour-on acaricide		18000	18000	18000	18000	18000	18000	18000	18000	18000	18000	18000
TOTAL		18168	18168	18168	18168	18168	18168	18168	18168	18168	18168	18168
COST PER HEAD PER YEAR		18.17	18.17	18.17	18.17	18.17	18.17	18.17	18.17	18.17	18.17	18.17
PERIOD AVE. COST/HEAD/YEAR		18.17	18.17	18.17	18.17	18.17	18.17	18.17	18.17	18.17	18.17	18.17

Stop dipping: ad hoc treatment of ECF cases with (bu-)parvaquone

		Cost (MK)										
ITEM	Year	1	2	3	4	5	6	7	8	9	10	25
Calf treatment		809	8085	12128	8085	0	0	0	0	0	0	0
Adults/followers		35530	53295	17765	8883	0	0	0	0	0	0	0
TOTAL		36338.5	61380	29893	16967.5	0	0	0	0	0	0	0
COST PER HEAD PER YEAR		36.34	61.38	29.89	16.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PERIOD AVE. COST/HEAD/YEAR		36.34	48.26	42.71	37.17	31.08	27.05	24.20	22.08	20.46	19.17	12.98

Stop dipping + Theileria immunization

		Cost (MK)										
ITEM	Year	1	2	3	4	5	6	7	8	9	10	25
Professional fees		2793	985	0	0	0	0	0	0	0	0	0
Farm labour		0	0	0	0	0	0	0	0	0	0	0
Cost of stabilate		13045	2291	0	0	0	0	0	0	0	0	0
Cost of tetracycline		31010	2079	0	0	0	0	0	0	0	0	0
Cost of adverse reactions		7564	1328	0	0	0	0	0	0	0	0	0
TOTAL		54412	6683	0	0	0	0	0	0	0	0	0
TOTAL/HEAD/YEAR		54.41	6.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PERIOD AVE. COST/HEAD/YEAR		54.41	31.68	22.11	17.35	14.51	12.63	11.29	10.31	9.55	8.95	6.06

Cessation of dipping

		Cost (MK)										
ITEM	Year	1	2	3	4	5	6	7	8	9	10	25
Losses cf long term non dipped area		-2980	28480	45850	10000	0	0	0	0	0	0	0
COST PER HEAD PER YEAR		-2.98	28.48	45.85	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PERIOD AVE. COST/HEAD/YEAR		-2.98	12.00	22.23	19.59	16.38	14.26	12.76	11.64	10.78	10.11	6.84

APPENDIX 29. LPEC CALCULATIONS FOR TONDE AND LIKUNI FOR 1991, 1992 AND 1993 WITH A CONSTANT PARTURITION RATE OF 68%

Production system : LIKUNI91-C

MORTALITY AND CULLING RATES

CLASS OF ANIMAL	MORTALITY RATE (%/Y)	CULLING RATE (%/Y)
Breeding females	3.4	20
Replacement females (suckling)	11	
Replacement females (weaned)	7.6	
Surplus females 1 (suckling)	11	
Surplus females 1 (weaned)	7.6	
Surplus females 2 (suckling)	11	
Surplus females 2 (weaned)	7.6	
Breeding males	3.4	50
Replacement males (suckling)	11	
Replacement males (weaned)	7.6	
Surplus males 1 (suckling)	11	
Surplus males 1 (weaned)	7.6	
Surplus males 2 (suckling)	11	
Surplus males 2 (weaned)	7.6	

OFFTAKE AND REPLACEMENT STOCK

TYPE OF OFFTAKE	VALUE PER UNIT MK	OFFTAKE UNITS/CCU/YEAR	VALUE MK /CCU/YEAR
Culled breeding female	506	.212319	107.4338
Mature surplus female type 1	435	.002374	1.032707
Mature surplus female type 2	435	0	0
Barren replacement female	435	.013074	5.687378
Culled breeding male	754	.061011	46.00262
Mature surplus male type 1	671	.198702	133.3294
Mature surplus male type 2	671	0	0
1 kg of milk	.5	38.26004	19.13002
Salvage value of carcases			27.41576
Other (per animal) costs			-1.161755
Purchased feed costs			0
NET TOTAL			338.8699

HERD STRUCTURE

CLASS OF STOCK	% HERD	ME/DAY	No/CCU
Breeding females	35.70884	40.26255	1.061599
Suck repl. females	10.82395	15.15408	.321788
Wean repl. females	18.99864	35.67646	.564816
Suck srp.1 females	.098270	15.15408	.002921
Wean srp.1 females	.172487	35.77927	.005127
Suck srp.2 females	0	15.15408	0
Wean srp.2 females	0	33.72295	0
Breeding males	4.104464	44.31494	.122022
Suck repl. males	2.697215	16.08352	.080186
Wean repl. males	4.734259	40.10928	.140746
Suck srp.1 males	8.225008	14.75888	.244523
Wean srp.1 males	14.43686	37.39827	.429198
Suck srp.2 males	0	14.78445	0
Wean srp.2 males	0	37.34221	0
TOTAL			2.972931

Production system : TONDE91-C

MORTALITY AND CULLING RATES

CLASS OF ANIMAL	MORTALITY RATE (%/Y)	CULLING RATE (%/Y)
Breeding females	2.7	20
Replacement females (suckling)	31.4	
Replacement females (weaned)	5.7	
Surplus females 1 (suckling)	31.4	
Surplus females 1 (weaned)	5.7	
Surplus females 2 (suckling)	31.4	
Surplus females 2 (weaned)	5.7	
Breeding males	2.7	50
Replacement males (suckling)	31.4	
Replacement males (weaned)	5.7	
Surplus males 1 (suckling)	31.4	
Surplus males 1 (weaned)	5.7	
Surplus males 2 (suckling)	31.4	
Surplus males 2 (weaned)	5.7	

OFFTAKE AND REPLACEMENT STOCK

TYPE OF OFFTAKE	VALUE PER UNIT MK	OFFTAKE UNITS/CCU/YEAR	VALUE MK /CCU/YEAR
Culled breeding female	506	.230564	116.6657
Mature surplus female type 1	435	0	0
Mature surplus female type 2	435	0	0
Barren replacement female	435	.012135	5.278935
Culled breeding male	754	.066254	49.95564
Mature surplus male type 1	671	.172877	116.001
Mature surplus male type 2	671	0	0
1 kg of milk	.5	41.54774	20.77387
Salvage value of carcases			33.8047
Other (per animal) costs			-1.156836
Purchased feed costs			0
REPLACEMENT STOCK			
Mature replacement female	435	-.031116	-13.53573
Mature replacement male	754	0	0
NET TOTAL			327.7872

HERD STRUCTURE

CLASS OF STOCK	% HERD	ME/DAY	No/CCU
Breeding females	39.04156	40.31233	1.152823
Suck repl. females	10.82252	15.15408	.319568
Wean repl. females	17.41293	35.67643	.514170
Suck srp.1 females	0	15.15408	0
Wean srp.1 females	0	35.77925	0
Suck srp.2 females	0	15.15408	0
Wean srp.2 females	0	33.72295	0
Breeding males	4.487535	44.31494	.132508
Suck repl. males	3.113831	16.08352	.091945
Wean repl. males	5.01001	40.10926	.147936
Suck srp.1 males	7.708687	14.75888	.227622
Wean srp.1 males	12.40292	37.39826	.366234
Suck srp.2 males	0	14.78445	0
Wean srp.2 males	0	37.34221	0
TOTAL			2.952809

Production system : LIKUNI92-C

MORTALITY AND CULLING RATES

CLASS OF ANIMAL	MORTALITY RATE (%/Y)	CULLING RATE (%/Y)
Breeding females	9.9	20
Replacement females (suckling)	49.2	
Replacement females (weaned)	15.2	
Surplus females 1 (suckling)	49.2	
Surplus females 1 (weaned)	15.2	
Surplus females 2 (suckling)	49.2	
Surplus females 2 (weaned)	15.2	
Breeding males	9.9	50
Replacement males (suckling)	49.2	
Replacement males (weaned)	15.2	
Surplus males 1 (suckling)	49.2	
Surplus males 1 (weaned)	15.2	
Surplus males 2 (suckling)	49.2	
Surplus males 2 (weaned)	15.2	

OFFTAKE AND REPLACEMENT STOCK

TYPE OF OFFTAKE	VALUE PER UNIT MK	OFFTAKE UNITS/CCU/YEAR	VALUE MK /CCU/YEAR
Culled breeding female	506	.256023	129.5479
Mature surplus female type 1	435	0	0
Mature surplus female type 2	435	0	0
Barren replacement female	435	.009326	4.057077
Culled breeding male	754	.073569	55.47174
Mature surplus male type 1	671	.098395	66.02336
Mature surplus male type 2	671	0	0
1 kg of milk	.5	46.13544	23.06772
Salvage value of carcases			76.07494
Other (per animal) costs			-1.14961
Purchased feed costs			0
REPLACEMENT STOCK			
Mature replacement female	435	-.205549	-89.41402
Mature replacement male	754	0	0
NET TOTAL			263.6791

HERD STRUCTURE

CLASS OF STOCK	% HERD	ME/DAY	No/CCU
Breeding females	43.35888	39.90998	1.280118
Suck repl. females	11.06154	15.15408	.326578
Wean repl. females	14.76713	35.67536	.435981
Suck srp.1 females	0	15.15408	0
Wean srp.1 females	0	35.77818	0
Suck srp.2 females	0	15.15408	0
Wean srp.2 females	0	33.72295	0
Breeding males	4.983778	44.31494	.147139
Suck repl. males	5.226597	16.08352	.154308
Wean repl. males	6.977492	40.10809	.206001
Suck srp.1 males	5.834946	14.75888	.172269
Wean srp.1 males	7.789635	37.39716	.229979
Suck srp.2 males	0	14.78445	0
Wean srp.2 males	0	37.34221	0
TOTAL			2.952376

Production system : TONDE92-C

MORTALITY AND CULLING RATES

CLASS OF ANIMAL	MORTALITY RATE (%/Y)	CULLING RATE (%/Y)
Breeding females	2	20
Replacement females (suckling)	18.6	
Replacement females (weaned)	3.2	
Surplus females 1 (suckling)	18.6	
Surplus females 1 (weaned)	3.2	
Surplus females 2 (suckling)	18.6	
Surplus females 2 (weaned)	3.2	
Breeding males	2	50
Replacement males (suckling)	18.6	
Replacement males (weaned)	3.2	
Surplus males 1 (suckling)	18.6	
Surplus males 1 (weaned)	3.2	
Surplus males 2 (suckling)	18.6	
Surplus males 2 (weaned)	3.2	

OFFTAKE AND REPLACEMENT STOCK

TYPE OF OFFTAKE	VALUE PER UNIT MK	OFFTAKE UNITS/CCU/YEAR	VALUE MK /CCU/YEAR
Culled breeding female	506	.215811	109.2007
Mature surplus female type 1	435	.021552	9.375467
Mature surplus female type 2	435	0	0
Barren replacement female	435	.012494	5.435048
Culled breeding male	754	.062014	46.75919
Mature surplus male type 1	671	.206944	138.8598
Mature surplus male type 2	671	0	0
1 kg of milk	.5	38.88927	19.44464
Salvage value of carcases			20.80444
Other (per animal) costs			-1.162173
Purchased feed costs			0
REPLACEMENT STOCK			
Mature replacement female	435	0	0
Mature replacement male	754	0	0
NET TOTAL			348.7172

HERD STRUCTURE

CLASS OF STOCK	% HERD	ME/DAY	No/CCU
Breeding females	36.44939	40.36522	1.079059
Suck repl. females	9.890068	15.15408	.292788
Wean repl. females	17.43376	35.67646	.516114
Suck srp.1 females	.853019	15.15408	.025253
Wean srp.1 females	1.503663	35.77927	.044514
Suck srp.2 females	0	15.15408	0
Wean srp.2 females	0	33.72295	0
Breeding males	4.189584	44.31494	.124029
Suck repl. males	2.552608	16.08352	.075568
Wean repl. males	4.499622	40.10928	.133208
Suck srp.1 males	8.190481	14.75888	.242473
Wean srp.1 males	14.4378	37.39827	.427421
Suck srp.2 males	0	14.78445	0
Wean srp.2 males	0	37.34221	0
TOTAL			2.96043

Production system : LIKUNI93-C

MORTALITY AND CULLING RATES

CLASS OF ANIMAL	MORTALITY RATE (%/Y)	CULLING RATE (%/Y)
Breeding females	18.9	20
Replacement females (suckling)	114.6	
Replacement females (weaned)	12.8	
Surplus females 1 (suckling)	114.6	
Surplus females 1 (weaned)	12.8	
Surplus females 2 (suckling)	114.6	
Surplus females 2 (weaned)	12.8	
Breeding males	18.9	50
Replacement males (suckling)	114.6	
Replacement males (weaned)	12.8	
Surplus males 1 (suckling)	114.6	
Surplus males 1 (weaned)	12.8	
Surplus males 2 (suckling)	114.6	
Surplus males 2 (weaned)	12.8	

OFFTAKE AND REPLACEMENT STOCK

TYPE OF OFFTAKE	VALUE PER UNIT MK	OFFTAKE UNITS/CCU/YEAR	VALUE MK /CCU/YEAR
Culled breeding female	506	.313508	158.6352
Mature surplus female type 1	435	0	0
Mature surplus female type 2	435	0	0
Barren replacement female	435	.006230	2.710191
Culled breeding male	754	.090088	67.92676
Mature surplus male type 1	671	.062303	41.80546
Mature surplus male type 2	671	0	0
1 kg of milk	.5	56.49418	28.24709
Salvage value of carcases			135.5797
Other (per animal) costs			-1.157877
Purchased feed costs			0
REPLACEMENT STOCK			
Mature replacement female	435	-.491397	-213.7578
Mature replacement male	754	-.061838	-46.62644
NET TOTAL			173.3623

HERD STRUCTURE

CLASS OF STOCK	% HERD	ME/DAY	No/CCU
Breeding females	53.71109	39.61322	1.567541
Suck repl. females	10.32586	15.15408	.301356
Wean repl. females	9.731746	34.93571	.284017
Suck srp.1 females	0	15.15408	0
Wean srp.1 females	0	35.03506	0
Suck srp.2 females	0	15.15408	0
Wean srp.2 females	0	33.72295	0
Breeding males	6.173687	44.31494	.180177
Suck repl. males	5.16293	16.08352	.150678
Wean repl. males	4.865873	39.29607	.142008
Suck srp.1 males	5.16293	14.75888	.150678
Wean srp.1 males	4.865872	36.64064	.142008
Suck srp.2 males	0	14.78445	0
Wean srp.2 males	0	37.34221	0
TOTAL			2.918468

Production system : TONDE93-C

MORTALITY AND CULLING RATES

CLASS OF ANIMAL	MORTALITY RATE (%/Y)	CULLING RATE (%/Y)
Breeding females	6	20
Replacement females (suckling)	45.2	
Replacement females (weaned)	3.4	
Surplus females 1 (suckling)	45.2	
Surplus females 1 (weaned)	3.4	
Surplus females 2 (suckling)	45.2	
Surplus females 2 (weaned)	3.4	
Breeding males	6	50
Replacement males (suckling)	45.2	
Replacement males (weaned)	3.4	
Surplus males 1 (suckling)	45.2	
Surplus males 1 (weaned)	3.4	
Surplus males 2 (suckling)	45.2	
Surplus males 2 (weaned)	3.4	

OFFTAKE AND REPLACEMENT STOCK

TYPE OF OFFTAKE	VALUE PER UNIT MK	OFFTAKE UNITS/CCU/YEAR	VALUE MK /CCU/YEAR
Culled breeding female	506	.242468	122.6888
Mature surplus female type 1	435	0	0
Mature surplus female type 2	435	0	0
Barren replacement female	435	.011640	5.063526
Culled breeding male	754	.069674	52.53471
Mature surplus male type 1	671	.154770	103.8508
Mature surplus male type 2	671	0	0
1 kg of milk	.5	43.69273	21.84636
Salvage value of carcases			46.08356
Other (per animal) costs			-1.157652
Purchased feed costs			0
REPLACEMENT STOCK			
Mature replacement female	435	-.094042	-40.90863
Mature replacement male	754	0	0
NET TOTAL			310.0014

HERD STRUCTURE

CLASS OF STOCK	% HERD	ME/DAY	No/CCU
Breeding females	41.16064	40.10075	1.21234
Suck repl. females	10.69609	15.15408	.315041
Wean repl. females	16.35804	35.676	.481807
Suck srp.1 females	0	15.15408	0
Wean srp.1 females	0	35.77882	0
Suck srp.2 females	0	15.15408	0
Wean srp.2 females	0	33.72295	0
Breeding males	4.731108	44.31494	.139349
Suck repl. males	3.585289	16.08352	.105600
Wean repl. males	5.483154	40.10877	.161500
Suck srp.1 males	7.110797	14.75888	.209440
Wean,srp.1 males	10.87488	37.39781	.320307
Suck srp.2 males	0	14.78445	0
Wean srp.2 males	0	37.34221	0
TOTAL			2.945386

APPENDIX 30. CHI SQUARED ANALYSIS

Contingency table

		+ve	-ve	Expected cases
Group x		A	B	$\frac{A+C}{C+D}$
Group y		C	D	$\frac{A+C}{A+B}$
	Total	A+C	B+D	A+B

Incidence table

	No. cases	Animal years	Rate	Expected cases
Group x	A	C	A/C	$\frac{(A+B)\,C}{(C+D)}$
Group y	B	D	B/D	$\frac{(A+D)\,D}{(C+D)}$
Total	A+B	C+D	$\frac{A+B}{C+D}$	A+B

$\chi^2 = \sum \frac{(O-E)^2}{E}$ with 1 degree of freedom

Where: O = observed
E = expected

APPENDIX 31. JOURNAL REPRINT OF PAPER 5

STVM-93

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Seroconversion to *Cowdria ruminantium* of Malawi zebu calves, reared under different tick control strategies

SOLDAN (A.W.), NORMAN (T.L.), MASAKA (S.), PAXTON (E.A.), EDELSTEN (R.M.), SUMPTION (K.J.). Séroconversion à *Cowdria ruminantium* de veaux zébus au Malawi, soumis à des stratégies différentes de lutte contre les tiques. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 171-177

L'ELISA indirect a été utilisé au Malawi pour comparer la séroconversion *à Cowdria ruminantium* jusqu'à l'âge d'un an de 66 veaux zébus locaux nés dans des groupes passés au bain détiqueur 17 fois par an, à celle de 32 veaux nés dans des groupes non détiqués. Le nombre d'*Amblyomma variegatum* et les cas cliniques de la maladie ont été enregistrés dans chaque groupe de bovins. Aucun cas de cowdriose n'a été observé chez les veaux des 2 groupes pendant les 22 premiers mois de leur vie. Un seul cas fut enregistré chez une vache de 8 ans chez les 1 800 bovins suivis de façon intensive pendant la même période. Presque tous les veaux non détiqués étaient devenus séropositifs à l'âge d'un an et 50 p. 100 des séroconversions ont été attribuées à des infections par des nymphes. Par ailleurs, seulement 41 p. 100 des veaux dans les groupes passés au bain étaient devenus positifs à l'âge d'un an. Ce régime de détiquage a donc diminué le taux de séroconversion de façon significative chez ces veaux. Soixante quatorze pour cent des veaux avaient des anticorps maternels contre *C. ruminantium* pendant les quatre premières semaines de leur vie. Chez tous les veaux des groupes détiqués, le taux de ces anticorps était tombé sous le seuil considéré comme positif dans l'ELISA à l'âge de 8 à 12 semaines et il n'y avait pas de conversion de séronégatif à séropositif pendant cette période chez ces veaux. La proportion de séropositifs détectés par un seul test ELISA à l'âge de 12 mois n'était pas significativement différente de celle déterminée par les résultats cumulatifs des 9 mois précédents et semble donc utilisable comme indication de l'état de l'immunité du troupeau. Les auteurs concluent qu'une stabilité enzootique *à C. ruminantium* existe chez les zébus locaux non détiqués, caractérisée par une résistance à l'infection, innée et élevée, et une séroconversion entre 3 et 9 mois d'âge de la plupart des veaux nés en mai-juin.

Mots-clés : Zébu - Cowdriose - *Cowdria ruminantium* - *Amblyomma variegatum* - Tique - Lutte antiacarien - Infestation - Test ELISA - Anticorps - Malawi.

INTRODUCTION

The vector of *Cowdria ruminantium* in Malawi is considered to be *Amblyomma variegatum*, which has a widespread distribution in the country, except for lowland areas of southern Malawi such as the Lower Shire Valley (2). Heartwater has been frequently reported in unprotected *Bos taurus* cattle, but the disease is seldom reported in indigenous Malawi Zebu cattle *(Bos indicus)*. The number of taurine cattle in Malawi is very low (less than 20,000) in comparison with numbers of Malawi Zebu in the national herd of 800,000 (10). The status of traditionally managed Malawi Zebu to *Cowdria ruminantium* is not known, although a state of enzootic stability and/or genetic resistance in indigenous ruminants to the infection, is considered to be present in *Amblyomma variegatum* infested areas. The occurrence and timing of seroconversion to *C. ruminantium* in relation to *Amblyomma* infestations in cattle is important to understanding the nature of infection in both undipped and dipped cattle populations.

Dipping to control tick-borne disease was first made compulsory in certain areas of Malawi in the early 1920's (DE MAZA, 1925, cited in MARES (8)). Although current legislation provides for weekly dipping of cattle in arsenic trioxide it is estimated that only 20-40 % are dipped regularly. A survey of cases presented to veterinary assistants at diptanks in north and central regions showed that ECF morbidity varied between 0.5 and 1.8 % (all ages) ; clinical cases of babesiosis and anaplasmosis were rare and heartwater was not recorded. The dipping attendance over the year was only 50 % and therefore the low ECF morbidity was not attributed to the suppression of ticks through dipping (5).

A three year trial commenced in 1990, which undertook to investigate the effect of reduced intensity dipping and non-dipping in traditionally managed cattle, upon morbidity, mortality, productivity and economic indicators. Seroconversion to *C. ruminantium* in cohorts of calves born during this trial into study herds at six diptanks is reported here.

An indirect ELISA test was used to test serum samples for antibodies to detergent soluble antigens extracted from the purified elementary body of *C. ruminantium* (SUMPTION, MASAKA and PAXTON, unpublished results). The significance of positive test results in immunofluorescent antibody (IFA) tests upon sera from animals from areas where *Amblyomma* ticks are present is unclear, because positive reactions have been observed in IFA tests with sera from some *Amblyomma* free areas (3). The latter results are presumed to be the result of cross-reactions caused by antibodies to *Ehrlichia* species, because serological cross-reactions in IFA tests have been observed between antisera raised to various *Ehrlichia* species and *C. ruminantium* antigens in infected mouse macrophages (3) or neutrophils (6, 7). The

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indirect ELISA used to test sera from Malawi utilized detergent soluble elementary body antigens, because a number of cross-reactive antigens to *E. phagocytophila* and *E. ondiri* were removed during the detergent extraction process ; the ELISA test consequently has a low level of detection of antibodies to these pathogens in comparison with IFA using *Cowdria* infected goat neutrophils or infected endothelial cells (SUMPTION and PAXTON, unpublished). The presence of *E. ondiri* or *E. bovis* has not been demonstrated in Malawi, and therefore antibody reactions in this study are assumed to be to *C. ruminantium*.

MATERIALS AND METHODS

Location of study area

Malawi is located between 9° - 17° South and 33° - 36° East in Central Africa. As part of a larger study, six diptanks in the same ecological zone were chosen in the Lilongwe area. This area is on the Central African Plateau with an undulating, almost flat topography about 1,100 m above sea level. Four of the tanks chosen were in good repair and had been using arsenic trioxide up to the start of the study in November 1991. Cattle at two diptanks which were to act as non-dipped controls had effectively not been regularly dipped as a result of tank disrepair, or because there was a large group of farmers who did not dip their cattle.

Organization of study

Approximately 300 animals at each of the six tanks were tagged in November 1990. Each of the cattle were Malawi-Zebu and belonged to smallholders, and were communally grazed. No alteration in management was instituted and no prophylactic treatments were given during the trial. Dipping was carried out in four tanks at 2 weekly intervals in the rainy season (December 1990 to March 1991 and December 1991 to March 1992) and at 4 weekly intervals through the dry season (April 1991 to November 1991). Dipping at two tanks was in chlorfenvinphos (Supona 30, Shell Chemicals Ltd.) and at the other two in amitraz (Triatix TR, Coopers Animal Health).

Acaricide concentrations and replenishment were as recommended by the manufacturers, and the total replacement method was used for amitraz. Dipping of cattle was not carried out at the two control tanks. An active disease monitoring system was set up with the aim of identifying the specific cause of each case of death or disease in cattle at each dip tank in the trial. The routine samples collected from dead animals were faeces, blood, lymph node, spleen, and brain crush smears. These were examined by staff of the protozoology section of the Central Veterinary Laboratory and a project veterinary officer. The veterinary assistant associated with each diptank visited owners with tagged cattle every week and project staff visited every 2 weeks throughout the study period.

Calf cohort study

The peak calving season in the Lilongwe area occurs between May and July each year. As part of a productivity study 15 calves born in May/June 1991 had serum samples collected at 8 week intervals with the first sample being collected at the first visit after birth. Samples were frozen at -20 °C and aliquots for *Cowdria* serology were forwarded on ice by airfreight to the Centre for Tropical Veterinary Medicine, Edinburgh, where they were tested by indirect ELISA. Chi squared tests were used to compare proportions.

Indirect ELISA for the detection of antibodies to *Cowdria ruminantium*

An indirect ELISA developed at the CTVM was used to test sera at a dilution of 1 in 50. The ELISA uses soluble antigens extracted from the elementary body (EB) stage of *C. ruminantium* (Welgevonden stock) following release from cell cultures, and has an extremely low reactivity to antibodies present in antisera raised to *Ehrlichia phagocytophila*, in comparison with immuno-fluorescent antibody tests (IFA) using Welgevonden infected neutrophils or infected endothelial cells. It also has an excellent sensitivity in the detection of experimentally infected animals (SUMPTION and PAXTON, unpublished results). Cross-reactions with *Ehrlichia* spp. present considerable difficulties in the interpretation of IFA tests for heartwater (3, 4). The indirect ELISA was developed using detergent soluble fractions of the *Cowdria* elementary body because these fractions have a reduced number of antigens with cross-reactivity to *E. phagocytophila* and *E. ondiri* sera, than is found in whole EB or infected cell preparations (SUMPTION and PAXTON, manuscript in preparation). Soluble antigen is prepared from EB's semipurified from culture medium by centrifugation for 20 min at 1,000 g for the pelleting of endothelial cells, followed by centrifugation at 10,000 g for the pelleting of EB's. Pellets were washed in sterile phosphate buffered saline (PBS, pH 7.4), and recentrifuged at 10,000 g for 20 min. The procedure was repeated two times, followed by detergent lysis of the elementary body in 0.5 % nonidet NP40 and 0.5 % sodium deoxycholate in 50 mM Tris-HCL (pH 8.0), 2 mM EDTA, 150 mM sodium chloride and 1 mM phenylmethylsulfonylfluoride for 2 min at room temperature followed by rapid passage through a 26 g needle to disaggregate elementary bodies, and incubation at 37 °C for 30 min. After a further round of needle passage, insoluble antigen was removed by centrifugation at 4 °C for 30 min at 16,000 g. Soluble antigen extracts were then stored at -20 °C until