Interactions Amongst the Community of Endemic Pathogens of African Cattle

A Longitudinal Study in South East Uganda

by

Olga Tosas i Auguet, BVM

Submitted in Total Fulfilment of the Requirements of the Degree of Doctor Philosophy

January 2007

The Royal (Dick) School of Veterinary Studies

Division of Animal Health and Welfare

University of Edinburgh

Abstract

The work presented in this thesis is focused upon the community of endemic pathogens of African cattle in Sub-Saharan Africa, which has long constrained livestock production in these areas. The first aim of this work is to investigate whether the pathogen community as a whole shapes the ensuant epidemiology and morbidity which are currently attributed to any of its individual pathogens. The second aim is to determine if a greater understanding of the interactions present amongst genetically distinct parasites of the same species can be used to better explain epidemiological features that are at present poorly understood. Emphasis is placed on examining spatial variation in the epidemiology of *Theileria parva*, a tick-transmitted protozoan that causes East Coast Fever.

To achieve these aims, this work examines field data collected from a large and comprehensive study conducted in south east Uganda. Through application of apposite statistical techniques and mathematical modelling, aspects of the complex relations amongst the pathogen community and their environment are explored. Evidence is presented that demonstrates the paramount role of the pathogen community as a whole in shaping the infection dynamics and pathogenicity of any of its individual components. By focusing on a single member of this pathogen community (*Theileria parva),* some of the influences of host, vector, geographical location, temporal dynamics and intra-species pathogen interactions are elucidated. Application of a polymorphic molecular marker to *Theileria parva* infected blood samples and the use of Cox proportional hazard analysis, show variability in the survival of infections in cattle in high and low tick challenge areas. Moreover infection survival, which plays a pivotal role in parasite transmission, is shown to be a function of the interactions established amongst genetically distinct co-infective parasites. In consequence, vector intensity alone is insufficient to develop reliable transmission models which can accurately predict the epidemiology of the parasite inside and outside enzootic belts. Finally, a theoretical model is developed which, based upon the field evidence obtained throughout this work, provides a possible explanation for the mechanics of *T. parva* survival in cattle. In summary, this thesis

makes a case that consideration of both inter- and intra-species pathogen interactions, can greatly augment understanding of the epidemiology of these pathogen communities. An integrated approach to pathogen dynamics can better equip an integrated approach to control of important diseases of African cattle.

Declaration

This is to certify that the thesis comprises only my original work towards the PhD. Due acknowledgement has been made in the text to all other material used. This work has not been submitted for any other degree or professional qualification.

Signed……

Al meu petit, gran home,

Com t'ho podria dir

perquè em fos senzill, i et fos veritat,

que sovint em sé tan a prop teu, si canto,

que sovint et sé tan a prop meu, si escoltes,

i penso que no he gosat mai ni dir-t'ho,

que em caldria agrair-te tant temps que fa que t'estimo

(Lluis Llach)

Acknowledgments

I would like to thank Drs. Susan Welburn and Mark Eisler, for providing me with the opportunity of studying for a doctorate, and for their guidance and advice during the course of the research work for this thesis. I am also grateful to Drs. Kim Picozzi, Eric Fevré and Aimee Tilley and all the students and staff at CTVM, University of Edinburgh, for their general encouragement in completing this work. I am immensely grateful to Dr Frank Katzer for his care and tactful advice. I am also indebted to Andrew Paul Cox, for incredibly motivational discussion of my work, and for the patience of reading and correcting many pages of this thesis. Many thanks are also addressed to Dr. Ewan Macleod for his generosity and his contribution towards the completion of this thesis. Thanks also to Dr Francis McOdimba, who has been a source of continuous encouragement and support. I would especially like to thank Dr. Ian Anderson for the assistance and guidance provided during the many hours spent collecting and compiling data in the field. The co-operation and willingness of many farmers from the villages of Bunghaji, Hitunga, Magoje and Ojilai in Tororo and Buyimini, Kubo, Nanjeho and Sitengo in Busia districts who volunteered their cattle and made this study possible is highly appreciated. Also, thank you to all personnel from LIRI (Uganda), very specially John, Merino, Susanne, and Dr. Okello-Onen, with whom I shared uncountable hours of laboratory work, and who made me feel at home. I am immensely grateful to Dr. Otim (LIRI), who coped very well with my over-enthusiastic approach and helped me throughout the process of collecting information from numerous places in Uganda. I have not enough words to express my gratitude to Dr. D.J.Shaw. (CTVM), without whom large part of the work presented in this thesis, would have not been possible. Thanks also to Mr. Edelstein for his immense kindness and encouragement. Finally, I am indebted to those whom I love most for their unconditional patience, warmth and support.

This study was funded by research grants from the Department for International Development, (Animal Health Improvement Programme) of the British Government, whose generosity is greatly appreciated.

Table of Contents

List of Figures

Appendices

List of Tables

Appendices

Table of Abbreviations and Acronyms in the Main Text

ADE	Antibody Dependent Enhancement
AIC	Akaike Information Criterion
APL	Altered Peptide Ligand
AR	Autoregressive
BCT	Buffy Coat Technique
BIC	Bayesian Information Criterion
bp	Base Pairs
Chisq	Chi-Square
CI	Confidence Interval
CNS	Central Nervous System
Coef	Coefficient
CTL	Cytotoxic T-cell
CTVM	Centre for Tropical Veterinary Medicine
DAC	Development Assistance Committee
df	Degrees of Freedom
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
DPX	Distrine Plastisiser Xylene
ECF	East Coast Fever
EDTA	Ethylenedianinetetraacetic Acid
ELISA	Enzyme – linked Immunosorbent Serological Assay
EM	Expectation Maximisation
GI	Gastro Intestinal
Glm	Generalised Linear Model
Glmm	Generalised Linear Mixed-Effects Model
HBS	Half the Body Surface
HCT	Haematocrit Centrifugation Technique
HLA	Human Leukocyte Antigen
HR	Hazard Ratio
HSS	Human Sleeping Sickness
HTC	High Tick Challenge
ICPTV	Integrated Control of Pathogenic Trypanosomes and their Vectors
ICTTD	Integrated Control of Ticks and Tick-borne Diseases
ILRI	International Livestock Research Institute
IPCC	Inter-governmental Panel on Climate Change
ISMM	Isomethamidium Chloride
LA	Long-Acting
LIRI	Livestock Health Research Institute
LME	Linear Mixed-Effects Models
LR	Likelihood Ratio
LTC	Low Tick Challenge
mAbs	Monoclonal Antibodies
MCR	Microscopy
MHC	Major Histocompatibility Complex
ML	Maximum Likelihood
NLME	Non Linear Mixed-Effects Model
OAU	Organisation of African Unity
OECD	Organisation of Economic Co-operation and Development
OR	Odds Ratio

PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PIM	Polymorphic Immunodominant Molecule
PP	Percentage Positivity
PPO	Percentage of Positive Observations
PQL	Penalised Quasi-Likelihood
PSG	Phosphate Buffered Saline with Glucose
RA	Adult *Rhipicephalus appendiculatus* Instars
REML	Restricted Maximum Likelihood
RMHb	REML Estimated Mean Haemoglobin
RNA	Ribonucleic Acid
SE	Standard Error
SSA	Sub-Saharan Africa
TBDs	Tick-Borne Diseases
TC	Transformed Tick Count
TTBD	Tick and Tick-Borne Diseases
W	Wald

Chapter 1: Sub-Saharan Africa, Cattle and Endemic Diseases – Prioritising and Integrating Research

1.1 Introduction

In 2001 the World Bank estimated that 2.8 billion of the world's 6 billion population were living on less than US$ 2 per day, and 1.2 billion on less than US$ 1 per day (World-Bank, 2001). There is a considerable need to alleviate this situation, and the Development Assistance Committee (DAC) of the Organisation of Economic Co-operation and Development (OECD) has stated its intention to reduce the numbers of people living in poverty by half by 2015. Such a reduction will not be achieved by a single strategy. Only an integrated approach incorporating action in many areas is likely to bring about a successful realisation of such an ambitious goal. Nowhere is the problem of poverty more apparent than sub-Saharan Africa (SSA), where 46% of the population are believed to be living on less than US$ 1 per day. Furthermore, in SSA the number of people within this category has actually increased (World-Bank, 2001). The population in developing countries is projected to grow to 7.6 billion by the year 2050 (Perry *et al.*, 2002, Thornton *et al.*, 2002), with an estimated one-fifth of the world's population residing in SSA. The Inter-governmental Panel on Climate Change has, in addition, indicated that global average temperatures may rise by 1.4 to 5.8 ˚C in the present century (IPCC, 2001a, 2001b), and that such climate change is likely to have an impact on productions systems particularly in Africa. These factors will undoubtedly exacerbate the problem of poverty in SSA and worldwide (Perry *et al.*, 2002, Thornton *et al.*, 2002).

Failures in recent attempts to alleviate poverty, have led to a re-examination of the causes of poverty and the most suitable methods to alleviate them. There is now general agreement that measures targeted directly at the poor are those most likely to have an impact (Randhawa *et al.*, 1990, UNDP, 1997). Because of the importance of livestock to the peoples of SSA and other impoverished areas, investing in animal health research as a component of the strategy to directly alleviate poverty has become an important priority. Livestock play a pivotal role in the wellbeing of the poorest communities

worldwide, not only as a source of food, but as a valuable asset providing fertiliser for crop production, traction power, transport and a source of income through sale of animals and animal products (LID, 1999, Ellis *et al.*, 2003a, Ellis *et al.*, 2003b). Livestock form a component of the livelihoods of 70% of the world's population (LID, 1999). In view of the privatisation trends of veterinary services in SSA, it is recognised that much effort should now be targeted at improving prevention and control of endemic diseases of cattle, which have only received secondary attention compared to the so-called "epidemic diseases or diseases of trade".

Within the endemic diseases of cattle in sub-Saharan Africa, vector-borne diseases transmitted by either ticks or tsetse flies, are the major factor constraining animal health and production (Kuzoe, 1991). Some of these pathogens not only impose a risk to livestock, but are also of considerable importance in terms of public health, as they can readily be transmitted to the human population, where they cause severe illness and death (Onyango *et al.*, 1966). Indeed, cattle (and other livestock) in these areas act as reservoirs of a major tsetse-transmitted zoonotic pathogen (*Trypanosoma brucei rhodesiense*) which causes Human Sleeping Sickness (Onyango *et al.*, 1966, Waiswa *et al.*, 2003, Coleman *et al.*, 2004).

1.2 The Relative Importance of Livestock

Factors affecting the health and productivity of livestock are important in the development and wellbeing of poor communities (Torr *et al.*, 2002). The spatial projections of human population growth, particularly in sub-Saharan Africa, and the predicted changes in the length of crop growing period for the same territory due to climate change, have predicted an increasing demand for livestock products in sub-Saharan Africa as well as in Asia and Latin America (Delgado *et al.*, 1999). The need for adaptation and mitigation work to enhance animal production in these areas is predicted to be particularly large in Sub-Saharan Africa.

Although there exist some 3882 breeds for 28 species of domesticated animals worldwide (de Haan *et al.*, 1997), only some 12 species dominate global livestock production (Blench, 2000). In SSA the most important species are Cattle, Sheep, Goats, Poultry, Pigs and Horses, Donkeys and Mules. The

relative importance of each of these species is difficult to assess and depends largely on the region and agricultural production system.

During a workshop held in January 2001, the International Livestock Research Institute, in collaboration with governmental and non-governmental organisations, set out to evaluate the relative importance of each domestic livestock species in different farming systems and different regions (Perry *et al.*, 2002, Thornton *et al.*, 2002). For eastern, central and southern Africa, cattle were determined to be the most important livestock species in five of the eight production systems considered. Only in the Landless – Peri-urban, Agro Pastoral - Mixed irrigated arid / semi arid and the Pastoral – livestock only, rangeland-based arid / semi-arid production systems were cattle not the most important livestock species (in these systems cattle ranked as the 4th, 2nd and 2nd most important species respectively). For West Africa, cattle were less important ranking consistently as the third or fourth most important species. In this region the most important species were sheep and goats (Perry *et al.*, 2002). Clearly, if research is to focus on alleviating poverty through improving the health of a single species of domestic livestock, then for sub-Saharan Africa cattle or small ruminants are the most important species to target.

1.3 General Classification of Livestock Diseases According to Impact upon Poor Livestock Keepers

Prioritisation of the major livestock diseases according to their impact on poor livestock keepers is a difficult and complex task. Livestock diseases important in global terms, or those of most importance to the developed world may not necessarily be those diseases important to livestock owners in developing countries or in SSA. The importance of a particular disease is likely to vary depending upon the region or production system present (Perry *et al.*, 2002).

In order to better understand and prioritise the relative importance of the wide array of livestock diseases present worldwide, it is useful to distinguish four broad classifications of diseases, which are: Endemic, epidemic (trans-boundary), zoonotic and food-borne (Perry *et al.*, 2001). Epidemic diseases are those that occur periodically at a frequency above that expected. They are usually highly infectious

causing morbidity and mortality in a relatively short space of time. This group includes, amongst others, viral infections such as foot-and-mouth disease, rinderpest, classic swine fever, Newcastle disease and the influenzas. Epidemic diseases tend to spread rapidly, crossing international borders and causing high mortality. These diseases can have enormous impact and are usually the diseases of greatest importance in the developed world. In order to protect important commercial livestock markets, a great deal of investment and legislation is directed at preventing the spread of epidemic diseases in developed countries (Perry *et al.*, 2002). Moreover, the costs associated with control of these diseases do not usually fall to the community or individual livestock owners. Endemic diseases comprise, amongst others, the vector-borne haemoparasitic diseases, helminthoses, the enteric bacterial diseases of the neonate, and the bacterial and viral causes of reproductive failure. Endemic diseases can be further divided into those that are tropical and those that are tropical and temperate in distribution. Much of the focus in control and research has been with the latter division; whilst much less attention has been paid to the tropical group, as these diseases are not of major economic significance to the production systems of the developed world. The tropical endemic diseases are precisely those that tend to have their greatest impact at the level of the farm, village and community. They are the diseases that the livestock owners encounter on a day-to-day basis and, in addition to the morbidity and mortality caused by these diseases, the cost of treatment is borne by the livestock owners themselves. Practical and affordable control strategies for these diseases are completely lacking and general knowledge and information regarding the epidemiology and impact is also lacking in comparison to the epidemic diseases (Perry *et al.*, 2002).

In the same way as an assessment of the most important livestock species was conducted, the same workshop held by ILRI (2001) ranked the importance of a range of 76 syndromes, general diseases and specific disease entities occurring worldwide or regionally. These conditions were assessed as they impact on the wellbeing of the poorest livestock owners. The results of the priority impact rankings in areas of sub-Saharan Africa are reproduced in Table 1.1.

Table 1.1 Top 20 regionally ranked livestock diseases in sub-Saharan Africa.

Eastern, Central and Southern Africa	West Africa
East Coast Fever (ECF)	Anthrax
Ectoparasites	Black-leg
GI Parasitism	**Contagious bovine pleuro-pneumonia (CBPP)**
Haemonchosis	Dermatophilosis
Infectious coryza	**Ectoparasites**
Newcastle Disease	**GI Parasitism**
Neonatal mortality	**Heartwater**
Nutritional / micronutrient deficiencies	**Liver fluke (Fascioliasis)**
Respiratory complexes	**Respiratory complexes**
Rift Valley Fever (RVF)	**Trypanosomiasis**
Babesiosis	Anaplasmosis
Contagious bovine pleuro-pneumonia (CBPP)	Brucellosis
Coccidiosis	Contagious caprine pleuro-pneumonia (CCPP)
Foot problems	Foot-and-mouth disease (FMD)
Fowl pox	**Foot problems**
Heartwater	Haemorrhagic scepticemia (HS)
Liver fluke (Fascioliasis)	Newcastle Disease
Reproductive disorders	Peste des petits ruminants (PPR)
Tick infestation	Rift Valley Fever (RVF)
Trypanosomiasis	Sheep and goat pox

(Perry *et al.* , 2002)

The table shows the ranking of the twenty most important diseases and syndromes in SSA, as determined by a large panel of experts during a workshop held by ILRI (2001). Diseases and syndromes to which cattle are susceptible and which are common to both regions are shown in bold text. Those diseases transmitted by ticks to cattle are shown in blue text.

It is evident from the ranking in Table 1.1 that the relative importance of each disease varies greatly with region; however, the most important disease globally is GI helminth infection, which is also of great importance in SSA (Perry *et al.*, 2002). East Coast Fever has been regarded as the most important livestock disease compromising animal health and production in eastern, central and southern Africa (Perry *et al.*, 2002). One criticism made of the ranking system used in the workshop is that the tick and tick-borne diseases (TTBD) are underrated because each TTBD is treated separately, unlike other disease entities that are considered as a complex. It is therefore possible that a higher ranking to TTBD applies in sub-Saharan Africa (Perry *et al.*, 2002).

1.4 The Role of Research in Alleviating Poverty through Improved Animal Health - Priorities for Common Endemic Pathogens of African Cattle in Sub-Saharan Africa

In sub-Saharan Africa, a large range of common endemic pathogens of African cattle are transmitted by ticks and tsetse, and the geographical distribution of these two vectors largely overlaps. Today there are increasing efforts to integrate control of tsetse and trypanosomiasis and to integrate the control of TTBD (Holmes, 1997, Torr *et al.*, 2002, Eisler *et al.*, 2003). The two European Union Concerted Actions on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV) and Integrated Control of Ticks and Tick-borne Diseases (ICTTD), held a joint workshop at the Antwerp Institute of Tropical Medicine in 2002 (ICPTV, 2002). The aim of this workshop was to explore ways of integrating the control of TTBD and Tsetse-borne diseases. The workshop highlighted the three main reasons for the trend toward integration of tsetse and tick-borne disease control. Firstly, the privatisation of government veterinary and extension services has placed the responsibility for animal health with the owners of the livestock. Secondly, a general increase in recognition of the need to move away from individual disease control to an integrated approach. Finally, the existence of an increasing awareness and availability of control methods effective against both tsetse and ticks. The workshop also concluded that there exists very little background information on the epidemiological implications of control of one vector/disease on the other, and that research in this area should be undertaken before recommendations for integrated control could be made (ICPTV, 2002).

In the light of the conclusions of the ICTTD-2 / ICPTV joint meeting (2002) regarding the need for more research capable of informing integrated control strategies for tsetse and tick-borne diseases, together with the report of the workshop held by ILRI (2002) prioritising the importance of livestock species and their diseases to the poor of developing countries, the overall aim of this thesis is twofold. Firstly to investigate, for the indigenous cattle of south east Uganda, if the pathogen community as a whole shapes the ensuant epidemiology and morbidity which are currently attributed to any of its individual components (an inter-species level). This investigation should include pasture-transmitted pathogens

(helminths), which are paramount to the health of livestock in SSA. Secondly, to determine if a greater understanding of the interactions present amongst genetically distinct parasites of *Theileria parva* (an intra-species level), can be used to better explain epidemiological features which are at present, poorly understood.

1.5 Common Endemic Pathogens of African Cattle

In view of the importance of tsetse-, tick- and pasture-transmitted pathogens to the health of cattle in SSA, a brief account of the pathogenic species covered in this thesis is presented. The species covered include *Theileria parva*, Anaplasma spp.; Babesia spp., the pathogenic African Trypanosomes; the liver fluke Fasciola spp. and gastrointestinal (GI) nematodes.

1.5.1 Tick-Transmitted Pathogens

1.5.1.1 Theileria parva

Theileria parva is a tick-transmitted intracellular protozoan parasite causing a lymphoproliferative disease in cattle known as 'East Coast fever'. Together with Plasmodium spp. and Toxoplasma spp. it belongs to the class Sporozoa and has been placed within the sub-phylum Apicomplexa, although there is some debate about the latter classification (Bishop *et al.*, 2004). There are ten species of Theileria infecting ruminants. However only five are found in sub–Saharan Africa: *T. mutans* (Southern Africa), *T. velifera* (Western, Eastern Central and Southern Africa), *T. taurotragi* (Eastern, Central and Southern Africa), *T. buffeli* (Eastern Africa) and *T. parva* (Eastern, Central and Southern) Africa. The most important of these species infecting cattle in sub-Saharan Africa are *T. parva* and to a lesser extent *T. mutans*, although the economic impact of the latter species remains to be fully assessed. East Coast Fever constrains cattle production and improvement in eleven countries. The affected countries are Burundi, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe (Mukhebi *et al.*, 1992).

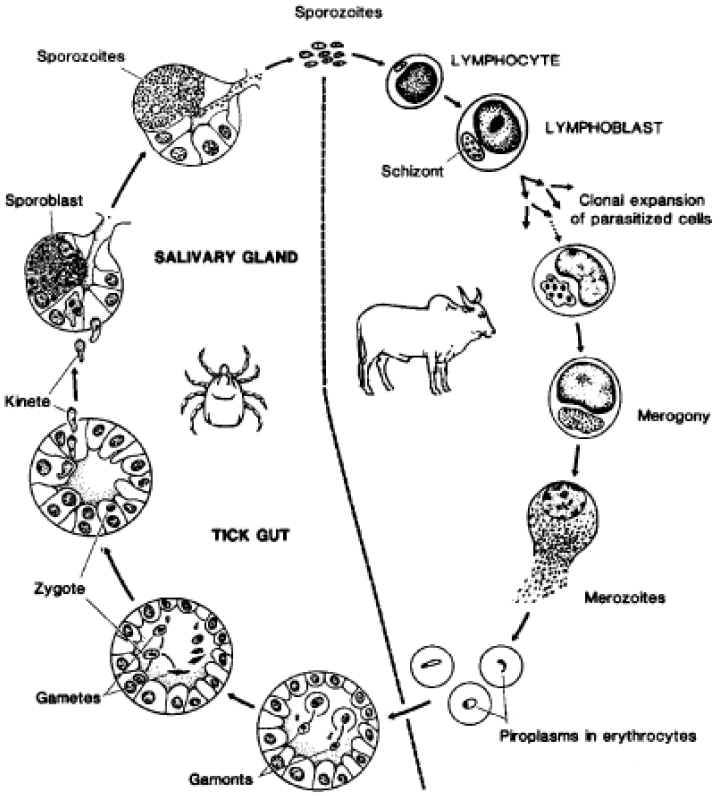
T. parva is predominantly transmitted by the three-host brown ear tick, *Rhipicephalus appendiculatus*. This species is classified as a three-host tick because the larvae, nymphs and adults feed on different

hosts (Reviewed by Bishop *et al.*, 2004) Transmission is trans-stadial, in that although larval and nymphal stages of the tick acquire infection upon feeding on an infected host, the infected tick can only transmit the infection after the nymphs or adults have moulted. In addition, transmission between ticks does not occur trans-ovarially.

East Coast Fever is one of the most economically important diseases of livestock in sub-Saharan Africa. In 1989 the disease was estimated to be responsible for U.S.$ 170 million worth of economic loss (Mukhebi *et al.*, 1992). Economic losses due to Theileriosis may arise from a number of different scenarios, the most obvious of which are production losses through morbidity and mortality (Minjauw *et al.*, 1998a, Minjauw *et al.*, 1998b) and through reduced productivity (Rumberia *et al.*, 1993, Pegram *et al.*, 1996, De Castro *et al.*, 1997). The disease also acts as a constraint to the ability of cattle owners to improve the productivity of their livestock through breeding programmes involving more productive, but more susceptible, 'exotic' breeds (Callow, 1983). Additionally, there are substantial costs involved with disease control, research, training and extension services (Mukhebi *et al.*, 1992). Kenya alone spent approximately US$10 million in 1987 on the importation of acaricides and drugs for the treatment of theileriosis and the provision of dipping and curative services (Young *et al.*, 1988). The cost of protecting animals by immunisation through infection and treatment may range from US$10 – US$20 per treatment per animal (Mutugi *et al.*, 1988, Young *et al.*, 1988), a price which is often too high to be a practical option for many of the farmers. It has been estimated that an annual ECF mortality of approximately one million cattle occurs in Kenya, Tanzania and Uganda (Mukhebi *et al.*, 1992).

The epidemiological situation for *T. parva* can be complex. Wild bovids act as a reservoir and can be infected with more than one species of the parasite. Additionally, east African cattle can be infected with up to five Theileria species at once, and more than one species of the parasite can be transmitted by the same tick species. Such is the case for *T. parva* and *T. taurotragi* by the vector *R. appendiculatus*.

Figure 1.1 Life cycle of *Theileria parva*.



(Figure from Bishop *et al*, 2004)
Cattle are infected by the bite of an infected tick; sporozoites from the salivary glands of the tick quickly invade host lymphocytes. Within the lymphocytes, the sporozoites develop into intracytoplasmic multinucleated schizonts. By mechanisms that are not fully understood, the parasite induces a malignant transformation of the lymphocyte. The parasite then undergoes clonal expansion in synchrony with the infected host cells. In this way, the parasite is able to multiply without leaving the host cell. Some parasites undergo merogony and merozoites are released into the bloodstream when host cells rupture. The newly released merozoites then invade erythrocytes and develop into the intra-erythrocytic forms known as piroplasms; this is the stage that is infective to the tick. Ticks ingest infected erythrocytes and, once in the tick's gut, gametes are formed. Sexual reproduction then takes place to form the zygotes. The zygotes subsequently invade the gut epithelial cells and differentiate into motile kinetes. The kinetes invade the tick salivary glands and eventually the cattle infective sporozoites are formed and the cycle is completed.

The transmission dynamics of *Theileria parva* has been shown to vary in different areas of sub-Saharan Africa. These variations depend on the levels of tick control, changes in the tick population, the proximity of a wildlife reservoir, cattle genotypes present, management regime and the interaction of *T. parva* genotypes (Reviewed by Bishop *et al*., 2004). Recovered animals have a solid cellular immunity against challenge with homologous isolates that may last for several years (Burridge *et al.*, 1972, Young *et al.*, 1973, Radley *et al*., 1975a, Morrison *et al.*, 1986). Experimental infections with

single strains suggest that acquired immunity is strain specific with limited cross-protection (Radley *et al.*, 1975b, Uilenberg *et al.*, 1976, Morrison, 1996, McKeever *et al.*, 2002). Initially, it was thought that once cattle experience an infection and recover, they develop sterile immunity. However more recent evidence has shown that once cattle become infected they remain carriers for life (Brocklesby *et al.*, 1966, Young *et al.*, 1981, Dolan, 1986, Bishop *et al.*, 1992, Kariuki *et al.*, 1995), ensuring continued transmission of the disease. Transmission models have shown, in addition, that the degree to which recovered carrier animals are able to transmit the infection to tick larvae or nymphs is a paramount determinant of infection in a herd (Medley *et al.*, 1993).

Sexual reproduction of *T. parva* occurs in the tick vector (Gonder, 1910, Melhorn *et al.*, 1984, Gauer *et al.*, 1995) and this may give rise to recombination between different genotypes (Allsopp *et al.*, 1989, Bishop *et al.*, 1993, Morzaria *et al.*, 1993). However, the frequency of these events in the field is not known. It is thought that one kinete infects one acinar cell in the tick salivary gland (Young *et al.*, 1981, Young *et al.*, 1986) and, in the field, only one acinar cell is usually found to be infected. These observations have led some to suggest that most cattle infections are derived from genetically identical sporozoites (Morzaria *et al.*, 1992).

The distribution of East Coast fever is strongly associated with the distribution of its vector. As tick populations are sensitive to climatic conditions, the prevalence of *T. parva* varies according to the suitability of the local climate to survival of the vector. Infection is often mild in Zebu calves and mortality in these animals is generally low (Latif *et al.*, 1995). This is in sharp contrast to the mortality rates in exotic breeds, which can be very high (Norval *et al.*, 1992).

While is it generally accepted that the notion of endemic stability applies to anaplasmosis and babesiosis (Norval *et al.*, 1983, Norval *et al.*, 1984), there currently exists a debate on whether the same notion can be applied to theileriosis due to ECF (Torr *et al.*, 2002, Eisler *et al.*, 2003). Endemic stability is an epidemiological state in which clinical disease is scarce despite high levels of infection in the population, and implies a climax relationship between host, [disease] agent, vector and environment (Norval *et al.*, 1992). The term was first coined to describe a history of empirical

observations of tick-borne diseases of livestock, in particular Babesiosis of cattle in Australia (Mahoney *et al.*, 1972) and ECF in Africa (Bruce *et al.*, 1910, Mettam *et al.*, 1936, Norval *et al.*, 1992). Central to the theory and practice of endemic stability, is that disruption of this climax relation, through control, results an increase of clinical disease incidence. In other words, if the force of infection (i.e. the per capita rate at which infection is acquired in a susceptible host population) falls, then disease incidence rises (Coleman *et al.*, 2001). The idea of endemic stability is used to guide the design of control programmes against tick-borne diseases of cattle (Norval, 1983, Cook, 1991, Deem *et al.*, 1993, Perry *et al.*, 1995, O'Callaghan *et al.*, 1998) and integrated control strategies for ticks and tsetse (Torr *et al.*, 2002).

Empirical observations suggest that ECF mortality and morbidity rather than continuing to rise, might start to plateau or even decline with increasing force of infection (Norval *et al.*, 1992). However, whether partial reduction in the force of infection through tick control practices might result in an overall increase of ECF mortality relative to preintervention levels, remains controversial as a result of disparity of empirical observations (Torr *et al.*, 2002, Eisler *et al.*, 2003). Moreover, it has been hypothesised that for the notion of endemic stability two criteria should be met (Coleman *et al.*, 2001). These are **(i)** that disease, at least over some age range, is a more likely (or more severe) outcome of infection in older than in younger individuals and **(ii)** that initial infection decreases the probability of subsequent infections, or the probability that subsequent infections result in disease. Whether effective inverse age immunity occurs, and hence whether the first criterion applies in the case of *Theileria parva*, is still debatable (Torr *et al.*, 2002).

The main clinical features of East Coast Fever are enlarged superficial lymph nodes, fever, anorexia, lachrymation, nasal discharge, depression and diarrhoea (Shannon, 1977, Kambarage, 1995, Mbassa *et al.*, 1998, Maloo *et al.*, 2001a). Anaemia is only weekly associated with infection with *T. parva*, as a result of the parasites tropism for leucocytes, which results in high degrees of leukopenia with neutropenia, lymphopenia and eosinopenia (Mbassa *et al.*, 1994).

Control of East Coast fever is largely effected by means of vector control through the use of acaricides, although this is an unsuitable method for the medium term (Tatchell, 1987). A method of immunization via infection and treatment of the cattle (Radley *et al.*, 1975a) has been in use for some time. However, this method presents problems related to the supply logistics (continuous cold chain required for storage of the vaccine), dangers of the use of potentially lethal live vaccines and the limited protection induced. Moreover, infection and treatment induces a carrier state in the cattle (Oura *et al*., 2004). The sporozoites surface protein p67 (Nene *et al.*, 1992) has been proposed as a candidate for inclusion in a sub-unit vaccine, and a form expressed in insect cells has been shown to induce 70% protection against lethal needle challenge with sporozoite stabilates in susceptible taurine cattle under laboratory conditions (Nene *et al.*, 1995). Several field trials with this vaccine candidate have been performed in Kenya in order to determine efficacy under different production systems and epidemiological situations. They indicated a reduction in severe disease of 47 – 50% (Reviewed by Bishop *et al*., 2004). Work towards a subunit vaccine is still ongoing.

1.5.1.2 Anaplasma spp.

Anaplasmosis, or so called gall sickness in Africa, is an arthropod-borne haemolytic disease of cattle, sheep, goats and some wild ruminant species caused by obligate intraerythrocytic rickettsial organisms of the genus Anaplasma (Schmidt, 1937, Bram, 1975, Kocan *et al.*, 2000, Dumler *et al.*, 2001). In the ninth edition of Bergey's Manual of Systematic Bacteriology, only four species of Anaplasma are recognised; three of which (*A. marginale* (Theiler, 1910); *A. centrale* (Theiler, 1911) and *A. caudatum* [or *Paranaplasma caudatum*]) occur in cattle (Kocan *et al.*, 2003). All isolations of *A. caudatum* have been from mixed infections with *A. marginale* (Kreier *et al.*, 1964). *A. caudatum* produces a mild to severe anaplasmosis in North America whilst both *A. marginale* and *A. centrale* occur in Africa.

In cattle, Anaplasmosis is endemic throughout tropical and subtropical regions of the world (McCallon, 1973). The disease occurs in the North and South Americas, Africa, Russia, European countries bordering the Mediterranean, the Middle East, the Far East and Australia. There is however, increasing evidence of the expansion of *A. marginale* into north European countries (i.e. Switzerland) over the

past years (Brulisauer *et al.*, 2004, Hofmann-Lehmann *et al.*, 2004). There is also serological evidence of the occurrence of *A. marginale* in different areas of Austria (Baumgartner *et al.*, 1992), although the estimated number of exposed cattle is very low (0.08%).

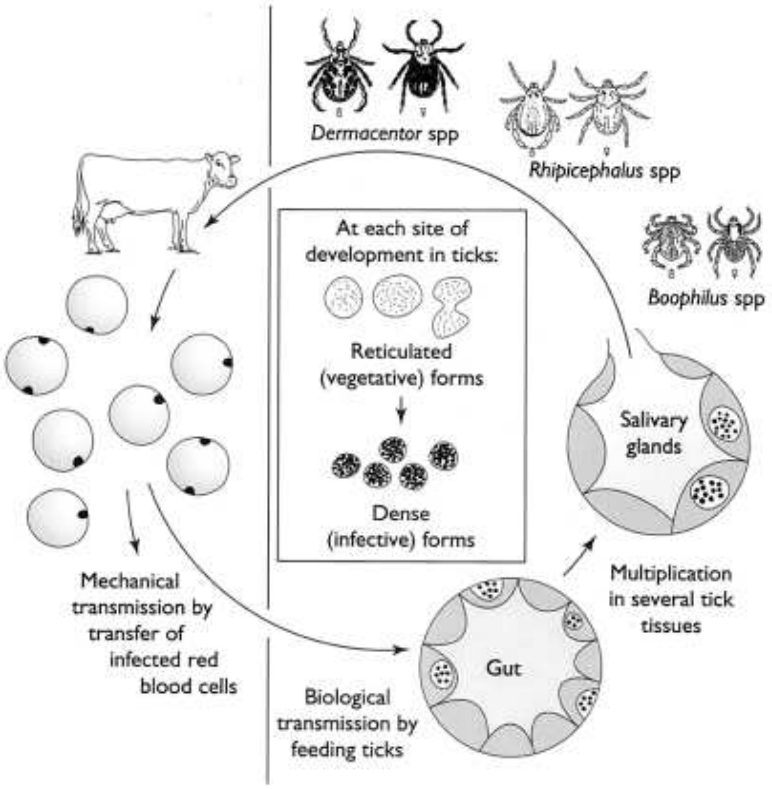
Transmission usually occurs via ticks with over 20 species being incriminated worldwide as biological vectors (Kocan, 1986). Ixodid ticks are the principal biological vectors of anaplasmosis, including the one-host tick genus Boophilus spp., but the argasid tick *Ornithodoros savignyi* can also transmit *A. marginale* (Kocan *et al.*, 2003). Mechanical transmission by biting flies of the genus Tabanus, Chrysops and Siphora (Ristic, 1968, Potgieter *et al.*, 1994) and iatrogenic means of transmission through blood-contaminated fomites may also occur (Dikmans, 1950).

Anaplasmosis causes economic losses in susceptible cattle herds in most countries in which it occurs. The losses due to anaplasmosis are measured through several parameters: reduced weight gain, reduced milk production, abortion, the cost of treatments, and mortality (Kocan *et al.*, 2003). There has been no direct assessment of the estimated losses in Sub-Saharan Africa, but in the USA loses due to bovine anaplasmosis had been estimated at US$ 300 million per year (McCallon, 1973) whilst in Latin America at US$ 800 million per year (Lonibardo, 1976). In Africa it has been estimated that economic losses due to both Babesia spp. and *Anaplasma marginale* in Kenya, Zimbabwe, Tanzania and South Africa total US$37.9 million. Anaplasmosis significantly constrains public and private cattle breeding programmes in the tropics, as programmes designed to improve the productivity of cattle breeds often utilise ‘exotic’ breeds which are highly susceptible to this and other tick borne diseases (Melendez, 2000).

Susceptibility of cattle to anaplasmosis is age related. Colostral and non-specific immunity largely protects calves up to the age of about nine months from the clinical effects of infection. Thereafter, susceptibility to disease generally increases. Infection causes a mild clinical picture in calves up to 1 year, including Zebu calves (Latif *et al.*, 1995), but animals of 2-3 years old often develop fatal anaplasmosis and cattle over 3 years develop a peracute and frequently fatal anaplasmosis (Potgieter

et al., 1994). Exceptions to this general pattern do occur. Age-specific seroprevalence of bovine anaplasmosis has occasionally been reported to decline with increasing age (Jongejan *et al.*, 1988).

Figure 1.2 Life cycle of *Anaplasma marginale*.



(Palmer *et al.*, 1999)
The erythrocytes infected with *A. marginale* are ingested with the blood meal as the tick feeds. The pathogen initially infects the cells of the gut as a reticulated or vegetative form. The reticulated stages divide by binary fission. When the tick takes a second blood meal many other tissues become infected, including the salivary glands. The 'dense' infective forms in the salivary glands transmit the infection back to cattle where the parasite invades red blood cells.

Following sub-clinical infection with Anaplasma spp., or on recovery from acute disease, cattle develop a persistent infection characterised by repetitive cycles of rickettsaemia (Eriks *et al.*, 1989) and animals usually remain carriers of the organism for life, thereby serving as reservoirs of infection for mechanical or biological transmission. Persistently infected carrier cattle are solidly immune to re-infection. As in the case of other haemopathogens reported here, indigenous adult cattle moved from Anaplasma spp. free areas into endemic areas are susceptible, and mortality rates of up to 80% may occur under such circumstances (Ajayi *et al.*, 1982).

Anaplasma spp. infections in mammalian hosts may range from unapparent infection to severe disease and mortality. In the clinical form of infection, disease is characterised by fever, progressive anaemia (haemolytic) with up to 70% or more of the erythrocytes becoming infected during the acute phase of infection (Ristic, 1968, Richey *et al.*, 1990), icterus, dehydration, dullness, laboured breathing, anorexia, weight loss, jaundice, drop in milk yield, abortion and death (Theiler, 1910, Ajayi *et al.*, 1987, Potgieter *et al.*, 1994, Egbe-Nwiyi *et al.*, 1997). Scanty hard faeces in anaplasma-infected cattle have been reported from Kenya, but not from an abattoir study conducted in Nigeria (Egbe-Nwiyi *et al.*, 1997). The severity of the disease is amongst other factors regarded as strain-dependant, and of unequal economical importance in different countries. Thus, contrary to the situation in Southern Africa, certain South American countries and Asia, anaplasmosis in Australia causes a relatively mild disease. Even within Africa, the clinical signs attributable to anaplasmosis appear to vary depending on the geographical location considered, possibly suggesting genetic variation in pathogen or host populations.

There are three methods currently used to control anaplasmosis: (i) Arthropod control by application of acaricides, although this is not always effective as anaplasma can be transmitted mechanically. (ii) Treatment of infected cattle with antibiotics, although this method is expensive and can give rise to resistant strains, and (iii) Vaccination. Both live and killed vaccines have been used for some time and are effective to control clinical anaplasmosis. However, current vaccines do not prevent cattle becoming carriers of the pathogen (Kocan *et al.*, 2000).

1.5.1.3 Babesia spp.

Babesiosis is caused by infection with intraerythrocytic parasites of the genus Babesia, which is of increasing importance as an emerging zoonosis worldwide (Homer *et al.*, 2000). Taxonomic classification of Babesia spp. places them in the sub-phylum Apicomplexa (class Sporozoa), order Piroplasmida and family Babesiidae. The bovine species of babesia include *B. bovis* (Europe, Russia, Africa); *B. berbera* (Russia, North Africa, Middle East); *B. divergens* (western and central Europe); *B. argentina* (South America, Central America, Australia); *B. major* (North Africa, Europe, Russia); *B.*

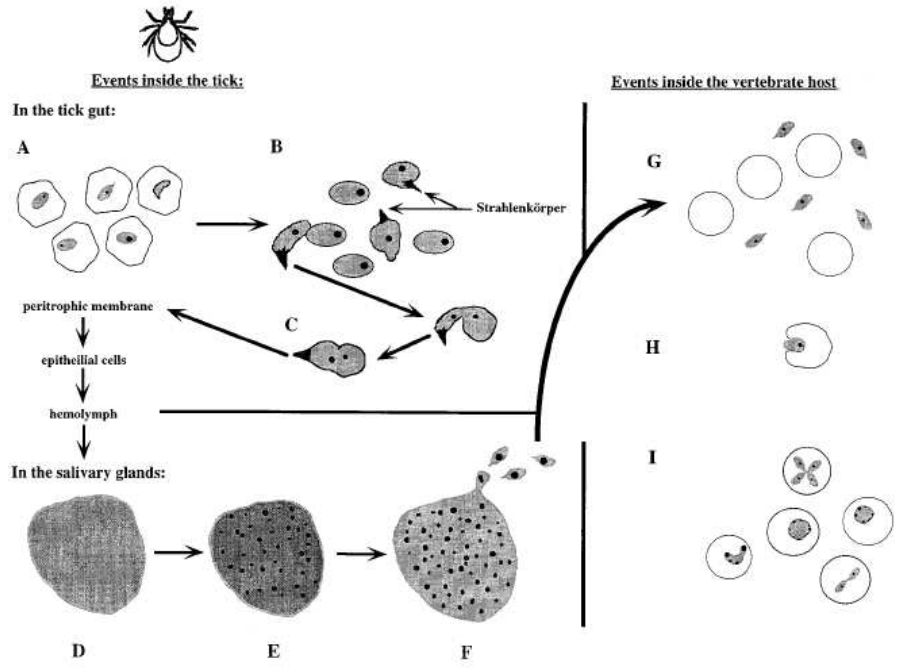
bigemina (America, Africa). The most important species infecting the bovine host in Sub-Saharan Africa are *B. bovis* and *B. bigemina*. The babesias are one of the most ubiquitous and widespread blood parasites in the world. Based on numbers and distribution of species in animals, they are second only to the trypanosomes in this respect (Levine, 1988, Telford *et al.*, 1993). Cattle seem particularly suitable as hosts to babesia piroplasms. Bovine babesiosis is a major problem in the tropics and sub-tropics of all continents where conditions favour large tick populations (De Vos, 1979).

Babesias can be found wherever certain species of ticks flourish. To date, only ixodid ticks have been identified as vectors for Babesia spp. except for one report that identified a non ixodes tick, *Ornithodoros erraticus* as a reservoir for *Babesia meri* (Gunders, 1977). Six or seven main genera of ixodid ticks have been demonstrated as experimental or natural vectors of Babesia spp. (Spielman, 1976, Schein *et al.*, 1981, Telford *et al.*, 1993). Some Babesia species, such as *B. bigemina* can infect more than one genus of tick (Kakoma *et al.*, 1993), whereas others such as *Babesia microti* can only infect ticks from the genus Ixodes. Several ticks can act as vectors of more than one *Babesia* species. *Ixodes dammini* (also known as *Ixodes scapularis*) (Spielman, 1979), for example, can harbour *B. microti* (Piesman *et al.*, 1986) in its nymphal stage (Spielman *et al.*, 1985, Piesman *et al.*, 1987), but also *Babesia odocoilei*. It is not known, however, if vectors can harbour or transmit mixed Babesia spp. (Armstrong *et al.*, 1998).

In Africa, *Babesia bovis* is transmitted only by *Boophilus microplus* whereas *Babesia bigemina* is transmitted by both *Boophilus microplus* and *Boophilus decoloratus* (De Vos, 1979, Norval *et al.*, 1983, Jongejan *et al.*, 1988). Although *B. bovis* is generally regarded as the most pathogenic species, *B. bigemina* has assumed greater importance in Africa as a result of the greater distribution of *B. decoloratus*.

B. bovis is highly pathogenic for non-indigenous breeds of cattle whereas *B. bigemina* is only moderately pathogenic (Mehlhorn *et al.*, 1984). The severity of bovine babesiosis is influenced by the abundance of competent vectors, virulence of the parasite species and the nature of the cattle population available for infection.

Figure 1.3 Life cycle of Babesia spp.



(Homer *et al.*, 2000)
The organisms are first detected in the tick about 10 hours after the tick begins to feed on an infected vertebrate. After about 46 to 60 hours of feeding, the parasites are still detectable within the consumed erythrocytes in the tick's gut, but begin to develop arrowhead-shaped organelles called Strahlenkörper or ray bodies (A). The gametes are then released from the consumed erythrocytes into the lumen of the ticks gut and begin to fuse through a process known as gamogony (B). The resulting zygote, or primary ookinete, enters the epithelial cells of the tick gut approximately 80 hours after the tick starts feeding. From the epithelial cells the ookinetes move to the salivary acini via the hemolymph (C), and undergo a process of sporogony giving rise to thousands of small sporozoites. This process of sporogony is divided in three stages. Initially, the parasite multiplies and fills the hypertrophied host cell forming a multinucleate undifferentiated sporoblast. The formation of specialized organelles only occurs after the tick begins feeding on the vertebrate host (D, E). Finally the mature sporozoites form through a budding process (F). Mature sporozoites are the infective stage of Babesia in the tick. Some 5,000-10,000 sporozoites can be produced within a single sporoblast. Several thousand sporozoites are deposited in the dermis around the tick's mouth during the final hours of attachment and feeding, and are finally injected into the vertebrate host (G). Sporozoites contact host erythrocytes and infect them by a process of invagination (H). Within the host erythrocytes, most sporozoites become trophozoites and divide by binary fission. This asexual reproduction (merogony) produces merozoites, which lyse the cell and go on to infect additional erythrocytes, building up an immense population in a short time. Erythrocytic phases, however, are reduced or apparently absent in resistant hosts. This asexual cycle continues indefinitely or until the host succumbs. Four parasites can form at the same time, giving rise to a Maltese cross form sometimes seen on stained blood smears (I).

Where *Bos indicus* cattle predominate and babesiosis is enzootically stable, the effect of bovine babesiosis is relatively small and no control measures are usually indicated. Such is the case in large

parts of Asia and Africa. In contrast, the disease is particularly severe when indigenous naïve cattle, or exotic *Bos Taurus* breeds, are introduced into endemic areas (De Vos, 1979, Norval *et al.*, 1983).

The clinical signs and the pathological changes in infections with *B. bovis* and *B. bigemina* are similar. The first symptom of disease is a sudden rise in temperature to 41 - 42 ˚C due to parasite multiplication in the bloodstream. Fever can persist for a week or more and infected animals rapidly become dull and listless and lose their appetite. The parasites destroy infected blood cells and invade new erythrocytes causing anaemia. Up to 75% of the erythrocytes may be destroyed in fatal cases, but even in milder infections so many erythrocytes are destroyed that a severe anaemia results (Hildebrandt, 1981).

The release of parasites and host constituents from destroyed red blood cells results in various physiological disturbances. In addition to anaemia, shock, tissue anoxia, icterus (jaundice), dehydration and general weakness can occur. Severe cases of *B. bovis* infection may end with central nervous system (CNS) signs (ataxia, paddling of limbs, coma) associated with sludging of parasitized red blood cells in brain capillaries. A typical peracute picture, often results in death within a week after the appearance of clinical signs (Hildebrandt, 1981). Chronically infected animals remain thin, weak, and out of condition for several weeks before recovering. Animals that survive acute early disease may have diarrhoea due to alimentary disturbances whilst later, animals may become constipated.

For unknown reasons drugs that are effective against trypanosomes are also effective against Babesia spp. A number of chemotherapeutic agents are available, some allowing recovery but leaving latent infection whilst others effecting a complete cure (Homer *et al.*, 2000). However, elimination of all parasites also eliminates premunition. Successful treatment depends on an early diagnosis and prompt administration of the appropriate drugs. Imidocarb is one of the most recent drugs and its efficacy and safety has resulted in it being the drug of choice in many instances. A vaccine has been developed by passaging *B. bovis* in splenectomised calves which are then used as donors. This attenuates the parasite so that it loses virulence but retains its antigenicity and can therefore be used as a vaccine with minimal risk of clinical reactions necessitating post-vaccinal babesicidal treatment (Callow, 1971,

Callow *et al.*, 1997). The most common form of control encountered is via tick repellents, vector avoidance, tick removal and acaricide application (Mather *et al.*, 1987, Stafford, 1991).

1.5.2 Tsetse-Transmitted Pathogens

1.5.2.1 Trypanosoma spp.

The African trypanosomes comprise a group of important protozoan pathogens affecting animal (domestic and wild) and human health in much of sub Saharan Africa. The causative organisms are represented by a variety of species and sub-species of heteroxenous haemoparasites of the genus Trypanosoma, some of which are zoonotic.

Four species of trypanosome are found in African cattle, but only three are thought responsible for causing "*Nagana*" disease in cattle. The species included in the 'Nagana' complex are *T. congolense* (Nannomonas), *T. vivax* (Duttonella), and *T. brucei* (Trypanozoon), but important pathogenic effects and economic losses in cattle are generally confined to infections with the first two (Williamson, 1970). In African cattle, *Trypanosoma brucei* is present in three sub-species: (i) *Trypanosoma brucei gambiense*, a human infective variant that causes the chronic form of the human disease known as 'sleeping sickness', (ii) *Trypanosoma brucei rhodesiense* which causes the acute form of sleeping sickness (Welburn *et al.*, 2001) and (iii) *Trypanosoma brucei brucei* which is not human infective and thought not to cause serious disease in cattle (Killick-Kendrick, 1971).

Tsetse flies belonging to the order Diptera, family Glossinidae and genus Glossina are the main vectors for African trypanosomiasis. There are 31 species and sub-species of this genus and the degree of transmission by these species and sub-species varies. *Trypanosoma vivax* can be transmitted mechanically, particularly by other bloodsucking insects such as horse flies and stable flies (Hoare, 1947, Wells, 1972).

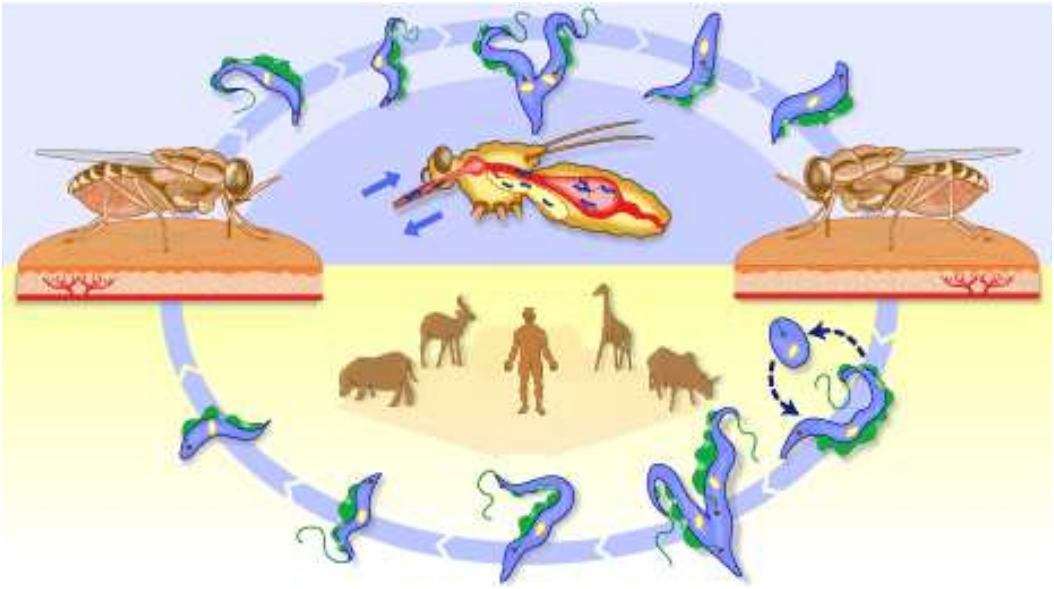
There are an estimated 46 million cattle at risk of trypanosomiasis in Sub-Saharan Africa (Kristjanson *et al.*, 1999) and the disease affects some 37 countries, some of which present with the lowest gross national product and the poorest ratio of veterinarians per livestock unit (Braend, 1979). There are 10 million square kilometres of arable land infested by tsetse flies (a third of the African continent), which

severely constrains livestock production by preventing the full use of land to feed a rapidly increasing human population (Murray *et al.*, 1991). Trypanosomiasis in domestic livestock has directly contributed to widespread malnutrition, by limiting the development of draught animal power in addition to reducing the amount of animal protein and by-products available to man (Mulligan *et al.*, 1970).

This disease has been cited as an important constraint if not the most important constraint to mixed crop and livestock farming in tropical Africa (Kristjanson *et al.*, 1999). Estimates of the total losses attributable to trypanosomiasis vary from US$1.3 – 5 billion and are dependent on the methodology, assumptions made and type of loss estimated. Losses for the 10 African countries completely infested by tsetse have been estimated to be in the range of US$192 to US$960 million. In terms of the potential benefits of eradication of trypanosomiasis from Sub-Saharan Africa, Swallow (1997) estimated that in sub humid zones the numbers of livestock would increase by around 37% and in humid zones the livestock numbers would increase by about 70%. In Uganda some 2.2 million of the 5.4 million cattle present are estimated to be at risk of trypanosomiasis. It is also estimated that trypanosomiasis has prevented the keeping of an extra 3.3 million cattle. Losses attributable to trypanosomiasis are clearly significant in a country where livestock are estimated to contribute 17% of the country's gross domestic product. Animal Trypanosomiasis is also particularly important because of its status as a zoonosis; wild and domestic animals are thought to act as a reservoir for the human infective forms of the disease (Onyango *et al.*, 1966, van Hoeve *et al.*, 1967, Hide *et al.*, 1996).

The occurrence of African Trypanosomiasis is largely coincident with the range of the vector species (Leak *et al.*, 1993). There are a number of other factors that are known to determine the distribution and transmission of the disease within the range of the vector. Different breeds of cattle show differing susceptibility to disease and the prevalence is often highest in high tsetse challenge areas where taurine cattle predominate (Hendrickx *et al.*, 2000). Other factors shown to influence the distribution of Nagana are density of cattle, cattle management practices (Kalu, 1995), climate, season (Kalu *et al.*, 1996) and host nutritional status (Holmes *et al.*, 2000).

Figure 1.4 Life cycles of the African Trypanosomes.



(WHO, 2003)

The life cycles of *Trypanozoon*, *Duttonella* and *Nannomonas* trypanosomes can be divided into two phases in the fly: The establishment of the infection and the maturation into vertebrate infective forms. *Duttonella* trypanosomes have the simplest lifecycle, as both infection and maturation take place in the mouthparts (proboscis). In contrast, both for *Trypanozoon* and *Nannomonas* the infection takes place in the midgut, while maturation occurs in the salivary glands or mouth parts (proboscis) respectively. For the establishment of infection in the fly's midgut, trypanosomes must change from bloodstream forms to procyclics, loosing their surface glycoprotein coat, and move to the ectoperitrophic space to form an actively dividing population. For the maturation of the procyclic forms into vertebrate infective forms, trypanosomes return to the endoperitrophic side of the midgut, move to the salivary glands (*Trypanozoon*) or to the mouthparts (*Nannomonas*), transform to dividing epimastigotes and subsequently into infective metacyclics. Differences in the transmissibility of *Nannomonas* and *Trypanozoon* trypanosome stocks from the vector to the host are thought to reflect differences in lectin (agglutinins) contents in the tsetse flies, and numbers of lectin binding sites in the surface coat of the parasites (Welburn and Maudlin, 1990). When the vector bites a host, metacyclic forms (already covered by a monolayer of a variable surface glycoprotein) are inoculated. Immediately after, local asexual multiplication of the parasites in the interstitial space of the subcutaneous tissue takes place. The parasites then invade the lymphatic system and the blood stream (Haematic [*Nannomonas* and *Duttonella*] and Humoral [*Trypanozoon*] groups) but they also invade other organs and tissues where they multiply extracellularly (mainly Humoral group [*Trypanozoon*]). At this stage short stumpy (infective to Glossina spp), intermediate and long-slender flagellated trypomastigote forms (actively multiplying forms) of the parasites might be observed in blood samples or lymph node samples.

The main clinical sign of infection is anaemia. Red and white blood cell counts are markedly decreased within one week of infection and the levels continue to drop during the later stages of the disease (Murray, 1978). Other symptoms include oedema, watery eyes and nose, loss of condition and fever

(Boyt, 1984). Susceptible animals become emaciated very quickly followed by deterioration in co-ordination and later paralysis and abortion; infertility may also be seen (Fiennes, 1970). More commonly, *T. vivax* and *T. congolense* infection in cattle present a chronic course, clinically characterised by progressive emaciation, intermittent periods of pyrexia coinciding with intermittent and scanty parasitaemia, and anaemia (Masake *et al.*, 1981).

The phenomenon of antigenic variation is responsible for the relapsing parasitaemia seen in the clinical disease. The pathology and course of the disease depends to a great extent on the host species infected and even on the genetic make up of individuals and the initial condition of the animals (Boyt, 1984). Immunologic lesions are significant in animal trypanosomiasis, and immunosuppression has been demonstrated following infection with *T. congolense* (Flynn *et al.*, 1993).This may lead to death from secondary infections.

In spite of intensive efforts, trypanosomiasis has proven difficult to control and many attempts to eradicate the disease from certain areas have failed (Holmes, 1997). The most widespread method of trypanosomiasis control is the administering of trypanocidal drugs. The two main drugs to control Nagana (isometamidium and diminazene) have been available for over thirty years, but are relatively cheap and are still widely used (Holmes *et al.*, 1982). The use of these drugs by cattle owners has had an enormous positive impact on livestock keeping (Holmes *et al.*, 1988). Methods to control tsetse have included ground and aerial spraying of insecticides, but at present the most widely employed method is the use of tsetse traps (Holmes, 1997).

1.5.3 Pasture-Transmitted Pathogens

1.5.3.1 Fasciola spp.

Fascioliasis is caused by platyhelminthes of the class trematoda, family Fasciolidae. The genus is spread worldwide, but in the cattle of tropical Africa the disease is mainly caused by *Fasciola gigantica*. The intermediate hosts are snails, and for *F. gigantica* the intermediate snail host is *Lymnea natalensis.* Consequently the distribution of the disease is restricted by the range of this vector. Fascioliasis causes economic losses in livestock as a result of mortalities, abortions, retarded growth,

reduced meat and milk production, condemnation of infected livers, emaciated carcasses, and cost of animal treatment. In cattle, modest fluke infections can result in significant reductions in performance. Infections as low as 54 flukes per animal have been shown to reduce weight gain by 8-9% (Cawdery *et al.*, 1977), even though this degree of infection results in no clinical signs of disease. Because of the high prevalences, fascioliasis is regarded as the most economically important helminth infection of cattle (Spithill *et al.*, 1998). In Africa few studies have quantified losses due to the disease, but Kenya is thought to have lost US$2.6 million over a ten year period from liver condemnations alone, whilst the losses in the Jos Plateau area of Nigeria have been estimated at US$40 million annually (Fabiyi, 1987).

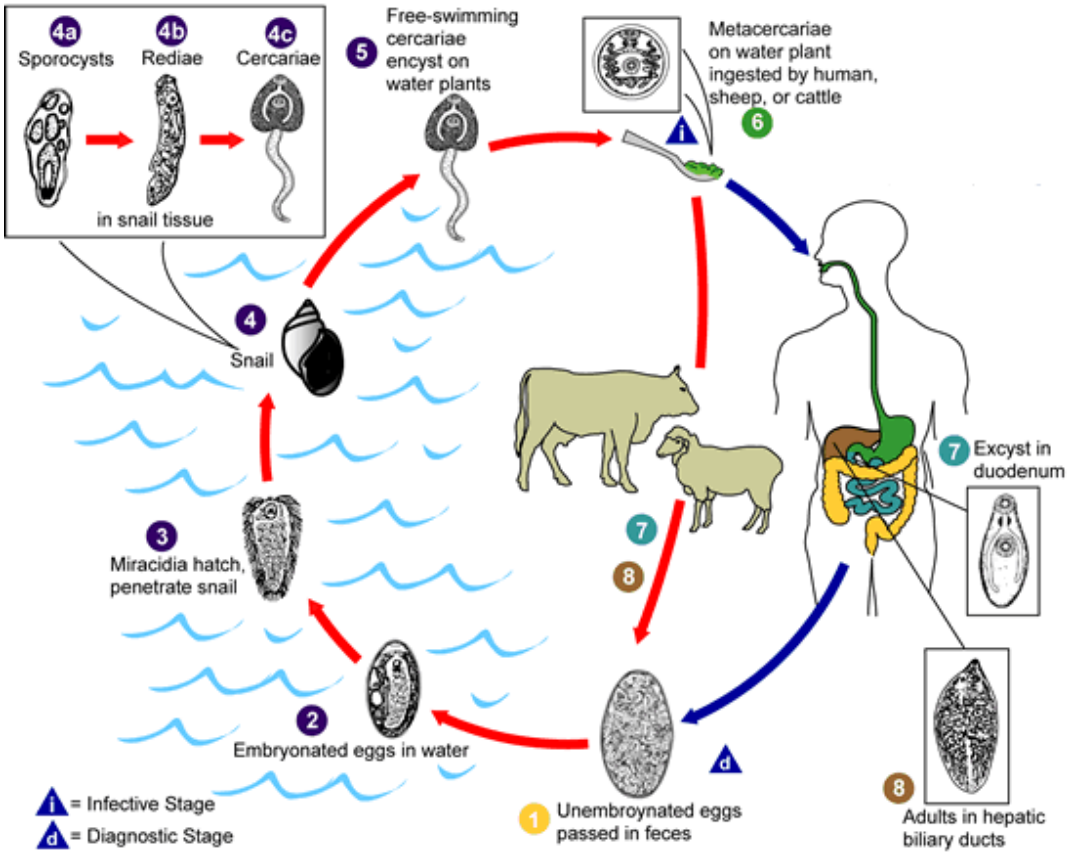
Infection with Fasciola spp. may result in a degree of acquired resistance that varies depending on the host species. In addition some animal species show a degree of innate resistance. Such is the case of horses, which are known to be less susceptible than ruminants (Nansen *et al.*, 1975). There appears to be little evidence of immunity preventing re-establishment of new infection. The analyses of age-related prevalence data in cattle, suggest that there is an increasing prevalence of Fasciola spp. as the cattle grow older (Baldock *et al.*, 1985, Gonzalez-Lanza *et al.*, 1989, Sanchez-Andrade *et al.*, 2002).

However, when the population reaches the age of 6-8 years, the prevalence approaches an asymptote of approximately 40%, suggesting that an equilibrium has been reached between rate of parasite acquisition and parasite death (Gonzalez-Lanza *et al.*, 1989). *Bos indicus* cattle appear to be more resistant to *Fasciola gigantica* than *Bos taurus*, and there is evidence that there is variation in susceptibility between breeds of *B. indicus* (Malone, 1986). The risk of fascioliasis is associated with the degree of soil moisture, surplus water and extended rainfall (Yilma *et al.*, 1998). This risk is further increased where cattle are allowed to graze around permanent water or in areas that are at times flooded (Yilma *et al.*, 1998).

Fasciolosis is characterised by anaemia, wasting, dehydration, weakness and oedema in the intermandibular space and over the abdomen (Egbe-Nwiyi *et al.*, 1996). Most infections are subclinical in nature, making infected cattle less productive and more susceptible to infections with other

pathogens (Waruiru *et al.*, 2000). Death of infected cattle often results from severe anaemia and loss of liver functions. These infected cattle often show an enlarged necrotic liver at necropsy (Boray, 1985).

Figure 1.5 Life cycle of Fasciola spp.



(CDC, 2002)

The adult forms live in the bile ducts of the host and produce unembryonated eggs which pass into the small intestine. The eggs are passed out of the host in the faeces; (1) these embryonate in water (2) and the first stage miracidia are released (3). Each miracidium must find and infect a snail within 24 hours of hatching. Once a snail host is located, they infect by penetrating the body of host (4). Inside the snail the miracidium loses its cilia and develops into the sporocyst stage. Each sporocyst divides and becomes a redia. Eventually, the cercariae are released from the snail (5). Once released, they attach to plants, lose their tails and encyst as metacercariae. The latter are infective to the vertebrate host. The vertebrate host becomes infected when ingesting plant material to which the metacercariae are attached (6). The metacercariae excyst in the small intestine (7) and migrate through the gut wall to the liver. The immature flukes tunnel through the liver parenchyma for around 6 – 8 weeks before entering the bile ducts. Type example *Fasciola hepatica*.

Eradication of parasitic infections is rarely practical, and measures most commonly aim at reducing the levels of disease to allow economic livestock production, prevent the build up of parasites in the environment and avoid areas of heavily contaminated pasture (Brunsdon, 1980). Reduction of pasture

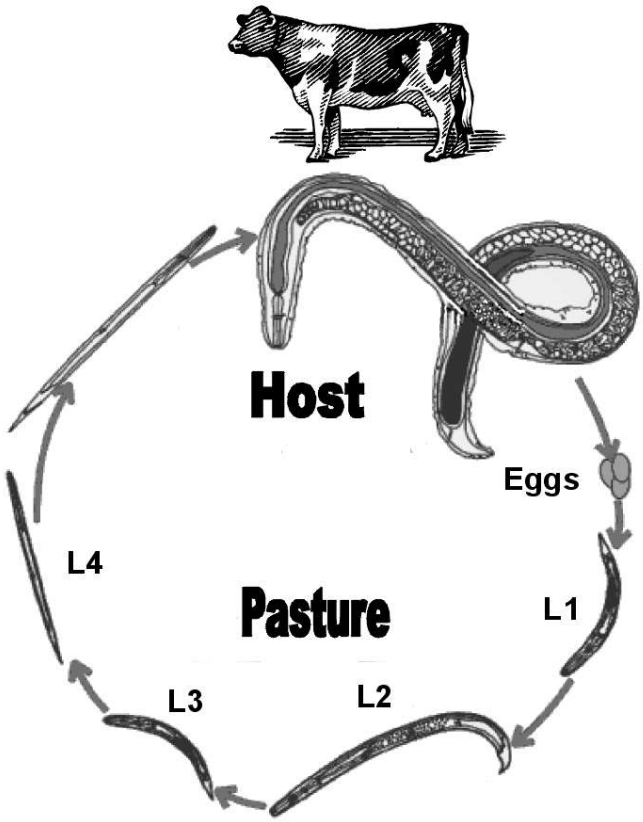
contamination might be achieved by the use of anthelmintics, management practices, molluscicides and biological competition, or through an integrated program utilising several of these options. In addition, the use of resistant animals is a potentially beneficial practice where the application of treatments is not cost effective (Roberts *et al.*, 1996). Today, models which can predict the likely impact of fascioliasis in particular years have been developed (McIlroy *et al.*, 1990) and can be used in the preventive planning of fascioliasis. However, their use in a variety of ecosystems requires further development. The principal method for controlling Fasciolosis in livestock is through the use of anthelmintics, which differ widely in price, availability, efficacy (depending of the fluke stage targeted) and safety. Most of the available products have equal efficacy against *F. hepatica* and *F. gigantica* (Brunsdon, 1980).

1.5.3.2 Gastrointestinal (GI) Nematodes

The main species of gastrointestinal nematodes present in Africa belong to the order Strongylida; these are *Haemonchus placei, Trichostrongylus axei*, *Cooperia pectinata, Oesophagostomum radiatum, Cooperia punctata* and *Bunostomum phlebotomum* (Mango *et al.*, 1974, Sauvage *et al.*, 1974, Omara-Opyene, 1985, Kaufmann *et al.*, 1989, Waruiru *et al.*, 1998, Waruiru *et al.*, 2000). These nematodes are widespread throughout Africa, where on occasions prevalences of 97.7% have been reported (Kaufmann *et al.*, 1990). The strongyle nematodes infecting the gastrointestinal tract of cattle are transmitted directly from vertebrate host to vertebrate host via the soil or pasture, and do not require a vector.

The most pathogenic of the nematodes commonly present are *H. placei* and *O. radiatum.* The others, being of limited pathogenicity, are therefore of less importance (Fabiyi, 1987, Waruiru *et al.*, 1998). Production losses are highest where the climate is favourable to survival of the free living stages (warm and humid) and cattle graze in high densities (Fabiyi, 1987).

Figure 1.6 Life cycle of gastrointestinal nematodes.



(Kieave, 2001)

Adult worms live in the true stomach of the ruminant host (the abomasum). The female nematode may lay up to 10,000 eggs per day which pass out of the host with the faeces. First stage juveniles (L1 stage) hatch from the eggs. The L1 and L2 stage juveniles feed on bacteria. The juveniles then progress to L3 stage keeping the cuticle of the L2 stage as a sheath. The L3 stage juveniles do not feed and are infective to the vertebrate host. The ruminant becomes infected when grazing and ingesting the infective L3 stage. The L3 stage exsheath in the rumen of the host and the young worms pass into the abomasum where they burrow into the mucosa. The L3 stage then undergoes another moult to produce the L4 stage juveniles. A final moult takes place to produce the mature nematodes. Then, mating of adults and egg production takes place. Type example *Haemonchus contortus.*

Mortality due to gastrointestinal helminthiasis can be severe under favourable conditions for the parasite. For example, 10 – 20% mortality has been reported in a study in Senegal - a semi arid climate - (Vassiliades, 1974), whilst in a survey in Botswana extensive deaths and condemnation of carcases due to severe emaciation reached 30% (Carmichael, 1972). Similarly high losses have been

reported outside Africa. In Burma overstocking resulted in 30% mortality (Griffiths, 1957), and in North Queensland wet conditions and overstocking resulted in a mortality of 33.5% (Copeman *et al.*, 1979).

Since the free living nematode stages require a moist environment, the prevalence of gastrointestinal nematodes is strongly influenced by season, with an increasing prevalence during the wet seasons (Waruiru *et al.*, 2000). Consequently in arid regions nematode infections are not as problematic (Omara-Opyene, 1985). Hot and humid climates, in contrast, can lead to severe contamination of pastures with nematodes such as *Haemonchus* spp. (Waruiru *et al.*, 1993). Cattle management practices can also contribute significantly to the degree of gastrointestinal nematode infection within an area. Increased stocking rates, communal grazing and the degree of helminth control are all important factors (Kaufmann *et al.*, 1990, Waruiru *et al.*, 2000). Nutrition also affects morbidity. A poor nutritional status leads to higher infection intensity and therefore the occurrence of clinical signs (Waruiru *et al.*, 1993). Similarly, concurrent infection with other pathogens can also result in higher worm burdens and greater morbidity (Kaufmann *et al.*, 1990, Kaufmann *et al.*, 1992, Waruiru *et al.*, 1998). Worm burdens are highest in the 6 month to 2 year age group, when calves pick up the infection as they begin to graze in the post-weaning period (Omara-Opyene, 1985, Rubaire-Akiiki *et al.*, 1999). Surviving cattle develop resistance to infection and the worm burden is controlled as the cattle mature, so that adult cattle generally have low worm burdens (Omara-Opyene, 1985).

The main clinical signs caused by gastrointestinal nematode infection in cattle include staring coat, 'pot belly', poor body condition, weakness, progressive emaciation and anaemia (Kaufmann *et al.*, 1989). Morbidity due to the two most pathogenic species, *H. placei* and *O. radiatum*, is generally due to the blood loss and trauma caused during migration of the nematodes (Waruiru *et al.*, 1998). Pathological features include fat degeneration, oedema, anaemia and haemorrhagic gastritis with large numbers of worms recovered from the lumen of the gastrointestinal tract (Kaufmann *et al.*, 1989, Waruiru *et al.*, 1993).

There are few instances of planned prophylactic helminth control regimens, except on large ranches and government farms. The treatments that are carried out are largely curative and in response to

evidence of morbidity in infected animals (Fabiyi, 1987). Most control measures revolve around regular treatment with anthelmintics. Treatment with drugs such as the benzimidazoles, levamisole, pyrantel, morantel and ivermectin are all effective against strongyles. Although resistance to anthelmintic drugs has been widely reported for small ruminants, the extent of development of resistance in cattle is as yet unknown (Kaplan, 2004). Treatment intervals vary depending on climate and season (Lee, 1955, Bergeon, 1968, Copeman *et al.*, 1979). Other methods of control include regularly moving stock to new pastures and rotation grazing, although many indigenous pastoralists are reluctant to deviate from traditional practices (Lee, 1955, Griffiths, 1957, Le Roux, 1957).

Although no commercially available vaccine is currently available, there are a number of candidate antigens that are producing high levels of protection. Further development of these potential vaccines is required (Dalton *et al.*, 2001).

1.6 The Importance of the Wider Community of Pathogens in Epidemiological Studies

Hosts with multiple infections are more common than hosts with either no infection or single infections (Petney *et al.*, 1998, Cox, 2001) and this has been the case since the earliest of times, as evident from the analysis of human coprolites and other human remains in prehistoric sites (Brothwell *et al.*, 1967, Cockburn *et al.*, 1998). Multiple parasite infections by different species are particularly frequent in animal and human populations from developing countries (Petney *et al.*, 1998). In Cote d'Ivoire (Africa) for example, two thirds of human patients harbour three or more pathogen genera (Raso *et al.*, 2004), whilst in Papua New Guinea, 82% of Plasmodium spp. infected children harbour multiple parasite species (Bruce *et al.*, 2003). For the case of endemic pathogens of cattle, few studies specifically report prevalence of mixed infections either in Africa or elsewhere (Hotter *et al.*, 1995, Ceci *et al.*, 1999, Magona *et al.*, 2002, Tassi *et al.*, 2002, Hofmann-Lehmann *et al.*, 2004, Muraguri *et al.*, 2005, Keyyu *et al.*, 2006), but high prevalences reported for individual pathogen species in the field (Deem *et al.*, 1993, Carrique Mas *et al.*, 2000), indirectly suggest that concurrent infections must occur frequently.

There is abundant evidence from laboratory studies that concurrent infections can have marked effects on the pathogenicity of - and susceptibility to - other pathogens (Dallas, 1976, Hughes *et al.*, 1977, Fakae *et al.*, 1994, Rose *et al.*, 1994, Goossens *et al.*, 1997, Yoshida *et al.*, 1999, Onah *et al.*, 2003). Although the number of studies illustrating the pivotal role of concomitant infections in field settings is currently increasing (Briand *et al.*, 2005), the bulk of research is still focused upon single infections (Cox, 2001) and there is a lack of information on the effects of natural mixed infections.

An understanding of these aspects by means of thorough field studies is hindered by the many confounding variables involved (Keusch *et al.*, 1982). However, there is increasing recognition that host population dynamics are affected by both single pathogen species and the wider pathogen community (Holmstad *et al.*, 2005), and that effective control strategies will require an integrated understanding of the whole pathogen community in addition to its individual components (Torr *et al.*, 2002).

Chapter 2: Longitudinal Study Design

2. 1 Introduction

To address the specific objectives of this thesis (Section 1.4), a study was conducted to monitor the infection dynamics of endemic pathogens of African cattle and how these relate to animal health. To recap, the specific objectives were defined upon assertions that (i) endemic diseases of African cattle are a major constraint to sustainable rural livelihoods in sub-Saharan Africa, (ii) the control of such diseases is increasingly compromised by shrinkage of government veterinary services in wake of privatisation, and that (iii) future control strategies will rely on integrated approaches. Collection of epidemiological data on the community of endemic pathogens was conducted by means of a longitudinal observational study. This type of study was chosen to better inform on the dynamics of pathogen infections in relation to cattle age and to avoid the deficit of mixed infections reported when cross-sectional studies are conducted (Bruce *et al*., 2003). It was also chosen to meet the requirements of a larger integrated research project co-funded by the Farming in Tsetse Control Areas (FITCA) project and the Department for International Development (DFID) Animal Health Programme, which was set to assess, in addition, the short and long term consequences of the use of veterinary drugs in indigenous herds. The following sections describe the design of the longitudinal observational study.

2.2 Study Population

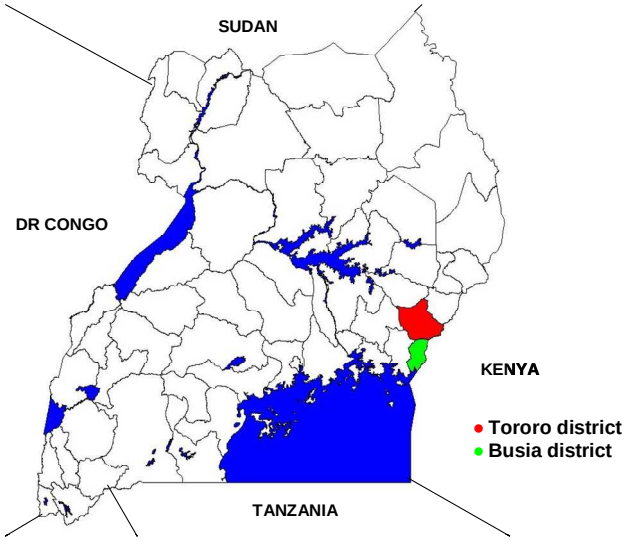
The population of interest consisted of smallholder rural farmers in Busia and Tororo districts from south east Uganda. These farmers mainly undertake agricultural production under a traditional mixed crop-livestock system. They produce a number of different food and cash crops and integrate crop production with livestock keeping (mainly cattle in small to medium sized herds but also sheep, goats, chicken and turkeys) for self-consumption and trade (Okello-Onen *et al.*, 2003).

The sustainability of rural livelihoods in these areas, is considered to be experiencing significant constraint due to the presence of the tsetse species *Glossina fuscipes fuscipes* and to a limited extend

G. pallidipes (Lancien *et al.*, 1990, Okoth *et al.*, 1991, Magona *et al.*, 1997, Okuna *et al.*, 1999). Tick species responsible for the transmission of major tick-borne diseases (TBDs) have been reported in these areas, including *R. appendiculatus*, *R. evertsi evertsi*, *B. decoloratus* and *A. variegatum* (Okello-Onen *et al.*, 1998, Magona *et al.*, 1999, Magona, 2004). In addition, the presence of swamps and marshland together with the climatic conditions in the area, favour the survival of *Lymnaea natalensis* (Ogambo-Ongoma, 1972) and the continuous development of helminth larvae in the pasture. Furthermore, faecal contamination of communal pastures by cattle from different herds ensures year round transmission of helminth parasites. Together, trypanosomiasis, tick-borne diseases and helminthosis are responsible for the bulk of cases of sick cattle reported to the government by field veterinarians in south east Uganda (Anon, 1992, Magona *et al.*, 2002).

Tororo and Busia districts, are located just North to the Equator and have an estimated area of 1592 and 744 km^2 respectively.

Figure 2.1 Geographical positions of Tororo and Busia districts.



Busia (Lat. 0˚27 ‘ 16N – Long. 34˚4 ‘ 33E) ; Tororo (Lat. 0˚41 ‘ 5N – Long. 34˚10 ‘ 52E)

Only 5 – 10% of the population lives in urban areas (Census, 2002a, 2002b). The remaining population lies in rural areas distributed in savannah grassland, inter-spaced by bush islands with a high proportion of *Lantana camara* shrubs (Magona *et al.*, 2000, Magona *et al.*, 2002, Okiria *et al.*, 2002).

The main ethnic group in Busia are the Bantu, which were the first people to move into Uganda, about 1000 A.D. from the Democratic Republic of Congo. In Tororo the population is mainly River-Lake Nilotes and Plain Nilotes. River-Lake Nilotes moved into Uganda around 1400 – 1500 A.D. from Sudan, although they were originally from Ethiopia. Plain Nilotes were traditionally cattle keepers who arrived to Uganda in the 17th century from north western Kenya (OAU, 1998).

Geographically, the districts are spread over altitudes ranging between 1060 and 1220 meters above sea level (high plain), although Tororo is slightly more elevated than Busia (1097-1219m vs. 1000-1128m). The rainfall in both districts is bimodal in distribution, with two wet seasons; March to May and September to November, and two dry seasons; December to February and June to August. The hottest and wettest months of the year are March, April and May (OAU, 1998).

Tororo and the northern area of Busia receive 1000 to 1500 mm of rainfall annually. The mean annual maximum and minimum temperatures range from 27.5 to 30 °C and 15.0 to 17.5 °C respectively. The Southern part of the Busia district receives 1500 – 2000 mm of rainfall each year, and the mean annual maximum and minimum temperatures range from 25 to 27.5 °C and 15.0 to 17.5 °C respectively (OAU, 1998). Small seasonal variations occur in rainfall and temperature between the districts (Ford *et al.*, 1976).

The natural vegetation in the eastern half of Tororo, and the eastern and southern parts of Busia is dry savannah, with the remaining area being covered with wet savannah. Forest vegetation type is limited to a small area to the north west area of Busia district. The major cash crops correspond to large millet and cotton plantations. Plantations of *Robusta* coffee are also common in south west Busia, and rice and papyrus plantations are increasingly important in Tororo (OAU, 1998). The major food crops are cassava and finger millet, although bananas, maize, wheat, sweet potatoes, groundnuts and beans are also grown (Okiria *et al.*, 2002).

Zebu and Sanga breeds constitute over 95% of the cattle population in Uganda (Magona *et al.*, 2002). Under traditional management, the majority of cattle owners neither undertake tick control, nor organise regular treatments for trypanosomiasis or helminthiasis. The cattle are tied up around the

homesteads at night and grazed on communal pastures during the day, often in swamp areas where tsetse flies, ticks and pasture-transmitted helminths are common. Young calves are usually tethered close to the homestead all the time.

2.3 Methods for Longitudinal Study Design

2.3.1 Selection of the Study Population

The study population was selected by means of a multistage cluster sampling process, but the sampling technique at each stage was either purposive or convenient. Purposive (judgemental) selection is the non-probabilistic choice of a sample, the average of whose quantitative characteristics or distribution of whose qualitative characteristics are similar to those of the target population (Thrusfield, 1995). Purposive selection presents the disadvantage that the sample might underestimate the variability of the population that is being sampled and hence result in bias (Yates, 1981). Convenience sampling, on the other hand, is the non-probabilistic collection of easily accessible sampling units, and presents the disadvantage that it may not truly represent the study population, for which extrapolation of results may be inappropriate (Thrusfield, 1995). Despite the mentioned disadvantages, purposive and convenience selection methods may be the only means of obtaining sufficient information to investigate aspects of the epidemiology of multiple livestock diseases in difficult field settings.

2.3.1.1 Selection of Busia and Tororo Districts

The Busia and Tororo districts were chosen for **(i)** the evidence of a persistent sleeping sickness focus since 1985, with more than 12,000 parasitologically confirmed cases since 1988 (Odiit *et al.*, 1999), **(ii)** the evidence of the role of cattle as major reservoirs of human sleeping sickness in south east Uganda (Fèvre *et al.*, 2001), **(iii)** the documented presence of tsetse and animal trypanosomes (*T. brucei*, *T. congolense* and *T. vivax*) in these areas (Lancien *et al.*, 1990, Okuna *et al.*, 1996, Magona *et al.*, 1997), **(iv)** the occurrence of major tick-borne diseases (TBDs) (Anon, 1996) and pasture transmitted gastrointestinal helminths in indigenous cattle (Magona *et al.*, 1998, Magona *et al.*, 1999), and **(V)** the

reported presence of mixed infections within the bovine hosts (Magona *et al.*, 2002), all of which offered a unique opportunity for the integrated investigation of these pathogens.

2.3.1.2 Selection of Rural Villages

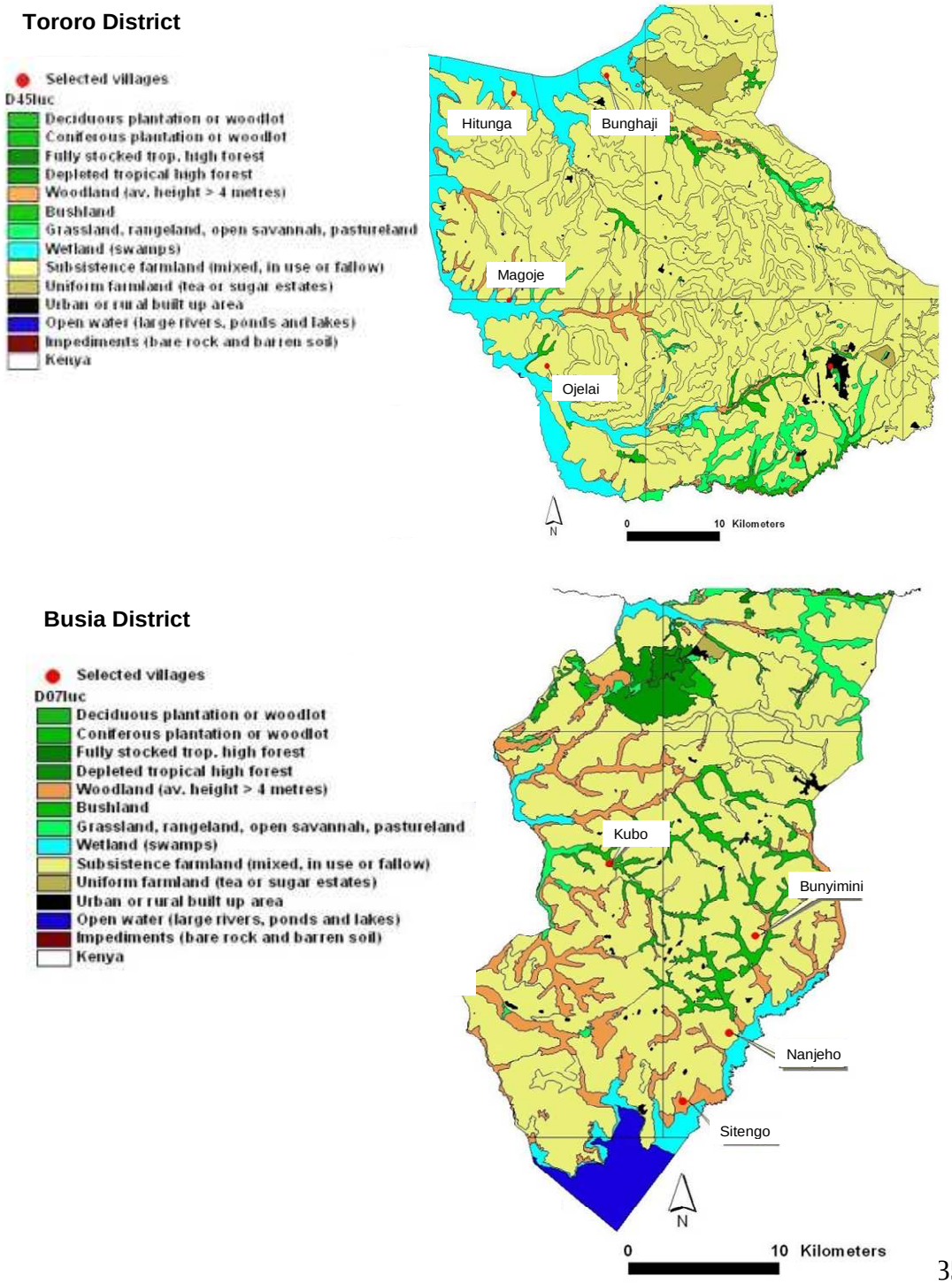
On the basis of the resources and manpower available, four rural villages capable of contributing a sufficient number of cattle were selected within each district.

The selection process followed a non probabilistic purposive method to satisfy the following criteria **(i)** Rural villages were to be located in high risk human sleeping sickness (HSS) areas, that is, close to swampland or bushland suitable for *Glossina fuscipes fuscipes* as suggested from observations that a positive correlation between HSS and proximity to these habitats exists (Odiit *et al.*, 2006). **(ii)** The traditional livestock management practices should not differ across villages, consisting fundamentally of agro-pastoralist sedentary farmers who feed their herds on communally grazed land, fallow land and crop land after the harvest, with no access to zero-grazing units. **(iii)** All villages, as well as the grazing areas utilised by the farmers within a village, had to be physically isolated from each other (e.g. by means of physical barriers such as woodland or swampland). **(iv)**There should be no cattle trade across villages in the study. **(v)** The villages should not be participating in other governmental or non-governmental programs benefiting animal or human health, including cattle dipping activities. **(vi)** The villages, or alternatively an area where farmers from the same village could bring their cattle throughout the longitudinal survey, had to be reasonably close to a route accessible by car. **(vii)** Cattle availability within each village had to be sufficient as to ensure that between 50 and 80 animals would be sampled each month throughout the longitudinal study, and additionally large enough to guarantee a sufficient contingency list from which to replace animals passively or actively withdrawn from the study.

Detailed maps of the territories of interest were obtained from the mapping unit in Entebbe and the planning units in Tororo and Busia. A number of villages were then selected on the basis of their geographical location and visited by team personnel after approval of the district veterinary officers. During these visits farmer and cattle censuses were conducted with the help of local administrators,

and the fulfilment of the recruitment criteria were assessed to guide the selection process. A contact person (preferably a local administrator) was chosen by the community in each village to ensure organised communication with cattle owners. The objectives of the observational longitudinal study were then explained to farmers in selected villages in a series of meetings organised through the contact person. The final recruitment step depended on the willingness of farmers to contribute in the study with their cattle. The four villages selected from Busia (Bunyimini, Sitengo, Nanjeho, Kubo) and Tororo (Hitunga, Magoje, Bunghaji, Ojelai) are shown in Figure 2.2.

Figure 2.2 Geographical distributions of study villages in Tororo and Busia districts.



2.3.1.3 Selection of Cattle

After village selection, and before the official start of the longitudinal observational study, a more detailed list of the total herd size of each farmer in the village was created by stratifying the number of cattle into age and sex groups. From this list 80 cattle from each village were selected purposively to take part in the study. The remaining cattle on the list served as a reserve for the later purposive recruitment of cattle to replace animals actively or passively withdrawn from the study. Depending on the owner's willingness to participate, the selection process took into consideration representation from all herds in the village. Recruitment of animals was age weighted to compensate for higher rates of drop out in younger animals (O'Callaghan, 1998) and to ensure a satisfactory sample size of younger cattle to assess the prevalence of major TBDs. The eighty recruited cattle from each village were ear-tagged and identified with the first two initials of the village's name and a number ranging from one to 80. Whenever an animal was permanently withdrawn from the longitudinal study, a new purposively selected animal was ear-tagged (with the first two initials of the village's name a number greater than 80) and introduced in the study. The ages, sex, breed and coat colours were systematically recorded at the time of ear–tagging.

Estimation of Cattle Age

Cattle age was estimated using the dentition score method developed for zebu cattle under a low plane of nutrition (Kikule, 1953), and by asking individual farmers the estimated time of birth of each tagged animal. The dentition score method is based on counting the number of permanent incisors (front teeth) that have erupted in the lower jaw. Typically, calves present with a complete set of deciduous incisors a few days after birth. Pairs of permanent (adult) incisors erupt together at particular times replacing these deciduous teeth, until a full set of adult teeth consisting of four pairs permanent incisors emerge. Eruption is assumed to have taken place as soon as the permanent incisor pair can be observed through the gum. Permanent can be distinguished from deciduous incisors in that the former are typically worn down whereas the latter are normally pointed. The presence of cheek teeth also indicates that the incisors are permanent.

The dentition score method is only approximate. Age at eruption can vary by several months from animal to animal, and accurate estimations are complicated by inter-breed variability and the tendency for higher planes of nutrition to lead to earlier eruption. Nevertheless dentition score remains, at present, the only available practical objective indicator of cattle age. Moreover, the estimated age was supported by information provided by the owner, which was usually very close to the project's estimate.

Cattle age was estimated for each individual at the time of ear-tagging only. The information was then recorded in a field collection data sheet as an ordered categorical variable where A, B, C or D corresponded to cattle ages of 1-6 (calves), 7-12 (calves), 13-24 (juveniles) or >24 (adults) months of age respectively. The precise age in months was recorded for calves of up to six months of age only. Hence, except for the case of younger calves, the initial recording system was unfortunate in that the age category into which animals should be classified on subsequent follow-up observations, could not be estimated. To overcome this limitation, cattle age was re-estimated for all animals presented by the farmers on September 2002 that is, two months before the end of the study period. The precise age of the animals was then recorded in months. This allowed calculation both prospectively and retrospectively (up to the time cattle were first recruited) of animals' age at the time of each visit round. A limitation of this system is that age could not be estimated for follow-up observations from cattle removed promptly from the study.

2.3.2 Longitudinal Study Logistics

2.3.2.1 Main Village Visits

The collection of data to address the objectives of the study took place during the main visits, which were carried out by a senior field based veterinarian (Ian Anderson) and team personnel from the Livestock Health Research Institute (LIRI) in Uganda. *"Main"* village visits were conducted on a regular rotating pattern from Monday to Thursday for two weeks so that, after initial visits, a fixed inter-main-visit interval of approximately 28 days (1 month) was maintained for individual villages. Only a single village was visited per day, and prior notice was passed on to cattle owners through the contact person

in each village. The period of surveillance in Tororo and Busia villages started at the end of July 2001 and on mid October of 2001 respectively, and ceased in November 2002. In total, Busia and Tororo villages were surveyed for 15 and 18 months respectively. Data collection, which involved the assessment of cattle health and the collection of biological samples, started at 8 a.m. and finished approximately by 1 p.m. Ear-tagged cattle were either sampled and examined within the owner's homesteads or at designated collection centres where owners gathered their animals. Veterinary treatment was provided when necessary, after sample collection.

2.3.2.2 Intermediate Village Visits

All villages were revisited two weeks after the corresponding main visit date. Parallel secondary visits, referred to as intermediate visits, served to assess the condition of sick animals detected at each main visit, and provide veterinary assistance when needed. These visits, however, were not restricted to ear–tagged cattle. Village intermediate visits were also conducted on a regular rotating pattern from Monday to Thursday for two weeks such that, after initial visits a fixed inter-intermediate-visit interval of approximately 28 days (1 month) was maintained for individual villages. Only one village was visited per day, and prior notice was passed on to cattle owners through the contact person in each village.

2.3.2.3 Experimental Block Veterinary Interventions

As part of the wider study, all cattle in three randomly selected villages from each district were block treated with either isometamidium chloride (ISMM [Samorin ®]), long-acting (LA) oxytetracycline (Retardoxi 20 L.A.), or both. Experimental block treatments were applied to all cattle presented at the time of the initial main visit in Tororo (July 2001) and Busia (October 2001), immediately after the systematic sample collection and the physical examination of the ear–tagged cattle. L.A. oxytetracycline was administered intramuscularly, at a dose of 20 mg/kg body weight. The serum half life of this drug above the minimum inhibitory concentration is 3 days (VMD, Arendonk, Belgium), for which clinically effective concentrations were not sustained at the time of follow-up observations. ISMM chloride, was also administered intramuscularly, at a dose of 1 mg/kg body weight to achieve chemoprophylactic effect even in areas of intense tsetse challenge (Uilenberg, 1998).

Given the long chemoprophylactic effect of ISMM in cattle serum (from 2 to more than four months depending on the level of tsetse challenge (Matovu *et al*., 2001)), a dilution effect of the initial block treatment impact may have occurred as untreated cattle were recruited in the study from contingency lists during follow-up visits.

Combined ISMM and L.A. oxytetracycline block treatments were applied to the villages of Kubo (Busia) and Hitunga (Tororo). "Single drug" block treatments involving ISMM only, were applied to the villages of Nanjeho (Busia) and Magoje (Tororo), while "Single drug" block treatments involving L.A. oxytetracycline only, were applied to the villages of Sitengo (Busia) and Bunghaji (Tororo). The villages of Bunyimini (Busia) and Ojelai (Tororo) received no experimental block treatments and were regarded as controls.

2.4 Village Questionnaires

Village questionnaires were conducted in February 2003 by the field veterinarian and field personnel from LIRI, aided by one contact person chosen by the community in each village. The questionnaire combined open and closed questions, and was designed as recommended by Thrusfield (Thrusfield, 1995). Each farmer in the study was interviewed individually. Data from questionnaires has only been used partially in this thesis, mainly for the description of tick-control practices and the cash and food crops of the farmers in the study in (Chapter 4 and Appendix 4.4 to 4.5).

Chapter 3: General Materials and Methods

3.1 Data Collection

3.1.1 Clinical Examinations

General physical examinations were conducted on all the ear-tagged cattle presented by farmers during the main visits, and involved the systematic recording of a number of conditions. At the time of the physical examinations, the clinical history of each individual animal - including information on stillbirths, abortions and deaths - was obtained from cattle owners. In addition, information on the owner's and the veterinarian's health assessment for each individual animal was recorded separately.

Body weight was recorded at strategic sampling times interspaced by either 4 or 5 months (November 2001, March 2002, July 2002 and November 2002). Cattle weight was estimated using a weight band. Each individual animal was then restrained on lateral recumbency for close physical examination by the field veterinarian. To assess the state of emaciation of animals, body condition scoring was performed (Nicholson *et al.*, 1986). The outline characteristics of this method are described in Section 3.1.2.1. Oral, vulval and conjunctival mucous membranes were immediately inspected for pallor, petechial haemorrhages or icterus. Animals were examined for the presence, intensity and aspect of lachrymation and nasal or vaginal discharge. The skin was inspected craneo-caudally for lesions including traumatic wounds or fungal and bacterial dermatitis, while the hair coat was assessed for signs of roughness or staring. Parotid, prescapular and prefemoral superficial lymph nodes were palpated for consistency and size. The precise length of the prescapular lymph node was also recorded in centimetres using a standard calliper measuring device. Rectal temperature was taken using a digital thermometer during the clinical examination. The presence of diahorrea was assessed visually. The collection of a faecal sample from the rectum using a plastic bag for helminth egg counts, provided an opportunity to assess the consistency, smell and the features of the faeces.

Each individual animal, was inspected for presence and intensity of louse infestation. The number of skin-attached adult stages of *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Boophilus*

decoloratus on half the body surface (HBS) of each individual, was also recorded. All male and female, engorged and non-engorded adult instars were considered in the count. The data was then recorded in the form of a "tick-score" as follows: zero (0), 1-10 (1), 11-50 (2) > 50 (3) adult instars / HBS.

During the process of physical examination the haemoglobin concentration in blood (g/dl) was measured using the HemoCue (HemoCue AB, Ängelholm, Sweden) haemoglobinometer system (Section 3.1.2.2). In addition, blood samples were collected for the evaluation of the packed cell volume (PCV) at the Livestock Health Research Institute (LIRI) in Tororo, using a standard micro-haematocrit reader (Hawksley, England).

3.1.2 Specific Field Methodologies

3.1.2.1 Condition Scoring

The nutritional plane to which an animal has been exposed over a reasonable length of time, is reflected by the extent to which fat is stored or muscle mass has diminished. Measuring changes in weight does not, per se, reflect an animal's condition, and has a number of disadvantages, including the variation associated to skeletal size, gut and bladder fill, pregnancy and parturition and tissue hydration (Nicholson *et al.*, 1986). The extent to which fat is stored or muscle mass has diminished, can be assessed visually using the method of condition scoring, for which high level of repeatability and reproducibility has been reported (Croxton *et al.*, 1976). Condition scoring is more important than live weight in assessment of reproductive physiology and behaviour in cattle, resource availability in farms or expected saleable meat (Elliot, 1964, Ward, 1968, Reed *et al.*, 1974, Steenkamp *et al.*, 1975). Scoring has been found to be least reliable in the case of young calves and weaners, as growing animals tend not to have heavy deposits of fat (Nicholson *et al.*, 1986).

Condition scoring was performed as described by Nicholson and Butterworth (1986) who specifically designed a system for *Bos Indicus* (Zebu) cattle. The score of an animal depends on the visibility of the anatomical parts, and the flesh and fat cover at strategic topographic points on the animal's body. Nine scores are used in which three main conditions (Fat [F], Medium [M], and Lean [L]) are subdivided into three categories. The scores are abbreviated as F+, F, F-; M+, M, M-; L+, L, L- when using a

categorical scoring system, as is the case in this study. Each scoring can alternatively be given a number ranging from 1 (L-) to 9 (F+). In a borderline case a half point is added to the lower score, so that a cow described as M- / L+ is scored as 3.5. The advantage of the nine-point system is that an approximation to a continuous distribution can be achieved, and the data analysed using analysis of variance (Anova), regression or Ancova. Condition scoring is an accepted system for comparing herds of cattle or individual animals under differing management systems, experimental treatments, seasons or environments, and to detect differences between and within groups over time.

3.1.2.2 Measurement of Haemoglobin (g/dl)

HemoCue (HemoCue AB, Ängelholm, Sweden) is a portable haemoglobinometer used in human medicine, and has recently been evaluated by for its suitability in measuring haemoglobin of bovine blood under field and laboratory conditions (Magona, 2004). This method involves a drop of blood (10 µl) obtained by ear marginal vein by prick, being drawn directly into the cavity of a microcuvette by capillary action, for on-spot determination. The microcuvette is then placed into the HemoCue photometer and a reading taken and recorded. Care was taken to ensure that there were no visible air bubbles inside the microcuvettes, which could lead to discrepant results, and that both the microcuvettes and the photometer device were kept below 30 ˚C.

HemoCue measures haemoglobin at two wavelengths as azide methaemoglobin, without dilution (von Schrenck *et al*., 1986). This method is based on an optical measuring microcuvette of a small volume (10 µl) and short light path (0.13 mm distance between the parallel walls of the optical window). Dry reagents are deposited on the inner wall of the microcuvette cavity, the blood sample is drawn into the cavity by capillary action and is mixed with the reagents spontaneously. The microcuvette is then placed in a HemoCue photometer, in which absorbance is measured at 565 and 880 nm. The instrument calculates the concentration of haemoglobin in the sample and displays the result. The advantadges of the HemoCue system are as follows: (i) No technical skills are required, (ii) the unit is battery or mains powered, (iii) it is accurate and robust (Magona, 2004), (iii) it compensates for turbidity in samples by measuring at two wavelengths and (iv) is easy to use in field settings.

3.1.3 Biological Sample Collection

Blood and faecal samples were collected from each animal presented during the main visits, while still in lateral recumbency. Lymph node aspirates were additionally taken from selected animals only.

3.1.3.1 Blood Samples

20 ml of blood were taken from the jugular vein using a 16 gauge blood collection needle (Terumo Corporation, USA), a standard Vacutainer® holder (Becton-Dickenson, UK) and a Venoject® multisample luer adaptor (Terumo Corporation, USA). Blood was directly dispensed into two (one heparinised and one non-heparinised) 10 ml vacutainer tubes (Becton-Dickenson, UK), previously identified with the date of collection and the animal's ear-tag. An additional blood sample was taken from the marginal ear vein by prick, for the on–spot making of thin and thick blood smears and for the determination of haemoglobin (g/dl). For the preparation of thick and thin blood smears, a drop of blood was applied on a clean slide using a microhaematocrit capillary tube, and spread out with the corner of another slide. Thick and thin blood smears were labelled with the date of collection and the animal's ear-tag using a diamond – pointed pen.

3.1.3.2 Lymph Node Samples

Biopsies were taken from enlarged parotid lymph nodes using a 5 cc syringe and a 16 gauge needle and immediately placed on a microscope slide. A smear was made and labelled with the date of collection and the animal's ear-tag using a diamond – pointed pen. Lymph node biopsies were taken only from ear-tagged cattle with pyrexia greater than 40 ˚C, enlarged lymph nodes and other clinical signs compatible with acute East Coast Fever.

3.1.3.3 Faecal Samples

Faecal samples were taken directly from the rectum and placed in separate plastic bags, labelled with the ear-tag number of the individual animal and the date of collection.

3.1.4 Transport of Biological Samples

All biological samples collected during the main visits were immediately dispatched to LIRI (Tororo) for storage, sample preparation or laboratory analysis. Lymph node and blood smears were air–dried, packed in separate slide boxes, transported at room temperature, and stored at LIRI for later processing. Blood samples collected in vacutainers were transported in a cool box and immediately processed on arrival. Faecal samples were dispatched in a cool box to the laboratory, where they were immediately preserved with 1% formalin and kept at 4˚C until examination. Faecal samples were examined within five days of collection.

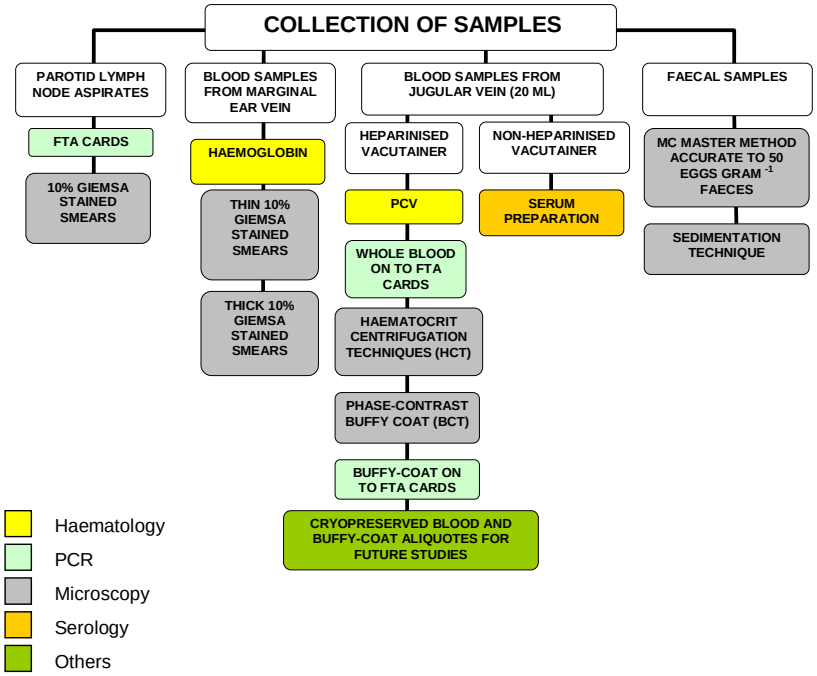
3.2 Laboratory Analysis

3.2.1 Haematology, Serology and Parasitological Screening of Blood, Lymph Node and Faecal Samples

3.2.1.1 Overview of Sample Processing

A general overview of the sample processing on arrival at LIRI (Tororo) is given in Figure 3.1. Blood in each heparinised tube was used for determining the Packed Cell Volume (PCV) of the corresponding ear-tagged animal and for the screening for *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei* using two standard field methods, the phase contrast buffy coat technique, BCT (Murray *et al.*, 1977) and the haematocrit centrifugation technique, HCT (Woo, 1969). In addition, blood and buffy-coat aliquots prepared from samples in non-heparinised tubes were stored in liquid nitrogen and on FTA cards (Whatman Bioscience, Cambridge, UK) for the later transport to the International Livestock Research Institute (ILRI, Nairobi) and the Centre for Tropical Veterinary Medicine (CTVM, Scotland) respectively. Samples collected in non-heparinised tubes served for the preparation of serum, which was later transported to ILRI (Nairobi) for serology screening of antibodies against major tick-borne pathogens.

Figure 3.1 Collection and processing of biological samples obtained at the main village visits.



Thin and thick blood films were examined for *Trypanosoma vivax*, *Trypanosoma congolense*, *Trypanosoma brucei*, Anaplasma spp., Babesia spp. and Theileria spp. infections after appropriate processing. Lymph node aspirates from selected animals were examined for Theileria spp. schizonts. Finally, faecal samples were screened for nematode eggs (mainly of the "*Strongyle*-type") and trematode eggs (mainly Fasciola spp.) using the McMaster method and the sedimentation technique respectively (Hansen *et al.*, 1994).

3.2.1.2 Haematology: Packed Cell Volume Measurement (PCV)

One microhaematocrit capillary tube was filled with 70 µl of blood from the corresponding heparinised tube, leaving approximately one fourth of the tube empty. The open end of the capillary tube was then sealed with plasticine, wiped clean with tissue paper, and spun for five minutes at 12,000 g in a microhaematocrit centrifuge. The PCV (measured as the length of the tube portion containing the concentrated cells, and expressed as a percentage of the total length of blood constituents in the tube) was read using a microhaematocrit reader (Hawksley, England).

3.2.1.3 Parasitological Screening of Blood and Lymph Node Samples

Haematocrit Centrifugation Technique (HCT)

The HCT was performed as described by Woo (1970). Capillary tubes were placed on a microscopy slide after measuring and recording the PCV. A maximum of twelve capillary tubes (each corresponding to a different animal) were placed on the slide and the spaces between the tubes flooded with immersion oil. The buffy–coat plasma junction of each capillary tube was examined by microscopy using the 100x oil immersion objective, and the presence or absence of either *T. brucei*, *T. congolense* and/or *T. vivax* recorded according to the observed morphology and the presence of pleomorphic forms (Uilenberg, 1998).

Buffy Coat Technique (BCT)

The phase-contrast buffy coat technique was performed as described by Murray *et al.* (1977). After examining the capillary tubes by means of the HCT technique, each tube was cut with a diamond pointed-pen 1 mm below the buffy coat/red cell junction to include the upper layer of blood cells, and 1 cm above to include part of the plasma. The contents of the tube were then applied on to a clean slide, mixed and covered with a coverslip. The sample was examined under dark ground phase contrast microscopy using the 100x oil immersion objective to detect the absence or presence of *T. brucei*, *T. congolense* and *T. vivax*. Trypanosome speciation was based on examining morphological traits and the presence or absence of pleomorphic forms (Uilenberg, 1998). Fifty microscopic fields were examined per sample.

Giemsa-Stained Thick and Thin Blood Smears

Thick blood films were placed in distilled water for 5 minutes to lyse the erythrocytes while thin films were dipped in methanol for 3 minutes for fixation. The blood smears were air dried and stained later in 10% Giemsa stain diluted in phosphate buffer pH 7.2 for 30 minutes. The slides were then rinsed with tap water, air dried and covered with DPX mountant and a coverslip. Examination of Theileria spp., Babesia spp., Anaplasma spp., *T. brucei*, *T. congolense* and *T. vivax* was carried out under the microscope using the 100x oil immersion objective. Trypanosome speciation was based on examining

morphological traits and the presence or absence of pleomorphic forms (Uilenberg, 1998). Fifty microscopic fields were examined for each sample. The intensity of parasitaemia was assessed for each organism using a scoring system, whereby + represented one organism found in more than 10 fields, ++ represented 1 organism found in more than one field and less than 10 fields, and +++ represented one or more organisms found per field.

Giemsa-Stained Lymph Node Aspirate Smears

Lymph node biopsy smears were fixed in methanol for 5 minutes. The smears were air dried and stained in 10% Giemsa stain diluted in phosphate buffer pH 7.2 for 30 minutes. The slides were then rinsed with tap water, air dried and covered with DPX mountant and a coverslip. Examination for the presence of Theileria spp. schizonts in the bovine lymphocytes was carried out under the microscope using the 100x oil immersion objective, and the presence or absence of schizonts recorded. Fifty microscopic fields were examined per sample. The presence of schizonts, accompanied by the presence of clinical signs compatible with ECF was considered confirmatory for *T. parva* infection (Urquhart *et al.*, 1996).

3.2.1.4 Enzyme-linked Immunosorbent Serological Assays

Blood in non-heparinised tubes served for the separation of serum at LIRI, Uganda. Serum was later tested for antibodies against *T. parva* (Katende *et al.*, 1998), *A. marginale* (Morzaria *et al.*, 1999) and *B. bigemina* (Tebele *et al.*, 2000) using ELISA. Enzyme – linked immunosorbent serological assays (ELISA) of test samples were conducted at the International Livestock Reseach Institute (ILRI), Nairobi (Magona, 2004).

3.2.1.5 Parasitological Screening of Faecal Samples

McMaster Quantitative (Counting) Technique

Faecal samples were examined for the abundance of "*Strongyle*-type" nematode eggs using the McMaster counting technique as described by Hansen and Perry (1994). The McMaster method allows

determining the number of eggs present per gram of faeces, and has a detection limit of 50 eggs per gram of sample (Hansen *et al.*, 1994).

The flotation fluid necessary to separate nematode eggs from faecal material was prepared by dissolving 400 g of sodium chloride in 1000 ml of water, and then adding 500 g of sucrose to the salt saturated solution. The suspension was then stirred until the sugar was dissolved. Four grams of sample material were subsequently weighed, placed into a plastic container and covered with 56 ml of flotation fluid. The contents were homogenised thoroughly and filtered through a tea strainer into a second container. After stirring the filtrate, a sub-sample was taken and transferred into both sides of a McMaster counting chamber using a Pasteur pipette. The filtrate was examined by microscopy using the 10x objective, after allowing the chamber to stand for 5 min. All nematode eggs within the engraved area of both chambers were counted. The number of eggs per gram of faeces was calculated by adding the egg count of the two chambers and multiplying the total by 50 (Hansen *et al.*, 1994). Egg counts of ≤400 and > 400 grams^{-1} faeces were considered indicative of sub-clinical and clinical nematode infection intensities respectively (Hansen *et al.*, 1994). Egg counts of ≤1000 and > 1000 grams^{-1} faeces were considered indicative of (clinical) moderate and (clinical) heavy nematode infection intensities respectively (Urquhart *et al.*, 1996).

Sedimentation Qualitative Technique

Faecal samples were examined for the presence or absence of trematode eggs using the sedimentation qualitative technique as described by Hansen and Perry (1994). To separate trematode eggs from sample material, 3 g of faeces were weighted and covered with 40 ml of water. The contents were homogenised and filtered through a tea strainer into an assay tube. The filtrate was allowed to sediment for 5 min before the supernatant was drawn off, transferred to a flat-bottomed tube and re-suspended in 5 ml of water. After sedimentation for a further 5 min, the supernatant was again drawn off and discarded. The sediment was stained with 5% methylene blue, transferred to a microslide and covered with a coverslip. The sediment was then examined for trematode eggs by microscopy. Animals were considered positive for fluke infections when Fasciola spp. eggs were detected or when

Schistosoma spp. eggs were found irrespective of the count. The latter were hardly reported and have not been considered in this thesis.

3.2.2 Preparation of Biological (Blood and Buffy-Coat) Samples for Transport and Storage

3.2.2.1 Cryopreservation of Blood Samples

1 ml whole blood aliquot from each heparinised vacutainer was placed in a separate cryo-tube appropriately labelled with the animal's ear-tag and containing a special buffer prepared *a priori*. The remaining sample volume in each heparinised tube was centrifuged at 10,000 g for 5 min. 1 ml buffy-coat aliquot was then removed with a plastic pipette and placed in a separate cryo-tube appropriately labelled with the animal's ear-tag and containing a special buffer prepared *a priori*. The process of cryopreservation in liquid nitrogen involved mixing the aliquot (either whole blood or buffy-coat) with 10% glycerol v/v, and phosphate buffered saline with 10% glucose (PSG) contained in the cryo-tube. Cryo-tubes were then suspended at the vapour phase over night before being lowered into liquid nitrogen tanks, which were later transported to the International Livestock Research Institute (ILRI, Nairobi), where samples from the longitudinal study remain stored for future studies.

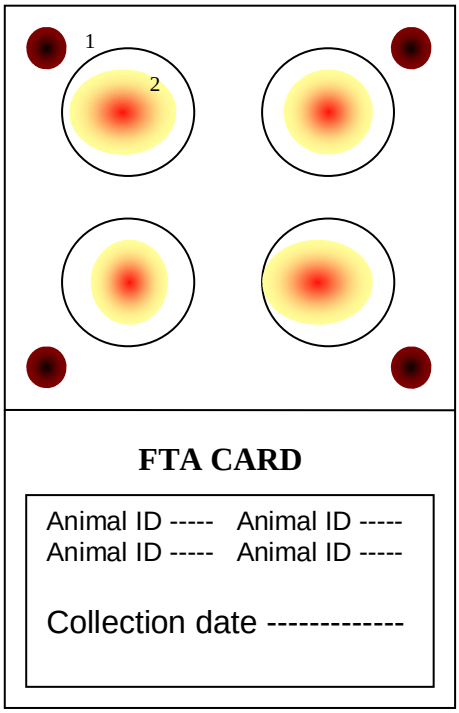
3.2.2.2 FTA cards (Whatman Bioscience, Cambridge, UK)

100 µl buffy-coat aliquot prepared from the heparinised vacutainer were separated and spotted onto a filter paper FTA card (Whatman Bioscience, Cambridge, UK) for the storage and later molecular screening of pathogen–specific DNA sequences. Heparin is less inhibitory to PCR reactions than EDTA (Solano *et al.*, 2002).

Buffy-coat samples were collected from the corresponding vacutainer with a plastic pipette, and applied to the cards in a spiral pattern with care not to contact nor over saturate the filter paper, in accordance with the recommendations of the manufacturer. In addition, one drop of whole blood from each centrifuged heparinised tube, was applied on to the FTA card, next to the buffy-coat sample

drawn from the same tube (Figure 3.2). Sample-saturated FTA cards were labelled with the date of collection and the animals' ear-tag and allowed to air-dry for 24 hours.

Figure 3.2 FTA card showing the storage method for buffy-coat and blood samples collected from cattle, at the time of the main visits.



[1, 2] Represent blood and buffy-coat samples from a same individual.
Samples were collected at the main visit specified in "*Collection date*".

Cards were then placed in foil pouches with a silica desiccant, and mailed by regular post CTVM. Pouches and silica desiccants were obtained from Whatman Bioscience (Cambridge, UK).

3.2.3 Molecular Screening of Blood and Buffy-Coat Samples

Molecular diagnosis for *Theileria parva*, *T. congolense (Savannah)*, *T. brucei* and *T. vivax* was carried out at CTVM, using Polymerase Chain Reaction amplification (PCR) for the target parasites' DNA.

3.2.3.1 General PCR Methodologies

Characteristics of the FTA Card Technology

FTA cards (Whatman Bioscience, Cambridge, UK) have been developed for the simplified collection, archive, purification and analysis of pure DNA and RNA obtained from a wide array of biological sources including blood and tissue. The card contains a filtration matrix impregnated with protein

denaturants, chelating agents and a free–radical trap which protects and entraps nucleic acids while preventing the growth of bacteria and other microorganisms. When specimens are spotted to the FTA matrix, cell membranes and organelles are lysed and the nucleic acids released and entrapped without fracture. Nucleic acids remain immobilised within the matrix during purification, eliminating the potential for cross–contamination between samples. Cards can be sent through the mail safely, without the need for temperature control or hazardous / restricted packaging, because these are non toxic and any pathogenic organisms in the sample are inactivated when in touch with the card. PCR products do not bind to the matrix and because the DNA remains matrix bound, the punch can be consistently and repetitively amplified.

Storage of Test Samples

Nucleic acids collected on FTA matrices can be stored at room temperature without degradation for more than 11 years if kept in dark, resulting in a convenient archival storage media for DNA. FTA cards containing the samples of interest were therefore stored at room temperature in foil pouches with silica desiccants (Whatman Bioscience, Cambridge, UK) to protect against moisture and strong light.

Control DNA Stocks

The stocks of Fasciola spp. and *Theileria parva* DNA, used as positive controls in parallel with the screening of cattle blood samples, were provided by Dr. Majiwa and Dr. Skilton (ILRI, Nairobi).

Table 3.1 Control DNA stocks.

Species	Stock Code
T. parva (Muguga) [1]	BJ273
T. brucei [2]	ILTat 1.2
T. vivax [3]	ILDat 1.2
T. congolense (Savannah) [4]	IL1180

[1] Piroplasm stage isolated from Kenya (Brocklesby *et al.*, 1961). [2] Initially isolated from bovine blood in Kenya (Onyango *et al.*, 1966). Passaged in mice (Nantulya *et al.*, 1984). [3] Initially isolated from a cow in Nigeria (Leeflang *et al.*, 1976). Passaged in mice and rats (Barry *et al.*, 1984). [4] Initially isolated from a lion in Tanzania (Geigy *et al.*, 1973). Passaged in mice (Nantulya *et al.*, 1982).

Storage and Processing of Control DNA Samples

Trypanosoma Control DNA

Trypanosoma control DNA was kept in 1x Tris-EDTA buffer and frozen at – 20 ˚C. A dilution series of each original stock was created by subsequently diluting the template in fresh 1x Tris-EDTA buffer. 1 µl of each test dilution was then amplified using the appropriate species-specific set of primers (25 µl reaction volume). The dilution series which resulted in the optimum band visualisation and intensity (BioRad Gel Doc 2000) was chosen as the working template concentration to be screened in parallel with the test samples. The working DNA solutions for the everyday use were kept at 4 ˚C, and placed into an ice box when in use.

Theileria parva Control DNA

T. parva purified control DNA, was stored onto an FTA card (Whatman Bioscience, Cambridge, UK) at room temperature. Control DNA contained in a circular 2 mm portion of the matrix was amplified using species-specific primers after appropriate treatment of the filter matrix (See FTA purification protocol). The resulting amplicon was visualised on a Fotodyne Transilluminator, separated from the gel using a surgical blade and placed in a clean eppendorf tube. Parasite-specific control DNA was extracted from the agarose gel using the QIAquick Gel Extraction Kit (Quiagen Ldt., West Sussex, UK) following the instructions of the manufacturer. The final step of the DNA purification protocol involved eluting the control template in 50 µl of 1xTris-EDTA. This solution was kept frozen at - 20 ˚C. 1 µl of each test dilution was then amplified using the appropriate species-specific set of primers (25 µl reaction volume). The dilution series which resulted in the optimum band visualisation and intensity (BioRad Gel Doc 2000) was chosen as the working template concentration to be screened in parallel with the test samples. The working DNA solutions for the everyday use were kept at 4 ˚C, and placed into an ice box when in use.

At least one species-specific positive control was included for each PCR run.

Negative Controls

A 2 mm circular portion of matrix from a clean FTA card (not containing sample material) was purified and processed in parallel with each batch of test samples. This ensured that no DNA contamination had occurred during the cutting and processing of the FTA matrices. The PCR master mix was prepared for all the samples of a batch in a common eppendorf tube, and later aliquoted into the PCR tubes containing each test sample. The negative control matrix therefore ensured that no contamination had occurred throughout the process of preparing the PCR solution.

At least one negative control was included for each PCR run.

FTA Purification Protocol

The FTA card was positioned on a mat and using a Harris Micro Punch ™ Tool (Whatman Bioscience, Cambridge, UK) a 2 mm circular portion of sample-saturated matrix was removed from the card, and placed in a 1.5ml eppendorf tube previously identified with the ear-tag of the animal. The disc was then prepared according the manufacturers instructions. Briefly, each disc was washed twice for 15 min in 200 µl of FTA purification reagent (Whatman Bioscience, Cambridge, UK) to remove any PCR inhibitors from the sample, and rinsed twice for 15 minutes in 200 µl of 1 x Tris-EDTA buffer (Sigma Aldrich, Dorset, UK) to remove traces of FTA buffer. Each test sample was then carefully transferred to a clean PCR tube identified with the animal's ear-tag and allowed to air-dry at room temperature for 60 min before a PCR reaction was performed.

Management and Storage of Stock Primers

Primers were obtained from Sigma Genosys (Pampisford, Cambridgeshire, UK). Oligonucleotides were suspended in 1xTris-EDTA buffer to a working concentration of 10 µM. The working solution was then aliquoted into 0.5 ml eppendorf tubes and stored at - 20˚C until use. Each eppendorf tube contained the volume of primer solution necessary for the PCR reactions performed in a day. When in use, primers were kept in an ice box.

Management and Storage of PCR Reagents

In all cases, the reagents necessary to carry out PCR amplification of parasite DNA, were provided with the RedTaq™ Superpak™ DNA Polymerase (Recombinant from *E. coli*) kit supplied by Sigma–Aldrich (Dorset, UK). This kit contains a red dye for tracking PCR products run in agarose gels, which is added directly to the PCR reaction mixture prior to amplification. The product served as a replacement for conventional agarose loading buffer. On arrival, all reagents were aliquoted into 0.5 ml eppendorf tubes and stored at - 20˚C until use. Each eppendorf tube contained the volume of reagent solution necessary for the PCR reactions performed in a day. When in use, PCR reagents were kept in an ice box.

Thermal Cycling

Thermal cycling was carried out using a Peltier Thermal Cycler (PTC – 220 DNA engine Dyad TM) manufactured MJ Research (Waltham, MA, USA).

Electrophoresis and Amplicon Visualisation

Electrophoresis was carried out in 1.5% agarose gels. Each gel was prepared by adding 10ml of Tris–Borate–EDTA 10x concentrate (Sigma Aldrich, Dorset, UK) to 90 ml of distilled water, then adding ethidium bromide to the buffer at 0.05 mg/L. To this buffer was added 1.5 g of Multi Purpose Agarose (Sigma Aldrich, Dorset, UK). The mixture was heated and allowed to cool until set. The full 25 µl of each test and control PCR reaction were dry loaded in separate wells in the gel. 20 µl of 100 bp molecular weight marker stock were also loaded at the first and last well in the gel (for the case of each row of wells). The molecular weight marker stock was prepared by mixing 120 µl of sterile water with 60 µl of gel loading solution and 40 µl of DNA ladder available from the PCR 100 bp low ladder kit from Sigma Aldrich (Dorset, UK). Electrophoresis was conducted at 80 volts. Amplicon visualisation was conducted using BioRad Gel Doc 2000.

3.2.3.2 Pathogen Species-Specific PCR Protocols

Whole blood and buffy-coat samples stored onto filter cards were used for the molecular screening for *T. parva* and Trypanosoma spp. (*T. brucei*, *T. vivax* and *T. congolense* [*Savannah*]) respectively.

Theileria parva

T. parva was amplified using the species-specific set of primers, namely forward and reverse IL3232 and IL4234 (Skilton *et al*., 2002). The set of primers target the 104 kDa rhoptry antigen (p104) single copy gene, first characterised by Iams (Iams *et al*., 1990), generating an amplicon of 276 bp. IL3232 and IL4234 amplify a wide range of cattle-derived and buffalo-derived stocks of *T. parva* and present no cross reactivity with other Theileria species including *T. taurotragi* (Skilton *et al*., 2002). This represents a major advantage over prior PCR techniques capable of detecting *T. parva* in field situations (Watt *et al*., 1998) but based on the highly polymorphic Tpr locus (Allsopp *et al*., 1989, Bishop *et al*., 1992, Bishop *et al*., 1997) thus failing to amplify all *T. parva* field isolates (Skilton *et. al*., 2002) in addition to presenting cross-reactivity with the closely related non-pathogenic *T. taurotragi* (Watt *et al*., 1997).

Protocol Using IL Forward and Reverse Primers

The PCR amplification was carried out using forward and reverse IL3232 and IL4234 primers with sequences 5'-GGCCAAGGTCTCCTTCAGAATACG-3' and 5'-TGGGTGTGTTTCCTCGTCATCTGC-3' respectively (Skilton *et. al*., 2002). PCR was conducted in reaction volumes of 25 µl which contained the following components: Sigma Aldrich PCR buffer (at final concentrations of 10 mM Tris–HCl, pH 8.0, 1.5 mM MgCl2, 50 mM KCl and 0.001% gelatin), 0.4 µM of each IL3232 and IL4234 primers (Sigma Genosys), 200 µM of each dNTP (Sigma Aldrich) and 5 U of RedTaq DNA Polymerase recombinant (Sigma Aldrich). Each reaction contained a single dried and washed 2mm punch from the relevant blood sample stored on a Whatman FTA card. One positive and one negative control were included with each PCR run (See earlier this section). 1 µl of *T. parva* (BJ273) control DNA was added in the positive control reaction. The thermal cycling conditions involved and initial denaturation step at

94 °C for 3 min followed by 30 cycles at 94 °C for 45 sec, 55 °C for 45 sec and 72 °C for 1 min and a final extension step at 72 °C for 5 min.

Trypanosoma brucei

Trypanosoma brucei was amplified using two sets of species specific primers, namely forward and reverse TBR and INGI oligonucleotide sequences, developed by (Moser *et al.*, 1989) and (Kimmel *et al.*, 1987) respectively. Forward and reverse TBR primers (TBR-1, TBR-2) target a 177 bp satellite DNA repeat with no apparent coding function (Sloof *et al.*, 1983). PCR targeted at the former DNA generates a species-specific amplicon 177 bp in size (Moser *et al.*, 1989). In contrast, forward and reverse INGI primers (INGI-1, INGI-2), target a repetitive DNA element known as INGI which is present approximately 200 times along the parasite's DNA; PCR targeted at INGI generates a species-specific amplicon of 590 bp in size (Kimmel *et al*., 1987).

Protocol using TBR Forward and Reverse Primers

The PCR screening for *Trypanosoma brucei* was carried out using reverse and forward TBR-1 and TBR-2 primers with sequences 5'-CGA ATG AAT AAA CAA TGC GCA GT-3' and 5'-AGA ACC ATT TAT TAG CTT TGT GC -3' respectively (Moser *et al*., 1989). PCR was conducted in reaction volumes of 25 µl which contained the following components: Sigma Aldrich PCR buffer (at final concentrations of 10 mM Tris–HCl, pH 8.0, 1.5 mM MgCl2, 50 mM KCl and 0.001% gelatin), 0.2 µM of each TBR-1 and TBR-2 primers (Sigma Genosys), 200 µM of each dNTP (Sigma Aldrich) and 5 U of RedTaq DNA Polymerase recombinant (Sigma Aldrich). Each reaction contained a single dried and washed 2 mm punch from the relevant blood sample stored on a Whatman FTA card. One positive and one negative control were included with each PCR run (See earlier this section). 1 µl of *T. brucei* (ILTat 1.2) control DNA was added in the positive control reaction. The thermal cycling conditions involved and initial denaturation step at 94 °C for 3 min followed by 30 cycles at 94 °C for 30 sec, 60 °C for 30 sec and 72 °C for 30 sec and a final extension step at 72 °C for 5 min.

Protocol Using INGI Forward and Reverse Primers

Parallel screening for *T. brucei* was carried out using reverse and forward INGI-1 and INGI-2 primers with sequences 5'-GAT CCG CAG CCG GGC CTG A-3' and 5'-GGG CCA AGG ACA GTT CCT TGT GG -3' respectively (Kimmel *et al*., 1987). PCR was conducted in reaction volumes of 25 µl following the same protocol as described when using TBR forward and reverse primers. The thermal cycling conditions involved and initial denaturation step at 94 °C for 3 min followed by 30 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min and a final extension step at 72 °C for 5 min.

Trypanosoma vivax

T. vivax was amplified using two sets of species specific primers, namely forward and reverse TVW (Masiga *et al.*, 1992) and forward and reverse TWJ (Masake *et al.*, 1997). Forward and reverse TVW primers (TVW-1, TVW-2) target genomic repeated DNA elements; the PCR generates a species-specific amplicon of 150 bp (Masiga *et al*., 1992). Forward and reverse TWJ primers (TWJ-1, TWJ-2) target a tandemly repeated sequence that encodes for an antigen recognised by the Tv27 monoclonal antibody. The antigen has been utilised for *T. vivax* diagnosis in Tv27 monoclonal antibody based ELISA tests (Masake *et al*., 1997), and contains a micro-satellite motif which is highly polymorphic across *T. vivax* populations (Morlais *et al.*, 2001). PCR targeted at the antigen encoding gene generates a species - specific amplicon of 400 bp (Masake *et al*., 1997). TWJ set of primers were used in parallel screening to detect *T. vivax* infection cases where TVW may have failed. The PCR utilizing the TVW primer set has been described as superior to that of TWJ in terms of sensitivity and specificity, but the former has been shown to fail in detecting *T. vivax* in some geographical areas were the parasite is known to be present (Morlais *et al*., 2001). TWJ primer set, in contrast, has been shown to amplify a wide range of *T. vivax* isolates from diverse areas of Africa and South America (Masake *et al*., 1997), both from vector mouth parts (Lehane *et al*., 2000) and animal hosts (Morlais *et al*., 2001).

Protocol using TVW Forward and Reverse Primers

The PCR screening for *Trypanosoma vivax* was carried out using reverse and forward TVW-1 and TVW-2 primers with sequences 5'-CTG AGT GCT CCA TGT GCC AC -3' and 5'-CCA CCA GAA CAC

CAA CCT GA -3' respectively (Masiga *et al*., 1992). PCR was conducted in reaction volumes of 25 µl which contained the following components: Sigma Aldrich PCR buffer (at final concentrations of 10 mM Tris–HCl, pH 8.0, 1.5 mM MgCl2, 50 mM KCl and 0.001% gelatin), 1 µM of each TVW-1 and TVW-2 primers (Sigma Genosys), 200 µM of each dNTP (Sigma Aldrich) and 5 U of RedTaq DNA Polymerase recombinant (Sigma Aldrich). Each reaction contained a single dried and washed 2 mm punch from the relevant blood sample stored on a Whatman FTA card. One positive and one negative control were included with each PCR run (See earlier this section). 1 µl of *T. Vivax* (ILDat 1.2) control DNA was added in the positive control reaction. The thermal cycling conditions involved and initial denaturation step at 94 °C for 3 min followed by 30 cycles at 94 °C for 30 sec, 60 °C for 30 sec and 72 °C for 30 sec and a final extension step at 72 °C for 5 min.

Protocol using TWJ Forward and Reverse Primers

Parallel screening for *T. vivax* was carried out using reverse and forward TWJ 1 and TWJ 2 primers with sequences 5'-TCG CTA CCA CAG TCG CAA TCG TCG TCT CAA GG -3' and 5'-CAG CTC GGC GAA GGC CAC TTG CTG GGG TG -3' respectively (Masake *et al*., 1997). PCR was conducted in reaction volumes of 25 µl following the same protocol as described when using TVW forward and reverse primers. The thermal cycling conditions involved and initial denaturation step at 94 °C for 3 min followed by 30 cycles at 94 °C for 60 sec, 55 °C for 120 seconds and 72 °C for 120 sec and a final extension step at 72 °C for 5 min.

Trypanosoma congolense (Savannah)

Screening for *Trypanosoma congolense* (*Savannah*) was as described by Masiga *et al*. (1992). This PCR is targeted to repetitive DNA elements unique to a previously described *T. congolense* (*Savannah*) type, differentiated on the basis of iso-enzyme typing (Young *et al*., 1983), and generates an amplicon of 316 bp in size.

Protocol using TCS Forward and Reverse Primers

The PCR amplification was carried out using reverse and forward TCS-1 and TCS-2 primers with sequences 5'-CGA GAA CGG CAC TTT GCG A-3' and 5'-GGA CAA ACA AAT CCC GCA CA-3'

respectively (Masiga *et al*., 1992). PCR was conducted in reaction volumes of 25 µl which contained the following components: Sigma Aldrich PCR buffer (at final concentrations of 10 mM Tris–HCl, pH 8.0, 1.5 mM MgCl2, 50 mM KCl and 0.001% gelatin), 1 µM of each TCS-1 and TCS-2 primers (Sigma Genosys), 200 µM of each dNTP (Sigma Aldrich) and 5 U of RedTaq DNA Polymerase recombinant (Sigma Aldrich). Each reaction contained a single dried and washed 2 mm punch from the relevant blood sample stored on a Whatman FTA card. One positive and one negative control were included with each PCR run (See earlier this section). 1 µl of *T. congolense* (*Savannah*) (IL1180) control DNA was added in the positive control reaction. The thermal cycling conditions involved and initial denaturation step at 94 °C for 3 min followed by 30 cycles at 94 °C for 1 min, 55 °C for 2 min and 72 °C for 2 min and a final extension step at 72 °C for 5 min.

3.3 Data storage and Management

Village and farmer details, veterinary treatments, laboratory results and individual animal data were entered and stored in Microsoft Access (2003). Data files were screened for proper coding, missing results and data entry mistakes. Datasets for analysis were then exported to Microsoft Excel (2003) and saved as a spreadsheet in a ".csv" file, or as a ".sdd" file. Both R 1.8.1 (Ihaka *et al.*, 1996) and S-plus 2000 (Insightful Corporation, Seattle, WA) were used for analysis of data depending on the applications required.

A summary of the variables recorded during clinical examinations conducted at main visit rounds is given in Table 3.2. The coding of the variables during data entering is also shown. Animals were considered anaemic if haemoglobin concentration was < 8 g/dl (direct indication) and/or if PCV was < 25% (indirect indication); (Schalm *et al.*, 1975). Animals with a temperature > 39.4 ^{o}C were considered to have fever (Minjauw *et al*., 1998a; Maloo *et al*., 2001a).

3.4 Data Analysis

A combination of classical tests, generalised linear modelling, linear mixed-effects modelling, generalised linear mixed-effects modelling (via Penalised Quasi-Likelihood estimation method [PQL])

and Cox regression analysis, has been used to address specific objectives in this thesis, in either one or several chapters. A general description of these statistical techniques follows.

Table 3.2 Variable coding for parameters collected from individual ear-tagged cattle at each round of observation.

Clinical examination	Variable levels
Lymph node enlargement	0 = Normal / 1 = Enlarged
Parotid	"
Prefemoral	"
Prescapular	"
Prescapular lymph node size	cm
Condition score	From 1 (- L) to 9 (+ F)
Weight	Kg
Coat Condition	0 = Normal / 1 = Rough or Staring
Skin lesions	A (None) / B (Light) / C (Mild) / D (Severe)
Colour of mucous membranes	0 = Normal / 1 = Pale
Oral	"
Vulval	"
Conjunctival	"
Discharge	A (None) / B (Light) / C (Mild) / D (Severe)
Vaginal	"
Ocular	"
Nasal	"
Oculo - Nasal	"
Diarrhoea	"
Rectal temperature	ºC
	0 = Normal / 1 = Fever (> 39.4ºC)
Packed cell volume (PCV)	%
	0 = Normal / 1 = Low (< 25%)
Haemoglobin	g / dl
	0 = Normal / 1 = Anaemic (< 8 g / dl)
Owner's health assessment	A (Healthy) / B (Poor) / C (Sick) / D (Severely sick)
Veterinarian's health assessment	"
Louse	A (None) / B (Light) / C (Mild) / D (Severe)
Adult tick stages / HBS	0 (None) / 1 (1 – 10) / 2 (11 – 50) / 3 (> 50)
Rhipicephalus appendiculatus	"
Bhoophilus decoloratus	"
Amblyomma variegatum	"

HBS = Half body Surface

Specific methodology details are given in the results chapters. Materials and methods for mathematical modelling are contained in Chapter 8.

3.4.1 Graphical Presentation – Transformed Tick Counts

A transformed tick count (TC) was constructed to simplify presentation of tick data, which was collected as a "tick score" comprising four categories. Transformed counts (See formula below) proved useful for reflecting, at the population level, both the proportion of tick-positive animals and the intensity of tick infestations from individual cattle. Accurate representation of tick data required different 'weights' to be applied to each of the tick scores, so that the higher tick categories received a greater weight than the lower tick scores. The weights chosen (See formula) were a result of comparing different types of weighting (including a weight derived from the median value of each tick score) in a Monte-Carlo simulation where populations of cattle with known numbers of ticks were generated and assigned the tick scores used in this study. The difficulty of using a weight derived from the median value of each tick score was in deciding the weight to apply to the category 50+. The formula shown below was found to be the best measure of temporal "vector-challenge" based on the results of the Monte-Carlo simulation:

$$TC = (a + 2b + 3c) * P$$

Where: TC = Transformed Count; a = Number of animals with a tick score of 1 - 10 ticks; b = Number of animals with a tick score of 11 - 50 ticks; c = Number of animals with a tick score of > 50 ticks; P = Proportion of animals presenting with ticks (Between 0 and 1).

3.4.2 Classical Tests

Classical tests conducted in this thesis included Chi-square, Fisher's exact tests, and Kolmogorov-Smirnov tests.

For a contingency table (x) specified as x <-matrix(c(a, c, b, d), nrow=2),

a	b
c	d

Chi-square tests (Chisq) were conducted using the function "chisq.test(x)", for as long as the expected value for a, c, b, d was > five and the total sample size considered in the contingency table (a+c+b+d)

was >30. In cases where these criteria were not met, the function “fisher.test(x)” was used instead. In this case, the contingency table (x) was constructed as: “x <-as.matrix(c(a, c, b, d))” followed by “dim(x)<-c(2,2)”. Odds ratios were calculated as OR = ad/bc, and the corresponding 95% confidence intervals as:

$$\log OR-(1.96*SE[\log OR]) \text{ to } \log OR+(1.96*SE[\log OR])$$

Where: SE = standard error. SE[log*OR*] was calculated as the square root of (1/a +1/c+1/b+1/d); (Altman, 1991).

Two-sample Kolmogorov-Smirnov tests were used for comparisons of distributions obtained from two sets of data (say “Data-1” and “Data-2”) using the function “ks.gof(Data-1, Data-2)”.

3.4.3 Generalised Linear Modelling

Generalised linear modelling (GLM) was used in cases of non-constant variance and non-normal distribution of errors, where repeated measures from individual cattle were averaged out. Broadly speaking, two types of GLMs have been used throughout the thesis depending on the nature of the response variable: (i) GLMs for proportion data, (ii) GLMs for count data.

The directive to fit a GLM in S-plus or R is “glm”. GLMs have in addition three important properties as follows: The error structure, the linear predictor and the link function. In GLMs for proportion or count data the error structure is defined by means of the “family” directive as:

Glm (y ~ z, family = binomial) Proportion Data.

Glm (y ~ z, family = poisson) Count Data.

Hence, where the response variable (y) is a proportion, the model should assume binomial error structure whereas where the response variable (y) is a count, poisson errors should be assumed instead. In the either case, the independent variable(s) may be all categorical, all continuous or a combination of both, in which cases the analyses are termed as follows:

Independent Variable(s) - Z	Binomial models with proportion data	Poisson models with binomial data
All categorical	Analysis of Deviance	Contingency Table
All continuous	Logistic Regression	Log-Linear Regression
Categorical and continuous	Analysis of Covariance	Ancova

In GLM models with proportion data, “y” is a two-vector response variable where the number of failures and successes are specified as y<-cbind(successes, total-successes). This method presents several advantages with respect to the modelling of percentage data, in that it takes into account the size of the binomial denominator (i.e. total sample size) from which the proportion is obtained. This property is termed “weighted regression”, given that individual sample sizes are considered in the analysis as weights.

In GLM, regression is not carried out on the response “y” but on a linearised version of the link function applied to “y”. GLMs extend linear models to accommodate transformations to linearity, by relating each observed “y” value to a predicted value obtained by transformation of the value emerging from the linear predictor (η), defined as the linear sum of the effects of one or more explanatory variables. GLMs with binomial errors use (by default) the “logit” link function to ensure that a linear relationship between the values of the response variable (y) and the linear predictor (η) is attained. The alternative link function “probit” may also be specified during data analysis. GLMs with poisson errors use (by default) the “log” link function to ensure a linear relationship between “y” and “η”. Link “sqrt” may alternatively be specified where it represents an improvement over the default model. Finally, since explicit expressions for the maximum likelihood estimators are not usually available in GLM, parameter estimation is iterative, weighted least squares (McCullagh *et al.*, 1989). In GLM parameter estimates obtained from the summary(model) directive represent differences between means. In addition, coefficients of models with binomial errors are given in “logits”, and hence require a process of back-transformation where “proportion” estimates are preferred. Back-transformations can be conducted automatically by applying the directive predict(model, type=”response”). Similarly, coefficients in

models with poisson errors are log-transformed. For obtaining differences between mean "counts" rather than mean "log(counts)", taking the antilogs is necessary.

3.4.3.1 Overdispersion, Hypothesis Testing and Model Criticism

There are two types of terms in statistical models: main terms (i.e. each of the explanatory variables in the model) and interaction terms (i.e interactions between main terms in the model). The notations to specify the inclusion of main terms, interaction terms or both are as follows:

y ~ a + b Consideration of main terms only.

y ~ a+ b+ [a : b] Consideration of main terms (+) and their interaction.

In S-plus and R, the latter model may also be specified as y ~ a*b, where '*' = a + b + [a : b].

In GLM, the assessment of the significance of main and interaction tests is carried out through deletion tests in step-down selection procedures. Hypothesis testing is then conducted on the basis of chi-square (Chisq), which represents the increase in scaled deviance that results from removal of the term of interest from the current model. This is carried out using the "anova" function as follows: "anova(model1, model2, test="Chi")". At each step of model simplification, the residual scaled deviance of the model (a) should be approximately equal to the model's residual degrees of freedom (b). In cases where the ratio a/b is >1, then the assumption that the dispersion (or scale) parameter of the model equals one, does not hold. In S-plus hypothesis testing, in cases of "overdispersion", is conducted on the basis of F-tests by specifying "anova(model1, model2, test="F")". In R, however, correction for overdispersion requires in addition specifying a different family for binomial and poisson errors: "family=quasibinomial" and "family=quasipoisson" respectively. The F-test uses the empirical scale parameter as an estimate equivalent to the error variance, and performs a test much more strict than the Chisq-test. Throughout this thesis, the dispersion (scale) parameter was taken to be different from one (i.e. overdispersion) when the a/b ratio was >1.3.(Crawley, 2002b, 2002c). In models with poisson errors, the possibility that data followed a negative binomial distribution in cases where the dispersion parameter was different from one, was also explored (Crawley, 2002c). It should be noted

that in GLM, underdispersion (a/b < 1) is also possible. The procedure to account for underdispersion during hypothesis testing is as explained in the case of a/b > 1.

A comprehensive introduction to GLMs is given by Firth (Firth, 1991) and by McCullagh and Nedler (McCullagh *et al.*, 1989). A wide range of variations can be applied to the basic model specifications described above, including the use of alternative families, link functions, variance functions and variable transformations. Alternative model specifications can in addition be compared to guide the selection of models best fitted to the data of interest. Finally, model criticism was conducted by assessing the distribution and trends of standarised and pearson residuals. Application of GLMs using S-plus or R software was in all cases as recommended by Crawley (Crawley, 2002b, 2002c) and Venables and Ripley (Venables *et al.*, 2002).

3.4.4 Linear Mixed-Effects (LME) Models

Mixed effect models provide a flexible and powerful tool for the analysis of grouped-data in cases where there is temporal and/or spatial pseudo-replication. Pseudo-replication implies that the observations are correlated within a group (whatever the grouping factor is), thus contravening one of the fundamental assumptions of standard statistical models: independence of errors. Mixed models take care of the non-independence of errors by modelling the covariance structure (within-group correlation) introduced by the grouping of the data, when the latter are specified as random-effects. They are also able to handle balanced and unbalanced data. Finally, they allow, by specifying appropriate nesting and grouping of data, for spatial autocorrelation between geographical areas, temporal correlation across repeated measures on the same individuals and across individuals within a particular area.

Mixed models are so called because the explanatory variables are a mixture of fixed-effects and random-effects, where the former influence the mean of the response variable and the latter its variance. Fixed-effects are used to estimate the influence of particular factors in the population, but it is only the random-effects that confer a predictive value for that estimation. The major benefit from a

random-effects model, is that it economises on the number of degrees of freedom used up by the factor levels.

The class of mixed-effects models which assume that both the random-effects and the errors follow gaussian (normal) distributions, and which are suitable for grouped data in which the response variable is continuous, are named linear (LME) or non linear (NLME) mixed models. LME are empirical models based only on the observed relationship between the response and the covariates and do not include any theoretical considerations about the underlying mechanism producing the data. In LME models, both the fixed- and the random-effects occur linearly in the model function. The general formulation for LME, was proposed by (Laird *et al.*, 1982). NLME models, in contrast, are often mechanistic. The parameters generally have a natural physical interpretation and are based on a model for the mechanism producing the response, which depends non linearly upon the parameters. LME and NLME have nevertheless far more similarities than differences. For the purposes of this thesis only LME models have been used. The directive to fit a LME in S-plus or R is "lme" from the nlme library (Pinheiro *et al.*, 2000). A comprehensive treatment of the theory of linear mixed-effect models can be found in (Searle *et al.*, 1992) and (Vonesh *et al.*, 1997).

The two general methods for parameter estimation in LME models are maximum likelihood (ML) and restricted maximum likelihood (REML). Maximum likelihood estimates of variance components tend to underestimate these parameters. In consequence, many analyses prefer the restricted (or residual) maximum likelihood (REML) estimates (Patterson *et al.*, 1971, Harville, 1977). In R, optimisation of the profiled log-likelihood or the profiled log-restricted-likelihood of an LME model is accomplished by an hybrid optimisation scheme involving both EM - an algorithm-type - iterations (Dempster *et al.*, 1977) and Newton-Raphson iterations (Thisted, 1988). Asymptotic results on the approximate distribution of the ML and REML estimators are then used to produce hypothesis tests and confidence intervals for the LME parameters (Laha *et al.*, 1967).

3.4.4.1 Random-Effects

The most appropriate grouped-data structure was selected by comparing models fit by REML (containing identical fixed-effects) where the levels of grouping - random-effects - differed. Comparison of models containing different random-effects was conducted through likelihood ratio (LR) tests (Lehmann, 1986). Briefly, models associated to a significantly lower Akaike Information Criterion (AIC) (Sakamoto *et al.*, 1986) and Bayesian Information Criterion (BIC) (Schwarz, 1978), according to the LR-test statistic, were preferred. In cases where specification of a more complex grouping-data structure did not improve significantly the initial model fit, the simpler model - containing a more basic grouping-data structure - was preferred.

The grouped-data structures tested in all cases were as follows:

Table 3.3 Random-effects tested in all LME and GLMM objects.

Random-Effects: Interpretation
random=~1|Animal Villages have a common intercept. Animals within villages have different intercepts. Villages and Animals within villages have a common time slope.
random=~Time|Animal Villages have a common intercept. Animals within villages have different intercepts. Time slope is common for all Villages. Animals within villages have different time slopes.
random=~1|Village/Animal Villages and Animals within villages have different intercepts. Villages and Animals within villages have common time slopes.
random=list (Village=~1,Animal=~Time) Villages and Animals within villages have different intercepts. Time slope is common for all Villages. Animals within villages have different time slopes.
random=list(Village=~Time,Animal=~1) Villages and Animals within villages have different intercepts. Villages have different time slopes. Time slope is common for all animals within a village.
random=list (Village=~Time,Animal=~Time) Villages and Animals within villages have different intercepts. Villages and Animals within villages have different time slopes.

Time, was included in the random-effects as an integer-valued variable that indexed the serial observations in 18 consecutive points (from 0 to 17).

The within-group error covariance structure can be flexibly modelled by combining correlation structures and variance functions. Correlation structures are associated with temporal and spatial dependence. The variance function structures are used to model heteroscedasticity in the within-group errors. In R and S-plus the optional arguments "correlation" and "weights" are used for specifying a correlation or a variance function respectively. By default, the within-group errors are assumed to be independent and homoscedastic. Throughout this thesis emphasis is placed at evaluating the influence of particular factors on the population (i.e. fixed-effects) whilst accounting for pseudo-replication. However, simple serial correlations including AR (autoregressive) were tested in models where inclusion of time in the random-effects represented an improvement over the simpler model (i.e. no different time slopes per animal or village). Whether inclusion of a particular correlation structure improved the model where no temporal correlation was assumed, was tested using LR-tests as described above. Serial correlation structures in the context of LME are described in detail in Jones (Jones, 1993). In all cases, selection of the models was as recommended by Pinheiro and Bates (Pinheiro *et al.*, 2000). Upon inspection of the residuals, heteroscedasticity was modelled using the function "VarIdent" when necessary. Whether inclusion of the "VarIdent" function –which allows different variances per level of a factor- improved the initial model (assumed as homoscedastic by default) was again tested by means of a LR-test comparing both models (See above).

3.4.4.2 Fixed-effects – Hypothesis Testing

LME models which differ in fixed-effects specifications can be compared through likelihood ratio tests following the same procedure as described above, only if both are fit by ML (Pinheiro and Bates, 2000). Through this process, the minimum model (that containing only significant terms) can be achieved by adding new terms or by removing non-significant terms from the initial full model. Likelihood ratio tests for achieving the minimum model are nevertheless not recommended by several analysts (Pinheiro and Bates, 2000) due to their "anticonservative" nature. This anticonservative nature is attributed to the fact that as the number of parameters being removed from the fixed-effects

becomes large, compared to the total number of observations, the inaccuracy of the p-values associated to the LR-test statistic can be substantial.

A better approach to performing hypothesis tests involving terms in the fixed-effects specification is to condition on the estimates of the random-effects variance-covariance parameters. For a fixed value of these, the conditional estimates of the fixed-effects are determined as standard least-square estimates. The approximate distribution of the ML or REML estimates of the fixed-effects is exact for the conditional estimates of the fixed-effects. Conditional tests for the significance of a term in the fixed-effects specification are then given by the usual F-tests and t-tests for linear regression models based on the REML of the variance. Conditional t-tests are included in the output of the "summary(model)" command applied to LME objects, and they test the marginal significance of each REML estimated coefficient for each variable level in the fixed-effects. The conditional F-tests are implemented in the single-argument form of the "anova(model)" command for fitted LME models, and the test the significance of variables fitted as fixed-effects, which may include several coefficients (depending on the number of levels in the variable).

Order of Explanatory Variables

Any data that are the object of statistical analysis, fall in two different categories: orthogonal and non-orthogonal data. In the first case, all possible explanatory variables are equally represented in the study and there are no missing values. Most commonly, observational studies are based on non-orthogonal data, where (i) the number of observations recorded from each subject varies, (ii) missing values are commonplace and (iii) correlations amongst explanatory variables are the norm. In orthogonal designs, the variation that is attributed to a given factor is constant and does not depend in the order in which that factor is removed from the maximal model. In contrast, with non-orthogonal data, the variance attributable to a given factor does depend upon the order in which the factor is removed from the model. The variation attributed to a factor removed from a null model, will be lower than the variation attributed to that same factor if it is fitted on its own (i.e. added to the null model).

Under all circumstances, the order of independent variables (fixed-effects) was specified following biological reasoning and considering the specific objectives of the analysis.

Contrasts

Main terms

Contrasts are the essence of hypothesis testing and model simplification. They are used to compare means or groups of means in what are known as single degree of freedom comparisons. Through this thesis the default "treatment" contrast of R has been used to specify the type of comparison preferred (also for GLM and glmmPQL). In S-plus, where "treatment" contrast is not the default, "options(contrasts=c("contr.treatment", "contr.poly"))" was specified prior to analysis. In "treatment" contrasts, all the means associated to a particular characteristic of interest are compared to a mean that is considered the standard for the population in the study. Then, in the output of the "summary" command applied to LME objects, the coefficients associated to each of the explanatory variables represent differences between the REML estimated mean for each term in the fixed-effects and the standard mean.

Interaction terms

LME models are based on the assumption that the effects of different explanatory variables are additive (Crawley, 2002). In other words, if factor "A" causes an increase of 1, and factor "B" causes an increase of 0.5, then the addition of factor "A" and factor "B" together will cause an increase of 1.5. If this is not the case then an interaction is said to occur because one way or another, the response to a factor "A" depends on the level of factor "B".

In treatment contrasts, the hypothesis testing in the case of interactions is based on comparing the mean associated to the interaction term, with that expected if the effect of the factors was merely additive. This observation also applies to other statistical models considered in this thesis.

REML Coefficients and Standard Errors

The standard errors (SE) associated to REML estimates are not SE of means, but of the differences between means. The only exception, is the case of standard errors associated to intercepts. This observation also applies to other statistical models considered in this thesis regardless of the method for parameter estimation.

3.4.4.3 Model Checking

Basic distributional assumptions for the mixed-effect models were checked as described by Pinheiro and Bates (2000), using diagnostic plots available in the nlme library. Briefly, these involve normal plots and scatter plots of residuals, fitted values and random-effects. The four basic assumptions assessed were that (i) the within-group errors are independent and identically normally distributed, (ii) the within-group errors are independent of the random-effects, (iii) the random-effects are normally distributed, with mean zero and covariance matrix Ψ (not depending on the group) and, (iv) the random-effects are independent for different groups, except as specified by nesting.

3.4.5 Generalised Linear Mixed-Effects Models Via PQL

Generalised linear mixed-effects models, with either binomial or poisson errors, were used to model binary and count data respectively using the "glmmPQL" directive available at the MASS library of R (Venables *et al.*, 2002). These models use penalised quasi-likelihood (PQL) estimation method (Schall, 1991, Breslow *et al.*, 1993, Wolfinger *et al.*, 1993). PQL can be viewed as an approximation to ML for GLMMs, and hence AIC and BIC information criteria cannot be used to guide model selection. Model selection with regard to grouping-data structure (random-effects) is still unclear. The protocol used involved fitting the simplest possible grouping-data structure and assessing sequentially, whether more complex grouping-data structures improved the fit of the model and resulted in lower log-likelihood values, although the reliability of the latter is doubtful. The significance of the fixed-effect terms was consistently assessed for various models where different random-effects were specified (See Table 3.3). The fixed-effect of interest was considered significant if results were robust amongst the biologically sound models tested (i.e. consisting of different random-effects). In general, unless a clear improvement of the model was apparent, the model containing the simplest grouping-data structure

(i.e. different intercepts for each individual animal) was preferred. Finally, calculation of the percentage variation attributed to each level of grouping, guided the selection of the most adequate random-effects.

The conditional F-tests implemented in the single-argument form of the "anova(model)" command for fitted models, were used to the test the significance of variables fitted as fixed-effects. These, in turn, were used for step-up and step-down selection of variables in the final (minimum) model. In practice, the methodology used was similar to that in LME objects (but see above) or GLM objects with either poisson or binomial errors.

In GlmmPQL with binomial errors, model coefficients representing differences between means were transformed for presentation of odds ratios (OR) and the corresponding 95% confidence intervals as follows:

exp(coefficient)	*OR*
qt(0.975, degrees of freedom)*SE	x
exp(coefficient-x)	Lower 95% confidence interval.
exp(coefficient+x)	Lower 95% confidence interval.

(SE corresponds to the standard error of the coefficient of interest).

3.4.6 Survival Analysis: Cox Proportional Hazards Model

"Survival time data" or "time-to-event" data arises when interest is focused on the time taken for some terminal event to occur. The term "survival", originated in the study and analysis of times to death (that is, survival times) for medical patients diagnosed with some fatal disease. The statistical treatment of survival times, known as survival analysis, is now a well developed field of statistical research and methodology pertaining to modelling and testing hypotheses of time-to-event data for humans as well as animals, or others.

An inherent feature of survival times is that subjects are nearly always followed for varying lengths of time and that the event of interest (also referred to as "terminal event"), is hardly ever observed in all subjects considered in the study. For those individuals where the terminal event is not recorded, all it is

known is that the survival time or time-to-event is longer than the length of observational period. Survival analysis, allows consideration of such individuals in the analysis by referring to each individual as a "censored observation", thus indicating that the period of observation was cut-off before the terminal event occurred.

3.4.6.1 Cox Models

A partially-parametric method for comparing the survival experience of two or more groups is the Cox Proportional Hazards model (Cox, 1972). The Cox model is the most commonly used regression model for survival data in view of assessing the prognostic significance of independent variables (Parmar *et al.*, 1996a). The Cox model offers several advantages with respect to other non-parametric analyses in that it allows (i) modelling survival time as a function of one or multiple independent variables, which may be metric or non-metric in nature, and (ii) modelling non-constant hazard rate, without making any assumption about the underlying distribution of the hazards in different groups, except that the hazards in the groups remain proportional over time (Parmar *et al*., 1996a).

The partially-parametric survival analysis was conducted using the survival5 [1] S-PLUS 2000 Statistical Library entry, produced by Terry Therneau of the Mayo Clinic.

The S-plus function "coxph ()", which implements the partial likelihood method described by Cox (1972), was used to fit the Cox Proportional Hazards model to the dataset:

Model<-(coxph(Surv(Time,Status)~X)

Where: x = explanatory variable of interest; Time = Time to terminal (or censored) event; Status = 0 (Censored) or 1 (Terminal event).

The Cox model assesses the potentially prognostic significance of a set of variables, by establishing comparisons with regards to the hazard ratio (HR) which is associated to each variable or variable level. The hazard ratio in survival analysis is a summary of the difference between two survival curves, representing the reduction in the risk of "terminal event" given a set of circumstances compared to others, over the period of follow-up. Hence, HR may be defined as a form of relative risk.

Survival functions from the Cox models were obtained using the "survfit()" directive.

3.4.6.2 Tied Observations

The S-plus function "*coxph ()* ", which implements the partial likelihood method described by Cox (1972), allows specifying one of three computational approximations (or partial likelihood methods) known as "Breslow", "Efron" and "Exact" These computational approximations are to account for, and handle, the presence of tied observations in the dataset. In the absence of ties, the three methods produce the same likelihood estimation. The Efron approximation was used in the analysis of survival data, in view of its greater accuracy when dealing with tied observations as compared to the Breslow method (which deflates the partial likelihood of the model when ties are present). Efron is also more computationally efficient than the Exact method (for which the computational time is excessive). The Efron partial likelihood method, is the default option of the S-plus "*coxph ()* " function.

3.4.6.3 Proportional Hazards

The Cox proportional hazards model assumes that the distribution of survival times is exponential, so that the hazard rate (or instantaneous event rate) λ, can be estimated by the ratio of the number of events observed divided by the total survival time (Parmar *et al*., 1996a). Under the assumption of the exponential distribution of survival time the hazard rate, λ, remains constant over time, a trait that is referred to as "proportional". The directive "cox.zph()" was used to assess the assumption of proportional hazards.

3.4.6.4 Contrasts, Hypothesis Testing and Variable Selection in the Cox Model

The significance of potentially prognostic variables was assessed through deletion tests using the likelihood ratio – test statistic. In all cases, variable selection was conducted as recommended by Parmer *et al*. (1996). Further details are contained in Chapter 7.

Chapter 4: Descriptive Statistics and Demographics of the Longitudinal Study

4.1 Introduction

The following sections provide a general overview of the characteristics of the longitudinal study in terms of the compliance of farmers, village demographics, cattle recruitment and withdrawal rates, rainfall and temperature patterns, and levels of tick challenge recorded in the study areas. The effects of experimental block treatments and the percentages of animals that required therapeutic (ad hoc) treatments over the course of the study are also described. Following this, the prevalences and geographical distributions of endemic pathogens of African cattle in the areas of interest are presented, along with a brief overview of the main epidemiological features of each pathogen. Emphasis is placed on investigating whether inter-specific pathogen associations can influence pathogen dynamics and shape the pathogen community, and on understanding the role of host factors (i.e. age) in the establishment, if at all, of pathogen interactions. In considering these aspects of the longitudinal study, how veterinary treatments affected infection status data collected during study is also described. Finally, a brief, introductory overview of the morbidity and mortality recorded from these cattle is presented.

4.1.1 Specific Objectives

It is hoped that a thorough description of aspects of the host population, pathogen biology, environmental, exogenous (i.e. drug use) and geographical factors, will enable a better understanding of the fundamental relationships that exist amongst all the variables considered. Such preliminary exploration is necessary to identify variables that will need consideration in later chapters, in order to avoid confounded analysis of the data. The following sections should inform the appropriateness of subsequent statistical methodologies and allow contextualisation and critical appraisal of the conclusions obtained in later chapters.

4.2 Methods

4.2.1 Veterinary Treatments

Two types of veterinary interventions took place during the study: block (experimental) and ad hoc (therapeutic).

A description of the distribution of block treatments across study villages is given in Section 2.3.2.3. Block treatments were given at the first main visit in selected villages, immediately after biological sample collection and the clinical examination of the ear-tagged cattle. Villages where block treatments were not administered were referred to as "*Control Villages*".

Ad hoc treatments were administered to cattle at any point during the study. These treatments were given for therapeutic or prophylactic reasons at each main visit or at the corresponding intermediate visit based on the judgment of the field veterinarian (Section 2.3.2). Ad hoc treatments included long-acting (LA) oxytetracycline (Retadoxi 20 LA ®), diminazene aceturate (Berenil ® or Veriben ®), albendazole (Valbazen ®), ivermectin (Ivomec ®), topical antiseptics in spray form (various trades) and vitamin supplementation. No ad hoc isometamidium (ISMM) treatments were administered. Ad hoc LA oxytetracycline was administered as explained in the case of experimental treatments (20 mg/kg body weight; Section 2.3.2.3). Diminazene aceturate was given intramuscularly at a dose rate of 7.5 mg/kg body weight, to ensure effectiveness against all Trypanozoon, Duttonella and Nannomonas trypanosomes in addition to Babesia spp. (Uilenberg, 1998). Albendazole was dispensed as a drench paste using automatic guns at a dose of 10mg/kg body weight. This anthelmintic was chosen on the basis of its broad spectrum effect as compared to other generic forms available in the market. Finally, ivermectin (Ivomec ®) was used as a pour-on to control louse or mite infestation at a dose of 500 µg/kg of body weight.

4.2.2 Endemic Pathogens of African Cattle

This chapter considers infection with tsetse-transmitted (*T. brucei*, *T. vivax* and *T. congolense*), tick-transmitted (*T. parva* and other Theileria spp., Anaplasma spp. and Babesia spp.) and pasture-

transmitted (Fasciola spp., "*Strongyle*-type" nematodes) pathogens of African cattle. In the following sections, positive observations of Theileria spp. are those obtained by microscopy that could not be classified as *T. parva* on the basis of the *T. parva*-specific PCR test. Although the terminology used for the classification of nematode infection intensities is as defined by Hansen *et al*. (1994) and Urquhart *et al*. (1996), see Section 3.2.1.5, no assumptions on the clinical manifestations should be drawn from the faecal egg counts reported here.

4.2.3 Clinical Signs

The clinical signs considered in this chapter are those recorded during the clinical examination of cattle as follows: Lymph node enlargement, poor condition score (either L +, L, L -; Section 3.1.2.1), rough or staring coat, pale mucous membranes (indirect indication of anaemia), discharge (vaginal, ocular, nasal, oculo-nasal), diarrhoea, fever (>39.4˚C), low packed cell volume (PCV < 25%; indirect indication of anaemia), anaemia (haemoglobin < 8 g/dl) and presence of skin lesions (i.e. external wounds, fungal or bacterial dermatitis). Other conditions considered are louse infestation and the veterinarian's and farmers' perception of the health of individual animals (i.e. whether animals were categorised as 'sick' or 'severely sick').

4.2.4 Graphical Presentation of Longitudinal Data

Longitudinal presentation of tick data was conducted using a Transformed Tick Count (TC; Section 3.4.1). The cumulative percentages of clinical signs, infection by any of the pathogens and ad hoc treatments administered to cattle, were calculated for each round of observation by dividing the cumulative number of cattle with the condition of interest "*to date*" by the cumulative number of cattle presented "*to date*" since the start of the study. The slopes of the resultant curves provide an indication of the incidence of each condition in the study population. Lines describing cumulative percentages may present small depressions when new cattle are recruited to the study and no additional animals present with the condition of interest. The percentages of cattle with the conditions of interest at each round of observation, were also shown graphically to enable a visual assessment of the implications of block treatments in Busia and Tororo villages.

4.2.5 Statistical Analysis

4.2.5.1 Age-related Distributions of Ad Hoc Treatments, Common Endemic Pathogens and Clinical Signs in the Study Population

The relationship between cattle age and ad hoc veterinary treatment, cattle age and infection status by any of the pathogens of interest, and cattle age and presence of abnormal clinical condition, was investigated using generalised linear mixed-effects modelling via penalised quasi-likelihood estimation method (glmmPQL) and binomial error structure. See Section 3.4.5. In all the analyses, cattle age was fitted as the independent variable with three categorical levels, indicating the age of the animals at each round of observation (either 1-12 [calf], 13-24 [juvenile] or more than 24 [adult] months of age).

Age-related Distributions of Ad Hoc Treatments Administered to Cattle

(Methodology for the Analysis Presented in Table 4.9)

A separate model was fitted to assess the need for ad hoc LA oxytetracycline, diminazene aceturate and albendazole in different cattle age groups. The number of animals and the number of initial and follow-up observations considered in the analyses was limited to the availability of age data. In each case, the dependent variable "Ad Hoc Drug" was coded "1" or "0" depending on whether veterinary treatment had been administered or not at the time of each visit round. The proportion of ad hoc veterinary treatments given to juvenile or adult cattle, was compared to that in calves (reference variable level). In all cases, the relationship between ad hoc veterinary treatments and cattle age was modelled considering different intercepts for each individual animal as random-effects.

Age-related Distributions of Endemic Pathogens

(Methodology for the Analysis Presented in Table 4.13)

A separate model was fitted to assess the age-related distribution of *T. parva*, Theileria spp., Anaplasma spp., Babesia spp., *T. brucei*, *T.vivax*, *T. congolense (Savannah)*, Fasciola spp. and "*Strongyle-type*" nematodes above the detection threshold of the available diagnostic techniques. The number of animals and the number of initial and follow-up observations considered in the analyses

were limited to the availability of age data and microscopy and PCR laboratory results. For the case of trypanosomes, only data obtained from PCR screening of the cattle was considered. This was because mis-speciation of trypanosomes during microscopy diagnosis may occur. In each model, the dependent variable was coded "1" or "0" depending on the presence or absence of the pathogen of interest in the test sample collected at each visit round. In the analyses, the proportion of pathogen-specific positive observations recorded from either juvenile or adult cattle, was compared to that in calves (reference variable level). The age-related distributions of Anaplasma spp., Theileria spp. and Trypanosoma spp. were modelled by considering different intercepts for each individual animal. The relationship between *T. parva* and cattle age by considering different intercepts and time slopes for each village, whereas that of Babesia spp. by allowing separate intercepts for village and for each animal within village. The analyses of the distribution of pasture-transmitted helminths across cattle age groups, considered separate intercepts and time slopes for each individual animal in the study.

In order to validate the results obtained in the analyses described above, the age-related distributions of pathogens were re-assessed by (i) correcting each model by the effect of pathogen species- (or genus-) specific treatments, and by (ii) re-analysing datasets where animals treated with the relevant drug had been removed as follows. *T. parva*, Theileria spp. other than *T. parva* and Anaplasma spp.: LA Oxytetracycline; Babesia spp.: LA oxytetracycline, diminazene or either; nematodes and Fasciola spp.: Albendazole; Trypanosoma spp.: Diminazene, ISMM or either. The analyses corrected by the effect of treatments were specified as follows: 'Pathogen of interest (PI) ~ Treatment (VT)*Cattle Age', where "VT" was a two-level categorical variable indicating, at each round of observation, whether treatment against the pathogen of interest (PI) had been administered.

Age-related Distributions of Clinical Signs

(Methodology for the Analysis Presented in Table 4.18)

Statistical analysis was conducted for clinical conditions with sufficient numbers of abnormal observations. A separate model was fitted to assess the age-related distribution of lymph node enlargement, poor condition score, rough or staring coat, pale mucous membranes, ocular discharge,

diarrhoea, fever (>39.4˚C), low PCV (<25%) and anaemia (haemoglobin < 8g/dl). The number of animals, and the number of initial and follow-up observations considered in the analyses, were limited to the availability of age and clinical data. In each model, the dependent variable was coded "1" or "0" depending on the presence or absence of the clinical sign of interest at each visit round. In the analyses, the proportion of sign-specific positive observations recorded from either juvenile or adult cattle, was compared to that in calves (reference variable level). The age-related distributions of rough/staring coat, pale mucous membranes, fever (>39.4˚C), anaemia (haemoglobin < 8g/dl) and diarrhoea were modelled considering different intercepts for each individual animal. The relationship between ocular discharge and cattle age was investigated by allowing separate intercepts for village, and separate intercepts and time slopes for each animal within village. The analyses of low PCV (<25%) and poor condition, considered separate intercepts and time slopes for each village, and separate intercepts for each animal within each village. Finally, the distribution of lymph node enlargement was modelled by considering separate intercepts for each village, and separate intercepts for each animal within village.

4.2.5.2 Frequency of Ad Hoc Treatments Administered to Cattle in Block Treated as Compared to in Control Villages

(Methodology for the Analysis Presented in Table 4.10)

Contingency tables with poisson errors (GLM; Section 3.4.3) were used to investigate whether the administration of block treatments affected the need for subsequent ad hoc treatments in the study population. A separate model was fitted to evaluate the need for ad hoc LA oxytetracycline, diminazene aceturate and albendazole as follows:

Maximal Model: 'N˚ of Cattle ~ Ad Hoc Treatment + Block Treatment + (Ad Hoc Treatment : Block Treatment)'

Where: 'Ad Hoc Treatment' was a two-level categorical variable ("Treatment Given" or "No Treatment Given") and 'Block Treatment' was a four-level categorical variable ("None", "ISMM", "LA oxytetracycline" or "ISMM plus LA oxytetracycline").

The dependent variable consisted of the number of cattle falling into each category (i.e. cross-tabulation between the need for ad hoc treatment [Yes/No] and the type of block treatment in each study area [None, ISMM, LA oxytetracycline or both]).

Block treatments affected the need for subsequent drug-specific ad hoc treatments where the interaction term '*Ad Hoc Treatment : Block Treatment*' was significant. The significance of the interaction term was assessed through a deletion test comparing the saturated model containing the two main terms and their interaction, with a reduced model containing only main terms. See Section 3.4.3.1. A classic Chi-square test of association (Section 3.4.2), was used in cases where the need for drug-specific ad hoc treatments was investigated across two, rather than four, levels of the "block treatment" variable. All analyses included only those animals which were present at the time of the first main visits and were given experimental (block) treatments in areas where these were administered. The analyses were then repeated by including, in addition, cattle that were recruited at the time of follow-up visits, and had not received experimental treatments.

4.2.5.3 Pathogen Associations

(Methodology for the Analysis Presented in Tables 4.14 & 4.15)

Pathogens influencing the distribution of a blood-borne pathogen of interest above the detection threshold of the diagnostic techniques, were investigated using glmmPQL with binomial errors (Section 3.4.5). A separate model was fitted to identify pathogens affecting the distribution of *T. brucei*, *T. vivax, T. congolense (Savannah)*, *T. parva*, Anaplasma spp. and Babesia spp. in different cattle age groups as follows:

Minimum Model: 'PI ~ Cattle Age + X_1 …+ X_n + (interaction terms amongst every two and every three main terms)'

Where: 'PI' was the binomial dependent variable indicating whether the blood-borne pathogen of interest was present (0) or absent (1) in each test sample. 'Cattle Age' was a three-level categorical variable indicating the age of the animals at each round of observation (1-12 [Calf], 13 – 24 [Juvenile] or >24 months of age [Adult]). 'X_1 to X_n' were pathogens other than the pathogen of interest (PI) that

had a consistently significant effect on the distribution of 'PI' in several exploratory analyses. 'X_1 to X_n' were two-level categorical variables with levels 'X Present' and 'X Absent'; only in the case of nematodes, 'X' consisted of a three-level categorical variable reflecting infection, and infection intensities, as follows: 'Negative', '≤ 400 eggs^{-1} faeces' and '>400 eggs^{-1} faeces'. *R. appendiculatus* scores recorded from individual cattle at each round of observation were also taken into account in the analysis of *T. parva*. Tick scores were specified as a four-level independent categorical variable with levels '0', '1- 10', '11-50' or '>50' *R. appendiculatus* / HBS.

Minimum models were achieved through a combination of procedures. Firstly, selection of main terms in each model involved the individual assessment of potentially significant variables, and both step-up and step-down selection of variables in models with no interactions. Secondly, only those variables that had a significant effect in all approaches were considered in the final analyses, which tested the significance of interaction terms amongst every two and every three significant variables. Selection of interaction terms for each minimum model, was carried out through both step-down and step-up selection procedures. After inclusion of interaction terms, main terms involved in such interactions may no longer be significant, but are necessarily retained in the minimum models. Cattle age was always specified as the first variable in the model, whilst pathogens ('X_1 …+ X_n') other than that of interest, were included by order of prevalence in the study areas (i.e. from highest to lowest).

The number of animals, and hence the number of observations considered in the analyses, were limited to the availability of data for the terms considered in each model. For the case of trypanosomes, only data obtained from PCR testing of samples was considered. This was because mis-speciation of trypanosomes during microscopy diagnosis may occur. Theileria spp. by microscopy probably represents a heterogeneous group of species that differ in the way they relate to the pathogens of interest; for simplicity, Theileria spp. was not considered in the analyses.

In all cases, 'PI' was modelled considering different intercepts for each individual animal in the study. Anaplasma spp. was modelled by considering in addition different time slopes for each individual animal.

4.2.5.4 Effect of Veterinary Interventions on Species-Specific and Genus-Specific Infection Status

(Methodology for the Analysis Presented in Table 4.16)

Transition models were used to assess veterinary treatments as protective factors against incident or persistent pathogen species-specific infections in cattle. Longitudinal data was analysed using glmmPQL with binomial error structure. The maximal model was specified as follows:

$$\text{Maximal Model: } `IS_{(t+1)} \sim IS_{(t)} + VT_{([t] \rightarrow [t+1])} + (IS_{(t)} : VT_{([t] \rightarrow [t+1])})'$$

Where: '$IS_{(t)}$' is the infection status for each individual at the current round of observation. '$IS_{(t+1)}$' is the infection status of each individual at the following round of observation (i.e. 28 days later). '$VT_{([t] \rightarrow [t+1])}$' indicates whether veterinary treatment was given at any time between two consecutive observations (i.e. either at the main or intermediate visit). '$IS_{(t-1)} : VT_{([t-1] \rightarrow (t))}$' is the interaction term between infection status at the current round of observation, followed - or not - by species-specific treatment.

The dependent variable was coded "1" or "0" depending on the presence or absence of the pathogen of interest, obtained from either microscopy or PCR testing of animals in the study. The independent variables were specified as two-level categorical variables. The effect of LA oxytetracycline, diminazene aceturate and albendazole on target pathogen species, was evaluated in nine separate models as follows: LA oxytetracycline (dispensed either at the time of block interventions or ad hoc) on (1) *T. parva*, (2) Theileria spp. other than *T. parva*, (3) Anaplasma spp. and (4) Babesia spp. Diminazene aceturate on (1) Babesia spp. and Trypanosoma spp. obtained by either (2) PCR or (3) microscopy, and albendazole on (1) Fasciola spp. and (2) "*Strongyle*-type" nematodes. The effect of ISMM chloride was not evaluated by means of transition models as a result that this drug was only given for experimental purposes at the start of the study period. The sample size for analysis was limited by the availability of complete parasitological and PCR data from observations inter-spaced by no longer than one visit round (28 days). Due to the small number of observations available for analysis when only samples with PCR data for all three Trypanosoma spp. were included, the analysis was also conducted by including samples with PCR data for at least one of the three Trypanosoma

spp. The effect of trypanocidal treatments on incident or persistent Trypanosoma spp. infections was not extended to the species level, due to the insufficient number of observations with infection status data for either Trypanosome spp. inter-spaced by no longer than 28 days. The minimum model containing only significant terms, was obtained through a process of step-down simplification of the maximum model. The sample size available for analysis increased as non-significant terms were removed from the model. This was achieved by specifying "na.action=na.omit", which allowed consideration of incomplete datasets during the process of model simplification. In the analyses, the probability that currently positive cattle are infected on the following month was compared to that in currently negative cattle (reference variable level). Similarly, the probability that currently treated cattle are infected on the following month was compared to that in currently untreated cattle (reference variable level). In all analyses, the interaction term ($IS_{(t)}$: $VT_{([t] \rightarrow [t+1])}$) provided information on whether a drug of interest had a different effect with regards to preventing new infections or curing existing ones (significant interaction), or on the contrary, the drug had a similar preventive and curative effect (no significant interaction). In all cases – except for those specified otherwise - the relationship between drug treatment and infection status on the following visit round, was modelled considering different intercepts for each individual animal as random-effects. The relationship between albendazole and the subsequent infection status by either Fasciola spp. or nematodes (as measured by the presence of eggs in faeces) was modelled considering separate intercepts and time slopes for each individual animal. Finally, the effect of LA oxytetracycline and diminazene on subsequent infection status by Babesia spp. was investigated considering different intercepts for each village, and for each animal within each village.

A limitation of the transition models described above, was that the sample size for analysis was substantially reduced as a result of the requirement for infection status data in observations inter-spaced by no longer than 28 days. For robustness, the analyses were repeated. In these parallel models, infection status data for the pathogen of interest at each round of observation (PI) was fitted as the dependent variable, whilst 'VT' was fitted as the independent variable. 'VT' was a two-level categorical variable indicating whether pathogen species- (or genus-) specific treatment had been

dispensed within the 28 days which preceded the collection of the test sample. These parallel models did not allow testing for whether a drug of interest had a different protective or curative effect, but allowed examination of a larger sample size to test whether a treatment of interest, had reduced the probability of infection by the pathogen of interest above the detection limit of the available diagnostic techniques. The effect of ISMM chloride on Trypanosoma spp. infection status data by PCR or microscopy was also evaluated during parallel analyses.

4.2.5.5 Interpretation of GlmmPQL Analyses

(Interpretation of Tables 4.9, 4.13, 4.14, 4.15, 4.16, & 4.18)

In the GlmmPQL analyses, F-tests evaluate the significance of each independent variable in the model by establishing whether at least one of the levels in the independent variable, is significantly different from the reference level concerning the dependent or response variable. In contrast, T-tests are used to establish the significance of level-specific comparisons with the reference level.

Odds ratios (OR) for the conditions of interest, were calculated from PQL coefficients obtained for each level of the independent variable as compared to the reference (Section 3.4.5). Finally, random-effects indicated the grouping-levels where considerable variation existed in the relationships amongst dependent and independent variables.

4.3 Results

4.3.1 Compliance of Farmers Participating in the Study

A total of 256 farmers participated in the longitudinal study (Table 4.1). Of these, 216 farmers were recruited at the beginning (85 from Busia and 131 from Tororo) whilst 40 farmers were recruited later from contingency lists (17 in Busia and 23 in Tororo). The mean number of animals contributed by each farmer in the study was 3.46, and ranged from 1 to 24 (Table 4.1).

Table 4.1 Total number of farmers and mean number of cattle contributed per farmer in the longitudinal study.

Area	Farmers			Number of Cattle Contributed				
	Initial recruitment	Later recruitment	Total	Mean	Median	Min	Max	SD
BUSIA								
Bunyimini	17	8	25	3.84	2	1	23	5.17
Kubo	33	5	38	2.68	3	1	7	1.54
Nanjeho	25	2	27	4.04	4	1	8	2.26
Sitengo	10	2	12	7.83	4	1	24	7.91
Total	85	17	102	3.93	3	1	24	4.21
TORORO								
Bunghaji	27	12	39	3.36	3	1	13	2.72
Hitunga	25	1	26	3.85	2	1	12	3.63
Magoje	42	5	47	2.7	2	1	16	2.6
Ojelai	37	5	42	3.02	2	1	9	2.25
Total	131	23	154	3.15	2	1	16	2.74
GRAND TOTAL	216	40	256	3.46	2	1	24	3.42

Information on cattle ownership was missing for five animals in Nanjeho, seven animals in Bunghaji and one animal in Ojelai. These animals are not included in the summary statistics listed in the table.

The participation rate of farmers in Busia was higher than that in Tororo (Table 4.2). Of the 102 (85 + 17) farmers recruited in Busia, 76.5% (78) were present in the last visit round of the longitudinal study, whilst of the 154 (131 + 23) farmers recruited in Tororo, only 54.5% (84) were present in the last visit round.

The majority of farmer withdrawals occurred as a result of participants electing to abandon the study (75.5% [71/94]). The field veterinarian reported that the majority of farmers that actively withdrew from the study complained of "*excessive blood sampling*" and were most commonly owners of the healthiest herds (i.e. those receiving few or no veterinary treatments). The second most common reason for farmers' withdrawal was sale of cattle (17% [16/94]). Farmer withdrawals due to loss of all their cattle (i.e. death or slaughter), dowry or other reasons were in contrast relatively uncommon. A description of the round of observation (visit number) at which farmers announced their withdrawal is given in Appendix 4.1 (Busia District) and Appendix 4.2 (Tororo District).

Table 4.2 Description of 94 out of the 256 farmers which left the longitudinal study before the final visit round.

Area	Total Farmers	REASON FOR CATTLE WITHDRAWAL						Loss to Follow-up (%)
		Death	Dowry	Slaughter	Sold	Unmanageable	Voluntary	
BUSIA								
Bunyimini	25	1			1		3	20
Kubo	38				2		9	28.9
Nanjeho	27				1		6	25.9
Sitengo	12				1			8.33
TORORO								
Bunghaji	39		1		2	1	8	30.8
Hitunga	26	1	1		3		7	46.2
Magoje	47	1		1	4		20	55.3
Ojelai	42				2		18	47.6
GRAND TOTAL	256	3	2	1	16	1	71	
PERCENTAGE		1.17	0.78	0.39	6.25	0.39	27.7	36.7

Farmer withdrawals occurred as a result of death, dowry, slaughter, selling or voluntary withdrawal of their cattle, or as a result of their cattle being unmanageable. Information on cattle ownership was missing for five animals in Nanjeho, one animal in Ojelai and seven cattle in Bunghaji. Unidentified owners are not included in the table.

4.3.2 Village Demographics

Farmers in the study were characterised by the traditional keeping of cattle and other livestock under a mixed crop – livestock production system. The total cattle herd size was available for 220 out of 256 participants in the study. The mean number of cattle owned per farmer was relatively low (9.2 cattle), but was higher in Tororo as compared to Busia (11.1 vs. 6.73; Appendix 4.3). In each district, each farmer contributed approximately 31.3% and 61.1% of their total cattle to the longitudinal study respectively. Farmers in the study also kept small numbers of sheep, goats, pigs and poultry.

Out of 231 interviewed farmers, 208 (90%) owned a maximum of 2 acres of land which was used to grow their main food or cash crop (Appendix 4.4). Spraying of crops was common in Tororo, where it was practiced by 56% of the farmers (83 out of 148 interviewed participants), but not in Busia (10.3% [10 out of 97 interviewed farmers]). This resulted from the predominant food/cash crop grown by farmers in each district (Appendix 4.5), which may or may not benefit from the distribution of chemicals to avoid crop pests. District Veterinary officers believed that application of crop pesticides to cattle to control major tick species was common practice in the study areas, although no precise evidence was provided. The main crop types grown by farmers in Tororo were millet (60.1% [89/148]), cotton (21.6%

[32/148]) and maize (14.2% [21/148]). In Busia, in contrast, the main crop type reported by farmers was cassava (93.8% [91/97]).

4.3.3 Disease Control Practices Exercised by Cattle Owners

All farmers in the study kept their cattle under traditional communal grazing management. The animals were typically tied up around the homesteads or kept in bomas at night and grazed on communal pastures during the day (Ganesan *et al.*, 1995, Magona *et al.*, 2000). Calves below 6 months of age, in contrast, remained permanently tied up around homesteads and separated from the dams during the day (Magona *et al.*, 2000).

Farmers considered TBDs as the major factor compromising the health of their cattle. Hence, the majority of the participants (82.4% [202 out of 245 interviewed farmers]) practiced some sort of tick control (Table 4.3).

Table 4.3 Tick control practices reported on final survey (February 2003) of 245 out of 256 farmers enrolled in the longitudinal study.

Area	Farmers	Tick removal	METHODS OF TICK REMOVAL			
			Manual	Spraying	Tick grease	Paraffin
BUSIA						
Bunyimini	25	5	2	3		
Kubo	33	31	9	4	6	11
Nanjeho	27	27	14	3		10
Sitengo	12	7	4		3	
Total	97	70	29	10	9	21
TORORO						
Bunghaji	39	34	34			
Hitunga	23	17	16	1		
Magoje	46	42	42			
Ojelai	40	39	33	5		1
Total	148	132	125	6		1
GRAND TOTAL	245	202	154	16	9	22
PERCENTAGE		82.4	62.9	6.53	3.67	8.98

Tick control was mainly based on traditional practices comprising manual removal of adult tick stages (62.9% [154/245]) and either topical application of paraffin (8.98% [22/254]) or tick grease containing cypermethin (3.67% [9/254]). See Table 4.3. Only 6.53% (16/254) of interviewed farmers sprayed their cattle by means of a hand pump/pressure operated spraying unit, but the dilutions used for the

available acaricides were never specified. Given the unavailability of functional dips in the study areas at the time of the study, none of the farmers practiced acaricide dipping as a measure to control ticks. The practice of tick control was always reported to be on an irregular basis according to tick burden. Farmers in Tororo preferred to remove ticks manually whereas those in Busia favoured paraffin, tick grease or spraying (Table 4.3).

The majority of farmers in the study attempted to control disease in cattle using traditional methods. Of the interviewed farmers, 42% (103/245) claimed to burn cow dung at sunset close to where cattle were confined in order to keep flies away from the animals. Anecdotal evidence suggested, in addition, that a widespread practice was the prompt removal or burning of parotid and/or prescapular superficial lymph nodes from calves when enlarged. Contact between farmers and veterinary officers from the corresponding districts was reported to be very sporadic, and limited to the occasional notification of seriously sick individual cattle.

4.3.4 Data Availability

In total, 640 cattle were ear-tagged at the start of the longitudinal study in Tororo and Busia villages whilst 261 animals were recruited over subsequent visit rounds to replace animals actively or passively withdrawn from the study (Table 4.4). Eight ear-tagged animals were lost to follow-up prior to initial examination, of which three were sold, one was withdrawn voluntarily by the owner and four were withdrawn for unspecified reasons. In consequence, a total of 893 cattle were examined and sampled for at least one visit round during the longitudinal study (Table 4.4).

All cattle in the study were of the East African short-horn Zebu (*Bos indicus*) type except for one bull in Kubo village, which was a Friesian (*Bos taurus*) x Zebu cross breed. This animal was recruited when older than 12 months of age and was sampled over a period of 9 months before it was voluntarily withdrawn from the study by its owner.

Table 4.4 Number and distribution of initial and total cattle recruited in the longitudinal study.

VILLAGES	Cattle tagged before initial visit [a]	Cattle tagged at follow-up visits [b]	Cattle lost to follow-up before initial visit [c]	Total cattle sampled [d]
BUSIA				
Buyimini	80	16		96
Kubo	80	22		102
Nanjeho	80	36	7 [1]	109
Sitengo	80	14		94
Total	320	88		401
TORORO				
Bunghagi	79	59		138
Hitunga	81	19		100
Magoje	80	47		127
Ojelai	80	48	1 [2]	127
Total	320	173		492
GRAND TOTAL	**640**	**261**	**8**	**893**

[1] Ear-tagged at follow-up visits. [2] Ear-tagged before the first village visits. [a] Number of cattle recruited and ear-tagged at the start of the study. [b] Number of cattle recruited and ear-tagged at follow-up visits. [c] Number of cattle recruited and ear-tagged at the start of the study, but which were withdrawn from the study before the initial visit was conducted. [d] Total number of cattle recruited and sampled in the study

Table 4.5 provides the temporal distribution of initial and follow-up observations recorded from calves, and cattle older than 12 months of age, over the course of the study period. An initial observation was that recorded at the time of each animal's recruitment. Recruitment, in turn, could have taken place at the start of the study period or later on, from contingency lists. Follow-up observations were those subsequently recorded from each animal, at visit rounds other than the first (i.e. other than the initial observation).

Overall, 320 animals were recruited as calves (up to 12 months of age) whereas another 563 animals were recruited as older than 12 months (i.e. 320 + 563 initial observations). An initial observation was also recorded from 10 animals for which age was not available. See Table 4.5. With respect to cattle older than 12 months of age, 410 initial observations took place at the time of the first visit in Tororo and Busia villages and 153 at visit rounds other than the than the first (i.e. animals recruited from contingency lists). In the case of calves, 219 initial observations were obtained at the time of the first visit in Tororo and Busia villages whilst a further 101 initial observations took place over the course of

subsequent visits. A total of 9240 observations on cattle were conducted in the study (893 plus 8347 initial and follow-up observations respectively; Table 4.5).

Table 4.5 Number and distribution of initial and follow-up observations on calves (up to 12 months of age) and older cattle (> 12 month of age) presented by visit round at which they were obtained.

Round of observation (Visit Number)	Initial observations on cattle >12 months	Follow-up observations on cattle >12 months	Initial observations on calves	Follow-up observations on calves	Initial observations on cattle of unknown age
1	201	-	108	-	10 [1]
2	1	184		102	
3	1	161	3	86	
4	240 [2]	155	132 [3]	85	
5	13	370	3	206	
6	1	354	4	191	
7	10	348	2	181	
8	33	329	14	174	
9	9	376	5	173	
10		363	1	181	
11	10	359	11	174	
12	13	373	3	188	
13	1	361		177	
14	5	365	8	173	
15	21	363	17	181	
16	4	371	9	181	
17		361		190	
18		335		176	
Totals	**563**	**5528**	**320**	**2819** [4]	**10**

Initial observations on cattle with no recorded age are also presented. [1] No follow-up observations were conducted on these animals. [2] 31 corresponded to observations from cattle in Tororo while 209 to observations from cattle in Busia villages, which were incorporated in the study at visit round 4. [3] 21 corresponded to observations from cattle in Tororo while 111 to observations from cattle in Busia villages, which were incorporated in the study at visit round 4. [4] 51.4% (1448) of calf follow-up observations took place when cattle were older than 12 months of age. Adding up initial observations gives the total number of cattle in the study (n = 893). Adding up initial and follow-up observations gives the total number of observations in the study (n = 9240)

Of the 320 initial calves recruited in the study, 167 were females and 148 were males. Sex was not recorded in the case of 5 animals. Concerning the 563 cattle recruited at ages greater than 12 months, 390 were females whilst 173 were males. Hence, the female to male ratio was greater amongst cattle recruited at ages greater than 12 months as compared to those recruited as calves (2.3 and 1.1 respectively). Sex data was not available for 10 animals for which age was also unknown at the time of recruitment.

Of the 563 animals older than 12 months at the time of recruitment, 230 (56.1%) of the 410 present at the initial village visits, were followed for the complete study period (i.e 15 months in Busia and 18 months in Tororo; Table 4.6). 105 (68.6%) of the 153 animals older than 12 months of age which were enrolled after the initial village visits, were also present at the last round of observation. A total of 228 animals recruited at ages older than 12 months were lost to follow-up before the study ceased.

Table 4.6 Frequency distribution of total number of observations obtained per animal during the longitudinal study, subdivided on the basis of the classification of the animal with respect to age at time of initial observation.

Number of observations	Initial cattle > 12 months	Initial calves [a]	Initial cattle with no recorded age
"0"	-	-	8 [1]
1	22	8	10
2	35	27	
3	26	18	
4	28	22	
5	21	20	
6	22	13	
7	24	14	
8	20	17	
9	13	9	
10	13	17	
11	35	12	
12	17	13	
13	32	16	
14	52	29	
15	111	44	
16 [2]	15	9	
17 [2]	27	10	
18 [2]	50	22	
Totals	563	320	10

[a] Up to 12 months of age. [1] Eight cattle lost to follow-up prior to initial examination. Two of these animals were recruited as calves, whereas for the remaining six animals age records were not available. [2] Given that the longitudinal study lasted for 15 and 18 months in Busia and Tororo respectively, only cattle from the latter district were sampled on more than 15 occasions.

Similarly, of the 320 animals initially identified as calves, 103 (47%) of the 219 present at the time of the initial village visits, were followed for the complete study period (Table 4.6). 73 (72.3%) of the 101 initial calves which were enrolled after the initial village visits, were present at the last round of observation. A total of 144 animals identified as calves at the time of recruitment were lost to follow-up

before the study ceased. With regard to 10 animals for which age was not available at the time of enrolment, loss to follow-up occurred immediately after the initial observation.

The reasons for passive or active follow-up loss in the case of 382 cattle presented in the study (i.e. 228 + 144 + 10 animals as explained earlier) are shown in Table 4.7. 50.7% of these cattle were voluntarily withdrawn by farmers, whilst 30.1% of losses to follow-up occurred as a result of animals being sold. Cattle death accounted for 10.5% of the animal losses throughout the study.

Table 4.7 Description of 382 animals from which an initial observation was recorded in the study and which were subsequently lost to follow-up. Losses are subdivided on the basis of the classification of the animal with respect to age at the time of loss to follow-up.

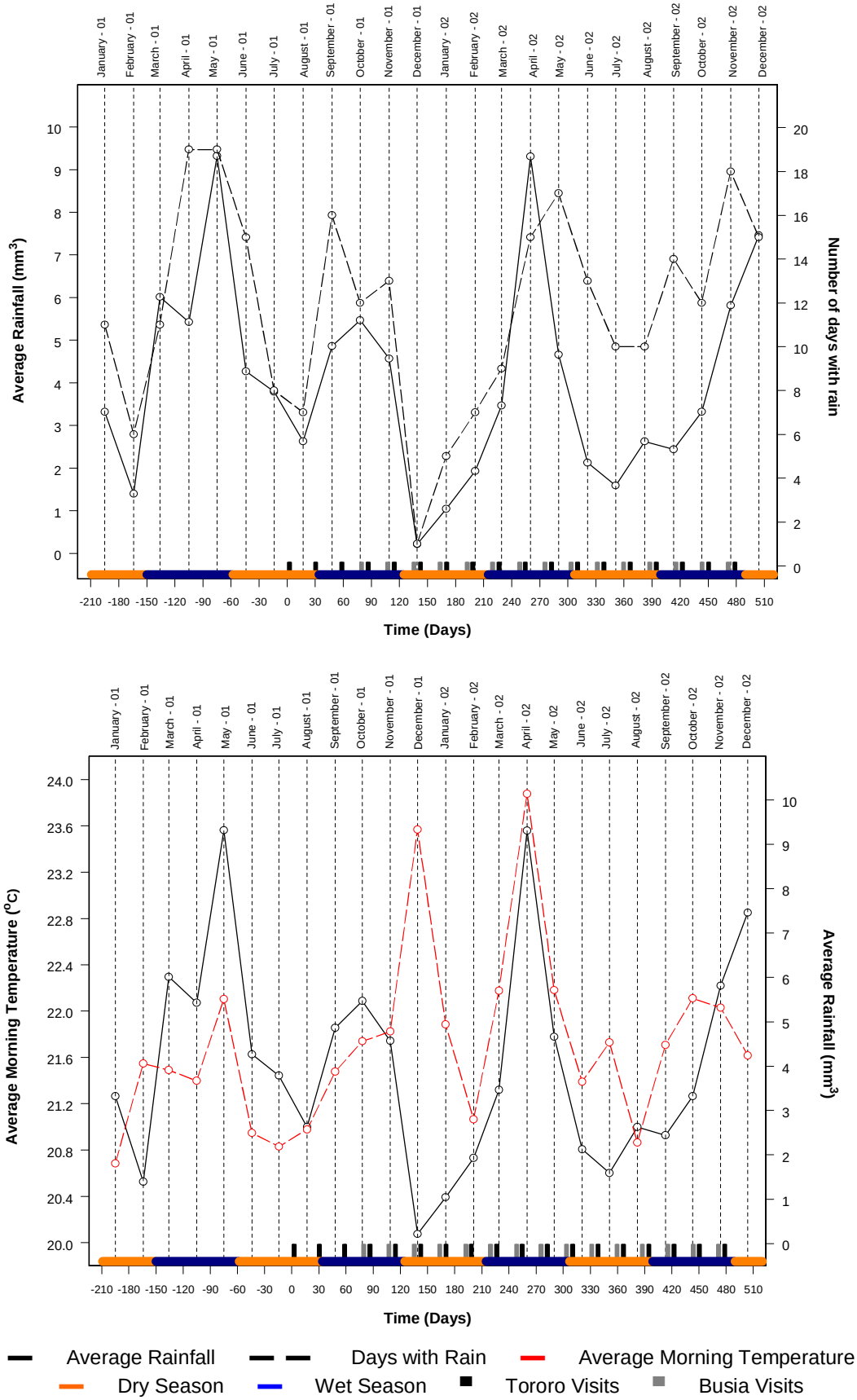
Reason for loss to follow-up	Cattle > 12 months	Calves [a]	Unknown	Total
Death	11	11	18 (1 [2] + 17 [1])	40
Dowry	6	0	11	7
Slaughter	5	0	11	6
Sold	72	11	321	115
Unmanageable	5	0	0	5
Voluntary	131 (129 +2 [1])	16	62 (9 [2]+ 53 [1])	209
Totals	230 (228 +2 [1])	38	114 (10 [2]+ 104 [1])	382

[a] Up to 12 months of age. [1] Animals recruited as calves. [2] Animals with no age record at the time of recruitment. Eight animals were in addition lost to follow-up prior to initial examination (of which three were sold, one was withdrawn voluntarily by the owner and four were unspecified). Two of the three sold animals were identified as calves. Age was not recorded in the case of the remaining six animals.

4.3.5 Seasonal Distribution of Village Visits in Busia and Tororo

The temporal distribution of Tororo and Busia village visits, in relation to dry-rainy seasons in Uganda and 2001-2002 rainfall and temperature data from Tororo Meteorological Station, is presented in Figure 4.1. The duration of the longitudinal study is shown in days and calendar months, rather than by visit rounds. Main visits in Tororo and Busia villages started at day 1 (30 July 2001) and day 78 (15 October 2001) respectively. The last round of observation in Tororo and Busia villages took place at days 480 (21 November 2002) and 473 (14 November 2002) after the start of the study respectively.

Figure 4.1 Temporal distribution of Tororo and Busia village visits, in relation to dry-rainy seasons in Uganda and 2001-2002 rainfall and temperature data from Tororo Meteorological Station.



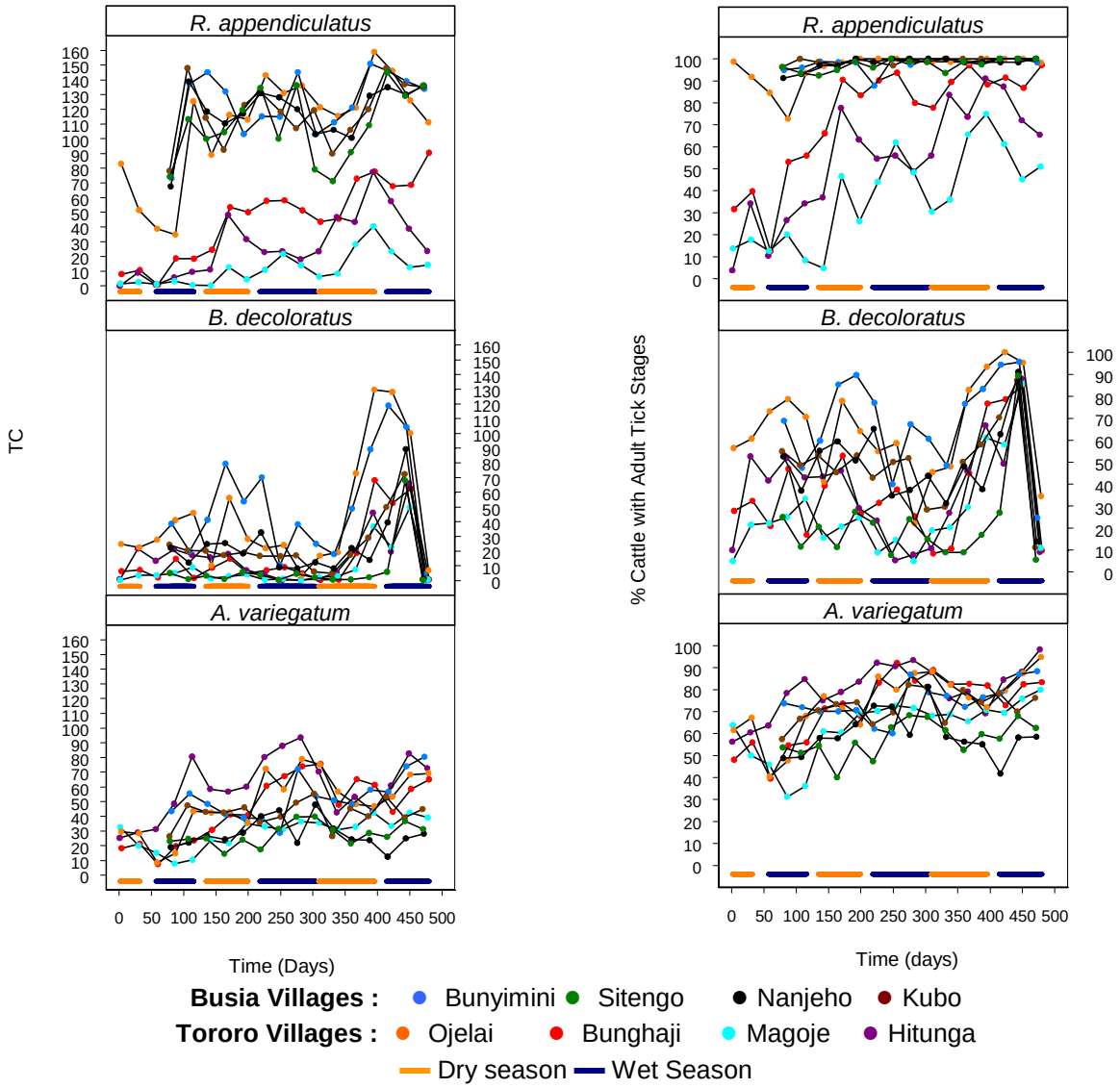
In the figure, calendar month labels are centred at day 15 within each month, whereas annual dry and wet seasons are indicated from **(i)** 1 December – 28 February, dry; **(ii)** 1 March – 31 May, wet; **(iii)** 1 June – 31 August, dry; and **(iv)** 1 September – 30 November, wet. Figure 4.1 is relevant to understanding subsequent figures, where longitudinal data obtained from initial and follow-up examinations on the study population is presented.

4.3.6 Vector Challenge

Over the course of the study, 94% of cattle were at some stage infested with adult *R. appendiculatus* instars on half the body surface (RA/HBS). Adult *A. variegatum* instars were observed in 93.1% of the animals, whereas *B. decoloratus* on 88.5% of cattle in the study. See Appendix 4.6. *R. appendiculatus* was the predominant tick species in Busia district, whilst *A. variegatum* was the most common tick species in Tororo. In either district, *B. decoloratus* was the least frequent tick species recorded from the coat of the animals (Appendix 4.6).

The prevalence of tick-positive cattle and the transformed species-specific tick counts (TC) at each visit round are shown in Figure 4.2 for each village. Several features are apparent upon visual inspection of these data. Firstly, all tick species were recorded from all villages. However, *A. variegatum* and *B.decoloratus* were homogeneously distributed across all areas in the study, whilst *R. appendiculatus* was patchily distributed across villages, both in terms of prevalence of tick-infested cattle and in terms of numbers of adult instars recorded from tick-positive animals. Secondly, visual inspection of the data showed continuous development of adult instars of all tick species all year round. However, seasonal fluctuations of tick infestation incidence and intensity were apparent for the three species monitored. An in depth description of *R. appendiculatus* dynamics in the study areas is given in Chapter 6.

Figure 4.2 Prevalences and transformed counts (TC) of *Rhipicephalus appendiculatus*, *Boophilus decoloratus* and *Amblyomma variegatum* adult stages.



4.3.7 Veterinary Interventions

Overall, 790 (88.5%) out of 893 cattle with an initial observation received veterinary treatment (experimental or ad hoc) throughout the longitudinal study (Table 4.8). Block interventions involving either ISMM, LA oxytetracycline or a combination of both drugs, were given to 639 cattle present at the first main village visit. ISMM block treatment was given to 35.9% (321) of total (893) cattle presented in the study, whilst LA oxytetracycline block treatment was given to 35.8% (320) of the study population.

Table 4.8 Percentages of cattle that received veterinary treatment.

Types of Treatment		Cattle		Observations	
		Treated	%	Treated	%
Block Treatments	Controls [1]	159	17.8	159	1.72
(Initial Village visits)	LA Oxytetracycline [2]	159	17.8	159	1.72
	ISMM [3]	160	17.9	160	1.73
	LA Oxytetracycline + ISMM [4]	161	18	161	1.74
Ad hoc Treatments	Albendazol	509	57	837	9.06
(Initial and Follow-up Village visits)	Diminazene	362	40.5	589	6.37
	LA Oxytetracycline	306	34.3	495	5.36
	Ivermectine	12	1.34	12	0.13
	Vitamin Complex	6	0.67	6	0.06
	Antiseptic Spraying	5	0.56	5	0.05
	Totals	**661**	**74.02**	**1705**	**18.45**
Totals	LA Oxytetracycline	530	59.4	811	8.78
(Block + Ad Hoc Treatments)	ISMM	321	35.9	321	3.47
	Albendazole	509	57	837	9.06
	Diminazene	362	40.5	589	6.37
	Ivermectine	12	1.34	12	0.13
	Vitamin Complex	6	0.67	6	0.06
	Antiseptic Spraying	5	0.56	5	0.05
	Totals	**790**	**88.5**	**2132**	**23.1**

The percentages of initial and follow-up observations (n = 9240) where veterinary treatment was administered, are also shown. The percentages of cattle were calculated considering all 893 animals presented in the study. [1] Corresponds to 80 cattle in Bunyimini (Busia) and 80 cattle in Ojelai (Tororo), which were present at the first round of observation. [2] Corresponds to 80 cattle in Sitengo (Busia) and 79 cattle in Bunghaji (Tororo), which were present at the first round of observation.[3] Corresponds to 80 cattle in Nanjeho (Busia) and 80 cattle in Magoje (Tororo), which were present at the first round of observation. [4] Corresponds to 80 cattle in Kubo (Busia) and 81 cattle in Hitunga (Tororo), which were present at the first round of observation.

With regard to the ad hoc (curative / prophylactic) treatments, ivermectin, vitamin complexes, and topical antiseptics in spray form were given once to twelve, six and five animals respectively (Table 4.8), either alone or combination with other drugs. These treatments were given to 2.6% (23) of the total cattle presented in the study. Ad hoc diminazene aceturate, albendazol, and LA oxytetracycline treatments were administered for therapeutic purposes to a larger number of cattle presented in the study as follows: 40.5% (362), 57% (509) and 34.3% (306) respectively (Table 4.8). Overall 59.4% (530) of the cattle were treated with LA oxytetracycline, either for the purposes of the experimental trial or ad hoc.

4.3.7.1 Age-related Distributions of Ad Hoc Treatments Administered to Cattle

The number of ad hoc treatments given to cattle varied significantly across age groups (Table 4.9).

Ad hoc administration of diminazene was more common at the time of initial or follow-up visits conducted on cattle older than 12 months of age (7.2% vs. 3.4%; Table 4.9). In contrast, LA oxytetracycline was more often administered at the time of initial or follow-up visits conducted on calves as compared to on juvenile or adult cattle (10.9% vs. 4.4%; Table 4.9).

Table 4.9 Age-related distributions of ad hoc treatments administered to cattle.

Ad Hoc drugs	Cattle age (months)	Observations			Model Estimates		F-value	p-value
		Total	Treated	% Treated	OR	95% CI		
LA Oxytetracycline [a]	1 - 12	1281	139	10.9			$F_{2, 7674} = 61.7$	<.0001
	13 - 24	2660	168	6.32	0.61	0.49 - 0.75		
	> 24	4618	152	3.29	0.24	0.18 - 0.31		
Diminazene	1 - 12	1281	44	3.43			$F_{2, 7674} = 22.5$	<.0001
	13 - 24	2660	160	6.02	1.91	1.42 - 2.58		
	> 24	4618	361	7.82	2.75	2.03 - 3.73		
Albendazole	1 - 12	1281	144	11.2			$F_{2, 7674} = 4.35$	0.013
	13 – 24 ▲	2660	248	9.32	0.81	0.65 – 1.01		
	> 24	4618	395	8.55	0.74	0.60 – 0.90		

[a] Excludes block (experimental) treatments. For each model, the number of initial and follow-up observations available for analysis was of 8559 on 883 cattle. Odds ratio (OR) values of less than one indicate that the age group of interest (13 to 24 or > 24 months of age) had a lower probability of needing veterinary treatment as compared to calves (up to 12 months of age). OR values of more than one indicate that the age group of interest had higher probability of needing veterinary treatment as compared to calves. ▲ Highlights cattle age groups where the proportion of treated observations was not significantly different from that in calves, according to the T-test statistics (Data not shown). In such cases, the 95% confidence interval of the OR includes the value "1".

The number of anthelmintic treatments given to calves or juveniles was not different, but was significantly lower amongst adult cattle (>24 months of age) as compared to calves of up to 12 months of age (8.8% vs. 11.2%; Table 4.9).

4.3.7.2 Frequency of Ad Hoc Treatments Administered to Cattle in Block Treated as Compared to in Control Villages

The percentages of cattle needing ad hoc trypanocidal or anthelmintic treatment over the course of the study did not differ in control villages as compared to in ISMM and/or LA oxytetracycline block treated villages (Table 4.10). Although the same was not true concerning the percentage of cattle needing ad hoc LA oxytetracycline (df = 3; Chisq = 11.33; p-value = 0.010; Table 4.10), this did not differ in control villages as compared to in villages where only LA oxytetracycline block treatment was dispensed (df = 1, Chisq = 2.295, p – value = 0.130).

Table 4.10 Percentages of cattle that received ad hoc treatment in block treated villages as compared to in control villages.

Classification of villages on the basis of the initial block treatment	Ad hoc treated cattle								
	LA Oxytetracycline			Diminazene			Albendazole		
	Treated	Total	%	Treated	Total	%	Treated	Total	%
Cattle present at initial main visit only									
Controls [1]	49	159	30.8	56	159	35.2	96	159	60.4
LA Oxytetracycline [2]	37	159	23.3	54	159	34	88	159	55.3
ISMM [3]	63	160	39.4	66	160	41.3	99	160	61.9
LA Oxytetracycline + ISMM [4]	59	161	36.6	72	161	44.7	95	161	59
Totals	208	639	32.6	248	639	38.8	378	639	59.2
Test Statistics	df = 3, Chisq = 11.33 p–value = 0.010			df = 3, Chisq = 5.20 p–value = 0.157			df = 3, Chisq = 1.54 p–value = 0.673		
Cattle present at initial main visit plus cattle recruited at follow-up visits									
Controls [1]	64	223	28.7	80	223	35.9	129	223	57.8
LA Oxytetracycline [2]	77	232	33.2	96	232	41.4	137	232	59.1
ISMM [3]	92	236	39	103	236	43.6	130	236	55.1
LA Oxytetracycline + ISMM [4]	73	202	36.1	83	202	41.1	113	202	55.9
Totals	306	893	34.3	362	893	40.5	509	893	57
Test Statistics	df = 3, Chisq = 5.88 p–value = 0.118			df = 3, Chisq = 3.07 p–value = 0.381			df = 3, Chisq = 0.91 p–value = 0.823		

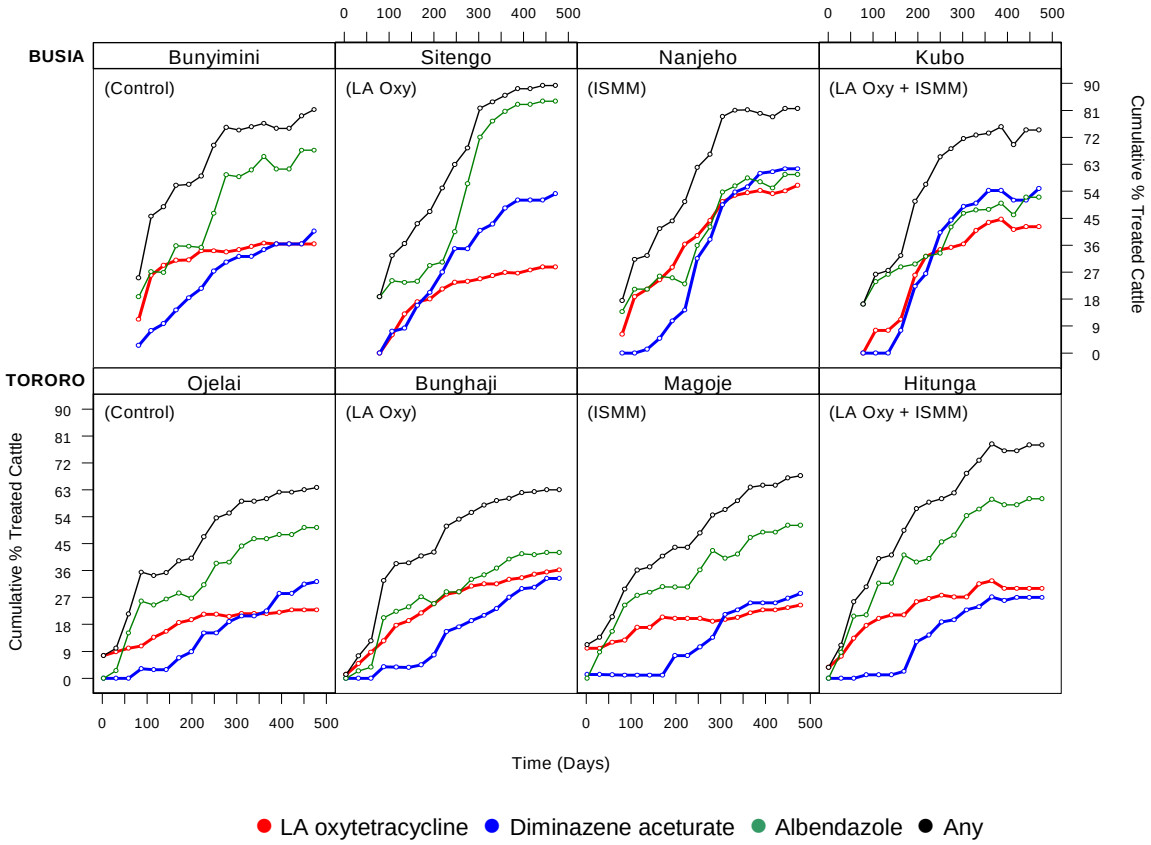
[1] Bunyimini (Busia) and Ojelai (Tororo), [2] Sitengo (Busia) and Bunghaji (Tororo), [3] Nanjeho (Busia) and Magoje (Tororo), [4] Kubo (Busia) and Hitunga (Tororo).

Those cattle receiving ad hoc interventions were given a maximum of five antibiotic treatments and a maximum of five trypanocidal ad hoc treatments over the course of the study. In addition, a maximum of four anthelmintic treatments were given per animal. However, the distribution of number of ad hoc treatments given per animal appeared independent of whether block treatments had been administered, and of the type of drug used in the experimental trials (Appendix 4.7). These observations may be confounded by different block treatments being administered to separate geographical areas, where the level of vector and pathogen challenge may differ substantially.

Figures 4.3 and 4.4 show the frequency of ad hoc treatments administered to cattle in each of the eight villages separately. These figures avoid pooling of data on the basis of the initial block interventions, although the type of block treatment dispensed to each village is still indicated in the panels. Antibiotic and trypanocidal ad hoc treatments were mainly given to cattle in Busia district, irrespective from the

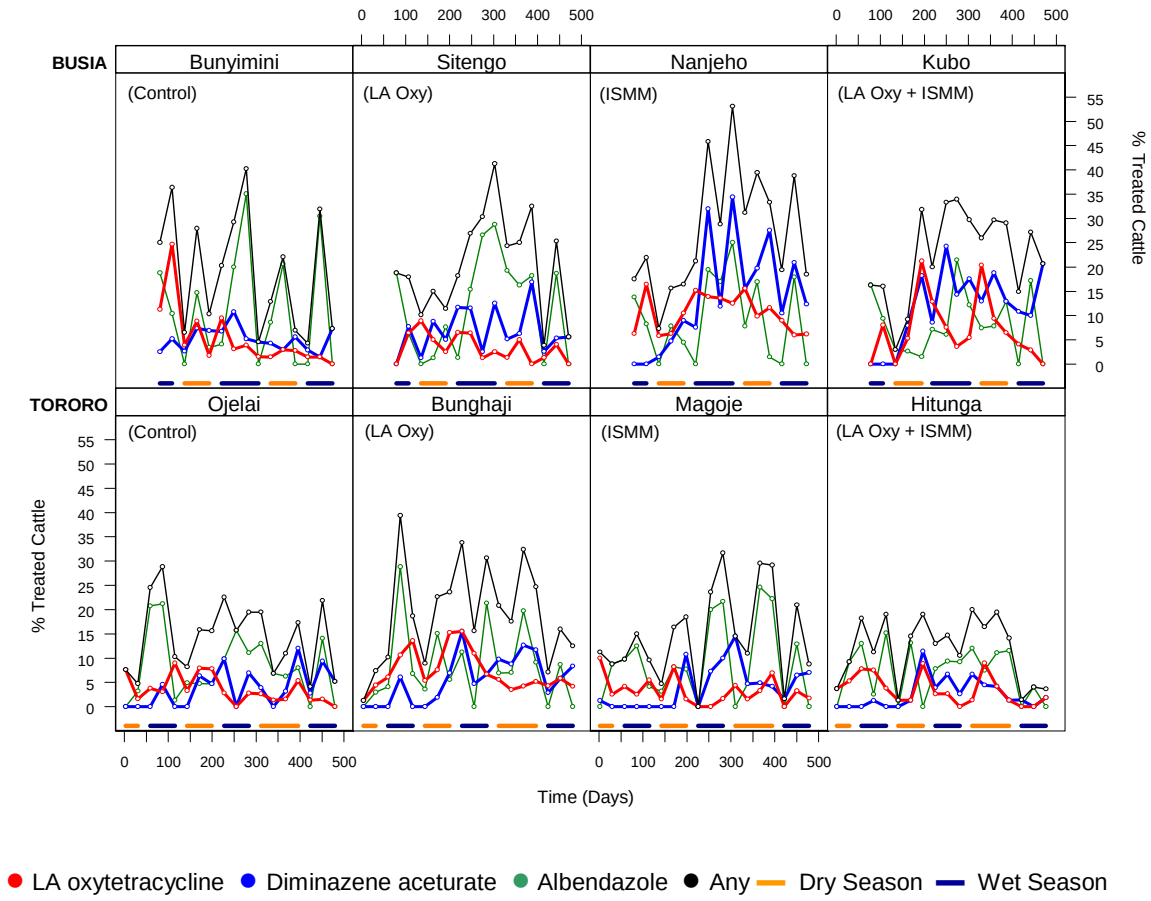
initial block treatment trials (Figure 4.3). Busia villages were in turn geographically closer to Lake Victoria.

Figure 4.3 Cumulative percentages of cattle to which ad hoc treatments were administered.



Notably, trypanocidal ad hoc treatments were not required for six months in ISMM block treated villages from Tororo, and for two months in villages from the same district where no ISMM was given (Figure 4.4). Conversely, trypanocidal support was not required for as short as one to two months in Busia villages where ISMM was dispensed, and was needed as soon as at the first or at the second visit rounds in Busia villages where no ISMM had been given (Figure 4.4). Altogether, there existed heterogeneity in ISMM associated chemoprophylactic effect, depending on the geographical distribution of districts in relation to Lake Victoria.

Figure 4.4 Longitudinal percentages of cattle to which ad hoc treatments were administered.



4.3.8 Prevalences of Tick-Transmitted, Tsetse-Transmitted and Pasture-Transmitted Pathogens

A detailed summary of the prevalence of endemic pathogens obtained from either microscopy or PCR screening of cattle is given in Table 4.11. Over the course of the study, 93.7% of cattle with an initial observation were diagnosed with helminthosis involving either Fasciola spp. or "*Strongyle*-type" nematodes, whereas 90.7% and 61.1% tested positive to tick- and tsetse-transmitted haemo-pathogens respectively. "Strongyle-type" nematodes, Theileria spp. and Anaplasma spp. affected 88.2%, 84.8% and 83.5% of the cattle population respectively, and were the most common pathogens over the course of the longitudinal studies.

Table 4.11 Prevalences of tick-transmitted, tsetse-transmitted and pasture-transmitted pathogens in cattle by either microscopy or PCR.

Pathogens	Diagnostic Technique	Cattle			Observations		
		Positive	Total	%	Positive	Total	%
TICK-TRANSMITTED							
Any Tick-Transmitted Pathogen [1]	PCR + MCR	801	883	**90.7**	2890	8014	**36.1**
T. parva	PCR	281	884	**31.8**	537	8094	**6.63**
Theileria spp.	MCR	757	893	**84.8**	2077	9206	**22.6**
Theileria spp. (but not *T. parva*) [2]	"	733	893	**82.1**	1935	9206	**21**
Anaplasma spp.	"	746	893	**83.5**	2281	9154	**24.9**
Babesia spp.	"	76	893	**8.51**	78	9206	**0.85**
TSETSE-TRANSMITTED							
Trypanosoma spp. [1]	PCR + MCR	487	797	**61.1**	856	3228	**26.5**
T. brucei	"	404	808	**50**	688	6650	**10.3**
T. vivax	"	345	840	**41.1**	448	4001	**11.2**
T. congolense	"	222	835	**26.6**	273	3913	**6.98**
Trypanosoma spp. [1]	PCR	460	798	**57.6**	785	3236	**24.3**
T. brucei	"	385	808	**47.6**	640	6660	**9.61**
T. vivax	"	290	842	**34.4**	366	4013	**9.12**
T. congolense (Savannah)	"	191	836	**22.8**	233	3925	**5.94**
Trypanosoma spp. [1]	MCR	297	893	**33.3**	416	9221	**4.51**
T. brucei	"	92	893	**10.3**	99	9221	**1.07**
T. vivax	"	238	893	**26.7**	320	9221	**3.47**
T. congolense	"	107	893	**12**	120	9221	**1.3**
PASTURE-TRANSMITTED							
Any Helminth [1]	"	834	890	**93.7**	3448	8667	**39.8**
Fasciola spp.	"	529	890	**59.4**	1214	8693	**14**
"Strongyle-type" nematodes	"	786	891	**88.2**	2589	8849	**29.3**
• Sub-clinical [3]	"	404	891	**45.3**	1991	8849	**22.5**
• Clinical [4]	"	382	891	**42.9**	598	8849	**6.76**
• Moderate [5]	"	286	891	**32.1**	474	8849	**5.36**
• Heavy [6]	"	96	891	**10.8**	124	8849	**1.4**
"ANY PATHOGEN" [1]	PCR+MCR	745	772	**96.5**	2209	2923	**75.6**

MCR = Microscopy. [1] Includes observations from which complete parasitological and PCR data were available, and cattle from which complete parasitological and PCR data were available for the case of at least one visit round during the study. [2] Theileria spp. positive observations which could not be classified as *T. parva* on the basis of the PCR test. [3] Up to 400 eggs gram^{-1} faeces. [4] More than 400 eggs gram^{-1} faeces. [5] More than 400 eggs gram^{-1} faeces up to 1000 eggs gram^{-1} faeces. [6] More than 1000 eggs gram^{-1} faeces. The classification of clinical and sub-clinical nematode infection intensities is based on that described by Hansen and Perry (1994), whereas that of moderate and heavy clinical infection intensities, on that described by Urquhart *et al*. (1996).

Clinical nematode infections characterised by intensities of more than 400 eggs g $^{-1}$ faeces (Hansen *et al.*, 1994) were only observed in 42.9% of the cattle, while heavy clinical nematode infection intensities [>1000 eggs g $^{-1}$ faeces (Urquhart *et al.*, 1996)] from only 10.8% of the animals. Fasciola spp., *T.brucei*

and *T. vivax*, were the second most common group of endemic pathogens in the study areas, and were diagnosed from 59.4%, 50% and 41.1% of the cattle population respectively. *T. parva* and *T. congolense* were moderately common, affecting 31.8% and 26.6% of the animals over the course of the study respectively, whereas Babesia spp. was only diagnosed from 8.5% of the cattle population and was therefore the least common pathogen in Busia and Tororo districts.

4.3.8.1 Mixed Pathogen Infections

Notably, mixed pathogen infections were diagnosed in 651 (87.4%) of 745 pathogen-positive animals with complete parasitological and PCR data for at least one visit round during the study (n = 772). See Table 4.12.

Table 4.12 Frequency distributions of the number of pathogens diagnosed from cattle.

Maximum number of pathogens	Cattle			Total number of pathogens	Observations		
	Frequency [3]	%	Cumulative %		Frequency	%	Cumulative %
0	27	3.5	3.5	0	714	24.4	24.4
1	94	12.2	15.7	1	951	32.5	57
2	238	30.8	46.5	2	713	24.4	81.4
3	227	29.4	75.9	3	343	11.7	93.1
4	135	17.5	93.4	4	150	5.13	98.2
5	43	5.57	99	5	44	1.51	100
6	8	1.04	100	6	8	0.27	100
Totals	**772** [2]	**100**			**2923** [1]	**100**	

The table shows the frequency distribution of the 'maximum' number of pathogen species (or genera) diagnosed from cattle, and the frequency distribution of the number of pathogen species (or genera) recorded from initial and follow-up observations. [1] Number of initial and follow-up observations where Anaplasma spp., Theileria spp., Babesia spp., *T. brucei*, *T. vivax*, *T. congolense*, Fasciola spp. and nematodes infection status was assessed by microscopy, and where *T. parva*, *T. brucei*, *T. vivax* and *T. congolense* (*Savannah*) infection status was assessed by PCR. [2] Number of cattle from which all parasitological and PCR data were available for the case of at least one visit round during the longitudinal study. [3] Number of cattle diagnosed with a maximum of either 0 up to 6 pathogen species (or genera) concomitantly (at the same round of observation), over the course of the study.

Only 94 (12.6%) of these 745 positive cattle were consistently diagnosed with a maximum of one pathogen at a time (Table 4.12). Moreover, considering 2923 initial and follow-up observations for which complete parasitological and PCR data were available (Table 4.12), concomitant infections involving two or more pathogens were recorded from a higher proportion of positive observations than single pathogen infections (1258 [56.9%] vs. 951 [43.1%]).

4.3.8.2 Age-Related Distributions of Endemic Pathogens

The probability of infection by any of the pathogens considered in the study was, in most cases, dependent on cattle age (Table 4.13).

Table 4.13 Age-related distributions of tick-transmitted, tsetse-transmitted and pasture-transmitted pathogens in cattle.

Pathogen	Cattle Age (months)	Observations Total	Observations (+)	Model Estimates OR	Model Estimates 95% CI	F-value	p-value	Total Cattle
Tick-Transmitted								
T. parva	1 - 12	1140	120			$F_{2, 7484} = 55.5$	<.0001	873
	13 - 24 ▲	2277	224	0.99	0.78 - 1.27			
	> 24	4077	145	0.33	0.25 - 0.43			
Theileria spp.	1 - 12	1275	330			$F_{2, 7652} = 18.1$	<.0001	883
*(*but not *T. parva)*	13 - 24	2654	585	0.8	0.68 - 0.93			
	> 24	4608	906	0.62	0.53 - 0.73			
Anaplasma spp.	1 - 12	1267	364			$F_{2, 7607} = 11.1$	<.0001	883
	13 - 24 ▲	2638	705	0.92	0.78 - 1.08			
	> 24	4587	1087	0.71	0.60 - 0.84			
Babesia spp.	1 - 12	1275	12			$F_{2, 7652} = 0.12$	>0.05	883
	13 - 24 ▲	2654	22	0.88	0.44 - 1.77			
	> 24 ▲	4608	43	0.99	0.52 - 1.88			
Tsetse-Transmitted								
T. brucei	1 - 12	1020	65			$F_{2, 5331} = 9.43$	0.0001	797
	13 - 24	1923	200	1.78	1.35 - 2.33			
	> 24	3187	318	1.76	1.34 - 2.33			
T. vivax	1 - 12	751	84			$F_{2, 2827} = 7.42$	0.0006	829
	13 - 24 ▲	1130	118	0.84	0.64 - 1.10			
	> 24	1777	136	0.6	0.45 - 0.79			
T. c. (Savannah)	1 - 12	747	53			$F_{2, 2752} = 20.5$	<.0001	823
	13 - 24 ▲	1120	83	0.85	0.64 - 1.14			
	> 24	1710	83	0.4	0.29 - 0.56			
Pasture-Transmitted								
Fasciola spp.	1 - 12	1221	71			$F_{2, 7177} = 33.3$	<.0001	879
	13 - 24	2510	315	2.23	1.71 - 2.91			
	> 24	4327	764	3.01	2.30 - 3.94			
Nematode eggs	1 - 12	1238	559			$F_{2, 7316} = 102$	<.0001	881
	13 - 24	2554	805	0.54	0.46 - 0.62			
	> 24	4407	994	0.34	0.29 - 0.39			

Odds ratio (OR) values of less than one indicate that the age group of interest (13 to 24 or > 24 months of age) had a lower probability of infection as compared to calves (up to 12 months of age). OR values of more than one indicate that the age group of interest had higher probability of infection as compared to calves. ▲ Highlights cattle age groups where the proportion of pathogen-positive observations was not significantly different from that in calves, according to the T-test statistics (Data not shown). In such cases, the 95% confidence interval of the odds ratio includes the value "1".

As they grew older, cattle were less likely to test positive to *T. parva* and other Theileria species, Anaplasma spp., Duttonella and Nannomonas trypanosomes, and "*Strongyle-type*" nematodes. However, the characteristics of the inverse relationship between pathogen diagnosis and cattle ageing were dependent on the species or genus of organism considered. The probability of infection with Theileria species other than *T. parva* and the probability of positive "*Strongyle-type*" nematodes observations, was significantly reduced once calves (up to 12 months) grew into juveniles (13 – 24 months of age). In contrast, the frequency of *T. parva*, Anaplasma spp., *T. vivax* and *T. congolense (Savannah)* positive observations, did not drop significantly until after animals reached adulthood (> 24 months old). See Table 4.13. Conversely, Fasciola spp. *and T.brucei* infections increased with cattle ageing. Fasciola spp. were more frequent amongst faecal samples from juvenile cattle compared to calves (12.5% vs. 5.8%), and more frequent amongst samples from adults compared to juveniles (17.7% vs. 12.5%). In contrast, the frequency of *T. brucei* positive observations increased significantly once calves grew into juveniles (6.4% vs. 10.4%), but remained stable thereafter (Table 4.13). Cattle age was not found to be a significant predictor of Babesia spp. infection, but the conclusiveness of this analysis was limited by the small number of Babesia spp. positive observations recorded from the study population.

The results presented in Table 4.13 were consistent with those obtained in parallel analyses corrected by the effect of treatments, and with those in analyses where treated animals had been removed (Data not shown).

4.3.8.3 Geographical Distributions of Endemic Pathogens

Anaplasma spp. and Theileria species other than *T. parva* were the predominant tick-transmitted pathogens in all the study villages (Figure 4.5). Babesia spp. was also reported from all the study villages, but consistently affected less than 10% of the population in each village over the course of the study. The geographical distribution of these pathogens was more homogeneous than that of *T. parva*, which was mainly reported from cattle in Busia district (Figure 4.5). The cumulative prevalence and incidence of helminths recorded from cattle in different study villages is shown in Figure 4.6.

Figure 4.5 Cumulative prevalences of tick-transmitted pathogens in cattle by either microscopy or PCR.

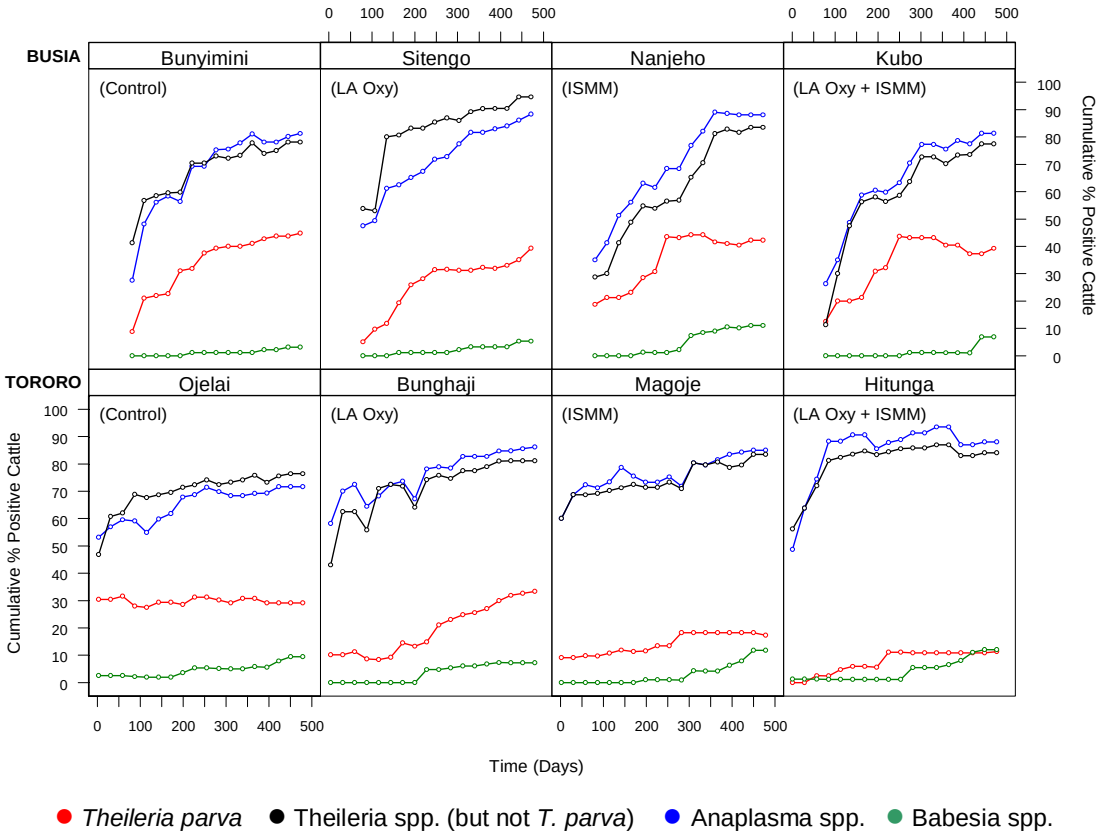
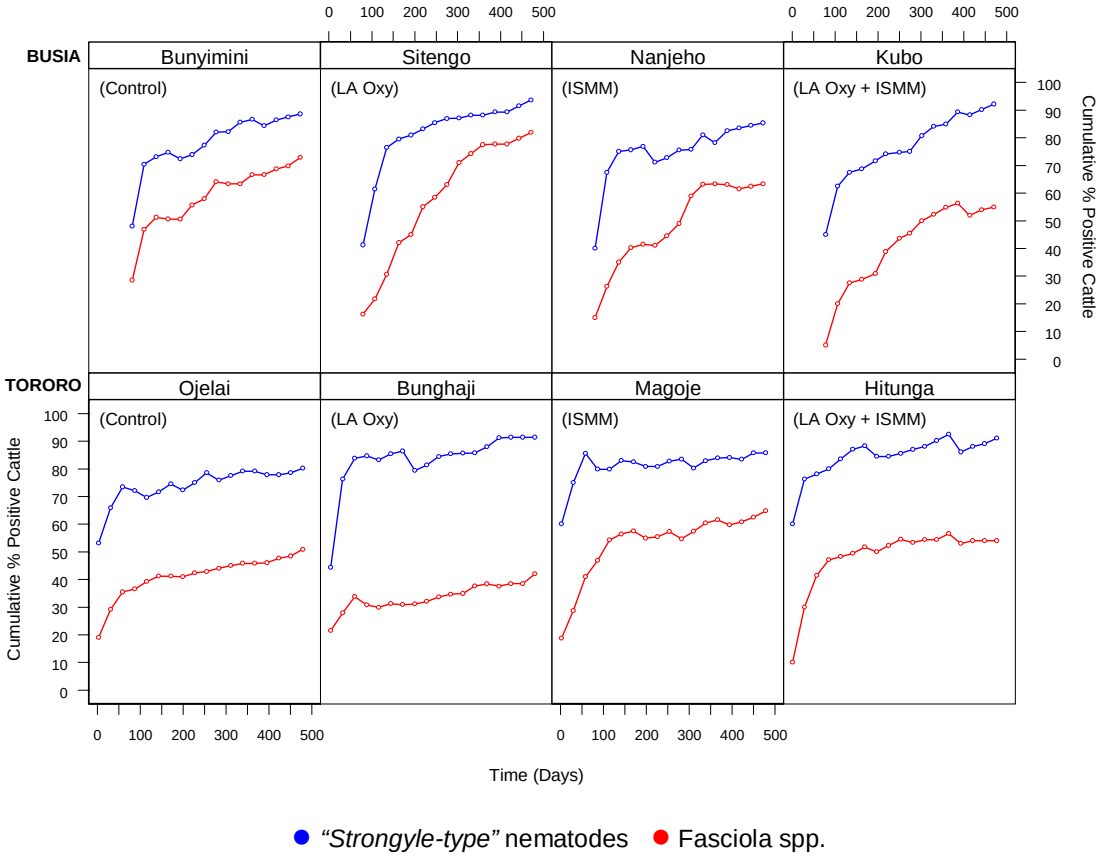
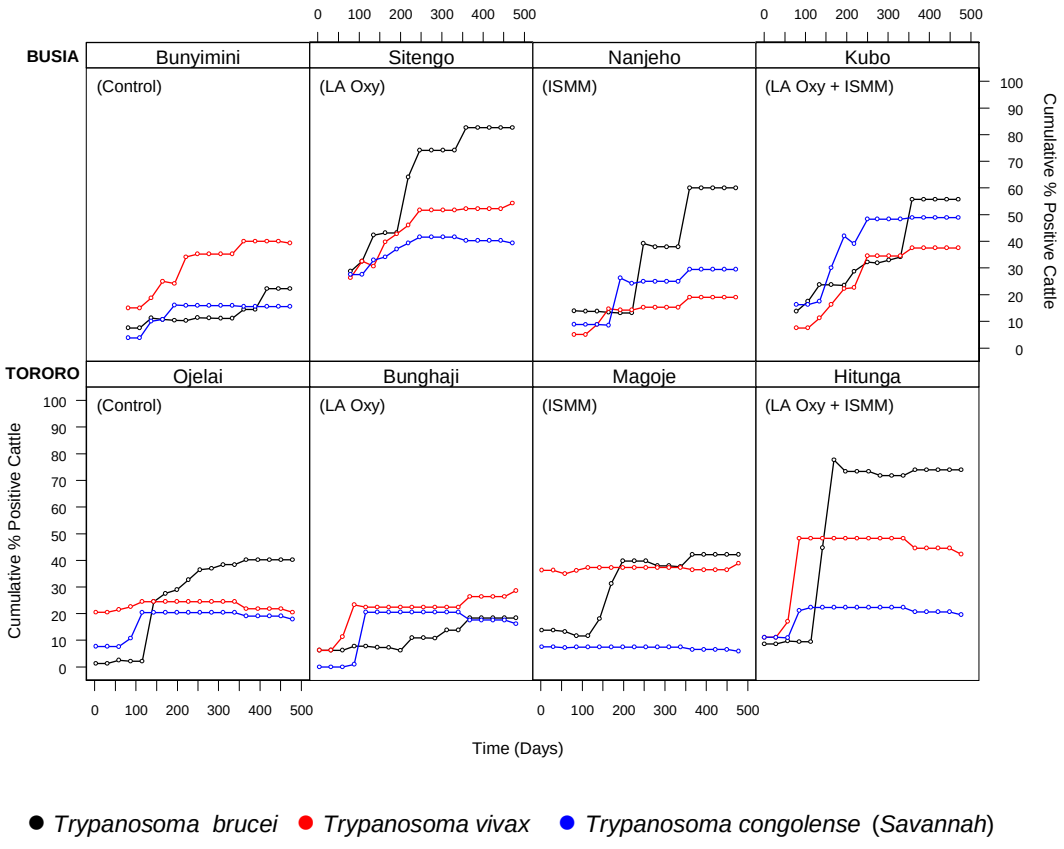


Figure 4.6 Cumulative prevalences of pasture-transmitted helminths in cattle, obtained by faecal egg counts.



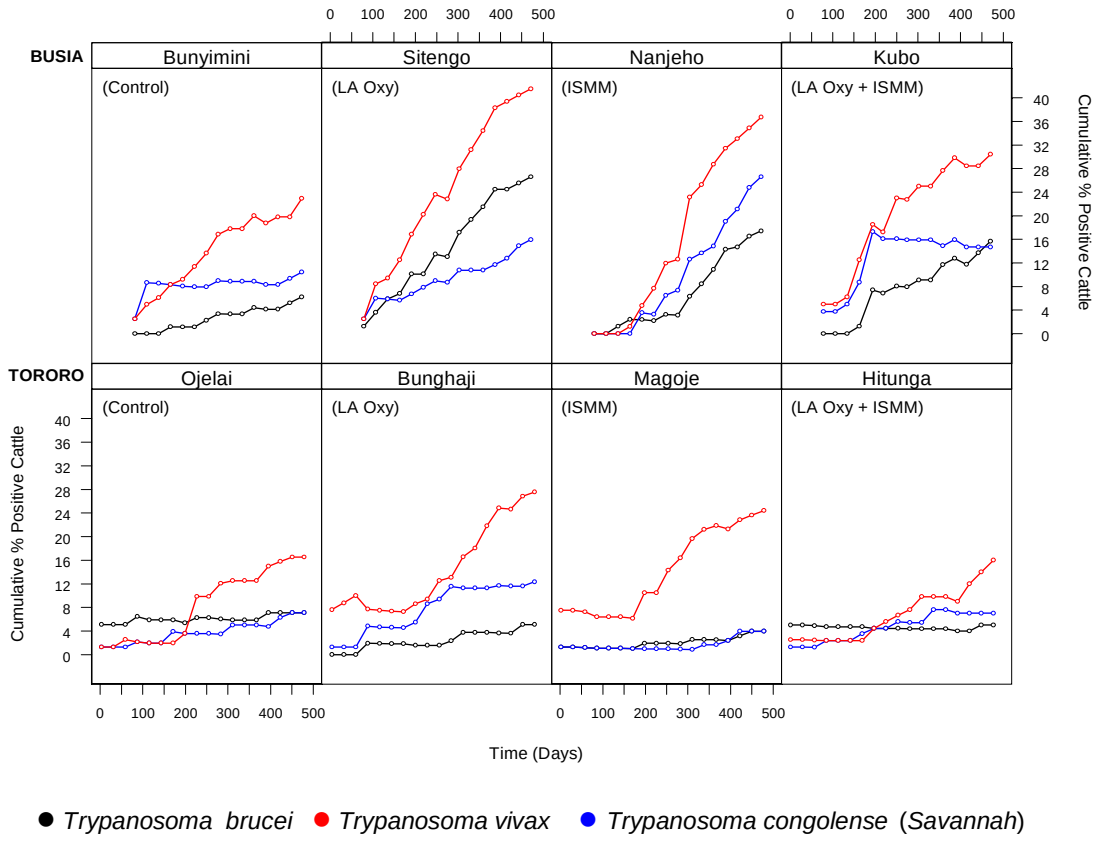
The prevalence of Trypanosoma spp. differed substantially depending on whether it was obtained from microscopy or PCR screening (Table 4.11). 57.6% of the cattle were affected by Trypanosoma spp. infections above the PCR detection threshold but only 33.3% presented with infection intensities above the microscopy detection threshold. Trypanosomes were therefore widely spread amongst the cattle population, whilst high infection intensities detectable microscopically were limited to a moderate proportion of positive individuals. However, Trypanosoma spp. cases by microscopy were treated six times more frequently with ad hoc trypanocidals (diminazene) and twice as frequently with either experimental (ISMM) or ad hoc (diminazene) trypanocidals than Trypanosoma spp. cases by PCR. Notably, the predominant Trypanosoma species in the study population depended on village and on whether the infection status of cattle in the study was determined by PCR or by microscopy. *T. brucei* was the most widespread species using PCR in all villages except for in Bunyimini (Busia) and Bunghaji (Tororo) where *T. vivax* was more widespread (Figure 4.7). In contrast, the predominant trypanosome species using microscopy was *T. vivax* in all villages (Figure 4.8).

Figure 4.7 Cumulative prevalences of tsetse-transmitted trypanosomes in cattle by PCR.



Overall, Trypanosoma spp. infections at levels above the microscopy detection threshold were more widespread in cattle from Busia than from Tororo. This was despite the fact that 66% (390/589) of diminazene treatments were given in the former district compared to only 34% (199/589) in the latter. In contrast, infections at levels above the PCR detection threshold were more evenly distributed across all areas in the study (Figures 4.7 & 4.8).

Figure 4.8 Cumulative prevalences of tsetse-transmitted trypanosomes in cattle by microscopy.



These observations may result from differing parasitaemias in trypanosome-positive cattle depending on geographical area, and suggest that while trypanosomes are widely spread, the distribution of high infection intensities (detectable microscopically) is patchy, and mainly concentrated in areas which are closer to Lake Victoria.

4.3.8.4 Prevalence of Endemic Pathogens in Block Treated as Compared to in Control Villages

Visual inspection of the longitudinal data suggested no clear association between the administration of block veterinary interventions and the proportion of cattle infected with tick-transmitted pathogens or helminths, at least at levels detectable by the techniques used (Figures 4.9 and 4.10). In contrast, ISMM block treatments appeared to exercise a degree of curative and chemoprophylactic effect on Trypanosomiasis. This effect, however, was highly heterogeneous depending on village, species of trypanosome, and on whether infection was determined by PCR (Figure 4.11) or microscopy (Figure 4.12).

Figure 4.9 Longitudinal prevalences of tick-transmitted pathogens in cattle by either microscopy or PCR.

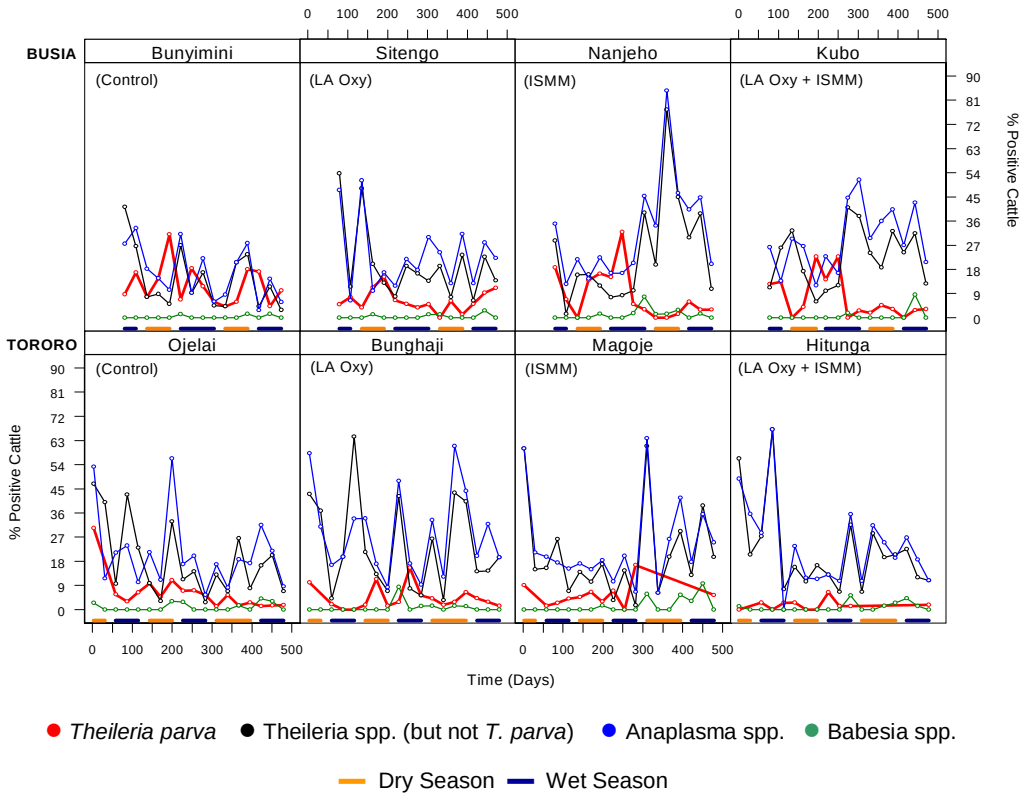
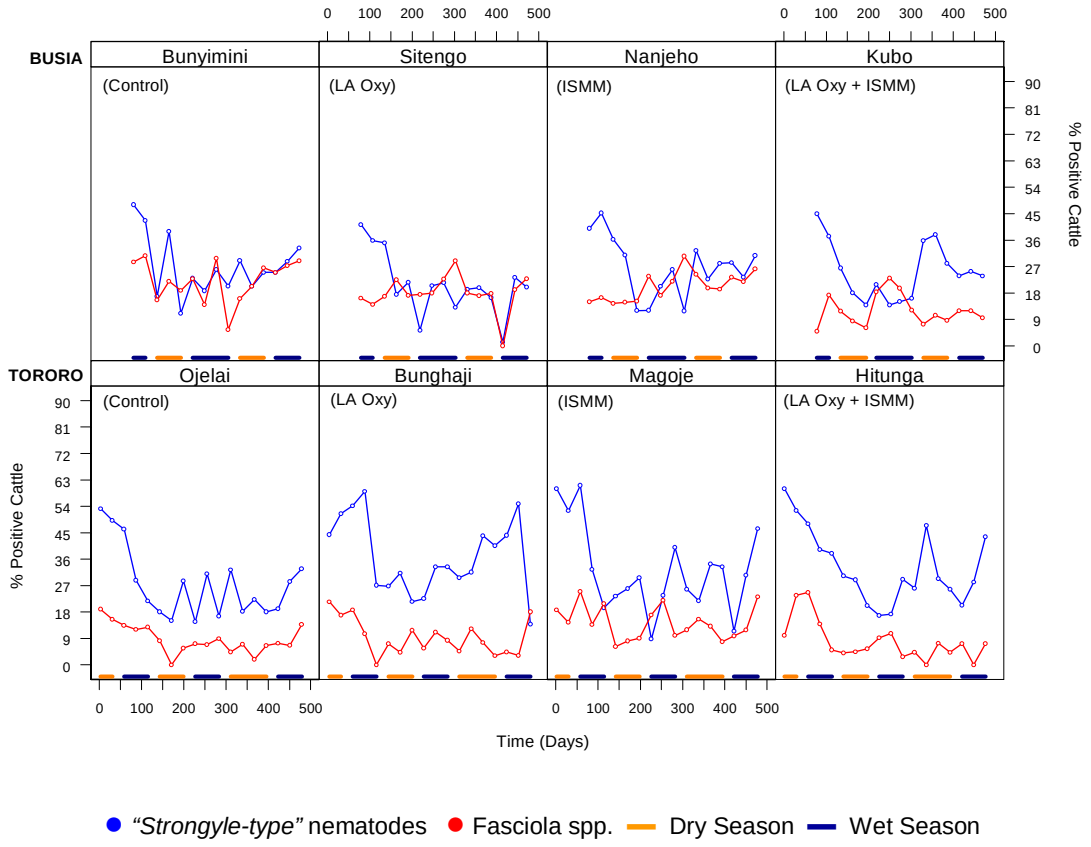
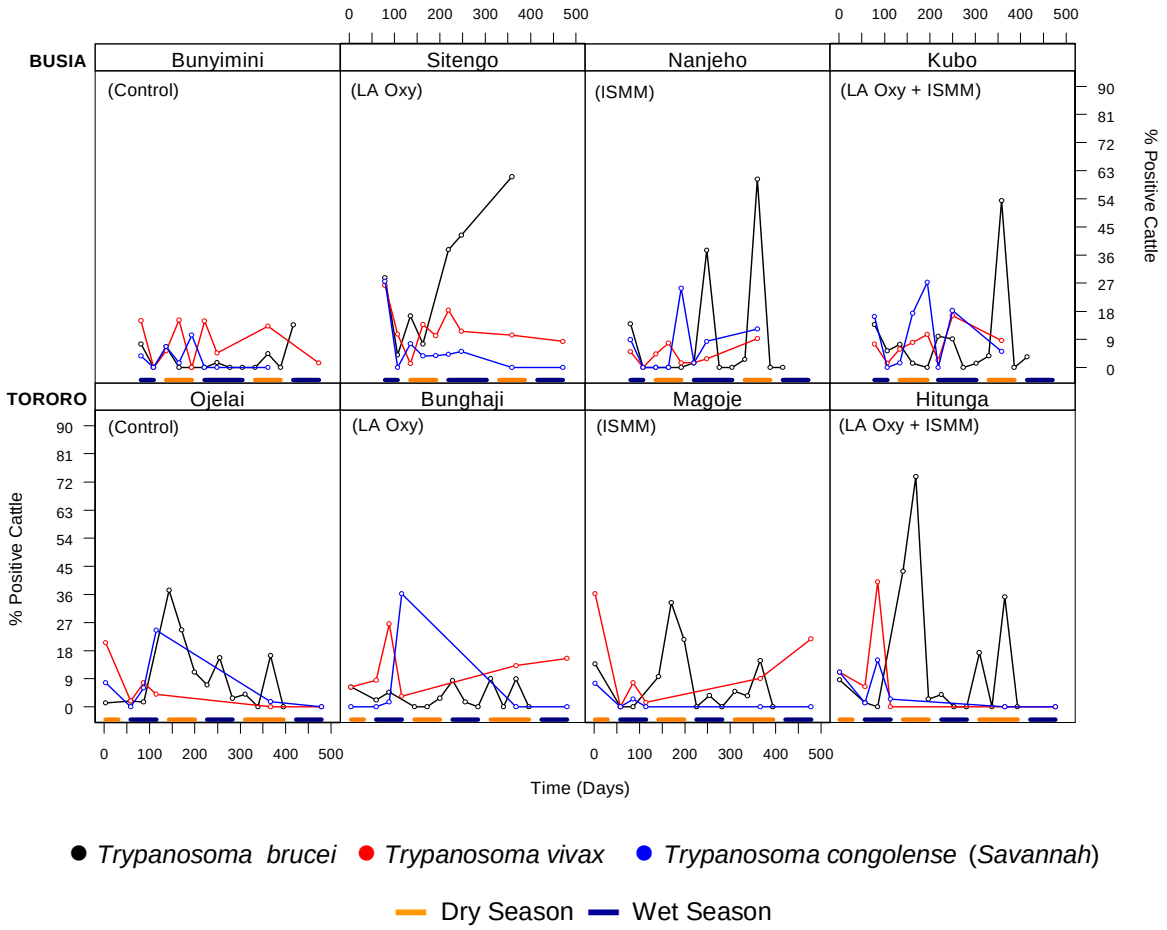


Figure 4.10 Longitudinal prevalences of pasture-transmitted helminths in cattle, obtained by faecal egg counts.



Trypanosoma spp. infection status data obtained from PCR screening (Figure 4.11) showed that, with the exception of in Bunghaji, the proportion of infected cattle dropped precipitously in all villages immediately after the initial visit, regardless of whether these had been exposed to ISMM block treatment or not. In all villages where ISMM was administered, the proportion of cattle diagnosed with trypanosomes increased from two months after chemoprophylaxis. Only in two villages (Kubo and Hitunga) did the number of Trypanosoma spp. infected cattle drop to zero, one month after the experimental intervention.

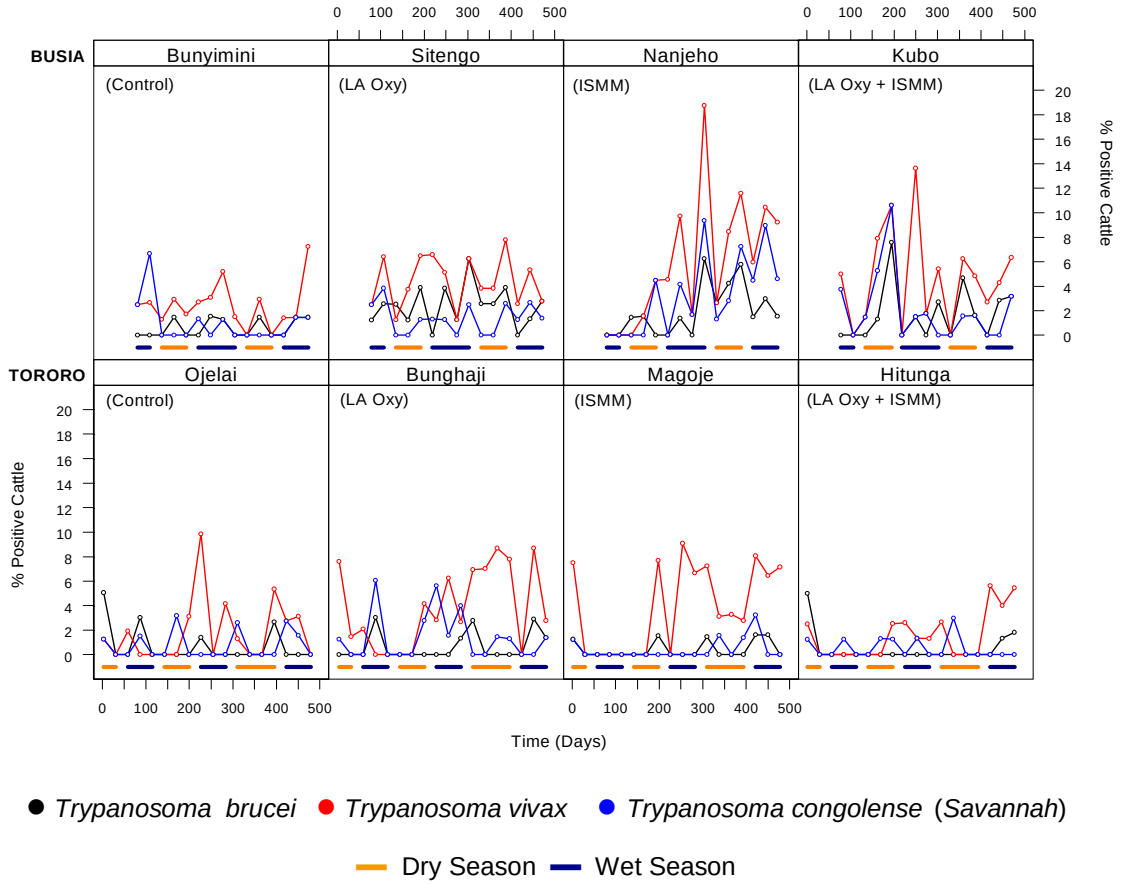
Figure 4.11 Longitudinal prevalences of tsetse-transmitted trypanosomes in cattle by PCR.



The effect of ISMM block treatment on Trypanosoma spp. infection status data using microscopic screening of cattle was more heterogeneous depending on the district considered (Figure 4.12). In all Tororo villages, the proportion of infected cattle dropped dramatically after the initial visit, regardless of whether or not they had been exposed to ISMM block treatment. However, only in ISMM-treated villages did the prevalence of trypanosomiasis remain at 0% (depending on trypanosome species) for a consistent period of time. In Magoje, breakthrough *T. congolense* and *T. vivax* infections were only diagnosed from cattle seven months after ISMM administration, whereas *T. brucei* was not recorded until after 12 months. In Hitunga, *T. congolense* breakthrough was recorded as soon as 2 months after administering ISMM, but no *T. vivax* or *T. brucei* infected cattle were observed until after 6 and 14 months respectively. In Busia, in contrast, the proportion of infected cattle dropped dramatically after the initial visit only in the ISMM-treated villages (Figure 4.12). However the prevalence of

trypanosomiasis was maintained at 0% for as little as one month, with trypanosomes breakthrough being recorded as soon as two months after treatment. In Nanjeho, *T. brucei*, *T. vivax* and *T. congolense* were diagnosed from cattle two, three and four months after administering ISMM respectively, whereas in Kubo, *T. vivax* and *T. congolense* were recorded two months and *T.brucei* three months after chemoprophylaxis.

Figure 4.12 Longitudinal prevalences of tsetse-transmitted trypanosomes in cattle by microscopy.



4.3.9 Pathogen Associations

The probability of infection by any given blood-borne pathogen appeared to be a function of endogenous cattle factors (i.e. host's age) and whether other pathogens were present in the host as measured by the available techniques. See Tables 4.14 and 4.15.

Table 4.14 Statistical associations amongst common endemic pathogens of African cattle - Factors affecting the distribution of African trypanosomes.

Minimum Model	Variable Levels	OR	95 % CI	F - value	p - value	Obs	Cattle
***T. brucei* (Ж)**						2924	783
Age	13 - 24	4.18	2.62 - 6.67	$F_{2,2133} = 17.9$	<.0001		
	> 24	5.33	3.35 - 8.47				
Anaplasma spp.	(+)	2.61	1.51 - 4.50	$F_{1,2133} = 15.0$	0.0001		
T. vivax	(+)	4	2.17 - 7.38	$F_{1,2133} = 8.18$	0.004		
Age : Anaplasma spp.	13 - 24 : (+)	0.38	0.19 - 0.73	$F_{2,2133} = 4.92$	0.007		
	> 24 : (+)▲	0.63	0.34 - 1.17				
Age : *T. vivax*	13 - 24 : (+)	0.31	0.14 - 0.71	$F_{2,2133} = 6.04$	0.002		
	> 24 : (+)	0.27	0.12 - 0.58				
***T. vivax* (Ж)**						2477	750
Age	13 – 24 ▲						
	> 24 ▲						
Nematodes	≤ 400	0.66	0.46 - 0.96	$F_{2,1712} = 6.98$	0.001		
	> 400 ▲	0.98	0.59 - 1.64				
Anaplasma spp.	(+)	1.44	1.06 - 1.95	$F_{1,1712} = 28.8$	<.0001		
T. brucei	(+)	2.56	1.16 - 5.66	$F_{1,1712} = 8.86$	0.003		
T. c. (Savannah)	(+)	4.46	2.25 - 8.87	$F_{1,1712} = 11.7$	0.001		
Nematodes : Anaplasma spp.	≤ 400 : (+)▲	1.44	0.83 - 2.50	$F_{2,1712} = 4.11$	0.017		
	> 400 : (+)	2.76	1.36 - 5.57				
Age : *T. brucei*	13 - 24 : (+)	0.32	0.13 - 0.80	$F_{2,1712} = 6.83$	0.001		
	> 24 : (+)	0.32	0.14 - 0.75				
Nematodes : *T. brucei*	1 -400 : (+)	2.49	1.23 - 5.04	$F_{2,1712} = 4.67$	0.01		
	> 400 : (+)	3.87	1.32 - 11.3				
Age : *T. c. (Savannah)*	13 - 24 : (+)	0.25	0.09 - 0.66	$F_{2,1712} = 4.25$	0.014		
	> 24 : (+)	0.34	0.13 - 0.92				
***T. c. (Savannah)* (Ж)**						2775	799
Age	13 – 24 ▲	1.07	0.76 - 1.51	$F_{2,1986} = 13.2$	<.0001		
	> 24	0.43	0.29 - 0.65				
Anaplasma spp.	(+)	1.5	1.14 - 1.96	$F_{1,1986} = 29.2$	<.0001		
Fasciola spp.	(+)▲						
T. brucei	(+)▲						
T. vivax	(+)	1.71	1.22 - 2.40	$F_{1,1986} = 10.5$	0.001		
Age : *T. brucei*	13 - 24 : (+)	0.34	0.14 - 0.85	$F_{2,1986} = 5.13$	0.006		
	> 24 : (+)▲	1.01	0.44 - 2.27				
Anaplasma spp. *: T. brucei*	(+) : (+)	2.5	1.34 - 4.68	$F_{1,1986} = 7.22$	0.007		
Fasciola spp. *: T. brucei*	(+) : (+)	0.32	0.13 - 0.78	$F_{1,1986} = 6.26$	0.012		

Non-significant main terms are those for which no variable levels are significantly different from the reference level according to the T-test statistics (▲; data not shown); in such cases OR and F-tests are not presented. See legend to Table 4.15.

Table 4.15 Statistical associations amongst common endemic pathogens of African cattle - Factors affecting the distribution of tick-transmitted pathogens.

Minimum Model	Variable Levels	OR	95% CI	F - value	p - value	Obs	Cattle
***T. parva* (Ж)**						3396	808
Age	13 – 24 ▲	1.23	0.93 - 1.63	$F_{2,2578} = 26.4$	<.0001		
	> 24	0.35	0.24 - 0.51				
Anaplasma spp.	(+)	1.45	1.16 - 1.81	$F_{1,2578} = 4.52$	0.034		
T. c. (Savannah)	(+)	4.2	2.14 - 8.25	$F_{1,2578} = 6.04$	0.014		
R. appendiculatus	1 - 10	1.66	1.13 - 2.42	$F_{3,2578} = 8.53$	<.0001		
	11 - 50	2.49	1.66 - 3.72				
	> 50	2.77	1.53 - 5.02				
Age : *T. c. (Savannah)*	13 - 24 : (+)	0.24	0.10 - 0.58	$F_{2,2578} = 5.13$	0.006		
	> 24 : (+)▲	0.71	0.29 - 1.69				
Anaplasma spp. *: T. c. (Savannah)*	(+) : (+)	0.3	0.14 - 0.65	$F_{1,2578} = 9.50$	0.002		
Anaplasma spp. (Ж)						3073	794
Age	13 – 24 ▲	0.88	0.69 - 1.11	$F_{2,2272} = 6.86$	0.001		
	> 24	0.74	0.59 - 0.94				
Nematodes	≤ 400	1.28	1.06 - 1.54	$F_{2,2272} = 7.42$	0.001		
	> 400	1.57	1.20 - 2.06				
T. vivax	(+)	1.96	1.52 - 2.53	$F_{1,2272} = 27.5$	<.0001		
T. c. (Savannah)	(+)	1.6	1.17 - 2.18	$F_{1,2272} = 8.89$	0.003		
Babesia spp.	(+)	18.5	2.33 - 147	$F_{1,2272} = 7.63$	0.006		
Babesia spp. (Ж)						9153	9153
Anaplasma spp.	(+)	14.7	8.20 - 26.2	$F_{1,8260} = 82.2$	<.0001		

Odds ratio (OR) values of less or more than one, indicate lower or higher probability of infection with the pathogen of interest (Ж) respectively. OR values for main terms show probability of infection with the pathogen of interest (Ж) in pathogen-positive (+) as compared to pathogen-negative samples or in animals of 13-24 or > 24 months of age as compared to calves (up to 12 months of age). OR values for "Age : Pathogen" interactions show the probability of infection with the pathogen of interest (Ж) in pathogen-positive (+) animals of 13-24 or >24 months of age as compared to in pathogen-positive (+) calves. OR values for "Pathogen (a) : Pathogen (b)" interactions show the probability of infection with the pathogen of interest (Ж) in animals infected with both pathogens (a + b) as compared to in animals infected with the second pathogen (b) only. ▲ Highlights variable levels where the probability of infection with the pathogen of interest (Ж) is not significantly different from that in the reference level according to the T-test statistics.

For example, the probability of patent *T. brucei* infection at levels above the PCR detection threshold was significantly higher in cattle infected with Anaplasma spp. or *T. vivax* detectable by PCR (See Table 4.14). Similarly, *T. vivax* patent infection above the PCR detection threshold was significantly higher in *T. brucei* or *T. congolense (Savannah)* positive animals by PCR, and in cattle infected with Anaplasma spp. Furthermore the probability of detecting *T. vivax* was higher if Anaplasma spp. or *T. brucei* infected animals were co-infected with nematodes, and even higher as the nematodes infection intensity increased (Table 4.14). The most important predictor of *T. congolense (Savannah)* patent

infection by PCR were Anaplasma spp. - *T. brucei* co-infections, although *T. vivax* by PCR was also a significant predictor of positive *T. congolense (Savannah)* diagnosis. Conversely, *T. congolense (Savannah)* infections above PCR detection threshold were less likely in *T. brucei* – Fasciola spp. co-infections as compared to in infections involving *T. brucei* only.

Clearly, not only trypanosome infections were a function of the host's infection status for other pathogens, but also tick-transmitted pathogens (Table 4.15). The distribution of *T. parva*, for example, was strongly affected by the distribution of *T. congolense (Savannah)* across animals in the study, and less so by that of Anaplasma spp. Notably, the probability of diagnosing *T. parva* by PCR was significantly higher in animals infected with either Anaplasma spp. or *T. congolense (Savannah)*. However, in hosts co-infected with both pathogens, the probability of patent *T. parva* infection was significantly reduced as compared to in hosts infected with *T. congolese (Savannah)* only. Anaplasma spp., in turn, was significantly more prevalent amongst cattle infected with trypanosomes (*T. vivax* and *T. congolense* [*Savannah*]), Babesia spp. or nematodes. Data exploration showed that Anaplasma spp. was always more prevalent in cattle infected with other pathogens (regardless of the genera or species involved) than alone (Data not shown). Again, Anaplasma spp. diagnosis was positively correlated with increasing nematode infection intensities. Finally, even Babesia spp. did not follow a distribution which could be regarded as independent from that of other pathogens. On the contrary, 82% of Babesia spp. positive observations (64/78) were recorded from Anaplasma spp. infected animals.

Altogether statistical associations were both apparent amongst pathogens sharing the same vector (e.g. tsetse-transmitted trypanosomes) and amongst pathogens whose transmission routes differed. Associations amongst pathogens were not necessarily symmetric. Positive associations between any two pathogens were common, but negative associations were also apparent when considering the effect of two co-infecting pathogens on a third. Notably, a number of pathogen associations were age-dependent (Tables 4.14 and 4.15). For example, the probability of *T. brucei* patent infection in *T.vivax* positive animals of 13-24 or >24 months of age, was significantly lower than in *T. vivax* positive calves (Table 4.14). Similarly, the probability of *T. brucei* patent infection in Anaplasma spp. positive animals,

decreased significantly in cattle of 13-24 months of age as compared to calves, but was not different in animals older than 24 months as compared to calves. All age-dependent pathogen associations were limited to calfhood (1-12 months of age) and, in some instances, adulthood (> 24 months of age), whilst such associations were not apparent in juvenile cattle (13 – 24 months of age). See Tables 4.14 and 4.15. Interaction plots showing age-dependent pathogen associations are shown in Appendix 4.8.

4.3.10 Effect of Veterinary Interventions on Species-Specific and Genus-Specific Infection Status

The statistical analyses of the effect of LA oxytetracycline, albendazole and diminazene, on subsequent infection status by potentially susceptible pathogen species, are presented in Table 4.16. To aid the interpretation of statistical analysis outputs, a summary of the raw data is presented in Appendix 4.9. The appendix presents a cross-tabulation of pathogen-specific infection status, veterinary intervention and their interaction, on subsequent infection status (i.e. at the following round of observation, 28 days later) obtained from either microscopy (MCR) or PCR screening of cattle over the course of the longitudinal study.

On the bases of the information presented in Table 4.16, there was no evidence to support the hypothesis that LA oxytetracycline affected the longitudinal incidence and/or prevalence of tick-transmitted pathogens. Antibiotic therapy was not shown to clear or prevent infection by target pathogens. Counter-intuitively, *T. parva*, Theileria spp. other than *T. parva* and Anaplasma spp. positive cases were significantly more frequent amongst observations which followed the decision of the field veterinarian to administer ad hoc LA oxytetracycline treatments. However, for *T. parva* and Anaplasma spp., infections were more frequently recorded in cattle that had positive laboratory tests at the previous visit (persistent infections). See Table 4.16. It is possible that LA oxytetracycline was mainly administered to positive cattle exhibiting high parasitaemias. These cattle, in turn, may have been more likely to sustain parasitaemia levels above the detection threshold on subsequent visit rounds.

Table 4.16 Effect of pathogen-specific infection status, drug treatment and their interaction, on subsequent infection status in cattle by either microscopy or PCR.

Target pathogen ($IS_{(t+1)}$)	Maximum Model		Minimum model		Significant Terms	Model Estimates		F-value	p-value
	Observations	Cattle	Observations	Cattle	(Minimum Model)	OR	95% CI		
T. parva	6460	769	6460	769	$IS_{(t)}$ = +	2.39	1.96 -2.91	$F_{1, 5689} = 79.3$	<.0001
					LA Oxytetracycline	1.33	1.04 -1.71	$F_{1, 5689} = 5.01$	0.025
Theileria spp. (but not *T.parva*)	7801	846	7830	847	LA Oxytetracycline	1.49	1.26 -1.77	$F_{1, 6982} = 21.0$	<.0001
Anaplasma spp.	7699	846	7699	846	$IS_{(t)}$ = +	1.32	1.18 -1.49	$F_{1, 6851} = 28.3$	<.0001
					LA Oxytetracycline	1.26	1.06 -1.50	$F_{1, 6851} = 7.10$	0.008
Babesia spp. [a]	7801	846	7830	847	Diminazene	2.43	1.20 -4.90	$F_{1, 6982} = 6.11$	0.013
Trypanosoma spp. (PCR) [b]	5483	714	5483	714	$IS_{(t)}$ = +	1.34	1.11 -1.62	$F_{1, 4767} = 8.63$	0.003
					Diminazene	0.72	0.52 -0.99	$F_{1, 4767} = 3.99$	0.046
Trypanosoma spp. (MCR) [c]	7831	846	7845	847	Diminazene	0.29	0.19 -0.42	$F_{1, 6997} = 39.3$	<.0001
Fasciola spp.	6981	835	7387	839	Albendazole	0.41	0.33 -0.52	$F_{1, 6547} = 56.1$	<.0001
Nematode eggs	7213	842	7213	842	$IS_{(t)}$ = +	1.35	1.20 -1.51	$F_{1, 6369} = 8.16$	0.004
					Albendazole	0.32	0.26 -0.39	$F_{1, 6369} = 109$	<.0001

MCR = Microscopy. In the analyses, the probability that currently positive cattle ($IS_{(t)}$ = +) are infected on the following round of observation ($IS_{(t+1)}$) is compared to that in currently negative cattle (reference variable level). The probability that currently treated cattle are infected on the following month is compared to that in currently untreated cattle (reference variable level).Given that the interaction term was not found significant in any of the analyses, each drug could be regarded as having the same effect on persisting or incident infections (i.e. both curative and preventive or neither). Odds ratio values of less or more than one, indicate a lower or higher risk of infection on subsequent visits respectively, as compared to the reference variable levels (uninfected, untreated).[a] LA oxytetracycline had no preventive or curative effect on Babesia spp. infections ($p > 0.05$; data not shown). [b] Includes samples with PCR data for at least one of the three Trypanosoma spp. Diminazene had no significant curative or preventive effect on Trypanosoma spp. cases by PCR when only samples with PCR data for all three Trypanosoma spp. were included in the analysis ($p > 0.05$; data not shown). This resulted from low numbers of observations available for analysis. [c] All samples were tested for all three Trypanosoma spp.

Neither a positive laboratory result, nor the requirement of LA oxytetracycline had a significant effect on the probability of diagnosing Babesia spp. on the following visit round. However, Babesia spp. was significantly more frequent amongst observations which followed the decision of the field veterinarian to administer diminazene aceturate (Table 4.16).

Diminazene aceturate had a significant effect with regard to both clearing and preventing Trypanosoma spp. infections at levels above the microscopy detection threshold, and only a marginally significant effect on Trypanosoma spp. infections by PCR when considering samples tested for at least one of the three species (Table 4.16). This resulted from the low number of consecutive observations (i.e. inter-spaced by no longer than 28 days) for which Trypanosoma spp. infection status by PCR was available for analysis (n = 5483 observations on 714 cattle). Only in the case of PCR data, positive observations were more frequent at visits that followed observation rounds where positive laboratory tests were obtained (Table 4.16). This suggested that high infection intensities in the study population were short-lived (< 28 days), while low Trypanosoma spp. infection intensities were likely to persist for at least two consecutive visit rounds.

Anthelmintic treatments had a significant clearing and preventive effect on infections by Fasciola spp. and gastrointestinal nematodes, but only in the latter, positive observations were more frequently recorded at visits which followed observation rounds where positive laboratory tests were obtained (Table 4.16).

The results presented in Table 4.16 were consistent with those obtained in parallel models where more observations were available for analysis (Section 4.2.5.4). Parallel analyses showed that Trypanosoma spp. infections above the PCR detection threshold were significantly reduced by treatment with ISMM (n = 7407 on 847 cattle; $F_{1, 6558} = 8.35$; p = 0.004; $OR_{(probability\ of\ positive\ observation\ after\ treatment)} = 0.63$ [0.46-0.86]) in addition to diminazene (n = 7407 on 847 cattle; $F_{1, 6558} = 8.35$; p = 0.004; $OR_{(probability\ of\ positive\ observation\ after\ treatment)} = 0.63$ [0.46-0.86]). The effect of ISMM on Trypanosoma spp. infections by microscopy could not be estimated reliably, as a result of the low number of infections above the microscopy detection threshold. Data from the longitudinal studies conducted in Busia and Tororo

districts, have enabled an in depth description of the effect of ISMM chloride on Trypanosoma spp. using appropriate statistical methods for "*time-to-event*" data (McOdimba, 2005). As suggested from visual inspection of data presented throughout this chapter, a prophylactic effect was only statistically demonstrated in areas where trypanosome challenge was lowest (i.e. Tororo District).

As veterinary treatments were only demonstrated to have an effect on the longitudinal incidence and prevalence of helminths and Trypanosoma spp., inclusion of LA oxytetracycline treated observations in chapters describing the epidemiology of *T.parva*, is unlikely to have resulted in serious bias.

4.3.11 Morbidity

Over the course of the study, 97.8% of the cattle presented clinical signs or were reported to be 'sick' or 'severely sick' by either the owners or the veterinarian (Table 4.17). The most common clinical finding in the study population was parotid, prefemoral or prescapular superficial lymph node enlargement, which affected 88.2% of the animals in the study. This was followed by rough / staring coat (69.8%), direct or indirect indication of anaemia (58.8%), ocular discharge (42.7%) and fever (30.6%). In contrast, cattle were rarely considered to have poor condition score (15.5%) or had presented with diarrhoea (9.21%), nasal (1.8%) or oculo-nasal (0.11%) discharge. Vaginal discharge was only reported from four cows. Lice, mainly low to moderate levels of infestation, were reported in 7.53% (67/890) of cattle in the study (Table 4.17). Ivermectin treatment, however, was only required for the case of 12 animals.

Despite the relatively high incidence of clinical signs in the study areas, few cattle were reported to be 'sick' or 'severely sick' by either the owner (12.8% [114/890]) or the field veterinarian (1.46% [13/890]), or were reported to have died (4.5% [40/893]) over the course of the study (Section 4.3.12). Subjective scoring of 'sick' or 'severely sick' animals was only conducted at the time of the main visits, but not at the time of the intermediate visits. However, the condition of animals with deteriorated health was re-assessed during intermediate visits, and it was at these visits when the majority of ad hoc treatments were given. In consequence, the cumulative percentage of 'sick / severely sick' cattle might have been

underestimated; the numbers of cattle needing ad hoc treatments may be a better indication of the numbers of sick cattle over the course of the study.

Table 4.17 Prevalences of abnormal clinical conditions in cattle.

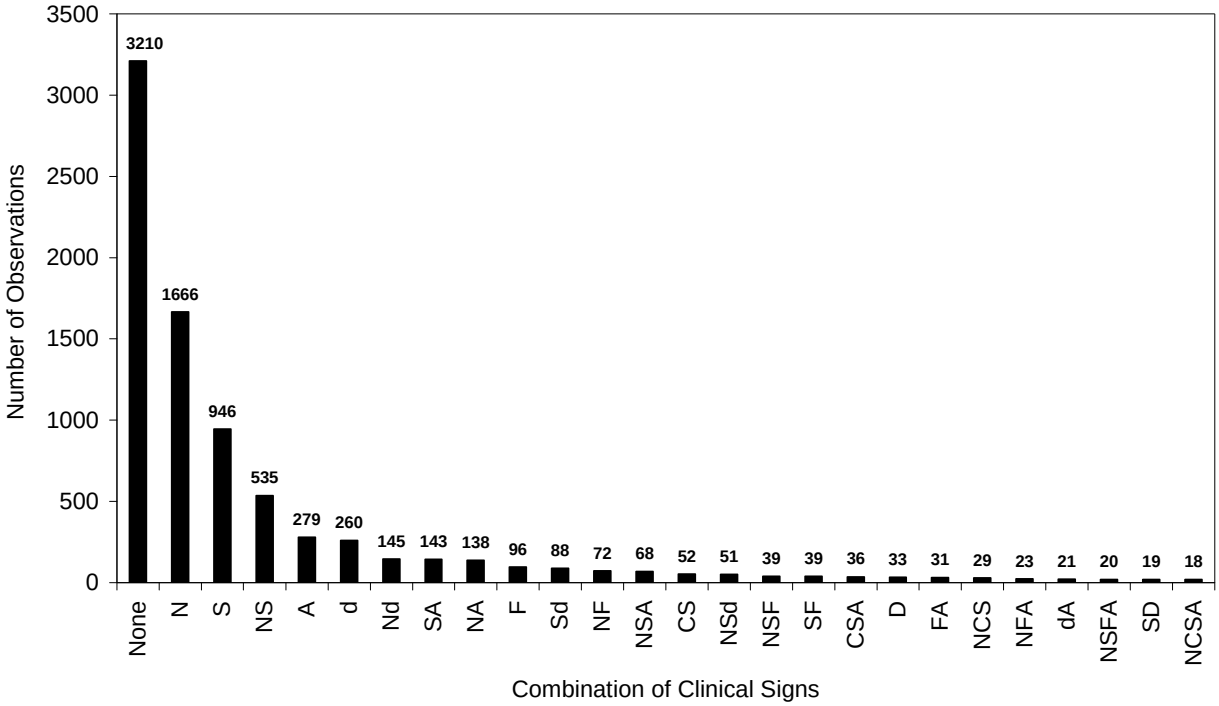
Clinical Signs	Cattle			Observations		
	Present	Total	%	Present	Total	%
Lymph node enlargement	785	890	88.2	3151	9173	34.4
Condition score (L+ ,L , L-)	138	890	15.5	239	9212	2.59
Staring coat	621	890	69.8	2474	9210	26.9
Pale mucous membranes (1)	282	890	31.7	452	9212	4.91
Discharge						
Vaginal	4	890	0.45	4	9206	0.04
Ocular	380	890	42.7	776	9206	8.43
Nasal	16	890	1.8	18	9206	0.2
Oculo-nasal	1	890	0.11	1	9206	0.01
Diarrhoea	82	890	9.21	119	9212	1.29
Fever (> 39.4ºC)	272	890	30.6	428	9205	4.65
PCV (<25%) (2)	303	892	34	567	9217	6.15
Haemoglobin (<8 g / dl) (3)	393	892	44.1	793	8974	8.84
Either (1), (2) or (3) [a]	523	889	58.8	1185	8967	13.2
Sick / Severely Sick (Owner's perception)	114	890	12.8	134	9212	1.45
Sick / Severely Sick (Veterinary perception)	13	890	1.46	13	9212	0.14
Skin lesions	5	890	0.56	5	9212	0.05
Any clinical sign [b]	**869**	**889**	**97.8**	**5477**	**8912**	**61.5**
Louse infestation	67	890	7.53	130	9212	1.41

[a] Includes observations from which for all direct and indirect indicators of anaemia were recorded (haemoglobin, colour of mucous membranes and PCV), and cattle from which all direct and indirect indicators of anaemia were recorded for the case of at least one visit round. [b] Includes observations from which complete clinical data was available, and cattle from which complete clinical data was available for the case of at least one visit round. "Any clinical sign" does not include louse infestation. Due to technical problems in the portable haemoglobinometer at the time of one visit round, haemoglobin readings were available for a lower number observations as compared to other clinical signs (n = 8974).

Multiple clinical signs were more frequently reported from calves, whereas 'no clinical signs' were mainly reported from physical examinations conducted on adult cattle (> 24 months of age). See Appendix 4.10. Multiple clinical signs were less frequent than a single sign in the case of observations conducted on juveniles (22.1% vs. 40%) or adults (16.5% vs. 40.2%), but as frequent as a single sign (37.9% vs. 37.4%) in physical examinations conducted on calves. Only one calf presented with six abnormal conditions simultaneously.

There existed no clear predominance of any particular combination of clinical signs, which could suggest non-random "*sign-specific*" associations into key clinical pictures amongst the study population (Figure 4.13).

Figure 4.13 Twenty-five most common clinical pictures recorded from initial and follow-up observations on cattle.



Where both subjective and objective indicators of the same clinical condition were available (i.e. pale mucous membranes vs. low PCV or low haemoglobin), only objective measures are taken into account. (N) Lymph node enlargement; (S) Staring coat; (A): Anaemia (PCV < 25% and / or haemoglobin < 8 g / dl); (d) Discharge (vaginal, ocular, nasal or oculo-nasal; No animals presented with more than one type of discharge at a time); (F) Fever; (C) Poor condition score. 'Skin lesions' and subjective scoring of 'sick / severely sick' animals are not included. The figure is based on observations where cattle age and complete data on the conditions of interest were available (n = 8261).

Lymph node enlargement or staring coat in the absence of other abnormal signs, were the two most common clinical pictures recorded over the course of the study, followed by the combination of these two conditions, either low PCV or low haemoglobin alone, and discharge alone. Fever was the ninth most common finding and was more commonly observed in the absence of other clinical signs or accompanied only by lymph node enlargement. Combined fever and anaemia was only observed in 31 out of 8261 repeated observations in the study population. Diarrhoea was more commonly observed in isolation of other clinical signs or in combination with staring coat, while poor condition score was

more commonly observed in combination with staring coat than alone. The 25 most common clinical pictures (i.e. combinations of clinical signs) recorded over the course of initial and follow-up observations are shown in Figure 4.13. Clinical signs, or combinations of clinical signs, not included in the figure, were reported in less than 18 repeated observations on the study population.

4.3.11.1 Age-related Distributions of Clinical Signs

Clinical signs, whose distribution across the study population was significantly dependent on the age of cattle at the time of initial and follow-up visit rounds, are listed in Table 4.18.

Table 4.18 Age-related distributions of clinical signs in cattle.

Clinical Signs	Cattle Age (months)	Observations Total	Observations (+)	Model Estimates OR	Model Estimates 95% CI	F-value	p-value	Total Cattle
Staring	1 - 12	1277	634			$F_{2, 7654} = 213$	<.0001	879
Coat	13 - 24	2651	623	0.21	0.18 - 0.25			
	> 24	4607	1013	0.11	0.08 - 0.13			
Pale mucous	1 - 12	1278	76			$F_{2, 7656} = 35.4$	<.0001	879
membranes	13 - 24	2651	111	0.69	0.52 - 0.90			
	> 24	4608	238	0.32	0.24 - 0.44			
Ocular	1 - 12	1278	127			$F_{2, 7651} = 9.18$	0.0001	879
Discharge	13 - 24 ▲	2650	230	0.83	0.66 - 1.06			
	> 24	4604	351	0.59	0.45 - 0.77			
Fever	1 - 12	1276	162			$F_{2, 7649} = 96.7$	<.0001	879
(> 39.4 ºC)	13 - 24	2649	143	0.39	0.32 - 0.48			
	> 24	4605	86	0.15	0.12 - 0.20			
PCV	1 - 12	1278	108			$F_{2, 7658} = 21.2$	<.0001	882
(< 25%)	13 - 24 ▲	2654	181	0.85	0.68 - 1.07			
	> 24	4610	249	0.46	0.35 - 0.61			
Haemoglobin	1 - 12	1236	158			$F_{2, 7437} = 18.3$	<.0001	881
(< 8 g/dl)	13 - 24	2605	242	0.73	0.59 - 0.91			
	> 24	4479	351	0.62	0.24 - 0.38			

Odds ratio (OR) values of less than one indicate that the age group of interest (13 to 24 or > 24 months of age) had a lower probability of presenting with the clinical sign of interest as compared to calves (up to 12 months of age). ▲ Highlights cattle age groups where the proportion of observations with the abnormal condition of interest was not significantly different from that in calves, according to the T-test statistics (Data not shown). In such cases, the 95% confidence interval of the odds ratio includes the value "1".

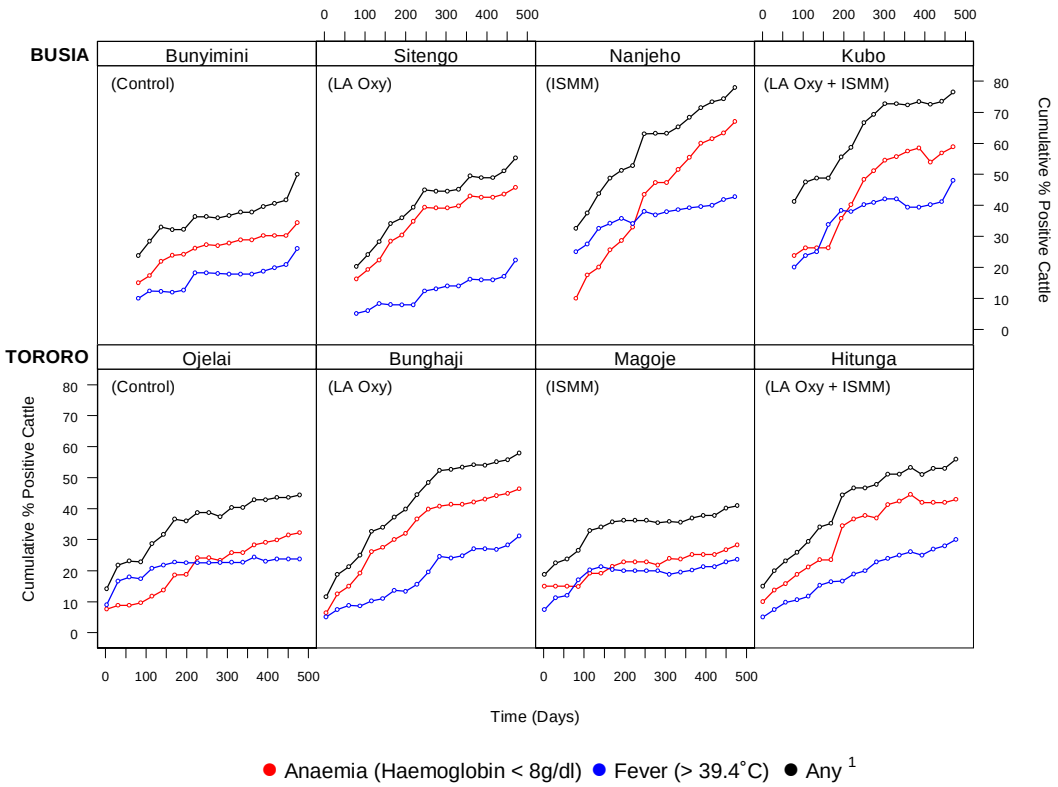
The proportions of lymph node enlargement and poor condition score observations, were uniformly distributed across age groups, although poor condition score was less common amongst adults ($p>0.05$; data not shown). Diarrhoea was the only clinical condition which was more frequent amongst

adult cattle (1.9% [89/4608]), but this finding was not statistically significant (p>0.05; data not shown). For the case of the remaining clinical signs, abnormal observations were mainly reported at the time of initial or follow-up physical examinations conducted on calves, and dropped significantly thereafter as cattle in the study grew older (Table 4.18).

4.3.11.2 Geographical Distributions of Anaemic and / or Pyretic Cattle

The cumulative prevalence of cattle diagnosed with either fever or anaemia (haemoglobin < 8 g/dl) in each study village, is shown in Figure 4.14. The slopes of the curves reflect the incidence of cattle presenting with either condition over the course of the longitudinal study. Both conditions were widespread amongst cattle from all villages, and were reported from 40 up to 80% of the animals depending on the village. The incidence of cattle presenting with fever or anaemia, was greatest in Nanjeho and Kubo (Busia), whereas lowest in Magoje, Ojelai (Tororo) and Bunyimini (Busia). In all villages, anaemia affected a greater percentage of cattle than fever.

Figure 4.14 Cumulative prevalences of anaemic and/or pyretic cattle.

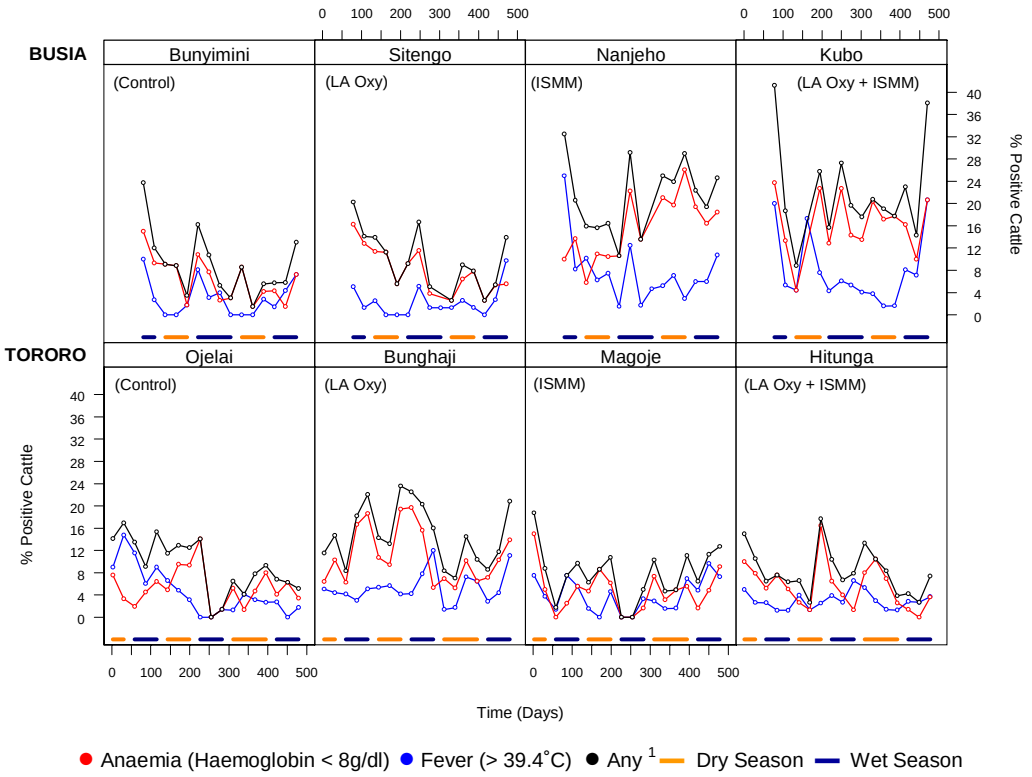


[1] Considers only those observations for which both haemoglobin and rectal temperature readings were available

4.3.11.3 Prevalence of Anaemic and / or Pyretic Cattle in Block Treated as Compared to in Control Villages

As suggested from the visual inspection of the data (Figure 4.15), the percentage of cattle affected by fever or anaemia at each visit round, appeared independent of whether block treatments had been administered, and of the type of drug used in the experimental trials.

Figure 4.15 Longitudinal prevalences of anaemic and/or pyretic cattle.



[1] Considers only those observations for which both haemoglobin and rectal temperature readings were available

4.3.12 Mortality

Mortality was reported in 40 (4.5%) of 893 cattle presented in the study. Unfortunately, post-mortem examination on cattle that died during the study was not possible, given that mortality losses occurred at times between main or intermediate visits, and no service was provided for farmers in remote villages to notify deaths immediately. In consequence, definitive diagnosis of cause of death (as notified by animal owners) was only available for the case of three animals (8%), of which two were reported to die due to strangulation and broken leg respectively, while a third died giving birth. Details

of clinical and laboratorial results obtained at the time of the last visit from 40 cattle which died during the longitudinal study, are given in Appendix 4.11.

Putative diagnosis of mortality losses, based on clinical examination and microscopy data obtained at the time of the last visit, was reported by the field veterinarian for only nine animals. The death of two clinically ill calves in Nanjeho, which presented severe signs of anorexia, abdominal enlargement or anaemia (haemoglobin = 5.5 g/dl), was attributed to anaplasmosis (5%). Another two animals from Magoje and a third animal from Bunghaji, were reported to die due to East Coast Fever (7.5%). However, *T. parva* infection was confirmed by PCR from only one animal at the time of the last visit. In these cattle, clinical illness was characterised amongst others, by moderate to severe anaemia with haemoglobin values ranging from 7.9 g/dl, 3.9 g/dl and 3 g/dl. The precise age of these individuals at the time of death, was unknown except for one animal from Magoje, which was identified as calf.

In four additional animals from Sitengo, death was attributed to multiple pathogen infections (10%). These animals presented severe clinical illness at the time of the last visit round. The first individual corresponded to a calf co-infected with Theileria spp., Anaplasma spp., *T. brucei*, *T. vivax*, *T. congolense* (at levels above the microscopy detection threshold) and gastrointestinal nematodes (moderate clinical infection: 1000 eggs gram $^{-1}$ faeces). The second corresponded to a calf co-infected with Theileria spp., Anaplasma spp., *T. brucei* (at levels above the PCR detection threshold) and gastrointestinal nematodes (sub-clinical infection: 900 eggs gram $^{-1}$ faeces). The third, was co-infected with *T. vivax* at levels above the microscopy detection threshold and gastrointestinal nematodes (heavy clinical infection: 1200 eggs gram $^{-1}$ faeces), whereas the fourth with Anaplasma spp. and *T. vivax* at levels above the PCR detection threshold. Age at the time of death was not available for the last two animals. Severe anaemia, with haemoglobin values of 4.6 g/dl, 2.4 g/dl, 3.7 g/dl and 5.4 g/dl respectively, was observed from all four individuals. No putative cause of death was specified by the field veterinarian for the remaining 28 mortality losses over the course the study. Infection accompanied by clinical signs, was observed in 19 of 28 mortality losses with no putative diagnosis at the time of the last visit (68%). Of these, eleven animals (58%) were co-infected with multiple pathogen

species, whereas eight (42%) were infected by a single pathogen. Notably, either PCR or microscopy data were incomplete for five of eight individuals from which a single pathogen was recorded.

4.4 Discussion

The discussion is centred on the results which are directly relevant to the bulk of work presented in the following chapters, and which stress the need for an integrated view of the community of endemic pathogens of African cattle as a whole.

4.4.1 Introduction

The bulk of research indicates that pathogens sharing a host may interact and result in infection outcomes that may be detrimental or beneficial to the host and/or the pathogens, in a manner which is not necessarily intuitive (Petney *et al.*, 1998). Synergistic and competitive interactions can influence the likelihood of a pathogen's successful transmission to other hosts and increase or decrease their overall pathogenic impact (Petney *et al.*, 1998). Co-infections involving protozoans (e.g. *T. congolense*) and helminths, can alter the maturation rates, egg shedding and weight of adult helminths stages (Griffin *et al.*, 1981b, Kaufmann *et al.*, 1992). Production of transmission stages of blood-borne protozoans may also increase or decrease when a second related parasite species is present (McKenzie *et al.*, 2002). Changes in chemoprophylactic or chemotherapeutic regimens may be required in mixed as compared to in single pathogen infections involving helminths and protozoans (Kaufmann *et al.*, 1992). Ongoing infection by one pathogen, can increase (Dobson *et al.*, 1995, Yoshida *et al.*, 1999) or decrease (Yacob *et al.*, 2004, Briand *et al.*, 2005) the probability of infection by, and the infection intensity of, a second pathogen, and determine whether a chronic or an acute form of infection will be established (Goossens *et al.*, 1997). Pathogen species interactions may also result in higher virulence or mortality (Dallas, 1976, Griffin *et al.*, 1981b, Kaufmann *et al.*, 1992, Fakae *et al.*, 1994, Goossens *et al.*, 1997, Sharma *et al.*, 2000) or reduced virulence or mortality (Palau *et al.*, 2003, Frontera *et al.*, 2005). Alteration of pre-patent periods in the presence of co-infectors have also been reported (Kaufmann *et al.*, 1992, Sharma *et al.*, 2000). In summary, the range and severity of

effects observed for interactions between pathogenic organisms is wide and, with reference to laboratory-based studies, few report no interactions between the pathogens under investigation.

The vast majority of research investigating the effect of inter-species interactions consist of laboratory-based experiments (Cox, 2001), but there are inherent problems in relating the findings of laboratory based experiments to the naturally occuring mixed infections (Christensen *et al.*, 1987). In laboratory studies, experimental hosts are often not the natural hosts of the pathogens in question, and the tightly controlled conditions that exist in laboratory based studies result in loss of the many external factors which may influence the course of infection in field settings.

4.4.2 Pathogen Species Interactions in the Study Areas

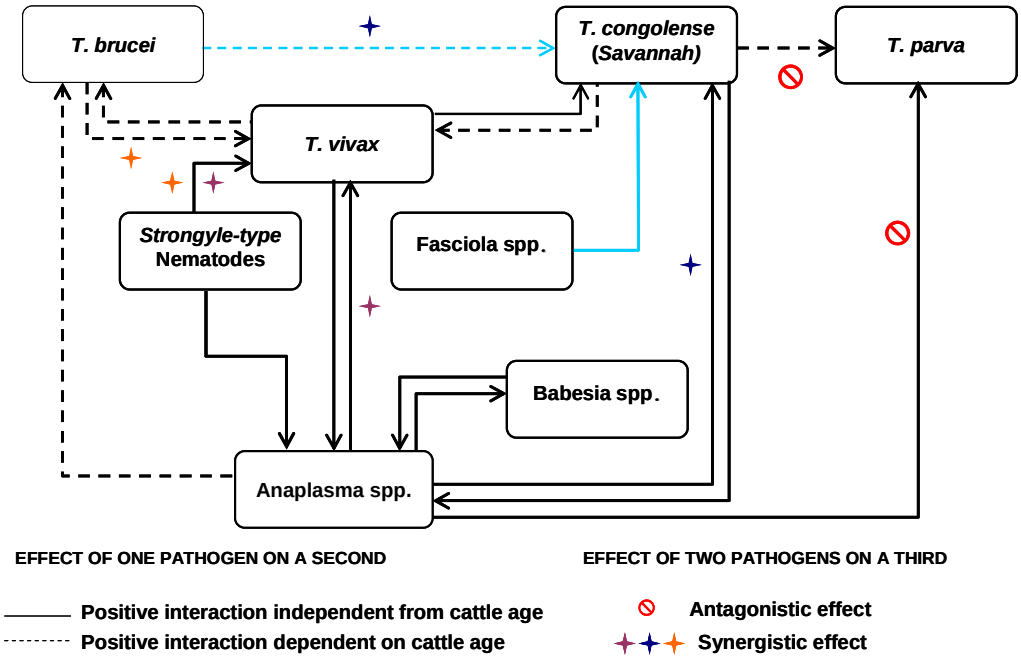
The work presented here has provided preliminary evidence that the distribution of pathogens amongst the bovine hosts, at infection intensities above detection threshold, is heavily influenced by the distribution of other co-circulating pathogens.

Positive associations – whereby the presence of a given pathogen increases the probability of patent infection by a second - were observed amongst pathogenic organisms sharing the same transmission route (i.e. Tsetse-transmitted trypanosomes) and amongst pathogens whose transmission routes differed. Negative associations were only observed in mixed infections involving at least three pathogens. In such cases, it was apparent that concomitant infection by two pathogenic organisms could decrease the probability of infection above the detection threshold of a third, as compared to when only one of the former pathogens was present.

The fact that negative associations were limited to interactions involving three pathogens, may have indicated competition for limited resources (Read *et al.*, 2001, Taylor *et al.*, 2005). Alternatively, negative associations may have been brought about by some density-dependent mechanism where the hosts, or the pathogens themselves, orchestrate infection intensities so that peaks of parasitaemia above the detection threshold occur for each species at different times. This has been reported in several longitudinal studies investigating the dynamics of mixed Plasmodium spp. infections. High parasitaemias of different Plasmodium spp. may alternate resulting in a statistically significant absence

(i.e. negative association) of mixed infections above the detection threshold of the diagnostic techniques (Earle, 1939, Bruce *et al.*, 2000a, Bruce *et al.*, 2003). Density-dependent mechanisms have been attributed to a combination of species-specific and species-trascending immunity, which is in turn thought to depend on the infection intensity attained by the various Plasmodium species (Bruce *et al.*, 2003).

Figure 4.16 Co-circulating pathogens shaping infection probability of other blood-borne pathogens in bovine hosts from the study areas.



The diagram presents the effect of infection by any pathogen species on the distribution of blood-borne pathogens only. Note the central role of Anaplasma spp. Arrows show the direction of the relationships. Blue arrows indicate pathogen pairs that decrease the probability of infection by a third when infecting the host contemporaneously, but that have no overall effect on the third pathogen when infecting the host separately. Black arrows indicate that the pathogen of interest is on its own capable of increasing the probability of infection by a second, and dashed arrows show that such effect is dependent on the age of infected hosts. Positive signs indicate pairs of pathogens that increase even further the probability of infection by a third in contemporaneous infections. Red circles highlight pathogen pairs for which the positive effect on a third is reduced when infecting the host concomitantly as compared to when infecting the host separately.

In contrast to the density-dependent mechanisms previously discussed, positive associations could have arisen from the absence of such mechanisms in infections involving two pathogens, if lower infection intensities were attained in such cases compared to when three pathogens were present (Figure 4.16). This is consistent with studies which report mixed Plasmodium species infections only

when the infection intensity of each species remains low (Ritchie, 1988). Positive associations may have alternatively reflected that presence of one pathogen increases the infection intensity or the production of transmission stages of a second (Dobson *et al.*, 1995, Yoshida *et al.*, 1999, McKenzie *et al.*, 2002), or that the presence of one predisposes to infection by the other. *T. congolense* infection, for example, is thought to increase the feeding time of *R. appendiculatus* on rabbits. This could increase the chances of *T. parva* transmission if the same were to be true when ticks feed on cattle (Heller-Haupt *et al.*, 1983). Similarly, reduced resistance to infestation with *A. variegatum* ticks, has been reported in cattle infected with *T. congolense* and *B. bigemina* (Dossa *et al.*, 1996).

A number of positive pathogen associations reported in this thesis, have been found to be dependent on the age of cattle, suggesting that inter-species interactions may, at least partially, be orchestrated by the immune system of the host (Read *et al.*, 2001, Bruce *et al.*, 2003, Lello *et al.*, 2004). A puzzling finding was that positive age-dependent associations were consistently limited to calves, or to a lesser extend adult animals, but were never recorded from juvenile cattle (13 – 24 months of age). Such age-dependent interactions invariably involved trypanosomes, although the patterns differed for associations involving *T. brucei* or *T. congolense (Savannah)* as compared to *T. vivax*. A deeper understanding of how pathogen associations vary with cattle age (particularly in relation to trypanosome species), may provide invaluable clues which can be used to explain this phenomenon.

Anaplasma spp. affected the distribution of the widest range of blood-borne pathogens in the study areas, increasing in most cases the probability of patent infection by a second pathogen (Figure 4.16). Anaplasma spp. was the only blood-borne pathogen which increased the probability of detecting nematodes and Fasciola spp. eggs in faeces, and which increased the intensity of nematodes infection, as measured by the eggs counts found in faecal samplings (Data not shown). These findings are of particular relevance, given that Anaplasma spp. was one of the most common pathogens in the study areas, with 83.5% of the cattle testing positive on at least one occasion over the study period. Hence, any effect exercised by this pathogen is likely to be present in a large proportion of individuals. Although gastrointestinal nematodes were more common than Anaplasma spp., the former did not appear to increase the probability of patent infection of such a wide range of pathogenic organisms

(Figure 4.16). This observation suggests that the range of associations detected in the case of Anaplasma spp. could not merely be explained by the widespread distribution of this organism amongst the study population.

4.4.3 Implications of the Field Results

While laboratory-based studies widely report significant interactions between pathogens, field studies often fail to report the existence of interactions, and conclude that interactions between species play no, or at most a minor role in shaping the dynamics of individual members of pathogen communities (Behnke *et al.*, 2001).

Statistical analysis of parasitological data provide powerful tools to investigate the biological processes underlying parasites infection and disease (Pacala *et al.*, 1988, Wilson *et al.*, 1996, Wilson *et al.*, 1997, Zinsstag *et al.*, 2000, Paterson, 2001). Surveys of statistical methods employed in field studies have nevertheless suggested that adequate statistical techniques are seldom employed in parasitology (Wilson *et al.*, 1997, Paterson *et al.*, 2003). In addition, an ideal setting to study natural inter-species interactions necessitates a high prevalence of mixed infections, a requirement which is rarely met in cross-sectional studies (Bruce *et al.*, 2003). The wide range of factors that can exert an influence upon the establishment of pathogen interactions, the requirement for longitudinal studies, the vast number of confounding variables and the under-use of appropriate statistical tools, all combine to hinder the understanding of natural pathogen interactions, and may have all contributed to the disparity between results obtained from field- and laboratory-based studies.

Chapter 5: Interactions Amongst Common Endemic Pathogens of African Cattle: Implications for Pathogenesis

5.1 Introduction

5.1.1 Multiple Pathogen Infections and Morbidity Studies

Our knowledge of the morbidity associated with tick-borne pathogens (Anderson *et al.*, 1972, Dolan *et al.*, 1984, Gale *et al.*, 1996, Bock *et al.*, 1997), trypanosomes (Akol *et al.*, 1986, Moloo *et al.*, 1999, Bengaly *et al.*, 2002, Nok *et al.*, 2003) and helminths (Bitakaramire *et al.*, 1969, Horchner, 1969, Knight, 1978) is often derived from 'single infection' experimental studies. Our understanding is also brought about by field studies containing reports of morbidity, but many of these studies attribute the morbidity findings to single pathogens without determining if others are present (Moll *et al.*, 1986, Mbassa *et al.*, 1994, Minjauw *et al.*, 1998b, Gitau *et al.*, 2001, Muraguri *et al.*, 2005).

In field settings, where mixed infections are the norm, morbidity is likely to be more complex than that found under experimental conditions (Christensen *et al.*, 1987) and probably less clear-cut than suggested by field studies attributing morbidity findings to the pathogen of interest (Petney *et al.*, 1998, Cox, 2001). For example, characteristically, only mild anaemia is reported in cattle-derived *T. parva* infections (Maxie *et al.*, 1982), although this is not a constant finding in ECF terminal stages (Norval *et al.*, 1992). Deviation of the clinical disease from the classical cattle-derived *T. parva* infection has nevertheless been acknowledged in many parts of Tanzania (Mbassa *et al.*, 1994). This parallel "syndrome" has been claimed to result from mixed infections involving *T. parva*, Anaplasma spp. and Babesia spp., but no formal work has been carried out to confirm these suspicions (Mbassa *et al.*, 1994). A second example is that of the so-called Bovine Cerebral Theileriosis (BCT), or turning sickness, which has been reported regularly but at a low incidence, throughout South Africa, Burundi, Uganda, Kenya and Tanzania (Mettam *et al.*, 1936, Flanagan *et al.*, 1957, De Vos *et al.*, 1981, Loomu, 1988, Machange, 1997, ITTBDP, 1999, 2000). BCT is characterised by nervous symptoms and uncontrollable movements (Mettam *et al.*, 1936), and has been associated to the presence of *Theileria*

taurotragi schizonts in brain capillaries (Lynen *et al.*, 2001). However, *T. taurotragi* is normally benign in cattle (Grootenhuis *et al.*, 1979). Several lines of preliminary evidence have suggested that BCT may be expressed in concomitant infections perhaps involving trypanosomes (Moll *et al.*, 1985), or that BCT may be closely linked to exposure with *T. parva* (Bakuname *et al.*, 2002). Co-factors responsible for the expression of BCT are currently being investigated, accounting for the potential effect of the community of co-circulating pathogens (Bakuname *et al.*, 2002).

5.1.2 Specific Objectives

There is currently increasing recognition that, with regards to animal health in developing countries, research and control efforts will require an integrated understanding of the whole community of pathogens and not just each of its individual components (Torr *et al.*, 2002). This work is the first to describe and quantify the relationship between number of co-infective pathogens and onset and severity of clinical signs in African cattle by means of objective indicators of animal health. It also compares the morbidity associated with the pathogen of interest when omitting information on co-circulating pathogens ("single pathogen focus"), with that observed when the whole community of pathogens is considered ("integrated focus"), and investigates whether certain pathogen species interact, causing a more - or less- detrimental effect on cattle health than would be expected from the mere sum of their individual effects in natural settings. The role of infection intensity on the establishment and outcome of natural pathogen interactions is taken into account by differentiating infection status data above the PCR or microscopy detection threshold. In summary, this chapter sets out to provide the first integrated view of the impact of the whole community of endemic pathogens on African cattle health.

5.2 Methods

5.2.1 Selection of Clinical Signs for Addressing the Specific Objectives

Haemoglobin depletion was found to be the optimum animal health indicator – to address the specific objectives of this chapter - on the basis of seven criteria as follows:

Pathognomonic Significance: Haemoglobin was one of the most pathognomonic indicators of infection by the pathogens considered. In contrast, rough / staring coat, poor condition score and some of the other clinical signs recorded in the study (i.e. ocular, nasal and oculo-nasal discharge) are frequently associated with non-systemic processes or infections by pathogens other than those considered. For example, a number of viral respiratory complexes can cause ocular and/or nasal discharge (OIE, 2004), whilst weight loss and poor condition score are also dependent on nutrition, season, management practices, calving (Rutter *et al.*, 1984, Wright *et al.*, 1986, Cissé *et al.*, 1995, Ezanno *et al.*, 2003) and even levels of tick infestation amongst other causes (Pegram *et al.*, 1990, Pegram *et al.*, 1991, Scholtz *et al.*, 1991).

Ubiquity: All the pathogens considered in this study have been associated with anaemia, either in natural or experimental hosts. Numerous examples exist in the literature for trypanosomes (Grootenhuis *et al.*, 1990, Connor, 1994, Akinbamijo *et al.*, 1998, Mwangi *et al.*, 1998, Moloo *et al.*, 1999, Magona *et al.*, 2000), *Theileria parva* (Shatry *et al.*, 1981, Maxie *et al.*, 1982, Moll *et al.*, 1986, Mbassa *et al.*, 1994), Anaplasma spp. (Jatkar *et al.*, 1967, 1969, Anderson *et al.*, 1972, Gale *et al.*, 1996), Babesia spp. (Omuse, 1978, Hildebrandt, 1981, Ristic, 1981, Losos, 1986); Fasciola spp. (Bitakaramire *et al.*, 1970, Yadav *et al.*, 1999, Ganga *et al.*, 2006) and gastrointestinal nematodes (Harness *et al.*, 1970, Harness *et al.*, 1971, Kaufmann *et al.*, 1989, Waruiru *et al.*, 1993, Gennari *et al.*, 1995).

Objectivity: Haemoglobin (g/dl), relying on a reading of indicated values (Section 3.1.2.2), did not require any subjective judgement on the part of the field veterinarians.

Direct Measurements: Animals with a packed cell volume (PCV) lower than 25% or haemoglobin concentration lower than 8 g/dl were considered anaemic (Schalm *et al.*, 1975), as were those presenting with pale mucous membranes. Haemoglobin, however, was the only direct measure of onset and severity of anaemia and was therefore preferred to indirect indicators of the same condition.

Sensitivity: Haemoglobin (g/dl) was recorded in a continuous scale, thus allowing a more sensitive quantification of the relative impact of one or several pathogens on animal health as compared to other health indicators.

Clinical Significance: Anaemia, as measured by circulating haemoglobin concentrations (g/dl), was more likely to be a finding of clinical significance (i.e. reflect disease) than other abnormal conditions recorded in the study. Lymph node enlargement, for example, may occur in infected cattle but may not necessarily reflect that the health of the animals is compromised. This was evident from data in this study, where transient superficial lymph node enlargement upon infection with *T. parva* was observed in otherwise healthy adult cattle (Data not shown). Equally, other signs such as poor condition score, could simply reflect time since calving or seasonal availability of food resources, rather than disease (Ezanno *et al.*, 2003).

Availability: Anaemia (i.e. < 8 g/dl) was recorded from 44% of cattle at some stage during the study, implying that a sufficient sample size of abnormal observations was available for statistical analysis. Other clinical signs, in contrast, were not prevalent enough to allow a reliable estimate of the effect of single and mixed pathogen infections on animal health (Table 4.17).

Rectal temperature fulfilled six of the seven criteria listed above, and was therefore preferred to investigate whether the conclusions obtained during the analysis of haemoglobin (g/dl) data may apply to other clinical signs. Rectal temperature allowed objective estimation of a condition of clinical significance in the population, fever, which was said to occur above 39.4ºC (Minjauw *et al.*, 1998b, Maloo *et al.*, 2001b).

5.2.2 Generalities of the Statistical Analyses

5.2.2.1 Pathogens Considered

A total of nine pathogens (or pathogen groups) have been considered to address the specific objectives of this chapter, including (1) *T. brucei*, (2) *T. vivax* and (3) *T. congolense* tsetse transmitted protozoans; (4) *T. parva* , (5) Theileria spp., (6) Anaplasma spp. and (7) Babesia spp. tick-transmitted pathogens; (8) Fasciola spp. and (9) "*Strongyle*-type" nematodes pasture-transmitted helminths.

In the following sections, Theileria spp. positive observations are those obtained by microscopy that could not be classified as *T. parva* on the basis of the *T. parva* – specific PCR test. Babesia spp. has been taken into account as an individual component of the pathogen community in all the analyses. However, due to the small number of Babesia spp. positive observations in the study, the individual effect of this pathogen on animal health has not been addressed.

5.2.2.2 Statistical Analyses

Linear mixed-effects models (LME; Section 3.4.4) were used to analyse haemoglobin and rectal temperature data in relation to infection with single or multiple pathogen species or genus. In the LME analyses, F-tests evaluate the significance of each independent variable in the model by establishing whether at least one of the levels in the independent variable, is significantly different from the reference level concerning the dependent or response variable. T-tests are in contrast used to establish the significance of level-specific comparisons with the reference level.

LME analyses excluded animals for which no repeated observations were available.

5.2.3 Evaluation of Bias

5.2.3.1 Effect of Drug Treatment on the Analyses of Haemoglobin (g/dl) and Rectal Temperature Data

(Methodology for the Analysis Presented in Table 5.2)

The two-sample Kolmogorov-Smirnov (K-S) goodness of fit test is a simple technique that tests whether two distributions are significantly different from each other in at least one of the parameters that define the distribution (i.e. mean, variance, skew or kurtosis). The K-S test works on cumulative distribution functions (CDFs), which give the probability that a randomly selected value of $X \leq x$:

$$F(x) = P[X \leq x]$$

In S-plus, the CDFs of both distributions can be plotted and visually compared, while the K-S goodness of fit test can be used to calculate the probability that the two distributions are actually the same (Crawley, 2002a).

Two-sample K-S tests were used to evaluate whether inclusion of treated cases in the datasets for analysis would alter the distribution of haemoglobin and rectal temperature values recorded from the study population or, on the contrary, withdrawal of treated cases was unnecessary. In each K-S test, the distribution of either haemoglobin (g/dl) or rectal temperature (ºC) values in samplings positive for the pathogen of interest, was compared to that found when positive cases, preceded by drug treatment within the previous 28 days, were excluded. No specific analysis was conducted for Babesia spp. because of the low number of positive cases recorded in the study (n = 78), and the even lower number of Babesia spp.-positive observations that were preceded by either LA oxytetracycline (n = 7) or diminazene aceturate (n = 9) administration in the previous 28 days.

5.2.3.2 Effect Diagnostic Technique on Pathogen Species-Specific Morbidity

(Methodology for the Analysis Presented in Table 5.3)

Prior to addressing the specific objectives, the effect of each trypanosoma species on haemoglobin (g/dl) was evaluated by considering the technique with which trypanosoma-positive observations were obtained (i.e. either microscopy or PCR).

A separate LME analysis was conducted for *T. brucei*, *T. congolense* and *T. vivax*. Samples included in each analysis, were those for which the infection status for either *T. brucei*, *T. vivax* or *T. congolense* had been tested using both PCR and microscopy. In each analysis, haemoglobin was fitted as the response variable while the infection status for Trypanosoma spp. was fitted as the fixed-effect in the form of a three-level categorical variable. The levels corresponded to whether (i) samples were negative by both PCR and microscopy, (ii) positive by PCR only or (iii) positive by microscopy regardless of the PCR diagnosis. In each analysis, the REML estimated mean haemoglobin (RMHb) for each variable level (ii, iii), was compared to that of uninfected samples (i), but the reference variable level was changed depending on the question addressed. For *T. brucei* and *T. vivax*, haemoglobin was modelled considering different intercepts and time slopes for village, and different intercepts and time slopes for each animal within each village. The impact of *T. congolense* on haemoglobin, was

modelled considering different intercepts for village and different intercepts and time slopes for each animal within each village.

Parallel Analysis of Two Datasets (A & B)

The effect of each trypanosome species on haemoglobin (g/dl), varied depending on the technique with which positive observations were obtained (Section 5.3.1.1). As a result, two datasets were analysed in parallel to address two objectives in this chapter (Sections 5.2.5 and 5.2.6). Both datasets considered observations for which infection status for all nine pathogens (or pathogen groups) was known, and for which haemoglobin concentration was also available. The first dataset (Dataset 'A'), however, only took into account PCR data for the three trypanosoma species, whilst the second (Dataset 'B') included only microscopy data for the three trypanosome species.

The parallel use of two datasets was justifiable. Because PCR testing for *T. vivax* and *T. congolense* was only conducted for a subset of samples in the study, the number of microscopy-positive observations available for analysis was reduced substantially in a data frame considering samples tested by both techniques. This, in turn, resulted in far less reliable estimates of the impact of microscopy-positive trypanosome infections on haemoglobin.

It is not possible to compare estimates derived from Dataset A and Dataset B, as the former was a non-random subset of the latter and the distribution of haemoglobin values in the two datasets was significantly different (Two-sample Kolmogorov-Smirnov test = 0.32; p <.0001). This resulted from Dataset A being restricted to samples tested for *T. vivax* and *T. congolonse (Savannah)* by PCR. Molecular testing for these species ceased relatively early in the study (except for the last two visit rounds where infection status by PCR was also available). As a result, the proportion of calves in Dataset A was higher than in Dataset B, as was the prevalence of pathogens and mixed infections (Data not shown).

Within each dataset (A or B), the estimated impact of each pathogen and any combination of pathogens on haemoglobin (g/dl) can be reliably compared. Where the interest is to establish

comparisons between effects observed in the different datasets, additional data analysis targeting specific questions was necessary (Section 5.2.7).

5.2.4 Relationship between Number of Co-Infecting Pathogens and Onset and Severity of Anaemia and Fever

5.2.4.1 LME Analysis of the Effect of Number of Pathogens on Haemoglobin

(Methodology for the Analysis Presented in Figure 5.2)

A separate LME analysis of the effect of number of pathogens on haemoglobin was conducted for cattle age groups for which the relationship of interest differed significantly. Each analysis considered those observations for which haemoglobin readings were available and for which cattle age and the infection status for all nine pathogens (or pathogen groups) was known. No distinction was made for Trypanosoma spp. infections above the PCR or the microscopy detection threshold. For each Trypanosoma spp., a test sample was either positive or negative if infection status was recorded by means of at least one of the two available techniques. In each analysis, haemoglobin was fitted as the dependent variable whilst the number of pathogens was fitted as a fixed-effect in the form of a five-level categorical variable. In the analyses, the REML estimated mean haemoglobin (RMHb) for each variable level (each level representing infections with one, two, three, or four or more pathogens) was compared to that in uninfected samples. Haemoglobin was modelled considering different intercepts and time slopes for village, and different intercepts and time slopes for each animal within village.

Variation across Cattle Age groups

Variation in the relationship of interest across age groups, was investigated through assessing the significance of the interaction term (:) in the following model:

Maximal Model: 'Haemoglobin ~ Cattle Age + Number of Pathogens + (Cattle Age : Number of Pathogens)'

Where: "Cattle Age" was specified as a three-level categorical variable (Calf [1-12 months], Juvenile [13-24 months] or adult [>24 months of age]), whilst "Number of Pathogens" as a five-level categorical

variable (0, 1, 2, 3 or 4 or more pathogens). Other level groupings were also explored for the variable "Cattle Age".

5.2.4.2 LME Analysis of the Effect of Number of Pathogens on Rectal Temperature

(Methodology for the Analysis Presented in Figure 5.4)

A separate LME analysis was conducted for different cattle age groups following the same methodology and criteria as explained for the case of haemoglobin data, with the following modifications. Each analysis considered those observations for which rectal temperature readings were available. Rectal temperature was fitted as the dependent variable. In the case of calves and adults, rectal temperature was modelled considering different intercepts and time slopes for each village, and different intercepts for each animal within each village. In the case of juveniles, rectal temperature was modelled considering different intercepts for each village and each animal within each village.

5.2.5 "A Single Pathogen Focus" – Implications for Pathogen Species-Specific Attributed Morbidity

5.2.5.1 Pathogen-Specific Impact on Haemoglobin when Infection Status for Other Pathogens is Omitted

(Methodology for the Analysis Presented in Table 5.4)

A separate LME analysis was conducted to evaluate the effect of each pathogen on haemoglobin. The analysis was repeated using Dataset A and Dataset B. In each pathogen-specific analysis, haemoglobin was specified as the response variable and the infection status for the pathogen of interest as the independent categorical variable (fixed-effect) with two levels (infected or uninfected). The REML estimated mean haemoglobin (RMHb) in infected samples, was in all cases compared to that in uninfected samples. In the case of Dataset A, haemoglobin was modelled considering different intercepts for village, and different intercepts and time slopes for each animal within village. Depending on the pathogen considered, different time slopes for village were also considered. In the case of

Dataset B, haemoglobin was always modelled considering different intercepts and time slopes for village, and different intercepts and time slopes for each animal within village.

5.2.5.2 Pathogen-Specific Impact on Haemoglobin when Infection Status for Other Pathogens is Considered – A Comparison

(Methodology for the Analysis Presented in Table 5.5)

A separate LME analysis was conducted to evaluate the effect of each pathogen on haemoglobin, whilst taking into account the infection status for other pathogens. The analysis was repeated using Dataset A and Dataset B. In these analyses, the infection status for the pathogen of interest was specified as a fixed-effect in the form of a five-level categorical variable. The five levels corresponded to whether samples were: (1) negative for all of the pathogens considered, (2) positive for the pathogen of interest but negative for all of the others considered, (3) positive for the pathogen of interest and at least a second pathogen, (4) positive for only one pathogen other than that of interest, or (5) positive for more than one pathogen other than that of interest. In each analysis, the REML estimated mean haemoglobin (RMHb) for each variable level, was compared to that in uninfected samples. In the case of Dataset A, haemoglobin was modelled considering different intercepts for village and different intercepts and time slopes for each animal within village. In the case of Dataset B, haemoglobin was modelled considering different intercepts and time slopes for village and different intercepts and time slopes for each animal within village.

(Methodology for the Analysis Presented in Table 5.6)

Re-examination of level-specific T-test statistics, when the RMHb in mixed infections involving the pathogen of interest was specified as the reference variable level, allowed assessment of the significance of differences between the mean haemoglobin in mixed as compared to in single infections involving the pathogen of interest, and in mixed infections involving or not the pathogen of interest.

5.2.6 Pathogen Species-Specific Interactions on Incidence and Severity of Anaemia

5.2.6.1 Definition of Interaction

An 'interaction' by the pathogens on haemoglobin was said to occur when concomitant infections resulted in significantly more haemoglobin reduction (positive interaction) or significantly less haemoglobin reduction (negative interaction) than the mere sum of the effect associated to each pathogen when infecting the host separately.

5.2.6.2 Analysis of Pathogen Species-Specific Interactions

(Methodology for the Analysis Presented in Table 5.7)

A separate LME analysis was conducted for Dataset A and Dataset B. Each analysis considered only those pathogens with a significant effect on haemoglobin, as determined from the analyses in Section 5.2.5.1. In each maximal model (i.e. for Dataset A & B respectively), haemoglobin was fitted as the response variable. The infection status for the pathogens of interest were fitted as fixed-effects in the form of two-level categorical variables (infected or uninfected), together with all the possible interactions between any two pathogens. The sample size of infected cases in Datasets A or B was insufficient to evaluate three - or more - way interactions. Pathogens were considered in the fixed-effects formula by decreasing REML estimated impact on haemoglobin, according to a model containing only main terms but no interactions. Minimum models containing only significant terms, were obtained after a process of step-down simplification of each maximal model. In each analysis, the REML estimated mean haemoglobin (RMHb) in samples infected by the pathogen of interest (main term), was compared to that in uninfected samples. In contrast, the RMHb in samples infected by any pathogen pair, was compared to that obtained when the individual effect of each pathogen in the pair (i.e. main terms) was subtracted from the RMHb in uninfected samples.

In the case of Dataset A, haemoglobin was modelled considering different intercepts for village and different intercepts and time slopes for each animal within each village. In the case of Dataset B, haemoglobin was modelled considering different intercepts and time slopes for village, and different intercepts and time slopes for each animal within each village.

5.2.6.3 Calculation of REML Estimated Mean Haemoglobin (RMHb) from Model Coefficients

(Methodology for Figure 5.6)

Model coefficients obtained in the analyses described in Section 5.2.6.2, represent differences between (i) the RMHb in uninfected samples and that in samples infected with the pathogen of interest, and (ii) the expected and observed RMHb in samples infected by any pathogen pair.

The RMHb associated to any pathogen was calculated by subtracting from the mean haemoglobin of uninfected samples, the coefficient associated to the pathogen of interest. The RMHb associated to any combination of pathogens was calculated by subtracting from the mean haemoglobin of uninfected samples, the effect of each pathogen individually and the effect of their interaction if significant. RMHb 95% confidence intervals, were obtained by performing the same calculations on the approximate 95% confidence intervals, obtained using the approximate distributions for the REML estimates and the conditional T-tests.

5.2.6.4 Parallel Analyses of Pathogen Species-Specific Interactions

For robustness, the analyses described in Section 5.2.6.2 were validated by conducting several other analyses as follows: (i) By correcting each model (i.e. for Dataset A and Dataset B) by cattle age (either calves, juveniles or adult cattle); (ii) By considering as well pathogens with no significant effect on haemoglobin as determined from the analyses in Section 5.2.5.1; (iii) By examining each possible combination of two pathogens separately, using all the data points for which the infection status of the pathogens in the combination were available (Table 5.1); and (iv) By analysing each possible combination of two pathogens as explained, whilst correcting the analysis by the effect of cattle age (either calves, juveniles or adult cattle). In cases were a confounding effect was suspected, two by two explorations were corrected by the infection status of additional pathogens as well as cattle age. (v) Interactions on haemoglobin brought about by any combination of two Trypanosoma spp. above either the PCR or the microscopy detection threshold, were also examined. This analysis considered six two-level (i.e. infected or uninfected) categorical variables as follows: *T. congolense* (microscopy data), *T.*

vivax (microscopy data), *T. brucei* (microscopy data), *T. congolense (Savannah)* (PCR data), *T. vivax* (PCR data) and *T. brucei* (PCR data). Only interactions between any two variables were explored. The model considered those samples where, in addition to haemoglobin readings, both microscopy and PCR data for the three trypanosome species were available. This analysis was finally repeated by correcting for cattle age, and for the presence of pathogens other than trypanosomes.

Table 5.1 Combinations of two pathogens tested for interactions on haemoglobin (g/dl).

	A	Tp	T	Tc (P)	Tv (P)	Tb (P)	Tc (M)	Tv (M)	Tb (M)	N	F
A		+	+	+	+	+	+	+	+	+	+
Tp			+	+	+	+	+	+	+	+	+
T				+	+	+	+	+	+	+	+
Tc (P)					+	+		+	+	+	+
Tv (P)						+	+		+	+	+
Tb (P)							+	+		+	+
Tc (M)								+	+	+	+
Tv (M)									+	+	+
Tb (M)										+	+
N											+
F											

The combinations of pathogens tested are indicated with a plus sign. The abbreviations correspond to: Anaplasma spp. (A), *T. parva* (Tp), Theileria spp. (T), *T. congolense* (Tc), *T. vivax* (Tv), *T. brucei* (Tb), nematodes (N), Fasciola spp. (F), PCR (P) and microscopy (M).

In all analyses (i to v), the infection status for the pathogens of interest was considered in the fixed-effects formula by decreasing REML estimated impact on haemoglobin, according to a model containing only main terms but no interactions. Whenever cattle age was accounted for in the model, it was always fitted as the first main term.

To avoid mistaking confounded pathogen species-specific interactions on haemoglobin as true interactions, potential age-dependent associations involving any two pathogens were necessarily examined in some instances, if these had not been previously characterised in Section 4.3.9. Potential associations were investigated using GlmmPQL with binomial error structure as explained in Section 4.2.5.3.

5.2.7 Effect of Infection Intensity on Pathogen Species-Specific Interactions

(Methodology for the Analysis Presented in Table 5.9)

Assuming that positive microscopy cases are representative of higher mean trypanosome parasitaemias as compared to positive PCR cases, the following analyses tested the null hypothesis that infection intensity (either lower [above PCR threshold] or higher [above microscopy threshold]), is a factor determining the establishment of interactions between trypanosomes and other pathogens on haemoglobin. Because Dataset A and Dataset B were technically not comparable (Section 5.2.3.2), the data was necessarily subjected to further analysis in order to accept or reject the null hypothesis. Independent data frames were created to investigate "apparent" infection-intensity dependent interactions (as suggested from the analyses in Section 5.2.6.2) separately. Model specification was as follows:

Maximal Model: 'Haemoglobin ~ T_y m + X + T_y p + (T_y m : X) + (X : T_y p)'

Where the abbreviations correspond to: **T_ym** = Trypanosoma spp. infection above the microscopy threshold (either *T. brucei*, *T. vivax* or *T. congolense*)*;* **X** = Infection by a second pathogen; **T_yp** = Trypanosoma spp. infection above the PCR detection threshold (either *T. brucei*, *T. vivax* or *T. congolense [Savannah]*).

Main terms were specified as two-level (infected or uninfected) categorical variables. Pathogens were considered in the fixed-effects formula by decreasing REML estimated impact on haemoglobin, according to a model containing main terms but no interactions. Minimum models (i.e. those containing only significant terms), were obtained through a process of backwards simplification of the maximal models. In all cases, the number of observations considered was substantially higher than in Dataset A. Greater numbers of observations available for analysis were achieved by considering all samples for which the infection status for the pathogens of interest was known, regardless of the availability of PCR or microscopy data for other pathogens. Greater sample sizes were also achieved by allowing missing values throughout the process of model simplification, by means of the "na.action = na.omit" directive. Removal of non-significant terms automatically enabled the analysis of a larger number of

observations in the simplified model, thus maximizing the number of trypanosome cases by microscopy available for analysis. Infection intensity-dependant pathogen interactions were said to occur only when either (T_y m : X) or (X : T_y p) were retained in the minimum models, but not both. Depending on the model, haemoglobin was modelled considering different intercepts for village and different intercepts and time slopes for each animal within each village, or different intercepts and time slopes for village, and different intercepts and time slopes for each animal within each village.

5.3 Results

5.3.1 Evaluation of Bias

5.3.1.1 Effect of Drug Treatment on the Analysis of Haemoglobin (g/dl) and Rectal Temperature Data

Inclusion of treated cases did not alter the distributions of haemoglobin or rectal temperature values obtained from pathogen-positive observations (Table 5.2). The p-value associated to each K-S test was in all cases equal to or greater than 0.05. A marginally significant effect ($p = 0.04$) on the distribution of haemoglobin values was only observed when Trypanosoma spp. cases by PCR, preceded by combined ISMM and diminazene treatment, were excluded. In consequence, and because exclusion of treated cases would have reduced substantially the number of positive observations available for analysis, treated cases were not withdrawn from the datasets used in subsequent analyses.

Table 5.2 Effect of drug treatment on the analysis of haemoglobin (g/dl) and rectal temperature (ºC) data.

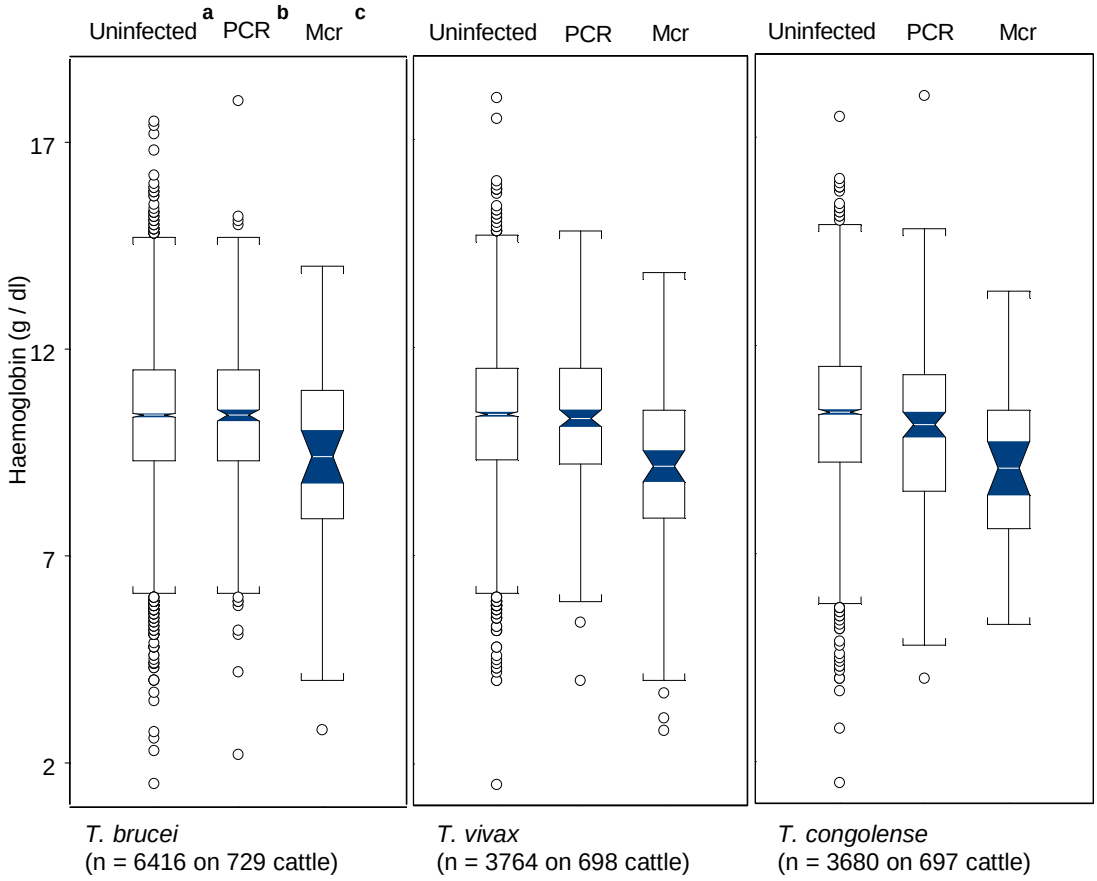
Pathogens	Positive observations [a]	Withdrawal criteria [b]	Withdrawn positive observations [c]	K-S test p- value [d]
Haemoglobin (g / dl)				
Anaplasma spp.	2142	LA Oxytetracycline	234	0.083
Theileria spp.	1830	LA Oxytetracycline	196	0.073
T. parva	526	LA Oxytetracycline	54	0.075
Trypanosoma spp. (PCR)	761	ISMM + Diminazene	40	0.040
Trypanosoma spp. (MCR)	388	ISMM + Diminazene	16	0.056
Nematodes	2536	Albendazole	103	0.048
Fasciola spp.	1166	Albendazole	70	0.066
Any pathogen (PCR – T) [1]	1962	Any treatment	368	0.109
Any pathogen (MCR - T) [2]	4233	Any treatment	846	0.123
Rectal Temperature (º C)				
Anaplasma spp.	2216	LA Oxytetracycline	236	0.100
Theileria spp.	1885	LA Oxytetracycline	201	0.085
T. parva	535	LA Oxytetracycline	54	0.114
Trypanosoma spp. (PCR)	779	ISMM + Diminazene	40	0.047
Trypanosoma spp. (MCR)	414	ISMM + Diminazene	18	0.047
Nematodes	2554	Albendazole	105	0.087
Fasciola spp.	1212	Albendazole	75	0.075
Any pathogen (PCR - T) [1]	1967	Any treatment	369	0.180
Any pathogen (MCR - T) [2]	4333	Any treatment	874	0.135

MCR = Microscopy. [b] Criteria for exclusion of positive observations. [c] Positive observations preceded by the administration of treatment within the previous 28 days. [d] Compares the distribution of haemoglobin (g/dl) or rectal temperature (ºC) values in [a] versus [a - c]. [1] Samples positive to any of the following: Anaplasma spp., Babesia spp., Theileria spp., *T. parva*, nematodes, Fasciola spp. or Trypanosoma spp. by PCR. [2] Samples positive to any of the pathogens above, but includes Trypanosoma spp.-positive observations by microscopy, rather than by PCR.

5.3.1.2 Effect of Diagnostic Technique on Pathogen-Specific Morbidity

The distribution of haemoglobin values in samples from uninfected animals, and in samples from animals infected with either *T. brucei*, *T. vivax* or *T. congolense* –above microscopy or PCR detection thresholds - are shown in the form of box-plots in Figure 5.1. Each box-and-whisker plot indicates the lower and upper quartiles representing the haemoglobin values below which 25% and 75% of the observations lie respectively. The median haemoglobin concentration is indicated within each box by a white horizontal bar. The whiskers show the range of observations excluding outliers, which are extreme haemoglobin values shown as white dots.

Figure 5.1 Box – and – Whisker plots of the haemoglobin concentration (g/dl) in *T. brucei*, *T. vivax* and *T. congolense* positive samples by either PCR or microscopy.



[a] Haemoglobin values from samples negative by PCR and microscopy. [b] Haemoglobin values from samples positive by PCR only. [c] Haemoglobin values from samples positive by microscopy, regardless of the PCR diagnosis. In each panel, observations from animals for which no repeated samplings were available are excluded. In the case of *T. congolense,* PCR was conducted for the *Savannah* type only.

Finally, the blue notched areas indicate the 95% confidence intervals (CI) for the median haemoglobin concentration. The 95% CI show that the median haemoglobin concentration in samples positive by PCR only, does not differ from that in uninfected samples except for the case of *T. congolense (Savannah)*. The median haemoglobin concentration in Trypanosoma spp. samples positive by microscopy is clearly lower than in negative samples and samples positive by PCR only.

Statistical analysis of the data presented in Figure 5.1, showed that all three trypanosome species are associated with significant haemoglobin depletion in infected hosts as indicated by the F-test statistics for each analysis (Table 5.3). In the table, the REML estimated difference between the mean haemoglobin in uninfected samples and that in samples positive by PCR only [a] or by microscopy [b], is

shown in the column labelled as "COEF". The asterisks (*) highlight those cases where, according to the T-test of each variable level [a, b], the mean haemoglobin was significantly different from that in uninfected samples.

Table 5.3 REML estimates of the effect of *T. brucei*, *T. vivax* and *T. congolense* infections - above PCR or microscopy detection threshold - on haemoglobin (g/dl).

Trypanosoma spp.	PCR positives [a]			Microscopy positives [b]			F - value	p - value
	n [a]	Coef	SE	n [b]	Coef	SE		
T. brucei [1]	618	+0.08	0.06	57	-0.92 *	0.20	$F_{2, 5685} = 11.74$	<.0001
T. vivax [2]	302	-0.09	0.09	114	-1.08 *	0.14	$F_{2, 3064} = 30.64$	<.0001
T. congolense [3]	207	-0.35 *	0.11	48	-1.40 *	0.21	$F_{2, 2981} = 26.76$	<.0001

The numbers of observations available for analysis were as follows: [1] n = 6416 on 729 cattle; [2] n = 3764 on 698 cattle; [3] n = 3680 on 697 cattle.

According to this test, haemoglobin depletion was only associated with *T. brucei* and *T. vivax* infections above the microscopy detection threshold, but not above the PCR detection threshold. Only in the case of *T. congolense*, infections detectable by either technique were significantly associated with haemoglobin depletion, but haemoglobin depletion was significantly greater for infections above the microscopy threshold than above the PCR threshold (T-test = - 4.51; p <0.0001). This comparison was carried out by converting variable level [a] to reference level in the model (Data not shown). These results confirmed that the apparent levels of anaemia recorded for trypanosome species, vary depending on whether infections are above the PCR or the microscopy detection thresholds.

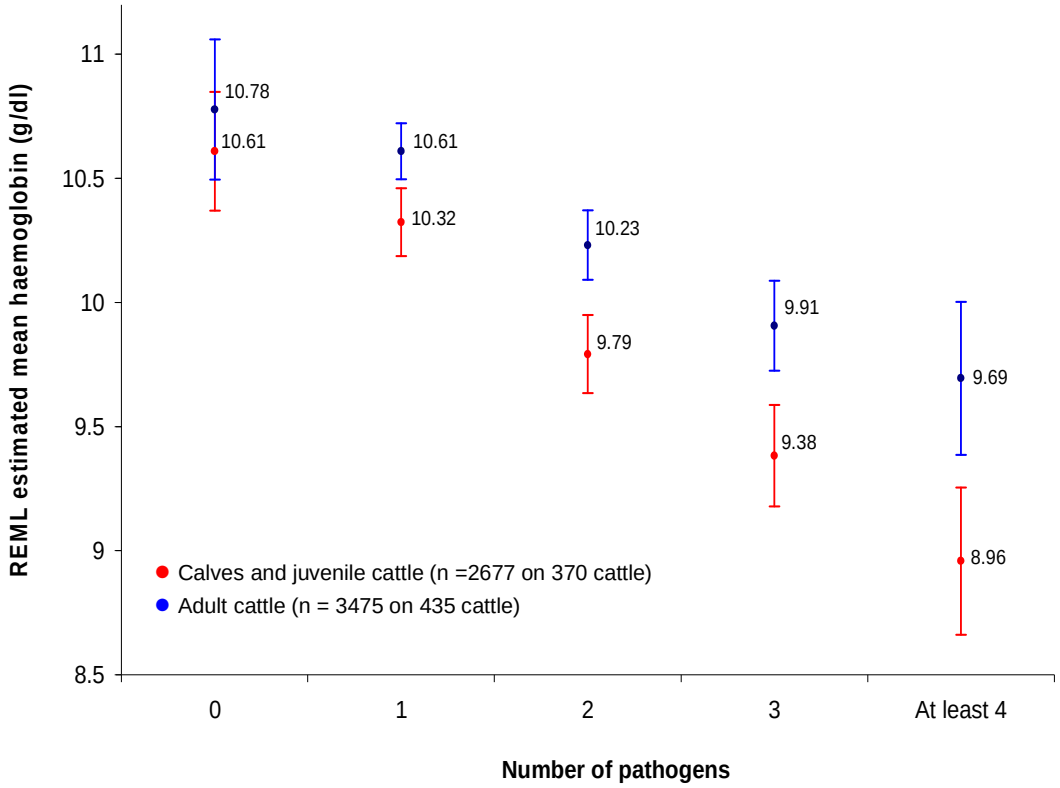
5.3.2 Relationship between Number of Co-Infecting Pathogens and Onset and Severity of Anaemia and Fever

5.3.2.1 LME Analysis of the Effect of Number of Pathogens on Haemoglobin

REML estimates of the effect of any given number of pathogens on haemoglobin - calculated for each age group using independent datasets - are presented in Figure 5.2. The coefficients shown in this figure, demonstrate a linear, age-dependent relationship between the number of pathogens co-infecting a host and the severity of haemoglobin depletion, with single pathogen infections causing only mild reduction of haemoglobin. Raw haemoglobin data in uninfected observations from calves and

juvenile cattle as compared to those from animals of the same age groups, infected with one, two, three or four or more pathogens are presented in Appendix 5.1.

Figure 5.2 REML estimates of the effect of one, two, three, or four or more pathogens on haemoglobin (g/dl).



Nº of pathogens	Calves and juveniles [a]		Adults [b]	
	Coef	SE	Coef	SE
1	-0.29 *	0.07	-0.17 *	0.06
2	-0.82 *	0.08	-0.55 *	0.07
3	-1.23 *	0.1	-0.87 *	0.09
At least 4	-1.65 *	0.15	-1.09 *	0.20

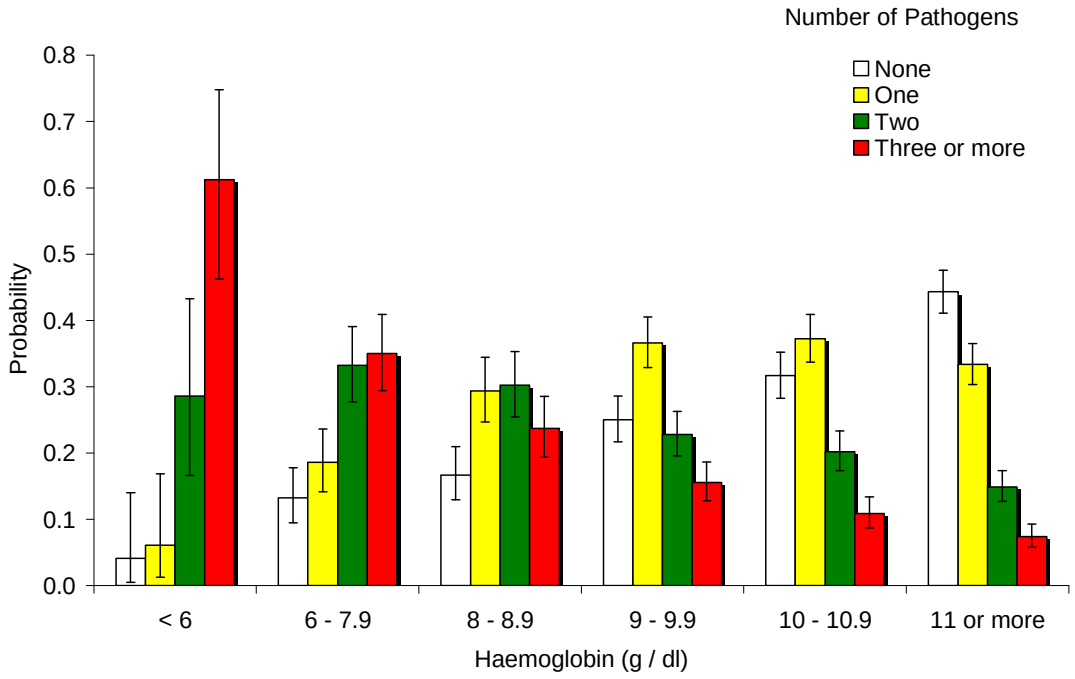
[a] n = 2677 observations on 370 cattle; $F_{4, 2303} = 65.19$; $p < 0.0001$; [b] n = 3475 observations on 435 cattle; $F_{4, 3036} = 36.95$; $p < 0.0001$. (*) In both analyses, p-values derived from level-specific T-tests showed that haemoglobin concentrations (i.e. REML estimates) in single and multiple pathogen infections, were significantly different from those in uninfected observations. Observations from animals for which no repeated samplings were available are excluded.

The REML estimates of the effect of one, two, three or four or more pathogens on haemoglobin, were significantly different in calves and juvenile cattle as compared to adults (n = 6347 on 667 cattle; $F_{4, 5671} = 2.89$; $p = 0.02$), but not different between calves and juvenile cattle ($p > 0.05$).

Single pathogen infections had a similar impact on the haemoglobin of animals of up to 24 months of age and adults (df = 5671; T-value = -1.23; p=0.22). However, concomitant infections involving two (df = 5671; T-value = -2.15; p = 0.03), three (df = 5671; T-value = -2.32; p = 0.02), or four or more pathogens (df = 5671; T-value = - 2.47; p = 0.01) were significantly worse in the younger age group.

Figure 5.3, shows that in animals of up to 24 months of age (i) haemoglobin readings of at least 11 g/dl were mainly recorded from uninfected observations; (ii) haemoglobin readings of 10 - 10.9 g/dl were mainly recorded from uninfected observations or single pathogen infections; (iii) haemoglobin readings of 9 - 9.9 g/dl were mainly recorded from observations infected by a single pathogen; (iv) values of 8 – 8.9 g/dl were almost equally associated to infections involving either one or two pathogens; (v) whilst mild anaemia (6 – 7.9 g/dl) and severe anaemia (< 6 g/dl) were mainly recorded from observations where multiple pathogens were diagnosed.

Figure 5.3 Bar plot of the probability of infection with zero, one, two, or three or more pathogens, at various intervals of haemoglobin concentrations (g/dl) in calves and juvenile cattle.



Vertical lines show exact binomial 95% confidence intervals. Observations from animals for which no repeated samplings were available are also included (n = 2968 observations on 661 cattle).

Mixed infections, were recorded from 71.4% of the observations where haemoglobin readings indicated anaemia (< 8 g/dl). In mild anaemia (6 – 7.9 g/dl), 68.2% of the observations corresponded to multiple pathogen infections, 18.6% to infections by only one pathogen and 13.2% to uninfected samplings. In severe anaemia (< 6 g/dl), 89.8% of the observations corresponded to mixed infections (mainly involving co-infection with three or more pathogens), 6.1% to single pathogen infections and 4.1% to uninfected samplings. See Figure 5.3.

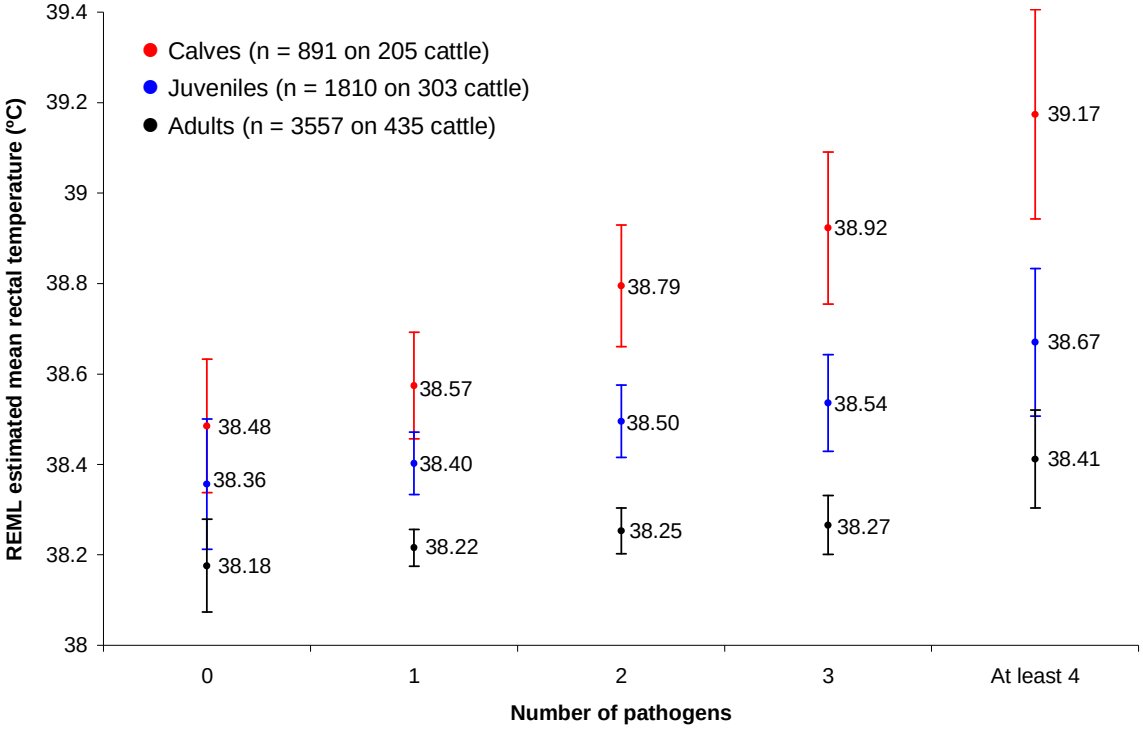
In the case of adult cattle, 72% (n = 169) of infections where anaemia was diagnosed and where PCR and microscopy data for all pathogens were available (n = 235), harboured multiple pathogens, whilst only 28% (n = 66) harboured a single pathogen (Data not shown).

In cattle of up to 24 months of age, the approximate haemoglobin value below which the probability of an animal being infected by two pathogens was greater than that of being infected by a single pathogen, was of 9.42 g/dl. The approximate haemoglobin value below which the probability of an animal being infected by three or more pathogens was greater than that of being infected by two pathogens was of 8.98 g/dl. See Appendix 5.2. Such cut-off points for increased probability of mixed infections, occurred at higher haemoglobin values than the cut-off point for anaemia (i.e. Haemoglobin < 8 g/ dl).

5.3.2.2 LME Analysis of the Effect of Number of Pathogens on Rectal Temperature

REML estimates of the effect of any given number of pathogens on rectal temperature - calculated for calves, juveniles and adults using independent datasets - are presented in Figure 5.4. The coefficients shown in this figure, demonstrate a linear, age-dependent relationship between the number of pathogens co-infecting a host and the severity of rectal temperature increase. Notably, only in the case of single pathogen infections, the REML estimated mean rectal temperature was not significantly different from that in uninfected observations, regardless of the age group considered. Raw rectal temperature data in observations from uninfected cattle of any age, compared to observations from cattle infected with one, two, three, or four or more pathogens are presented in Appendix 5.3.

Figure 5.4 REML estimates of the effect of one, two, three or four or more pathogens on rectal temperature (ºC).



Nº of pathogens	Calves [a]		Juveniles [b]		Adults [c]	
	Coef	SE	Coef	SE	Coef	SE
1	+ 0.09	0.06	+ 0.05	0.04	+ 0.04	0.02
2	+ 0.31 *	0.07	+ 0.14 *	0.04	+ 0.08 *	0.03
3	+ 0.44 *	0.09	+ 0.18 *	0.05	+ 0.09 *	0.03
At least 4	+ 0.69 *	0.12	+ 0.31 *	0.08	+ 0.24 *	0.06

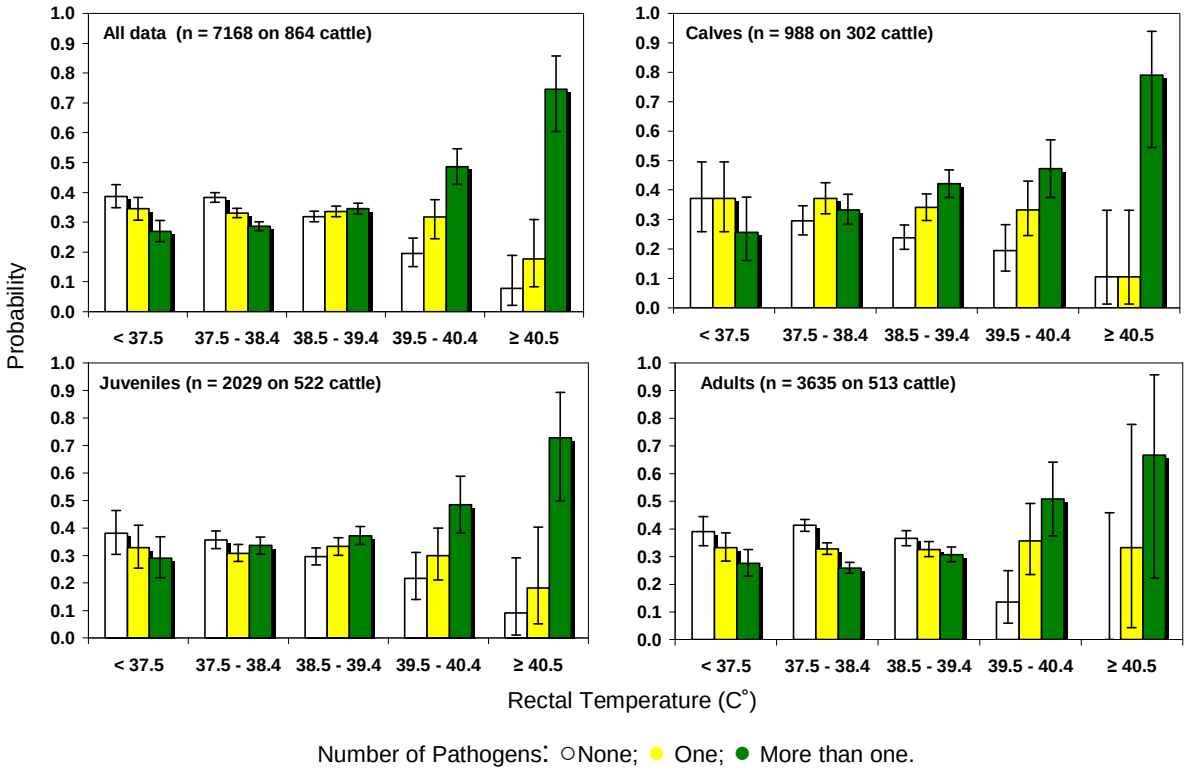
[a] n = 891 observations on 205 cattle; $F_{4, 682} = 15$; p <0.0001; [b] n = 1810 observations on 303 cattle; $F_{4, 1503} = 6.76$; p <0.0001; [c] n = 3557 observations on 435 cattle; $F_{4, 3118} = 6.5$; p <0.0001. (*) In all analyses, p-values derived from level-specific T-tests showed that rectal temperature readings (i.e. REML estimates) were significantly different in observations where two, three or at least four pathogens were diagnosed as compared to uninfected observations, but not different in observations harbouring a single pathogen as compared to uninfected observations. Observations from animals for which no repeated samplings were available are excluded.

REML estimates of the effect of one, two, three or four or more pathogens on rectal temperature were significantly different in calves, juveniles and adult cattle (n = 6477 on 667 cattle; $F_{8, 5796} = 5.3$; p = <0.0001). Single pathogen infections had a similar effect in all age groups (df = 5796; all level-specific T-values > -1.25; all level-specific p-values > 0.05). However, the effect of concomitant infections involving two, three or four or more pathogens was significantly worse in calves, followed by in juveniles, while milder in adults (df = 5796; all level-specific T-values < - 1.25; all level-specific p-values

< 0.05). Raw rectal temperature data from observations on uninfected cattle, compared to observations on cattle infected with infected with one, two, three, or four or more pathogens, are presented separately for calves, juveniles and adults in Appendix 5.4.

Whilst there was equal probability of diagnosing zero, one, or more than one pathogen in cases where normal rectal temperature had been recorded (≤ 39.4ºC), multiple pathogen infections were more probable than infections involving a single pathogen in mild (39.5 - 40.4 ºC) and severe (≥ 40.5 ºC) fever cases. Such trend was more obvious in the latter, and applied to all cattle age groups considered (Figure 5.5).

Figure 5.5 Bar plots of the probability of infection with zero, one, or more than one pathogen, at various intervals of rectal temperature (ºC).



Bar plots are shown for all cattle in the study, and for calves, juveniles and adult cattle separately. Vertical lines show exact binomial 95% confidence intervals. Exact binomial 95% confidence intervals of single and mixed infection probabilities in fever cases, overlapped for some of the age groups considered, but not when all cattle age groups were pooled together. This resulted from the small number of fever cases when considering each cattle age group separately. In adults, rectal temperatures of 40.5 ºC or more, were only recorded from six observations where infection status for all pathogens was available. Observations from animals for which no repeated samplings were available are also included.

The approximate rectal temperature value above which the probability of being infected by multiple pathogens was greater than that of being infected by a single pathogen, was of 38.81 ºC in calves, whilst 39.39ºC in juvenile cattle. See Appendix 5.5. Rectal temperature values defining greater probability of infection with zero, one or multiple pathogens, were far less clear-cut than when using haemoglobin readings for this purpose.

5.3.3 "A Single Pathogen Focus" – Implications for Pathogen Species-Specific Attributed Morbidity

5.3.3.1 Pathogen-Specific Impact on Haemoglobin when Infection Status for Other Pathogens is Omitted

The analysis of the impact of each pathogen on haemoglobin (Table 5.4) showed that neither Fasciola spp., nor *T.brucei* or *T.vivax* infections detectable by PCR, had a significant effect on haemoglobin.

Table 5.4 REML estimates of the effect of individual pathogens on haemoglobin (g/dl) when the infection status for other pathogens is omitted.

Pathogen	n [a]	Coef	SE	F-value	p-value	Obs	Cattle
Dataset A						2379	566
Anaplasma spp.	647	-0.97	0.07	$F_{1, 1812} = 190.3$	<.0001		
T. parva	238	-0.62	0.11	$F_{1, 1812} = 31.09$	<.0001		
Theileria spp.	573	-0.51	0.07	$F_{1, 1812} = 45.56$	<.0001		
T. c. (Savannah) (PCR)	156	-0.50	0.13	$F_{1, 1812} = 15.22$	0.0001		
Nematodes	878	-0.31	0.07	$F_{1, 1812} = 19.80$	<.0001		
T. brucei (PCR)	288	-0.19	0.11	$F_{1, 1812} = 3.250$	0.071		
T. vivax (PCR)	227	-0.07	0.11	$F_{1, 1812} = 0.470$	0.495		
Fasciola spp.	389	-0.07	0.09	$F_{1, 1812} = 0.730$	0.394		
Dataset B						6941	778
T. brucei (MCR)	76	-0.97	0.17	$F_{1, 6162} = 33.35$	<.0001		
T. congolense (MCR)	97	-0.91	0.15	$F_{1, 6162} = 36.98$	<.0001		
Anaplasma spp.	1624	-0.89	0.04	$F_{1, 6162} = 455.5$	<.0001		
T. vivax (MCR)	240	-0.77	0.10	$F_{1, 6162} = 64.27$	<.0001		
Theileria spp.	1344	-0.61	0.04	$F_{1, 6162} = 187.4$	<.0001		
T. parva	455	-0.50	0.08	$F_{1, 6162} = 42.09$	<.0001		
Nematodes	2066	-0.25	0.04	$F_{1, 6162} = 38.73$	<.0001		
Fasciola spp.	960	-0.08	0.05	$F_{1, 6162} = 2.400$	0.121		

[a] Number of pathogen-positive samples. Pathogens are listed by decreasing order of estimated impact on haemoglobin (g/dl). MCR = Microscopy.

The analysis also showed that tick-borne pathogens (Anaplasma spp., *T. parva* and Theileria spp.) were more pathogenic with regard to their effect on haemoglobin than trypanosome infections detectable by PCR (Dataset A). However, tick-borne pathogens were less pathogenic than trypanosome infections detectable by microscopy (Dataset B), except for Anaplasma spp., which caused haemoglobin depletion at levels intermediate to those caused by *T. vivax* and *T. congolense*. In either dataset, gastro-intestinal nematodes had only a mild effect on haemoglobin as compared to blood-borne pathogens.

5.3.3.2 Pathogen-Specific Impact on Haemoglobin when Infection Status for Other Pathogens is Considered

Pathogen-specific REML Coefficients in Table 5.4 Compared to REML Coefficients of Single Infections Involving the Pathogen of Interest in Table 5.5.

The estimated effect of each pathogen on haemoglobin was in most cases reduced when infection by pathogens other than that of interest was considered during data analysis. See Table 5.4 and REML coefficients of single infections involving the pathogen of interest in Table 5.5. For *T. parva*, for example, the estimated effect on haemoglobin when omitting information on co-infecting pathogens was twice that of when only single *T.parva* infections were considered (-0.62 / -0.26 [Dataset A]; -0.50 / -0.28 [Dataset B]). Omitting information on co-infecting pathogens in Dataset A, suggested that only *T. brucei* (by PCR), *T. vivax* (by PCR) and Fasciola spp. had no impact on haemoglobin (Table 5.4). However, accounting for the community of co-circulating pathogens during the analysis of Dataset A showed that on the top of these, *T. parva*, *T. congolense (Savannah)* infections by PCR and nematodes, had no significant impact on the haemoglobin when infecting the host alone (Table 5.5). Single infections by *T. parva* and nematodes were only found to have a significant albeit small impact on haemoglobin in Dataset B, where the standard errors of the coefficients were minimised as a result of the larger number of positive observations available in this dataset (Table 5.5). It is noteworthy that none of the trypanosome species had a significant effect on haemoglobin in single infections above the PCR detection threshold (Table 5.5). *T. congolense* and *T. vivax* single infections detectable by

microscopy were associated to a degree of anaemia, but the same could not be conclusively demonstrated for *T. brucei*, for which only three single infections were available in Dataset B (Table 5.5).

Table 5.5 REML estimates of the effect of individual pathogens on haemoglobin (g/dl) when infection status for co-circulating pathogens is considered. Uninfected samples are the reference.

Pathogen (Ж)	Single infections involving (Ж)		Mixed infections involving (Ж)		Single infections excluding (Ж)		Mixed infections excluding (Ж)		F - Value	Obs	Cattle
	Coef	SE	Coef	SE	Coef	SE	Coef	SE			
Dataset A [a]									$F_{4, 1809}$	2379	566
Anaplasma spp.	-0.81	0.18	-1.20	0.09	-0.2	0.08	-0.37	0.1	52.80		
Theileria spp.	-0.51	0.18	-0.88	0.10	-0.22	0.08	-0.79	0.09	31.58		
Fasciola spp.	-0.29 ▲	0.16	-0.57	0.11	-0.25	0.08	-0.95	0.09	33.94		
T. parva	-0.26 ▲	0.18	-1.31	0.14	-0.26	0.08	-0.73	0.08	34.95		
Nematodes	-0.18 ▲	0.11	-0.88	0.09	-0.29	0.09	-0.78	0.1	31.15		
T. vivax	-0.09 ▲	0.22	-0.67	0.13	-0.26	0.08	-0.87	0.08	31.41		
T. brucei	-0.08 ▲	0.18	-0.76	0.12	-0.27	0.08	-0.86	0.09	31.04		
T. c. (Savannah)	+0.21 ▲	0.24	-1.30	0.16	-0.27	0.08	-0.77	0.08	34.80		
Dataset B [b]									$F_{4, 6159}$	6941	778
T. brucei	-0.85 ▲ [c]	0.84	-1.32	0.17	-0.24	0.04	-0.92	0.04	117.5		
T. congolense	-0.84	0.33	-1.37	0.16	-0.24	0.04	-0.91	0.04	118.8		
Anaplasma spp.	-0.65	0.09	-1.11	0.05	-0.18	0.04	-0.55	0.07	139.4		
T. vivax	-0.54	0.21	-1.21	0.11	-0.23	0.04	-0.90	0.05	118.4		
Theileria spp.	-0.36	0.10	-0.94	0.05	-0.23	0.04	-0.92	0.06	116.4		
T. parva	-0.28	0.11	-1.23	0.10	-0.24	0.04	-0.89	0.05	118.8		
Nematodes	-0.12	0.05	-0.92	0.05	-0.35	0.05	-0.95	0.06	119.6		
Fasciola spp.	-0. 12 ▲	0.07	-0.64	0.07	-0.27	0.04	-1.04	0.05	124.2		

▲ Highlights variable levels where, according to the level-specific T-test, the REML estimate for haemoglobin (g/dl) is not significantly different from that in uninfected samples. The p–value associated to the F-test of each model was <0.0001 in all cases (Data not shown). [a] Includes only PCR data for Trypanosoma spp. [b] Includes only microscopy data for Trypanosoma spp. [c] In Dataset B, the significance of single *T. brucei* infections by microscopy should be dismissed, as only three samples harboured *T. brucei* alone (See standard error of coefficient). Pathogens are listed by decreasing order of estimated impact on haemoglobin (g/dl), when infections do not involve pathogens other than that of interest (Ж).

In all cases, the p-value associated to each F-test in Table 5.5 was <0.0001, suggesting that the mean haemoglobin for at least one variable level was significantly different to that in uninfected samples. Black triangles highlight single pathogen infections with no significant impact on haemoglobin.

REML Coefficients of Single Pathogen-Specific Infections Compared to REML Coefficients of Single Infections not Involving the Pathogen of Interest (Table 5.5)

Single infections causing the most severe haemoglobin drop were those involving Anaplasma spp. followed by those involving *T. congolense* above the microscopy detection threshold, along with Theileria spp. and *T. vivax* above the microscopy detection threshold. See Table 5.5. Single infections involving Anaplasma spp. were four times more pathogenic than those involving other pathogens (-0.81 / -0.20 [Dataset A]; 0.65 / 0.18 [Dataset B]). Single infections involving *T. congolense* above the microscopy threshold, were 3.5 times more pathogenic than those involving other pathogens (-0.84 / -0.24). Finally, single Theileria spp. infections or *T. vivax* infections above the microscopy detection threshold, were twice as pathogenic as single infections involving other pathogens (Theileria spp.: -0.51 / -0.22 [Dataset A]; -0.36 / -0.23 [Dataset B]; *T. vivax*: -0.54 / -0.23 [Dataset B]). Because only three single *T. brucei* infections above the microscopy detection threshold were available for analysis, the estimates of the relative impact of this pathogen by microscopy are given.

REML Coefficients of Single Pathogen-Specific Infections Compared to REML Coefficients of Mixed Infections Involving the Pathogen of Interest (Table 5.5 & 5.6)

Haemoglobin depletion was most enhanced in mixed as compared to single Trypanosoma spp. infections above the PCR detection threshold, followed by in mixed compared to single nematodes and *T. parva* infections (Table 5.5). Mixed infections involving *T. brucei* above the PCR detection threshold, for example, were 10 times more pathogenic than single *T. brucei* infections detectable by PCR (-0.78 / -0.08; Table 5.5). This was despite the fact that *T. brucei* infections above the PCR detection threshold were more prevalent amongst adult cattle (Section 4.3.8.2), which appeared to be more resistant to the effect of mixed infections (Section 5.3.2.1), and for which infection by other pathogens –except for the case of Fasciola spp. - was less frequent (Section 4.3.8.2). Similarly, haemoglobin depletion in mixed nematode infections was 5 -8 times more severe in mixed as compared to in single infections (-0.88 / -0.18 [Dataset A]; -0.92 / -0.12 [Dataset B]; Table 5.5). Equally, mixed *T. parva* infections caused 4 -5 times more haemoglobin depletion than single *T. parva* infections (-1.23 / -0.28

[Dataset B]; -1.31 / -0.26 [Dataset A]; Table 5.5). Less dramatic changes were observed for mixed as compared to single infections involving Anaplasma spp., Theileria spp. or trypanosome infections detectable by microscopy (Table 5.5). These were, in turn, the most pathogenic species when infecting the host alone.

Re-examination of level-specific T-test statistics when the mean haemoglobin in mixed infections involving the pathogen of interest was specified as the reference variable level, allowed assessment of the significance of the findings discussed in this section (Table 5.6).

Table 5.6 REML estimates of the effect of individual pathogens on cattle haemoglobin (g/dl) when infection status for other pathogens is considered. Mixed infections involving the pathogen of interest are the reference.

Pathogen (Ж)	Dataset A (n = 2379 on 566 cattle) [a]				Dataset B (n = 6941 on 778 cattle) [b]			
	Single infection by (Ж)		Mixed infection not (Ж)		Single infection by (Ж)		Mixed infection not (Ж)	
	Coef	SE	Coef	SE	Coef	SE	Coef	SE
Anaplasma spp.	+ 0.39	0.18	+ 0.83	0.10	+ 0.45	0.09	+ 0.55	0.07
Theileria spp.	+ 0.37	0.18	+ 0.09▲	0.10	+ 0.58	0.11	+ 0.03▲	0.07
Fasciola spp.	+ 0.28▲	0.17	- 0.38	0.11	+ 0.52	0.09	- 0.40	0.08
T. parva	+ 1.05	0.20	+ 0.57	0.14	+ 0.96	0.13	+ 0.34	0.10
Nematodes	+ 0.70	0.11	+ 0.10▲	0.10	+ 0.80	0.06	- 0.04▲	0.07
T. vivax	+ 0.57	0.24	- 0.20▲	0.13	+ 0.67	0.23	+ 0.31	0.11
T. brucei	+ 0.68	0.20	- 0.09▲	0.12	+ 0.47▲	0.86 [d]	+ 0.40	0.17
T. congolense [c]	+ 1.51	0.27	+ 0.53	0.15	+ 0.53▲	0.37	+ 0.46	0.16

The F-test statistic for each model is as listed in Table 5.5. Only the coefficients for single infections involving the pathogen of interest (**Ж**) and for mixed infections involving pathogens other than that of interest are shown. ▲ Highlights variable levels where, according to the level-specific T-test, the REML estimate for haemoglobin (g/dl) is not significantly different from that in mixed infections involving the pathogen of interest (**Ж**). [a] Includes only PCR data for Trypanosoma spp. [b] Includes only microscopy data for Trypanosoma spp. [c] Refers to *T. congolense (Savannah)* in the dataset with PCR data for trypanosomes. [d] The significance of the REML estimated impact of single as compared to mixed *T. brucei* infections should be dismissed, as only three samples in Dataset B harboured *T. brucei* alone (See standard error of coefficient).

All single infections involving the pathogen of interest had significantly less impact on the concentration of haemoglobin ($p<0.05$) than mixed infections involving the same pathogen (Table 5.6). The only case where this effect was not significant, was when considering *T. congolense* and *T. brucei* infections above the microscopy detection threshold. However, because of the low number of single infections involving either of these species above the microscopy threshold, such results were not conclusive

(See standard errors for REML estimates; Table 5.6). Single Fasciola spp. infections were not clearly less pathogenic than mixed infections involving Fasciola spp. This probably resulted from Fasciola spp. being significantly more prevalent in adult cattle, which were in turn less likely to test positive for the remaining pathogens except for *T. brucei* (PCR). See Section 4.3.8.2. Hence, Fasciola spp.-positive samples, were probably correlated with samples harbouring a lower number of co-infecting pathogens.

REML Coefficients of Mixed Pathogen-Specific Infections Compared to REML Coefficients of Mixed Infections not Involving the Pathogen of Interest (Table 5.5 & 5.6)

Mixed infections causing the most severe haemoglobin drop were those involving any trypanosome species above the microscopy detection threshold*, T. congolense (Savannah)* above the PCR detection threshold*, T. parva* and Anaplasma spp. See Table 5.5.

Anaplasma spp. was the most important determinant of the pathogenicity of mixed infections along with *T. congolense* and *T. parva*. Mixed infections involving Anaplasma spp. were 2-3 times more pathogenic than mixed infections not harbouring Anaplasma spp. (-1.11 / -0.55 [Dataset B]; -1.2 / -0.37 [Dataset A]). Mixed infections were also twice as pathogenic if these involved *T. congolense* above either the PCR (-1.3 / -0.77) or microscopy the detection threshold (-1.37 / -0.91) or *T. parva* (-1.31 / -0.73 [Dataset A]). See Table 5.5.

Mixed infections involving Trypanosoma spp. other than *T. congolense* resulted in slightly more haemoglobin depletion only when infection intensity was above the microscopy detection threshold. Nematodes, Fasciola spp. and Theileria spp. did not cause more haemoglobin depletion as compared to mixed infections not involving these pathogens (Table 5.5).

Re-examination of level-specific T-test statistics (Table 5.6), showed that mixed infections involving Anaplasma spp., *T. parva*, any Trypanosoma spp. above the microscopy detection threshold and *T. congolense (Savannah)* above the PCR detection threshold, caused significantly more haemoglobin depletion than those which did not involve these pathogens. In contrast, mixed infections involving Fasciola spp., were associated with significantly higher haemoglobin concentration as compared to

Fasciola spp.-negative samples (Table 5.6), but Fasciola spp.-positive samples were correlated with samples harbouring a lower number of co-infecting pathogens.

5.3.4 Pathogen Species-Specific Interactions on Incidence and Severity of Anaemia

5.3.4.1 Pathogen Interactions

Positive Interactions

Positive species-specific pathogen interactions on haemoglobin were only consistently demonstrated in four combinations of two pathogens in Dataset A and Dataset B (Table 5.7). Positive interactions on haemoglobin occurred in the cases of co-infections involving (i) Anaplasma spp. and *T. parva*, (ii) Anaplasma spp. and *T. congolense (Savannah)* infections above the PCR detection threshold, (iii) *T. parva* and *T. brucei* infections above the microscopy detection threshold and (iv) nematodes and *T. vivax* infections by microscopy.

Negative Interactions

A negative interaction on haemoglobin was only consistently demonstrated for one pair of pathogens in Dataset B. This was only the case of co-infections involving Anaplasma spp. and *T. vivax* parasitaemias detectable by microscopy (Table 5.7).

Parallel analyses (Section 5.2.6.4), did not reveal interactions other than those described in Table 5.7. Similarly, no interactions between low Trypanosoma spp. (PCR) and high Trypanosoma spp. (microscopy) species-specific parasitaemias were found (Data not shown).

5.3.4.2 Confounded Pathogen Interactions

Those interactions which were not consistently found significant during parallel or subsequent analyses - and should therefore not be regarded as "*true interactions*" - are highlighted with an asterisk in Table 5.7.

Table 5.7 Minimum models of the REML estimated effect of pathogens and their interactions on haemoglobin (g/dl).

Minimum Model	Coef	SE	F - values	p - values	Confounded interactions	Obs	Cattle
Dataset A [a]			$F_{1, 1803}$ =			2379	566
Anaplasma spp.	-0.86	0.11	196.9	<.0001			
T. parva	-0.31	0.13	28.80	<.0001			
T. congolense (Savannah)	+0.06	0.17	8.630	0.003			
Nematodes	-0.25	0.07	16.36	0.0001			
Theileria spp.	-0.38	0.11	5.010	0.025			
Anaplasma spp. *: T. parva*	-0.82	0.23	20.31	<.0001			
Anaplasma spp. *: T. c. (Savannah)*	-0.32	0.29	5.160	0.023			
T. parva : T. c. (Savannah)	-0.98	0.39	4.470	0.035	*		
Anaplasma spp. *:* Theileria spp.	+0.41	0.17	5.390	0.020	*		
T. c. (Savannah) : Theileria spp.	-0.63	0.31	4.060	0.044	*		
Dataset B [b]			$F_{1, 6151}$ =			6941	778
Anaplasma spp.	-0.78	0.06	468.8	<.0001			
T. congolense	-0.61	0.15	27.87	<.0001			
T. parva	-0.32	0.09	39.17	<.0001			
T. vivax	-0.51	0.14	26.95	<.0001			
T. brucei	-0.33	0.19	7.240	0.007			
Theileria spp.	-0.43	0.07	26.75	<.0001			
Nematodes	-0.17	0.04	26.26	<.0001			
Anaplasma spp. *: T. parva*	-0.59	0.16	19.38	<.0001			
Anaplasma spp. *: T. vivax*	+0.54	0.19	7.720	0.006			
T. parva : T. brucei	-1.26	0.53	5.790	0.016			
Anaplasma spp. *:* Theileria spp.	+0.27	0.11	6.230	0.013	*		
T. vivax : Nematodes	-0.57	0.21	7.710	0.007			

The difference between (i) the REML estimated mean haemoglobin (RMHb) in uninfected samples and that in samples infected with the pathogen of interest, and the difference between (ii) the expected and observed RMHb in samples infected by any pathogen pair, is shown in the column 'Coef' (i.e. Model coefficients). Minimum models are those which, after a process of step-down simplification, contain only significant terms as fixed-effects. [a] Includes only PCR data for Trypanosoma spp. [b] Includes only microscopy data for Trypanosoma spp.

Confounded Positive Interactions

The apparent positive interaction between *T.congolense (Savannah)* infections detectable by PCR and Theileria spp. on haemoglobin, depended on the sample size analysed. This interaction was therefore regarded as non-significant in view of the inconclusiveness of the results. The interaction between *T. parva* and *T. congolense* (*Savannah*) infections detectable by PCR, was significant in all the analyses described in Section 5.2.7, but not significant in the analysis designed to test whether density

dependant mechanisms limit the establishment of pathogen interactions on animal health (Section 5.2.8). These interactions are not considered further in this section.

Confounded Negative Interactions

The apparent negative interaction on haemoglobin in the case of Anaplasma spp. and Theileria spp. co-infections, resulted from several features of the data. Firstly, as Theileia spp. infections by microscopy corresponded to those which could not be classified as *T. parva* by PCR, this implied that all Theileria spp. – Anaplasma spp. co-infections were *T. parva* negative. Secondly, a strong association between Anaplasma spp. and Theileria spp. infections was observed ($n = 8941$; $F_{1, 7604} = 1805.3$; $p<0.0001$), which was mainly restricted to adult cattle ($n = 8941$; $F_{2, 7604} = 13.55$; $p<0.0001$). In consequence, the apparent negative interaction resulted from the increased likelihood of Anaplasma spp. - Theileria spp. co-infections in adults, where the overall prevalence of pathogens was lower. It also resulted from comparing the effect of Anaplasma spp. – Theileria spp. co-infections (highly correlated with Anaplasma spp. positive – *T.parva* negative cases) to Anaplasma spp. cases co-infected or not with *T.parva*. This was confirmed by testing the apparent Theileria spp. – Anaplasma spp. interaction on haemoglobin, after correcting for the presence of *T. parva* in Anaplasma spp. infected samples, and accounting for cattle age in the analysis (Anaplasma spp. : Theileria spp.; $p>0.05$).

5.3.4.3 Strength of Pathogen Interactions

Positive Interactions

The strongest positive interaction on haemoglobin was observed the case of *T. parva* and *T. brucei* infections detectable by microscopy (See REML coefficients of pathogen interactions in Table 5.7). In the absence of an interaction, mixed infections involving these pathogens would have reduced haemoglobin by 0.65 g/dl as compared to uninfected samples, which corresponds to the sum of the effect associated to *T. parva* (-0.32 g/dl) and *T. brucei* infections detectable by microscopy (- 0.33 g/dl) (Table 5.7; Dataset B). Co-infections by these pathogens, however, reduced haemoglobin by an excess of 1.26 g/dl (See Table 5.7, Dataset B) as compared to the expected effect (-0.65 g/dl). The

second strongest positive interaction was found in co-infections involving *T. parva* and Anaplasma spp. (- 0.82 g/dl [Dataset A]; - 0.59 g/dl [Dataset B]) followed by those involving nematodes and *T. vivax* parasitaemias detectable by microscopy (-0.57 g/dl). The smallest positive interaction on haemoglobin occurred in co-infections involving Anaplasma spp. and *T. congolense (Savannah)* parasitaemias detectable by PCR (- 0.32 g/dl).

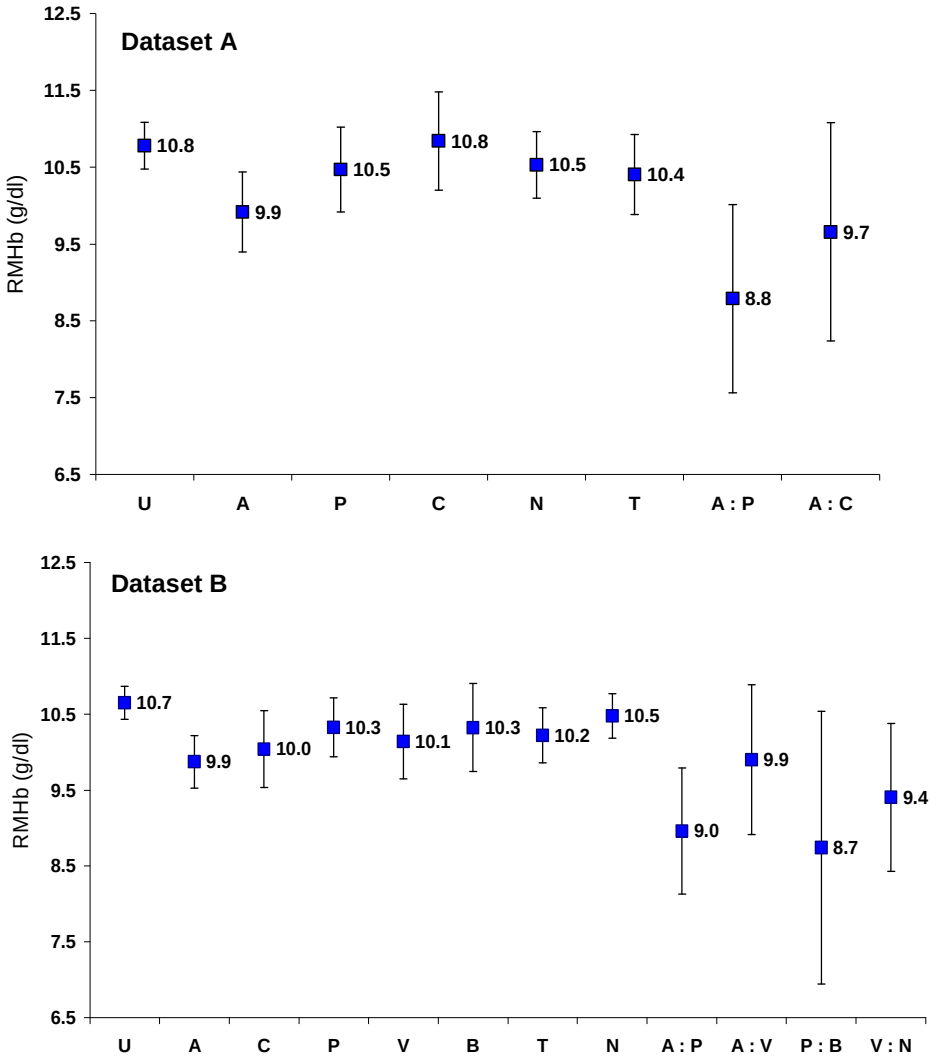
Negative Interactions

Co-infections involving Anaplasma spp. and *T. vivax* parasitaemias detectable by microscopy, reduced haemoglobin by 0.54 g/dl less than would be the case in the absence of an interaction between these pathogens (Table 5.7).

5.3.4.4 REML Estimated Mean Haemoglobin (RMHb) for Interactive Pathogen Pairs

Mixed infections involving either *T.parva* and *T.brucei* parasitaemias detectable by microscopy, or *T. parva* and Anaplasma spp., were associated with the lowest RMHb and were therefore the most pathogenic pairs of pathogens in the study population (Figure 5.6). These estimates, however, are best interpreted without omitting information on the associated 95% confidence intervals (Figure 5.6). The RMHb estimated for Anaplasma spp. – *T. parva* co-infections was more reliable than that estimated for *T. parva* – *T. brucei* (microscopy), as the RMHb 95% confidence interval of the latter was wider. The RMHb associated to any combination of pathogens was calculated as described in Section 5.2.7. Then, for example (given that the RMHb in uninfected samples was 10.78 g/dl and 10.65 g/dl in Dataset A and Dataset B respectively), the RMHb in samples co-infected with *T. parva* and Anaplasma spp. in Dataset A was 10.78 – (0.86 + 0.31+ 0.82) = 8.79 g/dl, and in Dataset B was 10.65 – (0.78 + 0.32 + 0.59) = 8.96 g/dl (See coefficients Table 5.7 and plotted RMHb values in Figure 5.6). The RMHb in samples co-infected with *T. parva* and *T. brucei* parasitaemias detectable by microscopy was 8.74 g/dl. No other pairs of pathogens were associated with RMHb values of less than 9 g/dl.

Figure 5.6 REML estimated mean haemoglobin (RMHb), for significant terms in minimum models explaining the effect of pathogen interactions on haemoglobin (g/dl).



The RMHb calculated from the coefficients of minimum models in Table 5.7, are shown with a blue square. Error bars show approximate 95% confidence intervals for the RMHb. In the X axis of the graphs, the labels stand for: uninfected (U), Anaplasma spp. (A), *T. congolense* (C), *T. parva* (P), *T. vivax* (V), *T. brucei* (B),Theileria spp. (T), "*Strongyle –type*" nematodes (N) and "interaction" (**:**). Only those interactions which were consistently found significant are shown. Dataset A includes only PCR data for Trypanosoma spp. (n = 2379 observations on 566 cattle). Dataset B includes only microscopy data for Trypanosoma spp.(n = 6941 observations on 778 cattle).

5.3.5 Effect of Infection Intensity on Pathogen Species-Specific Interactions

Five pathogen interactions on haemoglobin appeared to depend on the choice of the diagnostic technique used, according to the analysis of Datasets A and B (Table 5.7). The apparent "*technique-dependant*" interactions are summarised in Table 5.8, and are described graphically in Appendix 5.6.

Table 5.8 Interactions on haemoglobin that appear to depend on the choice of the diagnostic technique.

Anaplasma spp. ***: Trypanosoma congolense Theileria parva : Trypanosoma congolense***	**Positive interactions** on haemoglobin which appear to occur only at lower trypanosome parasitaemias (infections detectable by PCR), but not at higher trypanosome parasitaemias (infections detectable by microscopy)
"*Strongyle-type*" nematodes ***: Trypanosoma vivax Theileria parva : Trypanosoma brucei***	**Positive interactions** on haemoglobin which appear to occur only at higher trypanosome parasitaemias (infections detectable by microscopy), but not at lower trypanosome parasitaemias (infections detectable by PCR)
Anaplasma spp. ***: Trypanosoma vivax***	**Negative interactions** on haemoglobin which appear to occur only at higher trypanosome parasitaemias (infections detectable by microscopy), but not at lower trypanosome parasitaemias (infections detectable by PCR)

Assuming that infections above the microscopy detection threshold are representative of higher infection intensities as compared to infections above the PCR detection threshold, three infection intensity-dependant interaction types were confirmed to take place in the study population (Table 5.9). The first type is that where a positive interaction on haemoglobin occurs only at lower infection intensities above the PCR detection threshold, but where the effect of the pathogen combination is not different from the sum of the pathogens individual effects at higher infection intensities (i.e. Above the microscopy detection threshold). This was statistically demonstrated for the case of *T. congolense (Savannah)* and Anaplasma spp. co-infections (Table 5.9). The second type is where a positive interaction on haemoglobin is possible at sufficiently high infection intensities (i.e. above the microscopy detection threshold), but not at lower infection intensities above the PCR detection threshold only. Mixed infections involving *T. vivax* and "*Strongyle-type*" nematodes, or *T. brucei* and *T. parva* appeared to follow this pattern (Table 5.9). Finally, the third type is that where lower infection intensities detectable by PCR only, do not result in interaction, but where higher infection intensities

above the microscopy detection threshold, result in negative (rather than positive) interactions on haemoglobin. *T. vivax* and Anaplasma spp. co-infections appeared to interact in this manner (Table 5.9).

Table 5.9 LME analyses of infection intensity-dependant pathogen interactions on haemoglobin (g/dl).

Maximal model	Observations		Minimum model	F-value	p-value
	Maximal Model	Minimum Model			
Cm + A + Cp + (Cm : A) + (A : Cp)	3774	3774	Cm	$F_{1, 2936} = 46.87$	<.0001
			A	$F_{1, 2936} = 251.9$	<.0001
			Cp	$F_{1, 2936} = 7.48$	0.006
			A : Cp	$F_{1, 2936} = 4.42$	0.036
Cm + P + Cp + (Cm : P) + (P : Cp)	3687	3687	Cm	$F_{1, 2936} = 44.5$	<.0001
			P	$F_{1, 2936} = 27.61$	<.0001
			Cp	$F_{1, 2936} = 9.66$	0.002
Bm + P + Bp + (Bm : P) + (P : Bp)	5955	7835	Bm	$F_{1, 6950} = 31.74$	<.0001
			P	$F_{1, 6950} = 37.97$	<.0001
			Bm : P	$F_{1, 6950} = 4.47$	0.035
Vm + N + Vp + (Vm : N) + (N : Vp)	3526	8233	Vm	$F_{1, 7353} = 55.37$	<.0001
			N	$F_{1, 7353} = 43.99$	<.0001
			Vm : N	$F_{1, 7353} = 7.43$	0.006
Vm + A + Vp + (Vm : A) + (A : Vp)	3862	8908	Vm	$F_{1, 8013} = 62.06$	<.0001
			A	$F_{1, 8013} = 527.4$	<.0001
			Vm : A	$F_{1, 8013} = 4.20$	0.041

For each model, the independent variables are shown in the first column, where abbreviations correspond to *T. congolense* (C), Anaplasma spp. (A), *T. parva* (P), *T. brucei* (B), *T. vivax* (V), "*Strongyle-type*" nematodes (N), microscopy data (m), PCR data (p) and interaction (:).Minimum models are those which, after a process of step-down simplification, contain only significant terms as fixed-effects. For the purpose of these analyses, only the F-test statistics for the terms retained in the minimum models are shown.

The apparent infection intensity-dependant interaction on haemoglobin in the case of co-infections involving *T. parva* and *T. congolens*e (Table 5.7), was not statistically demonstrated in this analysis (Table 5.9). On the contrary, the analysis suggested that the apparent technique-dependent interaction resulted from the positive correlation between *T. congolense* cases by microscopy and *T. congolense (Savannah)* cases by PCR in *T. parva* infected samples.

5.4 Discussion

5.4.1 'Single-Pathogen' Focus in Epidemiological Studies

The longitudinal monitoring of indigenous cattle herds has shown that 87.4% of pathogen-positive animals harbour mixed infections. This is another example of the many studies that report concomitant infections involving closely or distantly related species in either humans or animals, (Christensen *et al.*, 1987, Ashford, 1991, Petney *et al.*, 1998, Viera *et al.*, 1998), including African cattle (Magona *et al.*, 2002). Despite the widespread evidence of concurrent infections, the bulk of research, along with the more specialised texts of epidemiology (Anderson *et al.*, 1991, Grenfell *et al.*, 1995, Isham *et al.*, 1996), evolution (Brooks *et al.*, 1993), immunology (Wakelin, 1996) and host-parasite relationships (Toft *et al.*, 1991), have been focused upon single pathogen infections (Cox, 2001).

The results presented in this chapter, show that there is now a very strong case that morbidity studies adopting a "single pathogen focus" can lead to false conclusions. Especially concerning the relative importance of individual pathogens with respect to herd health and subsequent prioritisation of control efforts in these areas. A "single pathogen focus" - whereby the impact of a single pathogen on animal health is assessed without consideration of co-circulating pathogens - results in substantial overestimation of the morbidity that is attributed to the pathogen in question. The inadequacy of adopting a single pathogen focus had previously been recognised in field studies attempting to quantify the morbidity associated with *T. parva* (Moll *et al.*, 1984), upon field evidence that *T. parva* associated morbidity was exacerbated in co-infections involving *T. mutans* (Moll *et al.*, 1986).

From the animal health point of view, it is noteworthy that single-pathogen infections appear to be relatively unimportant to zebu herds, as indicated by the analysis of haemoglobin and rectal temperature data. Such a statement appears to be true regardless of the pathogen species considered and the age of the infected cattle. Instead, the onset and severity of clinical signs follows a linear positive relationship with the number of pathogens co-infecting the host, which results in the vast majority of anaemia and fever cases being associated with multiple, rather than single, pathogen infections. Because mixed infections may consist of different pathogen combinations, and host

genotype is likely to influence the outcome of pathogen interactions, such a perfect linear relationship is worthy of further investigation. Variation in the relationship of interest is mainly a function of the age of infected cattle, and results from the number of co-infecting pathogens having a greater effect on the health of younger animals as compared to adults.

5.4.2 Pathogen-Species Interactions and Animal Health

Consistent with the bulk of research that indicates that pathogens sharing a host may interact and result in infection outcomes that can be detrimental or beneficial to the host and/or the pathogens (Petney *et al.*, 1998), certain pathogen combinations appear to impose a greater risk to cattle health than others. Amongst other factors, such a hierarchy depends on the virulence of the pathogens involved, but more importantly, on whether the pathogens possess the ability to interact with others, causing a more detrimental effect on animal health. It is noteworthy that not all pathogen pairs appear to possess such ability. However, mixed infections involving (i) Anaplasma spp and either *T. parva* or *T. congolense (Savannah)* by PCR, (ii) *T. parva* and *T. brucei* by microscopy or (iii) gastrointestinal nematodes and *T. vivax* by microscopy, can all cause a significantly worse clinical condition than expected from the sum of effects of each individual pathogen.

Although further work is needed before any conclusions can be made, preliminary evidence suggests that the establishment and outcome of pathogen interactions on animal health, is dependent on the parasitaemia of individual pathogens in concurrent infections. As higher infection intensities are positively associated with microscopy data (Bishop *et al.*, 1992, Morzaria *et al.*, 1999, Picozzi *et al.*, 2002), whether trypanosome infection intensities were above the PCR or the microscopy detection threshold, determined whether a protective, detrimental or neutral interaction on animal health occurred.

There is some evidence to suggest, at least in the geographical areas considered, that *T. congolense* may be more important to zebu cattle health than either *T. brucei* or *T. vivax*. Amongst the trypanosomes, *T. brucei* and *T. vivax* were only capable of interacting with other pathogens causing a worse clinical condition, when attaining parasitaemias above the microscopy detection threshold. In

contrast, *T. congolense* was able to interact with Anaplasma spp. to the detriment of the host, at lower parasitaemias (above the PCR detection threshold), which are more widespread. It is remarkable that *T. congolense* parasitaemias above the microscopy detection threshold did not cause more haemoglobin depletion in contemporaneous infections involving Anaplasma spp. as compared to in single infections. These observations may suggest that a virulence threshold may exist, beyond which higher parasitaemias of co-infecting pathogens no longer result in higher pathogenicity, or result in outcomes that can be beneficial to the host. For example, while no interaction was observed in concurrent infections involving Anaplasma spp. and *T. vivax* by PCR, mixed infections involving the former and *T. vivax* above the microscopy detection threshold, resulted in infection outcomes where haemoglobin depletion was less than expected.

Within the community of endemic pathogens considered in this work, Anaplasma spp. appeared to be the major component compromising the health of zebu herds, as determined by the haemoglobin (g/dl) readings. Single infections involving Anaplasma spp. were four times more pathogenic than those involving other pathogens. Likewise, Anaplasma spp. was the most important determinant of the pathogenicity of mixed infections in the study population, as multiple infections involving this organism were 2-3 times more pathogenic than mixed infections not harboring Anaplasma spp. Although, to a lesser extent, mixed infections were also more pathogenic if they involved *T. parva*, any Trypanosoma spp. above the microscopy detection threshold, or *T. congolense (Savannah)* infection intensities above the PCR detection threshold, these pathogens were far less widespread than Anaplasma spp. in the cattle herds. Hence, any effects associated to infection with the latter, are likely to impact on a larger population scale.

5.4.3 Limitations of the Field Study

A number of pathogen interactions reported in the literature could not be demonstrated in this study. Such is the case, for example, of the well documented interactions between *T. congolense* and gastrointestinal nematodes, which are thought to result in enhanced virulence and the requirement of frequent anthelmintic treatments (Griffin *et al.*, 1981a, Kaufmann *et al.*, 1992, Fakae *et al.*, 1994,

Goossens *et al.*, 1997). Inconsistencies with reports from other studies, has probably resulted from the relative availability of mixed infections with the pathogens of interest during data analysis, the large amount of confounding variables present in the field, and the complexity of factors influencing the outcome of pathogen interactions. Amongst these, prior residency (i.e. history of previous exposure to related or unrelated pathogens) and timing of infection by different pathogen species, can all determine whether an interaction will occur, and whether this will be antagonistic or synergistic (Millott *et al.*, 1985, Kaufmann *et al.*, 1992, Fakae *et al.*, 1994, Sharma *et al.*, 2000). The picture is further complicated by the existence of intra-species interactions on the top of those established between pathogens of different species. To cite an example of the many that there exist, "*concomitant immunity*" in helminthes, whereby infection by adult parasite stages inhibits the establishment of subsequent infections by larval forms of the same species, may hinder the investigation of interactions between helminthes and the remaining pathogen community (Smithers *et al.*, 1969, Heath, 1995).

It is possible that extensive inter-geographical variation exists across areas in East Africa, with regards to the hierarchy of importance of individual organisms in the community of endemic pathogens. Differences in virulence have been demonstrated for genetically distinct parasites of the same species, including *T. annulata* (Rafyi *et al.*, 1965, Pipano *et al.*, 1974, Darghouth *et al.*, 1996, Graham *et al.*, 2001, Taylor *et al.*, 2002), *Babesia bovis* (Gill *et al.*, 1987, Timms *et al.*, 1990, Nevils *et al.*, 2000), *Trypanosoma congolense [Savannah]* (Van den Bossche, 2001, Masumu *et al.*, 2006), *Schistosoma mansoni* (Davies *et al.*, 2001), *Giardia intestinalis* (Homan *et al.*, 2001) and others. For Theileria spp. parasites, there is anecdotal evidence of unusually avirulent *T. parva* strains (Barnett *et al.*, 1966), unusually virulent *T. mutans* strains (Irvin *et al.*, 1972) and virulence differences between *T. parva* isolates (Koch *et al.*, 1988). Hence, if the virulence of pathogens was to differ across areas depending on the genetic variants present, a different hierarchy concerning the relative importance of individual pathogens on herd health would possibly apply.

5.4.4 Summary

The number of studies illustrating the pivotal role of concomitant infections in field settings is currently increasing (Briand *et al.*, 2005), and is raising the hypothesis that host population dynamics are not only affected by single pathogen species, but also by the wider pathogen community (Holmstad *et al.*, 2005). In this study, single pathogen infections were found to be relatively unimportant in terms of animal health. Previous results suggested that the pathogen community as a whole was capable of shaping the probability of infection by – and possibly transmission of - any individual pathogen (Chapter 4). In this chapter, preliminary evidence has also been presented which shows that the whole pathogen community determines the pathogenicity of its individual components and the incidence of clinical cases in the herd. There is, however, a hierarchy in the relative importance of each individual pathogen in the community. Such a hierarchy is multi-factorial and depends, amongst other things, on the pathogens virulence and the ability of each pathogen to interact with others in a manner detrimental to the health of the host, or beneficial to the transmission of the pathogen(s). Moreover, the ability to interact causing worse clinical condition may at least partially depend on the parasitaemias of the co-infecting organisms. In this context, the widespread Anaplasma spp. infections appear to play a paramount role within the pathogen community. Anaplasma spp. influences the infection probability of the widest number of endemic pathogens, and is the organism that most exacerbates the pathogenicity of mixed infections. This is not only because of its higher pathogenicity (as measured by haemoglobin depletion), but also because of its ability to interact with co-circulating pathogens. These findings are consistent with observations derived from fatal disease outbreaks in Swiss cattle, where *A. marginale* has been regarded as the major component orchestrating the onset of clinical disease in multiple pathogen systems (Hofmann-Lehmann *et al.*, 2004).

CHAPTER 6: The Epidemiology of *Theileria parva* and its Relationship with Cattle Age in Areas which Differ in *Rhipicephalus appendiculatus* Challenge

6.1 Introduction

6.1.1 Transmission Models

TBDs transmission models have to date proved more useful for improving our understanding of *T. parva* epidemiology than as an aid for the design of control policies, or for accurate prediction of the outcome of control strategies (Young *et al.*, 1988, Medley *et al.*, 1993). This is largely as a result of the complexity of the parasite's epidemiology which remains to be fully understood. Particular emphasis has been placed on investigating the extent and dynamics of carrier state in the field which is a crucial determinant of infection transmission (Medley *et al.*, 1993), and is central to a description of the epidemiology of the disease (Maritim *et al.*, 1989b).

At present only one transmission model has been developed which is specific to *T. parva* (Medley *et al.*, 1993). The model has been used to derive conclusions with regard to **(i)** the role of carrier animals in infection transmission, **(ii)** the extent to which tick density should be reduced to attain appreciable transmission decline and **(iii)** the impact that the creation of "carrier" animals by the practice of infection and treatment immunization may have on transmission burden. Model outputs are nevertheless largely based on simplified assumptions (Medley *et al.*, 1993) and have not been validated in the field. A major limitation of Medley's transmission model is that it is based on only a cohort of calves born into an endemically stable area, and is limited in its applicability to more diverse scenarios. This reflects the aspects of the parasite's epidemiology which are best understood as a result of detailed field studies, such as those conducted by Moll and co-workers (Moll *et al.*, 1984, Moll *et al.*, 1986), but highlights areas where research effort is required.

Modelling transmission dynamics in the context of a herd age structure more applicable to the field has been compromised by the lack of empirical data reporting infection probabilities in the bovine hosts (Medley *et al.*, 1993), and how these may be shaped over time and/or by population ageing as a result of interactions between the parasite and the host's immune system (Gettinby *et al.*, 1989), or as a result of acquired immunity against the vector (Fivaz *et al.*, 1989). It is also compromised by the lack of field studies reporting the proportion of recovered animals which remain carriers, albeit it is thought to approach 100% in Kenyan endemic areas (Young *et al.*, 1986, Kariuki, 1990), and up to 10% in Kenyan epizootic areas (Maritim *et al.*, 1989b). What impact animal age has on the likelihood of developing the carrier state under continuous vector re-challenge is largely unknown.

6.1.2 Specific Objectives

This is the first longitudinal study conducted on traditionally managed Zebu cattle that uses PCR to monitor natural *T. parva* infection dynamics in bovine hosts. As the PCR method used was applied to blood samples, a positive diagnosis is likely to have resulted from the presence of intra-erythrocytic transmission stages (i.e. piroplasms) in the sample. PCR diagnosis has several advantages over alternative diagnostic techniques. Following recovery from ECF, cattle present persistent low-level piroplasm parasitaemias which are usually undetectable by light microscopy (Dolan, 1986, Kariuki *et al.*, 1995). In contrast, PCR has been found to be capable of detecting these low-level parasitaemias (Bishop *et al.*, 1992, Morzaria *et al.*, 1999). More importantly, positive PCR results in recovered animals broadly correlate with transmission to ticks and subsequent infection to new hosts (i.e. carrier state), whilst negative PCR results have been found to correlate with lack of parasite transmission and infectivity to new hosts (Bishop *et al.*, 1992). As a result, PCR has been regarded as a good indicator of recovered animals that become carriers and hence play a role in *T. parva* transmission.

The specific objectives of this study were twofold. Firstly, to investigate the relationship between *T. parva* patent infection above PCR detection threshold and cattle age in indigenous herds kept in areas which differ in *R. appendiculatus* challenge. Secondly, to investigate whether there is spatial

heterogeneity in transmission of *T. parva* that cannot be explained by spatial variation in *R. appendiculatus* challenge.

6.2 Methods

To address the specific objectives, a combination of generalised linear modelling (GLM) and generalised linear mixed-effects modelling (glmmPQL) was used. Glm methods utilised in this chapter include contingency tables with poisson errors and analysis of deviance with proportion data. For details of Glm methodology see Section 3.4.3. GlmmPQL with binomial error structure was used to model *T. parva* infection status throughout the longitudinal study. *R. appendiculatus* counts were modelled specifying GlmmPQL with poisson error structure. In the latter, counts of zero, one, two, or three were specified as the dependent variable, and corresponded to tick scores of zero, 1-10, 11-50, or >50 adult *R. appendiculatus* tick stages on half the body surface (RA/HBS) respectively. This method is valid assuming random distribution of tick scores amongst the cattle sub-populations considered in this study (i.e. from different villages), and has proved useful to establish comparisons of the level of tick infestation experienced by animals given any set of circumstances. For details of GlmmPQL methodology see Section 3.4.5. Although the statistical analysis of tick data was invariably based on untransformed tick scores, raw tick data is presented in some instances using transformed tick counts (TC) to aid interpretation of the *R. appendiculatus* trends. To recap, TC combines information on the proportion of tick-positive animals and the intensity of infestations in cattle herds at any round of observation. The formula used to calculate TC is given in Section 3.4.1.

6.2.1 Classification of Study Villages according to *R. appendiculatus* Numbers and *T. parva* Prevalence in Cattle

The first part of this chapter offers a classification of geographical areas in the study according to the level of *R. appendiculatus* challenge and the incidence and prevalence of *T. parva* in cattle (Section 6.3.1 and 6.3.2). Following the criteria defined by Yeoman (1966), villages were classified as either enzootic or non-enzootic depending on the numbers of ticks recorded from cattle. Throughout the following sections, villages which fall within the category of enzootic foci have been termed 'HTC (High

Tick Challenge) villages', whilst villages which fall within the category of non-enzootic (i.e. recently enzootic, epizootic or sporadic foci) have been referred to as 'LTC (High Tick Challenge) villages'. To establish a classification of villages according to *R. appendiculatus* challenge, and examine how this relates to the classification of villages with regard to the incidence and prevalence of *T. parva* in cattle, GlmmPQL was used in all cases. The exception was when the interest was to compare the cumulative percentage of parasite-positive cattle in different study villages. In such cases, analyses of contingency tables with poisson errors or classical tests (i.e. Chisq; Fisher's exact) were used instead, depending on whether comparisons were established between two or across more than two villages or areas.

In the following sections, RA/HBS, tick counts or tick numbers refer to 'Number of adult *R. appendiculatus* instars recorded on half the body surface'.

6.2.2 Relationship amongst *T. parva* Infection Status, *R. appendiculatus*, Cattle Age and Time

The second part of this chapter investigates the relationship amongst the following variables: *T. parva* infection status, tick infestation intensity, cattle age and time (Section 6.3.5). These relationships were investigated using GlmmPQL with either poisson or binomial errors. In all cases, cattle age consisted of a four-level independent categorical variable (1-6, 7-12, 13-24 or > 24 months of age) but alternative level-groupings were taken into account for robustness (Data not shown). In some instances, the levels of this variable were collapsed into three levels (1-12, 13-24 or > 24 months of age) to allow for sufficient numbers of observations within each level.

The analyses are then expanded to characterise variation in the relationships of interest across villages and between enzootic (HTC) and non-enzootic (LTC) foci as defied by Yeoman (1966). See Section 6.3.6. Inter-geographical variation was explored by fitting different level-groupings of the variable "Geographical Origin of cattle" as fixed-effects (rather than as random-effects), depending on the questions addressed. Emphasis was placed throughout on understanding how the relationships amongst the variables of interest vary, if at all, across villages than may differ in the level of *R. appendiculatus* challenge.

6.2.3 Number of *T. parva* Positive Observations Recorded From Infected Cattle

Because the prevalence of *T. parva* at any time point during the study is a composite of new and persisting infections, an analysis was conducted to investigate the number of positive observations recorded from infected animals, and how these vary depending on the geographical origin and the age of animals at the time of the first parasite-positive diagnosis (Section 6.3.7). Variation in the number of positive observations obtained from *T. parva* infected cattle was investigated in an analysis of deviance with proportion data, which took into account the total number of PCR tests conducted on each animal. See Section 3.4.3. The response variable was modelled as a function of 1/. the age of individual cattle at the time of the initial parasite-positive observation, and 2/. the village of origin of each animal while accounting for the age of individual cattle at the time of the initial parasite-positive observation. In the first case, several level-groupings of "Cattle age" were tested. In the second case, inter-village variation was investigated by considering all eight villages (i.e. by specifying an eight-level categorical variable), or by considering only specific sets of villages depending on the questions addressed. In the analyses of inter-village variation, cattle age at the first positive diagnosis consisted of a three-level categorical variable (1-12, 13-24 or > 24 months of age), but alternative level-groupings were also taken into account for robustness (Data not shown).

Although LA oxytetracycline had no apparent clearance effect on *T. parva* infections above the PCR detection threshold (Section 4.3.10), an effect of LA oxytetracycline on the number of positive observations recorded from infected cattle, was discarded prior to assessment of inter-village variation, effect of age at the time of the first parasite-positive observation, or presence of interaction between these terms.

6.2.4 Presentation of Statistical Analyses

Throughout the results sections, a superscript number is shown next to each statistical test. Supplementary information on all the statistical models used in this chapter is given in Appendix 6.1. In glmmPQL analyses with binomial error structure, odds ratios (OR) were calculated from PQL model

coefficients. Untransformed PQL model coefficients ('Coef') are presented for glmmPQL analyses with poisson errors.

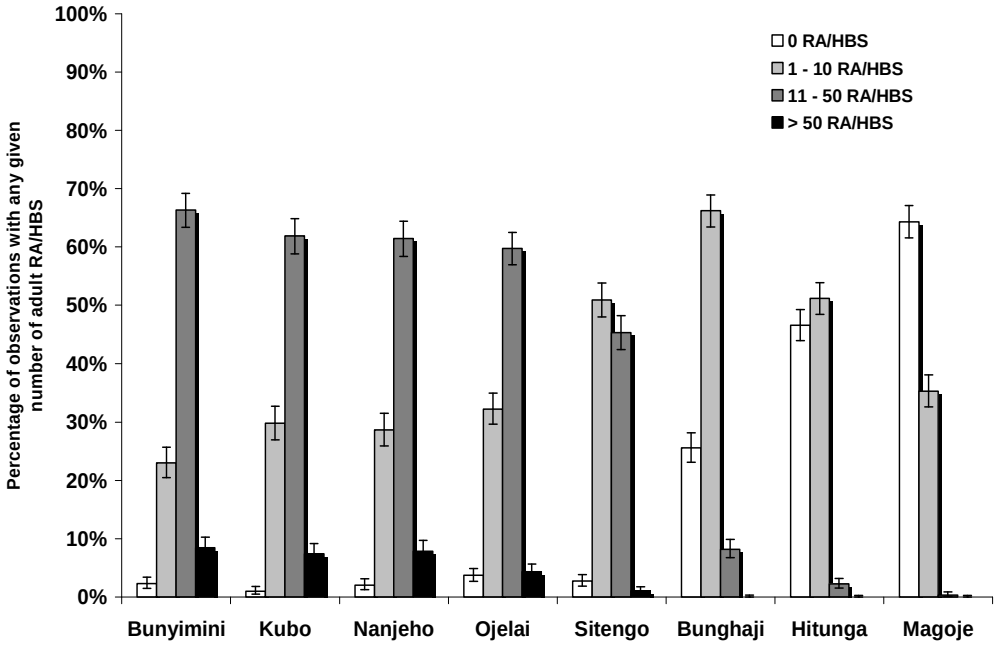
6.3 Results

6.3.1 Classification of Study Villages according to *R. appendiculatus* Numbers on Cattle

The number of adult *R. appendiculatus* stages on half the body surface (RA/HBS) was available for 9213 repeated observations on 893 cattle. Indigenous cattle from the study areas most commonly presented with 1-10 (40.4%; n = 3724) or 11-50 (36.4%; n = 3351) adult tick stages on half of their body surface. Tick-negative observations were also common (19.8%; n = 1828), whilst more than fifty ticks were rarely observed (3.4%; n = 310). See Figure 6.1.

Tick infestation rates, as well as tick infestation intensities, varied amongst cattle herds raised in the different villages (n = 9213 on 893 cattle; $F_{7, 855} = 525.9$; $p < 0.0001$; See Figure 6.1)[1]. In Bunghaji, Magoje and Hitunga (i) more than 50 RA/HBS were never recorded from cattle (n = 3726), whilst in the remaining villages – Bunyimini, Kubo, Nanjeho, Ojelai and Sitengo – more than 50 RA/HBS were recorded on 5.6% of the samplings (n = 5487). (ii) In the former villages the percentage of observations with 11-50 RA/HBS was low (3.6%; n = 3726) compared to in the latter study sites (58.7%; n = 5487). (iii) Tick infestations involving 1-10 RA/HBS were 1.5 times more common in Bunghaji, Hitunga and Magoje (50.9% vs. 33.3%) and (iv) tick-negative observations were almost 20 times higher in these study sites compared to in the remaining villages (45.5% vs. 2.4%).

Figure 6.1 Proportions of observations with zero, 1-10, 11-50 or more than 50 *Rhipicephalus appendiculatus* (RA/HBS) in the study villages.



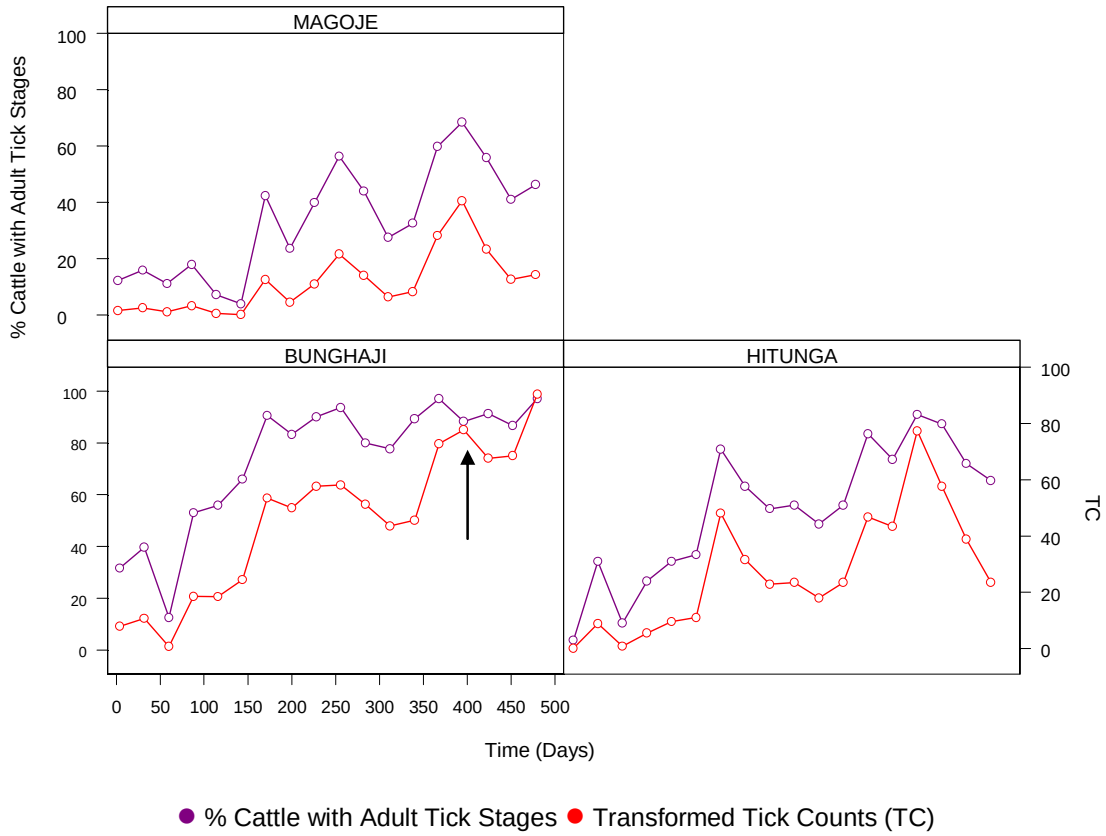
RA/HBS	Village								Total
	Bunyimini	Kubo	Nanjeho	Ojelai	Sitengo	Bunghaji	Hitunga	Magoje	
0	24	10	21	45	32	306	626	764	1828
1 to 10	243	302	295	393	593	792	687	419	3724
11 – 50	701	628	633	729	528	98	30	4	3351
> 50	89	75	81	53	12				310
Total	1057	1015	1030	1220	1165	1196	1343	1187	**9213**

The number of observations available in each village was as follows: Bunyimini [n = 1057 on 96 cattle]; Kubo [n = 1015 on 102 cattle]; Nanjeho [n = 1030 on 110 cattle]; Ojelai [n = 1220 on 126 cattle]; Sitengo [n = 1165 on 94 cattle]; Bunghaji [n = 1196 on 138 cattle]; Hitunga [n = 1343 on 100 cattle]; Magoje [n = 1187 on 127 cattle]. The table shows the number of observations with zero, 1-10, 11-50 or >50 adult tick stages on half the body surface (RA/HBS) in each village. Villages are arranged by decreasing level of *R. appendiculatus* challenge. Tick counts differed across villages (model [1]). Error bars show 95% exact binomial confidence intervals. Tororo villages: Ojelai, Bunghaji, Hitunga and Magoje. Busia villages = Bunyimini, Nanjeho, Kubo and Sitengo.

Almost all cattle had been exposed to *R. appendiculatus* on at least one visit round by the end of the study period (94%; n = 839). On the basis of the tick counts recorded from cattle in the study villages, and according to the criteria defined by Yeoman (1966), two areas representing different levels of *R.appendiculatus* challenge were apparent. Firstly, a "low tick challenge" area (LTC; Figure 6.2.a) where the prevalence of tick-infested cattle steadily built-up over the course of the study, but where a large proportion of animals were tick-free on multiple visit rounds. In this area, tick-infested cattle

presented only low numbers of ticks, and 9% to 20% of cattle, depending on the village considered, were never found infested with *R. appendiculatus* over the course of the study (Appendix 4.6).

Figure 6.2.a Transformed *Rhipicephalus appendiculatus* counts (TC), and proportions of *Rhipicephalus appendiculatus* infested cattle in low tick challenge villages (LTC).



TC broadly correlates with the prevalence of tick-infested cattle. This results from the absence of cattle with a tick score of three (i.e. >50 RA/HBS) and the low number of cattle with a tick score of two (i.e. 11-50 RA/HBS). Deviations from a perfect correlation between the two lines occur at visit rounds where tick scores of two were recorded. Note, for example, the fourth to last visit round in Bunghaji (highlighted with an arrow). In this visit round, the percentage of cattle infested with adult tick stages decreased, but TC increased as a result of higher tick counts being recorded from infested cattle. In the LTC villages, a strong upward trend was apparent for both TC and the proportion of tick-infested cattle over the course of the study. However, a large proportion of animals were tick-free or presented only low numbers of ticks (See Figure 6.2.b for comparison with HTC villages). The number of cattle for which tick data was available was 127 ($Magoje_{[Tororo]}$), 138 ($Bunghaji_{[Tororo]}$) and 100 ($Hitunga_{[Tororo]}$).

Secondly, a “high to intermediate tick challenge” area (HTC; Figure 6.2.b) where the percentage of tick-positive cattle was close to 100% throughout the study period, and where tick-infested cattle presented moderate to high numbers of ticks. All cattle from the HTC area were exposed to adult *R.*

appendiculatus instars at least once during the study except for two animals from Sitengo (Appendix 4.6).

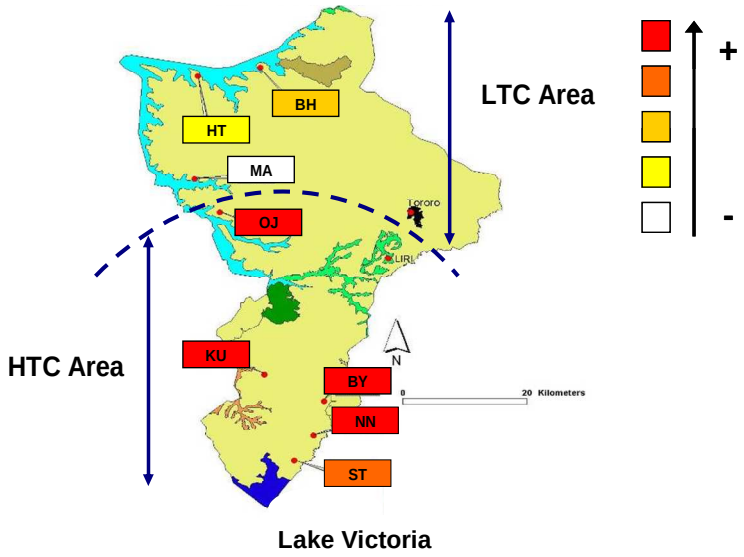
Figure 6.2.b Transformed *Rhipicephalus appendiculatus* counts (TC), and proportions of *Rhipicephalus appendiculatus* infested cattle in high tick challenge villages (HTC).

TC, unlike in LTC villages (Figure 6.2.a), does not correlate with the prevalence of tick-infested cattle. This resulted from the proportion of tick-infested cattle being close to 100% in all HTC villages over the course of the study. In contrast, TC increased or decreased as a function of the number of tick-infested cattle with high, moderate or low tick numbers (i.e. >50, 11-50 or 1-10 RA/HBS). In the HTC villages, tick-infested cattle presented moderate to high numbers of ticks (See values on TC axis compared to those in LTC villages [Figure 6.2.a]). The number of cattle for which tick data was available was 94 ($Sitengo_{[Busia]}$), 110 ($Nanjeho_{[Busia]}$), 126 ($Ojelai_{[Tororo]}$), 96 ($Bunyimini_{[Busia]}$) and 102 ($Kubo_{[Busia]}$).

The LTC territory was located to the north of the study area, and comprised three of the four Tororo villages (Bunghaji, Hitunga and Magoje). The HTC territory was located to the south of the study area, and was geographically closer to the shore of Lake Victoria. This comprised all Busia villages (Bunyimini, Kubo, Nanjeho and Sitengo) and Ojelai (Tororo). See Figure 6.3.

Statistical analysis revealed a greater level of detail to the gross LTC-HTC division (Figure 6.3). Significant differences in the level of tick challenge amongst cattle in HTC villages, occurred only when animals from Sitengo were considered in the analysis (n = 5487 on 528 cattle; $F_{4, 523} = 40.76$; $p < 0.0001$)[2]. Hence, within the HTC area, two distinctive clusters of villages were apparent with regard to the level of *R. appendiculatus* challenge. Firstly, tick numbers were highest and not different amongst cattle in Bunyimini, Kubo, Nanjeho and Ojelai (n = 4322 on 434 cattle; $F_{3, 430} = 1.87$; $p = 0.133$)[3]. Secondly, tick counts were lowest amongst cattle in Sitengo ($Sitengo_{[coef]} = -0.21$; n = 5487 on 528 cattle; $F_{1, 526} = 156.2$; $p < 0.0001$)[4].

Figure 6.3 Classification of villages, based on the level of *Rhipicephalus appendiculatus* challenge recorded during longitudinal studies in Tororo and Busia districts.



1:50.000 scale land use data

Depleted tropical high forest Woodland (average height > 4 m) Grassland, rangeland, open savannah, pastureland Wetland (swamps) Subsistence farmland (mixed, in use or fallow) Uniform farmland (rice plantations) Urban or rural built up area Open water (large rivers, ponds and lakes) Selected villages

The gross division between high (HTC) and low (LTC) tick challenge areas as defined by Yeoman (1966), is shown by a dashed blue line. The Busia district is situated south from the green area representing grassland, rangeland, open savannah and pastureland, whilst the Tororo district is located north from it. Those villages which did not differ significantly in "tick challenge" – as measured by the tick counts (RA/HBS) recorded from cattle – are highlighted with the same colour. Conversely, tick challenge was significantly different amongst villages highlighted with a different colour. In the right-hand side of the figure, the coloured boxes are arranged to represent the gradient of tick challenge across the study villages. $Bunyimini_{[Busia]}$ (BY), $Kubo_{[Busia]}$ (KU), $Nanjeho_{[Busia]}$ (NN), $Ojelai_{[Tororo]}$ (OJ), $Sitengo_{[Busia]}$ (ST), $Bunghaji_{[Tororo]}$ (BH), $Hitunga_{[Tororo]}$ (HT) and $Magoje_{[Tororo]}$ (MA).

Within the LTC area, each village was a separate cluster with regard to the level of *R. appendiculatus* challenge experienced by cattle. The level of challenge was higher in cattle from Bunghaji than from Hitunga (Hitunga$_{[coef]}$ = -1.13; n = 2539 on 238 cattle; $F_{1, 236} = 90.9$; p <0.0001)[5], and higher in cattle from Hitunga than from Magoje (Magoje$_{[coef]}$ = -0.45; n = 2530 on 227 cattle; $F_{1, 225} = 58.0$; p <0.0001)[6]. The overall proportion of tick-positive observations was also lower in the latter (Magoje$_{[OR]}$ = 0.46; n = 2530 on 227 cattle; $F_{1, 225} = 43.68$; p <0.0001)[7].

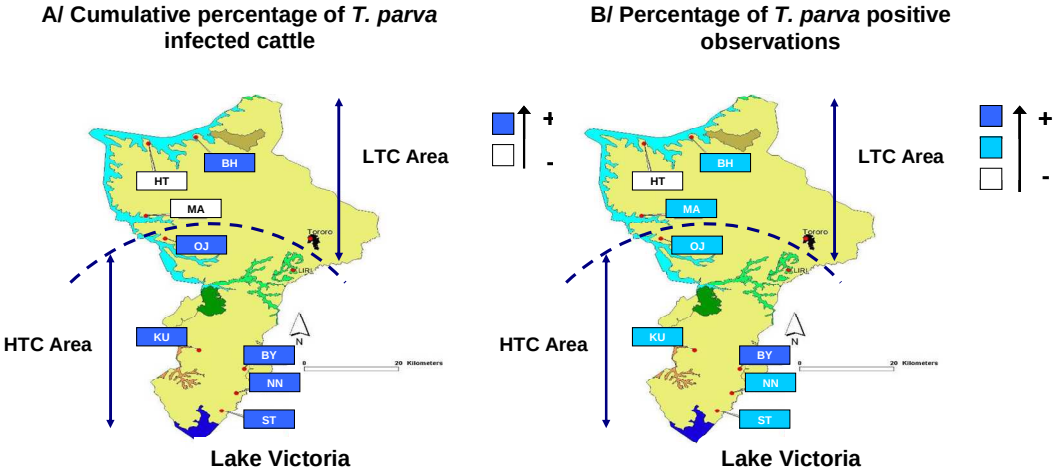
A comparison between the level of *R. appendiculatus* challenge in Bunghaji (i.e. LTC village where tick numbers were highest) and Sitengo (i.e. HTC village where tick numbers were lowest), showed that tick challenge was significantly higher in the latter study site (Sitengo$_{[coef]}$ = + 0.46; n = 2361 on 232 cattle; $F_{1, 230} = 262.6$, p<0.0001)[8].

6.3.2 Classification of Study Villages according to *T. parva* Prevalence in Cattle

The molecular screening of *T. parva* using IL3232 and IL4231 primers (Skilton *et al.*, 2002) was conducted on 884 out of 893 cattle recruited in the study, and on 8094 out of 9240 blood samples. *T. parva* was diagnosed in 31.8% (n = 281) of the study population, and was recorded from a differing proportion of cattle in each village. Infected animals were parasite-positive on one or multiple rounds of observation (up to 13 rounds). Overall, 537 out of 8094 samples tested were *T. parva* positive. The cumulative percentage of *T. parva* infected cattle, and the percentage of parasite-positive observations recorded over the course of the study in Tororo and Busia villages is summarised in Figure 6.4.

The cumulative percentage of cattle infected with the parasite differed significantly across villages (n = 884 cattle; Chisq = 53.13; df = 7; p<0001)[9]. However, the classification of villages with respect to the cumulative percentage of *T. parva* infected cattle - which is in turn a measure of parasite's incidence in the study population - was broadly consistent with the classification of villages on the basis of tick challenge (Figure 6.4).

Figure 6.4 Classification of villages, based on the cumulative percentage of *Theileria parva* infected cattle (A) or the percentage of *Theileria parva* positive observations (B), recorded during longitudinal studies in Tororo and Busia districts.



1:50.000 scale land use data

Depleted tropical high forest Woodland (average height > 4 m) Grassland, rangeland, open savannah, pastureland Wetland (swamps) Subsistence farmland (mixed, in use or fallow) Uniform farmland (rice plantations) Urban or rural built up area Open water (large rivers, ponds and lakes) Selected villages

Village	A/B	Cattle [A]			Observations [B]		
		n	% Positive	95 % CI	n	% Positive	95% CI
HTC							
Bunyimini	3.8	96	44.79	34.6 - 55.3	1060	11.9	10.0 – 14.0
Kubo	5.3	102	39.22	29.7 - 49.4	1017	7.37	5.84 - 9.16
Nanjeho	5	109	42.2	32.8 - 52.0	1029	8.45	6.83 - 10.3
Ojelai	4.5	127	29.13	21.4 - 37.9	1161	6.46	5.11 - 8.03
Sitengo	6.1	94	39.36	29.4 – 50.0	1170	6.41	5.08 - 7.97
LTC							
Bunghaji	7.5	138	33.33	25.5 - 41.9	1131	4.42	3.30 - 5.79
Hitunga	6.8	97	11.34	5.80 - 19.4	831	1.68	0.92 - 2.81
Magoje	3.4	121	17.36	11.1 - 25.3	695	5.04	3.53 - 6.93
Total		884	31.79	28.7 – 35.0	8094	6.63	6.10 – 7.20

'n' refers to total numbers of cattle (right-hand side) or observations (left-hand side) tested for *T. parva*. Villages that did not differ significantly with regard to 'A' or 'B', are highlighted with the same colour. The coloured boxes are arranged to represent the gradient of *T. parva* infections across the study villages. The classification of villages with respect to A, was broadly consistent with that on the basis of tick challenge. The same was not true concerning the classification of villages with respect to B. The ratio A/B provides a useful comparison of the cumulative percentage of infected cattle and the percentage of positive samples recorded from each village. In Bunghaji and Hitunga the cumulative percentage of infected cattle was 7-8 times higher than the percentage of positive samples. In Magoje, however, the former was only 3.4 times higher than the latter, suggesting that the few infected cattle, may have tested *T. parva* positive on multiple samplings. Abbreviations correspond to Bunyimini$_{[HTC]}$ (BY), Kubo$_{[HTC]}$ (KU), Nanjeho$_{[HTC]}$ (NN), Ojelai$_{[HTC]}$ (OJ), Sitengo$_{[HTC]}$ (ST), Bunghaji$_{[LTC]}$ (BH), Hitunga$_{[LTC]}$ (HT) and Magoje$_{[LTC]}$ (MA). 95% CI = 95% exact binomial confidence intervals.

The proportion of parasite-positive cattle recorded over the course of the study was lowest in villages where lower tick numbers were recorded. HTC villages and Bunghaji (LTC) presented similar numbers of parasite-exposed cattle towards the end of the study period, as measured by PCR (n = 666 cattle; Chisq = 8.38; df = 5; p=0.136)[10]. The cumulative percentage of *T. parva* infected cattle was also similar in Magoje and Hitunga (n = 218 cattle; Chisq = 1.56; df =1; p = 0.212)[11]. The proportion of parasite-positive cattle was significantly lower in these villages, where lower tick numbers had been recorded, than in the rest of study sites (n = 884; Chisq = 39.06; df = 1; p<.0001)[12].

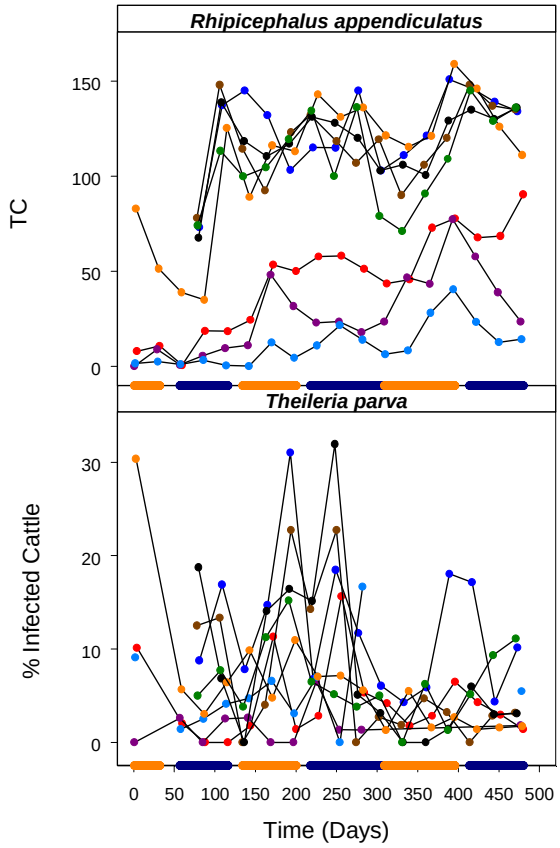
In contrast to the above observations, the classification of villages with respect to the percentage of *T. parva* positive observations (PPO), which represent a composite of new and persisting infections by PCR, was inconsistent with that based on tick challenge. For example in Bunghaji, the cumulative percentage of parasite-positive animals almost doubled that in Magoje (n = 259; Chisq = 8.58; df = 1; p = 0.003)[13]. This was consistent with observations that tick numbers were significantly higher in the former village. However, the percentage of *T. parva* positive observations (PPO) was similar in Magoje as compared to in Bunghaji (n = 1826 on 259 cattle; $F_{1, 257} = 1.238$; p = 0.267)[14].

Altogether, the classification of study villages with respect to the proportion of parasite-positive observations recorded during the study (PPO) was as follows: 1/. PPO was not different in Magoje (LTC) than in Bunghaji (LTC) or HTC villages other than Bunyimini (n = 6203 on 691 cattle; $F_{5, 685} = 1.292$; p = 0.265)[15]. This was despite tick numbers being substantially lower in Magoje and the cumulative prevalence of parasite-positive animals being significantly higher in the latter villages (Bunghaji & HTC villages other than Bunyimini). 2/. In Bunyimini, PPO was higher and significantly different to at least one of the villages in the main cluster (n = 7263 on 787 cattle; $F_{6, 780} = 9.69$; p <.0001)[16], whilst 3/. In Hitunga, PPO was lower and also significantly different from at least one of the villages in the main cluster (n = 7034 on 788 cattle; $F_{6, 781} = 4.31$; p = 0.0003)[17]. See Figure 6.4.

6.3.3 Temporal Distributions of *R. appendiculatus*

The transformed counts of *R. appendiculatus* (TC) and the percentage of *T. parva* infected cattle at any time point during the study are shown, for each village, in Figure 6.5.

Figure 6.5 Transformed counts (TC) of *Rhipicephalus appendiculatus* and percentages of *Theileria parva* infected cattle at each round of observation in each of the study villages.



HTC Villages: Bunyimini (B) Kubo (B) Nanjeho (B) Ojelai (T) Sitengo (B)
LTC Villages: Bunghaji (T) Hitunga (T) Magoje (T)
Dry season Wet Season

B = Busia village and T = Tororo village.

TC showed a continuous development of *R. appendiculatus* in all the study areas - with adult stages being recorded from cattle all year round - but the number of tick-positive cattle, and the intensity of tick infestations, varied significantly depending on the month of the year (n= 8363 on 825 cattle; $F_{14, 7426} = 90.16$; $p<0.0001$)[18]. In HTC villages tick numbers followed a cyclical pattern consisting of a TC fall during each seasonal dry spell, which was followed by a recovery of TC towards the onset of each rainy season. Three major peaks of adult *R. appendiculatus* were observed throughout the study period coinciding with the onset of three wet seasons. However, the TC peak recorded during the first wet season of the year 2002 (March to May) consisted in reality of two smaller consecutive peaks

inter-spaced by a shorter number of days, probably representing two generations of adult *R. appendiculatus* (Figure 6.5). In low tick challenge villages TC followed a strong upward trend which was far more obvious than in HTC villages.

The time length between TC peaks ranged from 56 to 112 days in the villages where tick challenge was highest (Bunyimini, Kubo, Nanjeho, and Ojelai), whilst from 56 to 140 days in Sitengo (HTC) and the LTC villages. TC started recovering approximately 28-56 days before reaching a peak, so that the time length between a peak and the start of TC recovery ranged invariably between 28 and 84 days (Figure 6.5). Temporal variation in RA/HBS was different depending on the village (*Village : Month*; n= 8363 on 825 cattle; $F_{98, 7426} = 9.13$; $p<0.0001$)[18]. In all areas, however, the time length between a TC peak and the start of TC recovery was of three months. A shorter time length (one month) was only observed during the first wet season of the year 2002 (March to May) coinciding with the highest average rainfall and average morning temperature records during the study period (Section 4.3.5).

6.3.4 Temporal Distributions of *T. parva* Infected Cattle

The prevalence of *T. parva* varied depending on the month of the year (n= 4991 on 806 cattle; $F_{8, 4121} = 16.17$; $p<0.0001$)[19] and temporal variation was in addition dependent on the village considered (*Village : Month*; n= 4991 on 806 cattle; $F_{56, 4121} = 4.98$; $p<0.0001$)[19]. Periodic fluctuations of *R. appendiculatus* were correlated with fluctuations in the prevalence of cattle infected with *T. parva*, with the greatest number of parasite-positive animals being recorded during the first wet season of 2002 (March to May). However, a consistent trait in all study areas was that peaks of parasite-positive cattle were recorded slightly earlier than peaks of adult tick numbers (Figure 6.5).

6.3.5 Relationship amongst *T. parva* Infection Status, *R. appendiculatus*, Cattle Age and Time

6.3.5.1 R. appendiculatus and Cattle Age

The number of adult RA/HBS increased significantly as cattle grew older (n = 8539 on 882 cattle; $F_{3, 7654} = 46.17$; $p <.0001$.See Appendix 6.1)[20]. Tick counts of more than 50 adult RA/HBS were almost

exclusively recorded from adult (77.4%) or juvenile cattle (19.5%). In adult cattle, the relative risk of zero RA/HBS was 0.40 that in calves of 1-6 months (705/4610 vs. 118/312), whilst the relative risk of 1-10 RA/HBS was 0.87 that in calves of up to six months (1683/4610 vs. 131/312). Conversely, in adult cattle, the relative risk of 11-50, or more >50 RA/HBS was 2.18 (1996/4610 vs. 62/312) and 15.3 (226/4610 vs. 1/312) that in calves of up to 6 months of age respectively. These calculations were based on raw data presented in Section 6.3.6.1 (Table 6.1; "Grand Total" rows). The relationship between transformed tick counts (TC) and cattle age is summarised in Figure 6.6 (Panel A).

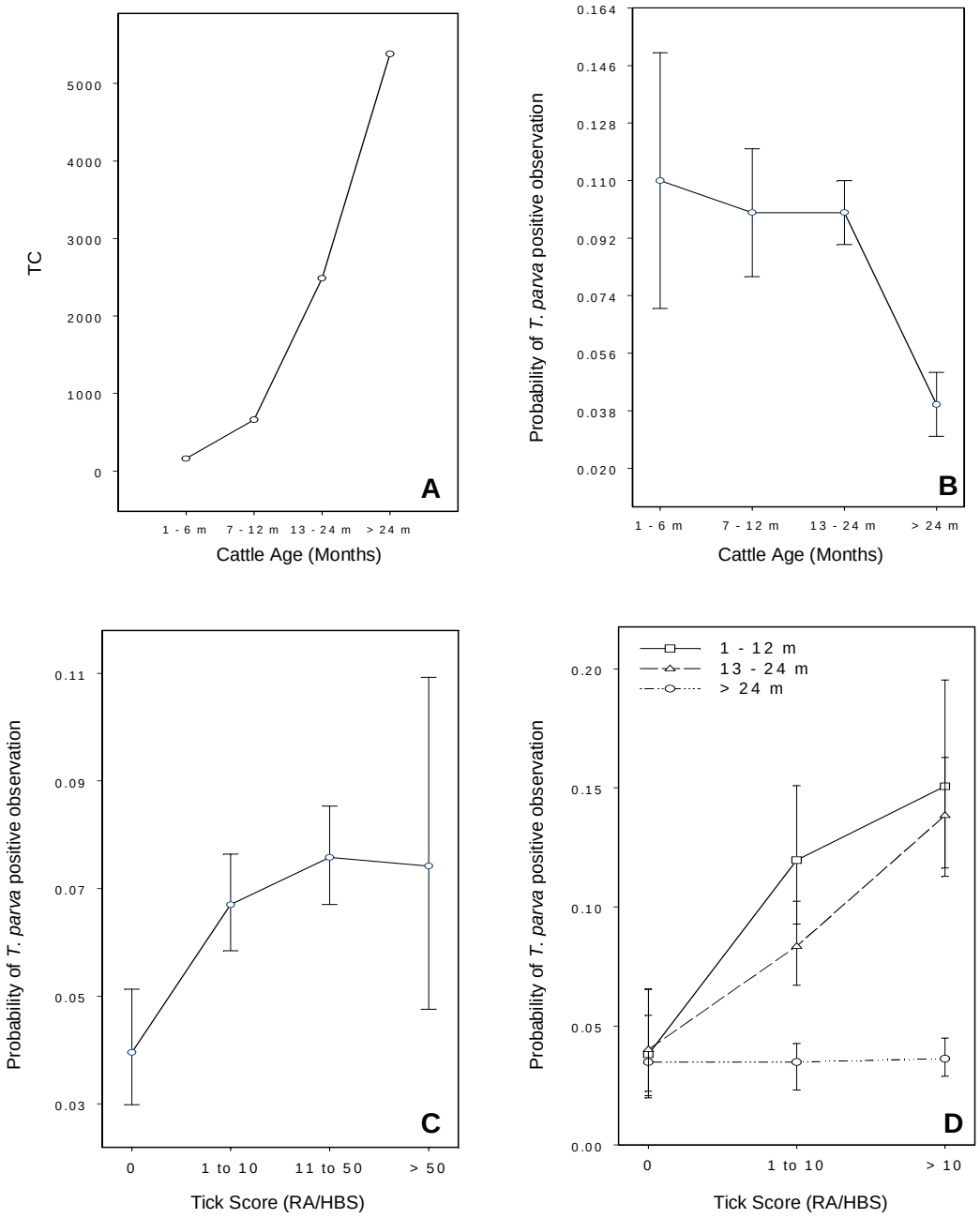
6.3.5.2 T. parva Infection Status and Cattle Age

Despite observations that tick-positive samplings as well as tick numbers increased as cattle grew older, the percentage of *T. parva* positive observations by PCR dropped significantly as soon as cattle grew into adults (n = 7494 on 8 villages; $F_{3, 7483} = 37.06$; $p < 0.0001$. See Appendix 6.1)[21], but was not different amongst calves of 1-6 and 7-12 months of age, and juveniles aged 13-24 months (n = 3417 on 719 cattle; $F_{2, 2696} = 2.087$; $p = 0.124$)[22]. The relationship between cattle age and *T. parva* infection status is presented graphically in Figure 6.6 (Panel B). In adult cattle (>24 months of age), the relative risk of *T. parva* infection by PCR was only 0.35 that in cattle of up to 24 months of age (145/4077 vs. 344/3417).

6.3.5.3 T. parva Infection Status, R. appendiculatus and Cattle Age

Tick infestation, and tick infestation intensity, had a predictive value on the probability of *T. parva* infection above the PCR detection threshold (n = 7479 on 872 cattle; $F_{2, 6599} = 9.16$; $p = 0.0001$. See Appendix 6.1)[23]. The probability of *T. parva* positive diagnosis increased with the number of adult RA/HBS recorded from cattle. This relationship, however, was non-linear (Figure 6.6. Panel C). This resulted from high tick scores being recorded mainly from adult cattle, which were in turn less likely to test *T. parva* positive by PCR.

Figure 6.6 Relationship amongst *T. parva* infection status, *R. appendiculatus* numbers and cattle age.



For simplicity, the relationship between cattle age and *R. appendiculatus* is represented using transformed tick counts (TC) rather than tick scores representing proportions of observations with zero, 1-10, 11-50 or >50 RA/HBS. The significance of this relationship was nevertheless tested considering untransformed tick scores. In order to minimise SE from model coefficients, the analysis of the relationships described in panels C and D (model [23]), considered only three tick scores (as in panel D). Error bars show 95% exact binomial confidence intervals.

The relationship between tick infestation (as well as tick infestation intensity) and *T. parva* infection above the PCR detection threshold, was dependent on the age of cattle (*Age : RA/HBS*; n = 7479 on

872 cattle; $F_{4, 6599} = 5.56$; p =0.0002)[23]. *T. parva* positive observations increased linearly with the number of ticks recorded from calves (1-12 months of age) and juveniles (13-24 months of age), but the same was not true in adult cattle (See Figure 6.6, Panel D). Analysis [23] took into account three cattle age groups and three tick scores as to reduce the standard errors of model estimates (Appendix 6.1; model [23]). However, visual inspection of the data suggested that the described trends were maintained when considering four age groups (1 – 6 , 7 – 12, 13 – 24 and > 24 months of age) and four tick scores (0, 1 – 10, 11 – 50 and > 50 adult RA/HBS); Data not shown.

6.3.5.4 Temporal Trends in R. appendiculatus and Cattle Age

Tick counts (RA/HBS) followed a significant upward trend throughout the study period ($Time_{[coef]}$ = +0.035; n = 9213 on 893 cattle; $F_{1, 8319} = 996.1$; p<0.0001)[24], which was still significant after considering the age of cattle at each round of observation ($Time_{[coef]}$ = +0.044; n = 8539 on 882 cattle, $F_{1, 7650} = 659.24$; p<0.0001)[25]. Notably, the rate at which the ticks increased every 28 days slowed down as animals grew older (*Age : Time*; n = 8539 on 882 cattle, $F_{3, 7650} = 20.18$; p<0.0001. See Appendix 6.1)[25]. The positive time-slope for tick numbers was not different in calves of up to 6 months of age or calves of 7-12 months of age (*Age : Time*; n = 1278 on 320 cattle; $F_{1, 955} = 0.256$; p=0.613)[26]. However, the slope recorded from calves (1-12 months) was greater than that in juveniles (*Juveniles : Time* = -0.01; n = 3929 on 737 cattle; $F_{1, 3189} = 8.14$; p=0.004)[27] or adults (*Adults : Time* = -0.03; n = 5888 on 821 cattle; $F_{1, 5064} = 46.38$; p<0.0001)[28]. The positive time-slope for tick numbers was also greater in juveniles than in adults (*Adults : Time* = -0.02; n = 7261 on 733 cattle; $F_{1, 6525} = 39.26$; p <.0001)[29].

The build-up of ticks in the study areas appeared to be attributable to a combination of the effect of cattle ageing and the occurrence of a genuine increase in tick numbers (i.e. on the top of that due to cattle growing older). The positive time-slope for tick numbers was greater when cattle age at each round of observation was taken into account (See model coefficient for "Time" in analyses [24 and 25]).

6.3.5.5 Temporal Trends in T. parva Infection Status and Cattle Age

The prevalence of *T. parva* followed a downward trend over the course of the study ($Time_{[OR]}$ = 0.94; n = 8094 on 884 cattle; $F_{1, 7209}$ = 5.329; p = 0.021)[30] . This trend, however, was not significant when the proportion of adult cattle at each round of observation was considered in the analysis (n = 7494 on 8 villages; $F_{1, 7483}$ = 0.478; p = 0.49)[31]. In consequence, and contrary to the case in ticks, the time-slope for *T. parva* infections by PCR, appeared to be explained mainly by the effect of cattle growing older over the course of the study. The time-slope for *T. parva* positive observations could not be regarded as different depending on the cattle age group considered (*Age : Time*; n = 7494 on 8 villages; $F_{1, 7483}$ = 3.575; p = 0.059)[31]. This analysis ([31]) considered only two age categories (up to 24 months of age versus adults [>24 months]), but was consistent with the results obtained when three or four age groups were taken into account (Data not shown).

6.3.6 Geographical Variation in the Relationship amongst *T. parva* Infection Status, *R. appendiculatus*, Cattle Age and Time

6.3.6.1 R. appendiculatus and Cattle Age

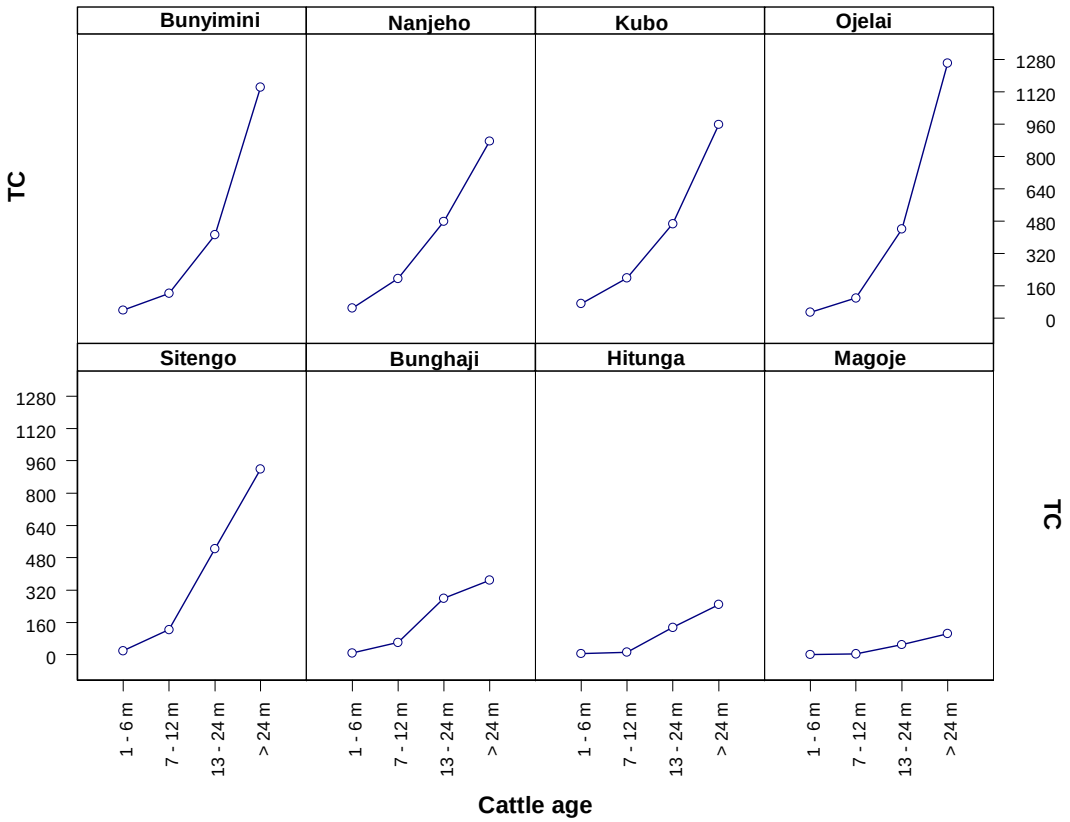
Host-vector contacts started at the age of 1-6 months in both HTC and LTC areas (Table 6.1). The relationship between cattle age and tick numbers (RA/HBS) was similar across geographical areas in that the general rule of increasing numbers with age applied in each study site (n = 8539 on 882; $F_{3, 7633}$ = 134.6; p<0.0001)[32]. However, the magnitude and rate at which tick numbers increased, varied across villages (*Village : Age*; n = 8539 on 882; $F_{21, 7633}$ = 5.69; p<0.0001)[32] and across HTC and LTC areas (*HTC or LTC : Age*; n= 8539 on 882 cattle; $F_{3, 7651}$ = 8.52; p<0.0001)[33]. See Figure 6.7. This resulted from tick score intercepts being different in calves of up to six months of age raised in either HTC or LTC areas. As animals grew older, tick scores moved from 1–10 to 11–50 RA/HBS or, less commonly, to > 50 RA/HBS in HTC villages. In LTC villages, tick scores moved from zero to 1-10 RA/HBS or, less commonly, 11-50 RA/HBS (See Table 6.1).

Table 6.1 Number of observations with zero, 1-10, 11-50 or > 50 adult *Rhipicephalus appendiculatus* (RA/HBS), classified on the basis of the geographical origin and the age of cattle at the time of each observation.

		Cattle Age (Months)			
Village	**RA/HBS**	**1 – 6 m**	**7 – 12 m**	**13 – 24 m**	**> 24 m**
Bunyimini	**0**	3	6	2	12
(HTC)	**1 - 10**	24	46	78	88
	11 - 50	9	41	152	434
	> 50		1	11	70
Kubo	**0**		6	1	3
(HTC)	**1 - 10**	26	55	81	118
	11 - 50	21	73	165	345
	> 50	1	2	19	52
Nanjeho	**0**	6	6	8	1
(HTC)	**1 - 10**	19	62	91	100
	11 - 50	19	65	170	315
	> 50		4	20	49
Ojelai	**0**		6	11	15
(HTC)	**1 - 10**	10	52	144	129
	11 - 50	9	27	149	510
	> 50			5	47
Sitengo	**0**		8	8	13
(HTC)	**1 - 10**	13	72	200	288
	11 - 50	3	29	165	313
	> 50		1	2	8
Bunghaji	**0**	15	52	77	117
(LTC)	**1 - 10**	17	84	289	352
	11 - 50		6	29	61
	> 50				
Hitunga	**0**	56	93	174	265
(LTC)	**1 - 10**	18	38	219	381
	11 - 50	1	1	10	16
	> 50				
Magoje	**0**	38	108	236	279
(LTC)	**1 - 10**	4	22	133	227
	11 - 50			2	2
	> 50				
Grand Total	**0**	118	285	517	705
	1 - 10	131	431	1235	1683
	11 - 50	62	242	842	1996
	> 50	1	8	57	226

The table shows the raw data used to construct the diagrams presented in Figure 6.6 (Panel A; See “Grand Total” rows) and Figure 6.7. Note the age at which host-vector contacts were established in each village.

Figure 6.7 Relationship between Transformed counts (TC) of *Rhipicephalus appendiculatus* and cattle age in high (HTC) and low (LTC) tick challenge villages.

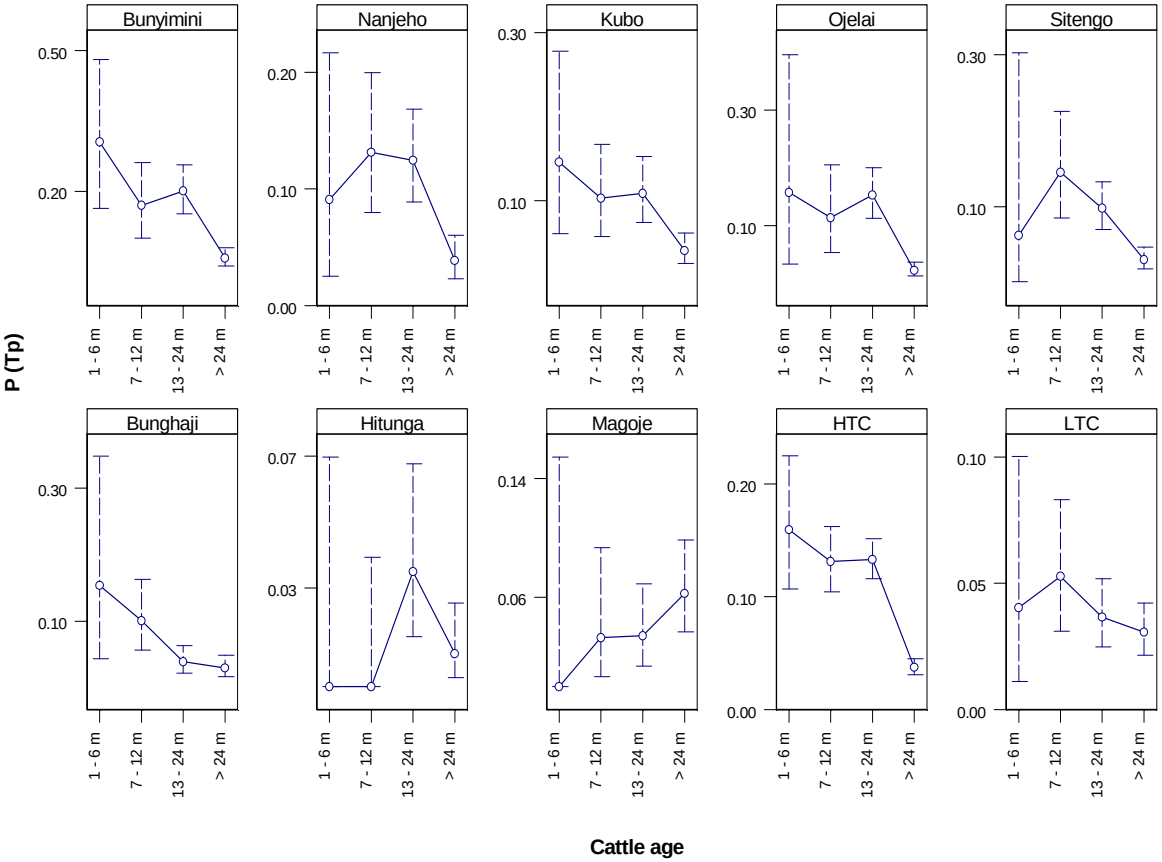


For simplicity, the relationship between cattle age and *R. appendiculatus* infestation, and infestation intensity, is represented using transformed tick counts (TC) rather than tick scores representing proportions of observations with zero, 1-10, 11-50 or >50 RA/HBS. However, the significance of inter-village variation with regard to the relationship of interest was tested considering untransformed tick scores. TC increased with cattle age in all HTC (Bunyimini, Kubo, Nanjeho, Ojelai, Sitengo) and LTC (Bunghaji, Hitunga and Magoje) villages. However, TC increase with cattle age was less pronounced in the LTC area as a result of tick-positive animals not attaining high infestation intensities, even during adulthood (>24 months of age).

6.3.6.2 T. parva Infection Status and Cattle Age

The distribution of *T. parva* across cattle age groups depended on the geographical origin of cattle (*Village : Age*; n = 7494 on 873; $F_{21, 6597} = 3.109$; $p < .0001$)[34] and on whether cattle were located in HTC or LTC areas (*HTC or LTC : Age*; n = 7494 on 873 cattle; $F_{3, 6615} = 5.301$; $p = 0.0012$)[35]. These analyses ([34, 35]) considered four age categories (1-6, 7-12, 13-24 and >24 months of age), but the results were consistent with those considering either two (≤ 24 or > 24 months of age) or three (1 – 12, 13 – 24, > 24 months of age) age groups. The relationship between *T. parva* infection above the PCR detection threshold and cattle age, is shown for each study village in Figure 6.8.

Figure 6.8 Relationship between *Theileria parva* infection status and cattle age in high (HTC) and low (LTC) tick challenge villages.



The figure presents raw percentages of *T. parva* positive observations recorded from different cattle age groups. The significance of the relationship between *T. parva* infection status and cattle age cannot be based on the degree of overlap between error bars (i.e. exact binomial 95% CI), as data analysis considered the identity of animals from which positive and negative observations were obtained, by specifying "Animal identity" as a random-effect (Appendix 6.1, models **34 to 50**).

In the HTC area, age was a significant predictor of *T. parva* infection regardless of whether the analysis was corrected by accounting for differences across villages (n = 5081 on 528; $F_{3, 4538} = 65.63$; p <.0001)**36** or not (n = 5081 on 528; $F_{3, 4550} = 69.58$; p <.0001)**37**. In this area, there existed heterogeneity in the relationship between cattle age and *T. parva* infection (*Village : Age*; n = 5081 on 528; $F_{12, 4538} = 2.467$; p = 0.003)**36**. However, in each HTC village the probability of detecting the parasite in adult cattle (> 24 months of age) was significantly lower than that in cattle of up to 24 months of age (all p-values < 0.05; Appendix 6.1; analysis **38**).

The prevalence of *T. parva* in calves of up to six months of age, seven to twelve months of age and juvenile cattle (13 – 24 months of age), was not significantly different in either of the HTC villages

except for the case of Ojelai (n = 379 on 100 cattle; $F_{2, 277} = 3.556$; p = 0.030) [39]. These results suggest that the risk of parasite infection does not decrease significantly until cattle reach adult age (> 24 months) in the majority of the HTC sites (four out of five villages). In Ojelai, however, the probability of parasite infection decreased slightly as soon as animals were seven months of age (n = 98 on 31 cattle; $F_{1, 66} = 9.604$; p = 0.003)[40], but a major fall in parasite-positive observations did not occur either until cattle reached adult age as compared to cattle aged 7-24 months (n = 1052 on 127 cattle; $F_{1, 924} = 87.18$; p <0.0001)[41]. See Appendix 6.1 and Figure 6.8. The overall probability of *T. parva* infection in adult cattle (>24 months old) from the HTC area, was 0.26 that in younger cattle ($Adults_{[OR]} = 0.26$; n = 5081 on 528 cattle; $F_{1, 4552} = 201.09$; p <.0001)[42].

In the LTC area, cattle age was not a significant predictor of *T. parva* infection (n = 2413 on 3 villages; $F_{3, 2407} = 1.142$; p = 0.331)[43] unless the analysis accounted for differences across villages in the relationship investigated (n = 2413 on 345 cattle; $F_{3, 2059} = 4.443$; p = 0.004)[44]. This observation, contrary to the case in HTC villages, resulted from the relationship between parasite infection and cattle ageing being highly heterogeneous (*Village : Age*; n = 2413 on 345 cattle; $F_{6, 2059} = 3.267$; p = 0.003)[44] and not inversely related in all LTC villages. These analyses considered four age categories ([43, 44]), but the results were consistent with those considering three age groups (1-12, 13-24 and >24 months of age; Data not shown). In the LTC area, when comparing the probability of parasite infection in animals of up to 24 months of age versus adults (> 24 months), cattle age did not appear as a significant predictor of *T. parva* infection even when differences across villages were taken into account (n = 2413 on 345 cattle; $F_{1, 2065} = 0.687$; p = 0.407)[45]. These analyses suggested altogether, that *T. parva* infection above the PCR detection threshold in LTC villages may follow a different relationship with cattle age compared to that expected from the analysis of HTC villages.

In Bunghaji, the probability of parasite infection decreased significantly at an earlier age as compared to the case in the majority of HTC villages. Despite the lower counts of adult RA/HBS in this village, the prevalence of *T. parva* dropped significantly as soon as calves grew into juveniles (>12 months of $age_{[OR]} = 0.27$; n = 1053 on 128 cattle; $F_{1, 924} = 18.63$; p <.0001)[46]. In contrast, the prevalence of *T. parva* could not be regarded as different amongst calves of 1 – 6 or 7 – 12 months of age (n = 165 on

52 cattle; $F_{1, 112} = 0.086$; $p = 0.770$)[47], nor amongst juveniles or adult cattle (n = 888 on 104 cattle; $F_{1, 783} = 0.571$; $p = 0.450$)[48].

In Hitunga, no cattle below 13 months of age were found to be infected with *T. parva* (Figure 6.8), but despite this delay in parasite exposure (as measured by PCR) the prevalence of parasite infection decreased significantly in adult cattle as compared to juveniles ($Adults_{[OR]} = 0.33$; n = 628 on 83 cattle; $F_{1, 544} = 10.39$; $p = 0.0013$)[49]. In this village, as in Bunghaji, a significant drop in *T. parva* prevalence occurred as soon as one year after the population became exposed to the parasite, in contrast with the case in the majority of HTC villages, where the probability of parasite infection did not fall significantly until after two years of exposure.

Magoje, where *R. appendiculatus* challenge was lowest, was the only village where a positive association between cattle ageing and parasite infection occurred, and where adult cattle were more likely to test positive to *T. parva* as compared to cattle of up to 24 months of age ($Adults_{[OR]} = 2.46$; n = 537 on 68 cattle; $F_{1, 468} = 6.129$; $p = 0.014$)[50]. The positive association between cattle age and *T. parva* infection in Magoje, occurred despite the population of cattle being exposed to *T. parva* from as early as seven months of age (Figure 6.8), and despite ticks being recorded from all age groups, including calves of 1-6 months of age (Table 6.1).

6.3.6.3 T. parva Infection Status, R. appendiculatus and Cattle Age

The relationship between *T. parva* infection status and tick infestation intensity (*HTC or LTC : RA/HBS*; n = 7479 on 872 cattle; $F_{2, 6595} = 0.805$; $p = 0.447$)[51], and the relationship between *T. parva* infection status and tick infestation intensity in different cattle age groups (*HTC or LTC : Age : RA/HBS*; n = 7479 on 872 cattle; $F_{4, 6591} = 1.043$; $p = 0.383$)[51], was not significantly different depending on whether cattle were located in HTC or LTC areas. This was tested in a model which considered three age categories (calves vs. juveniles vs. adults) and three possible tick scores (zero, 1-10 or >10 RA/HBS), to allow sufficient number of *T. parva* positive observations within each grouping. The results obtained from this analysis were consistent with those where different tick categories and/or age groups were tested, and were supported by visual inspection of the data in each case (Data not shown).

6.3.6.4 Temporal Trends in R. appendiculatus and Cattle Age

Despite accounting for the effect of cattle age during data analysis, a significant upward trend of adult RA/HBS was still apparent over the course of the study in each of the eight villages (all p-values <0.05; Appendix 6.1; analyses [52, 53]). However, the rate at which tick counts increased every 28 days was different depending on the village considered (*Village : Time*; n = 8539 on 882 cattle; $F_{7, 7617} = 49.06$; p<.0001)[54], and was higher in the LTC as compared to the HTC area (*HTC or LTC* : *Time*; n = 8539 on 882 cattle; $F_{1, 7647} = 305.1$; p<.0001)[55]. See Figure 6.5; Section 6.3.3.

The rate at which tick counts (RA/HBS) increased over time, was not different amongst Bunyimini, Kubo, Nanjeho and Sitengo (*Village : Time*; n = 4003 on 401 cattle; $F_{3, 3582} = 1.696$; p = 0.166)[56]. The estimated tick count increase recorded in these villages was smaller than that recorded in Ojelai (*Ojelai : Time*$_{[coef]}$ = +0.02; n = 5117 on 527 cattle; $F_{1, 4582} = 24.96$; p <.0001)[57], and the rate at which tick counts increased every 28 days in Ojelai was smaller than that in the LTC area (*LTC area : Time* $_{[coef]}$ = +0.04; n = 4536 on 481 cattle; $F_{1, 4047} = 74.34$; p <.0001)[58]. Amongst LTC villages, the rate at which the prevalence of tick-positive cattle increased every 28 days was not different in Hitunga and Bunghaji (*Village : Time;* n = 2371 on 228 cattle; $F_{1, 2137} = 1.01$; p = 0.3151)[59], but was smaller in Magoje (*Magoje: Time* $_{[coef]}$ = -0.052; n = 3422 on 355 cattle; $F_{1, 3063} = 7.762$; p = 0.005)[60].

The interaction "*Geographical area : Cattle age : Time* ", was significant when considering either the eight-level variable "*Village*" (n = 8539 on 882 cattle; $F_{14, 7617} = 2.823$; p=0.0003)[54] or the two-level variable "*HTC or LTC*" (n = 8539 on 882 cattle; $F_{2, 7647} = 4.468$; p=0.0115)[55]. This resulted from the fact that the build-up of ticks slowed down as animals grew older in areas where tick challenge was highest (i.e. Bunyimini [*Adults : Time*$_{[coef]}$ = -0.04]; Kubo [*Adults : Time*$_{[coef]}$ = -0.03], Nanjeho [*Adults : Time*$_{[coef]}$ = -0.03], Ojelai [*Adults : Time*$_{[coef]}$ = -0.03]; p <0.05 in all cases. See Appendix 6.1; analysis [52]). The same was not true in areas where the tick challenge was lower (i.e. Sitengo and LTC villages; *Age : Time*: p > 0.05 in all cases. See Appendix 6.1; analyses [52, 53]).

6.3.6.5 Temporal Trends in T. parva Infection Status and Cattle Age

A negative trend in the probability of parasite infection could not be explained by cattle ageing alone when correcting the analysis by accounting for the geographical origin of cattle (i.e. village). Accounting for variation across villages, showed a small - albeit significant - decrease in the probability of parasite infection in the study area, even after correcting for the effect of cattle ageing over the course of the study (n = 7494 on 873 cattle; $F_{1, 6565} = 31.21$; $p < .0001$)[61]. However, such effect depended on the village considered (*Village : Time;* n = 7494 on 873 cattle; $F_{7, 6565} = 10.68$; $p < .0001$)[61]. This analysis ([61]) considered four age categories, but the results were consistent with those considering either two (≤ 24 versus > 24 months of age) or three (1-12, 13-24 or > 24 months of age) age groups (Data not shown).

When conducting an analysis of the time-trend for *T. parva* infection status in each village, accounting for the age of animals at each round of observation, three different scenarios were observed: (i) villages where a negative trend for *T. parva* infection status was still significant after accounting for cattle ageing, (ii) villages where only cattle ageing was significant, thus reflecting a strong correlation between time and population ageing and (iii) villages where the interaction "Cattle age: Time" was significant, and where the probability of infection was characterised by a greater negative time-trend as cattle grew older (Data not shown). There was no apparent correlation between the classification of villages with regard to *R. appendiculatus* challenge and the classification of villages with respect to scenario i, ii or iii (See above). These findings reflected the complication of attempting to solve and quantify conundrums involving correlated variables (i.e. ageing versus time). Nevertheless, it was a notable exception that cattle herds raised in Magoje showed a positive - albeit small - time-trend for *T. parva* infection status after considering the age of cattle at each round of observation ($Time_{[OR]} = +1.09$; n = 537 on 68 cattle; $F_{1, 467} = 5.31$; $p = 0.0216$) [62].

6.3.7 Number of *T. parva* Positive Observations Recorded From Infected Cattle

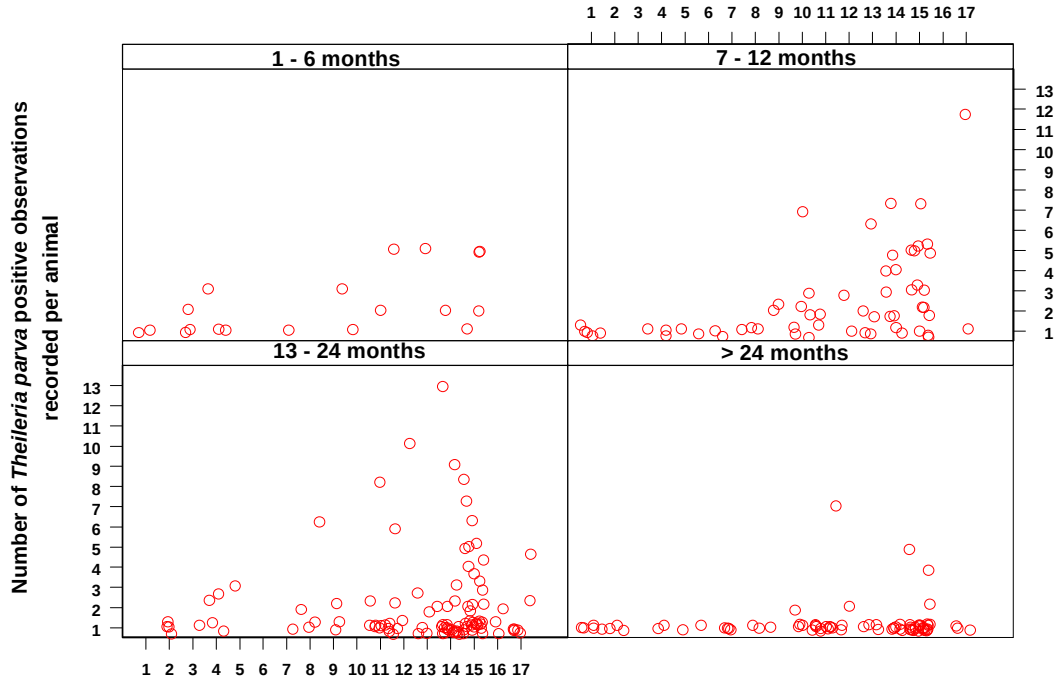
Supplementary information on the analyses conducted in this section, is contained in Appendix 6.1 (See analysis [63]). LA oxytetracycline did not have an effect on the number of *T. parva* positive

observations recorded from infected cattle in any of the 'one-variable' models tested or in any of the models that included cattle age, or cattle age and geographical origin of cattle, as independent variables (Data not shown).

6.3.7.1 Effect of Cattle Age at First T. parva Positive Observation

Age at first *T. parva* diagnosis was available for 259 out of 281 cattle with at least one p104 – positive observation over the course of the study. Taking into account the number of PCR tests conducted on each animal, cattle age at the time of the first *T. parva* positive observation had a significant effect on the number of positive observations recorded from infected cattle (n = 259; Cattle Age = 1 – 6 (n = 19), 7 – 12 (n = 59), 13 – 24 (n = 104) or > 24 months of age (n = 77); F-test = 8.31, df = 3, p<.0001). See Figure 6.9 for visual inspection of the raw data.

Figure 6.9 Number of PCR tests versus number of *T. parva* positive observations in individual cattle subdivided by age at the time of first positive diagnosis.



Each dot represents one animal. The scatterplots include animals that were parasite-positive in at least one visit round, and for which age was known at the time of the first positive test (n = 259). If the first positive observation was obtained from animals aged 1-24 months, more parasite-positive tests were obtained as more PCR tests were conducted. The same was not true in animals older than 24 months of age, which tended to test positive on only one occasion, regardless of the number of PCR tests conducted over the course of the study.

The number of *T. parva* positive observations recorded from infected cattle of either 1 – 6 or 7 – 12 months of age was not different (n = 78; F-test = 0.78, df = 1, p<0.380). However, calves of up to 12 months of age were positive to *T. parva* on more visit rounds than either juveniles (n = 182; F-test = 6.33, df = 1, p=0.013) or adults (n = 155; Chisq = 49.56, df = 1, p<.0001). Finally, cattle infected at adult age (>24 months) were parasite-positive on fewer subsequent tests than cattle infected as juveniles (n = 181; F-test = 7.07, df = 1, p= 0.009). These results were obtained from a dataset were 9.3% of the animals (24 out of 259) had a weight - or binomial denominator - equal or less than 3, a situation which is comparable to other datasets analysed in the literature (Crawley, 2002b). However, even when only cattle with a minimum weight of four were included in the analysis (n = 259 – 24 = 235), the results were consistent with those obtained when analysing the complete dataset (n = 259).

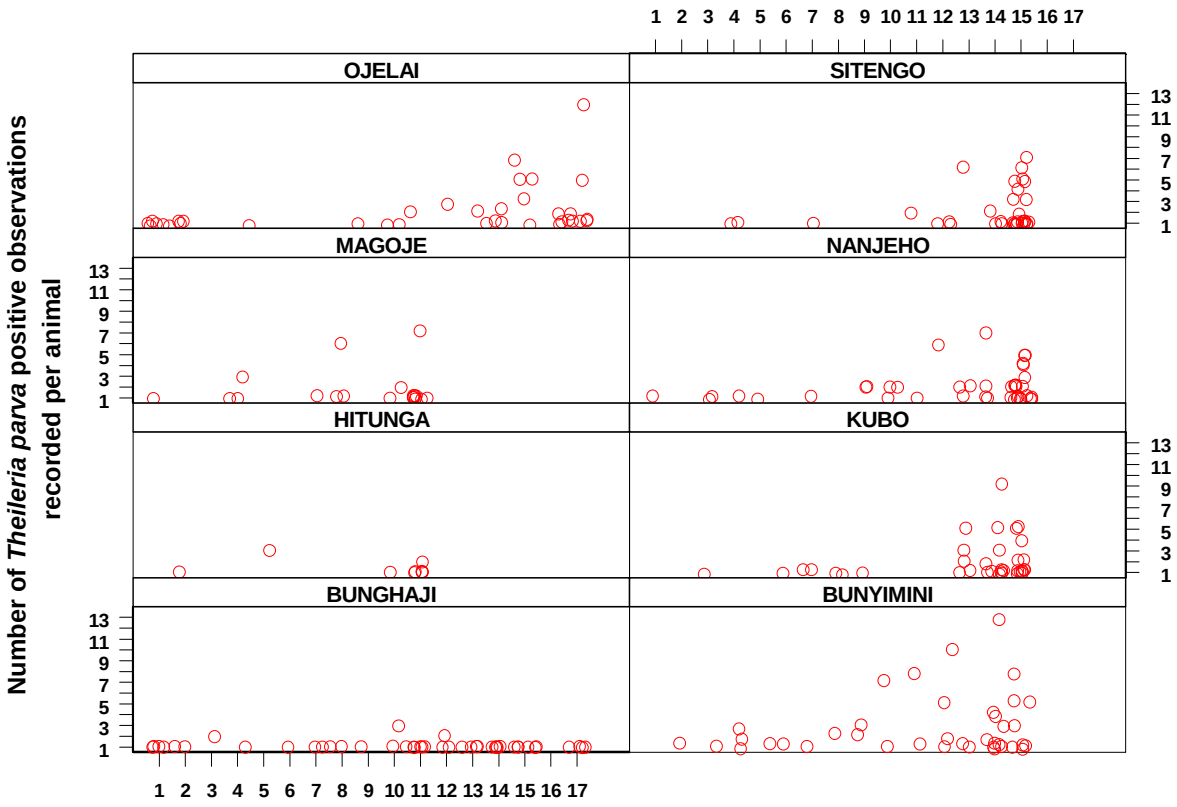
6.3.7.2 Geographical Variation

The number of positive observations recorded from infected cattle was found to vary significantly across study villages (n = 259; F-test = 2.85, df = 7, p = 0.007). See Figure 6.10 for visual inspection of the raw data. Cattle age was also a significant predictor of the percentage of positive observations, and was therefore retained in the model (n = 259; F-test = 12.52, df = 2, p <0.0001).

The percentage of positive observations recorded from infected cattle was similar amongst animals in the HTC study sites (n = 187; F-test = 2.34, df = 4, p = 0.057), but differed in LTC villages (n = 72; Chisq = 9.67, df = 2, p = 0.008). Cattle age at first *T.parva* positive observation was only a significant predictor of the number of subsequent positive observations in areas of HTC (n = 187; F-test = 12.7, df = 2, p <.0001), but not in villages of LTC (n = 72; Chisq = 1.55, df = 2, p = 0.461).

Exclusion of cattle from Magoje (LTC) in the analysis of the LTC areas, showed that the percentage of positive observations recorded from infected animals in the remaining LTC villages (Bunghaji and Hitunga) was not different (n = 53; Chisq = 1.1, df = 1, p = 0.295). Cattle age at first positive diagnosis had no influence on the outcome number of *T. parva* positive determinations in Bunghaji and Hitunga (p>0.05).

Figure 6.10 Number of PCR tests versus number of *T. parva* positive observations in individual cattle subdivided by village of origin.



HTC villages = Bunyimini, Kubo, Nanjeho, Ojelai and Sitengo.
LTC villages = Bunghaji, Hitunga and Magoje.

Each dot represents one animal. Only animals found *T. parva* positive in at least one visit round during the study, and for which age data was available at the time of the first positive diagnosis, are considered in the scatterplots (n = 259). Cattle from HTC villages and Magoje (LTC), showed more positive observations as more PCR tests were conducted. In contrast, animals from LTC villages other than Magoje, tended to test positive on only one occasion, regardless of the number of PCR tests conducted over the course of the study.

The number of *T. parva* positive observations recorded from infected cattle in Magoje – as suggested from the analyses described previously - was different to that in other LTC villages, but was in contrast comparable to that in areas of HTC (n = 206; F-test = 2.04, df = 5, p = 0.075). This was tested in a separate analysis where cattle age was retained as a significant term in the model (n = 206; F-test = 11.27, df = 2, p <0.0001), and where the five HTC villages and Magoje were included as categorical levels of the variable "Village".

In summary, cattle from the study villages could be classified into two separate groups regarding the number of *T. parva* positive observations recorded from infected cattle: (i) HTC villages and Magoje (LTC) and (ii) LTC villages other than Magoje. Such classification was not wholly consistent with that based on *R. appendiculatus* challenge across study villages (Figure 6.3). The number of *T. parva* positive observations recorded from infected cattle was higher HTC villages and Magoje (LTC), than in low LTC villages other than Magoje. This classification provided an explanation as to why in Magoje (where tick challenge was lowest), a similar percentage of positive observations was recorded as compared to in areas where tick challenge was higher, and where more cattle were infected over the study period (Figure 6.4). Only in HTC areas did the number of positive *T. parva* determinations recorded from infected cattle decrease with age. In contrast, the number of parasite-positive observations recorded from infected cattle was consistently low in Bunghaji and Hitunga (LTC), and higher in Magoje (LTC), regardless of the age of animals at the time of the initial *T. parva*-positive observation.

The interaction term 'Cattle age : Geographical origin of cattle' was never found significant regardless of the villages considered in the analyses and regardless of specifying age categories other than those mentioned throughout the text. Most likely, this resulted from the insufficient number of observations available, to reliably assess the significance of the interaction term, particulalry amongst the LTC villages. Finally, the results reported throughout this section were consistent with those where other level-groupings of the variable "cattle age at first parasite-positive observation" were considered (Data not shown). The results reported here concerning the classification of villages with regard to the number of positive observations recorded from infected animals, were consistent with the results obtained from 'one-variable' models that did not consider the age of cattle at the time of the first positive observation. The latter allowed for a greater number of animals / village in the analyses (Data not shown).

6.4 Discussion

6.4.1 Distribution of *R. appendiculatus* in Busia and Tororo Districts

R. appendiculatus was the most common adult tick stage recorded from the study population, followed by *A. variegatum* and *B. decoloratus*. This supports previous observations from tick surveys in southern (Kaiser *et al.*, 1982) and south east Uganda (Rubaire-Akiiki *et al.*, 2004). Unlike the rather uniform distribution of *A. variegatum* and *B. decoloratus*, there existed great heterogeneity of *R. appendiculatus* challenge across study sites. This heterogeneity correlated with reported *T. parva* seroprevalences amongst bovine subpopulations (i.e. from different villages) in the study (Magona, 2004), and with the number of cattle diagnosed with *T. parva* utilising PCR. Direct and indirect evidence of non-uniform distribution of *R. appendiculatus* across relatively close geographical areas, is well documented in Uganda (Rubaire-Akiiki *et al.*, 2004), Kenya (Deem *et al.*, 1993, Gitau *et al.*, 1994a, Gitau *et al.*, 1994b, O'Callaghan, 1998, Gitau *et al.*, 1999a, Gitau *et al.*, 1999b, Gitau *et al.*, 2000a), Tanzania (Yeoman, 1966) and Zambia (Speybroeck *et al.*, 2002, Fandamu *et al.*, 2005, Fandamu *et al.*, 2006) amongst others. The reasons for these variations are multifactorial although classifications of ecoclimatic conditions, agroecological zones, and grazing systems have been found to correlate with *R. appendiculatus* challenge.

In Uganda, detailed census data and seasonal patterns of *R. appendiculatus* numbers have been described for two vast ecoclimatic zones as defined by Pratt and Gwyanne (Pratt *et al.*, 1977). These ecoclimatic zones are described as **(i)** the humid to dry, sub-humid environment of southern Uganda (Kaiser *et al.*, 1982) and **(ii)** the dry sub-humid to semi-arid environment of northern Uganda (Kaiser *et al.*, 1991). Within the second ecoclimatic zone, the incidence of *T. parva* and the intensity of vector challenge are dependent on altitude (Rubaire-Akiiki *et al.*, 2004). *R. appendiculatus* and *T. parva* infections are highest in lowland zones (1100-1350 metres above sea level), which are characteristic of Tororo and Busia, and lowest in the midland and upperland zones (approximately 1800 metres above sea level) (Rubaire-Akiiki *et al.*, 2004). This observation is in keeping with the general statement that

climatically, lowlands are more suitable for *R. appendiculatus* than the midlands and uplands (Matthysse *et al.*, 1987). However, exceptions to this do occur (Fandamu *et al.*, 2005).

T. parva prevalence and hence vector challenge can also vary greatly across areas located within the same altitude, depending on the grazing systems and the agroecological zone considered (Deem *et al.*, 1993, Gitau *et al.*, 1994b, Gitau *et al.*, 1997, O'Callaghan, 1998, Gitau *et al.*, 2000a, Rubaire-Akiiki *et al.*, 2004). The latter has been well documented in Kenya following the agroecological zone classification defined by Jaetzold and Schmidt (Jaetzold *et al.*, 1983).

The classification of agroecological zones (Jaetzold *et al.*, 1983), is based on agro-climatic factors including rainfall and soil type, which ultimately determine cultivation of particular crop types. Village questionnaires (Chapter 4) and livestock census data (FITCA, 2002), have shown that Tororo (1097 – 1219 metres above sea level) and Busia (1000 – 1128 metres above sea level), fall within the category of "coastal lowland", and the "millet-livestock" and "cassava" sub-categorisations respectively (Jaetzold *et al.*, 1983). Cattle herds in Busia, presented characteristically moderate to heavy *R. appendiculatus* infestations all year round. Conversely in Tororo, with the exception of cattle from Ojelai which was geographically closer to Busia, animals presented low to very low *R. appendiculatus* infestations. These observations are in line with those reported by Deem *et al.* (1993) in the Coast Province of Kenya. Cassava systems are associated with high moisture conditions and average rainfall, and moderate to heavy numbers of *R. appendiculatus* (Deem *et al.*, 1993). Millet–livestock systems in contrast, are characterised by lower average rainfall. Low to very low numbers of *R. appendiculatus* are usually recorded from the coat of bovine hosts in millet–livestock systems (Deem *et al.*, 1993).

6.4.2 Enzootic, Recently Enzootic, Epizootic and Sporadic ECF Risk Zones - Extending Yeomans's Classification to South East Uganda.

The geographical distribution of high (HTC) and low (LTC) tick challenge areas did not strictly conform to a political barrier nor to a geographical division of agroecological zones. There existed a gradient of tick challenge which decreased as cattle herds were located further from the shore of Lake Victoria.

The most thorough description of the distribution of *R. appendiculatus* ticks amongst short-horned zebus raised around the shores of Lake Victoria has been given by a transect study in the Sukumaland area of the lake, near the south-east shore in Tanzania (Yeoman, 1966).

Table 6.2 Yeoman's classification of ECF risk zones based on *R. appendiculatus* challenge and proximity to Lake Victoria's shore

i	**Closer to Lake Victoria**	Permanently **enzootic belt** extending 40 miles inland around the shore. The average infestation rates are consistently in the order of 40-60 adult ticks per animal.
ii		**Recently enzootic** areas which must have been epizootic, but have only recently taken on an enzootic character. These are characterised by infestation rates of 5 to 20 adult ticks per animal.
iii		**Epizootic zones**, where the disease is maintained and spread in a more virulent manner by very low infestation rates of the order of 1 to 4 adult ticks per animal.
iv		**Sporadic zones** located only 16 miles apart from the high tick challenge belt, and where important outbreaks occur on non-constant basis. Infestation rates are in the order of 0.3 to 0.8 adult ticks per adult host.
v	**Further away from Lake Victoria**	Extensive **ECF free zones** (10-20 miles deep). Infestation rates of up to 0.2 adult ticks per animal may be found.

Yeoman defined five ECF risk areas around the lake's shore on the basis of adult *R. appendiculatus* counts recorded from the whole body surface of indigenous cattle (Table 6.2). He calculated average tick burdens by dividing the total number of ticks found, by the total number of cattle surveyed. Characteristically, the transition from high to low tick challenge areas took place within a zone of only 8 miles wide.

In spite of the fact that Yeoman's classification was developed some forty years ago, the current distribution of adult *R. appendiculatus* amongst cattle herds raised in study areas from south east Uganda, closely resembles Yeoman's earlier observations. However, increasing human population density over the second half of the 20th century might have decreased the extent of enzootic belts and transition belts towards low tick challenge areas.

Using the classification of ECF risk areas defined by Yeoman (1966), the Busia villages (Bunyimini, Kubo, Nanjeho, Sitengo) and the Ojelai village in Tororo, were located in a permanently enzootic belt extending approximately 25 miles around the shores of the lake (approximately half that reported by

Yeoman; Table 6.2). Within this area, almost 100% of the animals were infested with adult tick stages all year round, and 11-50 adult tick stages on half the body surface were reported on 60-66% of initial and follow-up observations depending on the village (Table 6.3).

Table 6.3 Application of the ECF risk zones of Yeoman (1966) to cattle herds in south east Uganda.

Numbers of adult tick stages	Percentages of examinations on cattle with 0, 1-10, 11-50 or > 50 adult *R. appendiculatus* on half body surface							
	Bunyimini	**Kubo**	**Nanjeho**	**Ojelai**	**Sitengo**	**Bunghaji**	**Hitunga**	**Magoje**
0	2	1	2	4	3	26	47	64
1 - 10	23	30	29	32	51	66	51	35
11 - 50	66	62	61	60	45	8	2	
>50	8	7	8	4	1			
	Enzootic belt					Recently Enzootic	Epizootic	Epizootic to Sporadic

Grey shaded areas highlight the most common tick infestation score in each study village. Within each village, percentages are based on more than 1000 observations.

Further inland, the transition from high to low tick challenge areas occurred within a zone of only some five miles wide (approximately three miles less as compared to Yeoman's observations). The latter was composed of a mosaic of recently enzootic, epizootic and sporadic zones as defined by Yeoman (1966), which did not necessarily conform to decreasing infestation rates with increasing distance from the lake. This may have resulted from the mechanisms by which foci outside the enzootic belt may have been generated.

Within the LTC belt, indigenous cattle from Bunghaji, presented with tick infestation rates which were compatible with those in recently enzootic zones (Yeoman, 1966). Within this area, zero or 1-10 adult tick stages on half the body surface were reported on 26% and 66% of examinations respectively (Table 6.3). This may approximate to half the tick count figures reported on the whole body surface of cattle in recently enzootic areas (Yeoman, 1966; Table 6.2). Cattle herds in Hitunga presented zero or 1-10 adult tick stages on half the body surface on 47% and 51% of the examinations respectively (Table 6.3). In Magoje, no ticks were recorded on 64% of the examinations, and 1-10 ticks were only recorded in 35% of the examinations (Table 6.3). Tick counts reported in these villages were therefore compatible with epizootic (Hitunga) and epizootic – sporadic (Magoge) ECF zones (Yeoman, 1966).

6.4.3 *R. appendiculatus* Dynamics and Cyclical Patterns

R. appendiculatus adult instars were observed on the coat of animals in the study all year round, suggesting that a continuous development of tick stages takes place in these areas. There existed a cyclical pattern in the intensity and prevalence of tick infestations in all the study villages, which was best represented using a transformed tick count (TC). Periodic fluctuations with a frequency of about three months consisted of a fall in the TC during each dry season, which was then followed by a recovery of TC towards the onset of each rainy season. During the wet season where highest average rainfall and average morning temperature was recorded, two consecutive peaks of adult *R. appendiculatus*, inter-spaced by a shorter time length (1 month) were observed. These observations indicated an interstadial development period from the adult to the larva instar, the larva to the nymph instar, and the nymph to the adult instar of three months. In the case of the wet season where higher average rainfall was accompanied by higher average morning temperature, the interstadial development period appeared to be as short as one month.

Periodic fluctuations of *R. appendiculatus* were correlated with fluctuations in the prevalence of cattle infected with *T. parva*. However, because peaks of *T. parva* infected cattle occurred slightly before peaks of adult stages, transmission of the parasite in the study areas must have been mainly conducted by nymphs rather than adult instars. Although larval / nymphal transmission of *T. parva* is less efficient than nymphal / adult transmission (Ochanda *et al.*, 1996) the results obtained in this study may be explained by the fact that for each adult instar, some 100 nymphs may be present in the field (Norval *et al.*, 1992).

6.4.4 Temporal Trends of *R. appendiculatus*

R. appendiculatus infestation rates increased with cattle ageing in all enzootic, recently enzootic, epizootic and epizootic-sporadic areas, although outside the enzootic belt, the rate at which host-vector contacts were established as animals grew older was slower. This well understood phenomena (Barnett *et al.*, 1955, Moll *et al.*, 1984, Moll *et al.*, 1986, Rubaire-Akiiki *et al.*, 2004) may be attributed to reported differences in grazing practices between calves and older animals (Magona *et al.*, 2000).

Temporal trends of tick numbers were stripped of the confounding influence attributable to the changing demographics of the host populations. This revealed a continuous increase of vector intensity in all areas above that expected by cattle aging alone. Such finding was evident from analyses showing that positive vector intensity slopes, were more pronounced when considering the effect of cattle ageing and when allowing different time slopes for each age group. The positive slope of adult tick infestation rates over the course of the study was more evident in epizootic or recently epizootic foci as compared to in the enzootic belt. This finding was in agreement with previous observations (Fandamu *et al.*, 2005).

Cattle surveys conducted from 1995 to 2002 in southern Zambia, have shown yearly fluctuations of *T. parva* incidence in traditionally managed cattle which are correlated with global weather changes (Fandamu *et al.*, 2005, Fandamu *et al.*, 2006). High *T. parva* seroprevalence, reduced age at initial vector contact and higher parasite transmission occur in calendar years which coincide with the presence of the periodic climatic phenomenon known as the El Niño Southern Oscillation (Fandamu *et al.*, 2005, Fandamu *et al.*, 2006). As expected, such El Niño dependent annual oscillations in *T. parva* incidence, coincide with reported *R. appendiculatus* abundance oscillations during the same periods (Speybroeck *et al.*, 2002). It is then possible that increasing *R. appendiculatus* numbers in cattle from south east Uganda, above those expected by cattle aging alone, may have resulted from the development of favourable conditions and the subsequent onset of the El Niño during 2001 and 2002 (Fandamu *et al*., 2005), a period that coincided with this longitudinal study.

While in the majority of enzootic areas the build-up of vector numbers slowed down as animals approached adulthood, this increased at a constant rate in bovines located in recently enzootic or epizootic areas, regardless of their age classification. The observations derived from this analysis suggest that acquired immunity against ticks (Bonsma, 1944, Barnett *et al.*, 1955, Sutherst *et al.*, 1979, Jongejan *et al.*, 1989, Fivaz *et al.*, 1990, Randolph, 1994) or a phenomenon of density-dependent regulation of tick numbers (Randolph, 1994), may take place within the enzootic belt but not in recently enzootic or epizootic foci.

6.4.5 Age-Related Dynamics of Patent *T. parva* Infections in Cattle Herds from different ECF Risk Zones

There existed great heterogeneity in the incidence of *T. parva* infections across ECF risk zones as defined by Yeoman (1966). However, the overall epidemiology of *T. parva*, and more specifically the relationship between cattle age and parasite infection, was strongly conserved in all cattle herds raised within the enzootic belt. It may therefore be possible that a transmission model representing an endemic area may also apply to other enzootic zones for as long as non-diapausing *R. appendiculatus* populations exist. In contrast, amongst other aspects of the parasite's epidemiology (See later in this section), the relationship between cattle age and *T. parva* infection was heterogeneous and rather unpredictable outside the enzootic belt. This observation suggests that factors other than tick challenge may be required before a transmission model can be extended to consider epizootic zones, other than those specifically characterised in terms of *T. parva* epidemiology. Given the diversity of epidemiological scenarios outside the enzootic belt, it is evident that pooling of cattle subpopulations (i.e. from different villages) to investigate how host-vector and host-parasite interactions are shaped with age, can lead to false epidemiological conclusions. A discussion follows on the generalities and exceptions with regard to the epidemiology of the parasite in high and low tick challenge areas as defined by Yeoman (1966).

6.4.5.1 High Tick Challenge Areas

Within 25 miles around the shore of Lake Victoria, host-vector contacts invariably started during early calfhood (1-6 months of age), as reflected by the percentage of tick-positive and *T. parva*-positive observations recorded from younger calves. Tick-positive examinations on animals of 1 - 6 months of age ranged from 86% to 100% depending on the village. Characteristically, infected animals tended to test parasite-positive over the course of multiple follow-up observations. This may be a result of continuous re-challenge, establishment of persistent infections or a combination of both. However, *T. parva*-positive observations dropped significantly once animals reached 24 months of age suggesting that effective immunity against circulating *T. parva* parasites, may be attained at a herd level after two

years of parasite exposure. In contrast, animals aged 1-6, 7-12 or 13-24 months exhibited higher, and not different, risk of presenting with *T. parva* parasitaemias above the PCR detection threshold. These observations are of utmost importance in the context of current *T. parva*-specific transmission models, which assume that the probability of infection is a constant modified only by tick challenge and the prevalence of *T. parva* in the tick population (Medley *et al*., 1993).

6.4.5.2 Low Tick Challenge Areas

As previously suggested, the relationship between cattle age and *T. parva* infection was heterogeneous outside the enzootic belt. Pooling of cattle data from different villages for subsequent analysis resulted in the erroneous conclusion that cattle age is not a significant predictor of *T. parva* infection above the PCR detection threshold, suggesting that acquired immunity is not effectively attained in ECF foci outside the enzootic belt. This result was far from that obtained when the analysis was conducted for each recently enzootic, epizootic or epizootic-sporadic area separately.

Recently Enzootic and Epizootic Foci

In the recently enzootic (i.e. Bunghaji) and epizootic (i.e. Hitunga) areas, host-vector contacts started during early calfhood (1-6 months of age), although fewer contacts were established at this age as compared to in enzootic areas. The percentage of tick-positive observations recorded from younger calves (1-6 months of age) ranged from 53% (Bunghaji) to 25% (Hitunga). As a result of lower tick challenge, initial host-parasite contacts started either during early calfhood (Bunghaji) or were observed to be delayed until animals were 13 – 24 months old (Hitunga). Observations were derived from the percentage of *T. parva* positive observations recorded from different cattle age groups in either focus. Contrary to the case in enzootic areas, infected animals did not tend to test parasite-positive on more than one observation. In addition, there existed statistical evidence which suggested that, at the herd level, effective immunity against circulating *T. parva* parasites may be attained at a faster rate as compared to in the enzootic foci. In herds raised within recently enzootic or epizootic areas, the risk of *T. parva* infection at levels above the PCR detection threshold dropped significantly at the herd level one year after initial host-parasite contacts were observed. Hence, outside the

enzootic belt, the relationship between patent infection and cattle age was shaped by the timing at which host-parasite contacts were initially established (i.e. in calves or in juveniles) and once such contact was established, a substantial reduction in *T. parva* positive diagnosis was observed in the population within 12 months.

Epizootic-Sporadic Foci

A notable exception to the general epidemiology of *T. parva* outside the enzootic belt was encountered in the epizootic-sporadic focus of Magoje, where tick challenge was lowest, and where the rate at which tick infestation rates increased with time and/or as cattle grew older was slowest.

In the epizootic-sporadic zone (Magoje), initial host-vector contacts were also established during early calfhood (1-6 months of age), albeit the percentage of tick-positive observations recorded from younger calves was low (10%) as compared to in other areas outside the enzootic belt. As a result of low tick challenge, initial host-parasite contacts were delayed until late calfhood (7-12 months old), as reflected by the percentage of *T. parva* positive observations recorded from calves of 1-6 months of age (0%). These observations were consistent with a general delay in host-parasite contacts in all areas located outside the enzootic belt as compared to in the enzootic foci. Such a delay in initial host-parasite contacts, was supported by serology-based observations which showed that under low tick challenge, seroconversion rates (i.e. initial parasite exposure) in cattle older than six months, were significantly higher than those of their counterparts under high tick challenge (Magona, 2004).

It is noteworthy that the epidemiology of *T. parva* in the Magoje epizootic-enzootic zone was different to that in areas outside the enzootic belt in that **(i)** infected animals were likely to test *T. parva* positive on multiple follow-up observations - a trait which was characteristic of enzootic foci - and that **(ii)** at the herd level, a significant drop in the number of animals presenting with patent parasitaemia did not occur. This was in contrast to other zones, where effective acquired immunity against circulating parasites appeared to have developed 12 months (outside enzootic zones) or 24 months (in enzootic zones) after initial parasite exposure. Within the epizootic-sporadic focus, the observed relationship between patent infection and cattle age was shaped by **(i)** the ability of infected animals to test-

parasite positive on multiple follow-up observations and **(ii)** the slow rate at which host-parasite contacts were established. This, in combination with factors discussed on the following chapters, could have delayed the build-up of acquired immunity against circulating parasites. Taken together, the combination of these epidemiological traits resulted in the accumulation of infections over time and hence a positive slope for *T. parva* infections with cattle age.

6.4.6 Summary

Modelling of the transmission dynamics of *T. parva* has been compromised by the lack of empirical data reporting infection probabilities and how these may be affected by interactions between the parasite and the host's immune system (Medley *et al*., 1993, Gettinby *et al*., 1989). This study has described the dynamics of *Theileria parva* infection in relation to cattle age in areas which differ in *R. appendiculatus* challenge, and has considered an age population structure which more closely resembles the age composition encountered in traditionally managed herds as compared to previous detailed works (Moll *et al.*, 1984, Moll *et al.*, 1986). Results reported in this study suggest that the probability of infection, at least at levels above the PCR detection threshold, may be strongly shaped by acquired immunity against circulating parasites, as is evident from the infection probabilities obtained from cattle as they aged. This study has also shown that the number of positive observations recorded longitudinally from infected cattle, is influential in shaping the relationship between *T. parva* prevalence and cattle age. In addition, the number of positive observations recorded from infected cattle is the one parameter that does not follow a linear relationship with the gradient of tick challenge recorded from the study sites. As a result, the rate at which effective immunity against circulating parasites is attained at the herd level, as apparent from the drop in parasite-positive cattle, also varies across areas in a manner that is not wholly concordant with geographical variation in tick challenge. Therefore, factors other than vector challenge, in particular the number of positive observations recorded longitudinally from infected cattle, might need to be incorporated in future transmission models for a full understanding of the relationship between parasite infection and population ageing.

Chapter 7: The PIM Gene – A Marker to Investigate Factors Affecting *Theileria parva* Infection Survival

7.1 Introduction

7.1.1 Specific Objectives

Previous results showed that the number of positive observations recorded from infected cattle differed across villages in a manner which was not wholly explained by geographical variation in vector challenge. Moreover, the number of positive observations recorded longitudinally from infected cattle contributed in shaping the cross-sectional prevalence of the parasite in cattle age groups from different locations (Chapter 6). The present chapter focuses on determining the factors which affect the survival of natural *T. parva* infections in cattle. The number of parameters considered as potential predictors of infection survival is extended to consider subpopulations of *T. parva* parasites as distinguished by a polymorphic molecular marker. It is hypothesised that differences in the survival of infections across villages, and hence differences in the epidemiology of the parasite, can be explained by variation in the composition of *T. parva* parasite types across locations.

7.1.2 The Polymorphic Immunodominant Molecule (PIM) and the PIM Gene

Amongst the antigen-encoding single copy genes of *T. parva*, the gene encoding the polymorphic immunodominant molecule (PIM), and the molecule itself (Toye *et al.*, 1991), have long been regarded as a 'potentially useful markers' of *T. parva* genetic diversity (Shapiro *et al.*, 1987, Toye *et al.*, 1991, Toye *et al.*, 1995a, Geysen *et al.*, 1999); PIM is present in all *T. parva* stocks examined (Shapiro *et al.*, 1987, Toye *et al.*, 1991).

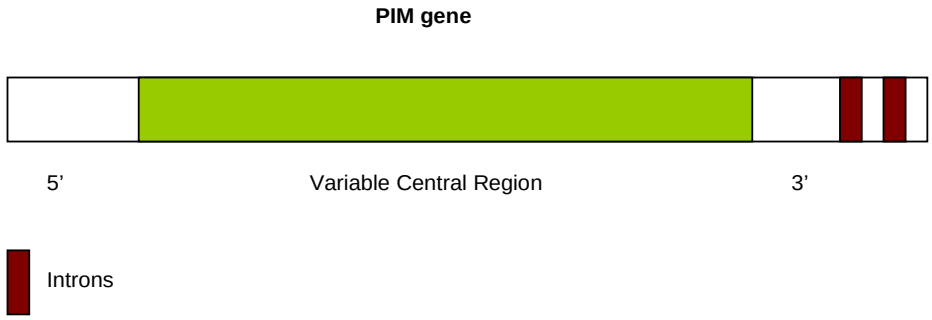
The PIM molecule has been extensively characterised (Toye *et al.*, 1991, Baylis *et al.*, 1993, Toye *et al.*, 1996). It is the predominant antigen recognised by monoclonal antibodies (mAbs) from infected mice (Shapiro *et al.*, 1987, Toye *et al.*, 1991) as well as antisera from immune cattle on western blot analysis of schizont-infected lymphocytes (Toye *et al.*, 1991). PIM is expressed in the schizont stage

and the sporozoite stage (Shapiro *et al.*, 1987, Toye *et al.*, 1991). Some mouse mAbs as well as cattle antisera reactive with PIM, block sporozoite entry into bovine lymphocytes in vitro (Toye *et al.*, 1995a). PIM exhibits strong variation in its molecular mass (Shapiro *et al.*, 1987, Toye *et al.*, 1991), and hence high size polymorphism across *T. parva* parasites, although some parasite stocks may be indistinguishable on the basis of PIM size polymorphism alone (Toye *et al.*, 1991). In addition, the molecule is highly polymorphic in the expression of antibody epitopes among different stocks of *T. parva*, although differences in the expression of antibody epitopes are not necessarily associated with differences in the size of PIM (Toye *et al.*, 1991).

As PIM is strongly immunogenic it is utilized in recombinant form for enzyme-linked immunosorbent assay (ELISA) detection of antibodies against *T. parva* (Katende *et al.*, 1998). The ELISA is widely used in field epidemiological studies (Gitau *et al.*, 1999a, Gitau *et al.*, 2000a, Gitau *et al.*, 2000b, Rowlands *et al.*, 2000, Gitau *et al.*, 2001, Kivaria *et al.*, 2004, Rubaire-Akiiki *et al.*, 2004, Swai *et al.*, 2005a, Swai *et al.*, 2005b). In addition, immunohistochemical staining of lymph node and blood smears with anti-PIM mAbs has proved useful for monitoring the kinetics of infection in cattle (Honda *et al.*, 1998).

In depth analysis of the overall structure of the PIM gene comes from sequence analysis of PIM products from different parasite stocks (Toye *et al.*, 1995a, Toye *et al.*, 1995b, Geysen *et al.*, 2004).

Figure 7.1 General structure of the PIM gene.



The PIM gene is located in chromosome 4 (Nene *et al.*, 1998), and presents a general structure of relatively conserved 5' and 3' termini flanking a more variable central region (Toye *et al.*, 1995a, Toye *et al.*, 1995b). The variable central region of the PIM molecule contains tetrapeptide repeats which are

rich in glutamine (Glu), Proline (Pro) and also Glycine (Gln); (Baylis *et al.*, 1993, Toye *et al.*, 1995b, Geysen *et al.*, 2004). PIM gene size polymorphisms across *T. parva* parasites are primarily attributable to complex variation in the number of tetrapeptide repeats within the central domain (Toye *et al.*, 1995b). Variation, as detected using a panel of mAbs and by nucleotide sequencing, also occurs in the relatively conserved 3' and 5' termini flanking the polymorphic central region, and includes substitutions of amino acid sequences within B cell epitopes (Toye *et al.*, 1995b, Toye *et al.*, 1996). PIM genes contain two small, highly conserved introns in the 3' conserved region (Toye *et al.*, 1995a).

7.1.3 Selective Pressure, Immunity and PIM

Analysis of *T. parva* isolates, has revealed high variability and a mosaic structure of the PIM gene which is exceptional for single-locus genes (Geysen *et al.*, 2004). These findings, along with observations that PIM is undergoing rapid evolution in the coding regions (Toye *et al.*, 1995b), suggest that strong immune selection pressure is being exerted on PIM (Bishop *et al.*, 2002, Geysen *et al.*, 2004). Establishing the underlying mechanisms generating this polymorphism is important in understanding the host-parasite interactions and its influence on parasite population structure (Geysen *et al.*, 2004).

Immunofluorescence assays (IFA) have shown that antibodies to the schizont are produced in infected cattle but they do not appear to be essential for immunity (Muhammed *et al.*, 1975, Emery, 1981). The schizont-infected cell-directed $CD8^+$ cytotoxic T lymphocytes (CTL) constitute then the dominant protective bovine immune response after a single exposure to infection (Morrison *et al.*, 1987, McKeever *et al.*, 1994). Currently, antigens that are the targets of MHC class I-restricted $CD8^+$ CTL from immune cattle are being characterised (Graham *et al.*, 2006).

7.1.4 Characterisation of *T. parva* Diversity – Application of a PIM Gene Specific PCR

In this study, characterisation of the PIM gene polymorphisms present in blood samples are assessed via variation in the amplicon size produced from a PIM gene specific PCR assay. The relative DNA

content of different life cycle stages of *T. parva*, including those morphologically identified in the tick as gametes and zygotes, is consistent with the parasite being haploid except for the diploid zygote and kinete stages (Gauer *et al.*, 1995). There exists a single copy of the PIM gene per haploid genome, implying that different versions of PIM are encoded by distinct alleles (Toye *et al.*, 1995b). Moreover, sexual recombination of *T. parva* takes place in the tick (Morzaria *et al.*, 1993). Hence, the PIM PCR assay will detect multiple PIM alleles if multiple parasite types are present in the bovine host, and a single PIM allele if the animal is infected with a single parasite type as distinguished by PIM gene polymorphisms.

The PIM gene represents only a single polymorphic allele in the genome, and as such it will under represent the true diversity of the parasite. However, this study constitutes the first attempt to examine the diversity and longitudinal expression of *T. parva* parasite types in the context of epidemiological features and infection survival. It is hoped that this approach can become a first step toward more complex studies involving a broader characterisation of the genetic diversity of *T. parva* in field populations.

7.2 Materials and Methods

7.2.1 Laboratory Analysis

7.2.1.1 Serial Testing

A strategy of serial testing (Fletcher *et al.*, 1988) was employed in the selection of samples for the molecular screening of the PIM gene using a semi-nested Polymerase Chain Reaction. Conventionally, only those animals that are positive to an initial test are selected for the second round of testing. This methodology has the advantage that more expensive techniques can be targeted to only those samples found to be positive in the initial screening test.

The PIM semi-nested PCR (the secondary screening technique) was applied to all blood samples stored onto FTA filter cards which were known to harbour *T. parva* (n = 537 samples on 281 cattle)

through PCR amplification of the conserved region of the 104 kDa rhoptry antigen (p104) gene (the initial screening technique) (Skilton *et al.*, 2002).

7.2.1.2 Template DNA

Secondary screening of 537 *T. parva* (p104 gene) positive samples stored onto FTA cards was conducted by two different methodologies dependent on when the initial screening for *T. parva* took place. The *T. parva* specific p104 gene PCR was available from the beginning of the study, whilst the PIM semi-nested PCR was developed in later stages of the longitudinal screening. The sample punches from *T. parva* (p104 gene) positive PCR reactions obtained before the development of the PIM amplification protocol, were not kept aside for further testing. In such cases, *T. parva* positive sample-saturated cards (n = 451) were screened *de-novo* by removing a new 2 mm circular punch. This punch was then used directly as template in the PIM semi-nested PCR reaction. Due to the uneven distribution of the parasite's DNA in positive sample-saturated cards, the presence of *T. parva* DNA in the new 2 mm filter card portion could not be guaranteed in all cases.

Once the PIM PCR was ready for use, p104 positive punches were set aside and stored for later re-use in the PIM gene PCR (n = 86). Since DNA remains bound to the card matrix, the same filter card 2 mm punch can be consistently re-used. This strategy had the advantage that the presence of the parasite's DNA was guaranteed in the PIM gene PCR reaction.

7.2.1.3 FTA Purification Protocol

De-novo Screening of T. parva (p104 gene) Positive Sample-Saturated FTA Cards

In cases where a new 2 mm disc was removed and used as template in the PIM gene amplification reaction, the purification protocol was as described in Section 3.2.3.1.

Re-amplification of T. parva (p104 gene) Positive Discs

The punches from samples positive in the initial round of screening, were placed into separate, labelled 1.5 ml eppendorf tubes, and stored at -20°C for a maximum of one week, if not used immediately. Negative control samples from the initial screening were also retained and stored in the

same way. When re-used, each disc was washed twice for 15 minutes in 200 µl of 1 x Tris-EDTA (Sigma Aldrich, Dorset, UK) to remove any traces of PCR reagents and amplification products. Each disc was then carefully transferred to a clean labelled PCR tube, and allowed to air-dry at room temperature for 60 minutes before the secondary PCR reaction was conducted. To reduce contamination, these steps were conducted in a separate laboratory from that where the PIM gene semi-nested PCR's were prepared, or where the transference of PCR products from initial to final reactions took place.

7.2.1.4 Storage and Processing of Control DNA Stocks

Positive controls consisting of *T. parva* stock DNA were tested in parallel with *T. parva* positive samples, as has been described for the case of the p104 gene PCR screening (Section 3.2.3.1). Storage and processing of the control DNA stock was conducted as explained earlier (Section 3.2.3.1). The dilution series which resulted in the optimum band visualisation and intensity (BioRad Gel Doc 2000) was chosen as the working template concentration to be tested in parallel with the PIM gene screening (Section 3.2.3.1). The positive control DNA solution for everyday use was stored at 4 ˚C, and placed on ice when in use. Two positive controls were included for every 20 test samples.

7.2.1.5 Negative Controls

De-novo Screening of T. parva (p104 gene) Positive Sample-Saturated FTA Cards

Two 2 mm circular punches from a clean FTA card (not containing sample material) were purified and processed in parallel with each batch of 20 test samples to ensure that no DNA contamination had occurred during the cutting and processing of the FTA matrices or throughout the process of preparing the PCR solution.

Re-amplification of T. parva (p104 gene) Positive Discs

Two negative controls were processed in parallel with each batch of 20 test samples. The first was the negative control carried over from the initial screening. The second was a new 2 mm punch from a clean FTA card (not containing sample material).

7.2.1.6 Management and Storage of Stock Primers and PCR Reagents

Primers were obtained from Sigma Genosys (Pampisford, Cambridgeshire, UK). On arrival, the oligonucleotides were suspended in 1xTris-EDTA buffer to a working concentration of 10 µM. The working solution was then aliquoted into 0.5ml eppendorf tubes and stored at – 20˚C until use. Each eppendorf tube contained the volume of primer solution necessary for the PCR reactions performed in a day. When in use, primers were kept on ice.

The reagents necessary to carry out PCR amplification of the PIM gene were provided with the BioTaq ™ MultiPack DNA polymerase from Bioline (London, UK), the 10x Custom PCR Master Mix-No Taq ™ from ABgene (Surrey, UK) and the *RediLoad* ™ Agarose Gel Loading Buffer from Invitrogen Ltd. (Paisley, UK). *RediLoad* ™ contains a red dye for tracking PCR products run in agarose gels, and is added directly to the PCR reaction mixture prior to amplification.

All reagents were aliquoted into 0.5 ml eppendorf tubes and stored at – 20˚C until use, except for the case of *RediLoad* ™, which was stored at 4 ˚C. Each eppendorf tube contained the volume of reagent solution necessary for the PCR reactions performed in a day. When in use, PCR reagents were kept in an ice box.

7.2.1.7 Semi-Nested PCR Protocol

The PIM gene was amplified using three species-specific primers, namely forward (For-1) and outer reverse (Rev-1) primers in a first reaction, followed by forward (For-1) and inner reverse (Rev-2) primers in the second reaction of a semi-nested PCR. For-1, Rev-1 and Rev-2 primers anneal to positions 365-385, 1326-1345 and 1311-1330 of the PIM gene published sequence respectively (accession number L41148, Genbank ™ database); (Toye *et al.*, 1995b). PCR targeted at the former DNA generates PIM-specific polymorphic amplicons ranging in base pairs size. For-1, outer Rev-1 and inner Rev-2 primers, with sequences 5'-CCA CTG GTT CTT CCG ATG TAA -3', 5'-GTT GTC CAG AAC CAT CAG CA -3' and 5'-CAG CAG CTG CAC CAG TCA TA -3' respectively, were developed by Dr. Frank Katzer (unpublished) at the Centre for Tropical Veterinary Medicine (Edinburgh, Scotland).

The first PCR was conducted in reaction volumes of 20 µl which contained the following components: ABgene 10x Custom PCR Master Mix-No Taq ™ (at final concentrations of 45 mM Tris–HCl [pH 8.8 at 25˚C], 11 mM $(NH_4)SO_4$, 4.5 mM $MgCl_2$, 0.113 mg/ml BSA, 4.4 mM EDTA, 1 mM of each dNTP), 0.5 µM of each For-1 and outer Rev-1 primers (Sigma Genosys), 0.5 U of BioTaq ™ MultiPack DNA polymerase (Bioline), and 2 µl of Rediload ™ (Invitrogen, Ltd.). Each reaction contained a single washed and air-dried 2 mm disc from the relevant blood sample stored on a Whatman FTA card. Two positive and two negative controls were included with each PCR run as explained earlier. 1µl of *T. parva* (*Muguga*, BJ273) control DNA was added in each positive control reaction.

The second round PCR was conducted in reaction volumes of 40 µl and contained the same components and final concentrations as those described for the first PCR, with the exception that the inner Rev-2 primer was added instead of the outer Rev-1 primer. Two positive and two negative controls were carried over as templates for the second round PCR. Only 0.5 µl of the first round products of the positive controls were transferred to the second round. For all the other samples, 1 µl of the first round product was carried over as template for the second round PCR.

The set up of the first and second PCR reactions was carried out in the same laboratory, but the transference of template from the first to the second reaction was performed within a UV chamber in a separate laboratory. The UV chamber was switched on half an hour before and half an hour after the transfer of amplicons from the first to the second PCR reactions to reduce contamination.

The thermal cycling conditions for the first and second PCRs consisted of initial denaturation step of 94 °C for 5 min followed by 40 cycles at 94 °C for 60 sec, 50 °C for 60 sec and 72 °C for 60 sec and a final extension step at 72 °C for 10 minutes. The thermal cycling was carried out on a Peltier Thermal Cycler (MJ Research, Waltham, MA, USA).

7.2.1.8 Electrophoresis and Amplicon Visualisation

Electrophoresis of First Round PCR Products

Electrophoresis and amplicon visualisation was carried out as described in Section 3.2.3.1. However, 2% standard agarose (Agarose Multi-Purpose ®; Bioline) gel was prepared. 19 µl of each first PCR

reaction were dry-loaded on to the gel. Two positive and two negative controls were included for every 20 test samples to confirm that there had been amplification and no contamination had occurred.

Electrophoresis of Second Round PCR Products

The conditions for the gel electrophoresis of the second PCR product were optimised to improve the separation and visualisation of bands. The conditions were as described in Section 3.2.3.1 with the following modifications: 40 µl of each sample were dry-loaded on to a 2% Metasieve agarose (Flowgen Bioscience) gel. Molecular weight marker, consisting of 40 µl of a 100 bp Hyperladder (Sigma), was loaded on to the far left and right lanes. Samples were run for 17 hours at 35 volts and for a further 7 hours at 80 volts to allow proper separation of the bands. Pictures were taken 15 hours and 24 hours after the loading of samples (BioRad Gel Doc 2000). Two positive and two negative controls were included in each gel (i.e. every 20 test samples).

7.2.1.9 Additional Validation of the PIM Semi-Nested PCR

The PIM semi-nested PCR was fully optimised and validated by Dr. Frank Katzer (unpublished) at the Centre for Tropical Veterinary Medicine (Edinburgh, Scotland). However, additional validating steps were developed during the process of screening for *T. parva* PIM types. Non-specific amplification products resulting from host DNA or excess DNA, were identified and differentiated from PIM gene amplicons. These bands were then excluded from analysis. The process of identifying these bands was as follows:

Bovine DNA

Blood from an uninfected Friesian cow of UK origin, was collected and stored in a heparinised vacutainer following the same methodology as explained earlier (Section 3.1.3.1). The tube was then centrifuged at 10,000g for 5 minutes. 25 µl of whole blood sediment was applied on to an FTA card (Whatman Bioscience, Cambridge, UK) and allowed to air-dry for 24 hours. Another 25 µl were transferred to an eppendorf tube and mixed with 1 µl of *T. parva* (Muguga; BJ273) control DNA. 25 µl of the mixture was then applied on to a separate FTA card (Whatman Bioscience, Cambridge, UK) and allowed to air-dry for 24 hours. Ten 2 mm discs of sample-saturated matrix (containing cow blood only)

were removed from the card, placed in separate eppendorf tubes and processed according to the manufacturer instructions (Section 3.2.3.1). Each disc was transferred to a separate PCR tube and utilised as template DNA in PIM-PCR reactions, following the methodology described earlier in this chapter. Two negative and two positive controls were included in the experiment. Negative controls corresponded to 2 mm circular discs from a clean FTA card (not containing sample material). Positives controls were obtained by adding a 2 mm disc of blood and *T. parva* control DNA saturated matrix (previously described) to the corresponding PCR reaction. Following appropriate processing of the filter card discs, semi-nested PIM PCR was conducted as explained in Section 7.2.1.7. Electrophoresis and amplicon visualisation was carried out as explained in Section 7.2.1.8.

Two non-specific bands were identified. The first (1311 bp) was detected in 9 out of 10 test reactions whereas the second (472 bp) was detected from 3 out of 10 test reactions Bovine DNA amplification was confirmed after appropriate isolation and sequencing of the second round PCR products. DNA fragments of these sizes, obtained over the longitudinal screening of *T. parva* positive samples using the PIM semi-nested PCR, were then disregarded from subsequent data analysis.

Separation, Extraction and Sequencing of PCR Products

Over the course of electrophoresis of second round PCR products, bands containing sufficient DNA were excised from the gel using a separate clean surgical blade, and placed into an eppendorf tube previously identified with the animal's ear-tag number, the date of sample collection and the estimated size of the band (bp). Separation of bands from the gel was carried out on a transilluminator (Fotodyne Incorporated, Hartland, WI, USA). Gel slices containing the DNA fragments of interest were processed immediately or frozen at -20ºC.

Selection of DNA fragments

Second round PCR products obtained from the screening of *T. parva* positive samples ranged from 300 bp up to 1311 bp. Over the course of the longitudinal screening for *T. parva* PIM types, two DNA fragments of each possible amplicon size (bp) were selected for sequencing in order to verify target bands. The criteria used to select gel slices for further processing were that (i) sufficient DNA, as

visually suggested from thickness and intensity of the band, was available to allow direct sequencing without re-amplification of agarose-extracted amplicons and that (ii) the DNA fragment was clearly separated from other bands when examined with the transilluminator.

Both criteria were consistently met by doubling the reaction volume of the second PCR round to 40µl, and by optimizing the electrophoresis protocol to ensure clear separation of DNA fragments.

DNA Extraction and Purification from Agarose Gels

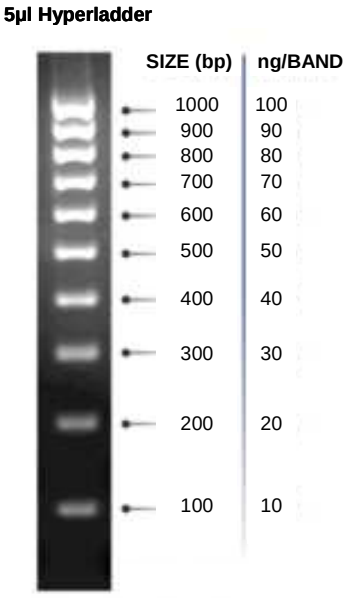
DNA fragments were immediately extracted from selected agarose slices, or extracted later from selected frozen slices, using the QIAquick Gel Extraction Kit (Qiagen Ltd., West Sussex, UK). Extraction and purification of DNA fragments was conducted following the instructions of the manufacturer. Briefly, each gel slice, containing as little excess agarose as possible, was weighed in a colourless tube. Slices weighing more than 400 mg were cut in two halves and processed separately. Three volumes of buffer QG were then added to the tube for each volume of gel (i.e. 300 µl of buffer for each 100 mg of gel). Tubes were subsequently transferred to a water bath and incubated at 50 ˚C for ten minutes, or until the gel slice was completely dissolved. To help dissolve the agarose gel, the solution was mixed by vortexing each tube every 2-3 minutes during the incubation. After incubation the colour of the mixture was checked, a yellow tint indicating that the pH was maintained below 7.5. In cases where an orange or violet colour was observed, 10 µl of 3 M sodium acetate (pH 5.0) was added and mixed to ensure that, in later steps, the adsorption of DNA to the QIAquick column membrane was efficient. One gel volume of isopropanol (100%) was then added to the tube and mixed. The sample was later applied to a QIAquick spin column previously placed in a 2 ml collection tube, and centrifuged at 13,000 rpm for one minute. Samples contained in agarose slices weighing more than 400 mg, and which had been incubated in separate tubes, were applied to the same column sequentially. In addition, as recommended by the manufacturer, no more than 800 µl of sample solution were added to each column at a time. Flow-through liquid in the collection tube was discarded after each centrifugation step, and the column placed *de-novo* into the same tube. To remove all traces of agarose from the column membrane, 0.5 ml of buffer QG was added to the QIAquick column which

was then centrifuged at 13,000 rpm for one minute. Flow-through liquid in the collection tube was discarded again. The membrane was then washed by repeating the previous step except in this case 0.75 ml of a solution containing buffer PE and 100% ethanol. The column was allowed to stand for 2-5 minutes in the collection tube after addition of buffer PE-ethanol solution, before centrifuging for one minute. After discarding the flow-through liquid, the column was placed again in the collection tube and centrifuged for one further minute, to remove traces of ethanol from the sample. The QIAquick column was then transferred into a clean 1.5ml eppendorf tube. To elute DNA, and following the recommendations of the laboratory where DNA fragments were sent for sequencing (Lark Technologies ™, Essex, UK), 25 µl of sterile water was applied to the centre of the column membrane. The column was allowed to stand for one minute and centrifuged for another minute at the maximum speed (>13,000 rpm).

Estimation of DNA Concentration

To estimate the concentration of purified DNA obtained from each gel extraction, 3 µl of eluted sample was mixed with 1 µl of gel loading buffer (Sigma-Aldrich, Dorset, UK) containing a bromophenol blue tracking dye.

Figure 7.2 Methodology to estimate the concentration of purified DNA.



Adapted from Bioline's online catalogue (*http://www.bioline.com*)

The 5 µl mixture was then loaded in to a standard 2% agarose gel, next to a lane containing 5 µl of Hyperladder IV from Bioline (London, UK). Electrophoresis was carried out following the protocol for first round PCR products described in Section 7.2.1.8. The concentration of purified DNA was estimated visually, using BioRad Gel Doc 2000, by comparing the intensity of the query band with that of bands from the ladder, for which the exact amount of DNA (ng/band) was known:

Sequencing

Samples containing sufficient DNA (≥ 20 ng for each 100 bp of amplicon with a sample volume = 15 µl), were sent for sequencing as recommended by Lark Technologies™ Sequencing Services (Essex, UK). In addition, 10 µl of primer "For-1" dilution and 10 µl of primer "Rev-2" dilution were sent per sequencing reaction, at a concentration of 50 ng/µl for a 20-mer primer. The remaining 7 µl of sample DNA was re-suspended in 1xTris-EDTA buffer and stored at – 20ºC.

Sequence Analysis

Sequences were aligned using Windows 32 SeqMan 5.03 software (Expert analysis software from DNA DNASTAR Inc. (1989-2001)). Thereafter, the Nucleotide-Nucleotide Basic Local Alignment Search Tool (nBLAST) from the National Center for Biotechnology Information (NCBI), was used to compare each single stranded test sequence individually, against other gene sequences found in public databases (e.g. nr database).(http://www.ncbi.nlm.nih.gov/Tools/). Where necessary, the nBLAST tool from The Institute for Genomic Research (TIGR), was used to cross-check the percentage identity of sequencing results against the *T. parva* Genome Database (http://www.tigrblast.tigr.org/). PIM amplicons ranged –mostly but not exclusively- between 1000 and 500 bp.

PCR products above 1000 bp were found to either correspond to unspecific amplification or did not produce any significant alignment with any known gene sequence. In contrast, sequences ranging from 1000 to 500 bp gave close to 100% identity - with high score (bits) and low e values - with the *T. parva* polymorphic immunodominant molecule gene of various isolates available in the Gene Bank database: *The Theileria parva glutamine rich protein mRNA* (THEGLUMEMP) (Baylis *et al.*, 1993), *The*

Theileria parva schizont / sporozite surface protein (THESSSP) (Toye *et al.*, 1995b) and *The Theileria parva polymorphic immunodominant molecule (PIM) mRNA* (THEPIM) (Toye *et al.*, 1995b), the Gene Bank accession numbers being L06323, L41148 and L41833 respectively. Between 400 and 500 bp, overlapping of bands resulting from both unspecific and PIM gene amplification were observed (two out of three sequenced bands corresponded with the expected target). Bands below 400 bp were unspecific or presented with too little percentage identity with PIM to provide a reliable match. These findings supported previous results that bands above 1000 bp invariably corresponded to amplification of host DNA while overlapping of PIM bands and bands not corresponding to PIM occurred between 500-400 bp.

7.2.1.10 Storage of PCR Products for Future Molecular Studies

DNA from 95 gel-extracted bands was stored at -20ºC to enable cloning and sequencing of PIM amplicons obtained from the study population in future studies.

7.2.1.11 Band Size Estimation, Data Storage and Management

The size of PIM gene amplicons (measured in base pairs [bp]) was estimated using The Discovery Series Quantity One Software (version 4.3.1) from Bio-Rad ®. (Available at: http://www. bio-rad.com). Band sizes were measured after running the gels for 15 hours, and re-measured after running the gels for 24 hours. Amplicon sizes were recorded in an excel spreadsheet for later analysis. Bands above 1000 bp, below 400 bp, and between 400 - 500 bp that corresponded to non specific amplification or amplification of host DNA, were not included in the final spreadsheet for statistical analysis.

7.2.2 Statistical Analysis

The purposes of the analyses were three-fold. Firstly, to determine which variables have a significant prognostic value for *T. parva* infection survival above the PCR detection threshold in cattle (Section 7.2.2.4). Secondly, to describe the manner in which potentially prognostic variables of infection survival relate to each other (Sections 7.2.2.1; 7.2.2.2; 7.2.2.5), and thirdly, to provide an overview of the general dynamics of infection in bovine hosts (Section 7.2.2.3). These parallel analyses should allow

critical appraisal of the conclusions obtained in the final Cox Proportional Hazards model/s of infection survival.

7.2.2.1 Diversity and Prevalence of T. parva Parasite Types

(Methodology for the Analysis Presented in Figure 7.3)

This analysis addressed whether certain parasite types were significantly more frequent than others among the study population, and quantified the extent of variation in the cumulative prevalence of parasite types in cattle. The cumulative prevalence of parasite types was defined as the proportion of type-positive animals recorded over the course of the study. Variation was investigated in an analysis of deviance with proportion data (GLM; Section 3.4.3). The dataset for analysis consisted of the number of type-positive cattle and the total number of cattle tested for each parasite type in each village. The dependent variable was defined as a *"cbind"* vector (y) where, the number of type-positive animals (a) and the total number of animals tested (b) were specified as *y*<−*cbind(a, b-a)*. Eight "y" vectors were available for each parasite type (one per each village). "Parasite Type" was fitted as a multi-level independent categorical variable identifying each of the parasite types as distinguished by PIM polymorphisms with a letter. The levels of the variable "Parasite Type" were replicated eight times (i.e. for each of the eight villages). The significance of the term "Parasite Type" was assessed through a deletion test where the initial model "y ~ Parasite Type", was compared with a model specified as "y ~ 1". F-test was chosen upon inspection of the dispersion parameter of the initial model (> 1.3), which was calculated by dividing the residual deviance by the residual degrees of freedom of the initial model. Variation in the cumulative prevalence of parasite types was estimated by dividing the deviance change obtained from the anova ([y ~ Parasite Type], [y ~ 1]) function, by the residual deviance of the simpler model (y ~ 1).

7.2.2.2 Geographical Distribution of T. parva Parasite Types

Inter-Village Variation in the Number of Cattle Infected with the Parasite Type of Interest

(Methodology for the Analysis Presented in Table 7.1)

Analysis of contingency tables with poisson errors (GLM) was conducted to investigate inter-village variation in the number of cattle infected with the parasite type of interest. A separate analysis was conducted for each parasite type. The significance of the interaction term "Parasite Type Exposure (a) : Village (b)", was assessed through a deletion test comparing a saturated model containing the two main terms and their interaction (a*b), with a reduced model containing only main terms (a + b). The dependent variable consisted of the number of cattle falling into each category (i.e. cross-tabulation between "*parasite-type exposure"* over the course of the study [Always negative/positive in at least one round of observation] and village [Bunyimini, Kubo, Nanjeho, Ojelai, Sitengo, Bunghaji, Hitunga or Magoje]). Chisq-test was chosen in view of the saturated nature of each initial model.

Inter-Village Variation in Richness and Relative Frequency of Circulating T. parva Types

To investigate inter-village variation in richness (i.e. Number of parasite types found in the area of interest) and relative frequency of circulating *T. parva* types, analysis of contingency tables with poisson errors (GLM) was conducted. The significance of the interaction term "Presence (a) : Parasite Type (b) : Village (c)", was assessed through a deletion test comparing a saturated model containing the three main terms and their interaction (a*b*c), with a reduced model containing only main terms (a + b + c). "Presence" was specified as a two-level categorical variable indicating either presence or absence of the parasite type of interest. "Parasite Type" was a multi-level categorical variable identifying each of the parasite types as distinguished by PIM polymorphisms with a letter. "Village" was specified as an eight-level categorical variable, with each level representing a different study village. The dependent variable consisted of the number of cattle falling into each category (i.e. cross-tabulation between a, b and c). A Chisq-test was chosen in view of the saturated nature of the initial model.

7.2.2.3 Super-Infection and / or Infection Recrudescence

(Methodology for the Analysis Presented in Figure 7.4)

The relationship between the logarithmic number of PIM positive follow-up observations, and number of new parasite types diagnosed by PCR, was investigated in an analysis of covariance with proportion data (GLM). The number of "new parasite types" referred to parasite variants which were not present at the initial PIM-positive observation but were diagnosed in subsequent visit rounds. The dependent variable was defined as a "*cbind* " vector (y) where, for each individual animal, the number of new parasite types identified over the course of follow-up PIM-positive observations (a), was modelled considering the total repertoire of parasite types not present at the initial PIM-positive sample (b), by specifying *y*<−*cbind(a, b-a)*. The response variable was modelled as a function of the age of individual cattle at the time of the initial PIM-positive observation, the logarithmic number of follow-up PIM-positive observations recorded from the same animals and the interaction between both terms. The maximal model can be summarised as "y ~ Cattle Age * log (Number of follow-up PIM-positive observations)". Only cattle with multiple PIM-positive observations and for which age at the time of the initial PIM-positive sample was available were included in the analysis. "Cattle age", was specified as a two-level categorical variable where each level corresponded to calves of up to 12 months of age or cattle older than twelve months. No other age categorisations could be tested as a result of low sample sizes within variable levels when other age groups were specified. The log-transformation of the number of follow-up PIM positive observations was chosen on the basis of diagnostic plots and the residual deviance of this as compared to alternative models. The significance of each term in the model (including the interaction term) was assessed through deletion tests.

7.2.2.4 Factors Affecting the Survival of T. parva Above PCR Detection Threshold

The Cox's proportional hazards model was used to investigate independent variables for prognostic significance of *T. parva* survival above the PCR detection threshold (Section 3.4.6). The analyses used all cattle for which PIM data by PCR was available. The dependent variable was defined as the duration (as measured in days) of *T. parva* infection above the PCR detection level, while the terminal

event was said to occur in cases where *T. parva* was no longer detectable from blood samples by PCR. In such cases "patent" phase of infection had reached an end. The use of PIM as a marker, together with the serology profiles obtained from each individual (Section 3.2.1.4), enabled discrimination of persisting infections from the onset of new infections sharing no parasite variants with the preceding infection, as distinguished by PIM gene polymorphisms. To avoid pseudo-replication, only the first infection from each animal was taken into account to investigate independent variables for prognostic significance of *T. parva* survival.

Dependent Variable and Terminal Event

The criteria used to define the survival time of *T. parva* above PCR detection threshold were as follows: Infection was said to (i) Start at the time of the first PIM-positive observation, (ii) Last for as long as at least one parasite type was shared with the preceding or the following PIM-positive observation, regardless of fluctuations around the detection threshold or missing observations over the course of patent infection, and (iii) End at the time of the last PIM-positive observation sharing at least one parasite type with preceding positive observations (terminal event).

Thereafter, follow-up PIM-positive observations presenting with a full new range of parasite variants as distinguished by the PIM PCR, failed to prove that a continuation of the preceding infection was taking place, and were therefore assumed to represent the onset of a new *T. parva* infection.

Censoring Criteria

Survival times were censored if: (i) The start of the infection coincided with the start of the observational period, or was immediately preceded by a visit round where no PCR (p104) test was performed, (ii) The period of observation ceased before the terminal event occurred, or the last PIM-positive observation was immediately followed by a visit round where no PCR (p104) test was conducted, and (iii) If patent infection was preceded or followed by one or more p104-positive observations where no PIM data was available, unless p104-positive observations with missing PIM data were recorded over the course of a subsequent new / independent infection sharing no parasite variants with the initial infection.

Independent Variables

The variables considered as potential predictors of infection survival were: (i) Cattle age at initial PIM-positive observation, (ii) Geographical origin of cattle (i.e. Village), (iii) Number of parasite types recorded over the course of patent infection (i.e. Infection multiplicity) and (iv) Parasite type (i.e. Presence or absence of the parasite type of interest as distinguished by PIM gene polymorphisms).

Individual Assessment of Potentially Prognostic Variables

(Methodology for the Analysis Presented in Tables 7.4 & 7.5)

Factors affecting infection survival were initially assessed individually for prognostic significance. In the case of multi-level variables, separate Cox models were fitted to investigate the hazard ratio (HR) of *T. parva* infections across biologically sound level-groupings. For the case of "Infection multiplicity" five level-groupings were tested. However, results are only presented for two possible level-groupings (either "single vs. mixed infections" or "infections with ≤ 3 vs. ≥ 3 PIM types"), given the consistency of results obtained in both individual and final Cox models for *T. parva* infection survival when taking into account other levels. The effect of "Village" was explored considering eight levels (each level representing a different village), and several other level-groupings consistent with the classification of villages with regards to *R. appendiculatus* challenge, or consistent with the classification of villages with regards to the epidemiology of *T. parva* in bovine hosts (Chapter 6). In the case of "Cattle age at first PIM-positive observation", levels-groupings were as follows: 1-6, 7-12, 13-24, >24 months old; 1-12, 13-24, >24 months old; ≤12 vs. > 12 months old and ≤24 vs. > 24 months old. Only one level-grouping ("0" = Not present in the infection; "1" = Present) was possible for assessing the prognostic significance of each parasite type with regard to *T. parva* survival by PCR. Variable levels were coded according to the methodology described by Parmar and Machin (Parmar *et al.*, 1996a) and in agreement with the S-plus 2000 manuals. Briefly, variables with two levels were specified as "dummy "(0/1) variables. All variables with more than two levels were categorical.

The significance of potentially prognostic variables of infection survival was assessed by means of both Likelihood Ratio (LR) and Wald (w) tests for purposes that are described later in this section. The

former, however, are preferred to the latter when assessing the prognostic significance of variables in a Cox model (Parmar *et al.*, 1996a). The assumption of proportional hazards was in all cases assessed as described in Section 3.4.6.3. Non-parametric Kaplan-Meier method was used instead when the assumption was not met, using either Log-rank (where Kaplan-Meier survival curves did not cross-over), or Generalised Wilcoxon's or Tarone–ware tests (where Kaplan-Meier survival curves did cross-over).

Tied observations were inevitable as the subjects were sampled monthly, rather than daily. The methodology used for the handling of tied observations during data analysis is described in Section 3.4.6.2.

Final Cox Model of Infection Survival

(Methodology for the Analysis Presented in Table 7.6)

Analysis of the factors influencing infection survival, took into consideration all variables which had a significant prognostic effect in the initial exploration (Parmar *et al.*, 1996b) as determined by both the Likelihood Ratio (LR) and Wald (W) test statistics. Selection of variables for the final Cox model was achieved through a step-up selection process, starting from a null model, as recommended by Parmar and Machin (Parmar *et al.*, 1996b). Briefly, at each step, whether introduction of a variable significantly improved the simpler model was assessed through a Likelihood Ratio (LR) test. The potentially prognostic variables were introduced in the model by order of increasing p-value obtained from the individual assessment of each variable (Parmar *et al.*, 1996b). As this procedure may be carried out by the use of either the Wald (W) or the Likelihood Ratio (LR) test (Parmar *et al.*, 1996b), the selection of variables in the final Cox model was conducted using both approaches for robustness. In addition, all possible one-variable models were fitted as the initial model prior to step-up selection of the remaining variables.

The number of variables and variable levels in a Cox model is limited by the availability of observations and terminal events in the dataset for analysis. Consideration of multi-level variables may lead to erroneous conclusions if a low number of observations are left available within each level, or may

compromise the inclusion of other variables during step-up selection procedures. Hence, all biologically sound, collapsed level-groupings (See previous section) with LR- and W-test p-values<0.05, were also considered in separate step-up selection procedures to overcome this limitation.

Parallel analysis was conducted to ensure that the final Cox models obtained as described above were consistent with those obtained when considering, in addition, variables (and level-groupings) with no significant effect on infection survival as determined by at least one of the two possible test statistics (i.e. LR and W). Given the consistency of results obtained by either approach, results from the parallel analyses are not shown.

7.2.2.5 Associations amongst Potentially Prognostic Variables of Infection Survival

Parasite Type - Infection Multiplicity

(Methodology for the Analysis Presented in Table 7.7)

Whether the parasite type of interest was more likely to be present in the host alone or in concomitant infections involving at least a second parasite variant was investigated using generalised linear mixed-effects modelling via penalised quasi-likelihood estimation method (glmmPQL) and binomial error structure (Section 3.4.5). Only cattle with multiple PIM-positive observations were considered in the analysis. A separate model was fitted for each parasite type. In each model, the dependent variable was coded “0” or “1” depending on whether the infection recorded from each blood sample comprised one or multiple parasite types. The independent variable consisted of two categorical levels indicating the presence or abcense of the parasite type of interest. Odds ratios were calculated from PQL model coefficients. All models considered different intercepts for each individual animal as random-effects. In two models, different time slopes for each individual animal were also considered. “Time” was included in the random-effects as an integer-valued variable with value “zero” at the time of the first PIM-positive observation. The inter-visit time length observed from the data was respected.

Cattle Age / Geographical Origin of Cattle - Infection Multiplicity

(Methodology for the Analysis Presented in Table 7.8)

Classical Chi-square tests were used to explore associations between other potentially prognostic variables of infection survival (Section 3.4.2). Two-sided Fisher's exact tests were used in cases where one or more expected frequencies in the contingency table were < 5 (Section 3.4.2).

7.3 Results

7.3.1 PIM Gene Amplification

The PIM gene was amplified from 207 out of 281 *T. parva* positive cattle (71.1%) diagnosed with the parasite from one up to 13 times during the period of observation, and from 382 out of a total of 537 p104 positive blood samples (73.3%). Re-using the same *T. parva* (p104 gene) positive 2 mm disc, was a better strategy for guaranteeing PIM gene amplification, than re-screening of *T. parva* positive sample-saturated cards *de-novo*. Amplification of the PIM gene was achieved in 100% of the cases (n=86) where the same 2 mm disc used as template in the p104 gene PCR, was then re-used as template in the PIM semi-nested PCR reaction. Where the 2 mm disc used for PIM gene testing did not correspond with that taken from the same sample-saturated card for the initial diagnosis of *T. parva* (p104 gene), the presence of the parasite's DNA was not completely guaranteed and consequently, amplification of PIM was only achieved in 65.6% (296/451) of the infected sample-saturated cards.

7.3.2 Data Availability

Appendix 7.1 provides a summary of the number and distribution of initial and follow-up *T. parva* positive observations as measured by PCR amplification of the p104 gene, in 207 cattle with at least one PIM positive sample in the study. The table shows the number of p104 positive observations for which the history of *T. parva* parasite types - as distinguished by the PIM PCR – was not available. The information is presented according to the age class (taken as the age of animals when the first *T. parva* [p104] positive sample was obtained) and the geographical origin of parasite-positive individuals. Of the 207 cattle for which PIM data was obtained, 66 were identified as calves (≤12 months old) and

125 were identified as older than 12 months of age at the time of the first p104-positive observation. Age at initial parasite-positive diagnosis was missing in the case of 16 animals. Information on *T. parva* parasite types as distinguished by the PIM PCR was missing for the first p104-positive observation in the case of 19 animals. In such cases, *T. parva* parasite types as distinguished by PIM gene polymorphisms were recorded only from p104-positive follow-up observations. Consequently, five initial calves at the time of the first p104-positive observation had grown into adults, while the age of one initial calf was no longer available at the time data on parasite types was recorded. Information on *T. parva* parasite types as distinguished by the PIM PCR, was missing in 54 of 248 p104-positive follow-up observations in 207 cattle where PIM data was obtained for at least one visit round.

The relationship between number of p104-positive observations recorded from cattle, and number of PIM-positive observations recorded from the same individuals is given in Appendix 7.2. Of the 207 cattle for which PIM data was available, 60 had multiple PIM-positive observations available for analysis whereas 147 had only one PIM-positive observation in the study. Of the 147 animals with only one PIM-positive observation, 126 were in fact parasite-positive on only one visit round during the study - as measured by the p104 PCR - whilst 21 had been parasite-positive on multiple samplings, but PIM data was only available for the case of one visit round. Of the 60 animals with multiple PIM-positive observations, PIM data was available for the totality of p104-positive samplings in 32 individuals. In contrast, for 28 of 60 cattle, the history of parasite types as distinguished by PIM gene polymorphisms was incomplete in relation with the total number of p104-positive observations recorded during the study.

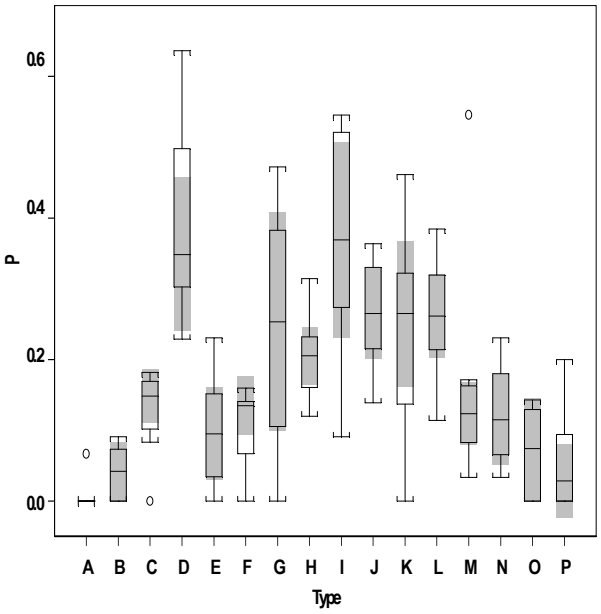
7.3.3 Diversity and Prevalence of *T. parva* Parasite Types

The PIM PCR differentiated sixteen *T. parva* parasite types on the basis of the amplicon length as measured in base pairs (bp). Parasite types were identified with a letter ranging from A to P according to increasing amplicon's length (Figure 7.3). The classification system was given in bp-intervals to account for inter-gel variation at the time of electrophoresis. *T. parva* parasite types with the smallest (439 – 451 bp) and largest (955-1000 bp) PIM sizes were types A and P respectively (Figure 7.3).

The cumulative prevalence of parasite types in the cattle population (n=207) was highly heterogeneous, and varied by 61.3% depending on the type considered (n = 207 cattle; F-value = 12.58; df = 15; p <0.0001). See Figure 7.3.

Figure 7.3 Cumulative prevalence of *Theileria parva* parasite (PIM) types in the 207 cattle tested across study villages.

Parasite Type	Length (bp)	Infected cattle		Statistical Test			
		Frequency	%	F-value	df	p-value	% Variation
A	439 - 451	2	0.97	12.58	15	<0.0001	61.3
B	500 - 519	9	4.35				
C	520 - 560	28	13.5				
D	561 - 609	78	37.7				
E	610 - 633	20	9.66				
F	634 - 662	24	11.6				
G	663 - 704	56	27.1				
H	705 - 724	43	20.8				
I	725 - 754	84	40.6				
J	755 - 784	52	25.1				
K	785 - 818	50	24.2				
L	819 - 855	50	24.2				
M	856 - 880	27	13.0				
N	881 - 919	25	12.1				
O	920 - 954	17	8.22				
P	955 - 1000	12	5.79				



The box-and-whisker-plot illustrates the variation in the proportion (P) of cattle positive to each parasite type across the eight study villages over the course of the observational period.

T. parva types whose PIM gene's length (bp) was close to the median bp length, were more widespread amongst cattle herds. Types I (725-754 bp) and D (561-609 bp), for example, were diagnosed in 40.6% (n = 84) and 37.7% (n = 78) of cattle respectively. In contrast, types A (439-451 bp) and P (955-1000 bp), were only recorded from two (0.97%) and 12 (5.79%) animals respectively.

7.3.4 Geographical Distribution of *T. parva* Parasite Types

The distribution of six parasite types varied significantly across cattle herds raised in different study villages (Table 7.1). Moreover, each study village could be regarded as a different diversity matrix with regard to richness (i.e. Number of parasite types [A to P] found in the area) and relative frequency of parasite types in cattle (n = 207 cattle; Chisq = 164.4; df = 105; p = 0.0002). Parasite type “A”, for example, was only recorded from two animals in Sitengo (Table 7.1).

Table 7.1 Cumulative prevalence of cattle positive to each *Theileria parva* parasite (PIM) type in each study village.

Parasite	Bunyimini		Nanjeho		Kubo		Ojelai		Sitengo		Bunghaji		Hitunga		Magoje		Statistical Tests		
Type	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	Chisq	df	p-value
A									2	6.67							7.84	7	0.347
B	2	5.71	1	2.78	2	9.09	2	8.00	2	6.67							7.53	7	0.376
C	6	17.1	3	8.33	4	18.2	3	12.0	5	16.7	5	14.3			2	15.4	5.22	7	0.633
D	10	28.6	22	61.1	8	36.4	8	32.0	10	33.3	8	22.9	7	63.6	5	38.5	**16.5**	**7**	**0.021**
E	2	5.71	6	16.7	3	13.6	1	4.00	4	13.3	1	2.86			3	23.1	11.1	7	0.134
F	5	14.3	5	13.9	3	13.6	4	16.0	4	13.3	2	5.71			1	7.69	5.36	7	0.617
G	16	45.7	17	47.2	4	18.2	6	24.0	8	26.7	1	2.86			4	30.8	**35.3**	**7**	**<.0001**
H	11	31.4	5	13.9	4	18.2	3	12.0	7	23.3	8	22.9	2	18.2	3	23.1	5.03	7	0.656
I	18	51.4	19	52.8	12	54.6	6	24.0	11	36.7	13	37.1	1	9.09	4	30.8	**15.1**	**7**	**0.035**
J	9	25.7	5	13.9	6	27.3	9	36.0	9	30.0	7	20.0	4	36.4	3	23.1	5.84	7	0.559
K	9	25.7	10	27.8	6	27.3	4	16.0	11	36.7	4	11.4			6	46.2	**16.4**	**7**	**0.022**
L	8	22.9	9	25	7	31.8	8	32.0	6	20.0	4	11.4	3	27.3	5	38.5	6.79	7	0.452
M	3	8.57	4	11.1	3	13.6	2	8.00	1	3.33	6	17.1	6	54.6	2	15.4	**16.1**	**7**	**0.024**
N	7	20	5	13.9	1	4.55	4	16.0	1	3.33	3	8.57	1	9.09	3	23.1	8.39	7	0.299
O	5	14.3	5	13.9	2	9.09	3	12.0			2	5.71					12.7	7	0.079
P	1	2.86	4	11.1			5	20.0			1	2.86			1	7.69	**16.3**	**7**	**0.023**

The table shows, for each parasite type (A to P), the number and percentage of cattle which were diagnosed with the *T. parva* type of interest in at least one round of observation in each village. For each parasite type, the significance of inter-village variation with regard to the cumulative percentage of positive cattle over the course of the study is listed in the latter columns. Significant tests are in bold. The total number of cattle sampled (207) classified on the bases of the village of origin, was as follows: Bunyimini (35), Nanjeho (36), Kubo (22), Ojelai (25), Sitengo (30), Bunghaji (35), Hitunga (11) and Magoje (13).

7.3.5 Single and Mixed Parasite Type Infections

Of the 382 PIM-positive observations, 40.3% (n = 154) were single parasite type infections whilst 59.7% (n = 228) were mixed *T. parva* type infections involving a maximum of seven parasite variants. The maximum cumulative number of parasite types recorded from an individual animal was 12 (Table 7.2).

Table 7.2 Frequency distribution of number of *Theileria parva* parasite types in 382 samples obtained from 207 PIM-positive cattle.

Number of Parasite Types	Observations			Cattle		
	Frequency	%	Cumulative %	Frequency	%	Cumulative %
1	154	40.3	40.3	76	36.7	36.7
2	104	27.2	67.5	45	21.7	58.5
3	69	18.1	85.6	29	14	72.5
4	29	7.59	93.2	19	9.18	81.6
5	16	4.19	97.4	12	5.8	87.4
6	8	2.09	99.5	12	5.8	93.2
7	2	0.52	100	5	2.42	95.7
8				5	2.42	98.1
9				2	0.97	99
11				1	0.48	99.5
12				1	0.48	100
Totals	**382**	**100**		**207**	**100**	

Parasite types were as distinguished by PIM gene polymorphisms. The frequency distribution of total number of parasite types recorded from individual animals over the course of the study is shown under the "Cattle" heading.

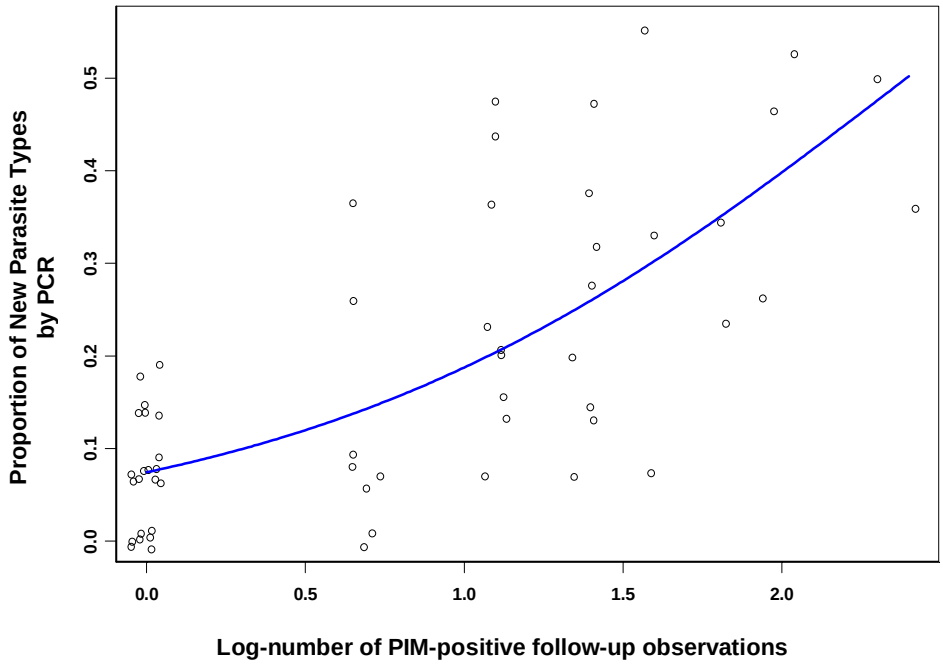
In the subset data frame of 60 cattle with multiple PIM-positive observations, 88.3% of animals presented mixed parasite type infections in at least one visit round (n = 53). In contrast, amongst the 147 cattle for which only one PIM-positive observation was available (of which 126 were p104-positive only once during the study), mixed-type infections were only recorded from 49.7% of the animals (n = 73). These observations, along with the fact that a cyclical pattern of mixed parasite type infections was apparent in the study population (Appendix 7.3), suggest fewer mixed species infections in cross-sectional compared to longitudinal data. A trend towards mixed infections being less frequent amongst

cattle where *T. parva* (p104) became undetectable by PCR within 28 days after initial diagnosis was also apparent.

7.3.6 Super-Infection and / or Infection Recrudescence

The number of new parasite types that each animal acquired over the course of the study, expressed as a proportion of the total circulating *T. parva* types not found in the initial PIM-positive sample, increased with the logarithmic number of PIM-positive follow-up observations recorded from each animal (n = 57, Coefficient = + 1.053 [S.E. = 0.14], Chisq = 63.67, df = 1, p<.0001). See Figure 7.4.

Figure 7.4 Relationship between logarithmic number of PIM-positive follow-up observations and the proportion of new *Theileria parva* parasite types.



The scatter-plot shows the model prediction (Section 7.2.2.3) for the relationship of interest. The proportion of "newly diagnosed parasite (PIM) types" refers to PIM types diagnosed in follow-up observations, expressed as a proportion of the total repertoire of PIM types not present at the initial PIM-positive observation. Each circle represents an animal with multiple PIM-positive observations and for which age was known at the time of the initial PIM-positive sample (n = 57).

The results obtained from this analysis were compatible with periodic super-infections taking place in cattle herds from the study areas, and/or with periodic recrudescence of parasite type infections initially below the PCR detection threshold. The number of new parasite types diagnosed in subsequent visit rounds was not different depending on whether animals were ≤12 months old (n = 27) or > 12 months

old (n = 30) at the time of the first PIM-positive observation ($p > 0.05$). Moreover, the rate at which the number of new parasite types increased with each PIM-positive follow-up observation was not dependent on whether animals were identified as calves or as older than 12 months of age at the first PIM-positive observation ($p > 0.05$). As no other age categorisations could be tested (Section 7.2.2.3), it cannot be concluded that the relationship of interest did not vary when considering other age groups.

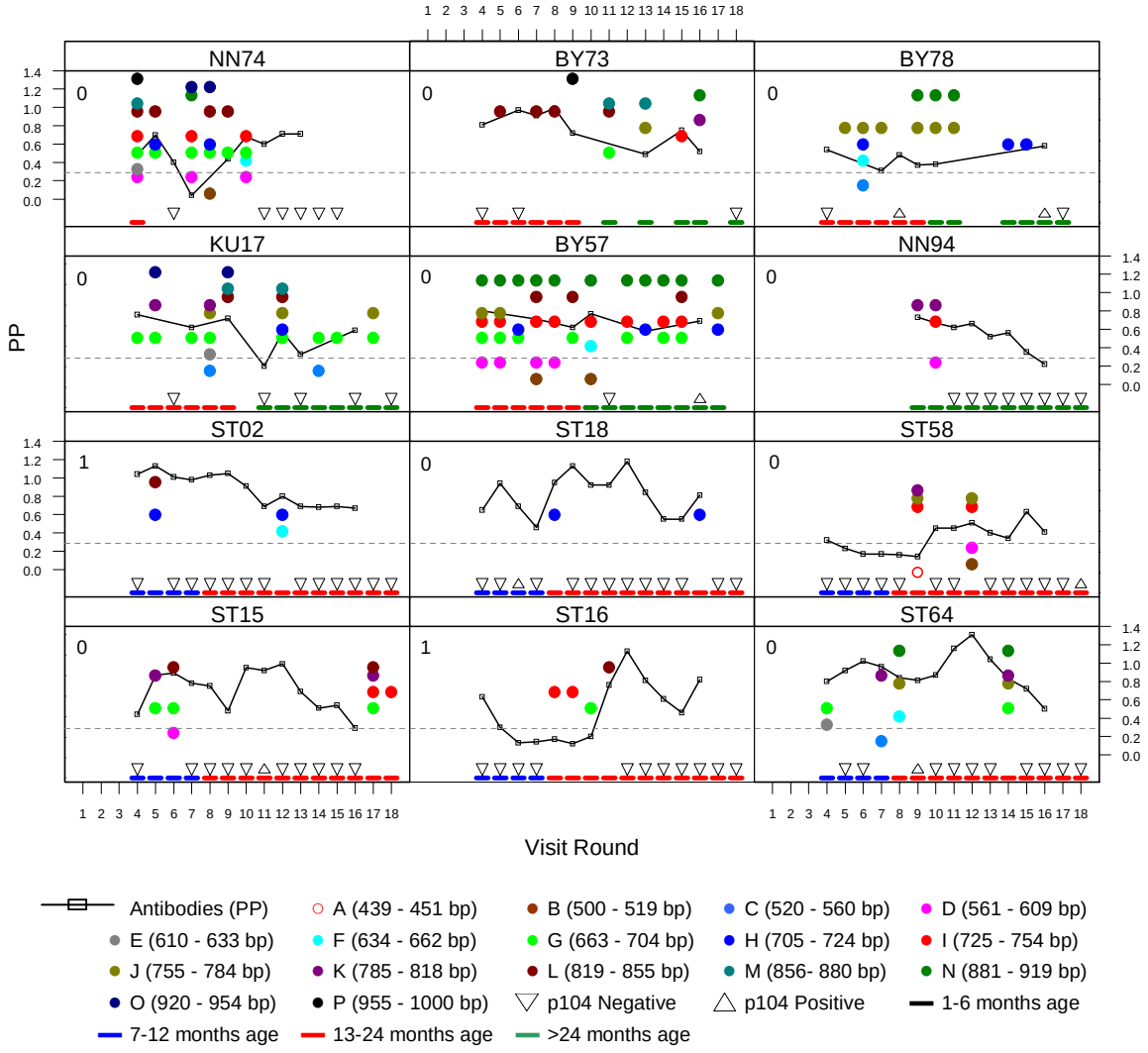
7.3.7 Factors Affecting the Survival of *T. parva* Above PCR Detection Threshold

7.3.7.1 Presentation of Raw Data - Longitudinal History of PIM-Positive Cattle

Figure 7.5 presents the longitudinal history of a sample of 12 of 60 cattle with multiple PIM-positive observations. The history of the remaining cattle included in the analyses (n = 207; 48 of 60 animals with multiple PIM-positive observations plus 147 animals with a single PIM-positive sampling) is given in Appendix 7.4.

In Figure 7.5, each panel presents the data from one animal. In the label for each animal, the two letters indicate the village of origin. The complete history of (i) *T. parva* positive observations above the p104 PCR detection threshold, (ii) infection multiplicity and composition of parasite types as distinguished by PIM gene polymorphisms at each round of observation and (iii) serology profiles obtained from the PIM ELISA (Katende *et al.*, 1998), is presented for each animal since the time of recruitment until the period of observation ceased. Dashed horizontal lines show the cut-off point for seropositivity (> 30 percentage positivity [PP]). The PP is presented in a scale of zero to one. For each animal, seroconversion or seroevents were said to take place when, given any two rounds of observation, the PP in the first visit round (PP_1) was ≤ 30, the PP in the following visit round (PP_2) was > 30 and PP_1-PP_2 ≥ 20. Seroconversion or primary immune response can only occur in an immunologically naïve animal, upon initial parasite-exposure during the entire life time. Secondary immune responses (seroevents) can occur after initial seroconversion, if the PP drops below the seropositivity threshold and increases *de-novo* upon re-infection with *T. parva*.

Figure 7.5 Longitudinal histories of 12 out of 60 cattle with multiple PIM-positive observations (animals 1 to 12 shown).



Coloured dots show different *T. parva* PIM types. BY73 and ST16 were considered to present with two independent *T. parva* infections over time (Section 7.2.2.4). Only the first infection was considered for analysis.

In each panel, "0" indicates that the first infection recorded from the animal of interest was censored and "1" that the terminal event had occurred. Animals which harboured multiple independent *T. parva* infections over the course of the study are indicated in the legend of the figure. Of the 207 cattle considered in the analysis, 18 harboured two (n =17) or three (n = 1) "*independent*" infections over time (Figure 7.5 & Appendix 7.4), sharing no parasite variants with the preceding infection as distinguished by PIM gene polymorphisms.

A summary of survival times and total number of censored and terminal events in the data frame for analysis (n = 207 cattle) is given below.

Table 7.3 *T. parva* infections in 207 cattle with PIM-positive observations - Survival times, censored observations and terminal events.

Survival Times	Total Observations	Censored Observations	Terminal Events
28	158	82	76
56	10	8	2
84	2		2
112	2	2	
168	3	1	2
192	1	1	
196	6	5	1
224	3	2	1
252	8	5	3
254	1	1	
279	1	1	
308	4	4	
310	1	1	
338	1	1	
392	3	3	
394	1	1	
422	1	1	
506	1	1	
Totals	**207**	**120**	**87**

Monthly samplings resulted in numerous tied observations. Tied observations are those where survival times (shown in days) are identical.

7.3.7.2 Individual Assessment of Potentially Prognostic Variables

Model estimates for individually tested variables which had a significant prognostic value on infection survival - as determined by both the Likelihood Ratio (LR) and the Wald (w) test statistics - are presented in Table 7.4. LR- and W- test statistics are shown, for significant variables, in Table 7.5 (Section 7.3.7.3).

Table 7.4 Cox models for eleven potentially prognostic variables for *Theileria parva* infection survival in 207 cattle with PIM-positive observations.

Variables	Levels	n	Terminal Events	ß	SE[ß]	z-test	p-value	HR	95% CI HR
Cattle Age	1-24 months	141	53	0				1	
	>24 months	49	25	0.55	0.25	2.25	0.0240	1.74	1.07-2.81
Village	Bunghaji	35	26	0				1	
	Bunyimini	35	15	-1.15	0.34	-3.43	0.0010	0.32	0.16-0.61
	Nanjeho	36	12	-1.24	0.35	-3.52	0.0004	0.29	0.15-0.58
	Kubo	22	8	-1.14	0.41	-2.79	0.0050	0.32	0.14-0.71
	Ojelai	25	5	-1.87	0.49	-3.80	0.0002	0.15	0.06-0.41
	Sitengo	30	14	-0.97	0.34	-2.83	0.0050	0.38	0.19-0.74
	Hitunga	11	6	-0.45	0.45	-0.99	0.3200	0.64	0.26-1.55
	Magoje	13	1	-2.85	1.01	-2.82	0.0050	0.06	0.01-0.42
Area	Bunghaji + Hitunga	46	32	0				1	
	HTC + Magoje	161	55	-1.20	0.23	-5.19	<.0001	0.30	0.19-0.47
Number (a)	Single	84	44	0				1	
	Mixed	123	43	-0.77	0.22	-3.44	0.0010	0.46	0.30-0.72
Number (b)	Up to three	157	78	0				1	
	> than three	50	9	-1.74	0.37	-4.66	<.0001	0.18	0.08-0.37
E	Absent	189	84	0				1	
	Present	18	3	-1.32	0.59	-2.24	0.0250	0.27	0.08-0.85
G	Absent	156	78	0				1	
	Present	51	9	-1.53	0.36	-4.24	<.0001	0.22	0.11-0.44
H	Absent	167	76	0				1	
	Present	40	11	-0.84	0.33	-2.54	0.0110	0.43	0.23-0.83
J	Absent	157	72	0				1	
	Present	50	15	-0.70	0.29	-2.43	0.0150	0.50	0.28-0.87
K	Absent	161	74	0				1	
	Present	46	13	-0.70	0.30	-2.31	0.0210	0.50	0.28-0.90
L	Absent	162	72	0				1	
	Present	45	15	-0.62	0.29	-2.15	0.0310	0.54	0.30-0.95
N [1]	Absent	185	81						
	Present	22	6						

The table presents only those variables that were of prognostic significance with regard to *T. parva* survival, as measured by both the Likelihood Ratio (LR) and the Wald (W) test statistics (Table 7.5; Section 7.3.7.3). The number of infections available for analysis within each variable level is shown in the column 'n', and the number of infections for which the terminal event was recorded is shown in the column 'Terminal Events'. Because only the first infection from each individual animal was considered in the analysis, the figures in these columns can also be taken to represent number of cattle. The variables 'Number (a)' and 'Number (b)' refer to number of parasite types present in the infection. The letters "E" to "N" indicate the identity of parasite types. 'HTC' refers to 'high tick challenge villages'.[1] In the case of PIM type N, the assumption of proportional hazards was not met. The significance of this variable was assessed using the Logrank-test statistic (Chisq = 4.5, df = 1, p = 0.033), after confirmation that Kaplan-Meier survival curves for infections where PIM type N was absent or present, did not overlap.

Interpretation of Table 7.4

Model estimates (coefficients) are shown in the column "ß" along with their associated standard errors (SE[ß]). Note that ß equals zero in the case of the reference level specified for each variable. The column "z-test" records the ratio of each coefficient (ß) to its standard error. The "z-test" is a Wald statistic which is asymptotically standard normal under the hypothesis that the corresponding ß is zero. For each variable level, the p-value derived from the z-test indicates whether the hazard ratio (HR) of *T. parva* is significantly different to that in the reference level. In each case, HR corresponds to the exponentiated ß coefficients ($e^{ß}$). Hence, in the case of reference variable levels HR = e^0 = 1. HR values of less than one indicate that the HR of *T. parva* infections is lower for the level of interest as compared to the reference. When HR is higher than 1, the HR of *T. parva* is higher for the level of interest as compared to the reference. For example, HR of *T. parva* increased by 74% (HR=1.74) in animals older than 24 months as compared to in younger cattle. In contrast, the HR of *T. parva* by PCR decreased by 82% ([1-0.18]*100 = 82%) in infections harbouring more than three parasite types as compared to in those harbouring up to three parasite variants. The HR for the level of interest is not significantly different from that of the reference level where the 95% confidence intervals overlap with "1". Finally, the HR is inversely related to infection survival above PCR detection threshold. HR <1 indicates greater infection duration in the level of interest as compared to the reference, whilst HR >1 indicates shorter survival of infection in the level of interest as compared to the reference.

Cattle Age

Age at first PIM-positive observation was not available for 17 out of 207 cattle with data on *T. parva* parasite types as distinguished by the PIM PCR. The numbers of cattle, classified on the basis of their age at the time of the first PIM-positive observation, were as follows: 16 (1-6 months of age); 44 (7-12 months of age); 81 (13-24 months of age) and 49 (>24 months of age).

Age at first PIM-positive diagnosis was a significant predictor of infection survival when considering all four age categories according to the LR-test (n = 190; LR-test= 13.3; df = 3; p=0.004), but not the Wald-test (n = 190; W-test= 7.8; df = 3; p=0.0502). This resulted from wide confidence intervals for exponentiated model coefficients when considering four age groups (Data not shown). Infection survival was not different amongst calves of 1-6 months, 7-12 months or juvenile cattle (13- 24 months); (n = 141; LR-test = 3.99; df = 1; p=0.05) and not different amongst cattle of up to 12 months or older than 12 months (n = 190; LR-test= 3.47; df = 1; p=0.063). However the hazard ratio of *T. parva* increased by 74% in animals older than 24 months of age as compared to animals of up to 24 months of age (n = 190; LR-test = 4.73; df = 1; p = 0.0297).

Village and Area

The number of cattle available for analysis in each village is shown in Table 7.4. The hazard ratio of *T. parva* varied significantly in cattle herds raised in different villages (n = 207; LR-test = 33.4; df = 7; p<0.0001). With regard to the survival time of *T. parva* infections at levels above the PCR detection threshold, cattle could be classified into two broad geographical groups which were not perfectly consistent with the classification of villages with respect to *R. appendiculatus* challenge. See Chapter 6. The duration of infections was similar in cattle from HTC villages (n = 148; LR-test = 3.55; df = 4; p=0.47), but differed significantly in cattle herds raised in different LTC villages (n = 59; LR-test = 19.2; df = 2; p<0.0001). Exclusion of cattle from $Magoje_{(LTC)}$ in the Cox model for LTC villages showed that the hazard ratio of *T. parva* in Bunghaji and Hitunga was not different (n = 46; LR-test = 0.94; df = 1; p=0.333) and hence that differences in infection survival amongst cattle in LTC villages were due to the $Magoje_{(LTC)}$ village. Moreover, whilst the survival of infections was different in $Bunghaji_{(LTC)}$ plus $Hitunga_{(LTC)}$ compared to in HTC villages (n = 194; LR-test = 24.8 on 5 df, p=0.000153), the hazard ratio of *T. parva* in cattle from $Magoje_{(LTC)}$ could not be regarded as different to that in areas of HTC (n = 161; LR-test = 8.28 df = 5; p=0.141). These results were supported by a number of other analyses (Data not shown).

When considering the variable "Village", only $Hitunga_{(LTC)}$ was not different from $Bunghaji_{(LTC)}$ (reference variable level) with regards to the HR of *T. parva* infections. See Table 7.4. Conversely, the HR was 68%-85% lower in HTC areas (depending on the villages considered) and was 94% lower in $Magoje_{(LTC)}$ compared to in $Bunghaji_{(LTC)}$. See Table 7.4. A limitation of the analysis was the low number of cattle available for analysis in Magoje and Hitunga, and the low number of terminal events in the former village (Table 7.4). However, these observations were consistent with the results reported in the previous chapter, where more animals and *T. parva* positive observations (as measured by the p104 PCR) were available for analysis (Chapter 6).

The HR of *T. parva* infections in cattle from Magoje$_{(LTC)}$ and HTC villages pooled together, was 70% lower than in Bunghaji and Hitunga (n = 207; LR-test = 23.9; df =1; p<0.0001). See variable "Area" in Table 7.4.

Parasite Type

Infection survival was also conditioned to the presence or absence of particular parasite types. Table 7.4 shows parasite types which had a significant effect on the survival of *T. parva* infections. Only parasite types diagnosed on >12 cattle (i.e. 13 out of 16 parasite types) were assessed for prognostic significance (types A, C, and P not tested). As suggested from the preliminary Cox models, parasite types as distinguished by PIM gene polymorphisms appeared to differ in their ability to induce persistent infections in that some parasite variants enhanced *T. parva* survival when present in the infection, while others did not. Seven out of 13 parasite types tested had a significant effect on infection survival, which was different depending on the type considered (Table 7.4; LR- and W-tests are shown in Table 7.5). For example, types E and G decreased the hazard ratio of *T. parva* infections by 73% and 78% respectively, whilst HR was reduced by 48% in infections harbouring parasite type L.

Infection Multiplicity

Multiple allele infections decreased the hazard ratio of *T. parva* by 54% compared to single allele infections (n = 207; LR-test = 31.4; df = 1; p<0.0001), and the hazard ratio of infections involving more than three PIM alleles was 82% lower than those involving up to three alleles (n = 207; LR-test = 11.8; df = 1; p = 0.0006).

7.3.7.3 Final Cox Models for T. parva Survival

Four possible final Cox models were obtained depending on whether Wald (W) or the Likelihood Ratio (LR) tests were used to guide the step-up selection process of variables with a significant prognosis effect for infection survival in the initial exploration (Table 7.5).

Table 7.5 Step-up variable selection procedure for the final Cox model for *Theileria parva* infection survival.

Likelihood Ratio (LR) Statistics				Wald (W) Statistics			
Variable order	**LR**	**df**	**p-value**	**Variable order**	**W**	**df**	**p-value**
Number (b)	31.4	1	2.05E-08	**Area**	26.9	1	2.12E-07
G	25.8	1	3.86E-07	**Number (b)**	21.7	1	3.20E-06
Area	23.9	1	1.00E-06	**G**	18	1	0.000022
Village	33.4	7	2.28E-05	**Village**	31.1	7	6.03E-05
Number (a)	11.8	1	0.000591	**Number (a)**	11.9	1	0.000571
H	7.83	1	0.00513	**H**	6.47	1	0.0109
E	7.67	1	0.00561	**J**	5.89	1	0.0152
J	6.76	1	0.0093	**K**	5.33	1	0.0209
K	6.22	1	0.0126	**Age**	5.07	1	0.0243
L	5.24	1	0.0221	**E**	5	1	0.0254
Age	4.73	1	0.0297	**L**	4.63	1	0.0313
N [1]				**N** [1]			

Each selection process considered either "Number" (a) or "Number" (b) and either "Area" or "Village". [1] In the case of PIM type N, the assumption of proportional hazards was not met (See Table 7.4). This variable was introduced at several possible steps during the selection procedure according to LR-tests and W-tests (i.e. assumption of proportional hazards met in all cases where N was not the only variable in the model). Area = [HTC villages and Magoje $_{[LTC]}$] versus [Bunghaji$_{[LTC]}$ and Hitunga$_{[LTC]}$]; Village [Bunghaji; Bunyimini; Nanjeho; Kubo; Ojelai; Sitengo; Hitunga; Magoje); Number (a) = [1 parasite type] versus [>1 parasite type]; Number (b) =.[≤ 3 parasite types] versus [>3 parasite types]; E to N = Parasite type identity. Age = [≤ 24 months] versus [> 24 months].

The four final Cox models were also a result of testing alternative level-groupings for the variables of interest in separate step-up selection procedures (Table 7.6). Final Cox models suggested that the survival of *T. parva* above the PCR detection is a function of "Geographical Origin of Cattle" and "Infection Multiplicity" (Table 7.6). This was regardless of whether "Village" or "Area" (i.e. either [HTC and Magoje$_{(LTC)}$] or [Bunghaji$_{(LTC)}$ and Hitunga$_{(LTC)}$]) were considered in the step-up selection process, or whether alternative level-groupings of "Infection multiplicity" were taken into account. Inspection of HR (or $e^{ß}$) obtained from final models, was consistent with *T. parva* infections lasting for longer in cattle from HTC villages and Magoje$_{(LTC)}$, and with the HR of *T. parva* being inversely related to increasing infection multiplicity (Table 7.6). The results presented in the table were consistent with those obtained from alternative step-up selection procedures (See Section 7.2.2.4).

Table 7.6 Final Cox models for two prognostic variables for *Theileria parva* infection survival in 207 cattle with PIM-positive observations.

Models	Levels	n	Terminal Events	ß	SE(ß)	z-test	p-value	HR	95% CI	Likelihood Ratio (LR) Statistics
Variable Selection (LR Test Statistics)										
Model 1										
Number of PIM types (b)	Up to 3	157	78	0				1		
	> than 3	50	9	-0.87	0.20	-4.46	<.0001	0.42	0.29 - 0.61	(LR = 60.3; df = 2; p <.0001)
Village	Bunghaji	35	26	0				1		
	Bunyimini	35	15	-0.78	0.34	-2.31	0.0210	0.46	0.24 - 0.89	
	Nanjeho	36	12	-0.93	0.35	-2.64	0.0084	0.39	0.20 - 0.79	
	Kubo	22	8	-1.01	0.41	-2.48	0.0130	0.36	0.16 - 0.81	
	Ojelai	25	5	-1.83	0.50	-3.71	0.0002	0.16	0.06 - 0.42	
	Sitengo	30	14	-0.71	0.34	-2.05	0.0400	0.49	0.25 - 0.97	
	Hitunga	11	6	-0.55	0.45	-1.21	0.2300	0.58	0.24 - 1.41	
	Magoje	13	1	-2.72	1.01	-2.69	0.0072	0.07	0.01 - 0.48	
Model 2										(LR = 46.7; df = 2; p <.0001)
Number of PIM types (b)	Up to 3	157	78	0				1		
	> than 3	50	9	-1.58	0.38	-4.12	<.0001	0.21	0.10-0.44	
Area	BH + HT	46	32	0				1		
	Others	161	55	-0.95	0.23	-4.07	<.0001	0.39	0.24-0.61	
Variable Selection (Wald Statistics)										
Model 1										
Village	Bunghaji	35	26	0				1		(LR = 41.6; df = 2; p <.0001)
	Bunyimini	35	15	-1.04	0.34	-3.05	0.0023	0.35	0.18 - 0.69	
	Nanjeho	36	12	-1.11	0.35	-3.14	0.0017	0.33	0.16 - 0.66	
	Kubo	22	8	-1.12	0.41	-2.74	0.0061	0.33	0.15 - 0.73	
	Ojelai	25	5	-1.82	0.49	-3.69	0.0002	0.16	0.06 - 0.43	
	Sitengo	30	14	-0.95	0.34	-2.79	0.0052	0.39	0.20 - 0.75	
	Hitunga	11	6	-0.33	0.46	-0.72	0.4700	0.72	0.30 - 1.76	
	Magoje	13	1	-2.66	1.02	-2.62	0.0087	0.07	0.01 - 0.51	
Number of PIM types (a)	Single	84	44	0				1		
	Mixed	123	43	-0.66	0.23	-2.86	0.0042	0.52	0.33 - 0.81	
Model 2										(LR = 33.6; df = 2; p <.0001)
Area	BH + HT	46	32	0				1		
	Others	161	55	-1.14	0.23	-4.93	<.0001	0.32	0.20-0.50	
Number of PIM types (a)	Single	84	44	0				1		
	Mixed	123	43	-0.7	0.23	-3.11	0.0019	0.5	0.32-0.77	

Interpretation of Table 7.6

Interpretation of the table is as explained in Table 7.4. Reference levels (or baseline groups) were as follows: Cattle from $Bunghaji_{(LTC)}$ infected with ≤ three PIM types (Model1; LR-test based step-up selection) or a single PIM type (Model 1; W-test based step-up selection); Cattle from $Bunghaji_{(LTC)}$ and $Hitunga_{(LTC)}$ infected with ≤ three PIM types (Model 2; LR-test based step-up selection) or a single PIM type (Model 2; W-test based step-up selection). Hence, the HR for villages or areas other than the reference is compared to the HR in the baseline group. Similarly, the HR in animals harbouring a greater number of parasite types is compared to the same baseline group.

7.3.7.4 Associations amongst Potentially Prognostic Variables of Infection Survival

With the inclusion of both infection multiplicity and area in the final survival analysis, neither cattle age nor the presence of any particular parasite type, were significant predictors of parasite's survival by PCR. The latter variables, however, were correlated with infection multiplicity.

Table 7.7 Distribution of *Theileria parva* parasite (PIM) types in single and mixed infections in 60 cattle with multiple PIM-positive observations.

Parasite Type	Positive Observations	Single	Mixed	OR	95% CI	$F_{1, 174}$	p-value
C	18	2	16	4.65	1.62 - 13.3	8.27	0.0050
D	50	5	45	7.49	2.99 - 18.7	18.8	<.0001
F	20	1	19	11.0	1.42 - 86.0	5.33	0.0220
G	84	14	70	4.10	2.00 - 8.41	15.1	0.0001
H	38	6	32	4.68	1.91 - 11.4	11.6	0.0010
I	86	19	67	2.51	1.30 - 4.84	7.66	0.0060
J	52	7	45	6.19	2.68 - 14.3	18.5	<.0001
K	40	4	36	7.24	2.85 - 18.4	17.5	<.0001
L	54	14	40	2.79	1.36 - 5.72	7.95	0.0050
N	39	5	34	6.41	2.61 - 15.7	16.7	0.0001
O	14	2	12	14.6	3.51 - 61.0	13.8	0.0003
A	1	0	1				
B	7	0	7				
E	14	0	14				
M	13	0	13				
P	7	1	6				

The analyses included 235 parasite-positive observations from 60 cattle with multiple PIM-positive observations. These showed a positive association between the parasite variant of interest and mixed infections in all cases. For example, if parasite type "H" was present, it was 4.7 times more probable that the infection contained co-infecting parasite types than that it contained the variant of interest only (See Odds Ratio [OR]). No analysis was conducted for parasite types A, B, E, M and P (Due to low numbers of positive observations), but visual inspection of the data suggested that a positive association between these and mixed infections also applied.

All parasite (PIM) types tested were more likely to infect cattle above the PCR detection threshold if at least a second parasite (PIM) variant was present in the host (Table 7.7). In addition, infections involving greater numbers of parasite types were significantly less prevalent amongst adult cattle (>24 months) than in cattle younger than 24 months of age (Table 7.8). Hence, exclusion of these terms from the final Cox models could be explained by correlations between these variables and infection multiplicity.

The classification of study villages with respect to *T. parva* survival was also correlated with the classification of study villages with respect to the number of parasite types present in parasite-positive cattle (i.e. infection multiplicity). For example, the number of infections involving more than three PIM alleles was greater, and not significantly different, in HTC villages and Magoje$_{(LTC)}$, but was lower in Bunghaji$_{(LTC)}$ and Hitunga$_{(LTC)}$. See Table 7.8.

Table 7.8 Chi-square and Fisher's exact tests showing associations between potentially prognostic variables for *Theileria parva* infection survival.

	Number of Parasite Types		OR	95% CI	Test of Association			
					Chisq			Fisher's
	≤3	>3			Chisq	df	p-value	p-value
Area								
HTC	104	44						
Magoje$_{[LTC]}$	9	4	1.05	0.31-3.59	-	1	-	1
HTC	104	44						
Bunghaji + Hitunga	44	2	0.11	0.03-0.46	12.9	1	<.0001	
HTC	104	44						
Bunghaji$_{[LTC]}$	33	2	0.14	0.33-0.62	8.68	1	0.003	
HTC	104	44						
Hitunga$_{[LTC]}$	11	0	0	-	-	1	-	0.035
Cattle Age								
Up to 24 months	101	40						
> 24 months	44	5	0.29	0.11-0.78		1	0.01	

The data analysed included the 207 initial infections, from the 207 cattle with PIM-positive observations, as considered in the Cox regression analyses. The figures in shaded boxes show cross-tabulations of the 207 initial infections for the conditions of interest. The results indicate a lower frequency of mixed infections involving > 3 parasite types in Bunghaji and Hitunga (LTC villages) compared to in HTC villages, and in adult cattle (>24 months of age) compared to in younger animals (See Odds Ratios [OR]). In Magoje, the frequency of mixed infections with > 3 parasite types was not different than in HTC villages. Age at initial PIM-positive observation was missing for 17 cattle.

A level of correlation between “Area” (or “Village”) and “Infection multiplicity” was also apparent upon inspection of the coefficients for one-variable Cox models (Table 7.4) as compared to the final Cox models (Table 7.6), or upon inspection of the survival curves obtained from individual (Figure 7.6) or final Cox models (Figure 7.7). For simplicity, the survival curves are only shown for “Area” and “Infection Multiplicity”. When variables are independent, model coefficients and survival curves do not vary depending on whether variables are considered independently or are considered simultaneously in a model (Parmar *et al.*, 1996b). However, coefficients and survival curves from final Cox models differed from those of initial one-variable models, indicating that a level of correlation existed between “Infection multiplicity” and either “Village” or “Area”. Despite these observations, neither variable (“Area / Village” or “Infection Multiplicity”) could be removed from the possible final Cox models. In addition, both terms appeared to have similar prognostic value with regards to the HR of *T. parva* infections.

In summary, patent infection by any parasite type (i.e. above detection threshold) appeared to be sustained for as long as co-infecting parasite types were also present. In contrast, where a single PIM allele or even two PIM alleles were present, infection became undetectable by PCR within 28 days (Figure 7.5 & Appendix 7.4). Inter-individual variations to this generality were apparent. For example, infection persistence of single allele infections beyond 28 days was recorded in four out of 207 cattle (2%), although in two of these animals patent infection lasted for only 56 days (two months). On the other hand, 17 out of 207 cattle (8%) which were infected with more than three PIM alleles at the time of the first PIM-positive observation cleared the infection within 28 days as detected by PCR (Figure 7.5 & Appendix 7.4). A second important prognostic variable of *T. parva* survival, was whether cattle were from Bunghaji$_{(LTC)}$ - Hitunga$_{(LTC)}$, or from HTC villages - Magoje$_{(LTC)}$.

Figure 7.6 Survival of *Theileria parva* infections in individual Cox models for two potentially prognostic variables – Parasite type multiplicity and area.

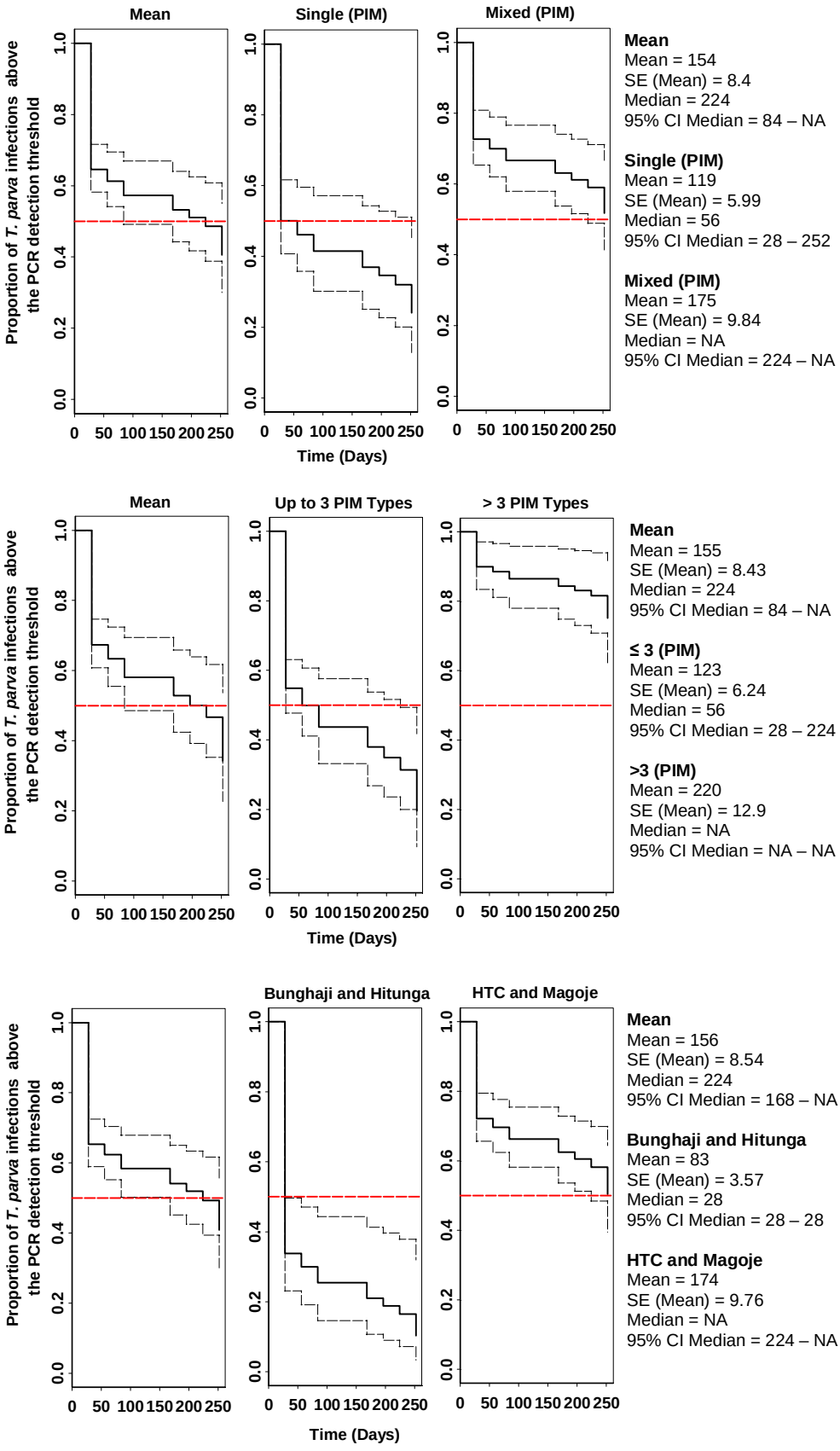
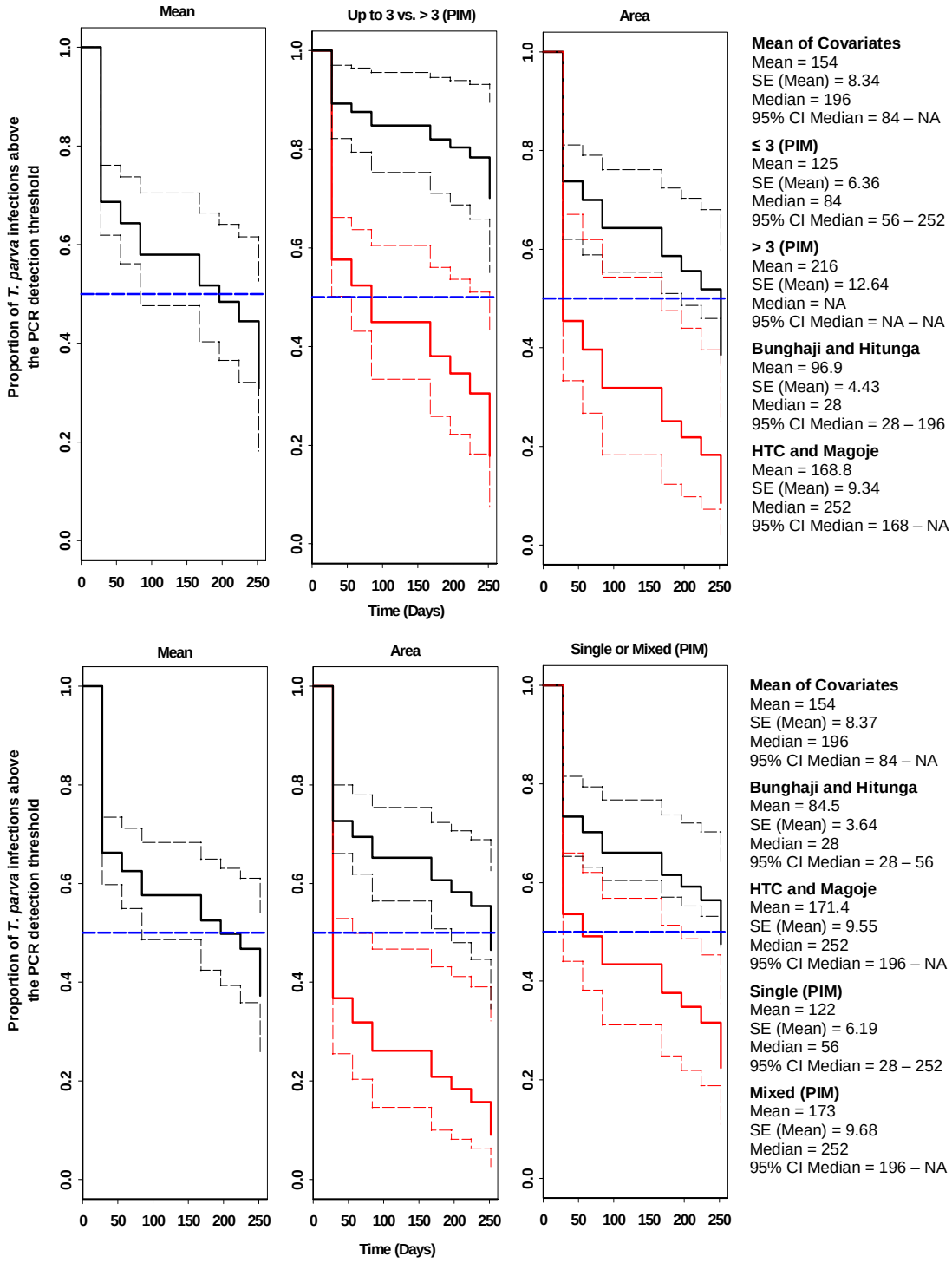


Figure 7.7 Survival of *Theileria parva* infections in two possible final Cox models for two prognostic variables - Parasite type multiplicity and area.



Interpretation of Figures 7.6 and 7.7

Survival functions are estimated in days, and are based on 207 cattle with PIM-positive observations. In each panel, the horizontal line indicates when 50% of infections were undetectable by PCR. The median of each survival function corresponds to the number of days at which the survival function crosses the median line. Broken lines show a point-wise 95% confidence envelope around each survival function. Note that "NA" indicates that the median survival time (and/or its 95% confidence interval) was longer than the maximum period of observation.

7.4 Discussion

7.4.1 Introduction

The analysis of *T. parva* infection dynamics was extended to consider the role of parasite antigenic diversity and pathogen survival in recently classified Enzootic, Epizootic and Epizootic-Sporadic Foci (Chapter 6). Polymorphisms in the PIM gene were used as a marker to distinguish parasite subpopulations and allow, to a certain extent, differentiation of persistent and incident infections. This provided a description of **(i)** the variation that exists regarding survival of natural infections across individuals and across herds within or outside the enzootic belt, **(ii)** the extent and epidemiological implications of co-infections by genetically distinct parasites due to continuous tick challenge and **(iii)** the predominant factors promoting infection persistence in the field. Consideration of parasite diversity could provide an explanation as to why some aspects of the parasite's epidemiology, which are influential in shaping the relationship between cattle age and patent infection, cannot be predicted from the level of tick challenge alone outside enzootic belts.

7.4.2 PIM Polymorphisms – Uses and Limitations

Diversity in PIM gene polymorphisms, which contrasts with the dimorphism of other microsphere-associated antigens (Skilton *et al.*, 1998, Geysen, 2000), has previously been used to subdivide field populations of *T. parva* into groups correlating with the concept of "strain" or "immunogenic type" (Geysen, 2000). Polymorphisms in PIM have been used in combination with other markers to characterise (Bishop *et al.*, 2001) and monitor (Oura *et al.*, 2004) the persistence of components of the "*Muguga cocktail*" in immunised and treated cattle and to measure the extent of *T. parva* diversity across Africa (Geysen *et al.*, 1999, Geysen, 2000, Geysen *et al.*, 2004).

The use of a single marker such as PIM, has clearly underestimated the extent of *T. parva* diversity in the study population. Parasite stocks, indistinguishable on the basis of PIM polymorphisms may be differentiated through sequencing, monoclonal antibodies, or by measuring diversity by means of other markers (Goddeeris *et al.*, 1990, Toye *et al.*, 1991, Morzaria *et al.*, 1995, Geysen *et al.*, 2004, Oura *et al.*, 2004). However, the use of a single marker has offered advantages in terms of expanding the knowledge on the field epidemiology of *T. parva* as compared to the limited insights offered by the species-specific PCR. Longitudinal monitoring of infected cattle to examine *T. parva* parasite subpopulations using PIM has improved our understanding of the nature and dynamics of persistent infections in the field. Longitudinal studies of this kind had been previously conducted to investigate the field epidemiology of human malaria parasites (Bruce *et al.*, 2000a, Bruce *et al.*, 2000b, Bruce *et al.*, 2000c, Owusu-Agyei *et al.*, 2002, Bruce *et al.*, 2003, Nassir *et al.*, 2005), but had never been applied to investigate the dynamics of natural *T. parva* infections.

7.4.3 Infection Survival and Piroplam Parasitaemia – Assumptions

Given that the population of lymphocytes is relatively small in the blood-stream, measurement of *T. parva* infection survival was based upon detection of piroplasm transmission stages. Piroplasms, undergo very little division in bovine erythrocytes (Conrad *et al.*, 1986, Fawcett *et al.*, 1987). Whether piroplasms can show differences in infection persistence above the detection threshold, or reflect how these may be shaped by the presence of other parasite variants in the host, may be dependent on the relationship that exists between these and the preceding replicating stages (shizonts).

Genetically distinct parasites of the same species may vary in fundamental aspects of their biology, including growth rates (Mackinnon *et al.*, 1999, Davies *et al.*, 2001), which ultimately determine infection intensities and impact on detection using available techniques. Growth rates differ across clones or isolates of *T. annulata* (Shiels *et al.*, 1992, Saravanan *et al.*, 2003, Taylor *et al.*, 2003), *Babesia bovis* (Nevils *et al.*, 2000) and *Plasmodium falciparum* (Chotivanich *et al.*, 2000) and *Plasmodium chabaudi* (Mackinnon *et al.*, 1999), *Trypanosoma brucei gambiense* (Diffley *et al.*, 1987), *Schistosoma mansoni* (Davies *et al.*, 2001) and *Plasmodium yoelli* (Knowles *et al.*, 1980) in vivo.

Genetically distinct parasites of the same species can also vary in their ability to differentiate into transmission stages when infecting a host. This has been demonstrated for *T. annulata* (Shiels *et al.*, 1992, Taylor *et al.*, 2003) and *P. falciparum* (Graves *et al.*, 1984) in vitro, and for *P. chabaudi* (Mackinnon *et al.*, 1999) and *S. mansoni* (Davies *et al.*, 2001) in vivo. Despite limited empirical evidence, a general assumption for parasites in general (Bremermann *et al.*, 1983, Bull, 1994, Frank, 1996) and for malaria parasites in particular (Hellriegel, 1992, Gupta *et al.*, 1996b), is that generation of transmission stages is correlated with within host replication rates of preceding stages. Hence, *T. annulata* clones presenting with faster schizont growth rates generally exhibit higher differentiation rates (Taylor *et al.*, 2003). A positive association has also been found across clones of *P. chabaudi* (Mackinnon *et al.*, 1999) and *S. mansoni* in mice (Davies *et al.*, 2001), and a across a wider range of infectious agents (Lipsitch *et al.*, 1997), although exceptions to this rule can occur (Shiels *et al.*, 1992, Lipsitch *et al.*, 1997, Taylor *et al.*, 2003).

In *T. parva*, the mechanisms that induce the shizont to undergo differentiation to produce merozoites, which then invade erythrocytes and transform into transmission stages (i.e. piroplasms), are unknown. Moreover, the relationship between schizont growth rates and differentiation rates, and how these may be shaped by host-pathogen interactions, is obscure. Despite these limitations, based on the bulk of research that there exists for other apicomplexans, it was hypothesised that detection of piroplasms would allow discrimination of parasites which differ in their ability to induce persistent infections. PCR detection of piroplasm DNA has previously shown that *T. parva* parasites as distinguished by PIM gene polymorphisms, can induce infections which differ substantially in their survival time above the detection threshold (Oura *et al.*, 2004). Based on findings with other apicomplexans, it is possible that parasite types exhibiting higher schizont growth rates, and attaining higher schizont infection intensities for longer time periods, might be capable of producing higher numbers of piroplasms over a longer period of time. For these parasites, piroplasms may be sustained above the PCR detection threshold for a longer interval of time. Conversely, in the absence of shizonts, or after a reduction of schizont parasitaemias as a result of host immune responses, piroplasmosis may eventually drop below detection threshold with a lag of time which is virtually unknown.

7.4.4 Multiple *T. parva* infections in the Field

PIM gene polymorphisms allowed discrimination of 16 parasite subpopulations circulating in the study areas, although a clear predominance of certain parasite types was observed. This may be representative of rapid expansion of one or more parasite variants (epidemic structure) reported in studies which have examined the genetic structure of *T. parva* parasites in Uganda, as detected using a wide range of neutral micro- and mini-satellite markers (Oura *et al.*, 2005).

Infection with multiple sub-populations of *T. parva* parasites, were observed in over 60% of the blood samples processed. This is consistent with observations in many infectious disease systems that hosts are infected with more than one genotype of the same pathogen (Lord *et al.*, 1999, Thompson, 2000, Read *et al.*, 2001), a fact particularly well documented for human malaria (Babiker *et al.*, 1999, Konate *et al.*, 1999, Magesa *et al.*, 2002) and in other Theileria species such as *T. annulata* (Miled *et al.*, 1994). High prevalence of multiple *T. parva* infections have been reported in Uganda of between 36% to 82% depending on the cattle age - despite regular acaricide application or vaccination with the "*Muguga cocktail*" (Oura *et al.*, 2004, Oura *et al.*, 2005).

7.4.5 The Role of "Parasite Type" in Mixed Infection's Survival

Parasite types, defined by their PIM polymorphisms differed in their ability to induce persistent infections - some parasite variants enhanced *T. parva* survival and infection persistence when present in the infection, while others did not. However when infection multiplicity was taken into account, the survival of *Theileria parva* at levels detectable by PCR could not be expressed as a function of the presence of a particular parasite variant. *T. parva* parasites are known to differ in their ability to induce persistent piroplasmosis above detection thresholds (Dolan, 1986, Young *et al.*, 1986, Bishop *et al.*, 1992, Kariuki *et al.*, 1995, Skilton *et al.*, 2002), also when distinguished on the basis of PIM gene polymorphisms (Oura *et al.*, 2004). In this study, all parasite types distinguished on the basis of PIM were more likely to infect the bovine host in combination with other PIM variants rather than alone.

Little is known about how competitive interactions of parasites within hosts affect the persistence and subsequent transmission of individual parasite types in mixed infections (Taylor *et al.*, 1997a, 1997b, Hood, 2003, de Roode *et al.*, 2005a). Multiple infections have been examined mathematically (Levin *et al.*, 1981, Bremermann *et al.*, 1983, Nowak *et al.*, 1994, May *et al.*, 1995, Van Baalen *et al.*, 1995, Frank, 1996, Mosquera *et al.*, 1998, Adler *et al.*, 2002, Nowak *et al.*, 2002), but the empirical understanding of mixed infections in hosts is hampered by limited experimental data (Read *et al.*, 2001, de Roode *et al.*, 2005a). Several recent studies have been undertaken in *T. annulata in vivo* or *in vitro* to unravel how parasites' growth rates, densities and differentiation rates are shaped in mixed as compared to in single clone infection (Ben Miled, 1993, Melrose, 1994, Graham *et al.*, 2001, Taylor *et al.*, 2002, Taylor *et al.*, 2005). This is also true in the case of the rodent malaria parasite *P. chabaudi in vivo* (Taylor *et al.*, 1997a, 1997b, Taylor *et al.*, 1998a, Taylor *et al.*, 1998b, Cheesman *et al.*, 2003, De Roode *et al.*, 2003, de Roode *et al.*, 2004a, de Roode *et al.*, 2004b, de Roode *et al.*, 2005a, de Roode *et al.*, 2005b). In mixed-genotype infections, patterns of asexual replication and transmission are expected to be different from those in single-genotype infections as a result of within host competition (Snounou *et al.*, 1992, Bull, 1994, Taylor *et al.*, 1997a, Hastings *et al.*, 2000, Kew *et al.*, 2002, de Roode *et al.*, 2004a, de Roode *et al.*, 2005a). Evidence suggests that competitive success of competing clones cannot be easily predicted from their growth rates in single infections (Nakamura *et al.*, 1992, Hodgson *et al.*, 2004) and that host genotype (Wille *et al.*, 2002, de Roode *et al.*, 2004b) and residency (i.e. previous history of exposure to genetically homologous or heterologous parasites) are also crucial determinants of within-host competition (Orcutt *et al.*, 1973, Morrison *et al.*, 1982, Duval-Iflash *et al.*, 1983, Allaker *et al.*, 1988, Barrow *et al.*, 2000, Ishii *et al.*, 2002, Hood, 2003, Thomas *et al.*, 2003). In this study, 60% of analysed samples harboured mixed *T. parva* infections and each bovine host may have presented a different history of exposure to parasite variants and a different composition of parasites in the monitored infections. Coupled with the fact that host genotype is a crucial determinant of infection outcome, this may explain why in natural infections, no particular parasite variant may be regarded as dominant in terms of its predictive value of infection survival.

7.4.6Infection Survival and Cattle Age

Infection multiplicity (as determined through PIM polymorphism) decreased with cattle ageing predictably as a result of build-up of acquired immunity to *T. parva* (Radley *et al.*, 1975a). This observation contrasts with data reported by other authors who, using a panel of micro- and minisatellite markers, have observed higher infection multiplicity in adult cattle as compared to in younger animals (Oura *et al.*, 2005). In that study, however, animals were regularly sprayed with acaricide, which will have delayed host-vector contacts. Since infection multiplicity is a crucial determinant of the establishment of persistent infections, decreased infection multiplicity in older age groups resulted in a significant reduction of infection survival above the detection threshold. Infection multiplicity and parasite densities have previously been shown to decrease with age in malaria patients from endemic areas (Bruce *et al.*, 2000b, Bendixen *et al.*, 2001). In these patients, infection survival above the detection thresholds was also found to decrease with age, correlating with the reduction of the number of parasite variants participating in the infection (Bruce *et al.*, 2000b).

7.4.7 Parasite Diversity in Enzootic, Recently Enzootic, Epizootic and Epizootic-Sporadic Areas

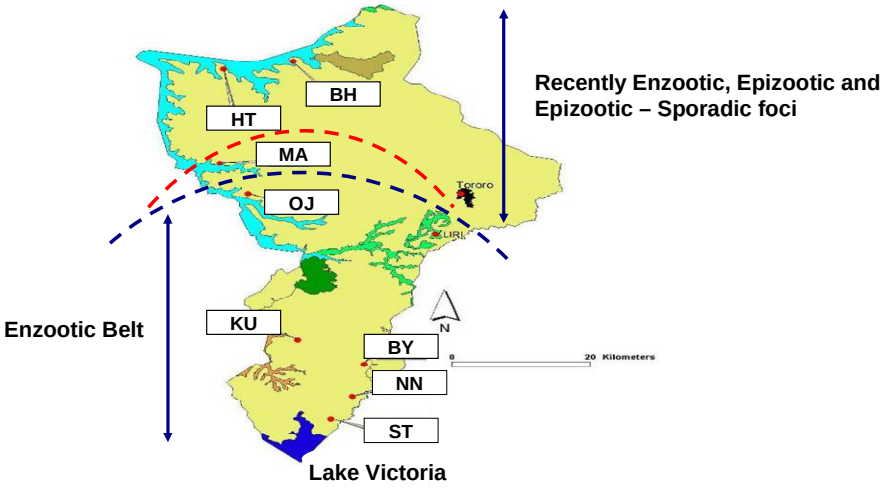
On the basis of the survival time of *T. parva* infections at levels above the PCR detection threshold, cattle could be classified into broad geographical groups which were not perfectly consistent with the level of *R. appendiculatus* challenge (Chapter 6). The duration of *T. parva* infections in cattle raised within the epizootic-sporadic area of Magoje, was not different to that in cattle raised within the enzootic belt. In contrast, *T. parva* infection survival in cattle from recently enzootic (Bunghaji) and epizootic (Hitunga) foci, was significantly lower as compared to in other areas in the study. These findings correlate with the classification of study areas with respect to the level of *T. parva* genetic diversity recorded from infected cattle, which was greater, and not significantly different, in the epizootic-sporadic area of Magoje and within the enzootic belt. Different levels of *T. parva* diversity have previously been reported across territories in Africa. High levels of diversity have been described in Kenya (Conrad *et al.*, 1987b, Allsopp *et al.*, 1988, Baylis *et al.*, 1991, Bishop *et al.*, 1997) and

Uganda (Oura *et al.*, 2005) while low levels of diversity have been reported in Zambia (Geysen *et al.*, 1999) and Zimbabwe (Bishop *et al.*, 1994). Unfortunately this information has not been described in the context of vector challenge and hence parasites transmission intensity.

In general terms, for parasites capable of sexual recombination within the vector, a positive relationship has been observed between transmission intensity and infection multiplicity (Bendixen *et al.*, 2001). Areas where more host-vector contacts are established, provide more opportunity for sexual recombination events to take place between circulating variants resulting in parasite systems which exhibit greater genetic diversity (Anderson *et al.*, 2000). The present study suggests, on the contrary, that infection multiplicity recorded from infected cattle in different areas may not always correlate with vector intensity, and hence host-vector contacts. These findings were based on very low numbers of *T. parva* infected cattle in the Magoje focus, and require validation through data collection in future field studies.

The epizootic-sporadic focus of Magoje is located only five miles from the enzootic belt compared to other non-enzootic foci (Bunghaji and Hitunga) which are approximately 20 miles further inland (Figure 7.8). The ticks found in Magoje may correspond to a recent extension of the tick population in the enzootic belt, harbouring a greater number of genetically distinct *T. parva* parasites. Advancing and retreating distribution of ticks coinciding with cyclical changes in climate suitability have been previously reported (Hoogstraal, 1956, Yeoman, 1966, Anon, 1974, Morzaria *et al.*, 1981, Chizyuka *et al.*, 1985, Julla, 1985, Tatchell *et al.*, 1986, Julla *et al.*, 1989, Norval *et al.*, 1990). Cattle movements from the enzootic belt may also have resulted in the introduction of ticks from this area into Magoje (Norval *et al.*, 1992). The recently enzootic (Bunghaji) and epizootic (Hitunga) foci, where infection multiplicity was lowest, may in contrast represent a separate *R. appendiculatus* population to that within the enzootic belt and Magoje. These foci may only occasionally be subject to the importation of a few infected animals, or animals carrying infected ticks, from other areas (either within the enzootic belt or others).

Figure 7.8 Hypothesised mechanism of generation of the ECF epizootic-sporadic focus (Yeoman, 1966) in Magoje



The black line shows the classification of villages according to *R. appendiculatus* challenge (Yeoman, 1966). The red line shows classification of villages according to multiplicity of infections and *T. parva* survival in cattle. It is hypothesised that Magoje may present epidemiological characteristics which are similar to those in the enzootic belt. This may come about through movement of ticks/cattle from the enzootic belt into Magoje.

7.4.8 Multiple Infection and Infection Survival

The survival of an infection at detectable levels is dependent on the number of antigenic variants participating in the infection. The number of parasite variants was observed to increase with the number of positive samples tested from infected animals, suggesting that super-infection was taking place. This was supported by serological analysis which showed multiple seroconversion events in some animals (Magona, 2004). The sequential detection of parasite types could also reflect oscillating parasitaemias (around the detection threshold) of parasite variants previously inoculated with the first infective tick bite (Dolan, 1986, Norval *et al.*, 1992, Skilton *et al.*, 2002). Patent infection by any *T. parva* parasite type was sustained for as long as co-infecting types were also present. In contrast, where a single PIM allele or even when only two PIM alleles were detected, chronic infections were not sustained, and parasites were cleared within 28 days (as measured by PCR). Persistence of a single allele infection beyond 28 days was only recorded in 2% of infected cattle (4 out of 207). In two of the four animals the patent infection lasted only 56 days. Similarly, only 8% of infected cattle (17 out of 207) which harboured more than three PIM alleles at the time of the first infective tick bite, were able to

clear the infection within 28 days as detected by PCR. Variation among animals has been reported previously (Oura *et al.*, 2004), and is consistent with the specificity of *T. parva*-specific CTL responses being determined by factors associated not only with the parasites (antigenic variability), but also with the hosts (MHC polymorphisms) (Goddeeris *et al.*, 1986, Taracha *et al.*, 1995a, Taracha *et al.*, 1995b, Morrison, 1996, McKeever *et al.*, 2002).

The majority of experiments investigating the dynamics of multiple or single infections with parasite variants of the same species have been conducted for the rodent malaria parasite *Plasmodium chabaudi*. These experiments have shown that the relative frequency of clones, as well as the overall density of asexual (schizont) stages, can vary dramatically over the course of an infection as a result of within host competition (Taylor *et al.*, 1997b, Taylor *et al.*, 1998b), and that host genotype appears to play a crucial role in the outcome of such competition (de Roode *et al.*, 2004b). Suppressive competition resulting in lower asexual (schizonts) and transmission (gametocytes) stages in mixed as compared to in single infections, appears to be restricted to the acute phase of infection (De Roode *et al.*, 2003). During the chronic phase of infection, however, mixed-clone infections sustain higher parasite densities (Taylor *et al.*, 1997a, Taylor *et al.*, 1998a, De Roode *et al.*, 2003) characterised by the production of more frequent and higher peaks of asexual parasites (De Roode *et al.*, 2003). Peaks of asexual stages recorded during the acute and chronic phases are in turn correlated with peaks of gametocytes (De Roode *et al.*, 2003). In consequence, transmission stages have been reported to be higher in mixed than in single -clone infections during the chronic phase (Taylor *et al.*, 1997a, Taylor *et al.*, 1998a, De Roode *et al.*, 2003), an observation which also appears to apply in *T. annulata* infections (Taylor *et al.*, 2002). In addition, in the later stages, mixed *P. chabaudi* infections have been found to produce over seven times as many oocysts as the sum of the corresponding single clone infections in the mosquito vector (Taylor *et al.*, 1997a). Gametocytaemia and the clonal composition of the oocyst infection in mosquitoes, reflect the size and composition of the asexual infection on that day (Taylor *et al.*, 1997a, Taylor *et al.*, 1998b, de Roode *et al.*, 2005b). These observations are strongly compatible with field and theoretical results reported in this thesis (See Chapter 8), whereby infection multiplicity enhances persistence of parasitaemias above the detection threshold.

The implications of mixed infections in transmission are in debate. It has been suggested that mixed clone infections present a transmission advantage since larger densities of asexual and transmission stages are sustained over the course of the chronic phase of infection (Taylor *et al.*, 1997a, Taylor *et al.*, 1998a, Taylor *et al.*, 1998b). It has also been argued that single clone infections are more successful in transmission terms because, in the absence of within host competition, they produce higher gametocyte densities as compared to mixed infections during the acute phase of infection (De Roode *et al.*, 2003). Given that, at least in enzootic foci, carrier animals appear to play a more important role in *T. parva* transmission than animals experiencing acute infection (Medley *et al.*, 1993), it is proposed here that genetically diverse infections present an advantage for *T. parva* transmission over single infections.

The importance of multiplicity of parasite strains in the survival and transmission of natural infections has been highlighted for human malaria in eastern Sudan, where the disease is characterised by limited seasonal transmission, with most of the year being transmission free. Monitoring of *P. falciparum* patients during the transmission-free season has suggested that infection multiplicity influences the longevity of asexual infection and its gametocyte production, and that transmission stages from mixed infections persist three times longer than those from single genotype infections (Nassir *et al.*, 2005). The inhabitants who contract genetically diverse infections during the transmission season retain long-lasting infections, and are then thought to initiate malaria transmission the following year (Nassir *et al.*, 2005).

Higher infection multiplicity in African Cape Buffalo may also explain its efficiency in sustaining carrier-state and *T. parva* transmission to ticks as compared to domestic bovines. Carrier buffalo are thought to be up to 100 times more efficient in producing infected ticks than carrier cattle infected with cattle-derived *T. parva* (Young *et al.*, 1985, Young *et al.*, 1986, Grootenhuis *et al.*, 1987, Maritim *et al.*, 1989a). In turn, the studies which have examined *T. parva* stocks directly isolated from African Cape Buffalo (Conrad *et al.*, 1987a, Conrad *et al.*, 1989, Bishop *et al.*, 1994, Toye *et al.*, 1995b, Nene *et al.*, 1996, Collins *et al.*, 1999, Nene *et al.*, 1999, Geysen *et al.*, 2004), suggest that buffalo-derived

parasites are generally much more diverse at the molecular level than the cattle-derived parasites (Bishop *et al.*, 2002).

7.4.9 Area and Infection Survival

Although a positive correlation existed between the survival of infections and the multiplicity of genetically distinct parasites in cattle from different study villages, the final analyses showed that infection persistence was a function of both "geographical origin" and "infection multiplicity". These results suggest that factors were at play other than infection multiplicity, which were characteristic from each area, and which equally played a significant role in determining infection persistence. The richness of the parasite pool and the relative proportion of cattle infected with any of the 16 PIM *T. parva* types varied significantly across villages considered in this study. In the absence of other factors explaining inter-geographical variation, each ECF focus was taken to represent a unique diversity matrix of *T. parva* parasites as distinguished by PIM gene polymorphisms, and possibly, host genotypes. Although the use of a single marker is inadequate to derive conclusions about the precise composition of genetically distinct parasites in each area, this observation is supported by previous studies reporting genetic sub-structuring of *T. parva* parasite populations across Uganda, where little cattle movement is assumed to have taken place (Oura *et al.*, 2005). In summary, *T. parva* infection survival could be expressed as a function of infection multiplicity and combination of parasite types circulating in infected cattle, in agreement with observations that the latter varies significantly across areas in the study.

7.4.10 Summary

Under field conditions where mixed infections are frequent, the survival of *T. parva* at levels detectable by PCR is not a function of the presence of a particular parasite variant, although parasite types as distinguished by PIM gene polymorphisms do differ in their ability to promote persistent infections. Instead, survival can be expressed as a function of number and combination of parasites present in the hosts, which may in turn be determined by the bovine genotypes. The hypotheses derived from the field observations are explored further in Chapter 8 using mathematical modelling. Mixed infections

persist for longer and longevity increases further with increasing numbers of co-infective parasites. However, parasites' multiplicity of infections decreases with age, correlating with an increase of the hazard ratio of patent parasitaemias by transmission stages. Notably, *T. parva* antigenic diversity in infected animals may not always correlate with vector intensity. Outside enzootic belts, a crucial determinant of the parasite's epidemiology and transmission may reside in the mechanism by which recently enzootic, epizootic or epizootic-sporadic foci are generated, as this may determine the extent of *T. parva* genetic diversity, and possibly the combination of parasites, circulating in the area.

Chapter 8: Theoretical Models for *Theileria parva* Infection Survival - Role of Multiplicity and Combination of Parasite Types

8.1 Introduction

8.1.1 Infection Dynamics of Genetically Diverse Pathogen Systems

Mathematical models of genetically complex pathogen systems, have proven useful in generating ideas on the dynamics of infection by pathogens other than *T. parva* at the single host level (Antia *et al.*, 1996, Frank, 1999, Bruce *et al.*, 2003, Lello *et al.*, 2004, Recker *et al.*, 2004) or at the population level (Nowak *et al.*, 1994, Haraguchi *et al.*, 1997, Recker *et al.*, 2005). These studies have invariably considered that, particularly in the case of protozoans, pathogen-to-pathogen interactions are ruled primarily by the hosts' immune system. Interactions between genetically distinct pathogens of the same species via direct interference - such as in the case of bacteria (Riley *et al.*, 1999) and viruses (Hart *et al.*, 1990), which produce interference molecules that actively modulate competitors - have not been conclusively demonstrated in protozoan parasites (Taylor *et al.*, 2005). However, parasite genotypes co-existing in the same host are expected to interact by competing for limited resources (Read *et al.*, 2001, Taylor *et al.*, 2005) and by strain-transcending immunity (Read *et al.*, 2001); within-host interactions will occur unless clone-specific host responses dominate and regulate pathogens at levels where resources are not limiting (Read *et al.*, 2001).

Studies of the infection dynamics of genetically diverse pathogen systems have mainly focused attention upon the effects of cross-immunity generated from recovery from infection with one antigenic pathogen variant, which acts to inhibit infection with another (Adler *et al.*, 1991, Gupta *et al.*, 1994a, Gupta *et al.*, 1996a, Andreasen *et al.*, 1997, Gupta *et al.*, 1998). Immune cross-reactivity, where enhancement of the immune response to one parasite type by another results in negative associations between the pathogen variants is widely reported, particularly for malaria (Marsh *et al.*, 1986, Giha *et al.*, 2000, Bull *et al.*, 2002). This is mediated by immunological responses to antigens shared by all pathogen variants. However, immune-mediated facilitation of infection can also occur. There is

evidence that both humoral and cellular immune-mediated mechanisms, can result in positive (cooperative) interactions amongst genetically distinct pathogens of the same species in terms of infection survival and transmission. For example, cross-reactive antibodies generated by a previous exposure to a heterologous strain can facilitate, rather than restrict, the within host replication and transmission of a second invading strain in some pathogen systems. 'Antibody dependent enhancement (ADE)', has been described for dengue (Halstead *et al.*, 1977a, Halstead *et al.*, 1977b, Kliks *et al.*, 1988, Ferguson *et al.*, 1999), HIV (Robinson *et al.*, 1988, Takeda *et al.*, 1988), a variety of flaviviruses and others (Peiris *et al.*, 1981, Porterfield, 1986). Evidence of cellular immune-mediated mechanisms of infection facilitation has been found in a number of cases. Studies of cytotoxic T-cell (CTL) responses to polymorphic antigens have shown that some naturally occurring variants of these antigens may specifically down-regulate the CTL response targeted against others through altered peptide ligand (APL) antagonism. APL antagonism has been described in *Plasmodium falciparum* (Gilbert *et al.*, 1998), hepatitis C and B viruses (Bertoletti *et al.*, 1994) and HIV (Klenerman *et al.*, 1994). In *P. falciparum* it has been proposed that antigen variants that are capable of APL antagonism (e.g. cp26 and cp29) are naturally selected in contemporaneous infections. This is because the use of an antagonist is a more efficient method of infection persistence (and immune evasion) than avoidance of human leukocyte antigen (HLA) binding (which causes class I restricted CTL attack), through antigenic diversity alone (Gilbert *et al.*, 1998). This hypothesis is supported by observations that strains containing antigen cp26 co-exist at a much greater prevalence than expected with strains containing antigen cp29 in infected patients from Gambia (Gilbert *et al.*, 1998). Because of the advantages of the APL antagonism, it has been proposed that such a strategy might be extended to other pathogens where cellular immunity plays a role (Williams, 1998).

8.1.2 Specific Objectives

Previous results have shown that infection multiplicity and infection duration in *T. parva* positive animals differs significantly across geographical areas, in a manner which is not completely concordant with the classification of villages according to the challenge of *R. appendiculatus* (Chapter 7). Field results also showed that *T. parva* infection survival can be expressed as a function of the number of

PIM types present in the infection and geographical area. The latter was interpreted as being representative of the combination of PIM types (and / or host genotypes) present in that location.

The objectives of this chapter are threefold. Firstly, to investigate whether the distribution of any *T. parva* parasite type as distinguished by PIM polymorphisms, is independent from that of other parasite types or, on the contrary, systematic positive and/or negative associations amongst parasite types occur in the field. Secondly, to investigate whether interactions amongst genetically distinct *T. parva* parasites sharing a host can explain preliminary field observations that 'infection multiplicity' and 'combination of parasite types' determine infection duration, at least at levels above the detection threshold of the available techniques. Finally, to test the hypothesis that *T. parva* infection survival is indeed a function of these parameters, rather than a function of infection with a particular parasite type.

To address the second and third objectives a generic mathematical model of infection within a single host is constructed to explore the dynamics of infection when assuming independence of parasite types compared to when assuming a range of interactions amongst co-infecting parasites. Although this work does not attempt to make any assumptions on the mechanisms that underlie the establishment of interactions, the model achieves pathogen-to-pathogen interactions via the host's immune system in agreement with the bulk of research that there exists for pathogens and protozoans in particular (Section 8.1.1). The work aims at exploring the consequences rather than the causes of intra-pathogen species interactions.

8.2 Methods

8.2.1 Analysis of Associations amongst *T. parva* Parasite (PIM) Types

(Methodology for the Analysis Presented in Table 8.2)

Generalised linear mixed-effects models (GlmmPQL; Section 3.4.5) with binomial error structure, were used to test the null hypothesis that *T. parva* parasite types as distinguished by PIM gene polymorphisms do not exhibit patterns of association. Only parasite-positive cattle with repeated PIM-positive observations were included in the analyses (n = 60); 147 out of 207 PIM-positive cattle were excluded due to absence of PIM-positive repeated measures. In the analyses, the presence or

absence of each parasite type was modelled as a function of the presence or absence of other *T. parva* (PIM) genotypes. A separate model was fitted for each PIM type as follows:

Maximal model: 'y ~ I + G + D + L + J + K + H + N + C + F + O + E + M + P + B'

Where: 'y' was a binary dependent variable with values '0' or '1' depending on the absence or presence of the PIM type of interest in the test blood sample. The independent variables (each representing a PIM type other than that specified as the dependent variable 'y'), were two-level categorical variables indicating presence or absence of types 'I' to 'B' in same test sample.

The independent variables were arranged in the model by decreasing order of cumulative prevalence amongst the 60 cattle considered in the analyses. Where the cumulative prevalence of parasite types was equal, the type with higher cumulative prevalence amongst all 207 PIM-positive cattle in the study was fitted first. No analysis was conducted to examine the parasite type interactions affecting the distributions of types 'A', 'B', 'E', 'M', 'O' and 'P'. This was because of the small number of samples that tested positive to any of these types in the subset data frame of 60 cattle with follow-up PIM positive observations.

Minimum models containing only significant main terms were achieved through a process of step-down simplification of the maximal models. See Section 3.4.5. In all cases, parasite type associations were modelled by considering separate intercepts for each animal in the study, except for the case of parasite type 'F', which was modelled by considering different intercepts and time slopes for each individual animal. "Time" was included in the random-effects as an integer-valued variable with value "zero" at the time of the first PIM-positive observation. The inter-visit time length observed from the data was respected.

For robustness, the analysis of parasite associations was repeated by accounting for the geographical origin of animals in the study (i.e. 'Village') and/or the age of cattle at each round of observation.

8.2.2 Modelling of the Effects of Interactions amongst PIM Types

The generic model was loosely based on that constructed by Lello and co-workers (Lello *et al.*, 2004).

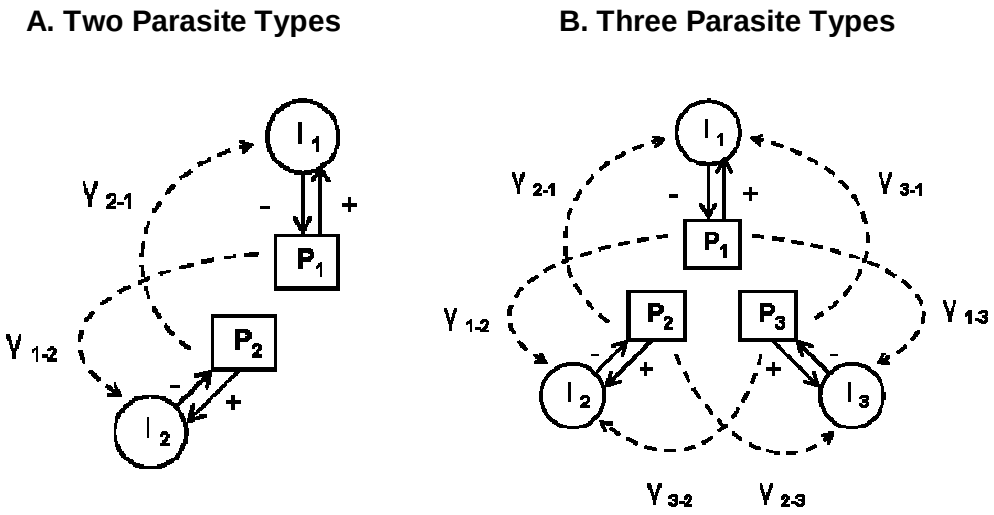
8.2.2.1 Model Assumptions

Model assumptions were as follows: (i) Parasite types interact through the immune system, (ii) Pathogen (parasite type) growth is exponential and (iii) The immune response is proportional to the density of the parasite type and the length of time that type has been present in the vertebrate host at levels detectable by the immune system.

8.2.2.2 Description of the Model

Schematic diagrams of the model are shown in Figure 8.1. The first diagram (A) shows a model of infection with two parasite types (P_1 & P_2) together with the immune responses for each parasite type (I_1 & I_2).

Figure 8.1 Schematic representation of the basic model with interactions between two or three parasite types.



P_1, P_2, P_3 = Parasite Types; I_1, I_2, I_3 = Immune response to each parasite type; $\gamma_{1\text{-}2}$, $\gamma_{2\text{-}1}$, $\gamma_{1\text{-}3}$, $\gamma_{3\text{-}1}$, $\gamma_{2\text{-}3}$, $\gamma_{3\text{-}2}$ = Parasite Interactions. Interactions are shown with dotted arrows. The immune response is proportional to the density of the parasite type and the length of time that type has been present in the vertebrate host at levels detectable by the immune system. Plus and minus signs, indicate that a parasite type stimulates the immune response, and that the immune response then decreases the density of that parasite type respectively.

Interactions are incorporated by allowing the density of one parasite type to modify the immune response to the other parasite type ($\gamma_{1\text{-}2}$ & $\gamma_{2\text{-}1}$). Each parasite type (e.g. P_1) modifies the immune response of another parasite type (e.g. I_2) at a rate proportional to the density of that parasite type (e.g. P_1). Interactions may be positive or negative but are always symmetric in that, for example, the

interaction from P_2 to I_1 is equal to that of the reciprocal interaction from P_1 to I_2. The second diagram (B) extends the simpler model to infection with three parasite types.

The model equations are presented in Figure 8.2 as a set of two difference equations and a memory function for each parasite type. The basic model describes the dynamics of a parasite of a particular type (P_j) infecting the host at time zero. This parasite type increases exponentially with a growth rate (λ), and is reduced by a mortality rate (μ). The parasite type stimulates a type specific immune response (I_j), which acts to decrease the density of that parasite variant by modification of the growth rate. This relation is described by equation 1.

The type specific immune response increases at a rate proportional to the density of that particular parasite type and is decreased by a decay rate (δ). The strength of the immune response is also modified by a memory function (M_j) which increases at a rate proportional to the length of time the parasite type has remained above a defined threshold (κ). The level of this memory function is maintained even if the parasite type falls below the threshold level. M_j acts to increase the strength of the immune response. See equations 2 & 3.

Each parasite type is governed by its own set of equations. In order to incorporate interactions (γ) between these parasite types, each pathogen is allowed to modify the immune response to another parasite type at a rate proportional to its own density. See equation 2. The relation is such that if the interaction value (γ) is positive, this will reduce the level of the immune response simulating a positive interaction for the influenced parasite type. Conversely, if the interaction value (γ) is negative, this will increase the level of the immune response simulating a negative interaction for the influenced parasite type.

Figure 8.2 Generic Model equations

Parasite type 1

$$P_1(t+1) = \lambda P\ (t)e^{-\beta I} - \mu P\ (t) \quad \textbf{(1)}$$

$$I_1(t+1) = (1+M\sigma)\alpha P\ (t) - \delta P\ (t) - \gamma P_3(t) - \gamma P_2(t) \quad \textbf{(2)}$$

$$M_1 = \sum_{t=0}^{n} P\ > \kappa \quad \textbf{(3)}$$

Parasite Type 2

$$P_2(t+1) = \lambda P\ (t)e^{-\beta I} - \mu P\ (t)$$

$$I_2(t+1) = (1+M\sigma)\alpha P(t) - \delta P(t) - \gamma P_1(t) - \gamma P_3(t)$$

$$M_2 = \sum_{t=0}^{n} P\ > \kappa$$

Parasite Type 3

$$P_3(t+1) = \lambda P\ (t)e^{-\beta I} - \mu P\ (t)$$

$$I_3(t+1) = (1+M\sigma)\alpha P(t) - \delta P(t) - \gamma P_2(t) - \gamma P_1(t)$$

$$M_3 = \sum_{t=0}^{n} P > \kappa$$

A set of two difference equations and a memory function for each parasite type are shown. The description of model constants and variables is given at the foot of the figure.
NOTE: Constants and variables in each equation for P_j, I_j and M_j refer to values specific to that parasite type unless the subscripts indicate otherwise.

Constants

λ = Growth rate of parasite type; **β** = Impact of immune response; **μ** = Mortality rate of parasite type; **σ** = Immune memory strength; **α** = Stimulation rate of immune response; **δ** = Decay rate of immune response; **γ** = Interaction of immune response; **κ** = Immune response memory threshold

Variables

$\mathbf{P_j}$ = Intensity of infection with parasite type; $\mathbf{M_j}$ = Time infection intensity of a particular parasite type is above the immune response memory threshold (κ); $\mathbf{I_j}$ = Immune response to parasite type.

Model equations were provided by Andy Paul Cox (CTVM, Edinburgh, unpublished).

For simplicity only three values of interaction (γ) were used in the model (γ = + 0.3 for a positive interaction, γ = 0 for no interaction and γ = - 0.3 for a negative interaction) and interactions were always symmetric in nature. Symmetric interactions are identical in both directions (e.g. $P_1 \leftarrow\!\!\!- P_2$ = $P_2 \rightarrow P_1$). Values used for each of the constants in the model are shown below.

Table 8.1 Details of the symbol, description and values used for each of the constants in the generic model.

Symbol	Description	Values
λ	Growth rate of parasite type	1.8 (P_1), 2.0 (P_2), 2.2 (P_3), 2.1 (P_4)
β	Impact of immune response	1.5
μ	Mortality rate of parasite type	0.1
σ	Immune memory strength	0.1
α	Stimulation rate of immune response	2
δ	Decay rate of immune response	0.1
γ	Interaction term	- 0.3, 0, + 0.3
κ	Immune response detection threshold	0.155

8.2.2.3 Application of the Model

The output of the model was recorded for a number of different 'baseline' scenarios. Initially, to determine the behaviour without interactions, three scenarios were examined: 1/. A single parasite type with medium growth rate (Figure 8.3); 2/. Two parasite types with low and medium growth rates but still without any interactions (Figure 8.4); 3/. Three parasite types with low, medium and high growth rates and no interactions (Figure 8.5). The time-course of the model outputs for each of these cases was then recorded.

In order to investigate the effect of interactions the model examined five different scenarios: 1/. Two parasite types with low and medium growth rates and negative symmetric interactions (Figure 8.6); 2/. Three parasite types with low medium and high growth rates and negative symmetric interactions (Figure 8.7); 3/. Two parasite types with low and medium growth rates and positive symmetric interactions (Figure 8.8); 4/.Three parasite types with low medium and high growth rates and positive symmetric interactions (Figure 8.9); 5/. The three-pathogen model was also subjected to a mixture of positive and negative symmetric interactions (Figure 8.10).

8.2.2.4 Introduction of a Fourth Parasite Type: Super-Infection

Finally, in order to investigate the effects of a new parasite type being introduced into an existing system of interactions, a model was constructed containing three parasite types present from time zero and a fourth new type (P_4) introduced into the model half way through the time course (at time (t) = 100). P_4 presented an intermediate growth rate between that of P_2 and P_3 (Table 8.1). The nature of symmetric interaction terms amongst the four types was allowed to vary randomly as $\gamma = + 0.3$, $\gamma = 0$ or $\gamma = - 0.3$ for 100 iterations. After exploring of the behaviour of each model upon introduction of the fourth new parasite type (assuming in each case different values for the interaction components [γ_j]), the responses were found to fall into five broad categories, namely "chaotic-chaotic", "stable-stable", "chaotic-stable", "stable-chaotic" and "collapse". Chaotic systems are here regarded as those where equilibrium between host's immune response and parasites' growth rate has not yet been achieved, and infection intensity fluctuates around a theoretical threshold for parasite detection or transmission. Stable systems are in contrast referred to those where equilibrium has been reached, and infection intensity is maintained constant below the theoretical threshold for parasite detection or transmission. A system is said to collapse, when the host's immune response has a complete success or failure over controlling the parasites' growth rate, and where parasite types' infection intensities approach infinity (host's death) or zero (self-cure). Each of these terms was applied to the infection behaviour recorded before and after introduction of the fourth antigenic type (P_4) to produce the five descriptive terms listed above. The frequency of each of these outcome classifications was determined by randomly varying the interactions between the four types for 100 iterations and classifying each outcome.

8.3 Results

8.3.1 Associations amongst *T. parva* Parasite (PIM) Types

Table 8.2 presents a summary of the results obtained in the analysis of parasite type associations / interactions.

Table 8.2 Significant associations amongst *T. parva* parasite (PIM) types.

Parasite Type	N° Positives		Minimum Model	Model Estimates		F-value	p-value
	Cattle	Samples		OR	95% CI		
I	39	86	**J**	0.20	0.08 - 0.52	$F_{1, 172} = 10.9$	0.001
			H	0.08	0.03 - 0.26	$F_{1, 172} = 16.2$	0.0001
			E	4.45	1.12 - 17.7	$F_{1, 172} = 4.55$	0.034
G	32	84	**B**	0.09	0.01 - 0.82	$F_{1, 174} = 4.62$	0.033
D	28	50	J	7.14	2.62 - 19.4	$F_{1, 169} = 4.50$	0.035
			K	0.06	0.01 - 0.35	$F_{1, 169} = 10.3$	0.002
			H	0.04	0.01 - 0.17	$F_{1, 169} = 8.17$	0.005
			C	8.06	2.27 - 28.6	$F_{1, 169} = 8.33$	0.004
			O	8.23	2.11 - 32.2	$F_{1, 169} = 7.92$	0.006
			P	32.5	2.54 - 416	$F_{1, 169} = 7.27$	0.008
J	24	52	**I**	0.08	0.03 - 0.21	$F_{1, 170} = 17.9$	<.0001
			D	2.81	1.05 - 7.54	$F_{1, 170} = 4.94$	0.028
			N	5.07	1.79 - 14.4	$F_{1, 170} = 4.27$	0.040
			F	6.28	2.03 - 19.4	$F_{1, 170} = 9.47$	0.002
			M	8.09	2.34 - 27.9	$F_{1, 170} = 11.1$	0.001
K	23	40	**D**	0.33	0.11 - 0.99	$F_{1, 174} = 3.97$	0.048
H	21	38	**I**	0.05	0.03 - 0.08	$F_{1, 170} = 24.2$	<.0001
			D	0.04	0.01 - 0.19	$F_{1, 170} = 6.14$	0.014
			K	5.19	1.91 - 14.1	$F_{1, 170} = 5.23$	0.023
			F	10.7	3.11 - 36.5	$F_{1, 170} = 9.55$	0.002
			M	61.9	12.4 - 308	$F_{1, 170} = 25.8$	<.0001
N	17	39	**H**	0.17	0.05 - 0.62	$F_{1, 174} = 7.43$	0.007
C	14	18	D	3.24	1.23 - 8.48	$F_{1, 173} = 4.46$	0.036
			E	3.67	1.03 - 13.1	$F_{1, 173} = 4.07$	0.045
F	14	20	J	6.58	2.89 -15.0	$F_{1, 171} = 9.41$	0.003
			H	2.91	1.23 - 6.90	$F_{1, 171} = 9.86$	0.002
			C	4.37	1.80 - 10.6	$F_{1, 171} = 9.36$	0.003
			B	7.73	1.80 - 33.2	$F_{1, 171} = 7.65$	0.006

No analysis was conducted to examine the parasite type interactions affecting the distributions of types “A”, “B”, “E”, “M”, “O” and “P”. No parasite types were found to affect the distribution of type “L”, which was present in 54 samples from 28 animals.

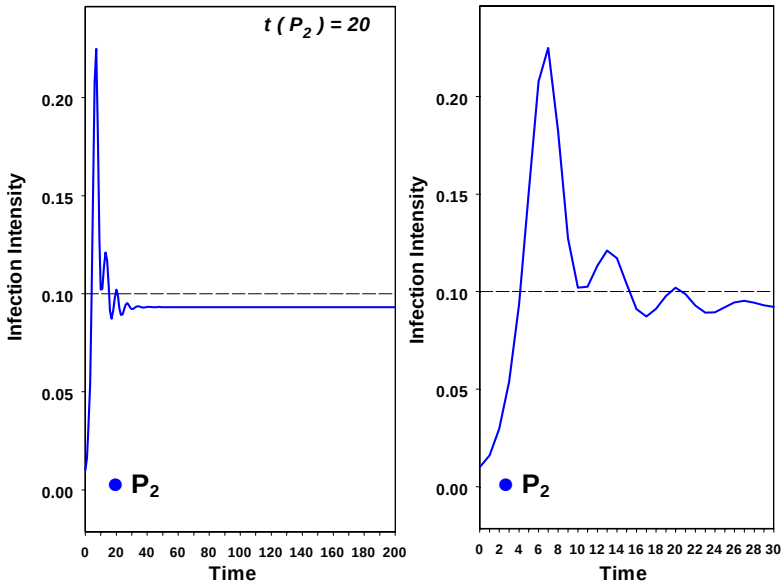
The "*Parasite Type*" column, lists the dependent variable specified in each model by decreasing order of cumulative prevalence amongst cattle considered in the analyses. These included 235 PIM positive samples from 60 cattle with initial and follow-up PIM positive observations. The significant terms in the reduced models are shown in the "*Minimum Model*" column. For example, the probability of parasite type 'I' patent infection as detectable by PCR, depends on whether types 'J', 'H' or 'E' are present in cattle blood. The extent to which the significant predictors affect the dependent variable, is given by the odds ratio (OR) calculated from the PQL model coefficients. Odds ratio values of less than one and of more than one indicate that the significant predictor reduces or increases the probability of patent infection with the parasite type of interest respectively. The former case (OR<1), reflects a "negative interaction or association" between the types considered, whereas the later (OR>1) reflects a "positive interaction or association" between the types. In the table, the parasite types that have a negative effect on the dependent variable are underlined.

Parallel analyses showed that even after accounting for the distribution of parasite types across villages and across cattle age groups (Section 8.2.1) PIM types exhibited patterns of association (Data not shown).

8.3.2 Dynamics of Infection with a Single *T. parva* Parasite Type

The single parasite type infection model (Figure 8.3), shows a rapid stabilisation of infection intensity below a theoretical threshold for parasite detection that is represented in the panels by a dotted horizontal line. The last opportunity for parasite detection occurs at time (t) = 20, when the infection intensity is last recorded as equal to or greater than 0.10. During the course of 'patent' infection, where equilibrium between the host's immune response and the parasite's growth rate has not yet been reached, the infection intensity is characterised by cyclical fluctuations around the limit of detection. P_2 represents a parasite type with intermediate growth rate as compared to parasite types P_1 (slower growth rate) and P_3 (faster growth rate), which are presented in subsequent figures.

Figure 8.3 Model output representing the dynamics of infection for a single *T. parva* parasite type.

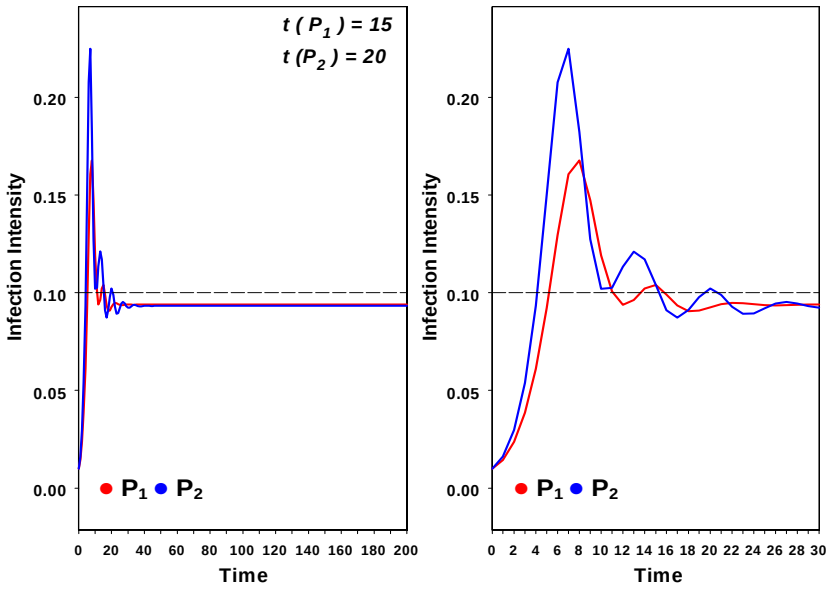


The left panel shows the long term dynamics of infection, limited to time = 200. The right panel shows a snap-shot of the infection limited to time = 30.

8.3.3 Dynamics of Infection with Two *T. Parva* Parasite Types and No Interactions

The mixed parasite type infection model with no interactions between types (Figure 8.4) shows a rapid stabilisation of both P_1 and P_2 infection intensities below a theoretical threshold for parasite detection. See dotted horizontal line. The model represents a hypothetical scenario where infection with P_1 and P_2 occurs at the same time point (t = 0) and where P_1 presents with a slower growth rate as compared to P_2. In the absence of interactions between the two parasite types, the last opportunity for P_2 detection remains unaltered with respect to the single parasite type infection model (Figure 8.3; t = 20). In addition, the infection intensity is last recorded as equal to or greater than 0.10 at an earlier time (t = 15) in the case of the parasite type which exhibits slower growth rate (P_1). During the course of 'patent' infection, where equilibrium has not yet been reached, P_1 and P_2 infection intensities are characterised by cyclical fluctuations around the limit of detection. Higher infection intensity peaks are associated with the parasite type exhibiting faster growth rate (P_2).

Figure 8.4 Model output representing the dynamics of infection for two *T. parva* parasite types with no interactions.

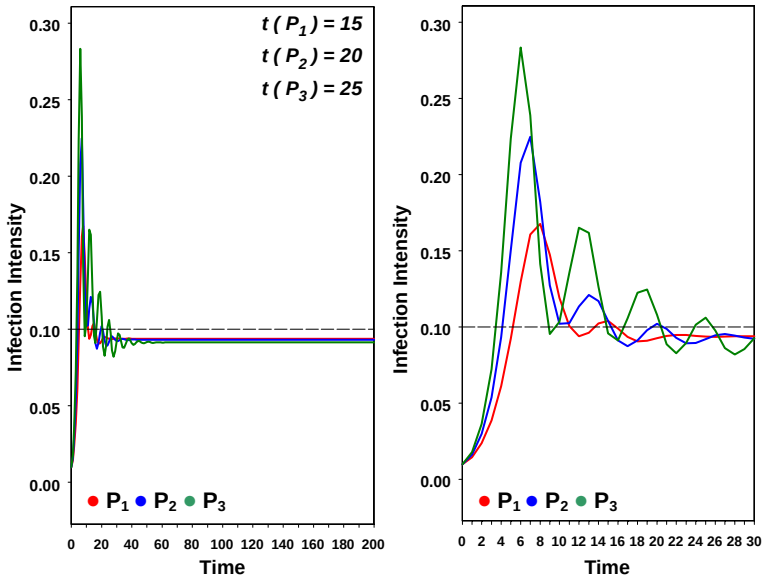


The left panel shows the long term dynamics of infection, limited to time = 200. The right panel shows a snap-shot of the infection limited to time = 30.

8.3.4 Dynamics of Infection with Three *T. Parva* Parasite Types and No Interactions

The mixed parasite type infection model assuming no interactions amongst types (Figure 8.5) shows a rapid stabilisation of P_1, P_2 and P_3 infection intensities below a theoretical threshold for parasite detection. See dotted horizontal line. The model represents a hypothetical scenario where infection with P_1, P_2 and P_3 occurs at t = 0, but where P_1 presents with a slower growth rate as compared to P_2 while P_2 presents with a slower growth rate as compared to P_3. In the absence of interactions amongst parasite types, the addition of P_3 in the model has no effect on the survival time of P_1 and P_2 infection intensities at levels above the detection threshold. See Figures 8.3 and 8.4. P_1 and P_2 are last detected at t = 15 and t = 20 respectively, which represent short lengths of 'patent' infection. The infection intensity of the parasite type which exhibits faster growth rate, P_3, is last recorded as equal to or greater than 0.10 at a comparatively later time (t = 25). During the course of 'patent' infection, where equilibrium has not yet been reached, P_1, P_2 and P_3 infection intensities are characterised by cyclical fluctuations around the limit of detection.

Figure 8.5 Model output representing the dynamics of infection for three *T. parva* parasite types with no interactions.



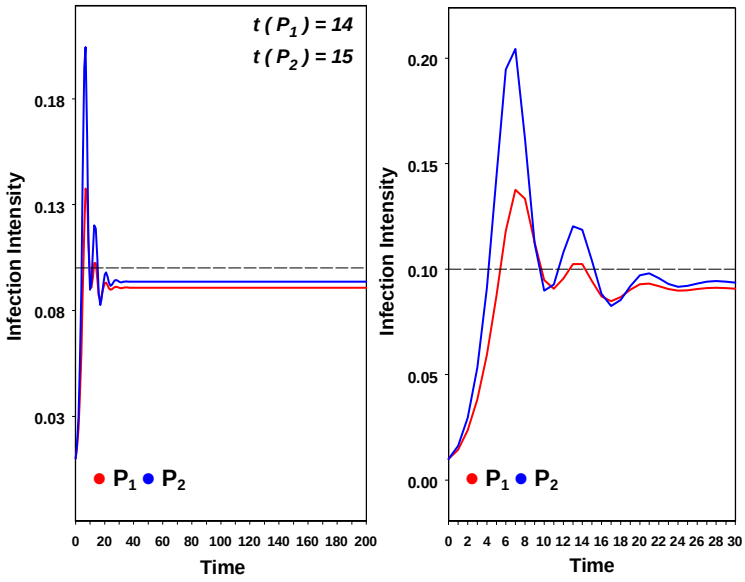
The left panel shows the long term dynamics of infection, limited to time = 200. The right panel shows a snap-shot of the infection limited to time = 30.

The model re-emphasizes that (i) higher infection intensity peaks are associated with parasite types exhibiting faster growth rates ($P_3 > P_2 > P_1$) and that (ii) the time length of 'patent' infection in the absence of interactions is short, and exclusively dependent on parasites' growth rate. The number of parasite types has no effect on the duration of the infection above the detection threshold.

8.3.5 Dynamics of Infection with Two *T. Parva* Parasite Types and Symmetric Negative Interactions

The mixed parasite type infection model with symmetric negative interactions between two types (Figure 8.6), shows that the infection intensities of P_1 and P_2 stabilise below the theoretical detection threshold at a faster rate as compared to the mixed parasite type infection model with no interactions. Under the assumption of negative symmetric interactions, the survival time above the theoretical detection threshold decreases from t =15 to t = 14 in the case of P_1 and from t = 20 to t = 15 in the case of P_2. See previous figures. Negative interactions have a greater effect on the parasite that exhibits faster growth rate (P_2).

Figure 8.6 Model output representing the dynamics of infection for two *T. parva* parasite types with symmetric negative interactions.



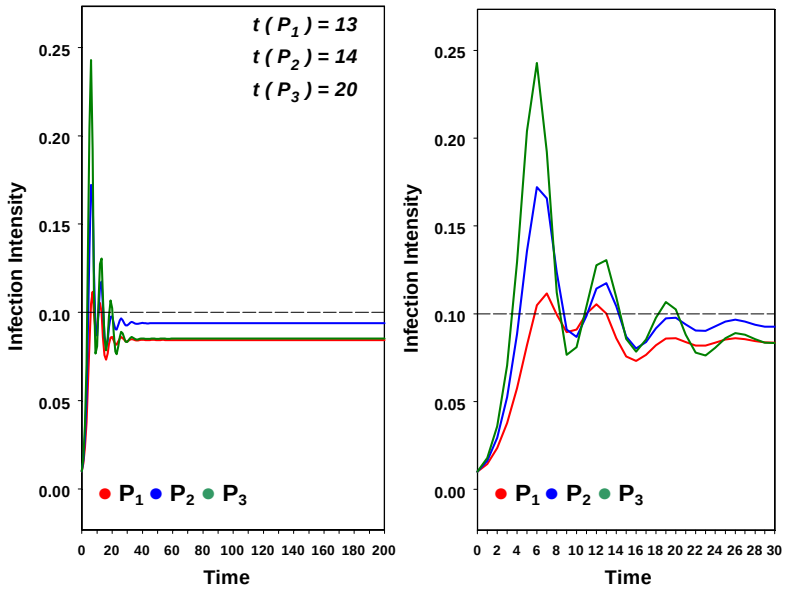
The left panel shows the long term dynamics of infection, limited to time = 200. The right panel shows a snap-shot of the infection limited to time = 30. The model represents a hypothetical scenario where infection with P_1 and P_2 occurs at t = 0 and where P_1 presents with a slower growth rate than P_2.

During the course of 'patent' infection, peaks of infection intensities for P_1 and P_2 occur synchronously. Higher infection intensity peaks are associated with the parasite type exhibiting faster growth rate, P_2. The mixed parasite type infection model with symmetric negative interactions, shows a case where interactions are not advantageous to the parasites' survival and may result in a greater chance of self-cure.

8.3.6 Dynamics of Infection with Three *T. parva* Parasite Types and Symmetric Negative Interactions

The mixed parasite type infection model with symmetric negative interactions amongst three types (Figure 8.7), shows that the infection intensities of P_1, P_2 and P_3 stabilise below the theoretical detection threshold at a faster rate than in the model of infection with only two types and symmetric negative interactions. Under the assumption of negative symmetric interactions involving three parasite types, the survival time above the theoretical detection threshold decreases further from t = 14 to t = 13 in the case of P_1, and from t = 15 to t = 14 in the case of P_2. See Figure 8.6 for comparison.

Figure 8.7 Model output representing the dynamics of infection for three *T. parva* parasite types with symmetric negative interactions.



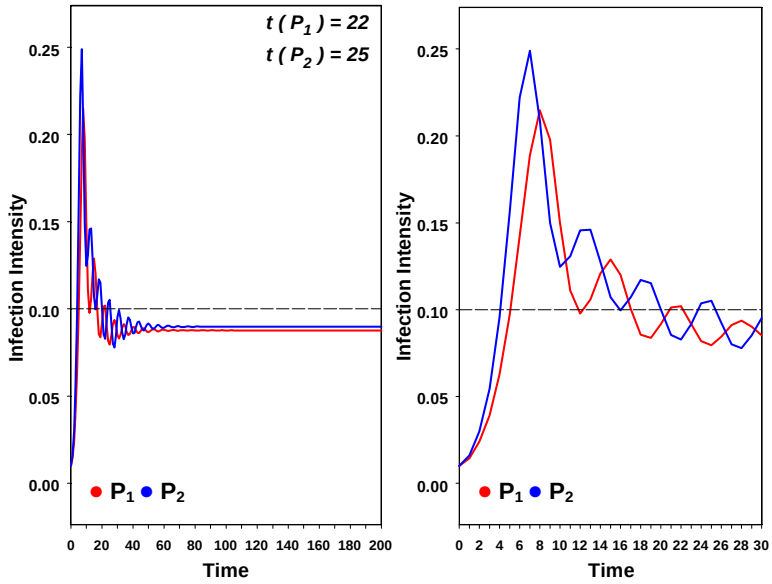
The left panel shows the long term dynamics of infection, limited to time = 200. The right panel shows a snap-shot of the infection limited to time = 30. The model represents a hypothetical scenario where infection with P_1, P_2 and P_3 occurs at t = 0 and where the growth rate of $P_1 < P_2 < P_3$.

The survival time of P_3 decreases from t =25 (when co-infecting the host with P_1 and P_2 and no interactions are assumed; Figure 8.5) to t = 20. During the course of 'patent' infection, peaks of infection intensities occur synchronously. Higher infection intensity peaks are associated with the parasite types exhibiting faster growth rates ($P_3 > P_2 > P_1$). The example of interactions amongst three rather than two parasite types, shows that the number of co-infecting types has an effect on infection survival for as long as these are not independent. The greater the number of parasite types with negative interactions, the shorter the time length of 'patent' infection and the greater chance of self-cure.

8.3.7 Dynamics of Infection with Two *T. parva* Parasite Types and Symmetric Positive Interactions

The mixed parasite type infection model with symmetric positive interactions between two types (Figure 8.8), shows that the infection intensities of P_1 and P_2 stabilise below the theoretical detection threshold at a slower rate as compared to the mixed parasite type infection model with no interactions.

Figure 8.8 Model output representing the dynamics of infection for two *T. parva* parasite types with symmetric positive interactions.



The left panel shows the long term dynamics of infection, limited to time = 200. The right panel shows a snap-shot of the infection limited to time = 30. The model represents a hypothetical scenario where infection with P_1 and P_2 occurs at t = 0 and where P_1 presents with a slower growth rate than P_2.

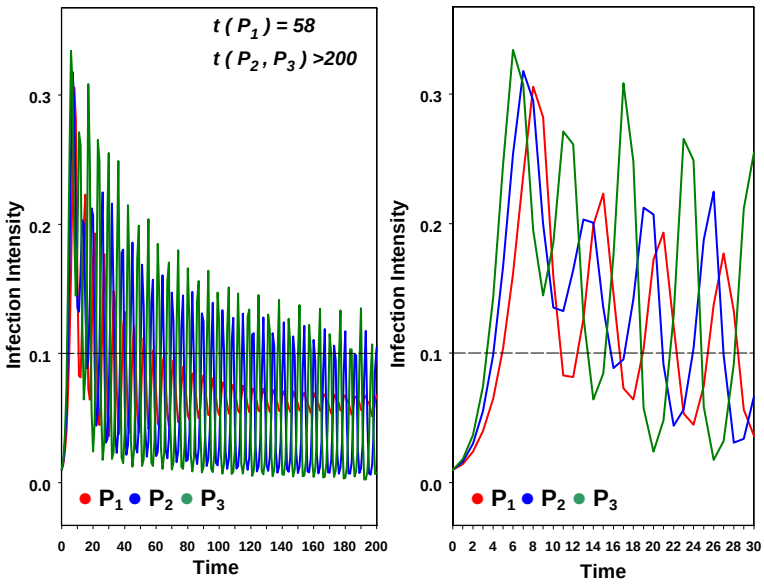
Under the assumption of a positive symmetric interaction, the survival time above the theoretical detection threshold increases from t =15 to t = 22 in the case of P_1 and from t = 20 to t = 25 in the case of P_2. See Figure 8.4. Positive interactions have a greater effect on the parasite that exhibits slower growth rate (P_1). During the course of 'patent' infection, infection intensities for P_1 and P_2 consistently peak at different times. Higher infection intensity peaks are associated with the parasite type exhibiting faster growth rate, P_2. The mixed parasite type infection model with symmetric positive interactions, shows a case where interactions are advantageous to the parasites' survival and may result in a greater chance of chronic infection.

8.3.8 Dynamics of Infection with Three *T. parva* Parasite Types and Symmetric Positive Interactions

The mixed parasite type infection model with symmetric positive interactions amongst three types (Figure 8.9), shows that the infection intensities of P_1, P_2 and P_3 stabilise below the theoretical

detection threshold at a slower rate as compared to the model of infection with only two types and symmetric positive interactions.

Figure 8.9 Model output representing the dynamics of infection for three *T. parva* parasite types with symmetric positive interactions.



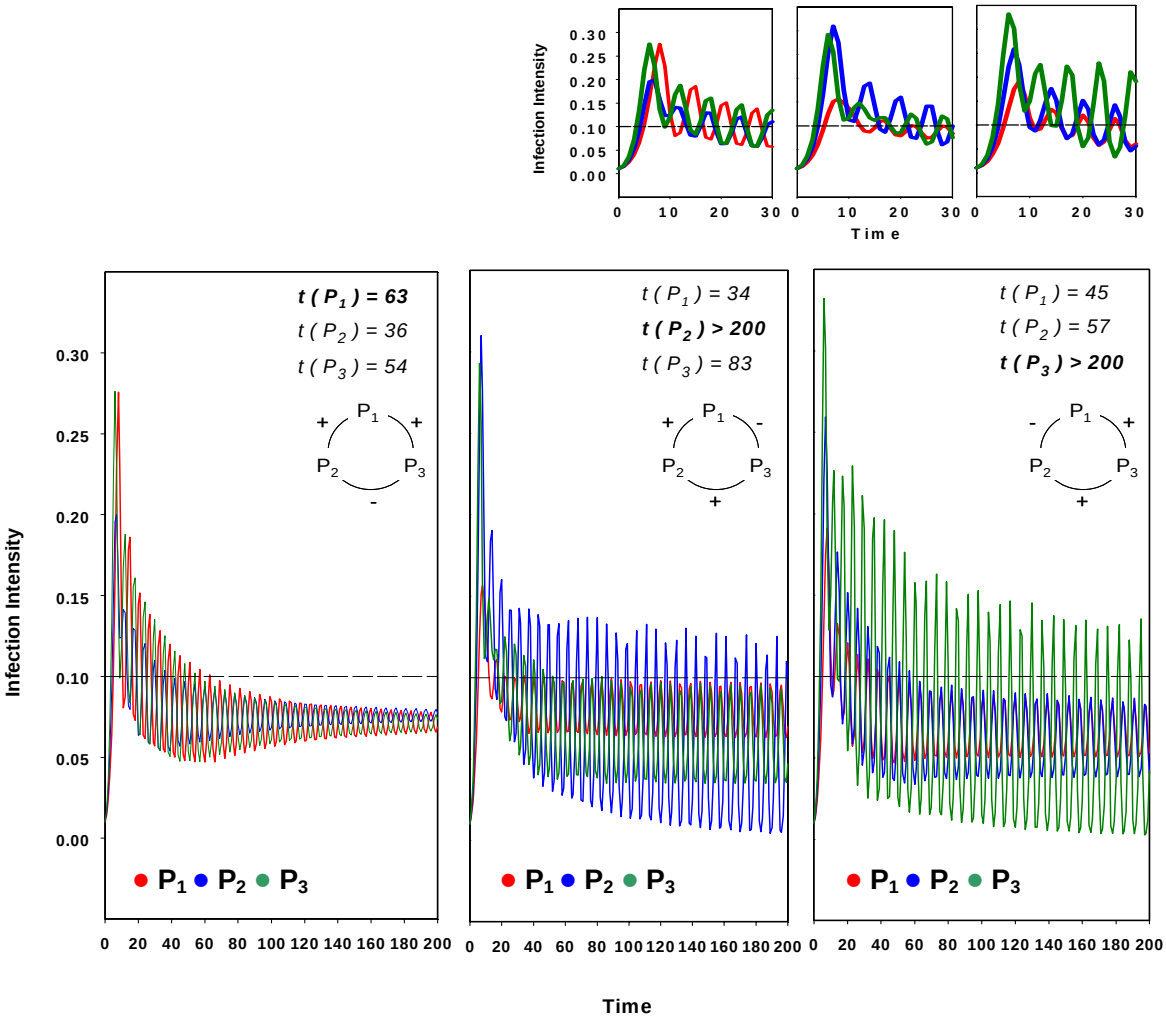
The left panel shows the long term dynamics of infection, limited to time = 200. The right panel shows a snap-shot of the infection limited to time = 30. The model represents a hypothetical scenario where infection with P_1, P_2 and P_3 occurs at t = 0 and where the growth rate of $P_1 < P_2 < P_3$.

Under the assumption of positive symmetric interactions involving three parasite types, the survival time above the theoretical detection threshold increases further from t = 22 to t = 58 in the case of P_1, and from t = 25 to t > 200 in the case of P_2. See Figure 8.8 for comparison. The survival time of P_3 increases from t =25 (when co-infecting the host with P_1 and P_2 and no interactions are assumed; Figure 8.5) to t >200. During the course of 'patent' infection, infection intensities for P_1, P_2 and P_3 consistently peak at different times. Higher infection intensity peaks are associated with the parasite types exhibiting faster growth rates ($P_3 > P_2 > P_1$). The example of interactions amongst three rather than two parasite types, shows that the number of co-infecting types has an effect on infection survival for as long as these are not independent. The greater the number of parasite types with greater interactions, the longer the time length of 'patent' infection and the greater chance of chronic infection.

8.3.9 Dynamics of Infection with Three *T. parva* Parasite Types and Various Symmetric Positive and Negative Interactions

Figure 8.10 shows three mixed parasite type infection models with two positive and one negative symmetric interaction.

Figure 8.10 Model outputs representing the dynamics of infection for three *T. parva* parasite types with both symmetric positive and negative interactions.



The larger panels show the long term dynamics of infection, limited to time = 200. The smaller panels show a snap-shot of the dynamics of infection limited to time = 30. Plus and minus signs represent positive and negative symmetric interactions respectively. The models represent hypothetical infection scenarios where infection with P_1, P_2 and P_3 occurs at t = 0 and where the growth rate of $P_1 < P_2 < P_3$.

The diagram in each panel shows the network of interactions for each infection model. The models show that, despite the inclusion of a negative interaction, the infection intensities of P_1, P_2 and P_3

stabilise below the theoretical detection threshold at a slower rate than in the mixed infection model with no interactions. Depending on the interactions present, and regardless of the growth rate of the parasites, it is possible that either P_1, P_2 or P_3 present with the longest survival time of 'patent' infection. In these models, the survival of any parasite type depends on the network of interactions established with other parasites. Only in models where no interactions are assumed, the parasite type with the fastest growth rate correlates to that with the longest survival time of 'patent' infection (Figure 8.5).

8.3.10 Dynamics of Infection in Case of Super-Infection with a New *T. parva* Parasite Type

The classification of model outputs in super-infections with a fourth *T. parva* parasite type at time =100 is shown below.

Table 8.3 Classification of model outputs in super-infections with a fourth *T. parva* parasite type at time = 100.

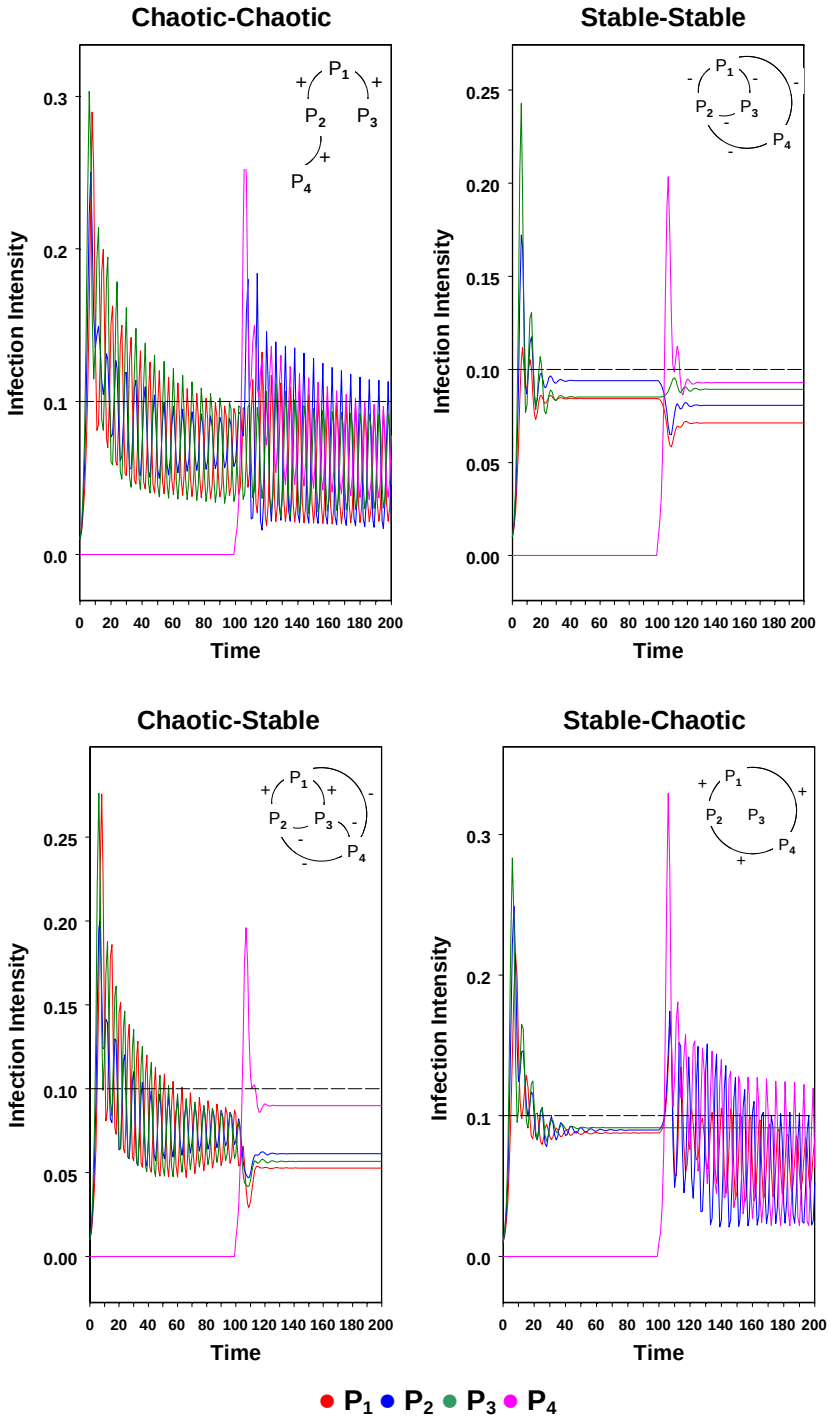
Output classification	%
Stable - Stable	45
Chaotic - Chaotic	20
Stable - Chaotic	16
Collapse	10
Chaotic - Stable	9

The frequency of each outcome classification was determined by randomly varying the nature of symmetric interaction terms amongst the four parasite types, as $\gamma = + 0.3$, $\gamma = 0$ or $\gamma = - 0.3$, for 100 iterations.

Figure 8.11 shows an example of "Chaotic-Chaotic", "Stable-Stable", "Chaotic-Stable" and "Stable-Chaotic" systems in the case of super-infection with a new parasite type (P_4). The diagram in each panel shows the network of interactions for each super-infection model.

Introduction of a new parasite type over the course of an established infection can allow previously recognised and stable parasite types to increase above the detection threshold or can result in the opposite effect. This depends on the network of interactions established. Super-infection can therefore result in markedly different effects making a stable system chaotic, a chaotic system stable or any degree of increase or decrease of the existing state.

Figure 8.11 Model outputs in super-infections with a fourth *T. parva* parasite type at time = 100.



Plus and minus signs represent positive and negative symmetric interactions respectively. Infection with P_1, P_2 and P_3 occurs at t = 0 and super-infection with P_4 takes place at t = 100. The growth rate of $P_1 < P_2 < P_4 < P_3$.

8.4 Discussion

8.4.1 Generic Model Outputs and their Relationship to Field Observations

This work has provided preliminary evidence for the existence of complex positive and negative associations amongst *T. parva* parasite types as distinguished by PIM gene polymorphisms. As suggested from field data (Chapter 7), a generic mathematical model assuming positive, negative or neutral interactions between distinct subpopulations of *T. parva* parasites co-existing in a host, has shown that it is possible that number and combination of parasite types (rather than infection with a particular type) are the key factors influencing *T. parva* survival. A pre-requisite, however, is that both competition (negative interactions) and facilitation (negative interactions) must occur amongst co-infecting parasites. Only under these assumptions can a generic model reproduce field observations that infection multiplicity and parasite combination, affect survival of infection above the detection threshold of the available techniques. Model outputs presented throughout this chapter are consistent with observations obtained from field data (Chapter 7).

The generic model has provided a number of important and consistent results. Single parasite type infections result in rapid stabilisation of infection intensity below a theoretical threshold for parasite detection (and/or transmission), and are characterised by a short length of the "patent" phase of infection. This observation is supported by analysis of field data, which suggests that single parasite type infections are unlikely to persist for longer than 28 days.

In mixed parasite type infections without cross-reactivity amongst parasite types, the duration of "patent" infection is independent of the number of parasite types present in the host and exclusively dependent on the growth rate of each parasite variant. The higher the growth rate of the parasite, the higher the infection intensity reached and the greater the duration of infection above a theoretical detection threshold. Growth patterns and survival times of parasite types remain unaltered with respect to single parasite-variant models, and hence the total duration of infection can be expressed as a function of infection with the parasite variant exhibiting greater growth rate. The duration of the "patent" infection phase in such cases is typically short. Independence of *T. parva* parasite types in this way is

not supported by the field data. This showed that parasite variants exhibit patterns of association and that the survival of mixed infections is not a function of infection with any particular parasite variant.

In mixed parasite type infections where cross-reactivity is assumed, the number of co-infecting types has an effect on infection survival which depends on the nature of the interactions. The model showed that the outcome of infection with parasite types exhibiting different types of interaction may be either detrimental or beneficial to the survival of a particular parasite type and therefore to transmission of the parasite. In the case where all of the interactions are negative, infection intensities stabilise below a theoretical threshold for parasite detection at a faster rate than is the case with no interactions present. Conversely, where all of the interactions are positive, infection intensities stabilise below a theoretical threshold at a slower rate than is the case with no interactions present and infection survival is increased. The greater the number of parasite types cross-reacting in a way which adds an advantage to parasite survival (i.e. positive interactions), the greater chance of chronic infection. Similarly, the more parasite types interacting in a way that does not benefit parasite survival (i.e. negative interactions), the greater chance of self-cure. Analysis of PIM data, however, suggests that positive (cooperative) associations are more common than negative (competitive) associations. Peaks of infection intensity for each parasite type may occur synchronously or may consistently peak at different times. Depending on the interactions present, variations in these patterns may occur. Model outputs assuming cross-reactivity amongst co-infecting parasite types are consistent with preliminary field observations that both number and combination of parasite types affect infection survival.

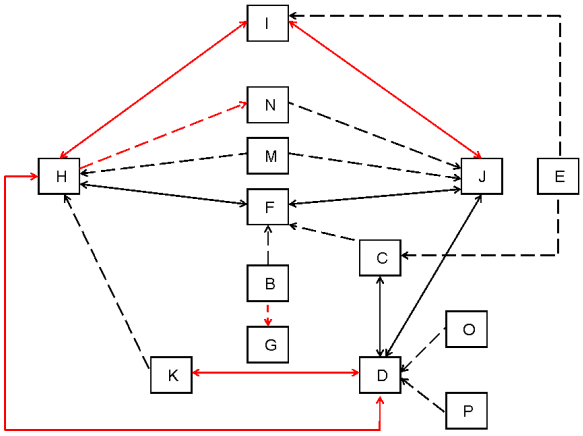
The survival time of a parasite type (above the theoretical threshold level), depends upon the network of interactions established with other parasite types, and the “perturbation” of the balance of the existing network of interactions via the introduction of new parasite types. The introduction of a new parasite type into an existing network of interactions can have markedly different effects, making a stable system chaotic, a chaotic system stable or any degree of increase or decrease of the existing state. Such an introduction may also cause the collapse of one or of all the other established types, allowing exponential increase of the parasite types (perhaps leading to death of the host) or eliminating the parasite types completely (self-cure). Super-infection with a new parasite type can also allow

previously established and stable parasite variants to increase above the threshold level. In models with cross-reactivity, the wide range of infection outcomes observed is consistent with the plasticity and variability of infections from animals with multiple *T. parva* variants in the field.

8.4.2 Associations amongst *T. parva* PIM Types

Re-examination of PIM data accounting for possible confounding variables during data analysis, has shown that parasite-type associations reported here cannot be explained by geographical variation in the distribution of PIM types, or by variation in the distribution of PIM types across cattle age groups.

Figure 8.12 Associations amongst *T. parva* parasite (PIM) types in cattle.



Symmetric and asymmetric interactions are represented with continuous and dashed lines respectively. Symmetric interactions occur in both directions [$P_x \leftarrow P_y = P_x \rightarrow P_y$]), whereas asymmetric interactions are not reciprocal. Negative and positive interactions are shown by means of red or black arrows respectively.

Positive associations cannot be explained either by the widespread distribution of certain parasite types in the study population. This is apparent from the fact that the PIM types involved in the greatest number of associations (i.e. types D, J, H & F) are not necessarily the most prevalent amongst cattle.

The analysis of parasite-type associations revealed three salient features from the data (Figure 8.12). Firstly, positive associations are more common than negative associations. Secondly, whilst most negative associations are symmetric in nature, most positive associations appear to be asymmetric. Thirdly, most *T. parva* PIM types involved in multiple associations exhibit a mixture of positive, negative, asymmetric and symmetric associations.

8.4.3 Mechanisms for Interactions amongst *T. parva* Parasite Types– Future work

The associations reported from the analysis of PIM data provide preliminary evidence that parasite types are not independent. Moreover, field and theoretical observations of the factors that determine *T. parva* infection survival, suggest that both competition (negative interactions) and facilitation (positive interactions; Lord *et al.*, 1999) amongst co-infecting parasites must occur. Observations that single–parasite type infections are relatively short lived, and that positive associations are more common than negative associations, suggest that the *T. parva* survival strategy may rely on the establishment of genetically complex infections where cooperative strategies are favoured.

The bulk of research indicates that, particularly for the case of protozoans, interactions amongst genetically distinct pathogens of the same species are mainly immune-mediated (Read *et al.*, 2001, Taylor *et al.*, 2005). See section 8.1. Cross-immunity generated from infection with one antigenic *T. parva* variant, which acts to inhibit infection with another, has been reported; cattle immunised with one field isolate of *T. parva* show some cross-protection against heterologous challenge (Radley *et al.*, 1975a, Radley *et al.*, 1975b, Radley *et al.*, 1975c, Uilenberg *et al.*, 1976) although protection is variable and limited (Morrison, 1996, McKeever *et al.*, 2002). At present, however, there is no evidence that mechanisms of facilitation amongst genetically distinct *T. parva* parasites (immune-mediated or not) can take place, and hence the mechanisms that enable immune evasion and carrier state remain obscure.

The most important immune response controlling infection with *T. parva* is cellular and mediated by class I MHC-restricted CD8+ cytotoxic T-cells targeted at the schizont stage (Emery *et al.*, 1981, Goddeeris *et al.*, 1986, Morrison *et al.*, 1987), although input is required from the CD4+ fraction (Taracha *et al.*, 1997). Infection and treatment of cattle with *T. parva* sporozoite stabilates has been used to induce protective immunity (Radley *et al.*, 1975a) and to analyse the nature of induced protection (Radley *et al.*, 1975a, Radley *et al.*, 1975b, Radley *et al.*, 1975c, Uilenberg *et al.*, 1976, Taracha *et al.*, 1995b). Cattle that have recovered from infection, whether naturally or from infection

and treatment, are thought to develop lifelong immunity to homologous challenge (Burridge *et al.*, 1972).

In *T. parva*, the efficiency and cross-reactivity of CTL responses appears to be mainly a function of the host's (MHC complex) phenotype (Goddeeris *et al.*, 1990), but there is evidence that in MHC identical animals *T. parva* parasite strains can influence the MHC restriction and therefore the specificity of CTL responses to *T. parva* (Reviewed by McKeever *et.al.*, 2002). As in the case of many viral (Ferrari *et al.*, 1991, Buseyne *et al.*, 1993, Chen *et al.*, 2000, Koelle *et al.*, 2003) and bacterial infections (Andersen *et al.*, 1992, Yewdell *et al.*, 1999), cellular immunity to *T. parva* schizonts possesses many of the characteristics of an immunodominant response (Taracha *et al.*, 1995a). In immunodominance there exists a hierarchy in dominance among different MHC – peptide combinations, and the CTL response is focused on the limited number of antigenic determinants that rank first in the hierarchy (Taracha *et al.*, 1995a). Effector CTL responses are capable of responding to antigens shared by two parasite populations (Taracha *et al.*, 1995b). However, the parasite strain specificity of bovine cytotoxic T-cell responses to *T. parva* is determined primarily by immunodominance, and results from the response being biased toward a limited number of immunodominant peptide-MHC determinants, which may or may not be shared by the genetically distinct parasite populations (Taracha *et al.*, 1995a, Taracha *et al.*, 1995b).

In general agreement with experimental and field data, it has been proposed that genetically distinct *T. parva* parasites may possess a mosaic structure of differing quantities of unique and overlapping epitopes (Morrison, 1996, McKeever *et al.*, 1998). If such were the case, a hypothesis that deserves further investigation is that the immunodominant features of the cellular response itself, bring about facilitation of the survival of one parasite variant by another (i.e. positive interaction). The presence of a parasite variant carrying immunodominant epitope/s may release, at least partially, the immune pressure exerted upon a second parasite variant presenting with no (currently) immunodominant epitope/s. Because parasite variants sharing no immunodominant epitopes would be advantageous to infection survival, a pathogen system presenting with this characteristic could be favoured. This hypothesis is compatible with the limited 'negative' cross-reactivity reported in *T. parva* (Morrison,

1996; McKeever *et al*., 2002), and with observations from this chapter that positive associations are 2.3 times more widespread than negative associations. Similarly, if genetically distinct parasites presented with shared immunodominant epitope/s, then the CTL response targeted against one variant, would reduce the chances of infection progression with the other. As expected, analysis of PIM data suggested that most negative associations are symmetric in nature, whilst somewhat surprisingly most positive associations are asymmetric in nature. The latter finding supports the hypothesis that immunodominance may explain facilitation of infection survival amongst parasite types. For example, if infection with parasite variant "A" resulted in MHC – peptide combination/s capable of inducing a variant-specific immunodominant CTL response, then infection with "A" could increase the chances of patent infection with a genetically distinct variant "B" not containing the immunodominant epitope/s, as a result of the CTL response being primarily biased towards "A". In contrast, parasite type "B" would have little or no effect on the chances of survival of parasite "A". Finally, the analysis of PIM data showed that a single parasite type may establish complex positive, negative, symmetric and asymmetric associations with several other genetically distinct parasites, which is compatible with the hypothesis that genetically distinct parasites may possess a mosaic structure of differing quantities of unique and overlapping immunodominant epitopes.

Preliminary inspection of a model that assumes that CTL response is primarily focused upon a limited number of immunodominant peptide-MHC determinants, and that *T. parva* parasite types possess varying degrees of shared and unique epitopes, suggests that immunodominance alone is capable of reproducing the time-series observations derived from field data and generic model outputs (Data not shown). Remarkably, given this set of assumptions infection duration and intensity correlate positively with the multiplicity of infection, and the diversity of epitopes amongst parasite types has a more important influence on infection survival than the rate of challenge with genetically distinct parasites. Infection survival of a genetically complex infection, and of each of its individual components, is equally dependent on the combination of parasite variants present, as variable compositions of unique and shared immunodominant epitopes amongst the parasite types, bring about variable positive and negative interaction networks that can facilitate or hinder infection progression.

8.4.4 Summary

For pathogens whose transmission relies on a '*Hit and Stay'* strategy the mechanisms that promote carrier state are generally multi-factorial (Huang *et al.*, 1970, Mims *et al.*, 1984, Ahmed *et al.*, 1990, Holland, 1990, Oldstone, 1991, Johnson *et al.*, 2002, Hilleman, 2004), but a common denominator for all pathogens is that mechanisms of within-host survival must somehow involve evasion of the host's immune system (Hilleman, 2004, Gupta, 2005). Generic model outputs support preliminary field observations that survival of *T. parva* can be expressed as a function of the 'number and combination' of parasites types present in the infection, rather than as a function of infection with a particular parasite variant. However, a requisite is that both competition (negative interactions) and facilitation (positive interaction) amongst co-infecting parasites must occur. Although mechanisms of within-host survival have not yet been characterised for *T. parva*, both theoretical and field observations further suggest that chronic infections may rely upon the establishment of genetically complex infections by parasite variants that result in facilitation of within-host replication of other co-infecting variants. In this respect, the question of whether immunodominance wholly of partially underlies the mechanism promoting immune evasion and parasites survival, is worthy of further investigation.

Chapter 9: General Discussion

9.1 Introduction

This study has provided preliminary evidence that the pathogen community as a whole (an inter-species level) is capable of shaping the probability of infection by - and possibly transmission of - any individual pathogen. In addition, the whole pathogen community determines the pathogenicity of its individual components and the incidence of clinical cases in the herd. From the animal health point of view, single-pathogen infections are relatively unimportant to zebu herds. Instead, the onset and severity of clinical signs follows a linear positive relationship with the number of pathogens co-infecting the host, which results in the vast majority of clinical cases being associated with multiple, rather than single, pathogen infections. There is, however, a hierarchy in the relative importance of each individual pathogen in the community. Such a hierarchy depends on the pathogens virulence and, more importantly, on the ability of each pathogen to interact with others in a manner detrimental to the health of the host, or beneficial to the transmission of the pathogen(s). In this context, the widespread Anaplasma spp. infections appear to play a paramount role in the community. Anaplasma spp. influences the infection probability of the widest number of endemic pathogens, and is the organism that most exacerbates the pathogenicity of mixed infections. This is not only because of its higher pathogenicity (as measured by haemoglobin depletion), but also because of its ability to interact with co-circulating pathogens.

This work has also shown that a greater understanding of the interactions present amongst genetically distinct parasites of *Theileria parva* (an intra-species level), can be used to better explain epidemiological features which were at present, poorly understood. Observations from this work suggest that acquired immunity strongly shapes the probability of *T. parva* infection over time. However, the rate at which effective immunity against circulating parasites is attained at the herd level (as apparent from the drop in parasite-positive cattle) varies across areas in a manner that is not wholly concordant with geographical variation in tick challenge. This is because the number of positive observations recorded longitudinally from infected cattle is influential in shaping the relationship

between parasite's prevalence and time, but the former is the one parameter that may not follow a linear relationship with the gradient of tick challenge recorded from the study sites. In consequence, the analysis of *T. parva* infection dynamics was extended to consider the role of parasite antigenic diversity and pathogen survival in recently classified Enzootic, Epizootic and Epizootic-Sporadic Foci. The results suggest that under field conditions where mixed infections are the norm, the survival of *T. parva* at levels detectable by PCR is a function of 'number and combination' of parasites present in the hosts. Moreover, although parasite types do differ in their ability to promote persistent infections, infection survival is not a function of the presence of a particular parasite variant. Mixed infections persist for longer and longevity increases further with increasing numbers of co-infective parasites. However, parasites' multiplicity of infections decreases with age, correlating with an increase of the hazard ratio of patent parasitaemias by transmission stages. Notably, *T. parva* antigenic diversity and infection survival in infected animals may not always correlate with vector intensity. Outside enzootic belts, a crucial determinant of the parasite's transmission and the rate at which effective immunity is attained at the herd level, may reside in the mechanism by which recently enzootic, epizootic or epizootic-sporadic foci are generated. This is because such mechanisms may determine the extent of *T. parva* genetic diversity and the combination of parasites circulating in the area, and hence the survival of *T. parva* in infected hosts.

A generic model supports preliminary field observations that survival of *T. parva* may be a function of the 'number and combination' of parasites types present in the infection rather than a function of infection with a particular parasite type. However, a requisite is that both competition (negative interactions) and facilitation (positive interaction) amongst co-infecting parasites must occur. Analysis of associations amongst *T. parva* (PIM) types in cattle with persistent infections has shown that parasites are not independent, and that cooperative associations are at least twice as frequent as competitive associations. Moreover, most positive associations are asymmetric in nature, whilst most negative associations are symmetric. These theoretical and field observations suggest that chronic infections may rely upon the establishment of genetically complex infections by parasite variants that result in facilitation of within-host replication of other co-infecting variants. In this respect, the question

of whether immunodominance wholly of partially underlies the mechanism promoting immune evasion and parasites survival, is worthy of further investigation.

The following sections will discuss the relevance of some of these findings in greater detail and will highlight areas that require further research.

9.2 Inter-Species Pathogen Interactions and the Control of Transmission and Disease – Future Research

This thesis has served to highlight that epidemiology and morbidity studies will necessarily need to account for the effect of interactions established amongst components of the pathogen community. Parameterisation of interactions between pathogen pairs across the multi-pathogen community can be used in models to explore the effect of increasing or decreasing the presence of one pathogen species on the pathogen community as a whole. These models are useful tools to identify counter-intuitive outcomes arising from interactions involving more than two pathogen species (Lello *et al.*, 2004).

Knowledge of inter-species interactions may also be used for the development of mathematical models that can predict the implications of control interventions directed against one pathogen species on the frequency of another (Lello *et al.*, 2004). For example, in a malaria control programme carried out in Tanzania during the 1950s, reduction of *P. falciparum* infections led to a doubling of *P. malariae* prevalence over two years (Bruce-Chwatt, 1963). As a result of interactions amongst Plasmodium spp. the use of single-species vaccines against *P. falciparum*, has implications in that reduction in the prevalence of this species can result in the increase of infection by other species normally suppressed by the former (Bruce *et al.*, 2003).

Investigation of pathogen species-interactions may also provide essential clues for the development of novel control measures which can more efficiently tackle transmission (and disease) of one or even several pathogen species. For example, consistent with recent field evidence that helminth infections can worsen clinical outcome of malaria (Druilhe *et al.*, 2005), and that the protective efficacy of a blood-stage malaria vaccine can be reduced by concurrent nematode infections (Su *et al.*, 2005, Su *et*

al., 2006), it has been suggested that anthelminthic treatments would offer an affordable means to reduce malaria (Druilhe *et al.*, 2005).

To date, the role of pathogen interactions, and how such interactions may be exploited for a better control of pathogenic organisms and diseases of African cattle, remains virtually unexplored. Yet, work described in this thesis raises the question of what the implications might be if only Anaplasma spp. infections were controlled in cattle herds from the study areas.

9.3 Intra-Species Pathogen Interactions and the Transmission Dynamics of *Theileria parva* – Future Research

9.3.1 Future Transmission Models for *Theileria parva*

Information presented on the dynamics of immunity and carrier state in enzootic and epizootic areas may prove useful for the development of a *T. parva* transmission model, and for building a framework for understanding the field applicability of the basis of endemic stability. This later point is particularly important as some authors question the validity of the concept of "endemic stability" for ECF (Torr *et al.*, 2002, Eisler *et al.*, 2003), and several gaps in current knowledge need to be filled before a general model for endemic stability can be applied to *T. parva.*

At present only one transmission model has been developed which is specific to *T. parva* (Medley *et al.*, 1993). This model is of limited applicability, because in the absence of accounting for the impact of acquired immunity on parasites transmission, it can only be extended to represent a cohort of calves in an endemic area. The four basic assumptions of the current model are (i) that host-vector contacts follow a linear relation with population ageing, (ii) that the probability of infection is a constant modified only by tick challenge and the parasite's prevalence in the tick population, (iii) that there is a constant probability that infected and recovered animals develop carrier state and (iv) that carrier state in enzootic areas is life-long as a result of continuous *R. appendiculatus* challenge.

Medley's assumption that host-vector contacts increase linearly as animals grow older was based on empirical data generated from a longitudinal study conducted in an endemic area, in which cattle were

monitored from calfhood up to 6 or 7 months old only (Moll *et al.*, 1984, Moll *et al.*, 1986). The results presented in this thesis have extended Moll's observations to incorporate time slopes for tick build-up across a broad range of age categories, and to consider bovine populations within or outside the enzootic belt. The observations derived from this analysis suggest that acquired immunity against ticks (Bonsma, 1944, Barnett *et al.*, 1955, Sutherst *et al.*, 1979, Jongejan *et al.*, 1989, Fivaz *et al.*, 1990, Randolph, 1994) or a phenomenon of density-dependent regulation of tick numbers (Randolph, 1994) may take place within the enzootic belt but not in recently developed enzootic or epizootic foci, and support previous suggestions that a non-linear function may be required to incorporate host-vector contact dynamics in *T. parva* transmission models (Floyd *et al.*, 1987, Byrom, 1990), depending on the ECF risk zone considered.

While it appears possible that a transmission model built to represent an endemic area may also apply to other enzootic zones, for as long as non-diapausing *R. appendiculatus* populations exist, factors other than tick challenge need to be taken into account before transmission models can be extended to consider epizootic zones other than those specifically characterised. In this respect, the mechanisms by which new enzootic, epizootic or epizootic-sporadic foci are generated, may be of utmost importance in determining the probability of parasite transmission in these areas.

The fourth of Medley's assumptions was derived from early studies conducted in the ECF endemic area of South Nyanza District, Kenya (Young *et al.*, 1986), where the carrier state of *T. parva* was found to approach 100% in adult cattle. This led the authors to suggest that carrier state in endemic areas might have been lifelong (Young *et al.*, 1986). Moreover, observations from other studies led to conclusion that infected cattle invariably develop a carrier state (Kariuki *et al.*, 1995).

Results reported in this thesis suggest that the probability of infection is strongly shaped by acquired immunity in cattle herds raised in and outside the enzootic belt, and that the ability to develop carrier state, at least as measured by the presence of *T. parva* parasites above the PCR detection threshold, depends on the 'infection multiplicity' and the 'combination of parasites' present. Although further research is needed, this work raises more arguments against rather that for the current view that

infected and recovered animals invariably become carries and that carrier state is life-long. Moreover, vector challenge alone cannot wholly explain the dynamics of *T. parva* transmission. These observations introduce enormous variability to current transmission model assumptions. The mechanisms underlying competitive and cooperative interactions amongst *T. parva* parasites, and the role these play in determining the outcome of infection in exposed and recovered cattle is perhaps one of the most important future research topics in *T. parva* epidemiology and control.

9.3.2 Transmission Dynamics of *Theileria parva* in South East Uganda

The results reported here suggest that there exist at least three epidemiological scenarios for *T. parva* in the study areas in south east Uganda. These epidemiological scenarios determine the rate at which acquired immunity will be attained at the herd level, and the potential for animals to develop chronic patent infections consisting of higher parasite densities as compared to sub-patent parasitaemias. They may in consequence result in quite different dynamics of transmission. The three scenarios are as follows (i) low tick challenge - low parasite diversity; (ii) low tick challenge – high parasite diversity and (iii) high tick challenge – high parasite diversity.

In epizootic and recently developed enzootic foci exhibiting low genetic diversity for *T. parva,* few animals are exposed to the parasite because of low vector challenge. Given the limited number of *T. parva* variants in these areas, acquired immunity against the full range of circulating parasites is rapidly attained by the few animals that become exposed to *T. parva*. Super-infection is limited by the lower repertoire of heterologous parasite variants. Moreover, the relatively low number of antigenically distinct parasites inoculated with each infective tick bite, coupled with the limited probability of increasing the antigenic multiplicity in primary infections, as a result of the slow rate of host-vector contacts, favours infection clearance within 28 days and failure to develop persistent infection. Given that persistent infections are rare, ticks feeding on *T. parva* exposed animals are unlikely to become infected and thereby boost the antigenic diversity of *T. parva* through sexual recombination (Morzaria *et al.*, 1993). Over the course of time, the majority of ticks end up feeding on naïve hosts or on previously exposed but non-infective hosts. As a result, *T. parva* patent infection at the population level self-limits and eventually dies-out, as was evident by the observed reduction in the number of PCR

positive results. This phenomenon was found to take place within the space of 12 months in recently enzootic and epizootic areas of south east Uganda characterised by low *T. parva* antigenic diversity.

Epizootic-sporadic areas which originate from the enzootic belt may in contrast be characterised by high *T. parva* antigenic diversity. The slow rate at which host-vector contacts are established coupled with the extensive number of parasite variants circulating in these areas may contribute to a delay in the build-up of acquired immunity to all circulating parasite variants as compared to the enzootic belt. A low rate of host-vector contacts ensures that a large proportion of the population remains naïve and hence susceptible to *T. parva*, while the wide range of circulating heterologous parasites ensures that infected cattle are susceptible to subsequent infections. Moreover, the inoculation of multiple parasite variants with the infective tick bites assists the establishment of persistent infections. In these epizootic-sporadic areas, characterised by low to very low tick challenge, it is likely that boosting of the antigenic multiplicity in primary infections through re-challenge with heterologous variants plays a minimal role in infection survival. However, given that persistent parasitaemia is a likely event, the few ticks which feed on infected animals are more likely to pick-up the multiple genotypes, thereby promoting parasite antigenic diversity through sexual recombination (Morzaria *et al.*, 1993). Over the course of time, ticks may feed on an increasing proportion of carrier animals. This, coupled with the fact that, as a result of low host-vector contacts, a large proportion of cattle remain naïve or susceptible to heterologous challenge with *T. parva*, may enable the parasite to continue to be successfully transmitted. As a consequence, *T. parva* infection at the population level increases over time, with the prevalence of positive observations being higher as animals approach adulthood. In this type of epizootic-sporadic scenario, it is expected that patent infection will eventually self-limit and die-out in the population at a slower rate (i.e. > 2 years) as compared to enzootic areas or recently enzootic and epizootic areas with low *T. parva* antigenic diversity, provided no new *T. parva* parasites are introduced in the area through immigration of ticks and or cattle.

Enzootic foci may represent an intermediate situation as compared to in areas outside the enzootic belt presenting with high or low antigenic diversity. Within the enzootic belt, where high *T. parva* genetic diversity occurs, almost 100% of the animals are continuously infested with *R. appendiculatus*,

and initial host-parasite contacts (as reflected by the occurrence of *T. parva* positive cases by PCR) occur at a faster rate as compared to in areas outside the enzootic belt. The fast rate of host-vector contacts ensures that a large proportion of the population is exposed to *T. parva*, reducing the availability of susceptible hosts as compared to in areas outside the enzootic belt. Successful transmission may be guaranteed for a given time length, as a result of the wide variety of antigenically distinct parasites circulating in the area, which are in turn capable of breaking through any acquired immunity against heterologous parasites. Together, these characteristics may result in conditions which are more favourable for transmission than those in low tick challenge areas, where low antigenic diversity occurs, but less favourable than those in epizootic foci where similar antigenic diversity is observed. The inoculation of multiple parasite variants with the infective tick bites promotes the establishment of persistent infections, and hence carrier state. Moreover, boosting of the *T. parva* multiplicity in primary infections through re-challenge with heterologous variants, contributes to survival of the infection. Over time the multiplicity of antigenic variants promoting infection survival and hence the carrier state, is reduced as a result of the build-up of an immune response against a broad spectrum of circulating variants. Remission of persistent infections and break-through infections as animals approach adulthood, may limit successful transmission to feeding ticks. This, in turn, may compromise the rate at which the recombination / diversity is generated, compared to the rate at which acquired immunity against circulating variants is built-up. Eventually, when the rate at which immunity against circulating variants overtakes the rate at which new variants are generated through sexual recombination, the prevalence of patent *T. parva* will drop and may eventually die-out at the population level, a phenomenon which occurred within two years in enzootic foci of south east Uganda.

9.3.3 Sustainability and Dynamics of ECF Foci

The extent of sexual recombination (McKeever *et al.*, 2002) and parasite-by-host genotype interactions (Dawkins, 1982, Lambrechts *et al.*, 2006) are likely to govern the sustainability and dynamics of ECF foci. In particular, the balance between the mechanisms that generate antigenic diversity and the forces that act to reduce it, will determine the genetic structure of the pathogen populations and how

this varies over time (Gupta *et al.*, 1996a, Gupta *et al.*, 1998, Gupta *et al.*, 1999a, Gupta *et al.*, 1999b, Recker *et al.*, 2005).

At present, the roles that sexual recombination and parasite-by-host genotype interactions play in shaping the population structure of *T. parva* are poorly understood, but it has been hypothesised by means of a simple model, that within enzootic belts *T. parva* infection at the population level may eventually reach a stable equilibrium with low-level carrier infections in adult animals and scarce disease (McKeever *et al.*, 2002). The equilibrium would arise partly from selection of cattle MHC genotypes consistent with effective control of schizont parasitosis and partly from the selection of parasites effective at maintaining adequate piroplasm parasitaemias, and this would not preclude sexual recombination and generation of new parasite variants (McKeever *et al.*, 2002). Only introduction of new cattle and/or foreign parasites would disturb the equilibrium and result in clinical ECF outbreaks (McKeever *et al.*, 2002).

Several limitations to this model emphasise the need for further reseach. Although *T. parva* meiotic division in the tick has been shown to permit inter-strain crossing over events and hence the generation of new parasite variants under laboratory conditions (Allsopp *et al.*, 1989, Morzaria *et al.*, 1990, Bishop *et al.*, 1993, Morzaria *et al.*, 1993), successful obtainment of recombinants in the laboratory have a low predictive value on the extend of sexual recombination in the field (Gauthier *et al.*, 2005). This is partially because the transmission intensity determines the prevailing population structure and the availability of parasite genotypes for sexual recombination (Anderson *et al.*, 2000, MacLeod *et al.*, 2000). In *P. falciparum* natural populations, for example, panmixia (Babiker *et al.*, 1994, Conway *et al.*, 1999), epidemic structure (Abderrazak *et al.*, 1999) and clonality (Rich *et al.*, 1997, Razakandrainibe *et al.*, 2005) correlate with high, low or very low transmission intensities respectively (Anderson *et al.*, 2000). Clonal populations arise when as a result of 'bottlenecked diversity' (Gauthier *et al.*, 2005), gametes that have an identical genetic make up mate in the vector producing a descent which is genetically identical to them (Dye, 1991, Awadalla *et al.*, 2001).

Observations from this thesis suggest that 'bottlenecked diversity' may also be possible in enzootic *T. parva* foci because of acquired immunity if limited movement of cattle and/or ticks takes place. Analysis of PIM data showed that infection multiplicity is inversely related to population ageing and/or time. Moreover, the former was important in maintaining persistent infections above the detection threshold of the available techniques. Because the level of piroplasm parasitaemia is a key factor influencing transmission (Young *et al.*, 1996), it cannot be discarded that a reduction in the multiplicity of infections may result in reduced transmission and less opportunities for boosting of *T. parva* genetic diversity through sexual recombination. What implications this may have concerning the dynamics and sustainability of ECF foci is unknown, but this possibility is consistent with observations that both clonal (Geysen *et al.*, 1999) and epidemic *T. parva* population structures characterised by the rapid expansion of one or more parasite variants (Oura *et al.*, 2005) can occur in the field.

Mathematical models for pathogen systems other than *T. parva*, suggest that when the degree of cross-immunity amongst pathogen genotypes is below a threshold value, all parasites may coexist in the host population with stable abundance and no strain structure (Gupta *et al.*, 1998); when the degree of cross-immunity exceeds an upper threshold, competition mediated through host immune responses may structure parasite populations into distinct strains despite recombination, but a stable population structure may still prevail (Gupta *et al.*, 1996a, Gupta *et al.*, 1998). At these two extremes, a stable equilibrium between hosts, parasites and vector genotypes, may be attained where they all coexist at high levels in the absence of disease (Gupta *et al.*, 1994b). This situation has some similarities with the proposed model for enzootic ECF foci persistence (McKeever *et al.*, 2002). However, at intermediate levels of cross-immunity amongst pathogen genotypes, the relative proportions of the different strains may exhibit very complex and chaotic dynamics with marked fluctuations over time (Gupta *et al.*, 1998). The latter can result in serial outbreaks of disease and can seriously hinder efforts to control transmission through vaccines (Gupta *et al.*, 1998). The extent of cross-immunity amongst natural *T. parva* parasites has not been fully characterised, but results from this thesis suggest that both cooperative and competitive pathogen-to-pathogen interactions occur and that the former are at least twice as frequent as the latter. Whether the interplay of these factors can

lead to unstable dynamics in some areas, with fluctuations of different parasite variants over time, will require further investigation before effective vaccination strategies to control *T. parva* can be applied in the field.

References

Abderrazak, S. B., Oury, B., Lal, A. A., Bosseno, M. F., Force-Barge, P., Dujardin, J. P., Fandeur, T., Molez, J. F., Kjellberg, F., Ayala, F. J. and Tibayrenc, M. (1999), *Plasmodium falciparum*: Population genetic analysis by multilocus enzyme electrophoresis and other molecular markers. *Experimental Parasitology,* **92**, *4*, pp. 232-238.

Adler, F. R. and Brunet, R. C. (1991), The dynamics of simultaneous infections with altered susceptibilities. *Theoretical Population Biology,* **40**, *3*, pp. 369-410.

Adler, F. R. and Mosquera, L. J. (2002), In *Adaptative dymanics of infectious diseases: In pursuit of virulence management*. Super- and co-infection: Filling the range. (Eds, Dieckmann, U., Metz, J. A. J., Sabelis, M. A. and Sigmund, K.) Cambridge University Press, Cambridge, pp. 139-149.

Ahmed, R. and Stevens, J. G. (1990), In *Virology*. Viral persistence. (Eds, Fields, B. N., Knipe, D. M., Chanick, R. M., Hirsch, M. S., Melnick, J. L., Monath, T. P. and Roizman, B.) Raven Press, Ltd, New York, pp. 241-265.

Ajayi, S. A., Fabiyi, J. P. and Umo, I. (1982), Clinical bovine anaplasmosis and babesiosis in Friesian cattle - An outbreak in Nigeria and its control. *World Animal Review,* **43**, pp. 41.

Ajayi, S. A., Oyetunde, I. L., Ogbonna, G. A. and Dipeolu, O. O. (1987), Bovine anaplasmosis: Clinical, haematological and blood biochemical changes in experimentally infected Nigerian cattle. *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux,* **40**, *1*, pp. 41-47.

Akinbamijo, O. O., Bennison, J. J., Jaitner, J. and Dempfle, L. (1998), Haematological changes in N'Dama and Gobra Zebu bulls during *Trypanosoma congolense* infection maintained under a controlled feeding regimen. *Acta Tropica,* **69**, *3*, pp. 181-192.

Akol, G. W., Authie, E., Pinder, M., Moloo, S. K., Roelants, G. E. and Murray, M. (1986), Susceptibility and immune responses of Zebu and Taurine cattle of West Africa to infection with *Trypanosoma congolense* transmitted by *Glossina morsitans centralis*. *Veterinary Immunology Immunopathology,* **11**, *4*, pp. 361-373.

Allaker, R. P., Lloyd, D. H. and Smith, I. M. (1988), Prevention of exudative epidermitis in gnotobiotic piglets by bacterial interference. *Veterinary Record,* **123**, *23*, pp. 597-598.

Allsopp, B. A. and Allsopp, M. T. (1988), *Theileria parva*: Genomic DNA studies reveal intra-specific sequence diversity. *Molecular and Biochemical Parasitology,* **28**, *1*, pp. 77-83.

Allsopp, B., Carrington, M., Baylis, H., Sohal, S., Dolan, T. and Iams, K. (1989), Improved characterisation of *Theileria parva* isolates using the polymerase chain reaction and oligonucleotide probes. *Molecular and Biochemical Parasitology,* **35**, *2*, pp. 137-147.

Altman, D. G. (1991), In *Practical statistics for medical research*. Comparing groups - Categorical data. Chapman & Hall/CRC, Boca Raton, Florida, pp. 229-276.

Andersen, P., Askgaard, D., Gottschau, A., Bennedsen, J., Nagai, S. and Heron, I. (1992), Identification of immunodominant antigens during infection with *Mycobacterium tuberculosis*. *Scandinavian Journal of Immunology,* **36**, *6*, pp. 823-831.

Anderson, I. L., Jones, E. W., Morrison, R. D., Holbert, D. and Lee, C. K. (1972), *Anaplasma marginale*: Haemoglobin patterns in experimentally infected young calves. *Experimental Parasitology,* **32**, *2*, pp. 265-271.

Anderson, R. M. and May, R. M. (1991), Infectious diseases of humans. Dynamics and control. Oxford University Press, Oxford.

Anderson, T. J., Haubold, B., Williams, J. T., Estrada-Franco, J. G., Richardson, L., Mollinedo, R., Bockarie, M., Mokili, J., Mharakurwa, S., French, N., Whitworth, J., Velez, I. D., Brockman, A. H., Nosten, F., Ferreira, M. U. and Day, K. P. (2000), Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*. *Molecular Biology and Evolution,* **17**, *10*, pp. 1467-1482.

Andreasen, V., Lin, J. and Levin, S. A. (1997), The dynamics of co-circulating influenza strains conferring partial cross-immunity. *Journal of Mathematical Biology,* **35**, *7*, pp. 825-842.

Anon (1974), Annual report of the Department of Veterinary and Tsetse Control Services for the year 1972. Government Printer, Zambia, Lusaka.

Anon (1992), Annual Report for the Department of Veterinary Services, 1992.

Anon (1996), Annual report for the department of Veterinary Services, Uganda, 1996.

Antia, R., Nowak, M. A. and Anderson, R. M. (1996), Antigenic variation and the within-host dynamics of parasites. *Proceedings of the National Academy of Sciences of the United States of America,* **93**, *3*, pp. 985-989.

Armstrong, P. M., Katavolos, P., Caporale, D. A., Smith, R. P., Spielman, A. and Telford, S. R. (1998), Diversity of Babesia infecting deer ticks (*Ixodes dammini*). *American Journal of Tropical Medicine and Hygiene,* **58**, *6*, pp. 739-742.

Ashford, R. W. (1991), The human parasite fauna: Towards an analysis and interpretation. *Annals of Tropical Medicine and Parasitology,* **85**, *1*, pp. 189-198.

Awadalla, P., Walliker, D., Babiker, H. and Mackinnon, M. (2001), The question of *Plasmodium falciparum* population structure. *Trends in Parasitology,* **17**, *8*, pp. 351-353.

Babiker, H. A., Ranford-Cartwright, L. C., Currie, D., Charlwood, J. D., Billingsley, P., Teuscher, T. and Walliker, D. (1994), Random mating in a natural population of the malaria parasite *Plasmodium falciparum*. *Parasitology,* **109**, *4*, pp. 413-421.

Babiker, H. A., Ranford-Cartwright, L. C. and Walliker, D. (1999), Genetic structure and dynamics of *Plasmodium falciparum* infections in the Kilombero region of Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **93**, *1*, pp. 11-14.

Bakuname, C., Lynen, G., Chonya, A., Shongon, B., Mollel, J. O., Jongejan, F. and Cleaveland, S. (2002), Bovine cerebral theileriosis in Tanzania: A report of a participatory appraisal in Arusha region. DFID.

Baldock, F. C. and Arthur, R. J. (1985), A survey of fascioliasis in beef cattle killed at abattoirs in southern Queensland Australia. *Australian Veterinary Journal,* **62**, *10*, pp. 324-326.

Barnett, S. F. and Bailey, K. P. (1955), A field survey of East Coast Fever and calf mortality in an enzootic ECF area. Annual Report, 1954-1955. East African Veterinary Research Organisation, Nairobi: East African Community, pp. 51-52.

Barnett, S. F. and Brocklesby, D. W. (1966), A mild form of East Coast Fever (*Theileria parva*) with persistence of infection. *British Veterinary Journal,* **122**, pp. 361-370.

Barrow, P. A. and Page, K. (2000), Inhibition of colonisation of the alimentary tract in young chickens with *Campylobacter jejuni* by precolonisation with strains of *C. jejuni*. *FEMS (Federation of European Microbiological Societies) Microbiology Letters,* **182**, pp. 87-91.

Barry, J. D. and Gathuo, H. (1984), Antigenic variation in *Trypanosoma vivax*: Isolation of a serodeme. *Parasitology,* **89**, *1*, pp. 49-58.

Baumgartner, W., Schlerka, G., Fumicz, M., Stoger, J., Awadmasalmeh, M., Schuller, W. and Weber, P. (1992), Seroprevalence survey for *Anaplasma marginale* infection of Austrian cattle. *Journal of Veterinary Medicine Series B-Zentralblatt Fur Veterinarmedizin Reihe B-Infectious Diseases and Veterinary Public Health,* **39**, *2*, pp. 97-104.

Baylis, H. A., Sohal, S. K., Carrington, M., Bishop, R. P. and Allsopp, B. A. (1991), An unusual repetitive gene family in *Theileria parva* which is stage-specifically transcribed. *Molecular and Biochemical Parasitology,* **49**, *1*, pp. 133-142.

Baylis, H. A., Allsopp, B. A., Hall, R. and Carrington, M. (1993), Characterisation of a glutamine- and proline-rich protein (QP protein) from *Theileria parva*. *Molecular and Biochemical Parasitology,* **61**, *2*, pp. 171-178.

Behnke, J. M., Bajer, A., Sinski, E. and Wakelin, D. (2001), Interactions involving intestinal nematodes of rodents: Experimental and field studies. *Parasitology,* **122**, pp. S39-S49.

Ben Miled, L. (1993), Population diversity in *Theileria annulata* in Tunisia. PhD Thesis. University of Edinburgh, Scotland.

Bendixen, M., Msangeni, H. A., Pedersen, B. V., Shayo, D. and Bodker, R. (2001), Diversity of *Plasmodium falciparum* populations and complexity of infections in relation to transmission intensity and host age: A study from the Usambara Mountains, Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **95**, *2*, pp. 143-148.

Bengaly, Z., Sidibe, I., Ganaba, R., Desquesnes, M., Boly, H. and Sawadogo, L. (2002), Comparative pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: Clinical observations and haematological changes. *Veterinary Parasitology,* **108**, *1*, pp. 1-19.

Bergeon, P. (1968), Retort to the Government of Ethiopia on a veterinary parasitology survey. No. T. A. 2458. F.A.O., Rome. 38 pages.

Bertoletti, A., Sette, A., Chisari, F. V., Penna, A., Levrero, M., De Carli, M., Fiaccadori, F. and Ferrari, C. (1994), Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. *Nature,* **369**, *6479*, pp. 407-410.

Bishop, R. P., Sohanpal, B., Kariuki, D. P., Young, A. S., Nene, V., Baylis, H., Allsopp, B. A., Spooner, P. R., Dolan, T. T. and Morzaria, S. P. (1992), Detection of a carrier state in *Theileria parva*-infected cattle by the polymerase chain reaction. *Parasitology,* **104**, *2*, pp. 215-232.

Bishop, R. P., Sohanpal, B. K., Allsopp, B. A., Spooner, P. R., Dolan, T. T. and Morzaria, S. P. (1993), Detection of polymorphisms among *Theileria parva* stocks using repetitive, telomeric and ribosomal DNA probes and anti-schizont monoclonal antibodies. *Parasitology,* **107**, *1*, pp. 19-31.

Bishop, R. P., Spooner, P. R., Kanhai, G. K., Kiarie, J., Latif, A. A., Hove, T., Masaka, S. and Dolan , T. T. (1994), Molecular characterisation of Theileria parasites: Application to the epidemiology of theileriosis in Zimbabwe. *Parasitology,* **109**, *5*, pp. 573-581.

Bishop, R., Musoke, A., Morzaria, S., Sohanpal, B. and Gobright, E. (1997), Concerted evolution at a multicopy locus in the protozoan parasite *Theileria parva*: Extreme divergence of potential protein-coding sequences. *Molecular and Cellular Biology,* **17**, *3*, pp. 1666-1673.

Bishop, R., Geysen, D., Spooner, P., Skilton, R., Nene, V., Dolan, T. and Morzaria, S. (2001), Molecular and immunological characterisation of *Theileria parva* stocks which are components of the 'Muguga cocktail' used for vaccination against East Coast Fever in cattle. *Veterinary Parasitology,* **94**, *4*, pp. 227-237.

Bishop, R., Geysen, D., Skilton, R., Odongo, D., Nene, V., Allsopp, B., Mbogo, S., Spooner, P. and Morzaria, S. (2002), In *Theileria*. Genomic polymorphism, sexual recombination and molecular epidemiology of *Theileria parva*. World Class Parasites. **3**, (Eds, Dobbbelaere, D. A. E. and Mc Keever, D. J.) Kluwer Academic Publishers, Boston, pp. 23-39.

Bishop, R., Musoke, A., Morzaria, S., Gardner, M. and Nene, V. (2004), Theileria: Intracellular parasites of wild and domestic ruminants transmitted by ixodid ticks. *Parasitology,* **129**, pp. 271-283.

Bitakaramire, P. K. and Bwangamoi, O. (1969), Experimental infection of calves with *Fasciola gigantica*. *Experimental Parasitology,* **25**, *1*, pp. 353-357.

Bitakaramire, P. K. and Bwangamoi, O. (1970), The pathology of experimental infection of cattle with *Fasciola gigantica*. *Bulletin of Epizootic Diseases of Africa,* **18**, *2*, pp. 149-157.

Blench, R. (2000), 'You can't go home again' - Extensive pastoral livestock systems: Issues and options for the future. ODI (Overseas Development Institute) & FAO (Food and Agriculture Organisation) Report, London.

Bock, R. E., de Vos, A. J., Kingston, T. G. and McLellan, D. J. (1997), Effect of breed of cattle on innate resistance to infection with *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*. *Australian Veterinary Journal,* **75**, *5*, pp. 337-340.

Bonsma, J. C. (1944), Hereditary heartwater-resistant characteristics in cattle. *Farming in South Africa,* **19**, pp. 71-96.

Boray, J. C. (1985), In *Parasites, pets and predators*. Flukes of domestic animals. (Eds, Gaafar, S. M., Howard, W. E. and Marsh, R. E.) Elsevier, New York, pp. 179-218.

Boyt, W. P. (1984), A field guide for diagnosis and prevention of African animal trypanosomiasis. Food and Agriculture Organisation, Rome.

Braend, M. (1979), World veterinary manpower: Comparative aspects. *Veterinary Record,* **105**, *4*, pp. 77-79.

Bram, R. A. (1975), Tick borne livestock diseases and there vectors. 1. The global problem. *World Animal Review,* **6**, pp. 1-5.

Bremermann, H. J. and Pickering, J. (1983), A game-theoretical model of parasite virulence. *Journal of Theoretical Biology,* **100**, *3*, pp. 411-246.

Breslow, N. E. and Clayton, D. G. (1993), Approximate inference in generalised linear mixed models. *Journal of the American Statistical Association,* **88**, pp. 9-25.

Briand, V., Watier, L., Le Hesran, J. Y., Garcia, A. and Cot, M. (2005), Coinfection with *Plasmodium falciparum* and *Schistosoma haematobium*: Protective effect of schistosomiasis on malaria in Senegalese children? *American Journal of Tropical Medicine and Hygiene,* **72**, *6*, pp. 702-707.

Brocklesby, D. W., Barnett, S. F. and Scott, G. R. (1961), Morbidity and mortality rates in East Coast Fever (*Theileria parva* infection) and their application to drug screening procedures. *British Veterinary Journal,* **117**, pp. 529-531.

Brocklesby, D. W. and Barnett, S. F. (1966), A mild form of East Coast Fever with persistence of infection. *British Veterinary Journal,* **122**, pp. 361-370.

Brooks, D. R. and McLennan, D. A. (1993), Parascript. Parasites and the language of evolution. Smithsonian Institution Press, Washington.

Brothwell, D. and Sandison, A. T. (Eds.) (1967), Diseases in Antiquity. Springfield, Illinois: C. T. Thomas.

Bruce, D., Hamerton, A. E. and Bateman, H. R. (1910), Amakebe: A disease of calves in Uganda. *Unknown*.

Bruce, M. C., Donnelly, C. A., Alpers, M. P., Galinski, M. R., Barnwell, J. W., Walliker, D. and Day, K. P. (2000a), Cross-species interactions between malaria parasites in humans. *Science,* **287**, *5454*, pp. 845-848.

Bruce, M. C., Donnelly, C. A., Packer, M., Lagog, M., Gibson, N., Narara, A., Walliker, D., Alpers, M. P. and Day, K. P. (2000b), Age- and species-specific duration of infection in asymptomatic malaria infections in Papua New Guinea. *Parasitology,* **121**, *3*, pp. 247-256.

Bruce, M. C., Galinski, M. R., Barnwell, J. W., Donnelly, C. A., Walmsley, M., Alpers, M. P., Walliker, D. and Day, K. P. (2000c), Genetic diversity and dynamics of *Plasmodium falciparum* and *P. vivax* populations in multiply infected children with asymptomatic malaria infections in Papua New Guinea. *Parasitology,* **121**, *3*, pp. 257-272.

Bruce, M. C. and Day, K. P. (2003), Cross-species regulation of Plasmodium parasitemia in semi-immune children from Papua New Guinea. *Trends in Parasitology,* **19**, *6*, pp. 271-277.

Bruce-Chwatt, L. J. (1963), The longitudinal survey of natural malaria infection in a group of West African adults. *West African Medical Journal,* **12**, pp. 199-206.

Brulisauer, F., Thoma, R., Cagienard, A., Hofmann-Lehmann, R., Lutz, H., Meli, M. L., Regula, G., Jorger, K., Perl, R. and Dreher, U. M. (2004), Anaplasmosis in a Swiss dairy farm: An epidemiological outbreak investigation. *Schweizer Archiv Fur Tierheilkunde,* **146**, *10*, pp. 451-460.

Brunsdon, R. V. (1980), Principles of helminth control. *Veterinary Parasitology,* **6**, pp. 185-215.

Bull, J. J. (1994), The evolution of virulence. *Evolution,* **48**, pp. 1423-1437.

Bull, P. C., Lowe, B. S., Kaleli, N., Njuga, F., Kortok, M., Ross, A., Ndungu, F., Snow, R. W. and Marsh, K. (2002), *Plasmodium falciparum* infections are associated with agglutinating antibodies to parasite-infected erythrocyte surface antigens among healthy Kenyan children. *Journal of Infectious Diseases,* **185**, *11*, pp. 1688-1691.

Burridge, M. J., Morzaria, S. P., Cunningham, M. P. and Brown, C. G. (1972), Duration of immunity to East Coast Fever (*Theileria parva* infection of cattle). *Parasitology,* **64**, *3*, pp. 511-515.

Buseyne, F., McChesney, M., Porrot, F., Kovarik, S., Guy, B. and Riviere, Y. (1993), Gag-specific cytotoxic T lymphocytes from human immunodeficiency virus type 1-infected individuals: Gag epitopes are clustered in three regions of the p24gag protein. *Journal of Virology,* **67**, *2*, pp. 694-702.

Byrom, W. (1990), Simulation models for investigating East Coast Fever and other parasitic diseases. PhD Thesis. Strathclyde University, UK.

Callow, L. L. (1971), The control of babesiosis with a highly effective attenuated vaccine. Proceedings of the 19th World Veterinary Congress. Lioate, Mexico, pp. 357-360.

Callow, L. L. (1983), Ticks and tick borne diseases as a barrier to the introduction of exotic cattle in the tropics. In: Ticks and Tick Borne Diseases. FAO Animal Health, Production and Health Paper No 36. Food and Agriculture Organisation, Rome, Italy, pp. 48-53.

Callow, L. L., Dalgliesh, R. J. and De Vos, A. J. (1997), Development of effective living vaccines against bovine babesiosis. The longest field trial? *International Journal for Parasitology,* **27**, *7*, pp. 747-767.

Carmichael, I. H. (1972), Helminthiasis in domestic and wild ruminants in Botswana: Preliminary investigations. *Tropical Animal Health and Production,* **4**, pp. 175-181.

Carrique Mas, J. J., Widdowson, M. A., Cuellar, A. M., Ribera, H. and Walker, A. R. (2000), Risk of babesiosis and anaplasmosis in different ecological zones of Santa Cruz Department, Bolivia. *Veterinary Parasitology,* **93**, *1*, pp. 29-38.

Cawdery, M. J., Strickland, K. L., Conway, A. and Crowe, P. J. (1977), Production effects of the liver fluke in cattle: 1. The effects of infection on livewieght gain, feed intake and food conversion efficiency in beef cattle. *British Veterinary Journal,* **133**, *2*, pp. 145-159.

CDC (2002), The lifecycle of *Fasciola hepatica* [Online]. Centres for Disease Control. Available at http:\www.dpd.cdc.gov/dpdx. Accessed 23/05/06.

Ceci, L., Jongejan, F., Carelli, G., Tassi, P. and Sparagano, O. (1999), Identification of *Theileria buffeli/orientalis* and *Babesia bigemina* in Apulian cattle using molecular techniques and study of changes in blood parameters. *Parassitologia,* **41**, *1*, pp. 31-32.

Census (2002a), Tororo District. 2002 Population and Housing Census.

Census (2002b), Busia District. 2002 Population and Housing Census.

Cheesman, S. J., de Roode, J. C., Read, A. F. and Carter, R. (2003), Real-time quantitative PCR for analysis of genetically mixed infections of malaria parasites: Technique validation and applications. *Molecular and Biochemical Parasitology,* **131**, *2*, pp. 83-91.

Chen, W., Anton, L. C., Bennink, J. R. and Yewdell, J. W. (2000), Dissecting the multifactorial causes of immunodominance in class I-restricted T cell responses to viruses. *Immunity,* **12**, *1*, pp. 83-93.

Chizyuka, H. G. B. and Mangani, M. P. C. (1985), Theileriosis in Zambia. Immunisation against theileriosis in Africa. Nairobi, (Ed, Irvin, A. D.) International Laboratory for Research on Animal Diseases, 1-5 October, 1984, pp. 12-15.

Chotivanich, K., Udomsangpetch, R., Simpson, J. A., Newton, P., Pukrittayakamee, S., Looareesuwan, S. and White, N. J. (2000), Parasite multiplication potential and the severity of Falciparum malaria. *Journal of Infectious Diseases,* **181**, *3*, pp. 1206-1209.

Christensen, N. O., Nansen, P., Fagbemi, B. O. and Monrad, J. (1987), Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infection of mammalian hosts. *Parasitology Research,* **73**, *5*, pp. 387-410.

Cissé, M., Fall, S. T. and Korréa, A. (1995), A view of monthly change in body condition in Zebu cattle during a fattening trial using agro-industrial bye-products. ISRA, pp. 18.

Cockburn, A., Cockburn, E. and Reyman, T. A. (Eds.) (1998), Mummies, Disease and Ancient Cultures. Second edition. Cambridge: Cambridge University Press.

Coleman, P. G., Perry, B. D. and Woolhouse, M. E. J. (2001), Endemic stability - A veterinary idea applied to human public. *The Lancet,* **357**, pp. 1284-1286.

Coleman, P. G. and Welburn, S. C. (2004), Are Fitness Costs Associated with Resistance to Human Serum in *Trypanosoma brucei rhodesiense*? *Trends in Parasitology,* **20**, *7*, pp. 311-315.

Collins, N. E. and Allsopp, B. A. (1999), *Theileria parva* ribosomal internal transcribed spacer sequences exhibit extensive polymorphism and mosaic evolution: Application to the characterisation of parasites from cattle and buffalo. *Parasitology,* **118**, *6*, pp. 541-551.

Connor, R. J. (1994), The impact of nagana. *Onderstepoort Journal of Veterinary Research,* **61**, *4*, pp. 379-383.

Conrad, P. A., Denham, D. and Brown, C. G. (1986), Intraerythrocytic multiplication of *Theileria parva* in vitro: An ultrastructural study. *International Journal for Parasitology,* **16**, *3*, pp. 223-229.

Conrad, P. A., Stagg, D. A., Grootenhuis, J. G., Irvin, A. D., Newson, J., Njamunggeh, R. E., Rossiter, P. B. and Young, A. S. (1987a), Isolation of Theileria parasites from African buffalo (*Syncerus caffer*) and characterisation with anti-schizont monoclonal antibodies. *Parasitology,* **94**, *3*, pp. 413-423.

Conrad, P. A., Iams, K., Brown, W. C., Sohanpal, B. and ole-MoiYoi, O. K. (1987b), DNA probes detect genomic diversity in *Theileria parva* stocks. *Molecular and Biochemical Parasitology,* **25**, *3*, pp. 213-226.

Conrad, P. A., ole-MoiYoi, O. K., Baldwin, C. L., Dolan, T. T., O'Callaghan, C. J., Njamunggeh, R. E., Grootenhuis, J. G., Stagg, D. A., Leitch, B. L. and Young, A. S. (1989), Characterisation of buffalo-derived theilerial parasites with monoclonal antibodies and DNA probes. *Parasitology,* **98**, *2*, pp. 179-188.

Conway, D. J., Roper, C., Oduola, A. M., Arnot, D. E., Kremsner, P. G., Grobusch, M. P., Curtis, C. F. and Greenwood, B. M. (1999), High recombination rate in natural populations of *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences of the United States of America,* **96**, *8*, pp. 4506-4511.

Cook, A. J. C. (1991), Communal farmers and tick control: A field study in Zimbabwe. *Tropical Animal Health and Production,* **23**, *3*, pp. 161-166.

Copeman, D. B. and Hutchinson, G. W. (1979), The economic significance of bovine gastrointestinal nematode parasitism in north Queensland. Proceedings of the 2nd International Symposium on Veterinary Epidemiology and Economics. Canberra, (Eds,

Geering, W. A., Roe, R. J. and Chapman, L. A.) Australian Government Publishing Service, **2**, pp. 383-387.

Cox, D. R. (1972), Regression models and life tables (with discussion). *Journal of the Royal Statistical Society,* **B34**, pp. 187-220.

Cox, F. E. G. (2001), Concomitant infections, parasites and immune responses. *Parasitology,* **122**, pp. S23-S38.

Crawley, M. J. (2002a), In *Statistical computing - An introduction to data analysis using S-plus*. Classical tests. John Wiley & Sons Ltd., The Atrium, West Sussex, England, pp. 169-194.

Crawley, M. J. (2002b), In *Statistical computing - An introduction to data analysis using S-plus*. Proportion data: Binomial errors. John Wiley & Sons Ltd, The Atrium, West Sussex, England, pp. 513-536.

Crawley, M. J. (2002c), In *Statistical computing - An introduction to data analysis using S-plus*. Count data: Poisson errors. Wiley & Sons, Ltd, The Atrium, West Sussex, England, pp. 513-536.

Croxton, D. and Stollard, R. J. (1976), The use of condition scoring as a management aid in dairy and beef cattle. *Animal Production,* **22**, pp. 146.

Dallas, A. B. (1976), Interaction between *Trypanosoma brucei* and *Plasmodium berghei* in concurrent infections in mice. *Journal of Tropical Medicine and Hygiene,* **79**, *8*, pp. 182-188.

Dalton, J. P. and Mulcahy, G. (2001), Parasite vaccines: A reality? *Veterinary Parasitology,* **98**, *1-3*, pp. 149-167.

Darghouth, M. A., Ben Miled, L., Bouattour, A., Melrose, T. R., Brown, C. G. and Kilani, M. (1996), A preliminary study on the attenuation of Tunisian schizont-infected cell lines of *Theileria annulata*. *Parasitology Research,* **82**, *7*, pp. 647-655.

Davies, C. M., Webster, J. P. and Woolhous, M. E. (2001), Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **268**, *1464*, pp. 251-257.

Dawkins, R. (1982), The extended phenotype. Oxford University Press.

De Castro, J. J., James, A. D., Minjauw, B., Di Giulio, G. U., Permin, A., Pegram, R. G., Chizyuka, H. G. B. and Sinyangwe, P. (1997), Long-term studies on the economic impact of ticks of Sanga cattle in Zambia. *Experimental and Applied Acarology,* **21**, *1*, pp. 3-19.

De Haan, C., Steinfeld, H. and Blackburn, H. (1997), Livestock and the environment: Finding a balance. WRE Media, Fressungfield, UK. 115 pages.

De Roode, J. C., Read, A. F., Chan, B. H. and Mackinnon, M. J. (2003), Rodent malaria parasites suffer from the presence of conspecific clones in three-clone *Plasmodium chabaudi* infections. *Parasitology,* **127**, *5*, pp. 411-418.

De Roode, J. C., Culleton, R., Bell, A. S. and Read, A. F. (2004a), Competitive release of drug resistance following drug treatment of mixed *Plasmodium chabaudi* infections. *Malaria Journal,* **3**:33.

De Roode, J. C., Culleton, R., Cheesman, S. J., Carter, R. and Read, A. F. (2004b), Host heterogeneity is a determinant of competitive exclusion or coexistence in genetically

diverse malaria infections. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **271**, *1543*, pp. 1073-1080.

De Roode, J. C., Helinski, M. E., Anwar, M. A. and Read, A. F. (2005a), Dynamics of multiple infection and within-host competition in genetically diverse malaria infections. *American Naturalist,* **166**, *5*, pp. 531-542.

De Roode, J. C., Pansini, R., Cheesman, S. J., Helinski, M. E., Huijben, S., Wargo, A. R., Bell, A. S., Chan, B. H., Walliker, D. and Read, A. F. (2005b), Virulence and competitive ability in genetically diverse malaria infections. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, *21*, pp. 7624-7628.

De Vos, A. J. (1979), Epidemiology and control of bovine babesiosis in South Africa. *Journal of the South African Veterinary Association,* **50**, pp. 357-362.

De Vos, A. J., Bessenger, R. and Banting, L. F. (1981), Theileria? *Taurotragi*: A probable agent of bovine cerebral theileriosis. *Onderstepoort Journal of Veterinary Research,* **48**, *3*, pp. 177-178.

Deem, S. L., Perry, B. D., Katende, J. M., McDermott, J. J., Mahan, S. M., Maloo, S. H., Morzaria, S. P., Musoke, A. J. and Rowlands, G. J. (1993), Variations in prevalence rates of tick-borne diseases in Zebu cattle by agroecological zone - Implications for East Coast Fever immunisation. *Preventive Veterinary Medicine,* **16**, *3*, pp. 171-187.

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. and Courbois, C. (1999), Livestock to 2020: The next food revolution. Food, Agriculture and Environment. Discussion Paper No 228. IFPRI (International Food Policy Research Institute). FAO, Rome & ILRI, Nairobi.

Dempster, A. P., Laird, N. M. and Rubin, D. B. (1977), Maximum likelihood from incomplete data via EM algorithm. *Journal of the Royal Statistical Society, Series B,* **39**, pp. 1-22.

Diffley, P., Scott, J. O., Mama, K. and Tsen, T. N. (1987), The rate of proliferation among African trypanosomes is a stable trait that is directly related to virulence. *American Journal of Tropical Medicine and Hygiene,* **36**, *3*, pp. 533-540.

Dikmans, G. (1950), The transmission of anaplasmosis. *American Journal of Veterinary Research,* **11**, *38*, pp. 5-16.

Dobson, R. J. and Barnes, E. H. (1995), Interaction between *Ostertagia circumcincta* and *Haemonchus contortus* infection in young lambs. *International Journal for Parasitology,* **25**, *4*, pp. 495-501.

Dolan, T. T., Young, A. S., Losos, G. J., McMilian, I., Minder, C. E. and Soulsby, K. (1984), Dose dependent responses of cattle to *Theileria parva* stabilate. *International Journal for Parasitology,* **14**, *1*, pp. 89-95.

Dolan, T. T. (1986), Chemotherapy of East Coast Fever: The long term weight changes, carrier state and disease manifestations of parvaquone treated cattle. *Journal of Comparative Pathology,* **96**, *2*, pp. 137-146.

Dossa, S. C., Kaaya, G. P., Essuman, S., Odulaja, A. and Assoku, R. G. (1996), Acquisition of resistance to the tick *Amblyomma variegatum* in Boran cattle, *Bos indicus* and the effects of *Trypanosoma congolense* and *Babesia bigemina* on host resistance. *Veterinary Parasitology,* **62**, *3-4*, pp. 317-330.

Druilhe, P., Tall, A. and Sokhna, C. (2005), Worms can worsen malaria: Towards a new means to roll back malaria? *Trends in Parasitology,* **21**, *8*, pp. 359-362.

Dumler, J. S., Barbet, A. F., Bekker, C. P., Dasch, G. A., Palmer, G. H., Ray, S. C., Rikihisa, Y. and Rurangirwa, F. R. (2001), Reorganisation of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: Unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *International Journal of Systematic and Evolutionary Microbiology,* **51**, *6*, pp. 2145-2165.

Duval-Iflash, Y., Chappuis, R., Ducluzeau, R. and Raibaud, P. (1983), Intraspecific interactions between *Escherichia coli* strains in human newborns and in gnotobiotic mice and piglets. *Progress in Food and Nutrition Science,* **7**, pp. 107-116.

Dye, C. (1991), Population genetics of nonclonal, nonrandomly mating malaria parasites. *Parasitology Today,* **7**, *9*, pp. 236-240.

Earle, W. C. (1939), Observations on the course of naturally acquired malaria in Puerto Rico. *Puerto Rico Journal of Public Health and Tropical Medicine,* **14**, pp. 391-406.

Egbe-Nwiyi, T. N. and Chaudrai, S. U. R. (1996), Observations on prevalence, haematological and pathological changes in cattle, sheep and goats naturally infected with *Fasciola gigantica* in arid zone of Borno State, Nigeria. *Pakistan Veterinary Journal,* **16**, *4*, pp. 172-175.

Egbe-Nwiyi, T. N., Salako, M. A. and Otukonyong, E. E. (1997), Observations on naturally occurring bovine anaplasmosis in arid zone of North-Eastern Nigeria: Prevalence, haematological and pathological changes. *Studies and Research in Veterinary Medicine,* **5**, pp. 95-99.

Eisler, M. C., Torr, S. J., Coleman, P. G., Machila, N. and Morton, J. F. (2003), Integrated control of vector-borne diseases of livestock - Pyrethroids: Panacea or poison? *Trends in Parasitology,* **19**, *8*, pp. 341-345.

Elliot, R. C. (1964), Some nutritional factors influencing the productivity of beef cattle in southern Rhodesia. PhD Thesis. University of London, UK.

Ellis, F. and Bahiigwa, G. (2003a), Livelihoods and Rural Poverty Reduction in Uganda. *World Development,* **31**, *6*, pp. 997-1013.

Ellis, F. and Mdoe, N. (2003b), Livelihoods and Rural Poverty Reduction in Tanzania. *World Development,* **31**, *8*, pp. 1367-1384.

Emery, D. L. (1981), Adoptive transfer of immunity to infection with *Theileria parva* (East Coast Fever) between cattle twins. *Research in Veterinary Science,* **30**, *3*, pp. 364-367.

Emery, D. L., Tenywa, T. and Jack, R. M. (1981), Characterisation of the effector cell that mediates cytotoxicity against *Theileria parva* (East Coast Fever) in immune cattle. *Infection and Immunity,* **32**, *3*, pp. 1301-1304.

Eriks, I. S., Palmer, G. H., McGuire, T. C., Allred, D. R. and Barbet, A. F. (1989), Detection and quantitation of *Anaplasma marginale* in carrier cattle by using a nucleic-acid probe. *Journal of clinical microbiology,* **27**, *2*, pp. 279-284.

Ezanno, P., Ickowicz, A. and Bocquier, F. (2003), Factors affecting the body condition score of N'Dama cows under extensive range management in southern Senegal. *Animal Research,* **52**, pp. 37-48.

Fabiyi, J. P. (1987), Production losses and control of helminths in ruminants. *International Journal for Parasitology,* **17**, pp. 435-442.

Fakae, B. B., Harrison, L. J., Ross, C. A. and Sewell, M. M. (1994), *Heligmosomoides polygyrus* and *Trypanosoma congolense* infections in mice: A laboratory model for concurrent gastrointestinal nematode and trypanosome infections. *Parasitology,* **108**, *1*, pp. 61-68.

Fandamu, P., Duchateau, L., Speybroeck, N., Marcotty, T., Mbao, V., Mtambo, J., Mulumba, M. and Berkvens, D. (2005), *Theileria parva* seroprevalence in traditionally kept cattle in southern Zambia and El Niño. *International Journal for Parasitology,* **35**, *4*, pp. 391-396.

Fandamu, P., Duchateau, L., Speybroeck, N., Mulumba, M. and Berkvens, D. (2006), East Coast Fever and multiple El Niño southern oscillation ranks. *Veterinary Parasitology,* **135**, *2*, pp. 147-152.

Fawcett, D. W., Conrad, P. A., Grootenhuis, J. G. and Morzaria, S. P. (1987), Ultrastructure of the intra-erythrocytic stage of Theileria species from cattle and waterbuck. *Tissue Cell,* **19**, *5*, pp. 643-655.

Ferguson, N., Anderson, R. and Gupta, S. (1999), The effect of antibody-dependent enhancement on the transmission dynamics and persistence of multiple-strain pathogens. *Proceedings of the National Academy of Sciences of the United States of America,* **96**, *2*, pp. 790-794.

Ferrari, C., Bertoletti, A., Penna, A., Cavalli, A., Valli, A., Missale, G., Pilli, M., Fowler, P., Giuberti, T., Chisari, F. V. and et al. (1991), Identification of immunodominant T cell epitopes of the hepatitis B virus nucleocapsid antigen. *Journal of Clinical Investigation,* **88**, *1*, pp. 214-222.

Fèvre, E. M., Coleman, P. G., Odiit, M., Magona, J. W., Welburn, S. C. and Woolhouse, M. E. (2001), The origins of a new *Trypanosoma brucei rhodesiense* sleeping sickness outbreak in eastern Uganda. *Lancet,* **358**, *9282*, pp. 625-628.

Fiennes, R. W. T. (1970), In *The African Trypanosomiases*. Pathogenesis and pathology of animal trypanosomiases. (Ed, Mulligan, H. W.) George Allen and Unwin, London, pp. 729-750.

Firth, D. (1991), In *Statistical theory and modelling. In honour of Sir David Cox*. Generalised linear models. (Eds, Hinkley, D. V., Reid, N. and Snell, E. J.) Chapman & Hall, London, pp. 183-187.

FITCA (2002), Livestock Census. Farming in Tsetse Control Areas (FITCA), Uganda.

Fivaz, B. H., Norval, R. A. and Lawrence, J. A. (1989), Transmission of *Theileria parva bovis* (Boleni strain) to cattle resistant to the brown ear tick *Rhipicephalus appendiculatus* (Neumann). *Tropical Animal Health and Production,* **21**, *2*, pp. 129-134.

Fivaz, B. H. and Norval, A. (1990), Immunity of the ox to the brown ear tick *Rhipicephalus appendiculatus*. *Experimental and Applied Acarology,* **8**, *1-2*, pp. 51-63.

Flanagan, H. O. and Le Roux, J. M. W. (1957), Bovine cerebral theileriosis - A report on two cases occurring in the Union. *Onderstepoort Journal of Veterinary Research,* **27**, pp. 453-461.

Fletcher, R. H., Fletcher, S. W. and Wagener, E. H. (1988), Clinical epidemiology: The essentials. Second edition. Williams and Wilkins, Baltimore.

Floyd, R. B., Maywald, G. F. and Sutherst, R. W. (1987), Ecological models. 2. A population model of *Rhipicephalus appendiculatus*. Ticks and tick-borne diseases: Proceedings of

an international workshop of the ecology of ticks and epidemiology of tick-borne diseases. Canberra, (Ed, Sutherst, R. W.) Australian Centre for International Agricultural Research.

Flynn, J. N. and Sileghem, M. (1993), Immunosuppression in trypanotolerant N'Dama cattle following *Trypanosoma congolense* infection. *Parasite Immunology,* **15**, *9*, pp. 547-552.

Ford, J. and Katondo, J. (1976), In *Atlas of Uganda*. The description of climatic conditions in Uganda. Department of Lands and Surveys, Uganda, pp. 16-21.

Frank, S. A. (1996), Models of parasite virulence. *The Quarterly Review of Biology,* **71**, *1*, pp. 37-78.

Frank, S. A. (1999), A model for the sequential dominance of antigenic variants in African trypanosome infections. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **266**, *1426*, pp. 1397-1401.

Frontera, E., Alcaide, M., Dominguez-Alpizar, J. L., Boes, J., Reina, D. and Navarrete, I. (2005), Evidence of interaction between *Ascaris suum* and *Metastrongylus apri* in experimentally infected pigs. *Veterinary Parasitology,* **127**, *3-4*, pp. 295-301.

Gale, K. R., Leatch, G., Devos, A. J. and Jorgensen, W. K. (1996), *Anaplasma marginale*: Effect of challenge of cattle with varying doses of infected erythrocytes. *International Journal for Parasitology,* **26**, *12*, pp. 1417-1420.

Ganesan, P. I., Appaji Rao, V. N. and Gunaseelan, I. (1995), A rare report of anaplasmosis in a calf. *Indian Veterinary Journal,* **72**, *5*, pp. 539.

Ganga, G., Varshney, J. P. and Sharma, R. L. (2006), Effect of *Fasciola gigantica* excretory secretory antigen on rat haematological indices. *Journal of Veterinary Science,* **7**, *2*, pp. 123-125.

Gauer, M., Mackenstedt, U., Mehlhorn, H. and Schein, E. (1995), DNA measurements and ploidy determination of developmental stages in the life cycles of *Theileria annulata* and *Theileria parva*. *Parasitology Research,* **81**, *7*, pp. 565-574.

Gauthier, C. and Tibayrenc, M. (2005), Population structure of malaria parasites: The driving epidemiological forces. *Acta Tropica,* **94**, *3*, pp. 241-250.

Geigy, R. and Kauffmann, M. (1973), Sleeping sickness survey in the Serengeti area (Tanzania) 1971. I. Examination of large mammals for trypanosomes. *Acta Tropica,* **30**, *1*, pp. 12-23.

Gennari, S. M., Abdalla, A. L., Vitti, D. M., Meirelles, C. F., Lopes, R. S. and Bressan, M. C. (1995), *Haemonchus placei* in calves: Effects of dietary protein and multiple experimental infection on worm establishment and pathogenesis. *Veterinary Parasitology,* **59**, *2*, pp. 119-126.

Gettinby, G. and Byrom, W. (1989), The dynamics of East Coast Fever: A modelling perspective for the integration of knowledge. *Parasitology Today,* **5**, *3*, pp. 68-73.

Geysen, D., Bishop, R., Skilton, R., Dolan, T. T. and Morzaria, S. (1999), Molecular epidemiology of *Theileria parva* in the field. *Tropical Medicine and International Health,* **4**, *9*, pp. A21-A27.

Geysen, D. (2000), The application of molecular biology techniques to analyse diversity in *Theileria parva* populations in Zambia. PhD Thesis. Brunel University, Uxbridge.

Geysen, D., Bazarusanga, T., Brandt, J. and Dolan, T. T. (2004), An unusual mosaic structure of the PIM gene of *Theileria parva* and its relationship to allelic diversity. *Molecular and Biochemical Parasitology,* **133**, *2*, pp. 163-173.

Giha, H. A., Staalsoe, T., Dodoo, D., Roper, C., Satti, G. M., Arnot, D. E., Hviid, L. and Theander, T. G. (2000), Antibodies to variable *Plasmodium falciparum*-infected erythrocyte surface antigens are associated with protection from novel malaria infections. *Immunology Letters,* **71**, *2*, pp. 117-126.

Gilbert, S. C., Plebanski, M., Gupta, S., Morris, J., Cox, M., Aidoo, M., Kwiatkowski, D., Greenwood, B. M., Whittle, H. C. and Hill, A. V. (1998), Association of malaria parasite population structure, HLA, and immunological antagonism. *Science,* **279**, *5354*, pp. 1173-1177.

Gill, A. C., Cowman, A. F., Stewart, N. P., Kemp, D. K. and Timms, P. (1987), *Babesia bovis* molecular and biological characteristics of cloned parasite lines. *Experimental Parasitology,* **63**, *2*, pp. 180-188.

Gitau, G. K., McDermott, J. J., Adams, J. E., Lissemore, K. D. and Waltner-Toews, D. (1994a), Factors influencing calf growth and daily weight gain on smallholder dairy farms in Kiambu district, Kenya. *Preventive Veterinary Medicine,* **21**, pp. 179-190.

Gitau, G. K., McDermott, J. J., Waltner-Toews, D., Lissemore, K. D., Osumo, J. M. and Muriuki, D. (1994b), Factors influencing calf morbidity and mortality on smallholder dairy farms in Kiambu district, Kenya. *Preventive Veterinary Medicine,* **21**, pp. 167-177.

Gitau, G. K., Perry, B. D., Katende, J. M., McDermott, J. J., Morzaria, S. P. and Young, A. S. (1997), The prevalence of serum antibodies to tick-borne infections in cattle in smallholder dairy farms in Murang'a district Kenya: A cross-sectional study. *Preventive Veterinary Medicine,* **30**, *2*, pp. 95-107.

Gitau, G. K., Perry, B. D. and McDermott, J. J. (1999a), The incidence, calf morbidity and mortality due to *Theileria parva* infections in smallholder dairy farms in Murang'a District, Kenya. *Preventive Veterinary Medicine,* **39**, *1*, pp. 65-79.

Gitau, G. K., McDermott, J. J., Katende, J. M. and Perry, B. D. (1999b), The epidemiology of *Theileria parva* infections on smallholder dairy farms in Kenya. Tropical veterinary diseases, control and prevention in the context of the new world order. Key West, FL, (Eds, House, J. A., Kocan, K. M. and Gibbs, E. P. J.) Society for Tropical Veterinary Medicine; New York, Jun, pp. 265-270.

Gitau, G. K., McDermott, J. J., Katende, J. M., O Callaghan, C. J. and Perry, B. D. (2000a), Differences in the epidemiology of theileriosis on smallholder dairy farms in contrasting agro-ecological and grazing strata of highland Kenya. *Epidemiology and Infection,* **124**, pp. 330-332.

Gitau, G. K., McDermott, J. J., Katende, J. M. and Perry, B. D. (2000b), The epidemiology of *Theileria parva* infections on smallholder dairy farms in Kenya. *Annals of the New York Academy of Sciences,* **916**, pp. 265-270.

Gitau, G. K., McDermott, J. J., McDermott, B. and Perry, B. D. (2001), The impact of *Theileria parva* infections and other factors on calf mean daily weight gains in smallholder dairy farms in Murang'a District, Kenya. *Preventive Veterinary Medicine,* **51**, *3-4*, pp. 149-160.

Goddeeris, B. M., Morrison, W. I., Teale, A. J., Bensaid, A. and Baldwin, C. L. (1986), Bovine cytotoxic T-cell clones specific for cells infected with the protozoan parasite *Theileria parva*: Parasite strain specificity and class I major histocompatibility complex restriction.

Proceedings of the National Academy of Sciences of the United States of America, **83**, *14*, pp. 5238-5242.

Goddeeris, B. M., Morrison, W. I., Toye, P. G. and Bishop, R. P. (1990), Strain specificity of bovine *Theileria parva*-specific cytotoxic T cells is determined by the phenotype of the restricting class I MHC. *Immunology,* **69**, *1*, pp. 39-44.

Gonder, R. (1910), The life cycle of *Theileria parva*: The cause of East Coast Fever in cattle in South Africa. *Journal of Comparative Pathology,* **23**, pp. 328-335.

Gonzalez-Lanza, C., Manga-Gonzalez, Y., Del-Pozo-Carnero, P. and Hidalgo-Arguello, R. (1989), Dynamics of elimination of the eggs of *Fasciola hepatica* Trematoda Digenea in the faeces of cattle in the Porma Basin Spain. *Veterinary Parasitology,* **34**, *1-2*, pp. 35-44.

Goossens, B., Osaer, S., Kora, S., Jaitner, J., Ndao, M. and Geerts, S. (1997), The interaction of *Trypanosoma congolense* and *Haemonchus contortus* in Djallonke sheep. *International Journal for Parasitology,* **27**, *12*, pp. 1579-1584.

Graham, S. P., Brown, D. J., Vatansever, Z., Waddington, D., Taylor, L. H., Nichani, A. K., Campbell, J. D. M., Adamson, R. E., Glass, E. J. and Spooner, R. L. (2001), Proinflammatory cytokine expression by *Theileria annulata* infected cell lines correlates with the pathology they cause in vivo. *Vaccine,* **19**, *20-22*, pp. 2932-2944.

Graham, S. P., Pelle, R., Honda, Y., Mwangi, D. M., Tonukari, N. J., Yamage, M., Glew, E. J., de Villiers, E. P., Shah, T., Bishop, R., Abuya, E., Awino, E., Gachanja, J., Luyai, A. E., Mbwika, F., Muthiani, A. M., Ndegwa, D. M., Njahira, M., Nyanjui, J. K., Onono, F. O., Osaso, J., Saya, R. M., Wildmann, C., Fraser, C. M., Maudlin, I., Gardner, M. J., Morzaria, S. P., Loosmore, S., Gilbert, S. C., Audonnet, J. C., van der Bruggen, P., Nene, V. and Taracha, E. L. (2006), *Theileria parva* candidate vaccine antigens recognised by immune bovine cytotoxic T-lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America,* **103**, *9*, pp. 3286-3291.

Graves, P. M., Carter, R. and McNeill, K. M. (1984), Gametocyte production in cloned lines of *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene,* **33**, *6*, pp. 1045-1050.

Grenfell, B. T. and Dobson, A. P. (Eds.) (1995), Ecology of infectious diseases in natural populations. Cambridge University Press, Cambridge.

Griffin, L., Allonby, E. W. and Preston, J. M. (1981a), The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in 2 breeds of goat. 1. Parasitology. *Journal of Comparative Pathology,* **91**, *1*, pp. 85-95.

Griffin, L., Aucutt, M., Allonby, E. W., Preston, J. and Castelino, J. (1981b), The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in 2 breeds of goat. 2. Haematology. *Journal of Comparative Pathology,* **91**, *1*, pp. 97-103.

Griffiths, R. B. (1957), Retort to the Government of Burma on parasitic diseases of livestock. Report No. 684. F.A.O., Rome.

Grootenhuis, J. G., Young, A. S., Dolan, T. T. and Stagg, D. A. (1979), Characteristics of Theileria species (eland) infections in elands and cattle. *Research in Veterinary Science,* **27**, pp. 59-68.

Grootenhuis, J. G., Young, A. S., Stagg, D. A., Leitch, B. L., Dolan, T. T. and Conrad, P. A. (1987), Infection of African buffalo (*Syncerus caffer*) and cattle with *Theileria parva lawrencei* after serial passage in cattle. *Research in Veterinary Science,* **42**, *3*, pp. 326-330.

Grootenhuis, J. G., Dwinger, R. H., Dolan, R. B., Moloo, S. K. and Murray, M. (1990), Susceptibility of African buffalo and Boran cattle to *Trypanosoma congolense* transmitted by Glossina morsitans centralis. *Veterinary Parasitology,* **35**, *3*, pp. 219-231.

Gunders, A. E. (1977), Piroplasmal sporozoites in the Argasid *Ornithodoros erraticus* (Lucas). *Experientia,* **33**, *7*, pp. 892-893.

Gupta, S., Swinton, J. and Anderson, R. M. (1994a), Theoretical studies of the effects of heterogeneity in the parasite population on the transmission dynamics of malaria. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **256**, *1347*, pp. 231-238.

Gupta, S., Trenholme, K., Anderson, R. M. and Day, K. P. (1994b), Antigenic diversity and the transmission dynamics of *Plasmodium falciparum*. *Science,* **263**, *5149*, pp. 961-963.

Gupta, S., Maiden, M. C., Feavers, I. M., Nee, S., May, R. M. and Anderson, R. M. (1996a), The maintenance of strain structure in populations of recombining infectious agents. *Nature Medicine,* **2**, *4*, pp. 437-442.

Gupta, S. and Day, K. (1996b), In *Models for infectious human diseases: Their structure and relation to data*. Virulence and transmissibility in *P. falciparum* malaria. (Eds, Isham, V. and Medley, G.) Cambridge University Press, Cambridge.

Gupta, S., Ferguson, N. and Anderson, R. (1998), Chaos, persistence, and evolution of strain structure in antigenically diverse infectious agents. *Science,* **280**, *5365*, pp. 912-915.

Gupta, S. and Galvani, A. (1999a), The effects of host heterogeneity on pathogen population structure. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* **354**, *1384*, pp. 711-719.

Gupta, S. and Anderson, R. M. (1999b), Population structure of pathogens: The role of immune selection. *Parasitology Today,* **15**, *12*, pp. 497-501.

Gupta, S. (2005), Parasite immune escape: New views into host-parasite interactions. *Current Opinion in Microbiology,* **8**, *4*, pp. 428-433.

Halstead, S. B. and O'Rourke, E. J. (1977a), Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *Journal of Experimental Medicine,* **146**, *1*, pp. 201-217.

Halstead, S. B., O'Rourke, E. J. and Allison, A. C. (1977b), Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. *Journal of Experimental Medicine,* **146**, *1*, pp. 218-229.

Hansen, F. and Perry, B. D. (1994), The epidemiology, diagnosis and control of helminth parasites of ruminants. ILRAD, Nairobi, Kenya.

Haraguchi, Y. and Sasaki, A. (1997). *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* **352**, pp. 11-20.

Harness, E., Fitzsimmons, W. M. and Sellwood, S. A. (1970), Experimental *Haemonchus placei* infection in calves. Blood picture at three levels of infection. *Journal of Comparative Pathology,* **80**, *2*, pp. 173-179.

Harness, E., Sellwood, S. A. and Young, E. R. (1971), Experimental *Haemonchus placei* infection in calves. Influence of anaemia and numbers of larvae on worm development. *Journal of Comparative Pathology,* **81**, *1*, pp. 129-136.

Hart, A. R. and Cloyd, M. W. (1990), Interference patterns of human immunodeficiency viruses HIV-1 and HIV-2. *Virology,* **177**, *1*, pp. 1-10.

Harville, D. A. (1977), Maximum likelihood approaches to variance component estimation and to related problems. *Journal of the American Statistical Association,* **72**, pp. 320-340.

Hastings, I. M. and D'Alessandro, U. (2000), Modelling a predictable disaster: The rise and spread of drug-resistant malaria. *Parasitology Today,* **16**, *8*, pp. 340-347.

Heath, D. D. (1995), In *Echinococcus and hydatid disease*. Immunology of Echinococcus infections. (Eds, Thompson, R. C. A. and Lymbery, A. J.) CAB International, Wallingford, Oxford, pp. 183-200.

Heller-Haupt, A., Varma, M. G., Langi, A. O. and Zetlin, A. (1983), The effect of *Trypanosoma congolense* infection on acquired immunity to the tick *Rhipicephalus appendiculatus*. *Annals of Tropical Medicine and Parasitology,* **77**, *2*, pp. 219-222.

Hellriegel, B. (1992), Modelling the immune response to malaria with ecological concepts: Short-term behaviour against long-term equilibrium. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **250**, *1329*, pp. 249-256.

Hendrickx, G., Napala, A., Slingenbergh, J. H., De Deken, R., Vercruysse, J. and Rogers, D. J. (2000), The spatial pattern of trypanosomosis prevalence predicted with the aid of satellite imagery. *Parasitology,* **120**, *2*, pp. 121-134.

Hide, G., Tait, A., Maudlin, I. and Welburn, S. C. (1996), The origins, dynamics and generation of *Trypanosoma brucei rhodesiense* epidemics in East Africa. *Parasitology Today,* **12**, *2*, pp. 50-54.

Hildebrandt, D. (1981), In *Babesiosis*. The organ and vascular pathology of babesiosis. (Eds, Ristic, M. and Kreier, J. P.) Academic Press, pp. 459-473.

Hilleman, M. R. (2004), Strategies and mechanisms for host and pathogen survival in acute and persistent viral infections. *Proceedings of the National Academy of Sciences of the United States of America,* **101**, *2*, pp. 14560-14566.

Hoare, C. A. (1947), Tsetse - borne trypanosmiasis outside their natural boundaries. *Annales da la Societe Belge de Medecine Tropicale (Liber Jubilaris J. Rodhain)*, pp. 267-277.

Hodgson, D. J., Hitchman, R. B., Vanbergen, A. J., Hails, R. S., Possee, R. D. and Cory, J. S. (2004), Host ecology determines the relative fitness of virus genotypes in mixed-genotype nucleopolyhedrovirus infections. *Journal of Evolutionary Biology,* **17**, *5*, pp. 1018-1025.

Hofmann-Lehmann, R., Meli, M. L., Dreher, U. M., Gonczi, E., Deplazes, P., Braun, U., Engels, M., Schupbach, J., Jorger, K., Thoma, R., Griot, C., Stark, K. D., Willi, B., Schmidt, J., Kocan, K. M. and Lutz, H. (2004), Concurrent infections with vector-borne pathogens associated with fatal haemolytic anaemia in a cattle herd in Switzerland. *Journal of clinical microbiology,* **42**, *8*, pp. 3775-3780.

Holland, J. J. (1990), In *Virology*. Defective viral genomes. (Eds, Fields, B. N., Knipe, D. M., Chanock, R. M., Hirsch, M. S., Melnick, J. L., Monath, T. P. and Roizman, B.) Raven Press, Ltd, New York, pp. 151-165.

Holmes, P. H. and Scott, J. M. (1982), In *Perspectives in trypanosomiasis research*. Chemotherapy against animal trypanosomiasis. (Ed, Baker, J. R.) Research Studies Press, Chichester, UK, pp. 59-69.

Holmes, P. H. and Torr, S. J. (1988), The control of animal trypanosomiasis in Africa: Current methods and future trends. *Outlook on Agriculture,* **17**, pp. 54-60.

Holmes, P. H. (1997), New approaches to the integrated control of trypanosomosis. *Veterinary Parasitology,* **71**, *2-3*, pp. 121-135.

Holmes, P. H., Katunguka-Rwakishaya, E., Bennison, J. J., Wassink, G. J. and Parkins, J. J. (2000), Impact of nutrition on the pathophysiology of bovine trypanosomiasis. Veterinary parasitology: Recent developments in immunology, epidemiology and control. (Eds, Martin, R. J. and Schallig, H. D. F. H.) Cambridge. S73-S86.

Holmstad, P. R., Hudson, P. J. and Skorping, A. (2005), The influence of a parasite community on the dynamics of a host population: A longitudinal study on willow ptarmigan and their parasites. *Oikos,* **111**, *2*, pp. 377-391.

Homan, W. L. and Mank, T. G. (2001), Human giardiasis: Genotype linked differences in clinical symptomatology. *International Journal for Parasitology,* **31**, pp. 822-826.

Homer, M. J., Aguilar-Delfin, I., Telford, S. R., Krause, P. J. and Persing, D. H. (2000), Babesiosis. *Clinical Microbiology Reviews,* **13**, *3*, pp. 451-469.

Honda, Y., Matsubara, Y., Morzaria, S. and McKeever, D. (1998), Immunohistochemical detection of parasite antigens in *Theileria parva*-infected bovine lymphocytes. *Veterinary Parasitology,* **80**, *2*, pp. 137-147.

Hood, M. E. (2003), Dynamics of multiple infection and within-host competition by the anther-smut pathogen. *American Naturalist,* **162**, *1*, pp. 122-133.

Hoogstraal, H. (1956), African *Ixodoidea*. 1. Ticks of the Sudan (with special reference to Equatorial Province and with preliminary reviews of the genera Boophilus, Margaropus and Hyalomma). Research Report NM 005.050.29.27. Department of the Navy, Bureau of Medicine and Surgery, Washington D. C. **1**, pp. 1-101.

Horchner, F. (1969), Clinical and immunological studies on cattle experimentally infected with *Fasciola hepatica*. *Berliner Und Munchener Tierarztliche Wochenschrift,* **82**, *11*, pp. 204-208.

Hotter, Edelhofer and Baumgartner (1995), Mixed infection with *Anaplasma marginale* and *Babesia divergens* in cattle. *Tierarztliche Umschau,* **50**, *4*, pp. 280.

Huang, A. S. and Baltimore, D. (1970), Defective viral particles and viral disease processes. *Nature,* **226**, *5243*, pp. 325-327.

Hughes, D. L., Purnell, R. E. and Brockles, D. W. (1977), The effect of initial *Fasciola hepatica* infection on the pathogenicity of subsequent *Babesia divergens* infections in intact and splenectomised calves. *Veterinary Record,* **100**, *15*, pp. 320-321.

Iams, K. P., Young, J. R., Nene, V., Desai, J., Webster, P., Ole-Moiyoi, O. K. and Musoke, A. J. (1990), Characterisation of the gene encoding a 104-Kilodalton micronemerhoptry protein of *Theileria parva*. *Molecular and Biochemical Parasitology,* **39**, *1*, pp. 47-60.

ICPTV (2002), Proceedings of the joint ICTTD-2 / ICPTV Workshop on integrated vector control including synergistic use of drugs and bait technologies for the control of trypanosomiasis and tick-borne diseases. Institute of Tropical Medicine, Antwerp, Belgium, 10 - 12 April 2002.

Ihaka, R. and Gentleman, R. (1996), R: A Language for data analysis and graphics. *Journal of Computational and Graphical Statistics,* **5**, pp. 299-314.

IPCC (2001a), The regional impacts of climate change: An assessment of vulnerability [Online]. The International Panel on Climate Change. Available at www..ipcc.ch. Accessed 01/06/06.

IPCC (2001b), Climate change 2001: The scientific basis [Online]. Working Group 1, Contribution to the IPCC Third Assessment Report. The International Panel on Climate Change. Available at www..ipcc.ch. Accessed 01/06/06.

Irvin, A. D., Brown, C. G., Burridge, M. J., Cunningham, M. P., Musoke, A. J., Pierce, M. A., Purnell, R. E. and Radley, D. E. (1972), A pathogenic theilerial syndrome of cattle in the Narok District of Kenya. I. Transmission studies. *Tropical Animal Health and Production,* **4**, *4*, pp. 220-229.

Isham, V. and Medley, G. (Eds.) (1996), Models for infectious human diseases: Their structure and relation to data. Cambridge University Press, Cambridge.

Ishii, T., Takatsuka, J., Nakai, M. and Kunimi, Y. (2002), Growth characteristics and competitive abilities of a nucleopolyhedrovirus and an entomopoxvirus in larvae of the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae). *Biological Control,* **23**, pp. 96-105.

ITTBDP (1999), Integrated tick and tick-borne disease control project. Training reports. Kiteto District, Tanzania.

ITTBDP (2000), Integrated tick and tick-borne disease control project. Training reports. Ngorongoro Conservation Authority, Tanzania.

Jaetzold, R. and Schmidt, H. (1983), In *Farm Management Handbook of Kenya*. Natural Conditions and Farm Management Information - Part C, East Kenya (Eastern and Coast Provinces). Ministry of Agriculture, Kenya, in Cooperation with the German Agricultural Team (GAT) of the German Agency for Technical Cooperation (GTZ), **2**, 411 pages.

Jatkar, P. R. and Kreier, J. P. (1967), Pathogenesis of anaemia in Anaplasma infection. *Indian Veterinary Journal,* **44**, *5*, pp. 393-399.

Jatkar, P. R. and Kreier, J. P. (1969), Pathogenesis of anaemia in anaplasma infection II - Auto-antibody and anaemia. *Indian Veterinary Journal,* **46**, *7*, pp. 560-566.

Johnson, W. E. and Desrosiers, R. C. (2002), Viral persistance: HIV's strategies of immune system evasion. *Annual Review of Medicine,* **53**, pp. 499-518.

Jones, R. H. (1993), Longitudinal data with serial correlation: A state-space approach. Chapman & Hall, London.

Jongejan, F., Perry, B. D., Moorhouse, P. D., Musisi, F. L., Pegram, R. G. and Snacken, M. (1988), Epidemiology of bovine babesiosis and anaplasmosis in Zambia. *Tropical Animal Health and Production,* **20**, *4*, pp. 234-242.

Jongejan, F., Pegram, R. G., Zivkovic, D., Hensen, E. J., Mwase, E. T., Thielemans, M. J. C., Cosse, A., Niewold, T. A., El Said, A. and Uilenberg, G. (1989), Monitoring of naturally acquired and artificially induced immunity to *Amblyomma variegatum* and *Rhipicephalus appendiculatus* ticks under field and laboratory conditions. *Experimental and Applied Acarology,* **7**, *3*, pp. 181-200.

Julla, I. I. (1985), Theileriosis in southern Sudan. Immunisation against theileriosis in Africa. Nairobi, (Ed, Irvin, A. D.) International Laboratory for Research on Animal Diseases, 1-5 October, 1984, pp. 27-30.

Julla, I. I., Tingwa, N. O. and Nwajok, V. L. (1989), Theileriosis in the Equatoria region of Sudan. Theileriosis in eastern, central and southern Africa. Lilongwe, Malawi, (Ed, Dolan, T. T.) International Laboratory for Research on Animal Diseases, Nairobi, 20-22 September, 1988, pp. 19-21.

Kaiser, M. N., Sutherst, R. W. and Bourne, A. S. (1982), Relationship between ticks and Zebu cattle in southern Uganda. *Tropical Animal Health and Production,* **14**, pp. 63-74.

Kaiser, M. N., Sutherst, R. W. and Bourne, A. S. (1991), Tick Acarina Ixodidae infestations on Zebu cattle in northern Uganda. *Bulletin of Entomological Research,* **81**, *3*, pp. 257-262.

Kakoma, I. and Mehlhorn, H. (1993), In *Parasitic Protozoa*. Babesia of domestic animals. **7**, (Ed, Kreier, J. P.) Academic Press, San Diego, California, pp. 141-216.

Kalu, A. U. (1995), Prevalence of trypanosomiasis among trypanotolerant cattle at the lower Benue River area of Nigeria. *Preventive Veterinary Medicine,* **24**, pp. 97-103.

Kalu, A. U. and Lawani, F. A. (1996), Observations on the epidemiology of ruminant trypanosomosis in Kano State, Nigeria. *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux,* **49**, *3*, pp. 213-218.

Kambarage, D. M. (1995), East Coast Fever as a continued constraint to livestock improvement in Tanzania: A case study. *Tropical Animal Health and Production,* **27**, *3*, pp. 145-149.

Kaplan, R. M. (2004), Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology,* **20**, *10*, pp. 477-481.

Kariuki, D. P. (1990), In *Progress towards the control of East Coast Fever (theileriosis) in Kenya*. Current status of theileriosis in Kenya, 1989. (Eds, Young, A. S., Mutugi, J. J. and Maritim, A. C.) Kenya Agricultural Research Institute, Nairobi, pp. 17-26.

Kariuki, D. P., Young, A. S., Morzaria, S. P., Lesan, A. C., Mining, S. K., Omwoyo, P., Wafula, J. M. and Molyneux, D. H. (1995), *Theileria parva* carrier state in naturally infected and artificially immunised cattle. *Tropical Animal Health and Production,* **27**, *1*, pp. 15-25.

Katende, J., Morzaria, S., Toye, P., Skilton, R., Nene, V., Nkonge, C. and Musoke, A. (1998), An enzyme-linked immunosorbent assay for detection of *Theileria parva* antibodies in cattle using a recombinant polymorphic immunodominant molecule. *Parasitology Research,* **84**, *5*, pp. 408-416.

Kaufmann, J., Komma, A. and Pfister, K. (1989), Studies on the epidemiology of gastrointestinal helminths in N'Dama cattle in The Gambia. Proceedings of the 16th International Conference of Institutes of Tropical Veterinary Medicine. Wageningen, The Netherlands, pp. 95-99.

Kaufmann, J. and Pfister, K. (1990), The seasonal epidemiology of gastrointestinal nematodes in N'Dama cattle in The Gambia. *Veterinary Parasitology,* **37**, *1*, pp. 45-54.

Kaufmann, J., Dwinger, R. H., Hallebeek, A., van Dijk, B. and Pfister, K. (1992), The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in trypanotolerant N'Dama cattle. *Veterinary Parasitology,* **43**, *3-4*, pp. 157-170.

Keusch, G. T. and Migasena, P. (1982), Biological implications of polyparasitism. *Reviews of Infectious Diseases,* **4**, *4*, pp. 880-882.

Kew, O., Morris-Glasgow, V., Landaverde, M., Burns, C., Shaw, J., Garib, Z., Andre, J., Blackman, E., Freeman, C. J., Jorba, J., Sutter, R., Tambini, G., Venczel, L., Pedreira, C., Laender, F., Shimizu, H., Yoneyama, T., Miyamura, T., van Der Avoort, H., Oberste, M. S., Kilpatrick, D., Cochi, S., Pallansch, M. and de Quadros, C. (2002), Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. *Science,* **296**, *5566*, pp. 356-359.

Keyyu, J. D., Kassuku, A. A., Msalilwa, L. P., Monrad, J. and Kyvsgaard, N. C. (2006), Cross-sectional prevalence of helminth infections in cattle on traditional, small-scale and large-scale dairy farms in Iringa district, Tanzania. *Veterinary Research Communications,* **30**, *1*, pp. 45-55.

Kieave, J. J. (2001), Lifecycle of *Haemonchus contortus* [Online]. Available at http://www.newrhabdias.kiev.ua/biology.htm. Accessed 22/05/04.

Kikule, S. B. (1953), Age changes in the teeth of Zebu cattle. *East African Agricultural Journal,* **19**, pp. 86-88.

Killick-Kendrick, R. (1971), The low pathogenicity of *Trypanosoma brucei* to cattle. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **65**, *1*, pp. 104.

Kimmel, B. E., ole-MoiYoi, O. K. and Young, J. R. (1987), *Ingi*, a 5.2-kb dispersed sequence element from *Trypanosoma brucei* that carries half of a smaller mobile element at either end and has homology with mammalian lines. *Molecular and Cellular Biology,* **7**, pp. 1465-1475.

Kivaria, F. M., Heuer, C., Jongejan, F., Okello-Onen, J., Rutagwenda, T., Unger, F. and Boehl, W. (2004), Endemic stability for *Theileria parva* infections in Ankole calves of the Ankole ranching scheme, Uganda. *The Onderstepoort journal of veterinary research,* **71**, *3*, pp. 189-195.

Klenerman, P., Rowland-Jones, S., McAdam, S., Edwards, J., Daenke, S., Lalloo, D., Koppe, B., Rosenberg, W., Boyd, D., Edwards, A. and et al. (1994), Cytotoxic T-cell activity antagonized by naturally occurring HIV-1 Gag variants. *Nature,* **369**, *6479*, pp. 403-407.

Kliks, S. C., Nimmanitya, S., Nisalak, A. and Burke, D. S. (1988), Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *American Journal of Tropical Medicine and Hygiene,* **38**, *2*, pp. 411-419.

Knight, R. A. (1978), Experimental cross infections of Fasciola hepatica in lambs and calves. *Journal of Parasitology,* **64**, *4*, pp. 601-605.

Knowles, G. and Walliker, D. (1980), Variable expression of virulence in the rodent malaria parasite *Plasmodium yoelii yoelii*. *Parasitology,* **81**, *1*, pp. 211-219.

Kocan, K. M. (1986), In *Morphology, Physiology and Behavioural Ecology of Ticks*. Development of *Anaplasma marginale* in ixodid ticks: Coordinated development of a rickettsial organism and its tick host. (Eds, Sauer, J. and Hair, J. A.) Ellis Horwood Ltd., England, pp. 472-505.

Kocan, K. M., Blouin, E. F. and Barbet, A. F. (2000), In *Tropical Veterinary Diseases*. Anaplasmosis control - Past, present, and future. Annals of the New York Academy of Sciences. **916**, pp. 501-509.

Kocan, K. M., de la Fuente, J., Guglielmone, A. and Melendez, R. D. (2003), Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. *Clinical Microbiology Reviews,* **16**, *4*, pp. 698-712.

Koch, H. T., Ocama, J. G., Munatswa, F. C., Byrom, B., Norval, R. A., Spooner, P. R., Conrad, P. A. and Irvin, A. D. (1988), Isolation and characterisation of bovine Theileria parasites in Zimbabwe. *Veterinary Parasitology,* **28**, *1-2*, pp. 19-32.

Koelle, D. M., Liu, Z., McClurkan, C. L., Cevallos, R. C., Vieira, J., Hosken, N. A., Meseda, C. A., Snow, D. C., Wald, A. and Corey, L. (2003), Immunodominance among herpes simplex virus-specific CD8 T cells expressing a tissue-specific homing receptor. *Proceedings of the National Academy of Sciences of the United States of America,* **100**, *22*, pp. 12899-12904.

Konate, L., Zwetyenga, J., Rogier, C., Bischoff, E., Fontenille, D., Tall, A., Spiegel, A., Trape, J. F. and Mercereau-Puijalon, O. (1999), Variation of *Plasmodium falciparum* msp1 block 2 and msp2 allele prevalence and of infection complexity in two neighbouring Senegalese villages with different transmission conditions. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **93**, *1*, pp. 21-28.

Kreier, J. P., Ristic, M. and Schroeder, W. (1964), Anaplasmosis .16. Pathogenesis of anaemia produced by infection with anaplasma. *American Journal of Veterinary Research,* **25**, *105*, pp. 343-352.

Kristjanson, P. M., Swallow, B. M., Rowlands, G. J., Kruska, R. L. and De Leeuw, P. N. (1999), Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. *Agricultural Systems,* **59**, pp. 79-98.

Kuzoe, F. A. S. (1991), Perspectives in Research on and Control of African Trypanosomiasis. *Annals of Tropical Medicine and Parasitology,* **85**, *1*, pp. 33-41.

Laha, R., Chakravarti, I. and Roy, J. (1967), Handbook of methods of applied statistics, **I**. John Wiley and Sons, Chichester.

Laird, N. M. and Ware, J. H. (1982), Random-effects models for longitudinal data. *Biometrics,* **38**, pp. 963-974.

Lambrechts, L., Fellous, S. and Koella, J. C. (2006), Co-evolutionary interactions between host and parasite genotypes. *Trends in Parasitology,* **22**, *1*, pp. 12-16.

Lancien, J., Muguwa, J., Lannes, C. and Bouvier, J. B. (1990), Tsetse and human trypanosomiasis challenge in south-eastern Uganda. *Insect Science and its Applications,* **11**, pp. 411-416.

Latif, A. A., Rowlands, G. J., Punyua, D. K., Hassan, S. M. and Capstick, P. B. (1995), An epidemiological study of tick-borne diseases and their effects on productivity of Zebu cattle under traditional management of Rusinga Island, western Kenya. *Preventive Veterinary Medicine,* **22**, *3*, pp. 169-181.

Le Roux, P. (1957), Retort to the Government of the Federation of Rhodesia and Nyasaland on the control of parasitic diseases in livestock. Report No. 696. F.A.O., Rome. 47 pages.

Leak, S. G., Mulatu, W., Authie, E., d'Ieteren, G. D., Peregrine, A. S., Rowlands, G. J. and Trail, J. C. (1993), Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. 1. Tsetse challenge and its relationship to trypanosome prevalence in cattle. *Acta Tropica,* **53**, *2*, pp. 121-134.

Lee, R. P. (1955), Report on a preliminary investigation of bovine parasitic - gastroenteritis in Western Region of Nigeria. Federal Department of Veterinary Research. 28 pages.

Leeflang, P., Buys, J. and Blotkamp, C. (1976), Studies on *Trypanosoma vivax*: Infectivity and serial maintenance of natural bovine isolates in mice. *International Journal for Parasitology,* **6**, *5*, pp. 413-417.

Lehane, M. J., Msangi, A. R., Whitaker, C. J. and Lehane, S. M. (2000), Grouping of trypanosome species in mixed infections in *Glossina pallidipes*. *Parasitology,* **120**, *6*, pp. 583-592.

Lehmann, E. L. (1986), Testing statistical hypotheses. Wiley, New York.

Lello, J., Boag, B., Fenton, A., Stevenson, I. R. and Hudson, P. J. (2004), Competition and mutualism amongst the gut helminths of a mammalian host. *Nature,* **428**, pp. 840-844.

Levin, S. and Pimentel, D. (1981), Selection of intermediate rates of increase in parasite-host systems. *American Naturalist,* **117**, pp. 308-315.

Levine, N. D. (1988), The protozoan phylum apicomplexa, **2**. CRC Press, Boca Raton, Florida.

LID (1999), Livestock in Poverty Focused Development. Livestock in Development (LID), Crewkerne, Somerset, UK. 95 pages.

Lipsitch, M. and Moxon, E. R. (1997), Virulence and transmissibility of pathogens: What is the relationship? *Trends in Microbiology,* **5**, *1*, pp. 31-37.

Lonibardo, R. A. (1976), Socioeconomic importance of the tick problem in the Americas. *PAHO Scientific Publication,* **316**, pp. 79.

Loomu, P. (1988), District veterinary officer: Report to veterinary investigation centre. Ref. VY/NGOR/1969/2 of 1988. Arusha.

Lord, C. C., Barnard, B., Day, K., Hargrove, J. W., McNamara, J. J., Paul, R. E. L., Trenholme, K. and Woolhouse, M. E. J. (1999), Aggregation and distribution of strains in microparasites. *Philosophical Transactions of the Royal Society of London B Biological Sciences,* **354**, *1384*, pp. 798-807.

Losos, G. J. (1986), Infectious tropical diseases of domestic animals. First edition. Churchill Livingstone Inc., New York.

Lynen, G., Gubbels, M.-J., Giulio, G., Bakuname, C., Mollel, J., Loomu, P., Bekker, C. and Jongejan, F. (2001), Cerebral theileriosis in African short-horn Zebu cattle is caused by *Theileria taurotragi* and not by *Theileria parva*. Proceedings of the International Joint Conference of the Society for Tropical Veterinary Medicine and the Wildlife Disease Association. Pilansberg, 22 to 27 July 2001.

Machange, J. (1997), In *Multiple land-use: The experience of the Ngorongoro conservation area, Tanzania*. Livestock and wildlife interactions. (Ed, Thompson, D. M.) IUCN - The World Conservation Union, Gland, Switzerland, pp. 127-141.

Mackinnon, M. J. and Read, A. F. (1999), Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. *Evolution,* **53**, pp. 689-703.

MacLeod, A., Tweedie, A., Welburn, S. C., Maudlin, I., Turner, C. M. and Tait, A. (2000), Minisatellite marker analysis of *Trypanosoma brucei*: Reconciliation of clonal, panmictic, and epidemic population genetic structures. *Proceedings of the National Academy of Sciences of the United States of America,* **97**, *24*, pp. 13442-13447.

Magesa, S. M., Mdira, K. Y., Babiker, H. A., Alifrangis, M., Farnert, A., Simonsen, P. E., Bygbjerg, I. C., Walliker, D. and Jakobsen, P. H. (2002), Diversity of *Plasmodium falciparum* clones infecting children living in a holoendemic area in north-eastern Tanzania. *Acta Tropica,* **84**, *2*, pp. 83-92.

Magona, J. W., Katabazi, W., Olaho-Mukani, W., Mayende, J. S. P. and Walubengo, J. (1997), Haemorrhagic *Trypanosoma vivax* outbreak in cattle in Mbale and Tororo districts in eastern Uganda. *Journal of Protozoology Research,* **7**, *2*, pp. 48-53.

Magona, J. W. and Musisi, G. (1998), Development of a strategic control method for helminthosis in cattle under communal grazing system. Conference for the Centenary Celebrations of 100 years of Agricultural Research in Uganda held at the Imperial Botanical Beach Hotel. Entebbe, Uganda, October 1998, pp. 5-8.

Magona, J. W., Olaho-Mukani, W., Musisi, G. and Walubengo, J. (1999), Bovine fasciola infection survey in Uganda. *Bulletin of Animal Health and Production in Africa,* **47**, pp. 9-14.

Magona, J. W., Greiner, M. and Mehlitz, D. (2000), Impact of tsetse control on the age-specific prevalence of trypanosomosis in village cattle in south east Uganda. *Tropical Animal Health and Production,* **32**, *2*, pp. 87-98.

Magona, J. W. and Mayende, J. S. P. (2002), Occurrence of concurrent trypanosomosis, theileriosis, anaplasmosis and helminthosis in Friesian, Zebu and Sahiwal cattle in Uganda. *Onderstepoort Journal of Veterinary Research,* **69**, *2*, pp. 133-140.

Magona, J. W. (2004), Point of care diagnosis of bovine trypanosomosis, tick-borne diseases and helminthoses with emphasis on portable anaemia-detection devices and decision support systems. PhD Thesis. Faculty of Veterinary Medicine; University of Glasgow, Scotland.

Mahoney, D. F. and Ross, D. R. (1972), Epizootiological factors in the control of bovine babesiosis. *Australian Veterinary Journal,* **48**, *5*, pp. 292-298.

Malone, J. B. (1986), Fascioliasis and cestodiasis in cattle. *Veterinary Clinics of North America - Food Animal Practice,* **2**, *2*, pp. 261-275.

Maloo, S. H., Rowlands, G. J., Thorpe, W., Gettinby, G. and Perry, B. D. (2001a), A longitudinal study of disease incidence and case-fatality risks on small-holder dairy farms in coastal Kenya. *Preventive Veterinary Medicine,* **52**, *1*, pp. 17-29.

Maloo, S. H., Ngumi, P., Mbogo, S., Williamson, S., Thorpe, W., Rowlands, G. J. and Perry, B. D. (2001b), Identification of a target population for immunisation against East Coast Fever in coastal Kenya. *Preventive Veterinary Medicine,* **52**, *1*, pp. 31-41.

Mango, A. M., Mango, C. K., Esamal, D. and Kariuki, D. (1974), Prevalence of selected common parasitic helminths of livestock in Kenya. *Veterinary Record,* **94**, *19*, pp. 432-435.

Maritim, A. C., Young, A. S., Lesan, A. C., Ndungu, S. G., Mutugi, J. J. and Stagg, D. A. (1989a), Theilerial parasites isolated from carrier cattle after immunisation with *Theileria parva* by the infection and treatment method. *Parasitology,* **99**, *1*, pp. 139-147.

Maritim, A. C., Kariuki, D. P., Young, A. S. and Mutugi, J. J. (1989b), In *Theileriosis in Eastern, Central and Southern Africa*. The importance of the carrier state of *Theileria parva* in the epidemiology of theileriosis and its control by immunisation. (Ed, Dolan, T. T.) International Laboratory for Research on Animal Diseases, Nairobi, pp. 121-128.

Marsh, K. and Howard, R. J. (1986), Antigens induced on erythrocytes by *Plasmodium falciparum* - Expression of diverse and conserved determinants. *Science,* **231**, *4734*, pp. 150-153.

Masake, R. A. and Morrison, W. I. (1981), Evaluation of the structural and functional changes in the lymphoid organs of Boran cattle infected with *Trypanosoma vivax*. *American Journal of Veterinary Research,* **42**, *10*, pp. 1738-1746.

Masake, R. A., Majiwa, P. A., Moloo, S. K., Makau, J. M., Njuguna, J. T., Maina, M., Kabata, J., Ole-MoiYoi, O. K. and Nantulya, V. M. (1997), Sensitive and specific detection of *Trypanosoma vivax* using the polymerase chain reaction. *Experimental Parasitology,* **85**, *2*, pp. 193-205.

Masiga, D. K., Smyth, A. J., Hayes, P. and Bromidge, T. J. (1992), Sensitive detection of trypanosomes in tsetse flies by DNA amplification. *International Journal for Parasitology,* **22**, *7*, pp. 909-918.

Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruysse, J., Dorny, P. and Van den Bossche, P. (2006), Comparison of the virulence of *Trypanosoma congolense* strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. *International Journal for Parasitology,* **36**, pp. 497-501.

Mather, T. N., Ribeiro, J. M. and Spielman, A. (1987), Lyme disease and babesiosis: Acaricide focused on potentially infected ticks. *American Journal of Tropical Medicine and Hygiene,* **36**, *3*, pp. 609-614.

Matovu, E., Seebeck, T., Enyaru, J. C. and Kaminsky, R. (2001), Drug resistance in *Trypanosoma brucei* spp., the causative agents of sleeping sickness in man and nagana in cattle. *Microbes and Infection,* **3**, *9*, pp. 763-770.

Matthysse, J. G. and Colbo, M. H. (1987), The ixodid ticks of Uganda. Ecological Society of America, College Park, Maryland, 426 pages.

Maxie, M. G., Dolan, T. T., Jura, W. G., Tabel, H. and Flowers, M. J. (1982), A comparative study of the disease in cattle caused by *Theileria parva* or *T. lawrencei*: II. Haematology, clinical chemistry, coagulation studies and complement. *Veterinary Parasitology,* **10**, *1*, pp. 1-19.

May, R. M. and Nowak, M. A. (1995), Coinfection and the evolution of parasite virulence. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **261**, *1361*, pp. 209-215.

Mbassa, G. K., Balemba, O., Maselle, R. M. and Mwaga, N. V. (1994), Severe anaemia due to haematopoietic precursor cell destruction in field cases of East Coast Fever in Tanzania. *Veterinary Parasitology,* **52**, *3-4*, pp. 243-256.

Mbassa, G. K., Kweka, L. E. and Dulla, P. N. (1998), Immunisation against East Coast Fever in field cattle with low infectivity *Theileria parva* stabilate - preliminary assessment. *Veterinary Parasitology,* **77**, *1*, pp. 41-48.

McCallon, B. R. (1973), Prevalence and economic aspects of anaplasmosis. Proceedings of the 6th National Anaplasmosis Conference. Stillwater, Oklahoma., (Ed, Jones , E. W.) Heratage Press, pp. 1 - 3.

McCullagh, P. and Nedler, J. A. (1989), Generalised linear models. Chapman & Hall, London.

McIlroy, S. G., Goddall, E. A., Stewart, S. M., Taylor, U. R. M. and McCracken, R. M. (1990), A computerised system for the accurate forcasting of the annual prevalence of fascioliasis. *Preventive Veterinary Medicine,* **9**, pp. 27-35.

McKeever, D. J., Taracha, E. L., Innes, E. L., MacHugh, N. D., Awino, E., Goddeeris, B. M. and Morrison, W. I. (1994), Adoptive transfer of immunity to *Theileria parva* in the CD8+ fraction of responding efferent lymph. *Proceedings of the National Academy of Sciences of the United States of America,* **91**, *5*, pp. 1959-1963.

McKeever, D. J. and Morrison, W. I. (1998), Novel vaccines against *Theileria parva*: Prospects for sustainability. *International Journal for Parasitology,* **28**, *5*, pp. 693-706.

McKeever, D. J. and Morrison, W. I. (2002), In *Theileria*. Epidemiological significance of strain-specific immunity to *Theileria parva*. World Class Parasites. **3**, (Eds, Dobbelaere, D. A. E. and McKeever, D. J.) Kluwer Academic Publishers, Boston, pp. 41-54.

McKenzie, F. E., Jeffery, G. M. and Collins, W. E. (2002), *Plasmodium malariae* infection boosts *Plasmodium falciparum* gametocyte production. *American Journal of Tropical Medicine and Hygiene,* **67**, *4*, pp. 411-414.

McOdimba, F. A. (2005), Epidemiology of vector-borne diseases in cattle from SE Uganda. PhD Thesis. The University of Edinburgh, Scotland.

Medley, G. F., Perry, B. D. and Young, A. S. (1993), Preliminary analysis of the transmission dynamics of *Theileria parva* in eastern Africa. *Parasitology,* **106**, *3*, pp. 251-264.

Mehlhorn, H. and Shein, E. (1984), The piroplasms: Life cycle and sexual stages. *Advances in Parasitology,* **23**, pp. 37-103.

Melendez, R. D. (2000), In *Tropical Veterinary Diseases*. Future perspectives on veterinary hemoparasite research in the tropics at the start of this century. Annals of the New York Academy of Sciences. **916**, pp. 253-258.

Melhorn, H. and Schein, E. (1984), The piroplams: Life cycle and sexual stages. *Advances in Parasitology,* **23**, pp. 37-103.

Melrose, T. R. (1994), Biochemical, immunological and genetic studies of apicomplexan parasites with special reference to *Theileria annulata*. PhD Thesis. Napier University, Edinburgh, Scotland.

Mettam, R. W. M. and Carcmichale, J. (1936), Turning sickness: A protozoan encephalitis of cattle in Uganda. Its relationship with East Coast Fever. *Parasitology,* **28**, pp. 254-283.

Miled, L. B., Dellagi, K., Bernardi, G., Melrose, T. R., Darghouth, M., Bouattour, A., Kinnaird, J., Shiels, B., Tait, A. and Brown, C. G. D. (1994), Genomic and phenotypic diversity of Tunisian *Theileria annulata* isolates. *Parasitology,* **108**, *1*, pp. 51-60.

Millott, S. M. and Cox, F. E. G. (1985), Interactions between *Trypanosoma brucei* and Babesia spp. and Plasmodium spp. in mice. *Parasitology,* **90**, pp. 241-254.

Mims, C. A. and White, D. O. (1984), Viral pathogenesis and immunology. Blackwell Scientific Publications, Oxford.

Minjauw, B., Otte, M. J., James, A. D., de Castro, J. J. and Sinyangwe, P. (1998a), Effect of different East Coast fever control strategies on disease incidence in traditionally managed Sanga cattle in Central Province of Zambia. *Preventive Veterinary Medicine,* **35**, *2*, pp. 101-113.

Minjauw, B., Otte, M. J., James, A. D., de Castro, J. J., Permin, A. and Di Giulo, G. (1998b), An outbreak of East Coast Fever in a herd of Sanga cattle in Lutale, Central Province of Zambia. *Preventive Veterinary Medicine,* **35**, *2*, pp. 143-147.

Moll, G., Lohding, A. and Young, A. S. (1984), Epidemiology of theileriosis in the Trans-Mara Division Kenya. Husbandry and disease background and preliminary investigations on theilerioses in calves. *Preventive Veterinary Medicine,* **2**, *6*, pp. 801-832.

Moll, G., Agan, L. and Lohding, A. (1985), In *Immunity against Theileriosis*. Bovine cerebral theileriosis in pure Boran and Sahiwal-cross cattle immunised against East Coast Fever and kept under continuous field challenge. (Ed, Irvin, A. D.) ILRAD.

Moll, G., Lohding, A., Young, A. S. and Leitch, B. L. (1986), Epidemiology of theileriosis in calves in an endemic area of Kenya. *Veterinary Parasitology,* **19**, *3-4*, pp. 255-273.

Moloo, S. K., Orinda, G. O., Sabwa, C. L., Minja, S. H. and Masake, R. A. (1999), Study on the sequential tsetse-transmitted *Trypanosoma congolense*, *T. brucei brucei* and *T. vivax* infections to African buffalo, eland, waterbuck, N'Dama and Boran cattle. *Veterinary Parasitology,* **80**, *3*, pp. 197-213.

Morlais, I., Ravel, S., Grebaut, P., Dumas, V. and Cuny, G. (2001), New molecular marker for *Trypanosoma* (Duttonella) *vivax* identification. *Acta Tropica,* **80**, *3*, pp. 207-213.

Morrison, W. I., Wells, P. W., Moloo, S. K., Paris, J. and Murray, M. (1982), Interference in the establishment of superinfections with *Trypanosoma congolense* in cattle. *Journal of Parasitology,* **68**, *5*, pp. 755-764.

Morrison, W. I., Goddeeris, B. M., Teale, A. J., Baldwin, C. L., Bensaid, A. and Ellis, J. (1986), Cell-mediated immune responses of cattle to *Theileria parva*. *Immunology Today,* **7**, *7-8*, pp. 211-216.

Morrison, W. I., Goddeeris, B. M., Teale, A. J., Groocock, C. M., Kemp, S. J. and Stagg, D. A. (1987), Cytotoxic T-cells elicited in cattle challenged with *Theileria parva* (Muguga): Evidence for restriction by class I MHC determinants and parasite strain specificity. *Parasite Immunology,* **9**, *5*, pp. 563-578.

Morrison, W. I. (1996), Influence of host and parasite genotypes on immunological control of Theileria parasites. *Parasitology,* **112**, pp. S53-S66.

Morzaria, S. P., Tatchell, R. J., Minor, R., Pedersen, V., Julla, I., Rahim, A., Dyson, D. and Aarle, P. A. M. (1981), Preliminary studies on the epidemiology of theileriosis in eastern Equatoria Province of Sudan. Advances in the control of theileriosis. ILRAD, Nairobi, (Eds, Irvin, A. D., Cunningham, M. P. and Young, A. S.) Martinus Nijhoff Publishers, The Hague, 9-13 February, pp. 83-85.

Morzaria, S. P., Young, J. R. and Batavia, T. (1990), Mapping the *Theileria parva* genome. Annual Scientific Report 1989. International Laboratory for Research on Animal Diseases, Nairobi, pp. 119.

Morzaria, S. P., Young, J. R., Spooner, P. R. and Dolan, T. T. (1992), Evidence of a sexual cycle in *Theileria parva* and characterisation of the recombinants. Tick-borne pathogens at the host-vector interface. Saint Paul, MN, (Eds, Munderloh, U. G. and Kurtti, T. J.) University of Minnesota College of Agriculture, September, pp. 71-74.

Morzaria, S. P., Young, J. R., Spooner, P. R., Dolan, T. T. and Bishop, R. P. (1993), In *Genome analysis of protozoan parasites*. *Theileria parva*: A restriction map and genetic recombination. (Ed, Morzaria, S. P.) ILRAD, Nairobi, pp. 67-73.

Morzaria, S. P., Dolan, T. T., Norval, R. A. L. and Bishop, R. P. (1995), Generation and characterisation of cloned *Theileria parva* parasites. *Parasitology,* **111**, *1*, pp. 39-49.

Morzaria, S. P., Katende, J., Musoke, A., Nene, V., Skilton, R. and Bishop, R. (1999), Development of sero-diagnostic and molecular tools for the control of important tick-borne pathogens of cattle in Africa. *Parassitologia,* **41**, *1*, pp. 73-80.

Moser, D., Cook, G., Ochs, D., Bailey, C., McKane, M. and Donelson, J. (1989), Detection of *Trypanosoma congolense* and *Trypanosoma brucei* subspecies by DNA amplification using the polymerase chain reaction. *Parasitology,* **99**, pp. 57–66.

Mosquera, J. and Adler, F. R. (1998), Evolution of virulence: a unified framework for coinfection and superinfection. *Journal of Theoretical Biology,* **195**, *3*, pp. 293-313.

Muhammed, S. I., Lauerman, L. H. and Johnson, L. W. (1975), Effect of humoral antibodies on the course of *Theileria parva* infection (East Coast Fever) of cattle. *American Journal of Veterinary Research,* **36**, *4 (1)*, pp. 399-402.

Mukhebi, A. W., Perry, B. D. and Kruska, R. (1992), Estimated Economics of Theileriosis Control in Africa. *Preventive Veterinary Medicine,* **12**, pp. 73-85.

Mulligan, H. W., Potts, W. H. and Kershaw, W. E. (1970), The African trypanosomiases. George Allen and Unwin, London.

Muraguri, G. R., McLeod, A., McDermott, J. J. and Taylor, N. (2005), The incidence of calf morbidity and mortality due to vector-borne infections in smallholder dairy farms in Kwale District, Kenya. *Veterinary Parasitology,* **130**, *3-4*, pp. 305-315.

Murray, M., Murray, P. K. and McIntyre, W. I. (1977), An improved parasitological technique for the diagnosis of African trypanosomiasis. *Transactions of the Royal of Society of Tropical Medicine and Hygiene,* **71**, *4*, pp. 325-326.

Murray, M. (1978), Anaemia of bovine African trypanosomiasis: Overview. Proceedings of a workshop on the pathogenicity of trypanosomes. Nairobi, Kenya, (Eds, Losos, G. J. and Chouinard, A.), 20-23 November 1978, pp. 121-127.

Murray, M., Stear, M. J., Trail, J. C. M., d'Ieteren, G. D. M., Agyemang, K. and Dwinger, R. H. (1991), In *Breeding for disease resistance in farm animals*. Trypanosomiasis in cattle. Prospects for control. (Ed, Owen, J. B. a. A., R. F. E.) CAB International, Wallingford, UK, pp. 203-223.

Mutugi, J. J., Young, A. S., Maritim, A. C., Ndungu, S. G., Mining, S. K., Linyonyi, A., Ngumi, P. N., Leitch, B. L., Morzaria, S. P. and Dolan, T. T. (1988), Immunization of cattle against theileriosis in Coast Province of Kenya: Laboratory evaluation of a *Theileria-Parva-Parva* stabilate for use in infection and treatment immunization in the field. *Research in Veterinary Science,* **47**, *2*, pp. 170-177.

Mwangi, E. K., Stevenson, P., Gettinby, G., Reid, S. W. and Murray, M. (1998), Susceptibility to trypanosomosis of three *Bos indicus* cattle breeds in areas of differing tsetse fly challenge. *Veterinary Parasitology,* **79**, *1*, pp. 1-17.

Nakamura, T., Konishi, T., Kawaguchi, H. and Imose, J. (1992), Estimation of relative fecundity in *Eimeria tenella* strains by a mixed infection method. *Parasitology,* **104**, *1*, pp. 11-17.

Nansen, P., Andersen, S. and Hesselholt, M. (1975), Experimental infection of the horse with *Fasciola hepatica*. *Experimental Parasitology,* **37**, *1*, pp. 15-19.

Nantulya, V. M., Musoke, A. J., Rurangirwa, F. R., Barbet, A. F., Ngaira, J. M. and Katende, J. M. (1982), Immune depression in African trypanosomiasis: The role of antigenic competition. *Clinical and Experimental Immunology,* **47**, *2*, pp. 234-242.

Nantulya, V. M., Musoke, A. J., Rurangirwa, F. R. and Moloo, S. K. (1984), Resistance of cattle to tsetse-transmitted challenge with *Trypanosoma brucei* or *Trypanosoma congolense* after spontaneous recovery from syringe-passaged infections. *Infection and Immunity,* **43**, *2*, pp. 735-738.

Nassir, E., Abdel-Muhsin, A. M., Suliaman, S., Kenyon, F., Kheir, A., Geha, H., Ferguson, H. M., Walliker, D. and Babiker, H. A. (2005), Impact of genetic complexity on longevity and gametocytogenesis of *Plasmodium falciparum* during the dry and transmission-free season of eastern Sudan. *International Journal for Parasitology,* **35**, *1*, pp. 49-55.

Nene, V., Iams, K. P., Gobright, E. and Musoke, A. J. (1992), Characterisation of the gene encoding a candidate vaccine antigen of *Theileria parva* sporozoites. *Molecular and Biochemical Parasitology,* **51**, *1*, pp. 17-27.

Nene, V., Inumaru, S., McKeever, D. and Morzaria, S. (1995), Characterisation of an insect cell-derived *Theileria parva* sporozoite vaccine antigen and immunogenicity in cattle. *Infection and Immunity,* **63**, *2*, pp. 503-508.

Nene, V., Musoke, A., Gobright, E. and Morzaria, S. (1996), Conservation of the sporozoite p67 vaccine antigen in cattle-derived *Theileria parva* stocks with different cross-immunity profiles. *Infection and Immunity,* **64**, *6*, pp. 2056-2061.

Nene, V., Morzaria, S. and Bishop, R. (1998), Organisation and informational content of the *Theileria parva* genome. *Molecular and Biochemical Parasitology,* **95**, *1*, pp. 1-8.

Nene, V., Gobright, E., Bishop, R., Morzaria, S. and Musoke, A. (1999), Linear peptide specificity of bovine antibody responses to p67 of *Theileria parva* and sequence diversity of sporozoite-neutralizing epitopes: Implications for a vaccine. *Infection and Immunity,* **67**, *3*, pp. 1261-1266.

Nevils, M. A., Figueroa, J. V., Turk, J. R., Canto, G. J., Le, V., Ellersieck, M. R. and Carson, C. A. (2000), Cloned lines of *Babesia bovis* differ in their ability to induce cerebral babesiosis in cattle. *Parasitology Research,* **86**, *6*, pp. 437-443.

Nicholson, M. J. and Butterworth, M. H. (1986), A guide to condition scoring of Zebu cattle. International Livestock Centre for Africa (ILCA), Addis Ababa.

Nok, A. J. and Balogun, E. O. (2003), A bloodstream *Trypanosoma congolense* sialidase could be involved in anaemia during experimental trypanosomiasis. *Journal of Biochemistry,* **133**, *6*, pp. 725-730.

Norval, R. A., Fivaz, B. H., Lawrence, J. A. and Daillecourt, T. (1983), Epidemiology of tick-borne diseases of cattle in Zimbabwe. I. Babesiosis. *Tropical Animal Health and Production,* **15**, *2*, pp. 87-94.

Norval, R. A. I. (1983), Arguments against intensive dipping. *Zimbabwe Veterinary Journal,* **14**, pp. 19-25.

Norval, R. A., Fivaz, B. H., Lawrence, J. A. and Brown, A. F. (1984), Epidemiology of tick-borne diseases of cattle in Zimbabwe. II. Anaplasmosis. *Tropical Animal Health and Production,* **16**, *2*, pp. 63-70.

Norval, R. A. and Perry, B. D. (1990), Introduction, spread and subsequent disappearance of the brown ear-tick, *Rhipicephalus appendiculatus*, from the southern lowveld of Zimbabwe. *Experimental and Applied Acarology,* **9**, *1-2*, pp. 103-111.

Norval, R. A. I., Perry, B. D. and Young, A. S. (Eds.) (1992), The epidemiology of theileriosis in Africa. Academic Press, London.

Nowak, M. A. and May, R. M. (1994), Superinfection and the evolution of parasite virulence. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **255**, *1342*, pp. 81-89.

Nowak, M. A. and Sigmund, K. (2002), In *Adaptative dynamics of infectious diseases: In pursuit of virulence management*. Super- and coinfection: The two extremes. (Eds, Dieckmann, U., Metz, J. A. J., Sabelis, M. A. and Sigmund, K.) Cambridge University Press, Cambridge.

OAU (Ed.) (1998), A new Macmillan social studies atlas for Uganda. Organisation of African Unity. Uganda, Kampala.

O'Callaghan, C. J., Medley, G. F., Peter, T. F. and Perry, B. D. (1998), Investigating the epidemiology of heartwater (*Cowdria ruminantium* infection) by means of a transmission dynamics model. *Parasitology,* **117**, *1*, pp. 49-61.

O'Callaghan, C. J. (1998), A study of the epidemiology of theileriosis on smallholder dairy farms in Kiambu District, Kenya. PhD Thesis. University of Guelph, Ontaio, Canada.

Ochanda, H., Young, A. S., Wells, C., Medley, G. F. and Perry, B. D. (1996), Comparison of the transmission of *Theileria parva* between different instars of *Rhipicephalus appendiculatus*. *Parasitology,* **113**, *3*, pp. 243-253.

Odiit, M. and Enyaru, J. (1999), Human African trypanosomiasis in south east Uganda, 1988 to 1998: A comparative epidemiological analysis. Proceedings of the XXV Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). Mombasa, Kenya, 27 September to 1 October.

Odiit, M., Bessell, P. R., Fevre, E. M., Robinson, T., Kinoti, J., Coleman, P. G., Welburn, S. C., McDermott, J. and Woolhouse, M. E. (2006), Using remote sensing and geographic information systems to identify villages at high risk for rhodesiense sleeping sickness in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **100**, *4*, pp. 354-362.

Ogambo-Ongoma, A. H. (1972), Fascioliasis survey in Uganda. *Bulletin of Epizootic Diseases of Africa,* **20**, *1*, pp. 35-41.

OIE (2004), Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Volumes I & II. World Organisation for Animal Health, Paris.

Okello-Onen, J., Rutagwenda, T., Unger, F., Bohle, W., Musinguzi, C., Mwayi, W., Erima, S. and Musisi, G. (1998), The status of ticks and tick-borne diseases under pastural farming system in Uganda. ICOPA 9th International Congress of Parasitology. Japan, Makuhari Messe, Chiba, pp. 537-541.

Okello-Onen, J., Tukahirwa, E. M., Perry, B. D., Rowlands, G. J., Nagda, S. N., Musisi, G., Bode, E., Heinonen, R., Mwayi, W. and Opuda-Asibo, J. (2003), The impact of tick control on the productivity of indigenous cattle under ranch conditions in Uganda. *Tropical Animal Health and Production,* **35**, *3*, pp. 237-247.

Okiria, R., Okuna, N. M., Magona, J. W. and Mayende, J. S. P. (2002), Sustainability of tsetse control by subsequent treatment of 10% of a previously treated Ugandan cattle population with 1% w/v deltamethrin. *Tropical Animal Health and Production,* **34**, *2*, pp. 105-114.

Okoth, J. O., Okethi, V. and Ogola, A. (1991), Control of tsetse and trypanosomiasis transmission in Uganda by applications of lambda-cyhalothrin. *Medical and Veterinary Entomology,* **5**, *1*, pp. 121-128.

Okuna, N. M., Katabazi, B., Omollo, P., Magona, J. W. and Mayende, J. S. P. (1996), The current status and epidemiological pattern of animal trypanosomiasis in different agroecological zones and farming systems in Uganda. Annual Report 1996. Livestock Health Research Institute, pp. 21-23.

Okuna, N. M., Okiria, R., Odiit, M., Mugenyi, A., Olaho-Mukani, W., Mayende, J. S. P. and Kisa, V. (1999), *Trypanosoma brucei* infection in cattle in Serere County, Soroti district at the onset of an outbreak of sleeping sickness. Twenty-fifth meeting of the International Scientific Conference on Trypanosomiasis Research and Control (ISCTRC). Mombasa, Kenya, pp. 126.

Oldstone, M. B. (1991), Molecular anatomy of viral persistence. *Journal of Virology,* **65**, *12*, pp. 6381-6386.

Omara-Opyene, A. L. (1985), A survey of gastrointestinal parasitism in cattle under nomadic management in Marsabit district of Northern Kenya. *Bulletin of Animal Health and Production in Africa,* **33**, pp. 107-112.

Omuse, J. K. (1978), A comparative haematological picture of field cases of East Coast Fever, anaplasmosis and babesiosis in bovines around Kabete. Proceedings of an international conference on tick-borne diseases and their vectors. Centre for Tropical Veterinary Medicine, Edinburgh, UK, (Ed, Wilde, J. K.).

Onah, D. N., Onyenwe, I. W., Ihedioha, J. I. and Onwumere, O. S. (2003), Enhanced survival of rats concurrently infected with *Trypanosoma brucei* and *Strongyloides ratti*. *Veterinary Parasitology,* **119**, pp. 165-176.

Onyango, R. J., Van Hoeve, K. and De Raadt, P. (1966), The epidemiology of *Trypanosoma rhodesiense* sleeping sickness in Alego location, Central Nyanza, Kenya. I. Evidence that cattle may act as reservoir hosts of trypanosomes infective to man. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **60**, *2*, pp. 175-182.

Orcutt, R. and Schaedler, R. W. (1973), In *Germfree research: Biological effect of gnotobiotic environments*. Control of staphilococci in the gut of mice. (Ed, Heneghan, J. B.) Academic Press, New York, pp. 435-440.

Oura, C. A. L., Bishop, R., Wampande, E. M., Lubega, G. W. and Tait, A. (2004), The persistence of component *Theileria parva* stocks in cattle immunised with the 'Muguga cocktail' live vaccine against East Coast Fever in Uganda. *Parasitology,* **129**, *1*, pp. 27-42.

Oura, C. A., Asiimwe, B. B., Weir, W., Lubega, G. W. and Tait, A. (2005), Population genetic analysis and sub-structuring of *Theileria parva* in Uganda. *Molecular and Biochemical Parasitology,* **140**, *2*, pp. 229-239.

Owusu-Agyei, S., Smith, T., Beck, H. P., Amenga-Etego, L. and Felger, I. (2002), Molecular epidemiology of *Plasmodium falciparum* infections among asymptomatic inhabitants of a holoendemic malarious area in northern Ghana. *Tropical Medicine and International Health,* **7**, *5*, pp. 421-428.

Pacala, S. W. and Dobson, A. P. (1988), The relation between the number of parasites and host age: Population causes and maximum likelihood estimation. *Parasitology,* **96**, pp. 197-210.

Palau, M. T., Mejia, A. J., Vergara, U. and Zuniga, C. A. (2003), Action of *Trypanosoma rangeli* in infections with virulent *Trypanosoma cruzi* populations. *Memórias do Instituto Oswaldo Cruz,* **98**, *4*, pp. 543-548.

Palmer, G. H., Rurangirwa, F. R., Kocan, K. M. and Brown, W. C. (1999), Molecular basis for vaccine development against the ehrlichial pathogen *Anaplasma marginale*. *Parasitology Today,* **15**, *7*, pp. 281-286.

Parmar, M. K. B. and Machin, D. (1996a), In *Survival analysis - A practical approach*. Cox's proportional hazards model. (Eds, Parmar, M. K. B. and Machin, D.) John Wiley & Sons Ltd, West Sussex, England, pp. 115-142.

Parmar, M. K. B. and Machin, D. (1996b), In *Survival analysis - A practical approach*. Selecting variables within a Cox model. (Eds, Parmar, M. K. B. and Machin, D.) John Wiley & Sons Ltd., West Sussex, England, pp. 145-159.

Paterson, S. (2001), The use of repeated measure linear modelling to analyse longitudinal data from experimental parasite infections. *Journal of Parasitology,* **87**, pp. 969-971.

Paterson, S. and Lello, J. (2003), Mixed models: Getting the best use of parasitological data. *Trends in Parasitology,* **19**, *8*, pp. 370-374.

Patterson, H. D. and Thompson, R. (1971), Recovery of interblock information when block sizes are unequal. *Biometrika,* **58**, pp. 545-554.

Pegram, R. G. and Chizyuka, H. G. (1990), The impact of natural infestations of ticks in Zambia on the productivity of cattle and implications for tick control strategies in central Africa. *Parassitologia,* **32**, *1*, pp. 165-175.

Pegram, R. G., James, A. D., Oosterwijk, G. P., Killorn, K. J., Lemche, J., Ghirotti, M., Tekle, Z., Chizyuka, H. G., Mwase, E. T. and Chizyuka, F. (1991), Studies on the economics of ticks in Zambia. *Experimental and Applied Acarology,* **12**, *1-2*, pp. 9-26.

Pegram, R. G., James, A. D., Bamhare, C., Dolan, T. T., Hove, T., Kanhai, G. K. and Latif, A. A. (1996), Effects of immunisation against *Theileria parva* on beef cattle productivity and economics of control options. *Tropical Animal Health and Production,* **28**, *1*, pp. 99-111.

Peiris, J. S., Gordon, S., Unkeless, J. C. and Porterfield, J. S. (1981), Monoclonal anti-Fc receptor IgG blocks antibody enhancement of viral replication in macrophages. *Nature,* **289**, *5794*, pp. 189-191.

Perry, B. D. and Young, A. S. (1995), The past and future roles of epidemiology and economics in the control of tick-borne diseases of livestock in Africa: The case of theileriosis. *Preventive Veterinary Medicine,* **25**, *1*, pp. 107-120.

Perry, B., McDermott, J. J. and Randolph, T. (2001), Can epidemiology and economics make a meaningful contribution to national animal disease control? *Preventive Veterinary Medicine,* **48**, pp. 231-260.

Perry, B. D., Randolph, T. F., McDermott, J. J., Sones, K. R. and Thornton, P. K. (2002), Investing in animal health research to alleviate poverty. A report commissioned by the UK Department for International Development, on behalf of the Inter Agency Group of Donors Supporting Research on Livestock Production and Health in the Developing World. International Livestock Research Institute (ILRI), Nairobi, Kenya.

Petney, T. N. and Andrews, R. H. (1998), Multiparasite communities in animals and humans: Frequency, structure and pathogenic significance. *International Journal for Parasitology,* **28**, *3*, pp. 377-393.

Picozzi, K., Tilley, A., Fèvre, E. M., Coleman, P. G., Magona, J. W., Odiit, M., Eisler, M. C. and Welburn, S. C. (2002), The diagnosis of trypanosome infections: Applications of novel technology for reducing disease risk. *African Journal of Biotechnology,* **1**, *2*, pp. 39-45.

Piesman, J., Karakashian, S. J., Lewengrub, S., Rudzinska, M. A. and Spielmank, A. (1986), Development of *Babesia microti* sporozoites in adult *Ixodes dammini*. *International Journal for Parasitology,* **16**, *4*, pp. 381-385.

Piesman, J., Lewengrub, S., Rudzinska, M. A. and Spielman, A. (1987), *Babesia microti*: prolonged survival of salavarian piroplasms in nymphal *Ixodes dammini*. *Experimental Parasitology,* **64**, *3*, pp. 292-299.

Pinheiro, J. C. and Bates, D. M. (2000), Mixed-effects models in S and S-plus. (Eds, Chambers, J., Eddy, W., Hardle, W., Sheather, S. and Tierney, L.) Springer-Verlag, New York.

Pipano, E., Weisman, Y. and Benado, A. (1974), The virulence of four local strains of *Theileria annulata*. *Refuah Veterinarith,* **31**, pp. 59-63.

Porterfield, J. S. (1986), Antibody-dependent enhancement of viral infectivity. *Advances in Virus Research,* **31**, pp. 335-355.

Potgieter, F. T. and Stoltz, H. H. (1994). Bovine anaplasmosis in infectious diseases of livestock with special reference to southern Africa. **1**, (Eds, Coetzer, J. A., Thompson, G. R. and Tustin, R. C.) Oxford University Press, Cape Town, pp. 408-430.

Pratt, D. J. and Gwynne, M. D. (1977), Rangeland management and ecology in East Africa. Hodder & Stoughton, London.

Radley, D. E., Brown, C. G. D., Burridge, M. J., Cunningham, M. P., Kirimi, I. M., Purnell, R. E. and Young, A. S. (1975a), East Coast Fever: 1. Chemoprophylactic immunisation of cattle against *Theileria parva* (Muguga) and five theilerial strains. *Veterinary Parasitology,* **1**, *1*, pp. 35-41.

Radley, D. E., Young, A. S., Brown, C. G. D., Burridge, M. J., Cunningham, M. P., Musisi, F. L. and Purnell, R. E. (1975b), East Coast Fever: 2. Cross-immunity trials with a Kenya strain of *Theileria lawrencei*. *Veterinary Parasitology,* **1**, *1*, pp. 43-50.

Radley, D. E., Brown, C. G. D., Cunningham, M. P., Kimber, C. D., Musisi, F. L., Payne, R. C., Purnell, R. E., Stagg, S. M. and Young, A. S. (1975c), East Coast Fever: 3. Chemoprophylactic immunisation of cattle using oxytetracycline and a combination of theilerial strains. *Veterinary Parasitology,* **1**, *1*, pp. 51-60.

Rafyi, A., Maghami, G. and Houshmand, P. (1965), On the virulence of *Theileria annulata* (Dschunkowsky and Luhs, 1904) and the premunition against bovine theileriasis in Iran. *Bulletin de l'Office International Des Epizooties,* **64**, pp. 431-446.

Randhawa, N. S. and Sundaram, K. V. (1990), Small Farmer Development in Asia and the Pacific: Some Lessons for Strategy Formulation and Planning. FAO Economic and Social Development Paper 87. Food and Agriculture Organisation, Rome. 104 pages.

Randolph, S. E. (1994), Population dynamics and density-dependent seasonal mortality indices of the tick *Rhipicephalus appendiculatus* in eastern and southern Africa. *Medical and Veterinary Entomology,* **8**, *4*, pp. 351-368.

Raso, G., Luginbuhl, A., Adjoua, C. A., Tian-Bi, N. T., Silue, K. D., Matthys, B., Vounatsou, P., Wang, Y. Y., Dumas, M.-E., Holmes, E., Singer, B. H., Tanner, M., N'Goran, E. K. and Utzinger, J. (2004), Multiple parasite infections and their relationship to self-reported morbidity in a community of rural Cote d'Ivoire. *International Journal of Epidemiology,* **33**, *5*, pp. 1092-1102.

Razakandrainibe, F. G., Durand, P., Koella, J. C., De Meeus, T., Rousset, F., Ayala, F. J. and Renaud, F. (2005), "Clonal" population structure of the malaria agent *Plasmodium falciparum* in high-infection regions. *Proceedings of the National Academy of Sciences of the United States of America,* **102**, *48*, pp. 17388-17393.

Read, A. F. and Taylor, L. H. (2001), The ecology of genetically diverse infections. *Science,* **292**, *5519*, pp. 1099-1102.

Recker, M., Nee, S., Bull, P. C., Kinyanjui, S., Marsh, K., Newbold, C. and Gupta, S. (2004), Transient cross-reactive immune responses can orchestrate antigenic variation in malaria. *Nature,* **429**, *6991*, pp. 555-558.

Recker, M. and Gupta, S. (2005), A model for pathogen population structure with cross-protection depending on the extent of overlap in antigenic variant repertoires. *Journal of Theoretical Biology,* **232**, *3*, pp. 363-373.

Reed, J. B. H., Doxey, D. L., Forbes, A. B., Finlay, R. S., Geering, W. I., Smith, S. D. and Wright, J. D. (1974), Productive performance of cattle in Bostwana. *Tropical Animal Health and Production,* **6**, pp. 23-29.

Rich, S. M., Hudson, R. R. and Ayala, F. J. (1997), *Plasmodium falciparum* antigenic diversity: evidence of clonal population structure. *Proceedings of the National Academy of Sciences of the United States of America,* **94**, *24*, pp. 13040-13045.

Richey, E. J. and Palmer, G. H. (1990), Bovine anaplasmosis. *Compendium on Continuing Education for the Practicing Veterinarian,* **12**, *11*, pp. 1661-1669.

Riley, M. A. and Gordon, D. M. (1999), The ecological role of bacteriocins in bacterial competition. *Trends in Microbiology,* **7**, *3*, pp. 129-133.

Ristic, M. (1968), An attenuated *Anaplasma marginale* vaccine. Proceedings of the 72nd Annual Meeting of the United States Livestock Sanitary Association, pp. 56-69.

Ristic, M. (1981), In *Diseases of cattle in the tropics*. Babesiosis. (Eds, Ristic, M. and McIntyre, I.) Martinus Nijhoff Publishers, The Hague, Netherlands, pp. 443-468.

Ritchie, T. L. (1988), Interactions between malaria parasites infecting the same vertebrate hosts. *Parasitology,* **96**, pp. 607-639.

Roberts, J. A. and Suhardono (1996), Approaches to the control of fasciolosis in ruminants. *International Journal for Parasitology,* **26**, *8-9*, pp. 971-981.

Robinson, W. E. J., Montefiori, D. C. and Mitchell, W. M. (1988), Antibody-dependent enhancement of human immunodeficiency virus type 1 infection. *Lancet,* **1**, *8589*, pp. 790-794.

Rose, M. E., Wakelin, D. and Hesketh, P. (1994), Interactions between infections with Eimeria spp. and *Trichinella spiralis* in inbred mice. *Parasitology,* **108**, *1*, pp. 69-75.

Rowlands, G. J., Musoke, A. J., Morzaria, S. P., Nagda, S. M., Ballingall, K. T. and McKeever, D. J. (2000), A statistically derived index for classifying East Coast Fever reactions in cattle challenged with *Theileria parva* under experimental conditions. *Parasitology,* **120**, *4*, pp. 371-381.

Rubaire-Akiiki, C. M., Opuda-Asibo, J. and Horchner, F. (1999), Gastrointestinal tract (GIT) nematode infections in dairy cattle in the lake Victoria basin (Uganda). *Bulletin of Animal Health and Production in Africa,* **47**, pp. 61-70.

Rubaire-Akiiki, C., Okello-Onen, J., Nasinyama, G. W., Vaarst, M., Kabagambe, E. K., Mwayi, W., Musunga, D. and Wandukwa, W. (2004), The prevalence of serum antibodies to tick-borne infections in Mbale District, Uganda: The effect of agro-ecological zone, grazing management and age of cattle. *Journal of Insect Science,* **4**, pp. 8.

Rumberia, R. M., Eley, R. M., Young, A. S. and Rowland, A. C. (1993), The effect of high and low dose *Theileria parva* infection on the reproductive function of Boran / Friesian heifers. *Theriogenology,* **40**, *5*, pp. 977-986.

Rutter, L. M. and Randel, R. D. (1984), Post-partum nutrient intake and body condition: Effect on pituitary function and onset of estrus in beef cattle. *Journal of Animal Science,* **58**, pp. 265-274.

Sakamoto, Y., Ishiguro, M. and Kitagawa, G. (1986), Akaike information criterion statistics. Reidel, Dordrecht, Holland.

Sanchez-Andrade, R., Paz-Silva, A., Suarez, J. L., Panadero, R., Pedreira, J., Lopez, C., Diez-Banos, P. and Morrondo, P. (2002), Influence of age and breed on natural bovine fasciolosis in an endemic area (Galicia, NW Spain). *Veterinary Research Communications,* **26**, *5*, pp. 361-370.

Saravanan, B. C., Sreekumar, C., Bansal, G. C., Ray, D., Rao, J. R. and Mishra, A. K. (2003), A rapid MTT colorimetric assay to assess the proliferative index of two Indian strains of *Theileria annulata*. *Veterinary Parasitology,* **113**, *3-4*, pp. 211-216.

Sauvage, J. P., Brown, J. R., Parkinson, J. G., Rossiter, P. B. and McGovern, P. T. (1974), Helminthiasis in cattle in the Ankole district of Uganda. *British Veterinary Journal,* **130**, *0*, pp. 120-127.

Schall, R. (1991), Estimation in generalised linear models with random-effects. *Biometrika,* **78**, pp. 719-727.

Schalm, O. W., Jain, N. C. and Carrol, E. J. (Eds.) (1975), Veterinary haematology. Lea and Febiger, Philadelphia.

Schein, E., Rehbein, G., Voigt, W. P. and Zweygarth, E. (1981), *Babesia equi* (Laveran 1901) 1. Development in horses and in lymphocyte culture. *Tropenmedizin Und Parasitologie,* **32**, *4*, pp. 223-227.

Schmidt, H. E. (1937), Anaplasmosis in cattle. *Journal of the American Veterinary Medical Association,* **90**, pp. 723-736.

Scholtz, M. M., Spickett, A. M., Lombard, P. E. and Enslin, C. B. (1991), The effect of tick infestation on the productivity of cows of three breeds of cattle. *Onderstepoort Journal of Veterinary Research,* **58**, *2*, pp. 71-74.

Schwarz, G. (1978), Estimating the dimension of a model. *Annals of Statistics,* **6**, pp. 461-464.

Searle, S. R., Casella, G. and McCulloch, C. E. (1992), Variance components. Wiley, New York.

Shannon, D. (1977), Field cases of East Coast Fever in grade cattle in Uganda. *Tropical Animal Health and Production,* **9**, *1*, pp. 29-35.

Shapiro, S. Z., Fujisaki, K., Morzaria, S. P., Webster, P., Fujinaga, T., Spooner, P. R. and Irvin, A. D. (1987), A life-cycle stage-specific antigen of *Theileria parva* recognised by anti-macroschizont monoclonal antibodies. *Parasitology,* **94**, *1*, pp. 29-37.

Sharma, D. K., Chauhan, P. P. and Agrawal, R. D. (2000), Interaction between *Trypanosoma evansi* and *Haemonchus contortus* infection in goats. *Veterinary Parasitology,* **92**, *4*, pp. 261-267.

Shatry, A. M., Wilson, A. J., Varma, S. and Dolan, T. T. (1981), Sequential study of lymph node and splenic aspirates during *Theileria parva* infection in calves. *Research in Veterinary Science,* **30**, *2*, pp. 181-184.

Shiels, B., Kinnaird, J., McKellar, S., Dickson, J., Miled, L. B., Melrose, R., Brown, D. and Tait, A. (1992), Disruption of synchrony between parasite growth and host cell division is a determinant of differentiation to the merozoite in *Theileria annulata*. *Journal of Cell Science,* **101**, *1*, pp. 99-107.

Skilton, R. A., Bishop, R. P., Wells, C. W., Spooner, P. R., Gobright, E., Nkonge, C., Musoke, A. J., Macklin, M. and Iams, K. P. (1998), Cloning and characterisation of a 150 kDa microsphere antigen of *Theileria parva* that is immunologically cross-reactive with the polymorphic immunodominant molecule (PIM). *Parasitology,* **117**, *4*, pp. 321-330.

Skilton, R. A., Bishop, R. P., Katende, J. M., Mwaura, S. and Morzaria, S. P. (2002), The persistence of *Theileria parva* infection in cattle immunised using two stocks which differ in their ability to induce a carrier state: Analysis using a novel blood spot PCR assay. *Parasitology,* **124**, *3*, pp. 265-276.

Sloof, P., Menke, H. H., Caspers, M. P. and Borst, P. (1983), Size fractionation of *Trypanosoma brucei* DNA: Localisation of the 177-bp repeat satellite DNA and a variant surface glycoprotein gene in a mini-chromosomal DNA fraction. *Nucleic Acids Research,* **11**, pp. 3889-3901.

Smithers, S. R., Terry, R. J. and Hockley, D. J. (1969), Host antigens in schistosomiasis. *Proceedings of the Royal Society of London Series B,* **171**, pp. 483-494.

Snounou, G., Bourne, T., Jarra, W., Viriyakosol, S., Wood, J. C. and Brown, K. N. (1992), Assessment of parasite population dynamics in mixed infections of rodent plasmodia. *Parasitology,* **105**, *3*, pp. 363-374.

Solano, P., Jamonneau, V., N'Guessan, P., N'Dri, L., Dje, N. N., Miezan, T. W., Lejon, V., Buscher, P. and Garcia, A. (2002), Comparison of different DNA preparation protocols for PCR diagnosis of human African trypanosomosis in Cote d'Ivoire. *Acta Tropica,* **82**, *3*, pp. 349-356.

Speybroeck, N., Madder, M., Van Den Bossche, P., Mtambo, J., Berkvens, N., Chaka, G., Mulumba, M., Brandt, J., Tirry, L. and Berkvens, D. (2002), Distribution and phenology of ixodid ticks in southern Zambia. *Medical and Veterinary Entomology,* **16**, *4*, pp. 430-441.

Spielman, A. (1976), Human babesiosis on Nantucket Island: Transmission by nymphal Ixodes ticks. *American Journal of Tropical Medicine and Hygiene,* **25**, *6*, pp. 784-787.

Spielman, A. (1979), Human babesiosis on Nantucket Island USA: Description of the vector *Ixodes* (Ixodes) *dammini*, n. sp. (Acarina: Ixodidae). *Journal of Medical Entomology,* **15**, pp. 218-234.

Spielman, A., Wilson, M. L., Levine, J. F. and Piesman, J. (1985), Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annual Review of Entomology,* **30**, pp. 439-460.

Spithill, T. and Dalton, J. (1998), Progress in development of liver fluke vaccines. *Parasitology Today,* **14**, pp. 224-228.

Stafford, K. C. (1991), Effectiveness of carbaryl applications for the control of *Ixodes dammini* (Acari: Ixodidae) nymphs in an endemic residential area. *Journal of Medical Entomology,* **28**, *1*, pp. 32-36.

Steenkamp, J. D. G., van der Horst, C. and Andrew, M., J, A. (1975), Reconception in grade and pedigree. Africander cows of different sizes: Post-partum factors affecting reconception. *South African Journal of Animal Science,* **5**, pp. 103-110.

Su, Z., Segura, M., Morgan, K., Loredo-Osti, J. C. and Stevenson, M. M. (2005), Imparment of protective immunity to blood-stage malaria by concurrent nematode infection. *Infection and Immunity,* **73**, *6*, pp. 3531-3539.

Su, Z., Segura, M. and Stevenson, M. M. (2006), Reduced protective efficacy of a blood-stage malaria vaccine by concurrent nematode infection. *Infection and Immunity,* **74**, *4*, pp. 2138-2144.

Sutherst, R. W., Utech, K. B. W., Kerr, J. D. and Wharton, R. H. (1979), Density-dependent mortality of the tick, *Boophilus microplus*, on cattle - Further observations. *Journal of Applied Ecology,* **16**, pp. 397-403.

Swai, E. S., French, N. P., Karimuribo, E. D., Fitzpatrick, J. L., Bryant, M. J., Brown, P. E. and Ogden, N. H. (2005a), Spatial and management factors associated with exposure of smallholder dairy cattle in Tanzania to tick-borne pathogens. *International Journal for Parasitology,* **35**, *10*, pp. 1085-1096.

Swai, E. S., French, N. P., Beauchamp, G., Fitzpatrick, J. L., Bryant, M. J., Kambarage, D. and Ogden, N. H. (2005b), A longitudinal study of sero-conversion to tick-borne pathogens in smallholder dairy youngstock in Tanzania. *Veterinary Parasitology,* **131**, *1-2*, pp. 129-137.

Swallow, B. M. (1997), Impacts of trypanosomosis on African agriculture. Twenty-fourth meeting of the International Scientific Council for Trypanosomiasis Research and Control. Maputo, Mozambique, Organisation of African Unity and International Scientific Council for Trypanosomiasis Research and Control, 29 September - 4 October 1997.

Takeda, A., Tuazon, C. U. and Ennis, F. A. (1988), Antibody-enhanced infection by HIV-1 via Fc receptor-mediated entry. *Science,* **242**, *4878*, pp. 580-583.

Taracha, E. L., Goddeeris, B. M., Teale, A. J. and Kemp, S. J. (1995a), Parasite strain specificity of bovine cytotoxic T cell responses to *Theileria parva* is determined primarily by immunodominance. *Journal of Immunology,* **155**, *10*, pp. 4854-4860.

Taracha, E. L., Goddeeris, B. M., Morzaria, S. P. and Morrison, W. I. (1995b), Parasite strain specificity of precursor cytotoxic T cells in individual animals correlates with cross-protection in cattle challenged with *Theileria parva*. *Infection and Immunity,* **63**, *4*, pp. 1258-1262.

Taracha, E. L., Awino, E. and McKeever, D. J. (1997), Distinct CD4+ T cell helper requirements in *Theileria parva*-immune and -naive bovine CTL precursors. *Journal of Immunology,* **159**, *9*, pp. 4539-4545.

Tassi, P., Carelli, G. and Ceci, L. (2002), Tick-borne diseases (TBDs) of dairy cows in a Mediterranean environment. A clinical, serological, and haematological study. *Annals of the New York Academy of Sciences*, **969**, *1*, pp. 314-317.

Tatchell, R. J. and Easton, E. (1986), (Acari: Ixodidae) ecological studies in Tanzania. *Bulletin of Entomological Research,* **76**, pp. 229-246.

Tatchell, R. J. (1987), Tick control in the context of ECF immunisation. *Parasitology Today,* **3**, *1*, pp. 7-10.

Taylor, L. H., Walliker, D. and Read, A. F. (1997a), Mixed-genotype infections of the rodent malaria *Plasmodium chabaudi* are more infectious to mosquitoes than single-genotype infections. *Parasitology,* **115**, *2*, pp. 121-132.

Taylor, L. H., Walliker, D. and Read, A. F. (1997b), Mixed-genotype infections of malaria parasites: within-host dynamics and transmission success of competing clones. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **264**, *1383*, pp. 927-935.

Taylor, L. H., Machkinnon, M. J. and Read, A. F. (1998a), Virulence of mixed-clone and single-clone infections on the rodent malaria *Plasmodium chabaudi*. *Evolution,* **52**, *2*, pp. 583-591.

Taylor, L. H. and Read, A. F. (1998b), Determinants of transmission success of individual clones from mixed-clone infections of the rodent malaria parasite, *Plasmodium chabaudi*. *International Journal for Parasitology,* **28**, *5*, pp. 719-725.

Taylor, L. H., Welburn, S. C. and Woolhouse, M. E. J. (2002), *Theileria annulata*: Virulence and transmission from single and mixed clone infections in cattle. *Experimental Parasitology,* **100**, *3*, pp. 186-195.

Taylor, L. H., Katzer, F., Shiels, B. R. and Welburn, S. C. (2003), Genetic and phenotypic analysis of Tunisian *Theileria annulata* clones. *Parasitology,* **126**, *3*, pp. 241-252.

Taylor, L. H., Matthews, L., Shaw, D. J. and Haydon, D. T. (2005), Competitive suppression in mixed-clone parasite cultures. *Biology Letters,* **1**, *1*, pp. 108-111.

Tebele, N., Skilton, R. A., Katende, J., Wells, C. W., Nene, V., McElwain, T., Morzaria, S. P. and Musoke, A. J. (2000), Cloning, characterisation, and expression of a 200-kilodalton diagnostic antigen of *Babesia bigemina*. *Journal of clinical microbiology,* **38**, *6*, pp. 2240-2247.

Telford, S. R. I., Gorenflot, A., Brasseur, P. and Spielman, A. (1993), In *Parasitic protozoa*. Babesia infections in humans and wildlife. **5**, (Ed, Kreier, J. P.) Academic Press, San Diego, Califormia, pp. 1-47.

Theiler, A. (1910), *Anaplasma marginale* (gen. spec. nov.). The marginale points in the blood of cattle suffering from a specific disease. Report of the government veterinary bacteriologist 1908-09. pp. 7-64.

Theiler, A. (1911), Further investigations into anaplasmosis of south African cattle. First Report of the Director of Veterinary Research. Department of Agriculture of the Union of South Africa. pp. 7-46.

Thisted, R. A. (1988), Elements of statistical computing. Chapman & Hall, London.

Thomas, M. B., Watson, E. L. and Valverde-Garcia, P. (2003), Mixed infections and insect-pathogen interactions. *Ecology Letters,* **6**, pp. 183-188.

Thompson, R. C. A. (2000), Molecular epidemiology of infectious diseases. (Ed, Thompson, R. C.A.) Arnold, London.

Thornton, P. K., Kruska, R. L., Henninger, N., Kristjanson, P. M., Reid, R. S., Atieno, F., Odero, A. N. and Ndegwa, T. (2002), Mapping poverty and livestock in the developing world. International Livestock Research Institute (ILRI), Nairobi, Kenya.

Thrusfield, M. (1995), Veterinary epidemiology. Second edition. Blackwell Sciences Ltd, Oxford, UK.

Timms, P., Stewart, N. P. and De Vos, A. J. (1990), Study of virulence and vector transmission of *Babesia bovis* by use of cloned parasite lines. *Infection and Immunity,* **58**, *7*, pp. 2171-2176.

Toft, C. A., Aeschlimann, A. and Bolis, L. (Eds.) (1991), Parasite-host associations: Co-existence or conflict? Oxford University Press, Oxford.

Torr, S., Eisler, M., Coleman, P., Morton, J. and Machila, N. (2002), Integrated control of ticks and tsetse. Project ZV0151. A Report for the DFID Advisory and Support Services Contract.

Toye, P. G., Goddeeris, B. M., Iams, K., Musoke, A. J. and Morrison, W. I. (1991), Characterisation of a polymorphic immunodominant molecule in sporozoites and schizonts of *Theileria parva*. *Parasite Immunology,* **13**, *1*, pp. 49-62.

Toye, P. G., Metzelaar, M. J., Wijngaard, P. L. J., Nene, V., Iams, K., Roose, J., Nyanjui, J. K., Gobright, E., Musoke, A. J. and Clevers, H. C. (1995a), Characterisation of the gene encoding the polymorphic immunodominant molecule, a neutralising antigen of *Theileria parva*. *Journal of Immunology,* **155**, *3*, pp. 1370-1381.

Toye, P. G., Gobright, E., Nyanjui, J., Nene, V. and Bishop, R. (1995b), Structure and sequence variation of the genes encoding the polymorphic, immunodominant molecule (PIM), an antigen of *Theileria parva* recognised by inhibitory monoclonal antibodies. *Molecular and Biochemical Parasitology,* **73**, *1-2*, pp. 165-177.

Toye, P. G., Nyanjui, J., Goddeeris, B. and Musoke, A. J. (1996), Identification of neutralization and diagnostic epitopes on PIM, the polymorphic immunodominant molecule of *Theileria parva*. *Infection and Immunity,* **64**, *5*, pp. 1832-1838.

Uilenberg, G., Schreuder, B. E., Silayo, R. S. and Mpangala, C. (1976), Studies on Theileriidae (Sporozoa) in Tanzania. IV. A field trial on immunisation against East Coast Fever (*Theileria parva* infection of cattle). *Tropenmedizin Und Parasitologie,* **27**, *3*, pp. 329-336.

Uilenberg, G. (1998), A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. Food and Agriculture Organisation of the United Nations, Rome.

UNDP (1997), Human Development Report,1997. United Nations Development Programme, Oxford University Press, New York. 256 pages.

Urquhart, G. M., Armour, J., Duncan, J. L. and Jennings, F. W. (1996), Veterinary parasitology. Second edition. Blackwell Sciences Ltd, Oxford, UK.

Van Baalen, M. and Sabelis, M. W. (1995), The dynamics of multiple infection and the evolution of virulence. *American Naturalist,* **146**, pp. 881-910.

Van den Bossche, P. (2001), Some general aspects of the distribution and epidemiology of bovine trypanosomosis in southern Africa. *International Journal for Parasitology,* **31**, *5-6*, pp. 592-598.

Van Hoeve, K., Onyango, R. J., Harley, J. M. and De Raadt, P. (1967), The epidemiology of *Trypanosoma rhodesiense* sleeping sickness in Alego location, Central Nyanza, Kenya. II. The cyclical transmission of *Trypanosoma rhodesiense* isolated from cattle to a man, a cow and to sheep. *Transactions of the Royal of Society of Tropical Medicine and Hygiene,* **61**, *5*, pp. 684-687.

Vassiliades, G. (1974), The helminthic infestations among cattle in Senegal River basin. *Bulletin of Epizootic Diseases of Africa,* **22**, pp. 69-74.

Venables, W. N. and Ripley, B. D. (2002), In *Modern and applied statistics with S*. Generalised linear models. (Eds, Chambers, J., Eddy, W., Hardle, W., Sheather, S. and Tierney, L.) Springer, New York, pp. 183-208.

Viera, L. Q., Oliviera, M. R., Newmann, E. and Nicoll, J. R. (1998), Parasitic infections in germ-free mice. *Brazilian Journal of Medical and Biological Research,* **31**, pp. 105-110.

Von Schrenck, H. and Falkensson, L. B. (1986), Evaluation of "HemoCue" a new device for determining haemoglobin. *Clinical Chemistry,* **32**, pp. 526-529.

Vonesh, E. F. and Chinchilli, V. M. (1997), Linear and nonlinear models for the analysis of repeated measures. Marcel Dekker, New York.

Waiswa, C., Olaho-Mukani, W. and Katunguka-Rwakishaya, E. (2003), Domestic Animals as Reservoirs for Sleeping Sickness in Three Endemic Foci in South-Eastern Uganda. *Annals of Tropical Medicine and Parasitology,* **97**, *2*, pp. 149-155.

Wakelin, D. (1996), Immunity to parasites: How parasitic infections are controlled. Cambridge University Press, Cambridge.

Ward, H. K. (1968), Supplementation of beef cows grazing on veld. *Rhodesian Journal of Agricultural Research,* **6**, pp. 93-101.

Waruiru, R. M., Ayuya, J. M., Weda, E. and Kimoro, C. O. (1993), Fatal haemonchosis in heifers in Kiambu district, Kenya: A case study. *Bulletin of Animal Health and Production in Africa,* **41**, pp. 263-265.

Waruiru, R. M., Nansen, P., Kyvsgaard, N. C., Thamsborg, S. M., Munyua, W. K., Gathuma, J. M. and Bogh, H. O. (1998), An abattoir survey of gastrointestinal nematode infections in cattle in the central highlands of Kenya. *Veterinary Research Communications,* **22**, *5*, pp. 325-334.

Waruiru, R. M., Kyvsgaard, N. C., Thamsborg, S. M., Nansen, P., Bogh, H. O., Munyua, W. K. and Gathuma, J. M. (2000), The prevalence and intensity of helminth and coccidial infections in dairy cattle in central Kenya. *Veterinary Research Communications,* **24**, *1*, pp. 39-53.

Watt, D., Sparagano, O., Brown, C. G. and Walker, A. R. (1997), Use of the polymerase chain reaction for identification and quantification of *Theileria parva* protozoa in *Rhipicephalus appendiculatus* ticks. *Parasitology Research,* **83**, *4*, pp. 359-363.

Watt, D., Kiara, H. and Sparagano, O. A. (1998), A PCR-based field evaluation of Theileria infections in cattle and ticks in Kenya. *Annals of the New York Academy of Sciences,* **849**, pp. 69-77.

Welburn, S. C., Fevre, E. M., Coleman, P. G., Odiit, M. and Maudlin, I. (2001), Sleeping sickness: A tale of two diseases. *Trends in Parasitology,* **17**, *1*, pp. 19-24.

Wells, E. A. (1972), The importance of mechanical transmission in the epidemiology of nagana: A review. *Tropical Animal Health and Production,* **4**, *2*, pp. 74-88.

WHO (2003), The lifecycle of *Trypanosoma brucei* [Online]. Special Programme for Research and Training in Tropical Diseases (TDR). Available at http:/www.who.org/TDR/Lifecycles/aa01123334k4k89fmmd. Accessed 23/05/06.

Wille, P., Boller, T. and Kaltz, O. (2002), Mixed inoculation alters infection success of strains of the endophyte *Epichloe bromicola* on its grass host *Bromus erectus*. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **269**, *1489*, pp. 397-402.

Williams, N. (1998), Malaria strains appear to gang up against immune defenses. *Science,* **279**, pp. 1136.

Williamson, J. (1970), In *The African Trypanosomiasis*. Review of chemotherapeutic and chemoprophylactic agents. (Ed, Mulligan, H. W.) Allen and Unwin, London, pp. 125-221.

Wilson, K., Grenfell, B. T. and Shaw, D. J. (1996), Analysis of aggregated parasite distributions: A comparison of methods. *Functional Ecology,* **10**, *5*, pp. 592-601.

Wilson, K. and Grenfell, B. T. (1997), Generalised linear modelling for parasitologists. *Trends in Parasitology,* **13**, *1*, pp. 33-38.

Wolfinger, R. and O'Connell, M. (1993), Generalised linear mixed models: A pseudo-likelihood approach. *Journal of Statistical Computation and Simulation,* **48**, pp. 233-243.

Woo, P. (1969), The haematocrit centrifugation technique for the detection of trypanosomiasis in blood. *Canadian Journal of Zoology,* **47**, pp. 921-923.

World-Bank (2001), World development report: Attacking poverty. Oxford University Press, New York, USA. 3556 pages.

Wright, I. A. and Russel, A. J. F. (1986), The use of body condition scoring to ration beef cows in late pregnancy. *Animal Production,* **43**, pp. 391-396.

Yacob, H. T., Dorchis, P., Jacquiet, P., Prevot, F., Grisez, C., Bergeaud, J. P. and Hoste, H. (2004), Concurrent parasitic infections of sheep: Depression of *Trichostrongylus colubriformis* populations by a subsequent infection with *Oestrus ovis*. *Veterinary Parasitology,* **121**, *3-4*, pp. 297-306.

Yadav, S. C., Sharma, R. L., Kalicharan, A., Mehra, U. R., Dass, R. S. and Verma, A. K. (1999), Primary experimental infection of riverine buffaloes with *Fasciola gigantica*. *Veterinary Parasitology,* **82**, *4*, pp. 285-296.

Yates, F. (1981), Sampling methods for censuses and surveys. Charles Griffin. Fourth edition. London and High Wycombe.

Yeoman, G. H. (1966), Field vector studies of epizootic East Coast Fever. I. A quantitative relationship between *Rhipicephalus appendiculatus* and the epizooticity of East Coast Fever. *Bulletin of Epizootic Diseases of Africa,* **14**, pp. 5-27.

Yewdell, J. W. and Bennink, J. R. (1999), Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. *Annual Review of Immunology,* **17**, pp. 51-88.

Yilma, J. M. and Malone, J. B. (1998), A geographic information system forecast model for strategic control of fasciolosis in Ethiopia. *Veterinary Parasitology,* **78**, *2*, pp. 103-127.

Yoshida, A., Maruyama, H., Yabu, Y., Amano, T., Kobayakawa, T. and Ohta, N. (1999), Immune response against protozoal and nematodal infection in mice with underlying *Schistosoma mansoni* infection. *Parasitology International,* **48**, *1*, pp. 73-79.

Young, A. S., Brown, C. G., Burridge, M. J., Cunningham, M. P., Kirimi, I. M. and Irvin, A. D. (1973), Observations on the cross-immunity between *Theileria lawrencei* (Serengeti) and *Theileria parva* (Muguga) in cattle. *International Journal for Parasitology,* **3**, *6*, pp. 723-728.

Young, A. S., Leitch, B. L. and Newson, R. M. (1981), In *Advances in the Control of Theileriosis: Proceedings of an International Conference Held at ILRAD, Nairobi, 9-13 February, 1981*. The occurrence of a *Theileria parva* carrier state in cattle from an East Coast Fever endemic area of Kenya. (Eds, Irvin, A. D., Cunningham, M. P. and Young, A. S.) Martinus Nijhoff Publishers, The Hague, pp. 60-62.

Young, A. S. and Grootenhuis, J. G. (1985), Influence of wildlife on immunisation of cattle against theileriosis in East Africa. Immunisation against theileriosis in Africa. Proceedings of a Joint Workshop. Nairobi, (Ed, Irvin, A. D.) International Laboratory for Research on Animal Diseases, 1-5 October, 1984, pp. 104-109.

Young, A. S., Leitch, B. L., Newson, R. M. and Cunningham, M. P. (1986), Maintenance of *Theileria parva parva* infection in an endemic area of Kenya. *Parasitology,* **93**, *1*, pp. 9-16.

Young, A. S., Groocock, C. M. and Kariuki, D. P. (1988), Integrated control of ticks and tick-borne diseases of cattle in Africa. *Parasitology,* **96**, *2*, pp. 403-432.

Young, A. S., Dolan, T. T., Morzaria, S. P., Mwakima, F. N., Norval, R. A. I., Scott, J., Sherriff, A. and Gettinby, G. (1996), Factors influencing infections in *Rhipicephalus appendiculatus* ticks fed on cattle infected with *Theileria parva*. *Parasitology,* **113**, *3*, pp. 255-266.

Young, C. J. and Godfrey, D. G. (1983), Enzyme polymorphism and the distribution of *Trypanosoma congolense* isolates. *Annals of Tropical Medicine and Parasitology,* **77**, pp. 467-481.

Zinsstag, J., Ankers, P., Njie, M., Itty, P., Monsan, V., Kaufmann, J. and Smith, T. (2000), Effect of strategic gastrointestinal nematode control on faecal egg count in traditional West African cattle. *Veterinary Research,* **31**, pp. 259-266.

Appendices

Appendix Chapter 4: Descriptive Statistics and Demographics of the Longitudinal Study

Appendix 4.1 Description of 24 out of the 102 farmers in Busia District which left the longitudinal study before the final visit round.

Round of withdrawal		REASON FOR CATTLE WITHDRAWAL			Cumulative Withdrawal (%)
	Farmers	Death	Sold	Voluntary	
1	0				0
2	1			1	0.98
3	0				0.98
4	0				0.98
5	2		1	1	2.94
6	1		1		3.92
7	1			1	4.9
8	0				4.9
9	2			2	6.86
10	2		1	1	8.82
11	2		1	1	10.8
12	3	1		2	13.7
13	3		1	2	16.7
14	7			7	**23.5**
Study end	78				100
GRAND TOTAL	**102**	**1**	**5**	**18**	

The round of observation (visit number) at which farmers announced their withdrawal is shown in the column “Round of withdrawal”. Information on cattle ownership was missing for five animals in Nanjeho. Unidentified owners are not included in the table.

Appendix 4.2 Description of 70 out of the 154 farmers in Tororo District which left the longitudinal study before the final visit round.

Round of withdrawal	Farmers	REASON FOR CATTLE WITHDRAWAL						Cumulative Withdrawal (%)
		Death	Dowry	Slaughter	Sold	Unmanageable	Voluntary	
1	1						1	0.65
2	12			1	1		10	8.44
3	3						3	10.4
4	5		1			1	3	13.6
5	7				1		6	18.2
6	3						3	20.1
7	7	1			1		5	24.7
8	4				1		3	27.3
9	2		1		1			28.6
10	1				1			29.2
11	1				1			29.9
12	3				2		1	31.8
13	1						1	32.5
14	0							32.5
15	3				1		2	34.4
16	7	1			1		5	39.0
17	10						10	**45.5**
Study end	84							100
GRAND TOTAL	**154**	**2**	**2**	**1**	**11**	**1**	**53**	

The round of observation (visit number) at which farmers announced their withdrawal is shown in the column “Round of withdrawal”. Information on cattle ownership was missing for one animal in Ojelai and seven cattle in Bunghaji. Unidentified owners are not included in the table.

Appendix 4.3 Cattle herd size owned by farmers enrolled in the longitudinal study and percentage of cattle recruited from each village.

Area	Farmers	TOTAL HERD SIZE					% Recruited
		Mean	Median	Min	Max	SD	Cattle
BUSIA							
Bunyimini	23	4.48	2	1	27	5.85	91.3
Kubo	36	4.50	3	1	15	3.84	61.7
Nanjeho	25	6.84	5	1	18	5.27	62.6
Sitengo	12	17.5	10.5	2	53	18.4	44.8
Total	96	6.73	3	1	53	8.80	61.1
TORORO							
Bunghaji	32	23.3	16	1	130	26.4	15.7
Hitunga	25	5.32	2	1	22	6.12	74.4
Magoje	41	8.63	6	1	35	8.29	32.5
Ojelai	26	5.73	4.5	1	16	4.24	67.8
Total	124	11.1	6	1	130	16.2	31.3
GRAND TOTAL	220	9.21	5	1	130	13.6	40.8

Information on cattle ownership was missing for five animals in Nanjeho, one animal in Ojelai and seven cattle in Bunghaji. Unidentified owners are not included in the table. Total cattle herd size was only available from 220 farmers out of 256 which participated in the longitudinal study.

Appendix 4.4 Main crop area reported on final survey (February 2003) of 231 out of 256 farmers enrolled in the longitudinal study.

Main crop area (Acres)	FARMERS		
	Busia	**Tororo**	**Both**
Less than 1	45	99	144
> 1 - 2	32	32	64
> 2 - 3	9	7	16
> 3 - 4	0	5	5
> 4 - 5	0	1	1
> 5 [1]	1	0	1
Total interviewed farmers	87	144	231

[1] Six acres

Appendix 4.5 Main crop type reported on final survey (February 2003) of 245 out of 256 farmers enrolled in the longitudinal study.

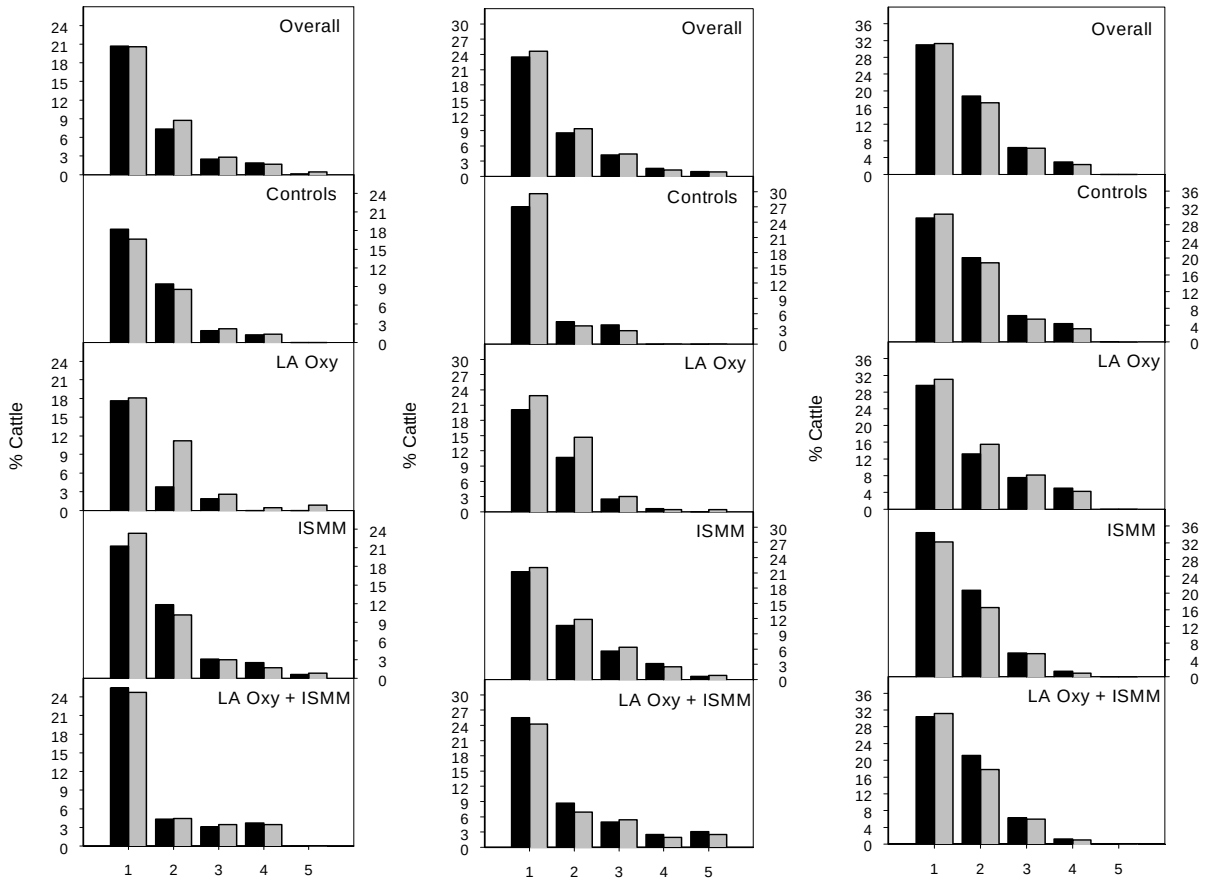
Main crop type	FARMERS		
	Busia	Tororo	Both
Millet		89	89
Cassava	70	1	71
Cotton		32	32
Maize	2	21	23
Cassava and Potatoes	12		12
Cassava and Maize	9		9
Rice		3	3
Cassava and Millet	2		2
Cassava and Sorghum	2		2
Tomatoes		1	1
Groundnuts		1	1
Total interviewed Farmers	97	148	245

Appendix 4.6 Percentages of cattle infested with Rhipicephalus appendiculatus, Boophilus decoloratus or Amblyomma variegatum adult stages.

	Cattle				Observations					
	n [1]	RA %	BD %	AV %	RA n [2]	RA %	BD n [2]	BD %	AV n [2]	AV %
BUSIA										
Bunyimini	96	100	96.9	97.9	1057	97.7	1056	67.7	1056	75.0
Kubo	102	100	95.1	99.0	1015	99.0	1016	49.4	1017	72.0
Nanjeho	109	100	90.8	89.9	1030	98.0	1030	47.9	1030	59.2
Sitengo	94	97.9	90.4	96.8	1165	97.3	1164	21.3	1165	57.5
Total	401	99.5	93.3	95.8	4267	98.0	4266	45.9	4268	65.7
TORORO										
Bunghaji	138	87.0	81.9	86.2	1196	74.4	1195	37.7	1196	72.8
Hitunga	100	91.0	90.0	95.0	1343	53.4	1343	36.6	1343	79.7
Magoje	127	80.3	74.8	90.6	1187	35.6	1187	26.8	1188	61.7
Ojelai	127	100	92.9	92.9	1220	96.3	1219	64.2	1219	74.4
Total	492	89.4	84.6	90.9	4946	64.8	4944	41.3	4946	72.4
GRAND TOTAL	**893**	**94.0**	**88.5**	**93.1**	**9213**	**80.2**	**9210**	**43.4**	**9214**	**69.3**

Percentages of initial and follow-up observations where tick infestation was recorded are also shown. [1] Total number of cattle from which *R. appendiculatus* (RA), *B. decoloratus* (BD) and *A. variegatum* (AV) counts were available for at least one visit round. [2] Total number of observations from which *R. appendiculatus*, *B. decoloratus* or *A. variegatum* data were available.

Appendix 4.7 Distribution of the number of ad hoc veterinary treatments administered to cattle in block treated villages as compared to in control villages.



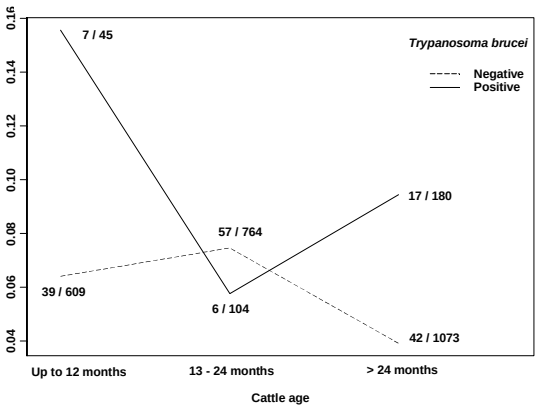
■ Considers cattle present at the initial main visit only (n = 639). All cattle received experimental treatments in areas where block interventions were dispensed.

■ Considers cattle present at the initial main visit, and cattle recruited at follow-up visits which did not received experimental treatments in areas where block interventions were dispensed (n = 893).

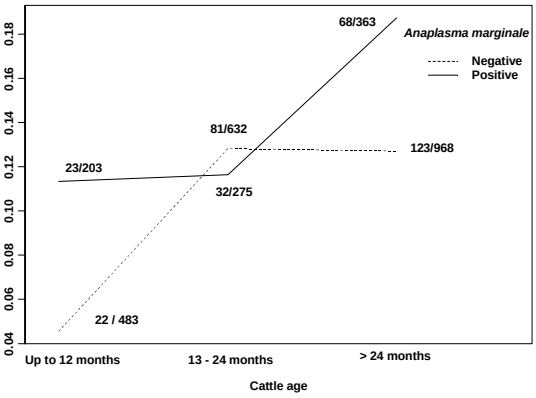
Controls: Bunyimini (Busia) and Ojelai (Tororo); **LA Oxytetracycline:** Sitengo (Busia) and Bunghaji (Tororo)**; ISMM:** Nanjeho (Busia) and Magoje (Tororo); **Both:** Kubo (Busia) and Hitunga (Tororo).

Appendix 4.8 Interaction Plots - Age-dependent associations amongst blood-borne pathogens of African cattle.

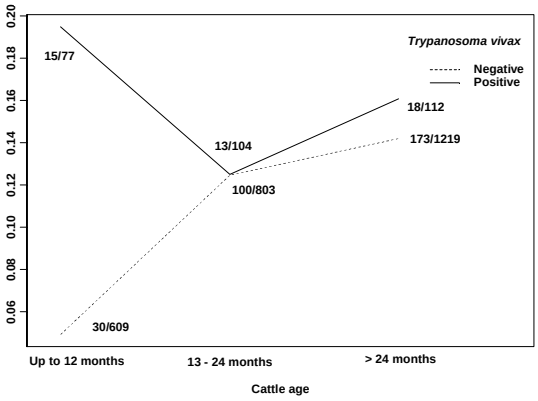
Y axis = Prevalence *T. congolense (Savannah)* ; n = 2775



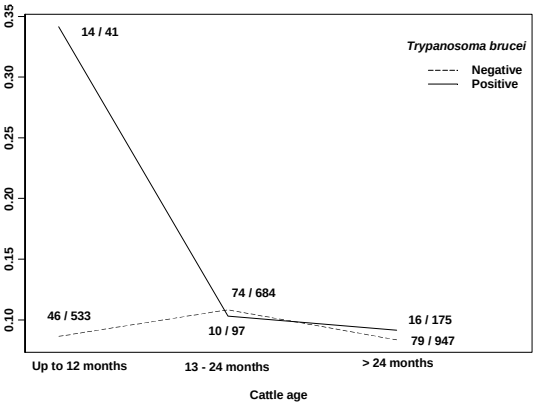
Y axis = Prevalence *T. brucei*
n = 2924



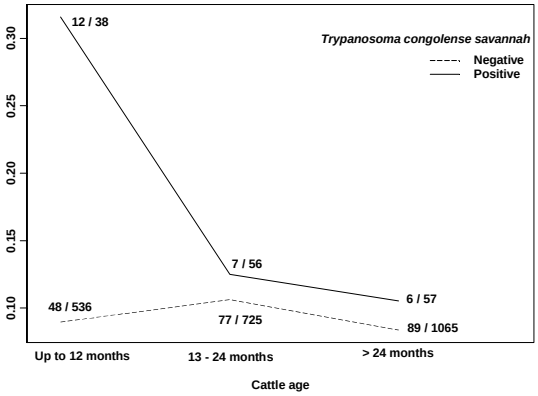
Y axis = Prevalence *T. brucei*
n = 2924



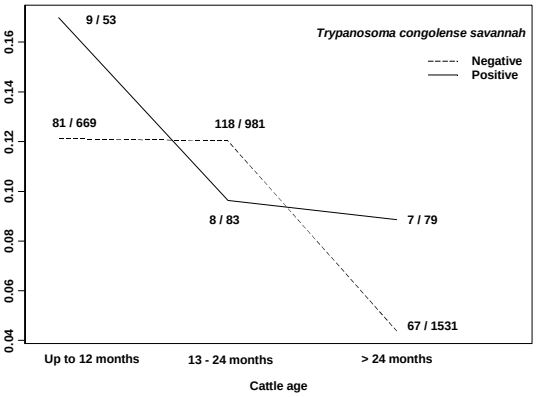
Y axis = Prevalence *T. vivax*
n = 2477



Y axis = Prevalence *T. vivax*
n = 2477



Y axis = Prevalence *T. parva*
n = 3396



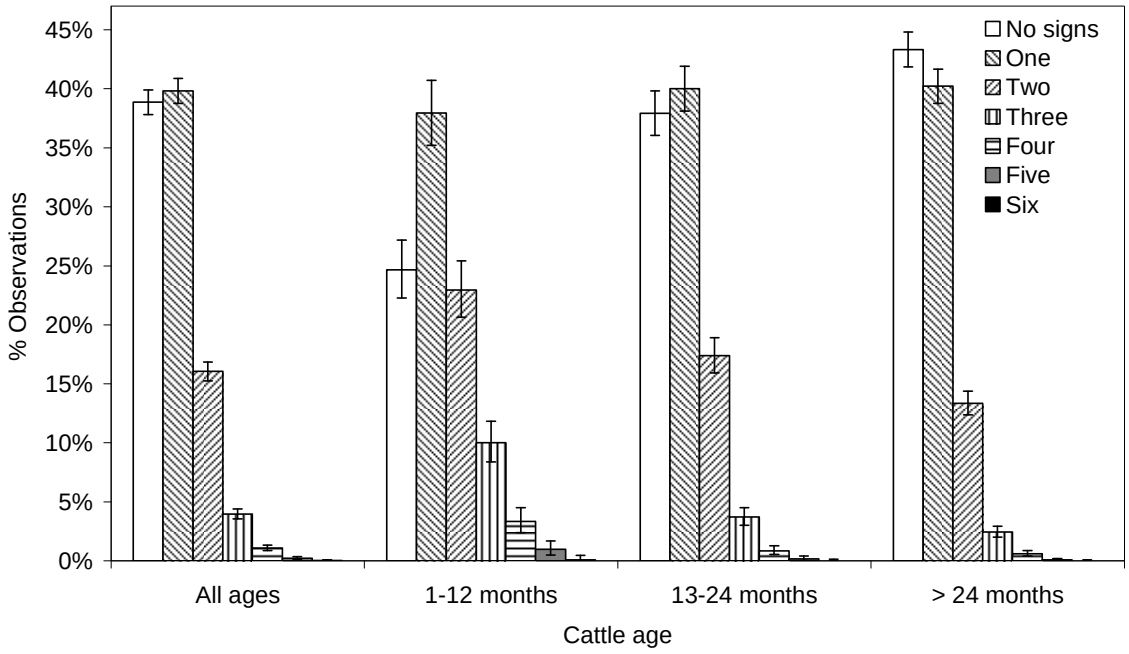
The plots include PCR data for trypanosomes only. See Tables 4.14 and 4.15.

Appendix 4.9 Cross-tabulation of pathogen-specific infection status, drug treatment and their interaction, on subsequent infection status by either microscopy or PCR.

Drug	Pathogen	Infection Status on Subsequent Observation ($IS_{(t+1)}$)	Current Infection Status ($IS_{(t)}$)		Treatment Between Infection Status Assessments		Interaction $IS_{(t)}$: Treatment			
							-		+	
			-	+	NT	T	NT	T	NT	T
OXY	*T. parva*	n [a]	6043	417	6257	550	5572	471	349	68
		Σ (+) [b]	261	150	372	50	234	27	127	23
		% (+) [c]	4.32	36.0	5.95	9.09	4.20	5.73	36.4	33.8
OXY	Theileria spp. (but not *T. parva*)	n	6130	1671	7108	722	5735	395	1345	326
		Σ (+)	1063	394	1271	194	970	93	293	101
		% (+)	17.3	23.6	17.9	26.9	16.9	23.5	21.8	31.0
OXY	Anaplasma spp.	n	5728	1971	7058	720	5402	326	1579	392
		Σ (+)	1133	628	1549	225	1048	85	488	140
		% (+)	19.8	31.9	21.9	31.3	19.4	26.1	30.9	35.7
OXY	Babesia spp.	n	7730	71	7108	722	7022	708	58	13
		Σ (+)	65		58	7	58	7		
		% (+)	0.84		0.82	0.97	0.83	0.99		
DIM	Babesia spp.	n	7730	71	7339	491	7253	477	57	14
		Σ (+)	65		56	9	56	9		
		% (+)	0.84		0.76	1.83	0.77	1.89		
DIM	Trypanosoma spp. (PCR) [d]	n	4679	804	5802	378	4399	280	726	78
		Σ (+)	613	154	817	43	581	32	146	8
		% (+)	13.1	19.2	14.1	11.4	13.2	11.4	20.1	10.3
DIM	Trypanosoma spp. (MCR) [e]	n	7482	349	7354	491	7223	259	117	232
		Σ (+)	308	24	318	14	302	6	16	8
		% (+)	4.12	6.88	4.32	2.85	4.18	2.32	13.68	3.45
ALB	Fasciola spp.	n	6016	965	6654	733	5654	362	605	360
		Σ (+)	748	227	959	68	729	19	178	49
		% (+)	12.4	23.5	14.4	9.28	12.9	5.25	29.4	13.6
ALB	Nematode Eggs	n	5111	2102	6781	735	4816	295	1664	438
		Σ (+)	1266	726	1956	103	1232	34	657	69
		% (+)	24.8	34.5	28.8	14.0	25.6	11.5	39.5	15.8

Microscopy (MCR); Uninfected (-); Infected (+); Not Treated (NT); Treated (T); LA oxytetracycline (OXY); Diminazene (DIM); Albendazole (ALB). [a] Number (sum) of observations over the course of the study, for which infection status was available for the current and the subsequent visit round (i.e. 28 days later). [b] Number (sum) of positive observations recorded on the subsequent visit round over the course of the study. [c] Proportion of positive observations obtained from dividing ([b]) by ([a]). [d] Includes samples with PCR data for at least one of the three Trypanosoma spp. [e] All samples were tested for all three Trypanosoma spp.

Appendix 4.10 Distribution of the number of clinical signs recorded from initial and follow-up observations, subdivided on the basis of the classification of the animals with respect to age at the time of each observation.



Where both subjective and objective indicators of the same clinical condition were available (i.e. pale mucous membranes vs. low PCV or low haemoglobin), only objective measures are taken into account as follows: Lymph node enlargement; Staring coat; Anaemia (PCV < 25% and / or haemoglobin < 8 g / dl); Discharge (vaginal, ocular, nasal or oculo-nasal; No animals presented with more than one type of discharge at a time); Fever; Poor condition score. 'Skin lesions' and subjective scoring of 'sick / severely sick' animals are not included. The figure is based on observations where cattle age and complete data on the conditions of interest were available (n = 8261). The sample size for calves, juveniles and adults was 1228, 2590 and 4443 observations respectively. Error bars represent exact binomial 95% confidence intervals.

Legend to Appendix 4.11

The 40 cattle which died during the longitudinal study are identified with an ear-tag that includes two letters and a number. Animals from Busia are identified with BY (Bunyimini), KU (Kubo), NN (Nanjeho) or ST (Sitengo), whereas those from Tororo with BH (Bunghaji), HT (Hitunga), MA (Magoje) or OJ (Ojelai). The age of each animal at the time of the initial (IV) and last (LV) visits, is shown in months. The abbreviations for infection status data are: Anaplasma spp. (A); Theileria spp. (Th); Fasciola spp. (F); "*Strongyle-type*" nematodes (N); Trypanosoma spp. (T); *Theileria parva* (P). None of these cattle were found to be infected with Babesia spp. Infection intensity data was available for Anaplasma spp., Theileria spp., Trypanosoma spp. (by microscopy) and nematode eggs. In the cases of Anaplasma spp., Theileria spp., Trypanosoma spp., '+' indicates that only one organism was found in > 10 microscopic fields, '++' that only one organism was found in > 1 field and < 10 fields, and '+++' that one or more organisms were found in one field. In the case of nematodes, a negative sign indicates that the infection intensity was below the detection limit of the McMaster Quantitative technique. The number of eggs per gram of faeces is shown for nematode-positive animals. The abbreviations for clinical signs are: Lymph node enlargement (N); poor (L-, L or L+) condition score (C); staring coat (S); ocular discharge (d); fever (> 39.4˚C), (F); Anaemia (direct [haemoglobin < 8g/dl] or indirect [pale mucous membranes or PCV < 25%] measurements), (A). Only ocular discharge was recorded from these cattle at the time of the last visit round. No animals presented with other clinical signs.

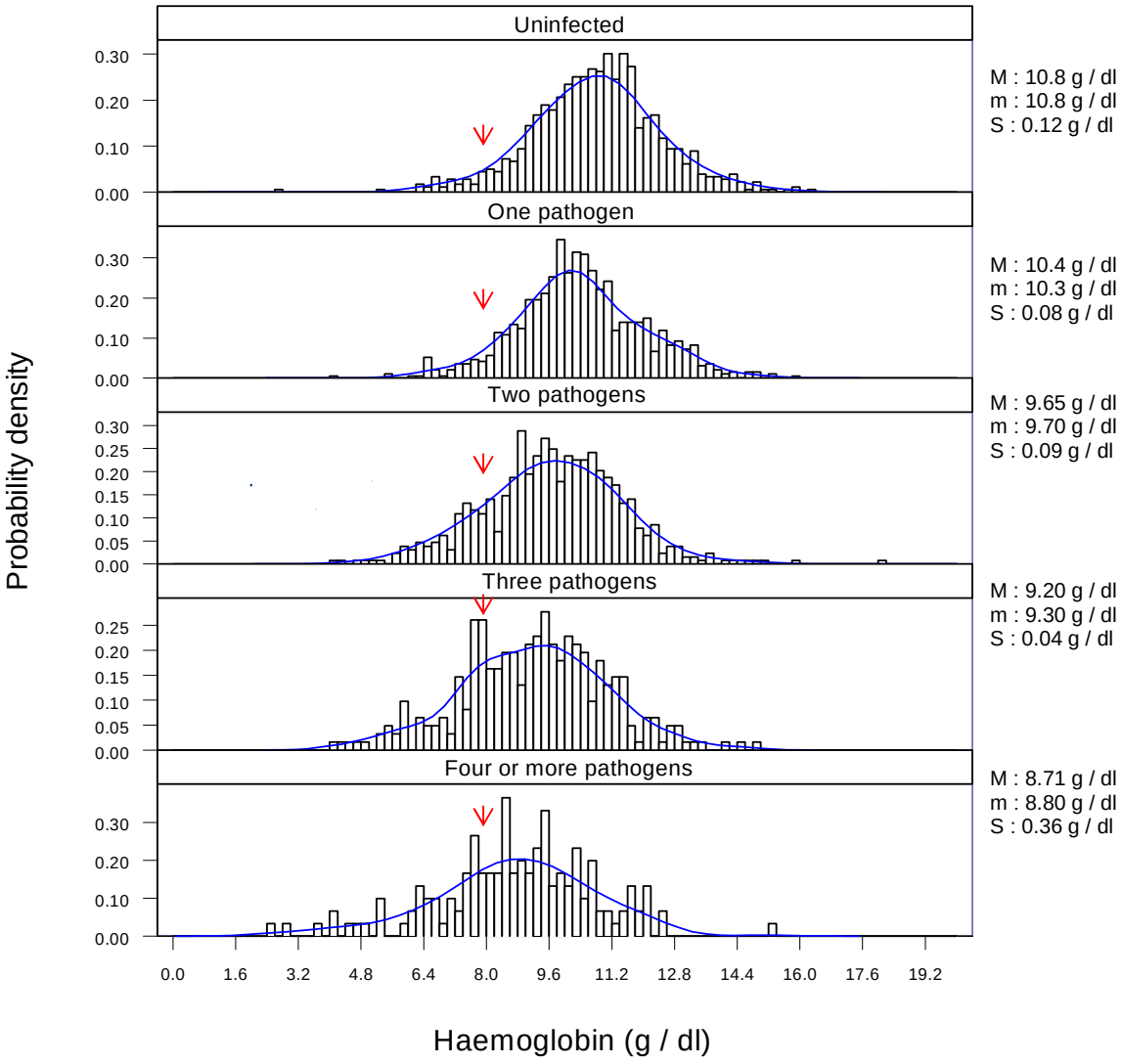
[1] The cause of death was strangulation. **[2]** The putative diagnosis of death was anaplasmosis. **[3]** Death was attributed to co-infection with multiple pathogens. These cattle presented with critical condition at the time of the last visit. **[4]** Other clinical signs included abdominal enlargement (possibly ascites) and anorexia. **[5]** The putative diagnosis of death was East Coast Fever. **[6]** The animal died while giving birth. **[7]** The cause of death was broken leg. **[8]** Mixed *T. brucei*, *T. vivax* and *T. congolense* infection. **[9]** *T. vivax* only. **[10]** *T. brucei* only.

Appendix 4.11 Description of clinical and laboratory results obtained at the last visit from 40 cattle that died during the longitudinal study.

Ear-Tag		Cattle Age (months)		Infection Status LV								Clinical Signs LV				
				MICROSCOPY					PCR							
		IV	LV	A	Th	F	N	T	T	Tp	N	C	S	d	F	A
BY	37	1-6	7-12	+++	+	+	-	-		-	+	-	+	-	+	-
	30	1-6	7-12	-	-	-	200	-		-	-	+	+	+	-	-
	91	>24	>24	-	+	-	-	-		-	+	-	-	+	-	-
KU	11	1-6	7-12	+	-	-	200	-	-	-	-	-	+	-	-	-
	35 [1]	>24	>24	-	-	+	-	-		-	-	-	-	+	-	+
	57	7-12		-	-	-	-	-	-	-	-	-	-	-	-	
	80	7-12		-	-	-	-	-	+[8]	-	-	-	-	-	-	+
	50	13-24		-	-	-	-	-	-	+	+	-	-	-	-	-
NN	76	1-6	1-6	-	-	+	-	-	-	-	+	-	-	-	-	-
	106	1-6	1-6	-	-	-	-	-		-	+	-	+	-	-	+
	18 [2]	7-12	7-12	++	-	-	-	-	-	-	+	+	+	+	-	-
	114	7-12	7-12	-	+	-	200	-		-	-	-	+	-	-	-
	73	>24	>24	+	+	-	100	-	-	+	+	-	-	-	-	-
	32	7-12		+	+	+	-	-	-	-	-	-	+	-	-	-
	12 [2, 4]	7-12		+	-	-	-	-	-	+		-	+	+	-	+
ST	35	1-6	1-6	-	-	-	-	-	+[9]	+	-	+	+	-	-	+
	8 [3]	7-12	7-12	++	+	-	1000	+++ [8]	+[9]	-	+	+	+	-	-	+
	41 [3]	7-12	7-12	+	+	-	900	-	+[10]	-	-	+	+	+	-	+
	9 [3]	7-12		++	-	-	-	-	+[9]	-	-	+	+	+	-	+
	43 [3]	7-12		-	-	-	1200	+++ [9]	-	-	-	+	+	-	-	+
BH	125	13-24	>24	+	-	-	100	-		-	+	-	-	-	-	+
	29 [4]	>24	>24	+	-		-	-			-	-	-	-	-	-
	7	7-12		+	+	+	-	-			-	-	-	-	-	+
	59	7-12		-	+	-	-	-			+	+	+	+	-	+
	73	7-12		-	-	-	-	-		-	+	-	-	-	-	-
	15	7-12		-	-	-	-	-		-	-	-	-	-	-	-
	16 [5]			-	+++	-	-	-	-	-	-	+	+	+	-	+
HT	58 [6]	13-24	>24	-	-	-	-	-			+	-	-	-	-	-
	7 [7]	>24	>24	+	-	-	-	-	-	-	-	-	-	+	-	-
	31	7-12		-	-	-	200	-		-	-	-	-	-	-	-
	69	7-12		-	-	-	200	-			-	-	-	-	-	-
MA	110 [5]	7-12	7-12	-	-	-	400	-			-	-	+	-	-	+
	125	7-12	13-24	++	-	-	-	-			+	-	+	-	-	-
	98	>24	>24	-	++	-	-	-		-	-	-	-	-	-	-
	114	>24	>24	+++	++	-	-	-			-	-	-	-	-	+
	46	7-12		-	-	-	200	-	-	-	-	-	-	-	-	-
	67	7-12		-	-	-	-	-		-	-	-	-	-	-	-
	93	7-12		+	-	-	400	-		-	-	-	+	-	-	-
	72 [5]	7-12		-	-	-	-	-		+	-	-	+	-	-	+
OJ	126	13-24	>24	+++	+			-		-	+	+	+	-	-	+
% Positive Cattle				**42.5**	**32.5**	**13.2**	**33.3**	**5.00**	**29.4**	**15.6**	**35.9**	**25.0**	**50.0**	**25.0**	**2.5**	**43.6**

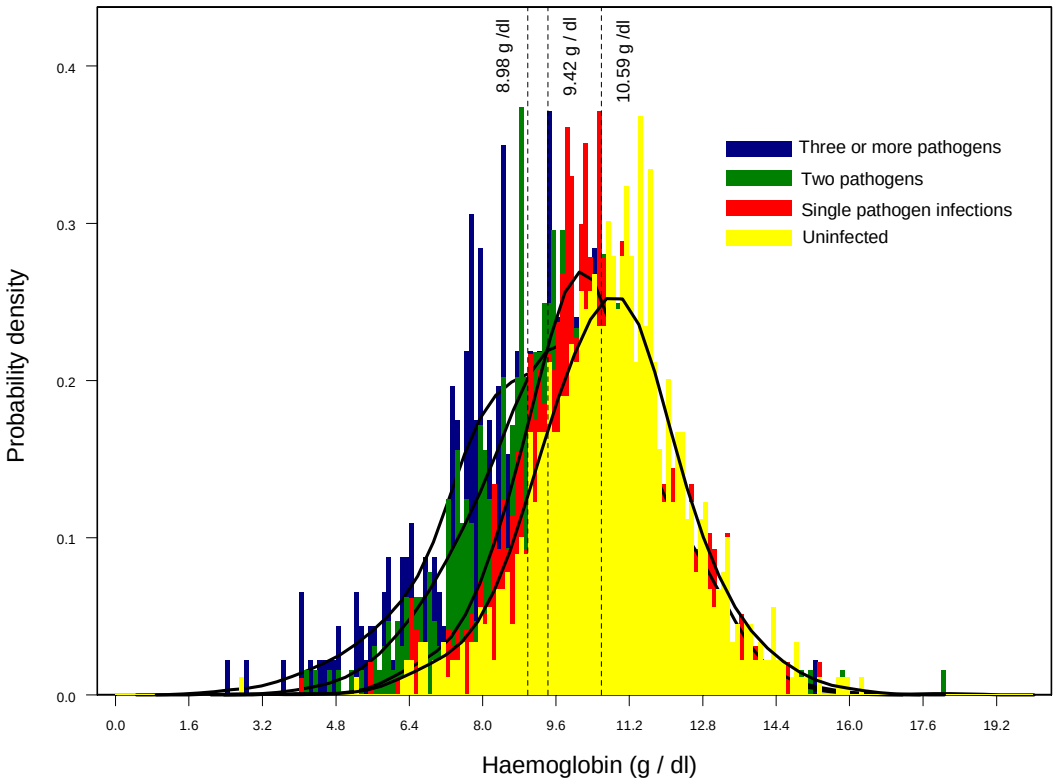
Appendix Chapter 5: Interactions Amongst Common Endemic Pathogens of African Cattle: Implications for Pathogenesis

Appendix 5.1 Histograms and probability densities of circulating haemoglobin concentrations (g/dl) from uninfected calves and juvenile cattle and cattle of the same age groups infected with one, two, three, or four or more pathogens.



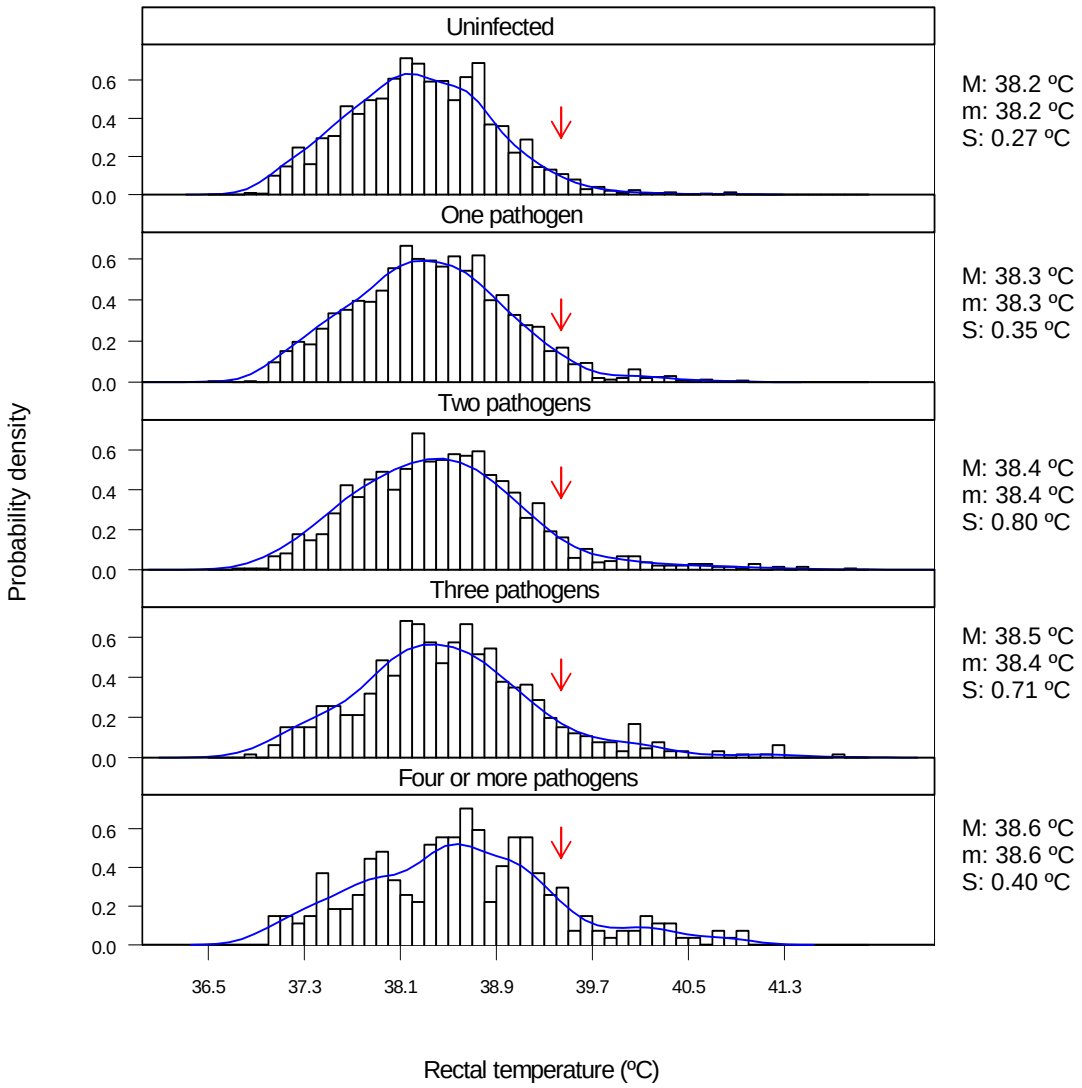
The range of haemoglobin concentrations is shown in the horizontal axis at interval widths of 0.2 g/dl. The density of samples within each interval is shown in the vertical axis. The height of the bars represents the relative concentration of data points within each interval, which is calculated by dividing the frequency of samples by the width of the interval. The blue lines show the probability density for each histogram. The sum of the area beneath the bars is equal to one. Basic descriptive statistics for each histogram (Mean “M”, median “m” and skewness “S”) are shown in the right-hand side. The red arrows highlight the cut-off point for anaemia, which occurs when haemoglobin values are below 8 g/dl. Observations from animals for which no repeated samplings were available are also included (n = 2968 observations on 661 cattle).

Appendix 5.2 Super-imposed histograms and probability densities of circulating haemoglobin concentrations (g/dl) from uninfected calves and juvenile cattle and cattle of the same age groups infected with one, two of three or more pathogens.



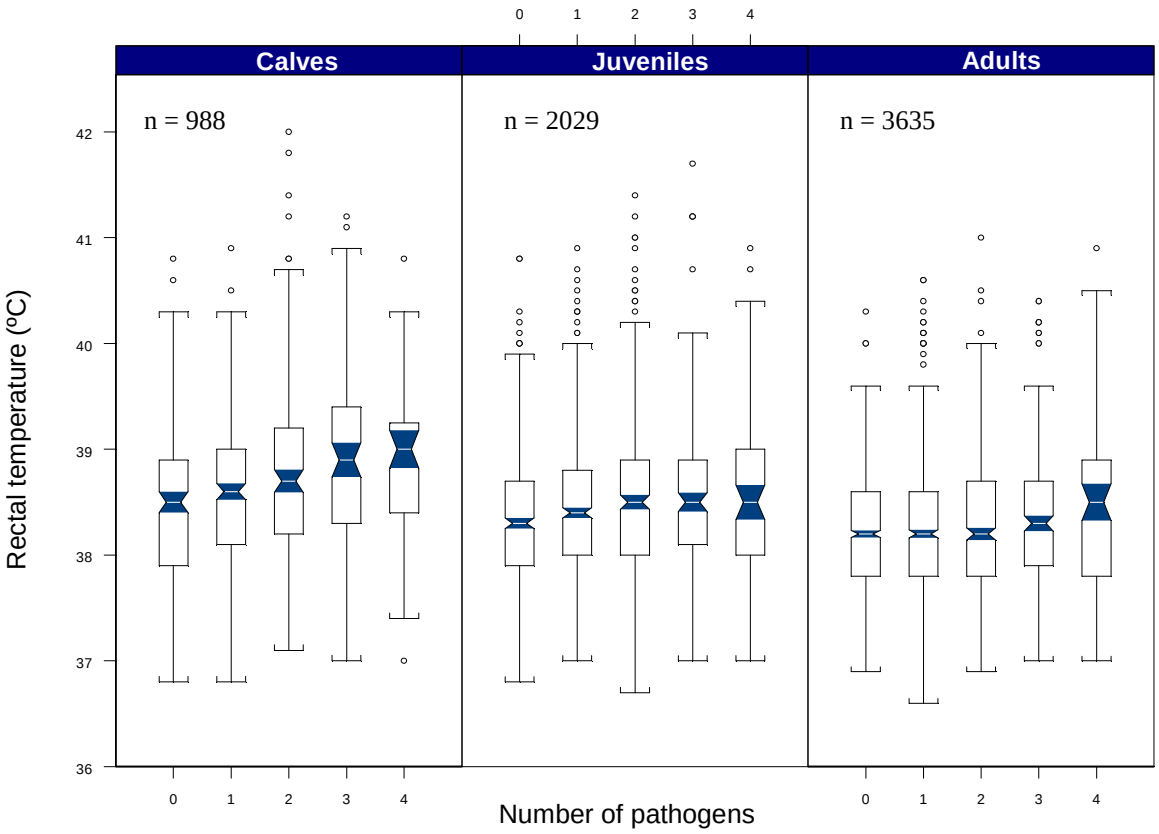
The super-imposed histograms highlight approximate values of haemoglobin below which infection with one, two, or three or more pathogens, is more likely. The range of haemoglobin concentrations is shown in the horizontal axis of the graph at interval widths of 0.1 g/dl. The height of the bars represents the relative concentration of data points within each interval of haemoglobin, which is calculated by dividing the frequency of samples by the width of the interval. The black lines highlight the probability density for each histogram. Observations from animals for which no repeated samplings were available are also included (n = 2968 observations on 661 cattle).

Appendix 5.3 Histograms and probability densities of rectal temperature (ºC) values from uninfected cattle and cattle infected with one, two, three, or four or more pathogens.



Rectal temperature values are from cattle of any age. Rectal temperatures are shown in the horizontal axis at interval widths of 0.1 ºC. The density of samples within each interval is shown in the vertical axis. The height of the bars represents the relative concentration of data points within each interval, which is calculated by dividing the frequency of samples by the width of the interval. The blue lines show the probability density for each histogram. The sum of the area beneath the bars is equal to one. Basic descriptive statistics for each histogram (Mean “M”, median “m” and skewness “S”) are shown in the right-hand side. The red arrows highlight the cut-off point for fever (> 39.4 ºC). Observations from animals for which no repeated samplings were available are also included (n = 7168 on 864 cattle).

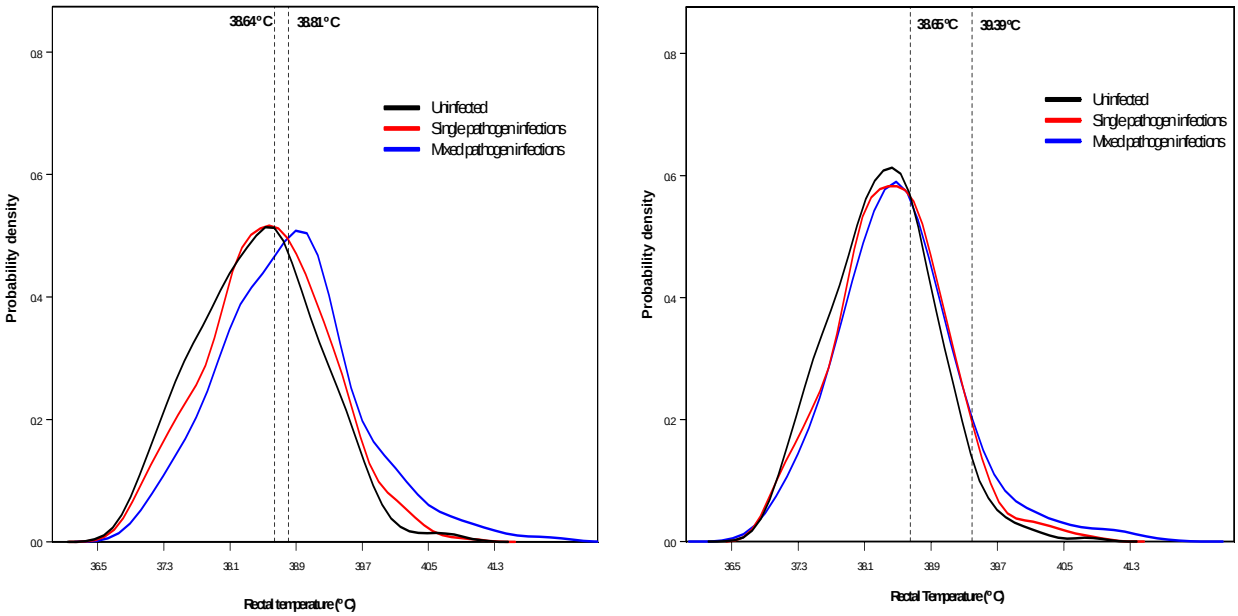
Appendix 5.4 Box-and-Whisker plots of rectal temperature (ºC) data from uninfected calves, juveniles, or adult cattle, and cattle of the same age groups infected with one, two, three, or four or more pathogens.



Each box indicates the lower and upper quartiles representing the temperature values below which 25% and 75% of the observations lie respectively. The median rectal temperature is shown within each box by a white horizontal bar. The whiskers show the range of observations excluding outliers, which are extreme temperature values represented by dots in the figure. The blue notched areas indicate the 95% confidence intervals for the median rectal temperature. Observations from animals for which no repeated samplings were available are also included (n = 302 calves, 522 juveniles and 513 adults).

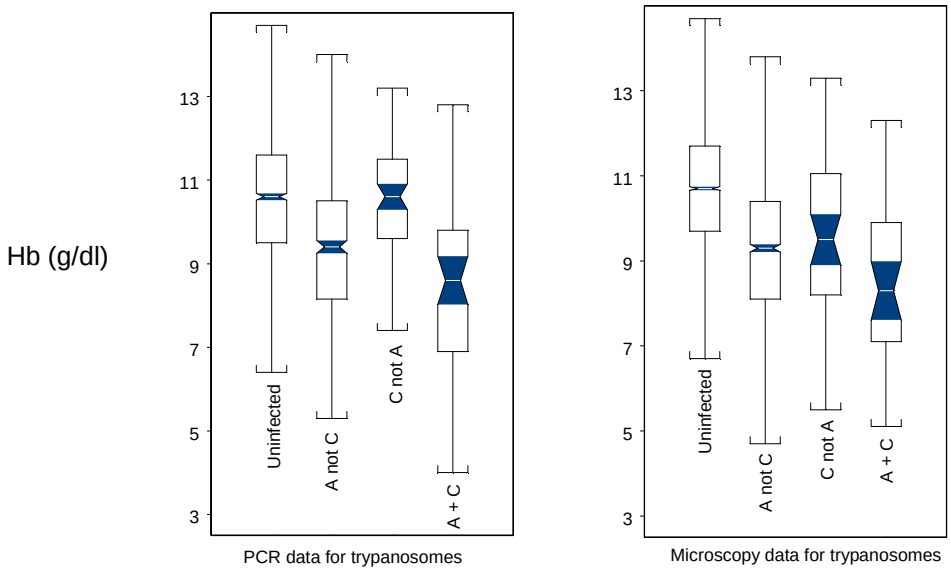
Appendix 5.5 Super-imposed probability densities of rectal temperature readings from uninfected calves or juvenile cattle, and from cattle of either age with single or mixed pathogen infections.

Calves (n = 988) **Juveniles (n = 2029)**



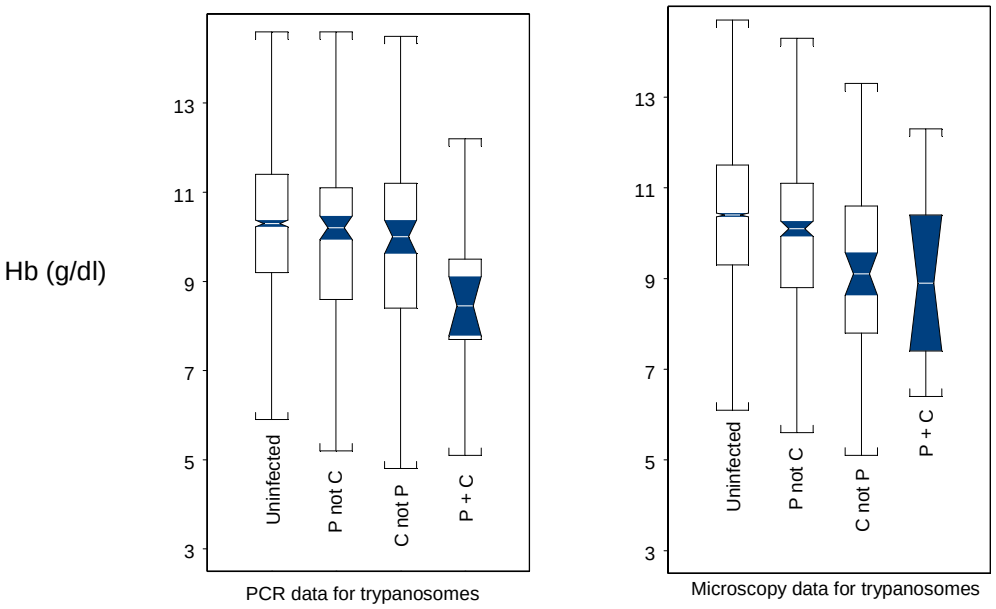
The rectal temperature values above which the probability of infection by any given number of pathogens changes, is far less clear-cut than when haemoglobin readings are used for this purpose.The range of rectal temperature values is shown in the horizontal axis of the graph. Histograms were built at temperature interval widths of 0.1 ºC. Probability density curves are shown in black, red or blue. These density curves are based on the height of the histogram bars (not shown), which represent the relative concentration of data points within each interval of rectal temperature. Observations from animals for which no repeated samplings were available are also included (n = 522 juveniles and 302 calves).

Appendix 5.6.a Box-and-Whisker plots of the haemoglobin concentration (g/dl) in separate and contemporaneous Anaplasma spp. and *T. congolense* infections.



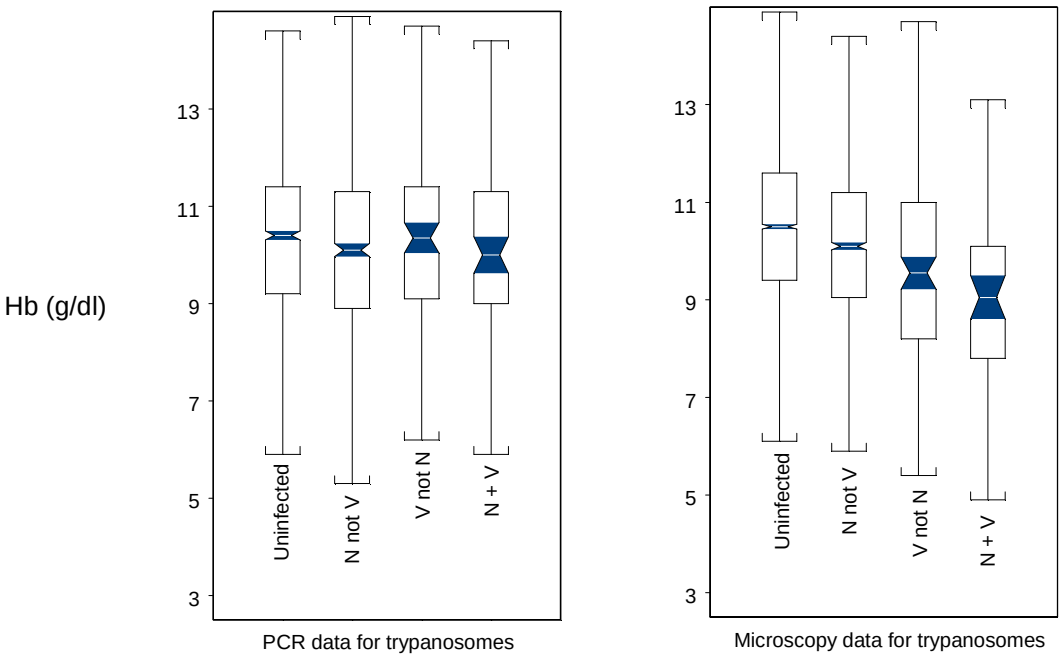
The box-and-whisker plots show the haemoglobin (Hb) concentration in samples infected with Anaplasma spp. alone (A), *T. congolense* alone (C) or both (A + C), compared to samples free of these pathogens. The first figure includes *T. congolense* infections by PCR, whilst the second includes *T. congolense* infections by microscopy. The median haemoglobin concentration is shown within each box by a white horizontal bar. When considering microscopy data for *T. congolense*, the median haemoglobin concentration in mixed infections is similar to that expected when summing the effect each individual pathogen (A + C [Expected = 8.1 g/dl vs. Observed = 8.3 g/dl]). In contrast, when considering PCR data for *T. congolense*, the median haemoglobin concentration in mixed infections is 0.8 g/dl lower to that expected in co-infections involving Anaplasma spp. In addition, the median haemoglobin concentration in *T. congolense* - Anaplasma spp. co-infections is similar regardless of whether microscopy or PCR data for the trypanosome is considered. This is an example of a positive interaction on haemoglobin which appears to occur only at lower trypanosome parasitaemias (infections detectable by PCR), but not at higher trypanosome parasitaemias (infections detectable by microscopy). Plotted observations are those included in Datasets A (n = 2379 observations on 566 cattle) and B (n = 6941 observations on 778 cattle).

Appendix 5.6.b Box-and-Whisker plots of the haemoglobin concentration (g/dl) in separate and contemporaneous *T. parva* and *T. congolense* infections.



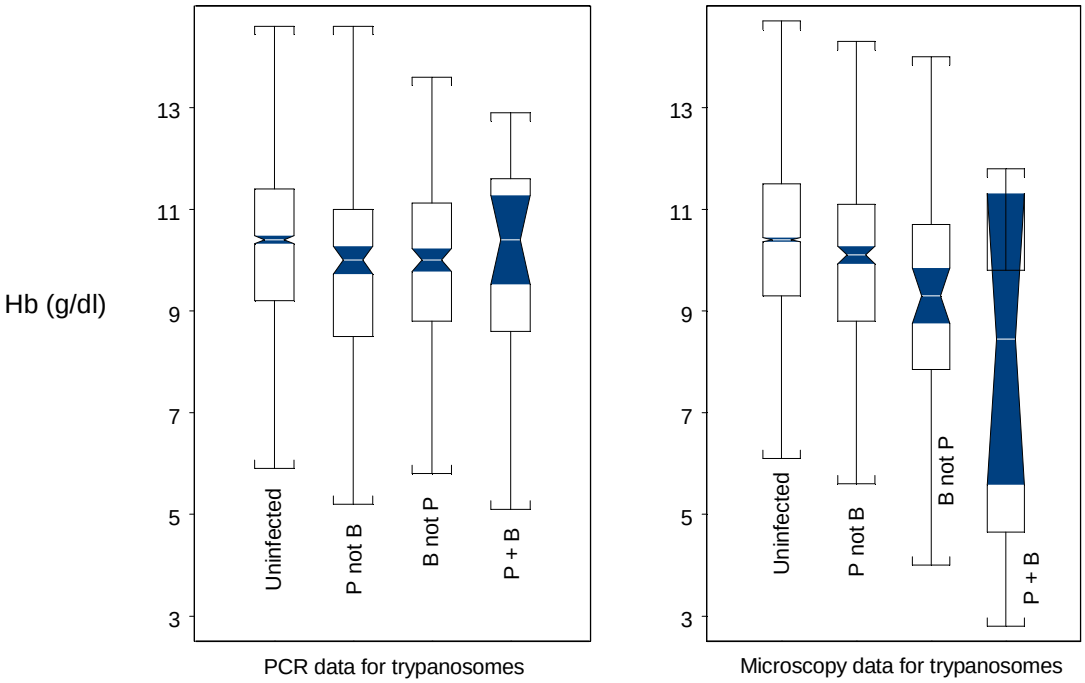
The box-and-whisker plots show the haemoglobin (Hb) concentration in samples infected with *T. parva* alone (P), *T. congolense* alone (C), or both (P + C), compared to samples free of these pathogens. When considering microscopy data for *T. congolense*, the median haemoglobin concentration in mixed infections is almost identical or even higher as compared to that expected when summing the effect each organism individually (P + C [Expected = 8.8 g/dl vs. Observed = 8.9 g/dl]). In contrast, when considering PCR data for *T. congolense*, the median haemoglobin concentration in mixed infections is 0.6 g/dl lower to that expected from the sum of the effect of each individual pathogen. In addition, the median haemoglobin concentration in *T. parva* co-infections is similar regardless of whether microscopy or PCR data for *T. congolense* is considered. This is another example of a positive interaction on haemoglobin which appears to occur only at lower trypanosome parasitaemias (infections detectable by PCR), but not at higher trypanosome parasitaemias (infections detectable by microscopy). Plotted observations are those included in Datasets A (n = 2379 observations on 566 cattle) and B (n = 6941 observations on 778 cattle).

Appendix 5.6.c Box-and-Whisker plots of the haemoglobin concentration (g/dl) in separate and contemporaneous "*Strongyle-type*" nematodes and *T. vivax* infections.



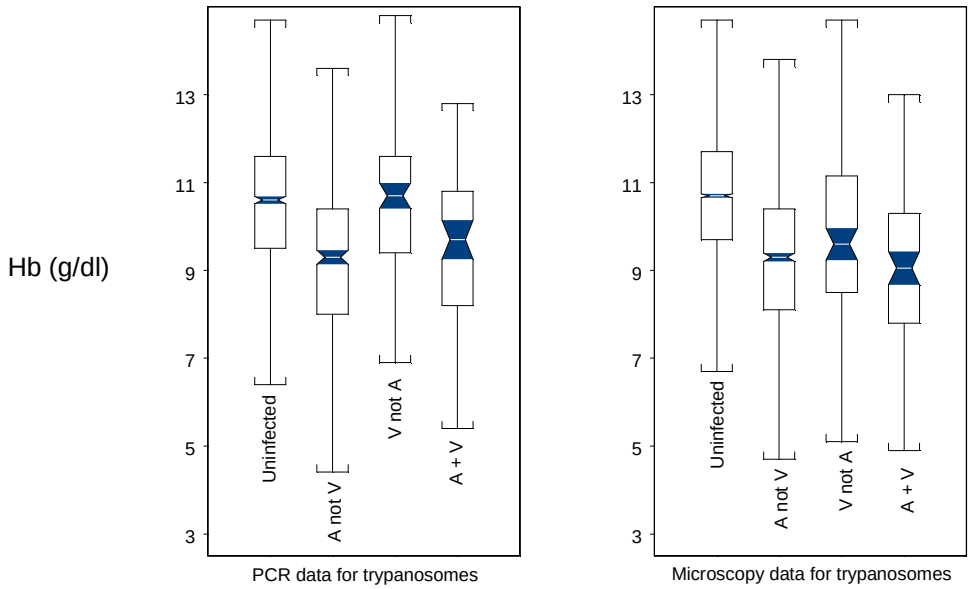
The box-and-whisker plots show the haemoglobin (Hb) concentration in samples from animals infected with *"Strongyle-type"* nematodes alone (N), *T. vivax* alone (V), or both (N + V), compared to samples from animals free of these pathogens. When considering PCR data for *T. vivax* the median haemoglobin concentration in mixed infections is not substantially different or is even higher than that expected when summing the observed effect of single nematode and *T. vivax* infections. In contrast, when considering microscopy data the median haemoglobin concentration in mixed infections is substantially lower as compared to the sum of effects in single pathogen infections. This is an example of a positive interaction on haemoglobin which appears to occur only at higher trypanosome parasitaemias (infections detectable by microscopy), but not at lower trypanosome parasitaemias (infections detectable by PCR). Plotted observations are those included in Datasets A (n = 2379 observations on 566 cattle) and B (n = 6941 observations on 778 cattle).

Appendix 5.6.d Box-and-Whisker plots of the haemoglobin concentration (g/dl) in separate and contemporaneous *T. parva* and *T. brucei* infections.



The box-and-whisker plots show the haemoglobin (Hb) concentration in samples from animals infected with *T. parva* alone (P), *T. brucei* alone (B), or both (P + B), compared to that in samples free of these pathogens. When considering PCR data for *T. brucei,* the median haemoglobin concentration in mixed infections is higher than that expected when summing the effect of single *T. parva* and *T. brucei* infections. This inding was not significant (See Table 5.7). In contrast, when considering microscopy data, the median haemoglobin concentration in mixed infections is substantially lower as compared to the sum of effects in single pathogen infections. This is another example of a positive interaction on haemoglobin which appears to occur only at higher trypanosome parasitaemias (infections detectable by microscopy), but not at lower trypanosome parasitaemias (infections detectable by PCR). Plotted observations are those included in Datasets A (n = 2379 observations on 566 cattle) and B (n = 6941 observations on 778 cattle).

Appendix 5.6.e Box-and-Whisker plots of the haemoglobin concentration (g/dl) in separate and contemporaneous *Anaplasma spp.* and *T. vivax* infections.



The box-and-whisker plots show the haemoglobin (Hb) concentration in samples infected with Anaplasma spp. alone (A), *T. vivax* alone (V), or both (A + V), compared to samples free of these pathogens. When considering PCR data for *T. vivax* the median haemoglobin concentration in mixed infections is similar to that expected when summing the effect each organism individually (Expected = 9.4 g/dl vs. Observed = 9.7 g/dl). In contrast, when considering microscopy data the median haemoglobin concentration in mixed infections is 0.9 g/dl higher to that expected in A+V co-infections. This is an example of a negative interaction on haemoglobin which appears to occur only at higher trypanosome parasitaemias (infections detectable by microscopy), but not at lower trypanosome parasitaemias (infections detectable by PCR). Plotted observations are those included in Datasets A (n = 2379 observations on 566 cattle) and B (n = 6941 observations on 778 cattle).

Appendix Chapter 6: The Epidemiology of *Theileria parva* and its Relationship with Cattlle Age in Areas which Differ in *Rhipicephalus appendiculatus* Challenge

Appendix 6.1 List of analysis methods, model specifications and results.

1 Tick counts (0, 1, 2, 3) ~ Village. Village = Eight-level categorical variable, where each level corresponds to a different village. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts for each individual animal. The significance of this test is shown below (coefficients not shown).

F-value	p-value	Observations
$F_{7, 855} = 525.9$	< .0001	9213 on 893 cattle

2 Tick counts (0, 1, 2, 3) ~ Village. Village = Five-level categorical variable, where each level corresponds to Bunyimini, Kubo, Nanjeho, Ojelai or Sitengo (i.e. HTC villages). Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts and time slopes for each individual animal. The significance of this test is shown below (coefficients not shown).

F-value	p-value	Observations
$F_{4, 523} = 40.76$	< .0001	5487 on 528 cattle

3 Tick counts (0, 1, 2, 3) ~ Village. Village = Four-level categorical variable, where each level corresponds to Bunyimini, Kubo, Nanjeho or Ojelai. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts and time slopes for each individual animal. The significance of this test is shown below (coefficients not shown).

F-value	p-value	Observations
$F_{3, 430} = 1.87$	0.133	4322 on 434 cattle

4 Tick counts (0, 1, 2, 3) ~ Village. Village = Two-level categorical variable, where the first level corresponds to Bunyimini + Kubo + Nanjeho + Ojelai (i.e. HTC villages other than Sitengo) whilst the second corresponds to Sitengo. "*HTC villages other than Sitengo*" was specified as the reference variable level. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts and time slopes for each individual animal. The significance and the coefficients of this analysis are shown below.

Variable level	Coef	SE	F-value	p-value	Observations
Sitengo	-0.21	0.02	$F_{1, 526} = 156.2$	<.0001	5487 on 528 cattle

5 Tick counts (0, 1, 2, 3) ~ Village. Village = Two-level categorical variable, where each level corresponds to either Bunghaji or Hitunga. Bunghaji was specified as the reference variable level. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts and time slopes for each individual animal. The significance and the coefficients of this analysis are shown below.

Variable level	Coef	SE	F-value	p-value	Observations
Hitunga	-1.13	0.12	$F_{1, 236} = 90.9$	<.0001	2539 on 238 cattle

6 Tick counts (0, 1, 2, 3) ~ Village. Village = Two-level categorical variable, where each level corresponds to either Hitunga or Magoje. Hitunga was specified as the reference variable level. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts and time slopes for each individual animal. The significance and the coefficients of this analysis are shown below.

Variable level	Coef	SE	F-value	p-value	Observations
Magoje	-0.45	0.06	$F_{1, 225} = 58.0$	<.0001	2530 on 227 cattle

7 Tick infestation [0 = No ticks present; 1 = Ticks present] ~ Village. Village = Two-level categorical variable, where each level corresponds to either Hitunga or Magoje. Hitunga was specified as the reference variable level. Method = GlmmPQL; binomial error structure. Tick infestation status was modelled considering different intercepts for each individual animal. The odds ratio and 95% confidence intervals, calculated from the model coefficient and the associated standard error, are shown in the table.

Variable level	OR	95% CI	F-value	p-value	Observations
Magoje	0.46	0.37 – 0.58	$F_{1, 225} = 43.68$	<.0001	2530 on 227 cattle

8 Tick counts (0, 1, 2, 3) ~ Village. Village = Two-level categorical variable, where each level corresponds to either Bunghaji (i.e. LTC village where tick challenge was highest) or Sitengo (i.e. HTC village where tick challenge was lowest). Bunghaji was specified as the reference variable level. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts and time slopes for each individual animal. The significance and the coefficients of this analysis are shown below.

Variable level	Coef	SE	F-value	p-value	Observations
Sitengo	+0.48	0.03	$F_{1, 230} = 262.6$	<.0001	2361 on 232 cattle

9 Analysis of contingency tables with poisson errors (Generalised linear modelling). The significance of the interaction term "(a) *T. parva* exposure : (b) Village", was assessed through a deletion test comparing a saturated model containing the two main terms and their interaction (a*b), with a reduced model containing only main terms. The dependent variable consisted of the number of cattle falling into each category (i.e. cross-tabulation between *T. parva* exposure over the course of the study [Always PCR negative/At least one PCR positive observation] and village [Bunyimini, Kubo, Nanjeho, Ojelai, Sitengo, Bunghaji, Hitunga or Magoge]). The significance of this test is shown below. Chisq-test was chosen in views of the saturated nature of the initial model.

Model Term	df	Chisq	p-value	Cattle
a : b	7	53.13	<.0001	884

10 Same as in model **9**, but "Village" was specified as a six-level categorical variable, where each level corresponded to a different village as follows: Bunyimini, Kubo, Nanjeho, Ojelai, Sitengo (HTC villages) and Bunghaji (LTC village). The significance of this test is shown below.

Model Term	df	Chisq	p-value	Cattle
a : b	5	8.38	0.136	666

11 A classical Chi-square test was used to compare the cumulative prevalence of parasite-positive cattle in Hitunga and Magoje. All expected values were > 5. The significance of this test is shown below.

Chisq	df	p-value	Cattle
1.56	1	0.212	218

12 A classical Chi-square test was used to compare the cumulative prevalence of parasite-positive cattle in [HTC villages + Bunghaji] versus [Hitunga + Magoje]. All expected values were > 5. The significance of this test is shown below.

Chisq	df	p-value	Cattle
39.06	1	<.0001	884

13 A classical Chi-square test was used to compare the cumulative prevalence of parasite-positive cattle in Bunghaji and Magoje. All expected values were > 5. The significance of this test is shown below.

Chisq	df	p-value	Cattle
8.58	1	0.003	259

14 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Village. Village = Two-level categorical variable, where each level corresponds to either Bunghaji or Magoje. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The significance of this test is shown in the table (Odds ratio not shown)

F-value	p-value	Observations
$F_{1, 257} = 1.238$	0.267	1826 on 259 cattle

15 Same as in model **14**, but "Village" was specified as a six-level categorical variable, where each level corresponded to Nanjeho, Kubo, Ojelai, Sitengo, Bunghaji or Magoje. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal. The significance of this test is shown below (Odds ratios not shown)

F-value	p-value	Observations
$F_{5, 685} = 1.292$	0.265	6203 on 691 cattle

16 Same as in model **14**, but "Village" was specified as a seven-level categorical variable, where each level corresponded to Bunyimini, Kubo, Nanjeho, Ojelai, Sitengo, Bunghaji or Magoje. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal. The significance of this test is shown below (Odds ratios not shown)

F-value	p-value	Observations
$F_{6, 780} = 9.69$	<.0001	7263 on 787 cattle

17 Same as in model **14**, but "Village" was specified as a seven-level categorical variable, where each level corresponded to Kubo, Nanjeho, Ojelai, Sitengo, Bunghaji, Hitunga or Magoje. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal. The significance of this test is shown below (Odds ratios not shown).

F-value	p-value	Observations
$F_{6, 781} = 4.31$	0.0003	7034 on 788 cattle

18 Tick counts (0, 1, 2, 3) ~ Village (a) + Month (b) + (a:b). Village = Eight-level categorical variable, where each level corresponds to a different village. Month = Fifteen-level categorical variable, where each level corresponds to a different round of observation (i.e. main visit), starting in October 2001 and ceasing in November 2002. Each visit was inter-spaced by 28 days (i.e. approximately one month). Before October 2001, main visits were only conducted in Tororo villages; rounds of observation prior to October 2001 were not included in the analysis. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts and time slopes for each individual animal. The significance of the terms in this model is shown below (Coefficients not shown).

Model Terms	F-value	p-value
Village (a)	$F_{7, 817} = 494.2$	<.0001
Month (a)	$F_{14, 7426} = 90.16$	<.0001
a : b	$F_{98, 7426} = 9.126$	<.0001

N = 8363 observations on 825 cattle

19 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Village (a) + Month (b) + (a:b). Independent variables were specified as in model **18**. However, month was specified as a nine-level categorical variable, where each level corresponded to a different round of observation (i.e. main visit), starting from October 2001. Between October 2001 and November 2002, visit rounds where PCR testing was not conducted in all eight villages were excluded from the analysis (i.e. six visit rounds). Method = GlmmPQL; binomial error structure. *T. parva* infection above PCR detection threshold was modelled considering different intercepts for each individual animal. The significance of the terms in this model is shown in the table (Odds ratios not shown).

Model Terms	F-value	p-value
Village (a)	$F_{7, 798} = 5.948$	<.0001
Month (a)	$F_{8, 4121} = 16.17$	<.0001
a : b	$F_{56, 4121} = 4.976$	<.0001

N = 4991 observations on 806 cattle

20 Tick counts (0, 1, 2, 3) ~ Cattle age. Cattle age = Four-level categorical variable, where each level corresponds to 1-6, 7-12, 13-24 or >24 months of age. One to six months of age was specified as the reference variable level. Method = GlmmPQL; poisson error structure.

Tick counts were modelled considering different intercepts and time slopes for village and different intercepts and time slopes for each animal within each village. Model coefficients were as listed in the table below. All level-specific p-values derived from level-specific T-tests were significant.

Model Terms	Variable levels	Coef	SE	F-value	p-value
Cattle Age	7-12 months	+0.13	0.04	$F_{3, 7654} = 46.17$	<.0001
	13-24 months	+0.29	0.04		
	> 24 months	+0.35	0.04		

N = 8539 observations on 882 cattle

21 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Four-level categorical variable, where each level corresponds to 1-6, 7-12, 13-24 or >24 months of age. One to six months of age was specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts and time slopes for each village, suggesting that cattle age accounted for the majority of inter-individual variation in the study. Odds Ratios and 95% confidence intervals, calculated from the model coefficients and associated standard errors, are shown in the table. ▲ highlights cattle age groups where, according to level-specific T-tests, the proportion of *T. parva* positive observations was not significantly different to that in calves of 1-6 months of age.

Model Terms	Variable levels	OR	95% CI	F-value	p-value
Cattle Age	7-12 months	0.91	0.58 - 1.42 ▲	$F_{3, 7483} = 37.06$	<.0001
	13-24 months	0.92	0.61 - 1.40 ▲		
	> 24 months	0.31	0.20 - 0.47		

N = 7494 observations on 8 villages

22 Same as in model **21**, but "Cattle age" was specified as a three-level categorical variable, where each level corresponded to 1-6, 7-12 or 13-24 months of age. *T. parva* infection above PCR detection threshold was modelled considering different intercepts for each individual animal. The significance of this test is shown below (Odds ratios not shown).

F-value	p-value	Observations
$F_{2, 2696} = 2.087$	0.124	3417 on 719 cattle

23 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age (a) + Tick counts (b) + (a:b). Cattle age = Three-level categorical variable, where each level corresponds to 1-12, 13-24 or >24 months of age. Tick counts = Three-level categorical variable, where each level corresponds to zero, 1-10 or >10 adult tick stages on half the body surface (RA/HBS). One to six months of age and zero ticks on half the body surface (RA/HBS) were specified as the reference variable levels. Method = GlmmPQL; binomial error structure. *T. parva* infection above PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. Odds Ratios and 95% confidence intervals, calculated from model coefficients and associated standard errors, are shown for the terms of interest ([b] and [a:b]). ▲ highlights variable levels where, according to level-specific T-test, the proportion of *T. parva* positive observations was not significantly different to that in the reference variable level.

Model Terms	Variable Levels	OR	95% CI	F-value	p-value
Cattle Age (a)				$F_{2, 6599} = 62.01$	<.0001
Tick Count (b)	1-10 RA/HBS	2.32	1.42 - 3.79	$F_{2, 6599} = 9.155$	0.0001
	> 10 RA/HBS	3.40	2.00 - 5.77		
a : b	13-24 months : 1-10 RA/HBS	0.81 ▲	0.43 - 1.52	$F_{4, 6599} = 5.564$	0.0002
	> 24 months : 1-10 RA/HBS	0.43	0.23 - 0.80		
	13-24 months : > 10 RA/HBS	0.78 ▲	0.41 - 1.50		
	>24 months : > 10 RA/HBS	0.27	0.14 - 0.51		

N = 7479 observations on 872 cattle

[24] Tick counts (0, 1, 2, 3) ~ Time. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts for each village and for each animal within each village. The significance and the coefficients of this analysis are shown below.

Model Term	Coef	SE	F-value	p-value	Observations
Time	+0.035	0.001	$F_{1, 8319} = 996.1$	<.0001	9213 on 893 cattle

[25] Tick counts (0, 1, 2, 3) ~ Cattle age (a) + Time (b) + (a:b). Cattle age = Four-level categorical variable, where each level corresponds to 1-6, 7-12, 13-24 or >24 months of age. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. Tick counts on animals of 1-6 months of age and at the start of the study period, were specified as the reference variable level. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts for each village and for each animal within each village. Model coefficients and associated standard errors, are shown below. ▲ highlights variable levels where, according to level-specific T-tests, tick counts were not significantly different to those in animals of 1-6 months of age, or where tick trends over the study period were not different to those observed in animals of 1-6 months of age.

Model Terms	Variable Levels	Coef	SE	F- value	p - value
Cattle Age (a)	7-12 months	+0.029▲	0.07	$F_{3, 7650} = 134.1$	<.0001
	13 – 24 months	+0.272	0.07		
	> 24 months	+0.471	0.07		
Time (b)	Time	+0.044	0.01	$F_{1, 7650} = 659.2$	<.0001
a : b	7-12 months : Time	+0.008▲	0.01	$F_{3, 7650} = 20.18$	<.0001
	13 – 24 months : Time	-0.006▲	0.01		
	> 24 months :Time	-0.021	0.01		

N = 8539 observations on 882 cattle

[26] Same as in model [25], but "Cattle age" was specified as a two-level categorical variable, where each level corresponded to either 1-6 or 7-12 months of age. Tick counts on animals of 1-6 months of age and at the start of the study period, were specified as the reference variable level. Tick counts were modelled considering different intercepts for each village and for each animal within each village. Model coefficients and associated standard errors, are shown in the table below. Note that the interaction term (a:b) was not significant.

Model Terms	Variable Levels	Coef	SE	F-value	p-value
Cattle Age (a)	7-12 months	+0.01	0.09	$F_{1, 955} = 6.985$	0.008
Time (b)	Time	+0.05	0.01	$F_{1, 955} = 130.2$	<.0001
a : b	7-12 months : Time	+0.01▲	0.01	$F_{1, 955} = 0.256$	0.613

N = 1278 observations on 320 cattle

[27] Same as in model [25], but "Cattle age" was specified as a two-level categorical variable, where each level corresponded to either 1-12 or 13-24 months of age. Tick counts on animals of 1-12 months of age and at the start of the study period, were specified as the reference variable level. Tick counts were modelled considering different intercepts for each village and for each animal within each village. Model coefficients and associated standard errors, are shown in the table below.

Model Terms	Variable Levels	Coef	SE	F-value	p-value
Cattle Age (a)	13-24 months	+0.24	0.044	$F_{1, 3189} = 175.8$	<.0001
Time (b)	Time	+0.05	0.004	$F_{1, 3189} = 430.0$	<.0001
a : b	13-24 months : Time	-0.01	0.005	$F_{1, 3189} = 8.145$	0.004

N = 3929 observations on 737 cattle

[28] Same as in model [25], but "Cattle age" was specified as a two-level categorical variable, where each level corresponded to either 1-12 or >24 months of age. Tick counts on animals

of 1-12 months of age and at the start of the study period, were specified as the reference variable level. Tick counts were modelled considering different intercepts for each village and for each animal within each village. Model coefficients and associated standard errors, are shown in the table below.

Model Terms	Variable Levels	Coef	SE	F-value	p-value
Cattle Age (a)	> 24 months	+0.48	0.039	$F_{1, 5064} = 286.3$	<.0001
Time (b)	Time	+0.05	0.004	$F_{1, 5064} = 375.7$	<.0001
a : b	> 24 months : Time	-0.03	0.004	$F_{1, 5064} = 46.39$	<.0001

N = 5888 observations on 821 cattle

29 Same as in model **25**, but "Cattle age" was specified as a two-level categorical variable, where each level corresponded to either 13-24 or >24 months of age. Tick counts on animals of 13-24 months of age and at the start of the study period, were specified as the reference variable level. Tick counts were modelled considering different intercepts for each village and for each animal within each village. Model coefficients and associated standard errors, are shown in the table below.

Model Terms	Variable Levels	Coef	SE	F-value	p-value
Cattle Age	> 24 months	+0.21	0.029	$F_{1, 6525} = 95.95$	<.0001
Time	Time	+0.04	0.002	$F_{1, 6525} = 540.2$	<.0001
a : b	> 24 months : Time	-0.02	0.003	$F_{1, 6525} = 39.23$	<.0001

N = 7261 observations on 733 cattle

30 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Time. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. Method = GlmmPQL; binomial error structure. *T. parva* infection above PCR detection threshold was modelled considering different intercepts and time slopes for each village, and different intercepts for each animal within village. Odds Ratios and 95% confidence intervals, calculated from the model coefficient and the associated standard error, is shown in the table below.

Model Term	OR	95% CI	F-value	p-value
Time	0.94	0.90 - 0.99	$F_{1, 7209} = 5.329$	0.021

N = 8094 observations on 884 cattle

31 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age (a) + Time (b) + (a:b). Cattle age = Two-level categorical variable, where each level corresponds to either 1-24 or >24 months of age. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. *T. parva* infection status in animals of >24 months of age and at the start of the study period was specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts and time slopes for each village, suggesting that cattle age accounted for the majority of inter-individual variation in the study. Odds Ratios and 95% confidence intervals, calculated from the model coefficients and associated standard errors, are shown in the table. Note that neither time (b), nor the interaction term (a:b) were significant.

Model Terms	Variable levels	OR	95% CI	F - value	p - value
Cattle Age (a)	Up to 24 months	2.10	1.36 - 3.26	$F_{1, 7483} = 121.3$	<.0001
Time (b)	Time	0.95▲	0.89 - 1.01	$F_{1, 7483} = 0.478$	0.490
a : b	Up to 24 months : Time	1.04▲	1.00 - 1.09	$F_{1, 7483} = 3.575$	0.059

N = 7494 observations on 8 villages

32 Tick counts (0, 1, 2, 3) ~ Village (a) + Cattle age (b) + (a:b). Village = Eight-level categorical variable, where each level corresponds to a different village. Cattle age = Four-level categorical variable, where each level corresponds to 1-6, 7-12, 13-24 or >24 months of age. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts for each individual animal in the analysis. The significance of each term in the model is shown below (coefficients not shown).

Model Terms	F-value	p-value
Village	$F_{7, 874} = 441.6$	<.0001
Cattle Age	$F_{3, 7633} = 134.6$	<.0001
a : b	$F_{21, 7633} = 5.689$	<.0001

N = 8539 observations on 882 cattle

33 Same as in model **32**, but "Village" was specified as a two-level categorical variable, where each level corresponded to either high tick challenge (HTC) area (i.e. Bunyimini + Kubo + Nanjeho + Ojelai + Sitengo) or low tick challenge (LTC) area (i.e. Bunghaji + Hitunga + Magoje). Tick counts were modelled considering different intercepts and time slopes for each village and different intercepts each animal within each village. The significance of each term in the model is shown below (coefficients not shown).

Model Terms	F-value	p-value
Village	$F_{1, 6} = 13.71$	0.010
Cattle Age	$F_{3, 7651} = 49.67$	<.0001
a : b	$F_{3, 7651} = 8.515$	<.0001

N = 8539 observations on 882 cattle

34 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Village (a) + Cattle age (b) + (a:b). Village = Eight-level categorical variable, where each level corresponds to a different village. Cattle age = Four-level categorical variable, where each level corresponds to 1-6, 7-12, 13-24 or >24 months of age. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The significance of each term in the model is shown below (coefficients not shown).

Model Terms	F-value	p-value
Village	$F_{7, 865} = 4.141$	0.0002
Cattle Age	$F_{3, 6597} = 50.78$	<.0001
a : b	$F_{21, 6597} = 3.109$	<.0001

N = 7494 observations on 873 cattle

35 Same as in model **34**, but "Village" was specified as a two-level categorical variable, where each level corresponded to either high tick challenge (HTC) area (i.e. Bunyimini + Kubo + Nanjeho + Ojelai + Sitengo) or low tick challenge (LTC) area (i.e. Bunghaji + Hitunga + Magoje). *T. parva* infection above the PCR detection threshold was modelled considering different intercepts and time slopes for each village and different intercepts for each animal within each village. The significance of each term in the model is shown below (coefficients not shown).

Model Terms	F-value	p-value
Village	$F_{1, 6} = 35.48$	0.001
Cattle Age	$F_{3, 6615} = 28.76$	<.0001
a : b	$F_{3, 6615} = 5.301$	0.001

N = 7494 observations on 873 cattle

36 Same as in model **34**, but "Village" was specified as a five-level categorical variable, where each level corresponded to a high tick challenge (HTC) village (i.e. Bunyimini, Nanjeho, Kubo, Ojelai, Sitengo). *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The significance of each term in the model is shown below (coefficients not shown).

Model Terms	F-value	p-value
Village	$F_{4, 523} = 4.855$	0.001
Cattle Age	$F_{3, 4538} = 65.63$	<.0001
a : b	$F_{12, 4538} = 2.467$	0.003

N = 5081 observations on 528 cattle

37 Same as in model **21**, but only cattle from HTC villages were considered in the analysis (i.e. Bunyimini, Nanjeho, Kubo, Ojelai, Sitengo). *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The significance of cattle age is shown below (coefficients not shown).

Model Terms	F-value	p-value
Cattle Age	$F_{3, 4550} = 69.58$	<.0001

N = 5081 observations on 528 cattle

38 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either up to 24 months of age or > 24 months of age. A separate analysis was conducted for each of the HTC villages. In each model, cattle of up to 24 months of age were specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was in all cases modelled considering different intercepts for each individual animal in the analysis. For each village, the significance of "Cattle Age" is shown below. Odds Ratios and 95% confidence intervals - estimated from model coefficients and their corresponding standard errors - are shown in all cases, as the comparison of interest was found significant in all HTC villages.

Village	Variable Levels	OR	95% CI	F-value	p-value	Observations	Cattle
Bunyimini	> 24 m	0.24	0.14 - 0.41	$F_{1, 881} = 27.03$	<.0001	978	96
Kubo	> 24 m	0.29	0.16 - 0.51	$F_{1, 867} = 18.11$	<.0001	970	102
Nanjeho	> 24 m	0.25	0.14 - 0.44	$F_{1, 825} = 23.56$	<.0001	935	109
Ojelai	> 24 m	0.09	0.06 - 0.15	$F_{1, 943} = 84.68$	<.0001	1071	127
Sitengo	> 24 m	0.32	0.17 - 0.58	$F_{1, 1032} = 14.11$	0.0002	1127	94

39 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Three-level categorical variable, where each level corresponds to 1-6, 7-12 or 13-24 months of age. A separate analysis was conducted for each of the HTC villages. In each model, cattle of 1-6 months of age were specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was in all cases modelled considering different intercepts for each individual animal in the analysis. For each village, the significance of "Cattle Age" is shown below. Odds Ratios and 95% confidence intervals - estimated from the model coefficients and their corresponding standard errors - are only presented where the main term was found significant.

Village	Variable Levels	OR	95% CI	F-value	p-value	Observations	Cattle
Bunyimini	(Cattle Age)			$F_{2, 285} = 2.508$	0.083	374	87
Kubo	"			$F_{2, 361} = 0.380$	0.684	451	88
Nanjeho	"			$F_{2, 378} = 0.834$	0.435	470	90
Ojelai	7-12 months	0.11	0.02 - 0.58	$F_{2, 277} = 3.556$	0.030	379	100
	13-24 months	0.16	0.03 - 0.88				
Sitengo	(Cattle Age)			$F_{2, 412} = 0.560$	0.572	503	89

40 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either 1-6 or 7-12 months of age. Only cattle from Ojelai (HTC) were considered in this analysis. Cattle of 7-12 months of age were specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The results from this analysis are shown below.

Model Terms	Variable Levels	OR	95% CI	F-value	p-value
Cattle Age	7-12 months	0.06	0.01 - 0.38	$F_{1, 66} = 9.604$	0.003

N = 98 observations on 31 cattle

41 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either 7-24 or > 24 months of age. Only cattle from Ojelai (HTC) were considered in this analysis. Cattle of 7-24 months of age were specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The results from this analysis are shown below.

Model Terms	Variable Levels	OR	95% CI	F-value	p-value
Cattle Age	> 24 months	0.09	0.06 -0.15	$F_{1, 924} = 87.18$	<.0001

N = 1052 observations on 127 cattle

42 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either up to 24 months of age or > 24 months of age. Only cattle from HTC villages were considered in this analysis (i.e. Bunyimini, Kubo, Nanjeho, Ojelai, Sitengo). Cattle of up to 24 months of age were specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The results from this analysis are shown below.

Model Terms	Variable Levels	OR	95% CI	F-value	p-value
Cattle Age	> 24 months	0.26	0.21 - 0.31	$F_{1, 4552} = 201.09$	<.0001

N = 5081 observations on 528 cattle

43 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Four-level categorical variable, where each level corresponds to 1-6, 7-12, 13-24 or >24 months of age. Only cattle from LTC villages were considered in this analysis (i.e. Bunghaji, Hitunga, Majoge). Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each village, suggesting that cattle age accounted for the majority of inter-individual variation in the study. The significance of cattle age is shown below (Odds ratios estimated from model coefficients not shown).

Model Terms	F-value	p-value
Cattle Age	$F_{3, 2407} = 1.142$	0.331

N = 2413 observations on 3 villages

44 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Village (a) + Cattle age (b) + (a:b). Village = Three-level categorical variable, where each level corresponds to a different LTC village (i.e. Bunghaji, Hitunga or Magoje). Cattle age = Four-level categorical variable, where each level corresponds to 1-6, 7-12, 13-24 or >24 months of age. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The significance of each term in the model is shown in the table (coefficients not shown).

Model Terms	F-value	p-value
Village (a)	$F_{2, 342} = 6.637$	0.0015
Cattle Age (b)	$F_{3, 2059} = 4.443$	0.0041
a : b	$F_{6, 2059} = 3.267$	0.0034

N = 2413 observations on 345 cattle

45 Same as in model **44**, but “Cattle Age” was specified as a two-level categorical variable, where each level corresponded to either up to 24 months of age or > 24 months of age. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts and time slopes for each individual animal in the analysis. The significance of each term in the model is shown below (coefficients not shown). Note that neither cattle age (b) nor the interaction term (a:b) were significant (only maximal model shown).

Model Terms	F-value	p-value
Village (a)	$F_{2, 342} = 8.329$	0.0003
Cattle Age (b)	$F_{1, 2065} = 0.687$	0.4072
a : b	$F_{2, 2065} = 2.967$	0.0517

N = 2413 observations on 345 cattle

46 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either 1-12 months or > 12 months of age. Only cattle from Bunghaji (LTC) were considered in this analysis. Cattle of up to 12 months of age were specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts and time slopes for each individual animal in the analysis. The results from this analysis are shown below.

Model Terms	Variable Levels	OR	95% CI	F-value	p-value
Cattle Age	> 12 months	0.27	0.15 - 0.49	$F_{1, 924} = 18.63$	<.0001

N = 1053 observations on 128 cattle

47 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either 1-6 or 7-12 months of age. Only cattle from Bunghaji (LTC) were considered in this analysis. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The results from this analysis are shown below (Odds ratios derived from model coefficients not shown).

Model Terms	F-value	p-value
Cattle Age	$F_{1, 112} = 0.086$	0.770

N = 165 observations on 52 cattle

48 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either 13-24 or >24 months of age. Only cattle from Bunghaji (LTC) were considered in this analysis. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts and time slopes for each individual animal in the analysis. The results from this analysis are shown below (Odds ratios derived from model coefficients not shown).

Model Terms	F-value	p-value
Cattle Age	$F_{1, 783} = 0.571$	0.450

N = 888 observations on 104 cattle

49 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either 13-24 or > 24 months of age. Only cattle from Hitunga (LTC) were considered in this analysis. Cattle of 13-24 months of age were specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The results from this analysis are shown below.

Model Terms	Cattle Age	OR	95% CI	F-value	p-value
Cattle Age	> 24 months	0.33	0.17 - 0.65	$F_{1, 544} = 10.39$	0.0013

N = 628 observations on 83 cattle

50 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age. Cattle age = Two-level categorical variable, where each level corresponds to either up to 24 months of age or > 24 months of age. Only cattle from Magoje (LTC) were considered in this analysis. Cattle of up to 24 months of age were specified as the reference variable level. Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The results from this analysis are shown below.

Model Terms	Cattle Age	OR	95% CI	F-value	p-value
Cattle Age	> 24 months	2.46	1.20 – 5.03	$F_{1, 468} = 6.129$	0.0137

N = 537 observations on 68 cattle

51 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Village (a) + Cattle age (b) + Tick count (c) + (a:b) + (a:c) + (b:c) + (a:b:c). Village = Two-level categorical variable where each level corresponds to either HTC village (i.e. Bunyimini + Kubo + Nanjeho + Ojelai + Sitengo) or LTC village (i.e. Bunghaji + Hitunga + Magoje). Cattle age = Three-level categorical variable, where each level corresponds to 1-12, 13-24 or >24 months of age. Tick Counts = Three level categorical variable where each level corresponds to zero, 1-10 or >10 adult stages on half the body surface (RA/HBS). Method = GlmmPQL; binomial error structure. *T. parva* infection above the PCR detection threshold was modelled considering different intercepts and time slopes for each village, and different intercepts for each animal within village. The table below shows the terms in the model which were eliminated in a process of step-down simplification, consisting of the withdrawal of a non-significant term at a time. In the main text, the p-value of a non-significant term was that obtained at the time the term of interest was withdrawn from the model. Hence the F-value and p-value may not correspond to that shown in the initial model.

Model Terms	Maximal Model		Minimum Model	
	F-value	p-value	F-value	p-value
Village (a)	$F_{1, 6} = 27.09$	0.002	$F_{1, 6} = 32.14$	0.001
Cattle Age (b)	$F_{2, 6591} = 42.54$	<.0001	$F_{2, 6597} = 43.32$	<.0001
Tick Count (c)	$F_{2, 6591} = 4.979$	0.007	$F_{2, 6597} = 4.012$	0.018
a : b	$F_{2, 6591} = 8.840$	0.000	$F_{2, 6597} = 7.681$	0.001
a : c	$F_{2, 6591} = 1.181$	0.307	-	-
b : c	$F_{4, 6591} = 1.965$	0.097	$F_{4, 6597} = 2.844$	0.023
a : b : c	$F_{4, 6591} = 1.043$	0.383	-	-

N = 7479 observations on 872 cattle

52 Tick counts (0, 1, 2, 3) ~ Cattle age (a) + Time (b) + (a:b). Cattle age = Three-level categorical variable, where each level corresponds to 1-12, 13-24 or >24 months of age. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. A separate analysis was conducted to investigate the relationship of interest in each HTC village (i.e. Bunyimini, Kubo, Nanjeho, Ojelai and Sitengo). Tick counts from animals of 1-6 months of age and at the start of the study period were specified as the reference variable level. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering different intercepts and time slopes for each individual animal in Bunyimini and Ojelai, and considering different intercepts for each animal in Kubo, Nanjeho and Sitengo. Model coefficients and associated standard errors, are shown in the table. ▲ highlights variable levels where, according to level-specific T-tests, tick counts were not significantly different to those in animals of 1-6 months of age, or where tick trends over the study period were not different to those observed in animals of 1-6 months of age.

Village	Minimum Model	Variable Levels	Coef	SE	F-value	p-value	Obs.	Cattle
Bunyimini	Age (a)	13-24	+0.24	0.11	$F_{2, 876} = 48.55$	<.0001	977	96
		> 24	+0.70	0.10				
	Time (b)	Time	+0.04	0.01	$F_{1, 876} = 19.94$	<.0001		
	a : b	13 – 24 : T	-0.01 ▲	0.01	$F_{2, 876} = 22.07$	<.0001		
		> 24 : T	-0.04	0.01				
Kubo	Age (a)	13-24	+0.08 ▲	0.08	$F_{2, 861} = 31.44$	<.0001	968	102
		> 24	+0.40	0.07				
	Time (b)	Time	+0.03	0.01	$F_{1, 861} = 64.39$	<.0001		
	a : b	13 – 24 : T	0.00 ▲	0.01	$F_{2, 861} = 12.99$	<.0001		
		> 24 : T	-0.03	0.01				
Nanjeho	Age (a)	13-24	+0.14 ▲	0.08	$F_{2, 821} = 33.54$	<.0001	935	109
		> 24	+0.50	0.08				
	Time (b)	Time	+0.03	0.01	$F_{1, 821} = 24.02$	<.0001		
	a : b	13 – 24 : T	-0.01 ▲	0.01	$F_{2, 821} = 10.82$	<.0001		
		> 24 : T	-0.03	0.01				
Ojelai	Age (a)	13-24	+0.22	0.08	$F_{2, 983} = 55.92$	<.0001	1114	126
		> 24	+0.47	0.08				
	Time (b)	Time	+0.05	0.01	$F_{1, 983} = 144.1$	<.0001		
	a : b	13 – 24 : T	-0.01 ▲	0.01	$F_{2, 983} = 8.953$	0.0001		
		> 24 : T	-0.03	0.01				
Sitengo	Age (a)	13-24	+0.12	0.04	$F_{2, 1026} = 12.97$	<.0001	1123	94
		> 24	+0.11	0.05				
	Time (b)	Time	+0.02	0.00	$F_{1, 1026} = 30.44$	<.0001		

"T" refers to "Time"

53 Tick infestation [0 = No ticks present; 1 = Ticks present] ~ Cattle age (a) + Time (b) + (a:b). Cattle age = Three-level categorical variable, where each level corresponds to 1-12, 13-24 or >24 months of age. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. A separate analysis was conducted to investigate the relationship of interest in each LTC village (i.e. Bunghaji, Hitunga, Magoje). Given the low number of observations with more than 10 ticks in these villages, a binomial model considering presence of ticks (yes/no) was preferred. Method = GlmmPQL; binomial error structure. Tick infestation status from animals of 1-6 months of age at the start of the study period was specified as the reference variable level. Tick infestation status was modelled considering different intercepts and time slopes for each individual animal in Bunghaji, and considering different intercepts for each animal Hitunga and Magoje. Odds ratios and 95% confidence intervals, calculated from model coefficients and associated standard errors, are shown in the table.

Village	Minimum Model	Variable Levels	OR	95% CI	F-value	p-value	Obs.	Cattle
Bunghaji	Time (b)	Time	1.31	1.25 - 1.36	$F_{1, 1057} = 145.9$	<.0001	1196	138
Hitunga	Age (a)	13-24	3.29	2.17 - 5.00	$F_{2, 1169} = 20.24$	<.0001	1272	100
		> 24	2.62	1.75 - 3.94				
	Time (b)	Time	1.21	1.17 - 1.24	$F_{1, 1169} = 183.6$	<.0001		
Magoje	Age (a)	13-24	2.65	1.53 - 4.60	$F_{2, 921} = 20.50$	<.0001	1051	127
		> 24	2.96	1.65 - 5.30				
	Time (b)	Time	1.14	1.11 - 1.18	$F_{1, 921} = 75.74$	<.0001		

54 Tick counts (0, 1, 2, 3) ~ Village (a) + Cattle age (b) + Time (c) + (a:b) + (a:c) + (b:c) + (a:b:c). Village = Eight-level categorical variable, where each level corresponds to a different village in the study. Cattle age = Three-level categorical variable, where each level corresponds to 1-12, 13-24 or >24 months of age. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. Method = GlmmPQL; poisson error structure. Tick counts were modelled considering

different intercepts for each individual animal in the analysis. The significance of each term in the model is shown below (coefficients not shown).

Model Terms	F-value	p-value
Village (a)	$F_{7, 874} = 553.7$	<.0001
Cattle Age (b)	$F_{2, 7617} = 167.2$	<.0001
Time (c)	$F_{1, 7617} = 625.3$	<.0001
a : b	$F_{14, 7617} = 5.939$	<.0001
a : c	$F_{7, 7617} = 49.06$	<.0001
b : c	$F_{2, 7617} = 26.64$	<.0001
a : b : c	$F_{14, 7617} = 2.823$	0.0003

N = 8539 observations on 882 cattle

55 Same as in model **54**, but "Village" was specified as a two-level categorical variable, where each level corresponded to either high tick challenge (HTC) area (i.e. Bunyimini + Kubo + Nanjeho + Ojelai + Sitengo) or low tick challenge (LTC) area (i.e. Bunghaji + Hitunga + Magoje). Tick counts were modelled considering different intercepts for each village and for each animal within each village. The significance of each term in the model is shown below (coefficients not shown).

Model Terms	F-value	p-value
Village (a)	$F_{1, 6} = 46.03$	0.001
Cattle Age (b)	$F_{2, 7647} = 180.7$	<.0001
Time (c)	$F_{1, 7647} = 642.6$	<.0001
a : b	$F_{2, 7647} = 21.91$	<.0001
a : c	$F_{1, 7647} = 305.1$	<.0001
b : c	$F_{2, 7647} = 26.06$	<.0001
a : b : c	$F_{2, 7647} = 4.468$	0.012

N = 8539 observations on 882 cattle

56 Same as in model **54**, but "Village" was specified as a four-level categorical variable, where each level corresponds to a different HTC village except Ojelai (i.e. Bunyimini, Kubo, Nanjeho, Sitengo). Tick counts were modelled considering different intercepts and time slopes for each individual animal in the analysis. The table below shows the terms in the model which were eliminated in a process of step-down simplification, consisting of the withdrawal of a non-significant term at a time.

Model Terms	Maximal Model		Minimum Model	
	F-value	p-value	F-value	p-value
Village (a)	$F_{3, 397} = 41.84$	<.0001	$F_{3, 397} = 41.13$	<.0001
Cattle Age (b)	$F_{2, 3582} = 107.0$	<.0001	$F_{2, 3582} = 106.2$	<.0001
Time (c)	$F_{1, 3582} = 131.8$	<.0001	$F_{1, 3582} = 130.9$	<.0001
a : b	$F_{6, 3582} = 2.773$	0.011	$F_{6, 3582} = 2.776$	0.011
a : c	$F_{3, 3582} = 1.696$	0.166	-	-
b : c	$F_{2, 3582} = 31.83$	<.0001	$F_{2, 3582} = 32.99$	<.0001
a : b : c	$F_{6, 3582} = 3.849$	0.001	$F_{9, 3582} = 2.927$	0.002

N = 4003 observations on 401 cattle

57 Same as in model **54**, but "Village" was specified as a two-level categorical variable, where each level corresponds to either a HTC village other than Ojelai (i.e. Bunyimini + Kubo + Nanjeho + Sitengo) or Ojelai. Tick counts were modelled considering different intercepts and time slopes for each individual animal in the analysis. The table below shows the minimum model after a process of step-down simplification. The coefficient, as well as the corresponding standard error, is shown for the term of interest. Note that tick trends in Ojelai are compared to those in other HTC villages.

Minimum Model	F-value	p-value	Variable level	Coef	SE
Village (a)	$F_{1, 525} = 7.262$	0.007			
Cattle Age (b)	$F_{2, 4582} = 156.3$	<.0001			
Time (c)	$F_{1, 4582} = 279.8$	<.0001			
a : b	$F_{2, 4582} = 7.001$	0.001			
a : c	$F_{1, 4582} = 24.96$	<.0001	*(Ojelai : Time)*	+0.02	0.003
b : c	$F_{2, 4582} = 43.86$	<.0001			

N = 5117 observations on 527 cattle

58 Same as in model **54**, but "Village" was specified as a two-level categorical variable, where each level corresponds to either a LTC village (i.e. Bunghaji + Hitunga + Magoje) or Ojelai (HTC). Tick counts were modelled considering different intercepts for each individual animal in the analysis. The table below shows the minimum model after a process of step-down simplification. The coefficient, as well as the corresponding standard error, is shown for the term of interest. Note that the tick trend in the LTC area (i.e. Bunghaji + Hitunga + Magoje) is compared to that in Ojelai.

Minimum Model	F-value	p-value	Variable level	Coef	SE
Village (a)	$F_{1, 479} = 560.7$	<.0001			
Cattle Age (b)	$F_{2, 4047} = 117.3$	<.0001			
Time (c)	$F_{1, 4047} = 492.8$	<.0001			
a : b	$F_{2, 4047} = 5.723$	0.003			
a : c	$F_{1, 4047} = 74.34$	<.0001	*(LTC :Time)*	+0.04	0.005
b : c	$F_{2, 4047} = 13.03$	<.0001			

N = 4536 observations on 481 cattle

59 Tick infestation [0 = No ticks present; 1 = Ticks present] ~ Village (a) + Cattle age (b) + Time (c) + (a:b) + (a:c) + (b:c) + (a:b:c). Village = Two-level categorical variable, where each level corresponds to either Bunghaji (LTC) or Hitunga (LTC). Cattle age = Three-level categorical variable, where each level corresponds to 1-12, 13-24 or >24 months of age. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. Given the low number of observations with more than 10 ticks in these villages, a binomial model considering presence of ticks (yes/no), rather than tick counts, was preferred. Method = GlmmPQL; binomial error structure. Tick infestation status was modelled considering different intercepts and time slopes for each individual animal in the analysis. The table below shows the terms in the model which were eliminated in a process of step-down simplification, consisting of the withdrawal of a non-significant term at a time. In the main text, the p-value of a non-significant term was that obtained at the time the term of interest was withdrawn from the model. Hence the F-value and p-value may not correspond to that shown in the initial model.

Model Terms	Maximal Model		Minimum Model	
	F-value	p-value	F-value	p-value
Village (a)	$F_{1, 226} = 72.24$	<.0001	$F_{1, 226} = 73.75$	<.0001
Cattle Age (b)	$F_{2, 2133} = 19.44$	<.0001	$F_{2, 2138} = 19.23$	<.0001
Time (c)	$F_{1, 2133} = 266.4$	<.0001	$F_{1, 2138} = 274.9$	<.0001
a : b	$F_{2, 2133} = 3.359$	0.035	-	-
a : c	$F_{1, 2133} = 0.997$	0.318	-	-
b : c	$F_{2, 2133} = 0.474$	0.622	$F_{2, 2138} = 3.455$	0.032
a : b : c	$F_{2, 2133} = 3.124$	0.044	-	-

N = 2371 observations on 228 cattle

60 Same as in model **59**, but "Village" was specified as a two-level categorical variable, where each level corresponds to either Magoje or a LTC village other than the former (i.e. Bunghaji + Hitunga). Tick infestation status was modelled considering different intercepts and time slopes for each individual animal in the analysis. The table below shows the minimum model after a process of step-down simplification. The coefficient, as well as the corresponding

standard error, is shown for the term of interest. Note that the tick trend in Magoje is compared to that in other LTC villages (i.e. Bunghaji + Hitunga).

Minimum Model	F-value	p-value	Variable level	Coef	SE
Village (a)	$F_{1, 353} = 67.65$	<.0001			
Cattle Age (b)	$F_{2, 3063} = 42.17$	<.0001			
Time (c)	$F_{1, 3063} = 346.5$	<.0001			
a : c	$F_{1, 3063} = 7.762$	0.005	*(Magoje:Time)*	-0.054	0.019

N = 3422 observations on 355 cattle

61 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Village (a) + Cattle age (b) + Time (c) + (a:b) + (a:c) + (b:c) + (a:b:c). Village = Eight-level categorical variable, where each level corresponds to a different village in the study. Cattle age = Four-level categorical variable, where each level corresponds to 1-6, 7-12, 13-24 or >24 months of age. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. Method = GlmmPQL; binomial error structure. *T. parva* infection status above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The significance of each term in the model is shown below (Odds ratio estimated from the model coefficient of the main term of interest [Time] was 0.94).

Model Terms	F-value	p-value
Village (a)	$F_{7, 865} = 4.534$	0.0001
Cattle Age (b)	$F_{3, 6565} = 50.52$	<.0001
Time (c)	$F_{1, 6565} = 31.21$	<.0001
a : b	$F_{21, 6565} = 2.603$	0.0001
a : c	$F_{7, 6565} = 10.68$	<.0001
b : c	$F_{3, 6565} = 4.306$	0.0048
a : b : c	$F_{21, 6565} = 2.335$	0.0005

N = 7494 observations on 873 cattle

62 *T. parva* [0 = Negative observation; 1 = Positive observation] ~ Cattle age (a) + Time (b) + (a:b) + (a:c) + (b:c) + (a:b:c). Cattle age = Two-level categorical variable, where each level corresponds to either cattle of up to 24 months of age or >24 months of age. Time = Continuous variable, where the unit of observation is expressed in number of months since the start of the study period. Only cattle from Magoje were considered in this analysis. The age categorisation chosen in this analysis, was that previously found significant with respect to the probability of diagnosing *T. parva* by PCR (See model **50**). Method = GlmmPQL; binomial error structure. *T. parva* infection status above the PCR detection threshold was modelled considering different intercepts for each individual animal in the analysis. The significance of each term in the minimum model (i.e. after a process of step-down simplification) is shown below. The odds ratio and 95% confidence intervals estimated from the model coefficient and the associated standard error, are shown for the term of interest (i.e. Time).

Minimum Model	F-value	p-value	OR	95% CI
Cattle Age (b)	$F_{1, 467} = 6.198$	0.0131		
Time (c)	$F_{1, 467} = 5.312$	0.0216	1.09	1.01 – 1.18

N = 537 observations on 68 cattle

63 Variation in the number of positive observations obtained from *T. parva* infected cattle was investigated in an analysis of deviance with proportion data, which took into account the total number of PCR tests conducted on each animal (GLM; Section 3.4.3). The dependent variable was defined as a *"cbind"* vector (y) where, for each individual animal, the number of parasite-positive observations by PCR (a), was modelled considering the total number of PCR tests conducted over the course of the study (b), by specifying *y<−cbind(a, b-a)*. The response variable was modelled as a function of 1/. The age of individual cattle at the time of the initial parasite-positive observation, and 2/. The village of origin of individual animals while accounting for the age of cattle at the time of the first parasite-positive observation. In

the first case, several level-groupings of “Cattle age” were tested. In the second case, all analyses were conducted in models specified as: ‘*y (response variable) ~ Cattle age at the time of the first parasite-positive diagnosis (a) + Village (b) + (a : b)*’. Inter-village variation was investigated considering all eight villages (i.e. by specifying an eight-level categorical variable) or by considering only specific sets of villages depending on the questions adressed. In the analyses of inter-village variation, cattle age at the first positive diagnosis consisted of a three-level categorical variable (1-12, 13-24 or > 24 months of age) but alternative level-groupings were also taken into account for robustness (Data not shown). The significance of terms in the models was assessed through deletion tests. Chisq-tests were chosen if the dispersion parameter was ≤ 1.3, whilst F-tests were chosen when the dispersion parameter was >1.3. The dispersion parameter was calculated by dividing the residual deviance of the model by the residual degrees of freedom of the same model before each deletion step. An effect of LA oxytetracycline on the number of positive observations recorded from infected cattle, was discarded prior to assessment of inter-village variation of “y” or variation of “y” across cattle age groups. Three different categorical “Treatment” variables were tested in either one-variable models or in models where cattle age and/or village were also fitted as independent variables. The “Treatment” variables were (i) Whether cattle were treated at any time since first diagnosed with the parasite (Yes / No), (ii) Whether cattle were never treated, treated once or treated on multiple visit rounds throughout the observational period and (iii) Whether cattle were not treated, treated once or treated on multiple visit rounds since the time of the first parasite-positive diagnosis until the end of the observational period.

Appendix Chapter 7: The PIM Gene – A Marker to Investigate Factors Affecting *Theileria parva* Infection Survival

Appendix 7.1 Number and distribution of initial and follow-up *Theileria parva* (p104 gene) positive observations, for which data on *T. parva* parasite (PIM) types was not available.

	Cattle age at initial *Theileria parva* (p104 gene) positive observation						Total	
	Calves		Cattle > 12 months		Unknown			
	Initial positive	Follow-up positive	Initial positive	Follow-up positive	Initial positive	Follow-up positive	Initial positive	Follow-up positive
Bunyimini	11 (1)	29 (7)	20 (2)	43 (11)	4 (1)	11 (2)	35 (4)	83 (20)
Kubo	9 (3)	20 (2)	11 (1)	11 (1)	2		22 (4)	31 (3)
Nanjeho	12 (1)	15 (6)	21 (1)	21 (2)	3 (1)	1	36 (3)	37 (8)
Ojelai	11 (2)	18 (4)	14 (3)	20 (10)			25 (5)	38 (14)
Sitengo	12 (2)	24 (5)	17	14 (1)	1		30 (2)	38 (6)
Bunghaji	10	3	23 (1)	1	2		35 (1)	4
Hitunga			9	3 (1)	2		11	3 (1)
Magoje	1	1	10	13 (2)	2		13	14 (2)
TOTALS [1]	66-(9) [3] = 57	110-(24) = 86	125-(8) =117	126-(28) = 98	16-(2) = 14	12-(2) = 10	207-(19) [2] = 188	248-(54) = 194

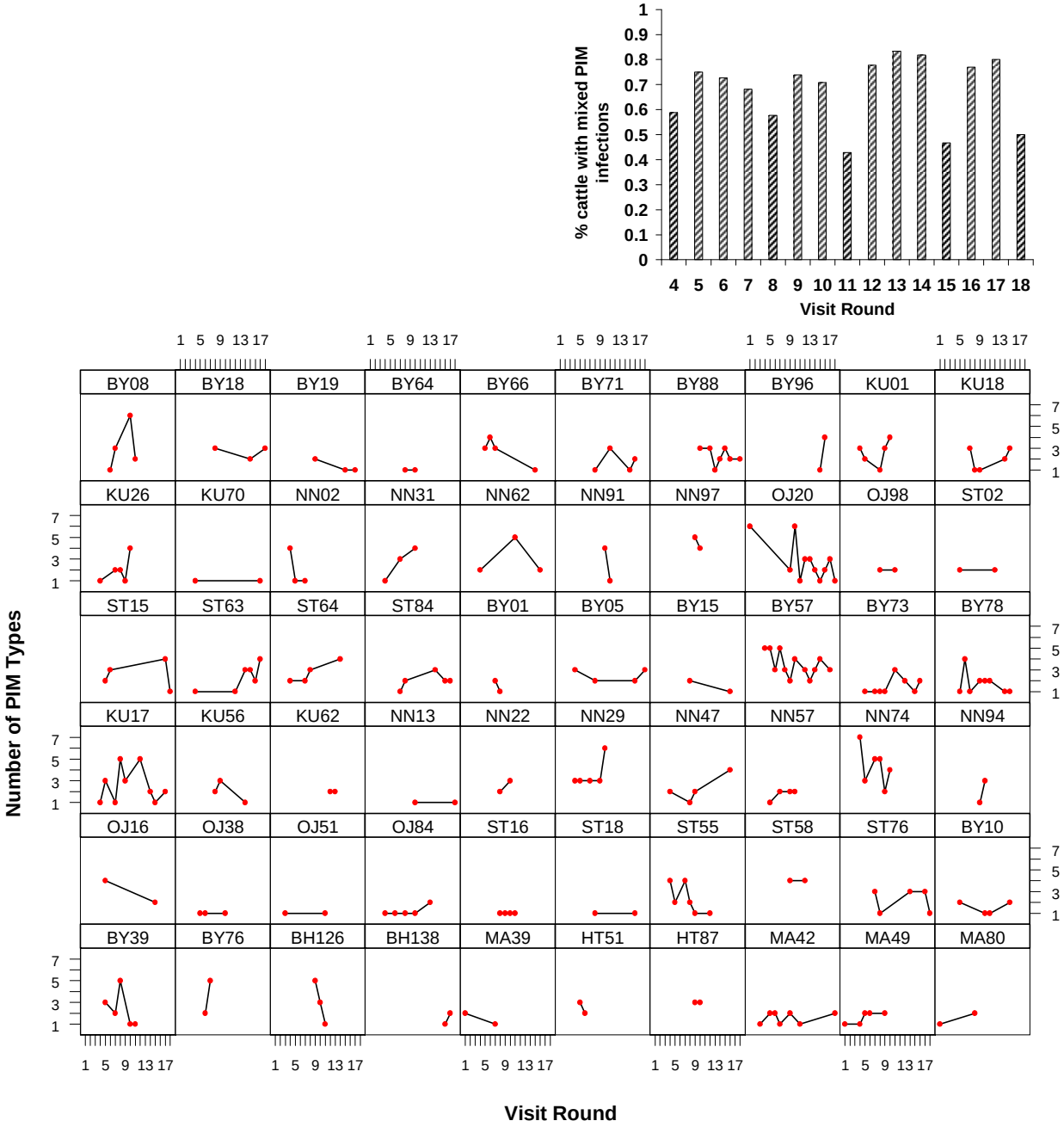
The table shows p104-positive observations for 207 cattle with PIM data. The observations are presented by age class at the time of the initial p104-positive sampling and by geographical origin of parasite-positive individuals. The number of p104-positive observations for which data on *T. parva* parasite (PIM) types was not available is shown in brackets. [1] The total number of PIM-positive observations is shown in the "Totals" row. For example, there were 66 initial p104-positive observations on calves, but PIM data was missing for the case of 9 observations. In consequence, PIM data was available for 57 out of 66 initial parasite-positive observations on calves. In total, information on parasite types as distinguished by PIM gene polymorphisms, was available for 382 (188 + 194) out of 455 (207 + 248) parasite-positive observations on 207 cattle. [2] In the case of 19 animals, the first PIM positive observation was recorded from *T. parva* positive observations other than the initial. [3] Five animals identified as calves at the time of the initial positive diagnosis were known to be older than 12 months at the time of the first PIM-positive observation, while the age of one animal initially identified as calf was no longer available.

Appendix 7.2 Relationship between number of p104-positive observations and number of PIM-positive observations recorded from cattle.

Number of p104 positive observations	Number of PIM positive observations											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	126											
2	15 (9)	14										
3	4 (2)	7 (2)	2									
4		2 (2)	2	3								
5	2 (1)		2 (1)	6 (1)	7							
6					2	2						
7			2 (1)			1	2					
8				1				1				
9									1			
10								1				
11												
12											1	
13												1
Totals	**147**	**23**	**8**	**10**	**9**	**3**	**2**	**2**	**1**		**1**	**1**

The figures correspond to numbers of cattle. Only 207 animals from which PIM data was obtained in at least one visit round are considered. Note that 126 cattle were positive to *T. parva* on only one round of observation, as apparent from the results of the p104 PCR. PIM data was available for the single parasite-positive observation in all 126 animals. In contrast, in the case of 15 animals, PIM data was available for only one round of observation, but these animals were parasite-positives on two visit rounds during the study. Figures in brackets correspond to numbers of cattle for which PIM data was only available for the case of follow-up p104-positive observations (i.e. parasite-positive observations other than the first recorded in the study).

Appendix 7.3 Number of *Theileria parva* parasite (PIM) types presented by round of observation in 60 cattle with multiple PIM-positive samples.



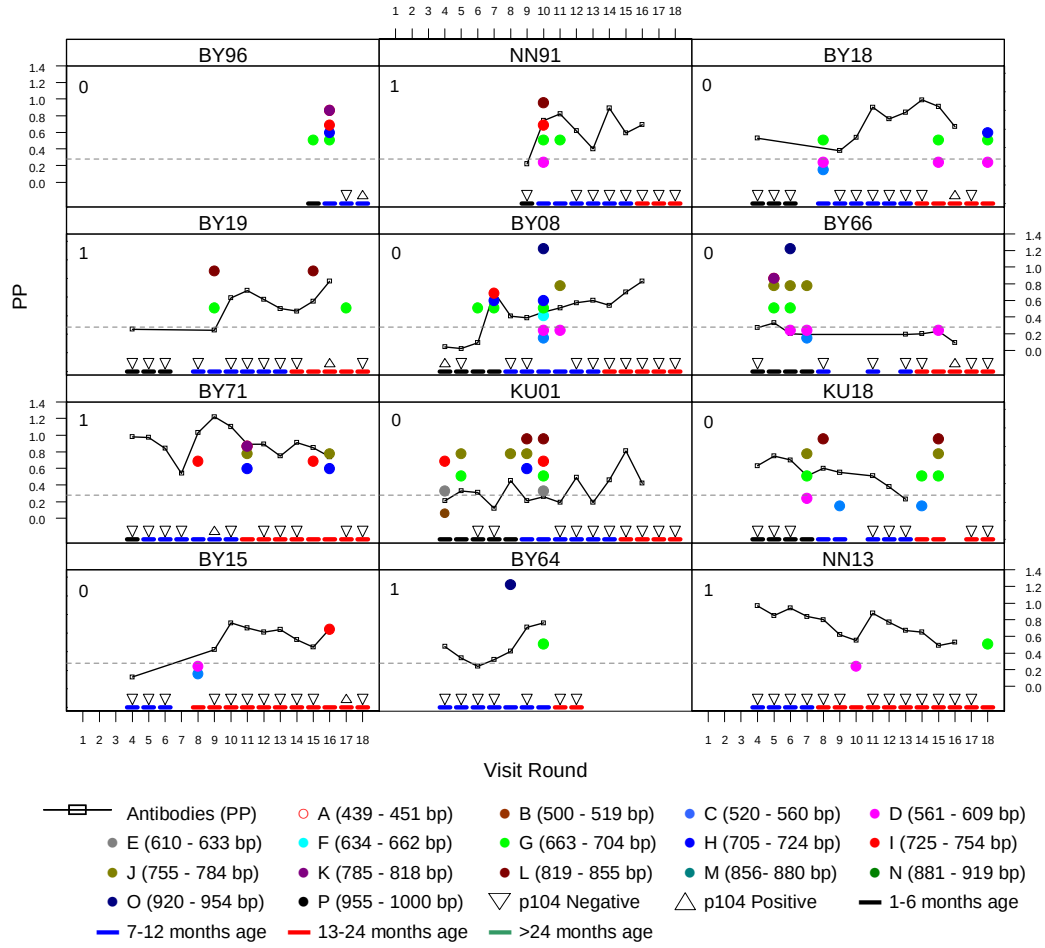
Each panel presents the data from one animal. In the label for each animal, the two letters indicate the village of origin. The bar graph was derived from the pooling of data obtained from the 60 animals, and presents the proportion of cattle with mixed *T. parva* infections at each round of observation. A cyclical pattern with regards to the prevalence of mixed infections over the course of the study is apparent upon visual inspection of the bar graph. The cyclical pattern did not follow a meaningful correlation with the onset of dry and rainy seasons.

Legend to Appendix 7.4.a to 7.4.g

The figures present the longitudinal history of the remaining PIM-positive cattle included in the analysis of *T. parva* infection survival above the PCR detection threshold (n = 207-12 = 195). The history of 12 animals with multiple PIM-positive observations is given in Section 7.3.7.1. Each panel presents the data from one animal. The complete history of (i) *T. parva* positive observations above the p104 PCR detection threshold, (ii) infection multiplicity and composition of parasite types as distinguished by PIM gene polymorphisms at each round of observation and (iii) serology profiles obtained from the PIM ELISA, is presented for each animal since the time of recruitment until the period of observation ceased. Dashed horizontal lines show the cut-off point for seropositivity (> 30 percentage positivity [PP]). The PP is presented in a scale of zero to one. Seroconversion or seroevents were said to take place when, given any two rounds of observation, the PP in the first visit round (PP_1) was ≤ 30, the PP in the following visit round (PP_2) was > 30 and PP_1-PP_2 ≥ 20. In each panel, “0” indicates that the first infection recorded from the animal of interest was censored and “1” that the terminal event had occurred. Only the first infection from each animal was considered in Cox models. In the label for each animal (shown only for animals with multiple PIM-positive observations) the two letters indicate the village of origin.

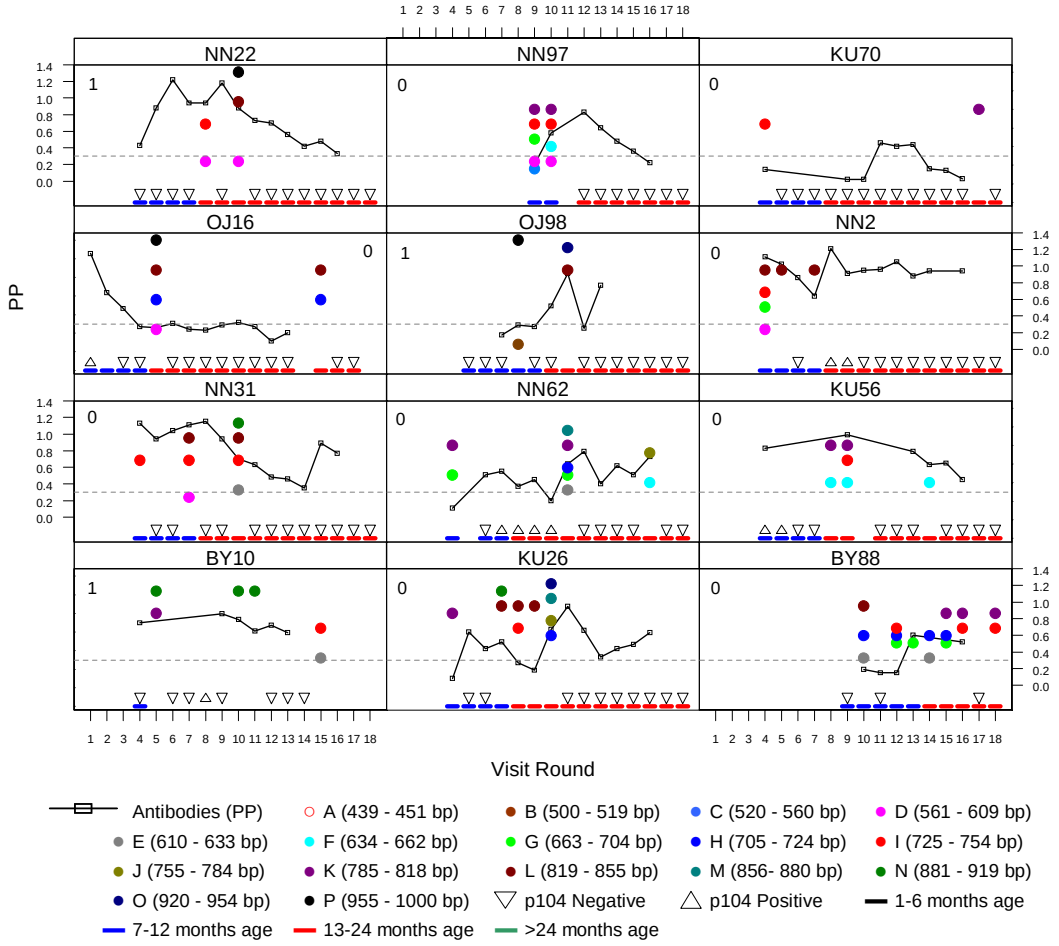
Appendix 7.4.a Longitudinal histories of 12 out of 60 cattle with multiple PIM-positive observations (animals 13 to 24 shown).

.



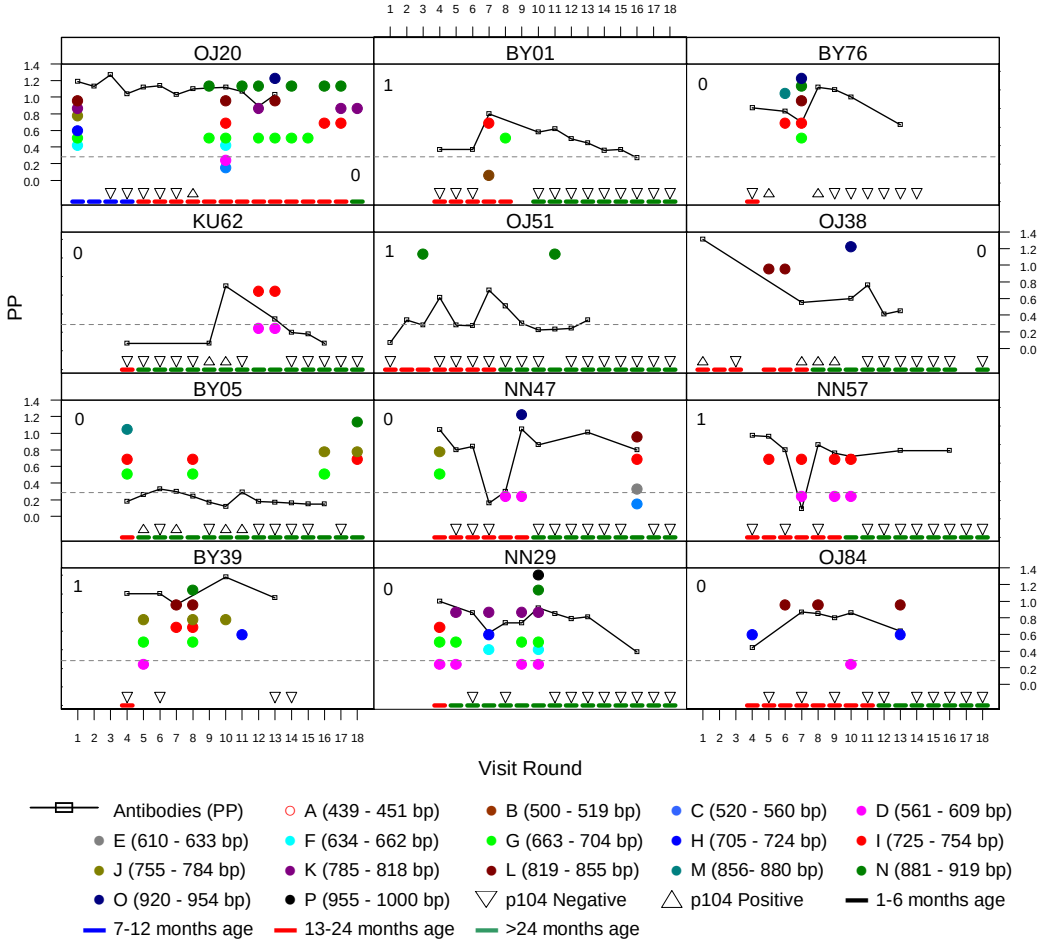
BY15, BY64 and NN13 were considered to present with two independent *T.parva* infections over time. Only the first infection was considered for analysis.

Appendix 7.4.b Longitudinal histories of 12 out of 60 cattle with multiple PIM-positive observations (animals 25 to 36 shown).



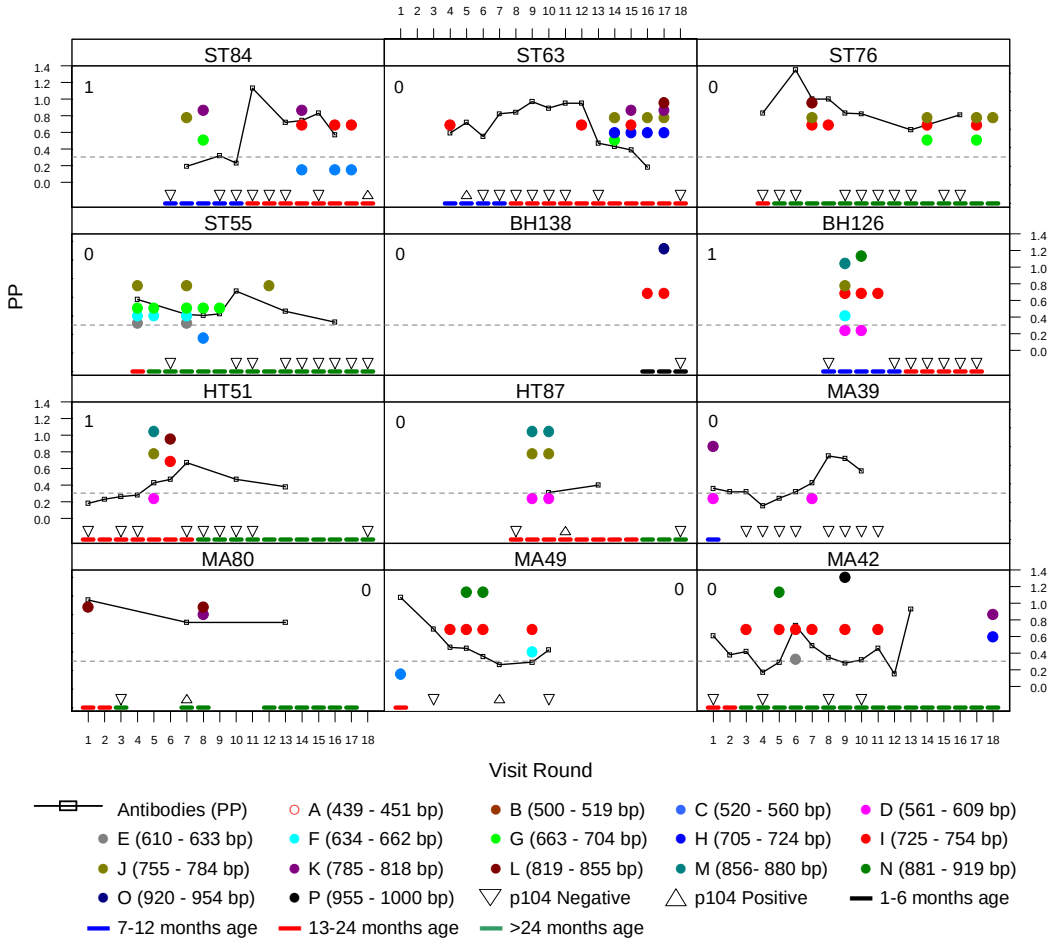
KU70, OJ98, NN62, BY10 and KU26 were considered to present with two independent *T.parva* infections over time. Only the first infection was considered for analysis.

Appendix 7.4.c Longitudinal histories of 12 out of 60 cattle with multiple PIM-positive observations (animals 37 to 48 shown).



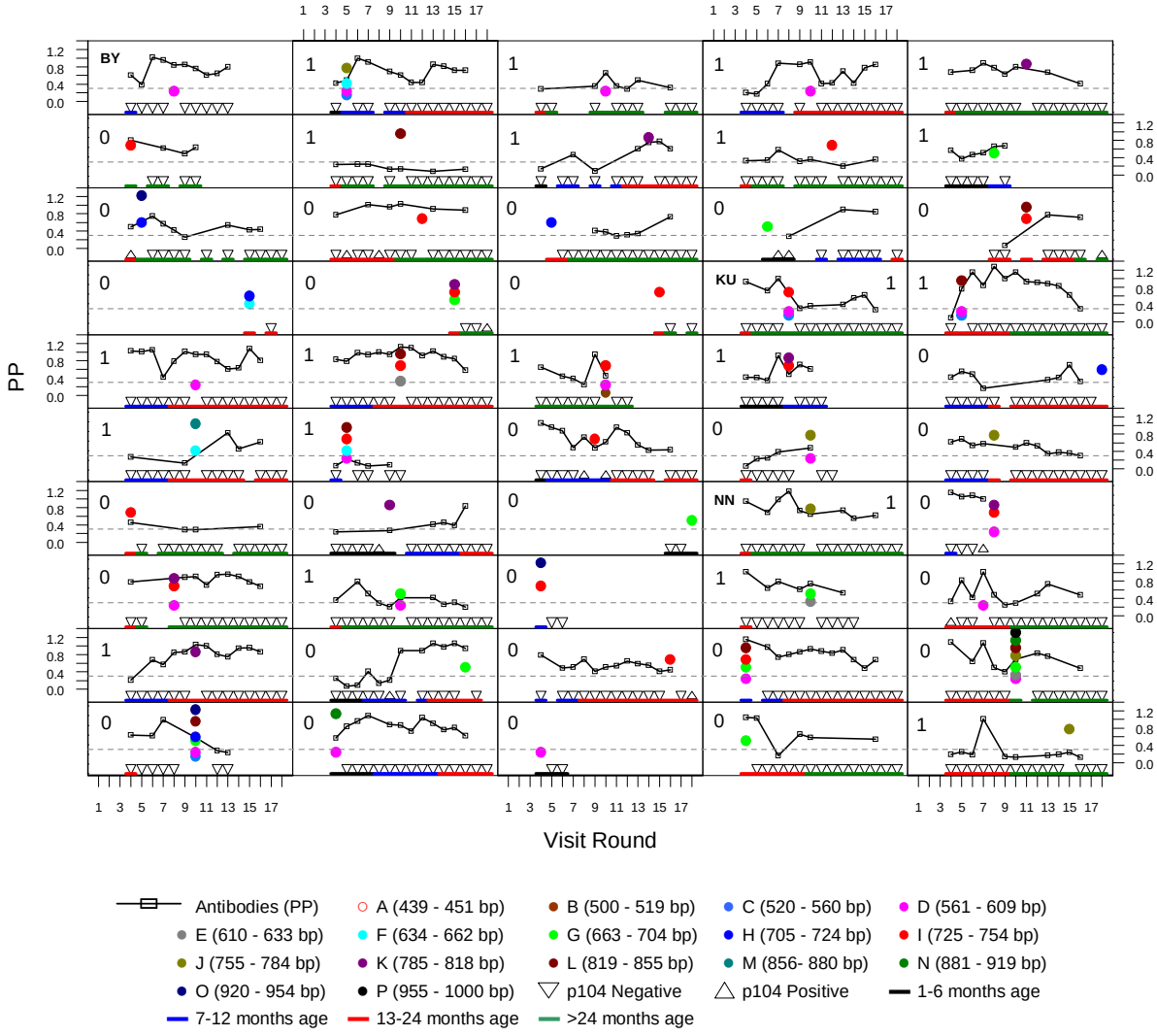
BY01, OJ38, and BY39 were considered to present with two independent *T.parva* infections over time, while NN47 was considered to present with three independent *T.parva* infections. Only the first infection was considered for analysis.

Appendix 7.4.d Longitudinal histories of 12 out of 60 cattle with multiple PIM-positive observations (animals 49 to 60 shown).

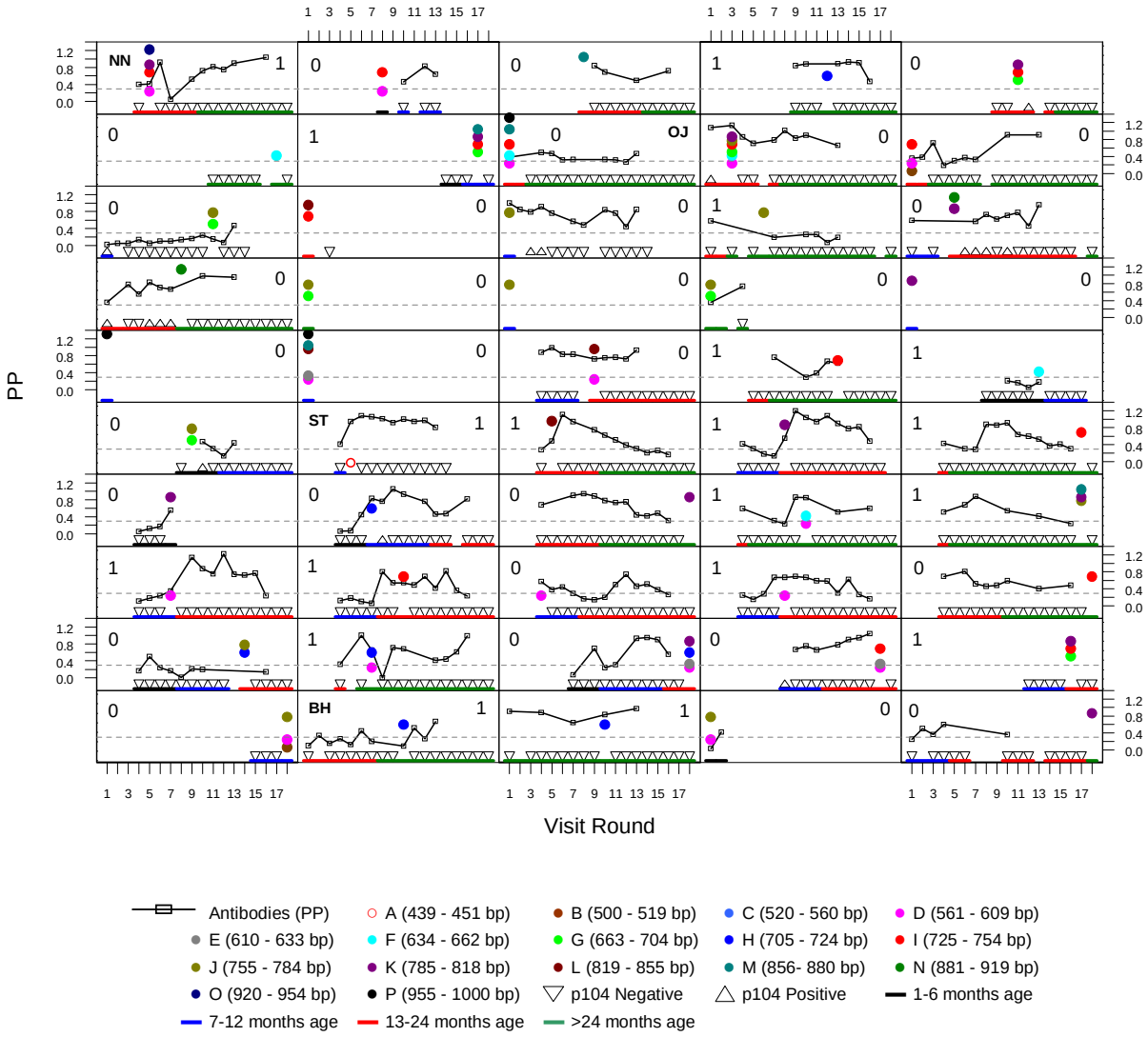


ST84, HT51, MA49 and MA42 were considered to present with two independent *T.parva* infections over time. Only the first infection was considered for analysis.

Appendix 7.4.e Longitudinal histories of 50 out of 147 cattle with one PIM-positive observation (animals 1 to 50 shown).



Appendix 7.4.f Longitudinal histories of 50 out of 147 cattle with one PIM-positive observation (animals 51 to 100 shown).



Appendix 7.4.g Longitudinal histories of 47 out of 147 cattle with one PIM-positive observation (animals 101 to 147 shown).