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Epidemiology of infections and co-infections: Impact on survival and growth of zebu cattle under one year

Samuel Thumbi Mwangi

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Dedication

kũrĩ maitũ Wangarĩ na awa Wambuthia aciari akwa nyenda

Declaration

This dissertation is submitted to the University of Edinburgh in accordance with the requirements for the degree of Doctor of Philosophy in the faculty of Science. Data generation has involved different people and laboratories, and I contributed to nearly all the field data collection and laboratory analysis. I have undertaken all the analysis presented in this thesis, and written it. This work is my own.

Thumbi Mwangi

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Abstract

In any host population, individuals may be infected with multiple pathogens concurrently or in sequence. The direction and strength of pathogen-pathogen interactions are often unknown and dependent on the mechanism of interaction. This thesis is concerned with the epidemiology of infections and co-infections in zebu cattle during their first year of life, and the consequences they have for hosts' survival probabilities and growth rates. Specifically, the study aims to: a) identify the many different pathogen infections occurring in zebu cattle under one year old, b) identify the main causes of mortality and reduced growth rates, c) test for evidence of effects of pathogen-pathogen interactions on mortality and growth, and d) determine the risk factors for infections with pathogens associated with increased mortality and reduced growth rates in zebu calves. To achieve these aims data collected from an epidemiological follow-up study of a cohort of 548 indigenous zebu cattle, recruited at birth and followed for the entire first year of life was used. Growth rates were enormously variable (52 to 704% of birth-weight) and 88 (16%) of the calves died during the first year, most from infectious disease. In total, 25,104 calf weeks of observation and data from 5,337 individual calf visits were analysed. Over 50 different pathogens were identified in the cohort. The thesis begins by providing an overview of zebu cattle and the importance of cattle diseases relevant to Sub-Saharan Africa, emphasising the importance of epidemiological studies taking into account co-infections, which are common in the natural populations, as opposed to a single-pathogen focus. A detailed description of the study design, data collection and descriptive analysis of non-infectious factors, including management and environmental factors, and a descriptive analysis of all pathogens screened for in the study are provided. Using Cox proportional models with frailty terms, the study then identifies infectious and non-infectious risk factors associated with mortality. Further, the role co-infections play in decreasing survival probabilities are investigated, revealing that the hazard for death from East Coast Fever (ECF) - the single most important disease associated with 40% of all deaths - increases 10 times in animals co-infected with *Trypanosoma* species, and 1.3 times for every 1000 eggs per gram faeces increase in strongyle egg count. Mixed-effect models are used to study growth rates and the impact of co-infections, revealing both synergistic interactions (lower host growth rates) of *T. parva* and *A. marginale* co-infections, and antagonistic interactions (relatively higher host growth rates) of *T. parva* and *T. mutans* co-infections

compared to single infections with *T. parva*. Further, this work shows that helminth infections can have a strong negative effect on the growth rates but this is burden-dependent. These findings provide baseline epidemiological data on the diseases with greatest impact on health and performance of young zebu cattle, information that is valuable in the prioritisation and control of diseases. Additionally, they provide evidence of co-infections affecting host growth and survival, and have important implications on disease control strategies, suggesting benefits of an integrated approach to control of worm, tick and tsetse-borne diseases.

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List of terminology and abbreviations

Name	Explanation
ADWG	Average Daily Weight Gain
AEZ	Agro-ecological zone
AIC	Akaike Information Criteria
BCS	Body condition score
BIC	Bayesian Information Criteria
corAR1	Autoregressive correlation structure
corARMA	Moving average correlation structure
CI	95% confidence interval
ECF	East Coast Fever
epg	egg per gram (of faeces)
exp(coef)	exponential of coefficients, used in survival analysis and represents the Hazard Ratio
HR	Hazard Ratio (HR)
ICC	Intra-class correlation
ID-mortality	Infectious Disease mortality
IQR	Inter-quartile range
L3	Larval stage 3 of helminths
LOESS	Local polynomial regression fitting
LogLik	Log likelihood
MAM	minimum adequate model
MCMC	Markov chain Monte Carlo
multivariable	Models with more than one explanatory variable
multivariate	Models with more than one outcome (response variable) including repeated measures/longitudinal studies where measures of the same attribute are taken repeatedly over time.
NDVI	Normalised Difference Vegetation Index
PCR	Polymerase Chain Reactions
RLB	Reverse Line Blot Hybridization
SEAZ	Small East African Shorthorn Zebu
SSA	Sub-Saharan Africa
TBD	Tick-borne diseases
univariable	Models with just one explanatory variable
univariate	Models involving a single outcome regardless of the number of explanatory variables

Chapter 1

General introduction

This thesis work focuses on the survival and growth performance of zebu cattle. Specifically it aims to establish the differential impact of infections and co-infections on two host outcomes: **survival probability** to one year and **growth rates** during the first year of life. The thesis aims to identify, and rank in order of importance, the infections with the greatest impact on these host outcomes, and risk factors for these infections. Further, by studying multiple parasite infections as opposed to single pathogen focus, the study seeks evidence of parasite-parasite interactions that may modify the host outcomes resulting either in increased or decreased severity in the outcome, as opposed to treating coinfecting pathogens as though they work independent of each other.

This chapter provides background information on the zebu cattle, their uses, the environment in which they are raised and the main constraints facing their utilization. It specifically provides background information identifying gaps in the knowledge of impacts and epidemiology of infectious diseases and their co-infections on host survival and productivity. Several topics are covered starting with the current knowledge on disease constraints on livestock production in Sub-Saharan Africa. Since this thesis work is interested in impact infections have on host outcomes and in cases of co-infections, their possible combined effect on host due to pathogen-pathogen interactions, the subject of coinfections and the challenges of doing such studies is explored.

The last section of this chapter lays the hypothesis and the specific scientific questions of this thesis. An outline of the remainder of the thesis chapters is also provided.

1.1 Zebu cattle and their uses

Zebu cattle (*Bos indicus*), indigenous to most of Sub-Saharan Africa, are cattle breeds characterised mainly by a thoracic hump, long legs and a large ventral dewlap, see Figure 1.1. Zebu are thought to have been introduced into Africa at various times, from as early as 1500 BC through initial contacts with Arabs or through the long distance Indian Ocean trade. The main introduction is however thought to have started in the 7th century AD, period coinciding with Arab settlement at the Coast of East Africa (Epstein, 1971; Hanotte et al., 2002).

The dispersal of zebus from the coast to inland may have followed pastoralist movements, and later accelerated in the late 19th century following rinderpest epidemics which affected *Bos taurus* (humpless) cattle more than the zebus (Epstein, 1971; Rossiter, 1994). In most of eastern and southern Africa, zebu have replaced the African taurine breeds (humpless) which date 2500-5000 BC, and which are now mainly limited to West and Central Africa (Rege, 1999).

The term "East African Zebu" is used to refer to the group of shorthorn zebu cattle inhabiting eastern and southern Africa. Based on their relative size, the East African Zebu are classified into two main subgroups; a) the Small East African Zebu (SEAZ) and b) Large East African Zebu. These differences are attributed to the different ecological niches the animals have been adapted to, with SEAZ occupying the wetter more agricultural environments, while the large type are mainly found in the drier areas of eastern Africa (Rege, 1999; Mwacharo et al., 2006). SEAZ, which are the subject of study in this thesis, are more abundant and more widely spread across eastern and parts of the south-central Africa.

Figure 1.1: Zebu cow with a suckling calf. Note the hump and its positioning in the thoracic region which is the main distinguishing characteristic of zebu cattle. They possess a large ventral dewlap and have long legs adapted for long distance walking (own image).

The habitats of Central and East African savannas are riddled with tsetse flies (which transmit the protozoan parasitic disease - trypanosomiasis) and with ticks which are vectors for a number of important livestock diseases including theileriosis, anaplasmosis, babesiosis and heartwater disease, and with many soil transmitted helminth infections. To a good extent, the ability of animals to survive and reproduce in the face of these infections has determined both the uptake of livestock farming and the choice of breeds to keep. In the absence of intense disease control measures, these environments of high disease pressure have been limiting to most breeds except for those adapted to the local environment.

A good account of this challenge of disease is given by Norval et al. (1992) reporting on the history of East Coast Fever (ECF) in Eastern and Central Africa. They detail how ECF, caused by the protozoan parasite *Theileria parva* and transmitted by the tick *Rhipicephalus appendiculatus*, thwarted the early development of beef and dairy ranches, a target of many European settlers in the former East African Protectorate (currently Republics of

Kenya and Uganda). A case in point is the attempt by Lord Delamere, who arrived in 1903 and acquired 500 cows which included local stock from drier parts of the Protectorate and Shorthorn bulls and heifers from England, to start a dairy farm in the Rift valley. After losing, to ECF, almost all the young stock raised on the farm and unable to control for the disease, he eventually abandoned the venture and sought to start the farm in a different location further down the Rift Valley (Norval et al., 1992).

The experiences with ECF in the early 1900's resulted in classifying the various parts of the Protectorate as either "clean" or "dirty" based on their ECF status. Areas around the Lake Victoria basin were considered the "dirtiest" and thought that all animals in the region had been survivors of ECF infection. In 1911 experimental work involving transfer of animals from the Lake Victoria region to an infected farm in Kiambu District (near Nairobi and where approximately 70% of the animals had previously died to ECF) to determine if they would survive the challenge confirmed the existence of acquired protection against ECF. Unlike the control cattle that all died, the animals from Western Kenya all survived and showed no clinical reaction even when infected further with known-infected ticks from Onderstepoort South Africa. Following this observation, a system was developed to provide immune cattle (branded with a "T", and referred to as the "T-brand oxen") to serve as transport oxen throughout Kenya (Norval et al., 1992).

This evidence of indigenous zebu cattle's ability to tolerate ECF may explain why zebu have remained the predominant cattle breed in the "very dirty" Lake Victoria Basin, known to be endemic for ECF to present time (Norval et al., 1992; Latif et al., 1995). Most other wet agricultural areas including the highland parts of Western Kenya managed to rear imported European breeds but on condition of intense tick control and clearance of bushes to remove the tsetse challenge.

Besides zebus being relatively resistant to killer diseases such as ECF compared to European breeds (Wambura et al., 1998; Ndungu et al., 2005), zebu animals have other adaptive features such as heat tolerance, ability to walk long distances, and feeding behaviour that have enabled them to cope effec-

tively in stressful environments, making them the only type of cattle able to survive over a large part of Africa (Rege, 1999).

Communities living in the shores of Lake Victoria prefer zebu over improved European breeds for various other reasons. A study by Amimo et al. (2011) for example reported farmers in Western Kenya preferred zebus over improved European breeds. Their main reasons for keeping zebu cattle were, in the order of importance, use as draft animals, for milk and as a store of wealth.

Specialised single purpose cattle breeds for exclusive production of beef or milk do not appeal to communities that keep cattle for multiple purposes. Besides meat and milk, Rege et al. (2001) report zebu cattle are kept for different other purposes including as a source of direct income through sales with the cash obtained used for purchasing food, medication and paying of school fees. For communities that practice mixed crop-livestock production systems, manure from these animals is used as fertiliser. For others, the manure is used as building material for houses, or used as fuel. The number of cattle owned is considered a measure of social standing, as well as a form of storing wealth. They serve a cultural role as well including the payment of dowry, as well as slaughter during specific occasions such as weddings, funerals, religious and cultural festivals (Rege et al., 2001).

1.2 Zebu cattle and livelihoods

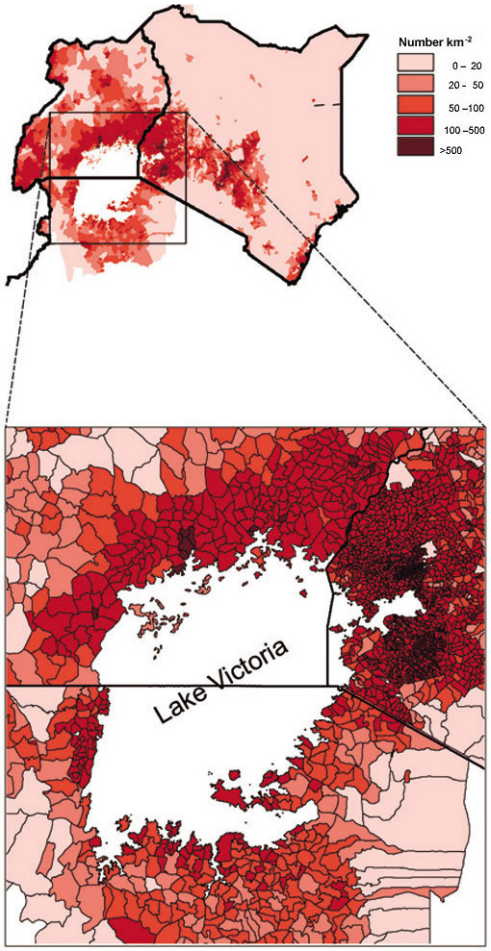
The ability of zebu cattle to survive and reproduce under harsh conditions, and their use for multiple purposes as described in the previous section has led to zebu cattle being increasingly viewed as one of the few options that can be utilised to help improve the livelihoods of livestock keepers (Kristjanson et al., 2004; Tarawali et al., 2011).

Western Kenya which falls by the shores of Lake Victoria is one of the most densely populated areas, with reported high levels of poverty, see Figure 1.2. Over 60% of the households are reported to earn less than US$15 per

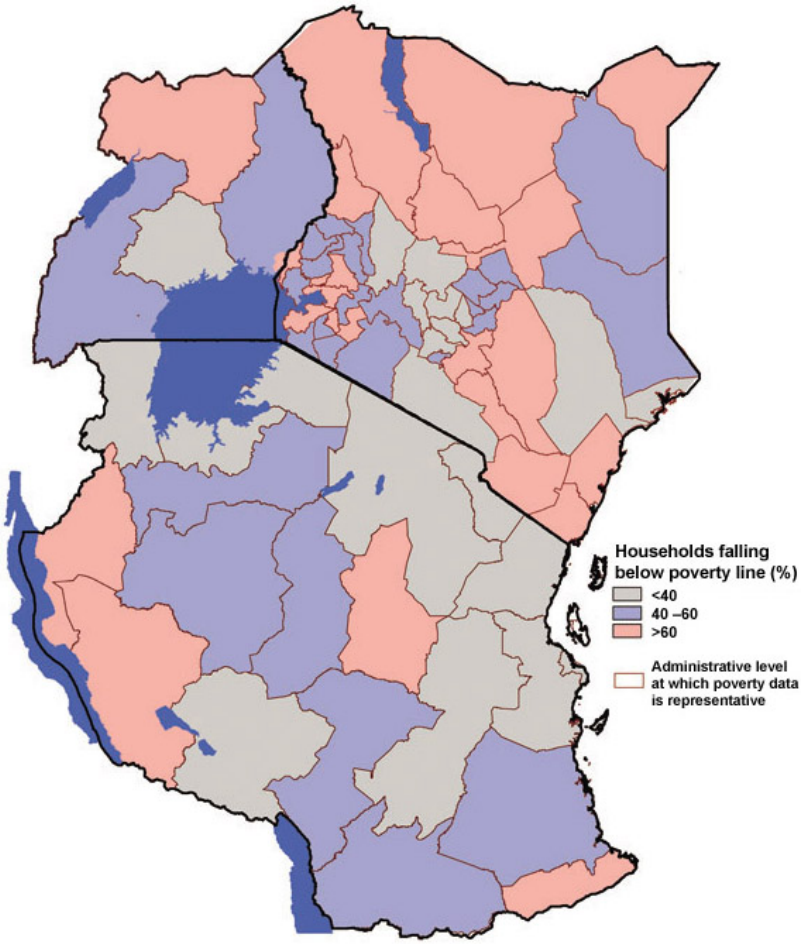
month which is insufficient to meet their basic needs (Thornton et al., 2002; Randolph et al., 2007). An estimated 68% and above of these people solely depend on livestock for their livelihoods.

A family with reproducing livestock has access to cash through direct sales of the animals, which would be used to meet among other needs including medical fees for family members and educational expenses for their children. In such cases, cattle are viewed as a pathway out of poverty. However, in the event of high disease and mortality rates, families that store their wealth and assets in the form of livestock are in the danger of falling right back into poverty (Kristjanson et al., 2004).

The dependence on livestock as a key source of livelihood is not unique to Western Kenya but extends to most of the Lake Victoria basin extending to Tanzania, Uganda and other communities in East Africa keeping SEAZ in smallholder livestock production systems. In this context, an understanding of the challenge of disease in a situation where many different diseases affect cattle at the same time and how best to prioritise and protect livestock assets through disease control has merit. In addition, the benefit of disease control in cattle may go beyond securing livestock assets to reducing vulnerability of livestock keepers by controlling zoonotic diseases such as brucellosis, Bovine Tuberculosis, Rift Valley Fever among others (Perry and Grace, 2009).



(a) Population density map



(b) Poverty map

Figure 1.2: Maps of East Africa highlighting a) the highly densely populated areas, and b) the poverty levels per administrative level. The poverty measure is based on local costs of a basket containing minimum food (calories per adult equivalent), and non food requirements. Households with monthly expenditures below the absolute poverty line are judged to be unable to afford the basket of food. The maps are adapted from the work by Thornton et al. (2002) on Mapping Poverty and Livestock in the Developing World.

1.3 Constraints to livestock production: the problem of disease

Infectious animal diseases pose the greatest threat to livestock production mainly through loss of animals through disease related mortality, use of resources for disease control, and denying livestock producers access to lucrative export markets for their livestock products (Perry, 2007; Ocaido et al., 2009). They are a hindrance to the transition from extensive to intensive livestock production (Rushton and Heffernan, 2002). In the context of growth and development, the impact is mainly thorough: a) diseases that kill and therefore remove livestock assets, and b) diseases that devalue livestock and constrain productivity and c) diseases that constrain market opportunities (Perry and Grace, 2009).

A comprehensive review of livestock diseases and their importance by region including SSA is provided by Rushton and Heffernan (2002). In this review, they classify animal diseases into 3 main groups: endemic diseases; zoonoses and food-borne diseases; and epidemic diseases. This thesis primarily focuses on endemic diseases, and little on zoonotic or epidemic diseases. The parasitic diseases affecting animals in small-scale traditional production systems are mainly endemic, rarely highly infectious, and do not cause epidemics. They occur as clinical or sub-clinical diseases and their main impact is considered to be through loss of productivity, lost potential and costs associated with their control (Perry and Randolph, 1999).

Endemic diseases of importance in Sub-Saharan Africa are broadly classified into; a) ticks and tick-borne diseases, b) trypanosomiasis, and c) gastrointestinal parasites (Rushton and Heffernan, 2002). Comprehensive reviews on these three main groups of endemic diseases have been provided, see (Hansen and Perry, 1994; Norval et al., 1992; Rushton and Heffernan, 2002). Besides these main groups of animal diseases, there are others including viral and fungal diseases whose impact and epidemiology remains largely unknown. Aided by climatic conditions that favour the survival of pathogens and that of pathogen-transmitting vectors, the environments in which zebus are raised

are endemic with a variety of pathogens.

Research on livestock health in the region has mainly been on tsetse and tick-borne diseases, not because these diseases have the greatest impact on zebu cattle but because, as noted earlier, they have been the major hindrance to introduction of improved breeds for commercial purposes. As a result, proper disease surveillance is not routinely carried out leading to a general lack of reliable epidemiological data on which prioritisation and design of disease control strategies can be based on.

Quantifying the burden of disease is further hampered by the lack of a consensus metric for animal disease and the limited information on prevalence and incidence of disease making it impossible to evaluate and prioritize disease (Perry and Grace, 2009). This is especially true in SSA where animal disease impact assessment has mainly been based on qualitative measures, for example, estimates obtained from farmers and veterinary experts. Although these qualitative data fill in where surveillance methods are absent, the data are rarely consistent and suffer biases especially against diseases that do not show dramatic clinical signs (Perry and Grace, 2009).

1.4 Co-infection studies

Hosts under field conditions are constantly exposed to, and infected with, a range of macro-parasites and micro-parasites at any single time (Petney and Andrews, 1998; Behnke, 2008). However, in studying infectious diseases both in humans and in animals, parasitologists have rarely considered more than the single organism that directly interests them (Cox, 2001; Lello and Hussell, 2008). Only recently for instance in human health has there been a renewed focus on poly-parasitism, with studies looking at, for example, multiplicity of *P.falciparum* infections in endemic areas (Tanner et al., 1999; Smith et al., 1999), anaemia burden in children with multiple helminth infections (Mupfasoni et al., 2009; Ezeamama et al., 2008), combined impact of malaria-helminth co-infections on child health (Mwangi et al., 2006; Brooker et al., 2007) and concurrent infections with HIV (Skinner-Adams et al., 2008;

Hamm et al., 2009). In animals, there have been a few coinfection studies looking at pathogen species interactions affecting parasite dynamics and susceptibility of infection in hosts (Lello et al., 2004; Telfer et al., 2008, 2010), and investigating coinfections as an indirect selective force within Soay sheep populations (Craig et al., 2008) - study investigating the effect different coinfection profiles have on the weight of Soay sheep at the beginning of winter which in turn influences the probability of survival over winter.

The impact these multiple infections have on a host is related to each infecting pathogen's virulence (measured by the severity of harm on the infected host attributable to the infecting pathogens), and the possible pathogen-pathogen interactions that may modify parasite densities or their effects on the host. Dependent on the mechanism of pathogen-pathogen interactions, coinfections may cause a) more harm on the host than the combined effect of the component infections, b) harm equal to the combined effect of component infections, or c) less harm than the combined effect of the component infections (Cox, 2001; Alizon and van Baalen, 2008). The mechanisms of interactions between parasites within a host may vary from interference competition when the parasites infect the same site in the host, to indirect interactions mediated by competition of resources or through the host immune system; see work by Pedersen and Fenton (2007) and Graham (2008) for detailed discussion on these possible mechanisms for pathogen-pathogen interactions.

From the above studies it is evident that pathogen-pathogen interactions occur, and that the effect observed on the hosts differs in strength and direction dependent on the specific coinfection combinations and the mechanisms by which pathogen-pathogen interactions occur. Knowledge of pathogen-pathogen interactions is still limited and we do not know which coinfections are important among domestic animals, the direction (synergistic or antagonistic) or strength (effect sizes) these may have on host survival, production and reproduction. Such information would potentially improve the design of disease control strategies, and ultimately their effectiveness in reducing mortality and other losses associated with infectious diseases.

1.5 Thesis structure

This thesis is concerned with establishing the burden of infectious diseases in zebu cattle under one year, specifically investigating the impact infections and coinfections have on two host outcome measures: a) **survival probability** to one year, and b) **growth rates** during the first year of life. By using a holistic approach considering multiple pathogen infections as opposed to focusing on a single-pathogen system, this thesis work aims at providing a comprehensive quantitative assessment of the entire infectious disease burden of zebu cattle during the first year of life. The study seeks evidence of pathogen-pathogen interactions with important effects on host survival and growth, and which would be a target in improving disease control in the population.

This study uses data obtained from the Infectious Diseases of East Africa Livestock (IDEAL) cohort study, which is fully described in the draft manuscript provided in Appendix A. Appendix B provides extra information on the farm management, environment, and factors related to the dam that is used in the later analysis chapters of this thesis.

The main objective of this thesis study is to:

- **Determine the differential impact infections and coinfections have on the survival probability of zebu calves to one year, and their growth rate during their first year of life.**

Specifically, this study aims at establishing the following, each of which forms a thesis chapter in the order below:

1. The range of pathogens infecting indigenous calves during their first year of life.

   This chapter explores and describes the ectoparasites, haemoparasites, viral and helminth pathogens infecting zebu cattle under one year. The temporal, age-related and spatial patterns of these pathogen infections are considered. These infection data are used in subsequent chapters

to determine the impact of both single and multiple infections on the survival probabilities and growth performance of the study animals.

2. The main aetiological causes and the risk factors associated with infectious disease mortality in zebu cattle under one year.

   This chapter identifies and ranks in order of importance, the risk factors and the main aetiological causes of infectious disease mortality. It estimates the mortality rates and the pathogens causing the greatest increase in the risk for death.

3. The role of coinfections in determining mortality of zebu cattle under one year.

   This chapter aims at testing for the effect size and direction of coinfections on the risk of cause-specific calf mortality. It provides information of pathogen-pathogen interactions influencing the risk of death with the specific causes of death identified in the previous chapter.

4. Impact of infections and coinfections on growth rates of zebu cattle that survive to one year.

   This chapter establishes the growth curve function that best describes growth of zebus during their first year of life. In addition, it investigates and quantifies the effect size and direction infections and coinfections have on growth rates.

5. Risk factors associated with selected infections found to have the greatest impact on calf growth and survival.

   This chapter investigates the risk factors of infection with the pathogens found to have the greatest impact on calf growth and survival, as identified in the previous chapters.

6. Main findings of the thesis work and a general discussion on the practical information gained and how the information can be used for improved disease control. This chapter also suggests interesting scientific questions arising from the work and offers suggestions on the future research direction.

Chapter 2

Parasitic infections in zebu calves under one year

2.1 Introduction

The survival and productivity of cattle under smallholder traditional management systems is affected by many factors including animal diseases, feed availability, management and environmental conditions. Through increased mortality and lowered production and reproduction, animal diseases pose the greatest threat to livestock production and are a hindrance to the transition from extensive to intensive livestock production (Rushton and Heffernan, 2002). Based on what most national-level disease control decisions and actions are based on, Perry et al. (2001) broadly classified animal diseases into four groups: zoonotic diseases, food-borne diseases, endemic diseases, and epidemic diseases. The parasitic diseases affecting animals in smallscale traditional production systems are mainly endemic, rarely highly infectious, and do not cause epidemics. They mainly occur as clinical or sub-clinical diseases and their main impact is through loss of productivity, lost potential and costs associated with their control (Perry and Randolph, 1999).

The endemic diseases of importance in most regions of Sub-Saharan Africa are ticks and tick-borne diseases, trypanosomosis and gastro-intestinal (GI) parasites (Rushton and Heffernan, 2002). Uilenberg (1995) cite *theileriosis, babesiosis, anaplasmosis* and *cowdriosis* as "the big four" tick-borne diseases with greatest economic importance in ruminants. Their distribution follows that of their respective tick vectors, but with a more complex interplay be-

tween host availability, susceptibility and immunity, ectoparasite abundance and seasonality, pathogen virulence and infection rates in the ticks, environmental conditions including farm management practices, and climate temperature, rainfall, humidity and vegetation cover (Norval et al., 1992; Bakheit and Latif, 2002; Rubaire-Akiiki et al., 2004; Kivaria, 2010; Gachohi et al., 2011). Tsetse-borne trypanosomiasis infections, although limited to regions falling within the tsetse belt, have a direct impact on livestock and an added burden to livestock keepers due their zoonotic potential (Thumbi et al., 2010; Maudlin et al., 2009).

The burden due to gastro-intestinal (GI) parasite infections is associated with damage in the gastric glands and/or mucus membranes of the GI tract caused during larval migration and attachment by adult worms. Dependent on the infecting helminth species, their effect on the host may include loss of appetite, reduced digestive and absorptive capacities, anaemia associated with blood-sucking worms, gastritis, diarrhoea, and loss of condition (Hansen and Perry, 1994). Helminth species occupying other body organs besides the GI tract, such as *Fasciola* spp. in the liver and *Dictyocaulus viviparus* in the lungs, are associated with damage and pathology observed in the respective organs and migratory routes (Kaufmann, 1996). The epidemiology of GI parasites, like that of vector-borne diseases, is dependent on host, pathogen and environmental factors, and their interactions.

This study focuses on zebu cattle under one year, the predominant cattle breed kept under the widely practised traditional small-holder livestock production system. They are raised in environmental conditions conducive for different types of vectors and parasites overlapping over large geographical areas. The co-occurrence of pathogens and subsequent mixed infections in hosts living under such conditions are therefore a rule rather than an exception (Petney and Andrews, 1998; Cox, 2001). This co-existence of zebus with parasites over years has resulted in animals with reduced susceptibility to endemic diseases and ability to survive in heterogeneous environments (Hanotte et al., 2010). This however has been at a cost of lowered productivity as measured using such indicators as weight gain and age at first calving

(Perry and Randolph, 1999). Attempts to introduce high-producing exotic breeds in regions such as the Lake Victoria basin have failed, with introduced animals quickly succumbing to disease in the absence of intense vector and disease control. Despite the knowledge of an existing disease problem, very little epidemiological data on these diseases is available, and fewer epidemiological studies have attempted to move from "single pathogen focus" into the more realistic "multiple-pathogen" system.

This present chapter aims at describing the infection data generated from this study and that is used in the subsequent chapters investigating the differential impact infections and coinfections have on the survival probability of zebu cattle to one year, and their growth rate during their first year of life. It explores and describes the ectoparasites, haemoparasites, viral and helminth pathogens infecting zebu cattle under one year. The age-related and spatial patterns of these pathogen infections are considered.

2.2 Materials and methods

Sample collection and diagnosis

A total of 548 animals, from 20 sublocations in 4 agro-ecological zones in the Lake Victoria basin of Western Kenya, were recruited into the study at birth and followed every 5 weeks until one year old.

During each visit, a complete clinical examination consisting of a systematic physical examination of the calf was carried out. Information on presence/absence of different tick species and other ecto-parasites was included.

2.2.1 Blood parasites diagnosis

Additionally, thin and thick blood smears obtained from marginal ear veins, and aspirate smears from swollen lymph-nodes were collected during each visit, and prepared for the purposes of parasitological screening of haemoparasites. The slides were stained with 10% Giemsa and examined under a 100x oil immersion objective lens. The data were entered both as a qualitative (recording individual species of parasite identified) and as a semi-quantitative (a measure of the infection intensity) value. An infection intensity scale of 3 (levels 1, 2 and 3) was used, defined as follows:

- Level 1 - One infected cell in more than 10 microscopic fields (low intensity infection)
- Level 2 - One or more infected cells for every 10 microscopic fields (medium intensity infection)
- Level 3 - Multiple infected cells in multiple microscopic fields (high intensity infection)

Blood samples from the jugular vein were collected into plain (without anticoagulant) vacutainers and separated through centrifugation to obtain serum. The serum samples from every calf visit were screened for antibodies

against four main tick-borne diseases (*Anaplasma marginale, Babesia bigemina, Theileria mutans* and *Theileria parva*). In addition, serum samples collected at one year were tested for antibodies against Epizootic Haemorrhagic Disease Virus (EHDV), Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhoea Virus (BVDV), *Neospora caninum* and Bovine Parainfluenza Virus type 3 (PIV3) using commercially available antibody ELISA kits (SVANOVIR^{TM}, Svanova Biotech AB, Sweden). Instructions from the manufacturer, as provided in manuals supplied together with the ELISA kits, were followed.

Blood samples from the jugular veins were also collected into EDTA bottles, and later used for DNA extraction. The DNA extracted from samples obtained at one year was screened for various pathogens using Polymerase Chain Reactions (PCRs) and Reverse Line Blot Hybridisation Techniques (RLBHT). Table 2.1 provides a summary of the samples collected, pathogens screened for, and the diagnostic methods used in each case.

The diagnosis of *Ehrlichia ruminantium* was only possible at post-mortem. A brain "squash" smear was prepared from all post-mortem examination cases by macerating a piece of the grey matter between two glass slides and spreading it like a blood smear. The slides were stained with 10% Giemsa and examined under a microscope for *E.ruminantium* bodies in the endothelial cells of brain capillaries.

2.2.2 Helminth diagnosis

Helminth parasites can be broadly classified into 4 groups: nematodes, cestodes, trematodes and protozoa. During each calf visit, two faecal samples were collected from the rectum of the study animal. The samples were processed and the diagnostic techniques described by Hansen and Perry (1994) used for the quantification and identification of helminth infections.

The first faecal sample was processed and McMaster counting technique used for the identification and quantitative scoring of the coccidia oocytes, nematode and cestode eggs per gram of faeces (epg). Sporulation to speciate

infecting *Coccidia* spp. was only done in samples with a coccidia count $\geq$ 2000 oocytes. To examine for the presence of trematode eggs (flukes), the sedimentation technique was used (Hansen and Perry, 1994). Isolation of lung-worms (*Dictyocaulus viviparus*) was done using the Direct Baermann's technique, which is based on the active migration of larvae from the faeces suspended in water (Hansen and Perry, 1994). Two faecal smears were prepared, one for Ziehl Neelsen (ZN) stain used to identify *Mycobacterium paratuberculosis*, and one for modified ZN for the identification of *Cryptosporidium* spp.

Since eggs from different nematode species are morphologically alike and difficult to differentiate at microscopy, the second faecal sample collected was prepared for faecal culture following the procedure described by Hansen and Perry (1994). A suitable environment was provided for the hatching and development of the nematode eggs to the infective larval stage 3 (L3), which were then used to differentiate between the infecting nematode species.

A summary flow chart showing the processing steps for each sample, and the diagnostic methods for the detection of the different helminth infections carried out in this study is presented in Figure 2.1.

Table 2.1: Summary table showing different samples collected, visit at which they were collected, the pathogens screened for and the diagnostic methods used. 7D is the recruitment visit, 5W - routine visits done every 5 weeks, Y - final visit at one year, PM - post-mortem visit. The lymph-node smear was taken conditional on swollen lymphnode.

Sample	Diagnosis	Pathogens	References
Blood smears (7D/5W/Y)	Microscopy (Giemsa stain)	*Anaplasma* spp., *Babesia* spp., *Theileria* spp. *Trypanosoma* spp.	(OIE, 2008)
Lymph-node smear (5W/Y)	Microscopy (Giemsa stain)	*Theileria* spp., *Trypanosome* spp.	(OIE, 2008)
Brain smear (PM)	Microscopy (Giemsa stain)	*Ehrlichia ruminantium*	(OIE, 2008)
Blood - EDTA (7D/5W/Y)	Haematocrit Centrifugation Technique (HCT)	*Trypanosoma* spp.	(Woo, 1970)
	Dark ground (DG) microscopy	*Trypanosoma* spp., Microfilaria	(Murray et al., 1977)
	Serology - ELISA	*Anaplasma marginale, Babesia bigemina, Theileria mutans, Theileria parva*	(Katende et al., 1998; Morzaria et al., 1999; Tebele et al., 2000)
(Y)	Serology - ELISA	Blue tongue virus (BTV), Epizootic Haemorrhagic Disease virus (EHDV)	(Anderson, 1984; Thevasagayam et al., 1996)
(Y)	Serology - ELISA	Bovine Viral Diarrhoea Virus (BVDV), Infectious Bovine Rhinotracheitis (IBR), *Neospora caninum*, Bovine parainfluenza virus type 3 (PIV3)	(SVANOVIR^{TM}BVDV-Ab, IBR-Ab, Neospora-Ab, PIV3-Ab, Svanova Biotech AB, Sweden)
(Y)	Reverse line blots (RLB)	*Anaplasma marginale, Babesia bigemina, Theileria mutans, Theileria parva, Theileria taurotragi, Theileria sable, Theileria velifera, Babesia bovis, Ehrlichia bovis, Ehrlichia ruminantium, Anaplasma phagocytophilum*	(Gubbels et al., 1999; Bekker et al., 2002)
(Y)	PCR	*Trypanosoma vivax, Trypanosoma congolense, Trypanosoma brucei*	(Njiru et al., 2005; Thumbi et al., 2008)
Faecal sample (Y)	Microscopy	*Mycobacterium paratuberculosis*	(OIE, 2008)

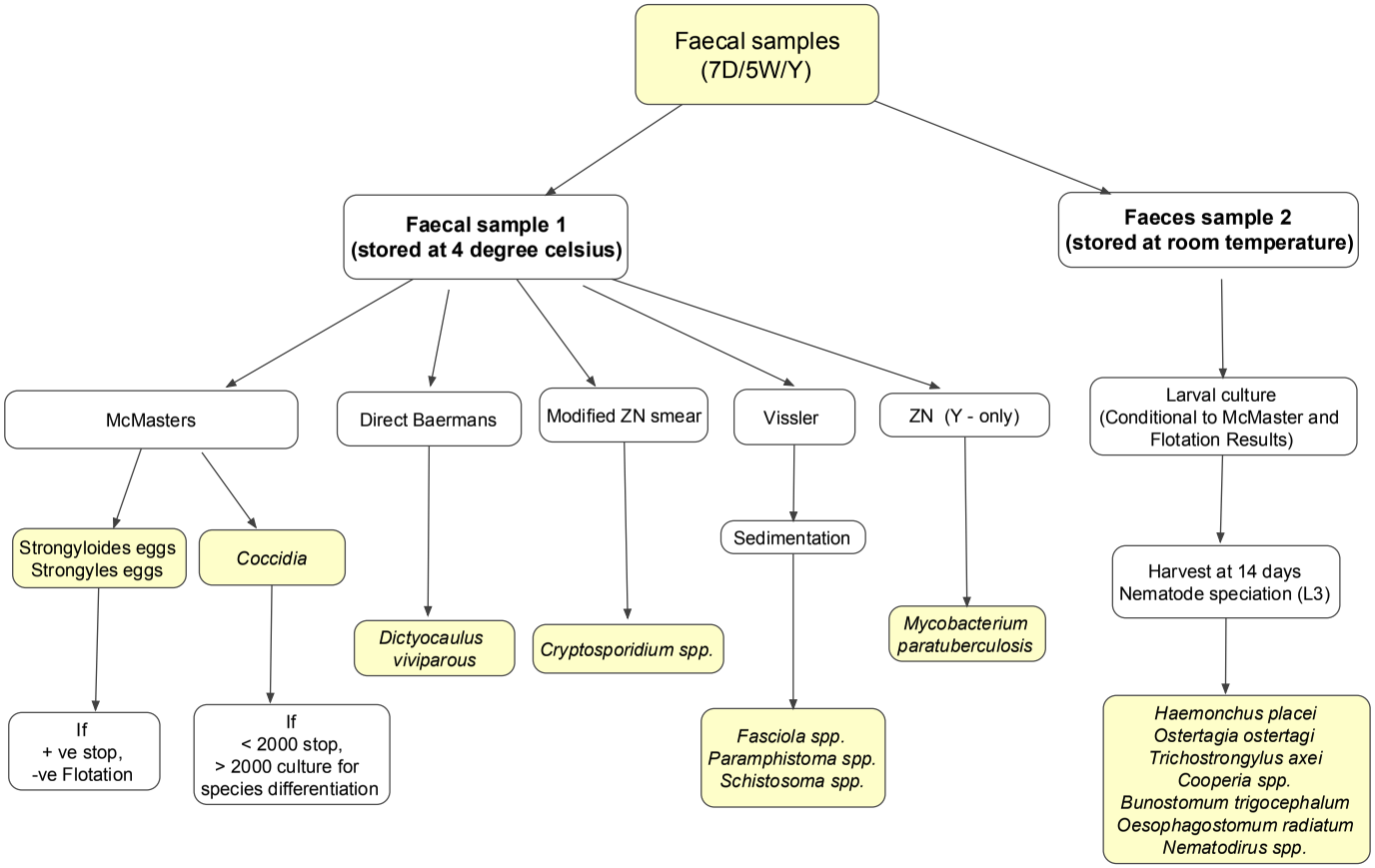


Figure 2.1: Flow chart showing the processing steps and procedures carried out on faecal samples collected during the recruitment (7D), 5 week (5W) and at one year (Y) calf visits, for the diagnosis of gastro-intestinal parasites. ZN - Ziehl Neelsen stain for *M.paratuberculosis* was done on samples collected in the visit at one year.

2.2.3 Data analysis

The frequency of occurrence for each of the pathogens investigated was determined by calculating their prevalence at every calf visit time point. These time points are equivalent to the age of the animals and correspond to the recruitment visit (the week of birth) and the 5 weekly calf follow up visits. Prevalences were calculated by dividing the number of calves infected with each pathogen at every of the 11 calf visit time points, over the total number of calves in the study at that corresponding age. Spatial and temporal patterns in prevalences for the pathogens were explored and graphical outputs used to investigate and describe these patterns.

2.3 Results

In total, 5,337 routine calf visits on the 548 animals in the study were made over the 3 year study period (equivalent to 25,104 calf weeks or 481.1 calf years of observation). The prevalence of each pathogen based on the actual number of samples tested, the specific diagnostic methods used in each case, and the normal location of the pathogen within the host are shown in Table 2.2.

Table 2.2: Summary table showing the prevalences of protozoan, helminth and viral pathogens infecting study calves, their location within the host, and the diagnostic methods used. ELISA results for the tick-borne diseases report the proportion of animals that complete the study and were ever seropositive.

Group	location	Diagnostic technique	Observations		
Protozoan parasites			positive	Total	%
Anaplasma spp.	Red blood cells	Microscopy	50	5337	0.9
Babesia spp.	Red blood cells	Microscopy	3	5337	0.1
Theileria spp.	Red blood cells	Microscopy	3113	5337	58.3
Trypanosoma spp.	Bloodstream	HCT + DG	62	5337	1.2
Trypanosoma theileri	Bloodstream	HCT + DG	3	5337	0.1
Trypanosoma vivax	Bloodstream	HCT + DG	33	5337	0.6
Coccidia spp.	Small intestine epithelium	McMaster	1521	4571	33.3
Anaplasma marginale	Red blood cells	ELISA	158	455	34.7
Babesia bigemina	Red blood cells	ELISA	102	455	22.4
Theileria mutans	Red blood cells	ELISA	292	455	64.2
Theileria parva	Red blood cells	ELISA	329	455	72.3
Trypanosoma vivax	Bloodstream	PCR	61	453	13.5
Trypanosoma congolense	Bloodstream	PCR	1	453	0.2
Trypanosoma brucei	Bloodstream	PCR	7	453	1.6
Ehrlichia bovis	Leucocytes	RLB	172	454	37.9
Ehrlichia ruminantium	Capillaries	RLB	2	454	0.4
Ehrlichia omatjenne	Leucocytes	RLB	187	454	41.2
Babesia bigemina	Red blood cells	RLB	1	454	0.2
Babesia bovis	Red blood cells	RLB	10	454	2.2
Theileria sable	Red blood cells	RLB	138	454	30.4
Theileria mutans	Red blood cells	RLB	314	454	69.2
Theileria parva	Red blood cells	RLB	55	454	12.1
Theileria taurotragi	Red blood cells	RLB	33	454	7.3
Theileria velifera	Red blood cells	RLB	287	454	63.2
Theileria ovis	Red blood cells	RLB	14	454	3.1
Theileria bicornis	Red blood cells	RLB	6	454	1.3
Anaplasma phagocytophilum	Neutrophils	RLB	2	454	0.4
Neospora caninum	Tissue and blood cells	ELISA	63	455	13.8
Helminths					
Strongyle-type					
Strongyle eggs	GIT	McMaster	3426	4571	75.3
Haemonchus placei	Abomasum	Faecal culture	2766	3604	76.7
Trichostrongylus axei	Abomasum	Faecal culture	1462	3604	40.6
Oesophagostomum radiatum	Colon	Faecal culture	649	3604	18
Ostertagia ostertagi	Abomasum	Faecal culture	10	3604	0.3
Cooperia spp.	Small intestine	Faecal culture	35	3604	1
Bunostomum trigonocephalum	Small intestine	Faecal culture	1	3604	0.03
Non-strongyles					
Nematodirus spp.	Small intestine	McMaster	29	4571	0.6
Strongyloides spp.	Small intestine	McMaster	533	4571	11.7
Trichuris spp.	Caecum	McMaster	61	4571	1.3
Dictyocaulus viviparus	Lungs	Direct Baermann's	254	4571	5.6
Toxocara vitulorum	Small intestine	McMaster	176	4571	3.9
Fasciola hepatica	Liver	Sedimentation	114	4571	2.5
Monieza expansa	Small intestine	McMaster	6	4571	0.1
Calicophoron	Small intestine,rumen,reticulum	Sedimentation	772	4571	16.9
Chabertia ovina	Small intestine, colon	McMaster	1	4571	0.02
Viruses					
Blue tongue virus		ELISA	430	455	94.5
Epizootic Haemorrhagic Disease virus		ELISA	291	455	64
Infectious Bovine Rhinotracheitis		ELISA	25	455	5.5
Bovine Viral Diarrhoea Virus		ELISA	79	455	17.4
PIV3		ELISA	80	455	17.6
Fungi					
Trichophyton spp.			38	5337	0.7%

2.3.1 Protozoan parasites

Infections with *Theileria* spp. were the most prevalent of protozoan parasites, with 58.3% of total calf visits being positive with either schizonts or piroplasms on microscopy. *Anaplasma* spp. and *Babesia* spp. infections were detected in only 0.9% and 0.1% of the total calf visits respectively. Levels of exposure to the pathogens as indicated by serology tests were much higher with 72.3%, 64.2%, 34.7% and 22.4% sero-conversion rates for *Theileria parva*, *Theileria mutans*, *Anaplasma marginale* and *Babesia bigemina* respectively, in calves reaching one year. Reverse line blots using oligonucleotide DNA probes for different pathogens species revealed the calves at one year were infected with different *Theileria, Ehrlichia, Anaplasma* and *Babesia* species. *T.mutans* and *T.velifera* were the most common theileria infections on RLB, and to a lesser extent infections with *T.sable*. Notably though, whereas the exposure levels to *T.parva* as indicated by the serology results were high (72.3%), the percentage of calves with detectable parasites at one year using RLB was 6 times lower (12%). This difference was not observed in *T.mutans* which had both serology and RLB results $> 60\%$. *B.bigemina* was detected in only one calf at one year (RLB) although serology results indicated 102 of the 455 calves had seroconverted by one year of age.

Results of PCR assays for *Trypanosoma* revealed 15.2% of the animals were infected with at least one species of trypanosome. *T.vivax* which is transmitted both biologically through tsetse flies and mechanically by biting flies was the most prevalent species. Haematocrit Concentration Technique (HCT) and Dark Ground (DG) microscopy methods picked 1.9% of the total calf visits as positive with *Trypanosoma* spp. infections.

Sporulation results of the samples with a coccidia count ≥ 2000 were positive for three species; *Eimeria bovis, Eimeria zuernii* and *Eimeria auburnensis*, which are all known to be associated with clinical disease in cattle.

Figure 2.2 is a plot showing the age-related prevalence for each of the 5 protozoan parasites diagnosed at microscopy. The shape of the curve shows

the relative incidence rate for each pathogen at each age period. A sharp rise in the curve during a time period indicates a higher incidence of infection during that period. Infections with *Theileria* spp. occurs from early on in life with 50% of the calves infected by the age of 4 months. The prevalence at each time point continues to steadily increase and appears to asymptote towards one year. *Coccidia* spp. infections are acquired early by 6 weeks of age, and the prevalence does not rise or decline much over the rest of the year. The survival curves plots for seroconversion to *T.parva, T.mutans, B.bigemina* and *A.marginale* are shown in Figure 2.3.

To explore the spatial patterns, prevalences for pathogens and study sublocations were calculated and plotted, see Figure 2.4. Prevalence differed between sublocations with some sublocations reporting no cases of trypanosome infections while others such as Magombe East had high ($> 40\%$) *Trypanosome* spp. prevalence in certain age groups, Figure 2.4. The age at which 50% of the calves in a sublocation are infected with *Theileria* spp., a measure associated with the force of infection, differed between study sublocations. For instance, this is reached by the age of 11 weeks in Bulwani and Kokare sublocations, and takes more than 36 weeks in Karisa sublocation. The prevalence of infections with *Coccidia* spp. also differed between sublocations, with some such as Karisa maintaining prevalences $< 30\%$ and others such as Bumala A recording prevalences $> 50\%$ at some calf ages.

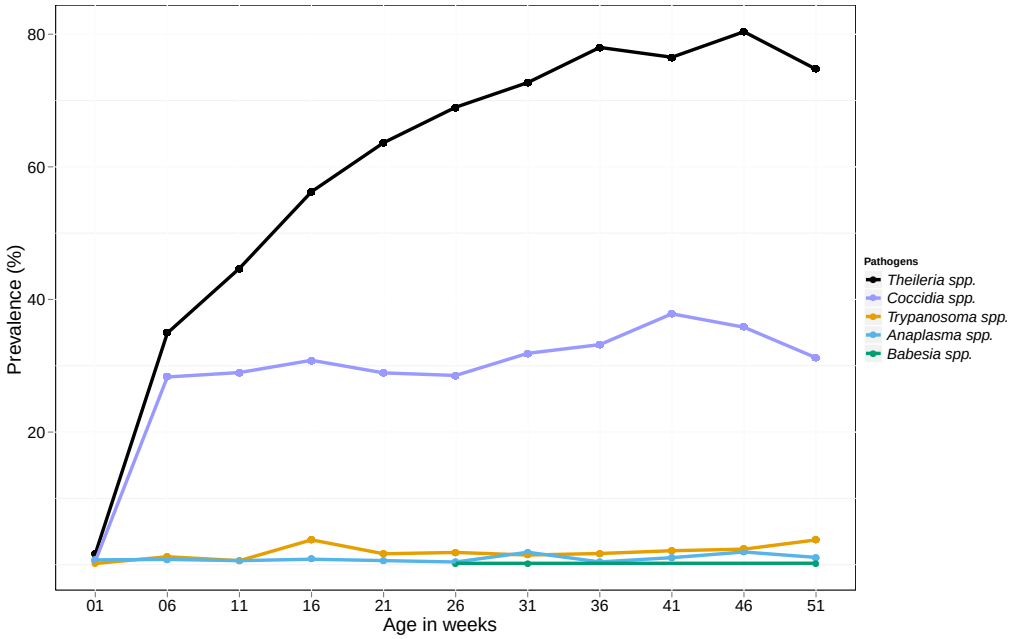


Figure 2.2: Prevalence of protozoan parasites by the age of calves; results based on microscopy.

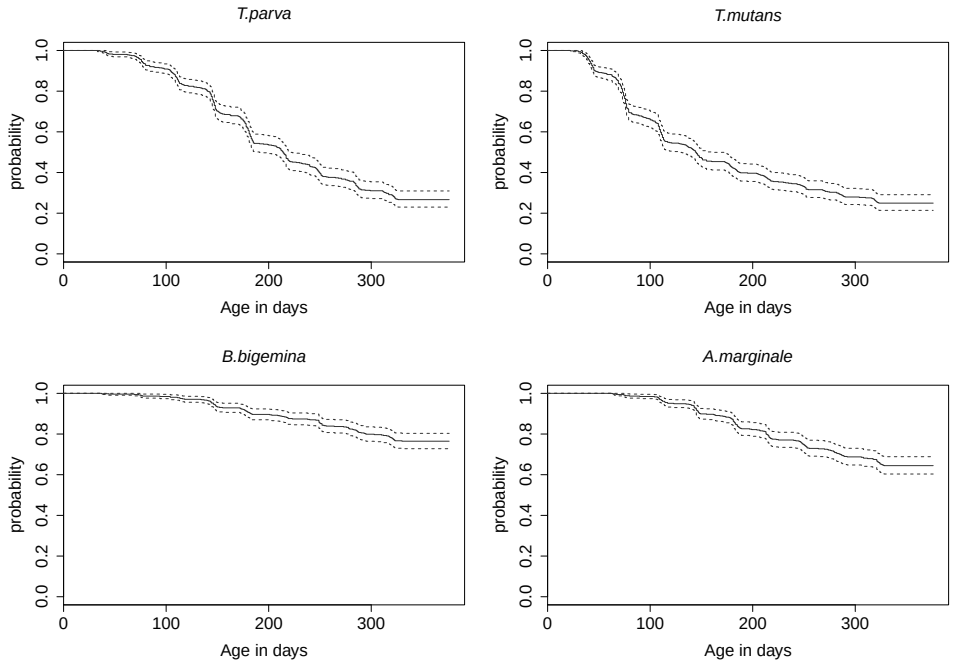


Figure 2.3: Survival plots for time to seroconversion to *T.parva, T.mutans, B.bigemina* and *A.marginale*.

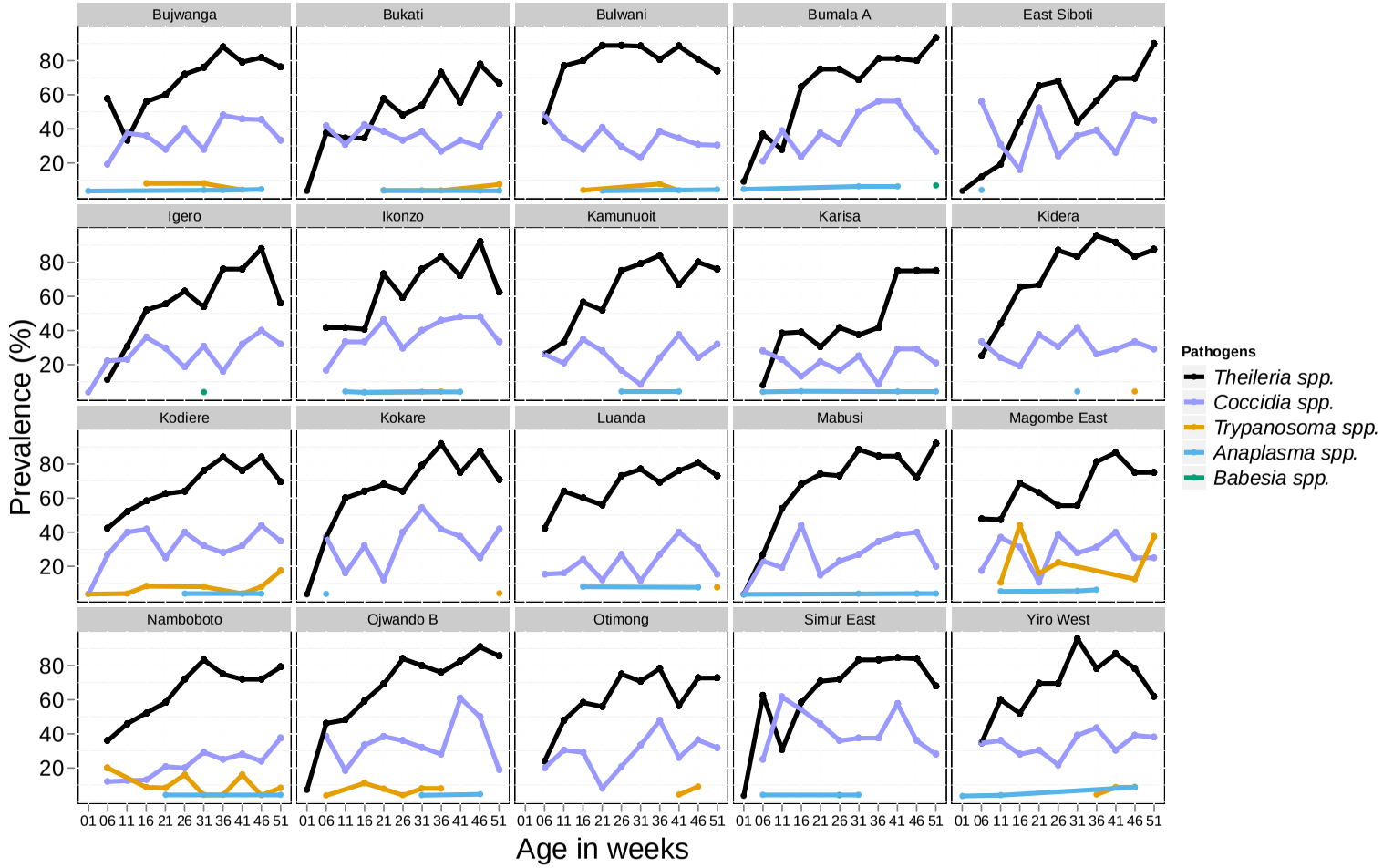


Figure 2.4: Prevalence of protozoan parasites by calf age and sub-location, identified by microscopy. *Theileria* spp. and coccidia were relatively the more common protozoan parasites identified. *Trypanosoma* spp. were found in only some sublocations, with high prevalence identified in Magombe East. *Anaplasma* spp. and *Babesia* spp. were rarely detected, sometimes only at certain ages (dots or short lines) or not detected at all in some sublocations.

2.3.2 Ectoparasites

The tick *Rhipicephalus appendiculatus*, the main vector for *T.parva, T.taurotragi* and *Anaplasma bovis*, was the most prevalent tick being present in > 90% of all calf visits. Infestation with *R.appendiculatus* occurred early in life, with 69% of all calves being infested by recruitment time which was done at the week of birth.

Amblyomma variegatum, the tick vector for *T.mutans, T.velifera* and *A.bovis* was the second most prevalent tick infesting up to 77% of animals at one year. *Ehrlichia ruminantium* (causative agent for heartwater disease) is thought to be transmitted by *Amblyomma* spp. although the infective blood stage of these parasites is not detectable by microscopy.

Unlike *R.appendiculatus*, the prevalence of *A.variegatum* infestation increased steadily with the age of calves, Figure 2.5. *Boophilus* spp. are important in the transmission of *Babesia bovis* and *Babesia bigemina*, which causes clinical disease (anaemia and haematuria) in cattle, and *Anaplasma marginale* also characterised by anaemia.

The prevalence of *Boophilus decolaratus* was low remaining under 10% in calves below 6 months, and less than 20% in calves above 6 months of age. *Rhipicephalus evertsi*, a secondary vector for *T.parva* was encountered at a low prevalence which increased with the age of the calf.

Flea infestation was highest in young calves, and decreased with the age of calves. The slopes of the prevalence plots are an indication of the incidence of infestations at each time period.

Calves from sublocations such as Magombe East and Kodiere had high *R.appendiculatus* infestation rates (> 90%) at recruitment time, whereas others as Bukati and Ikonzo took up to 21 weeks to reach a 90% prevalence level indicating differences in infestation pressure between sublocations, Figure 2.6.

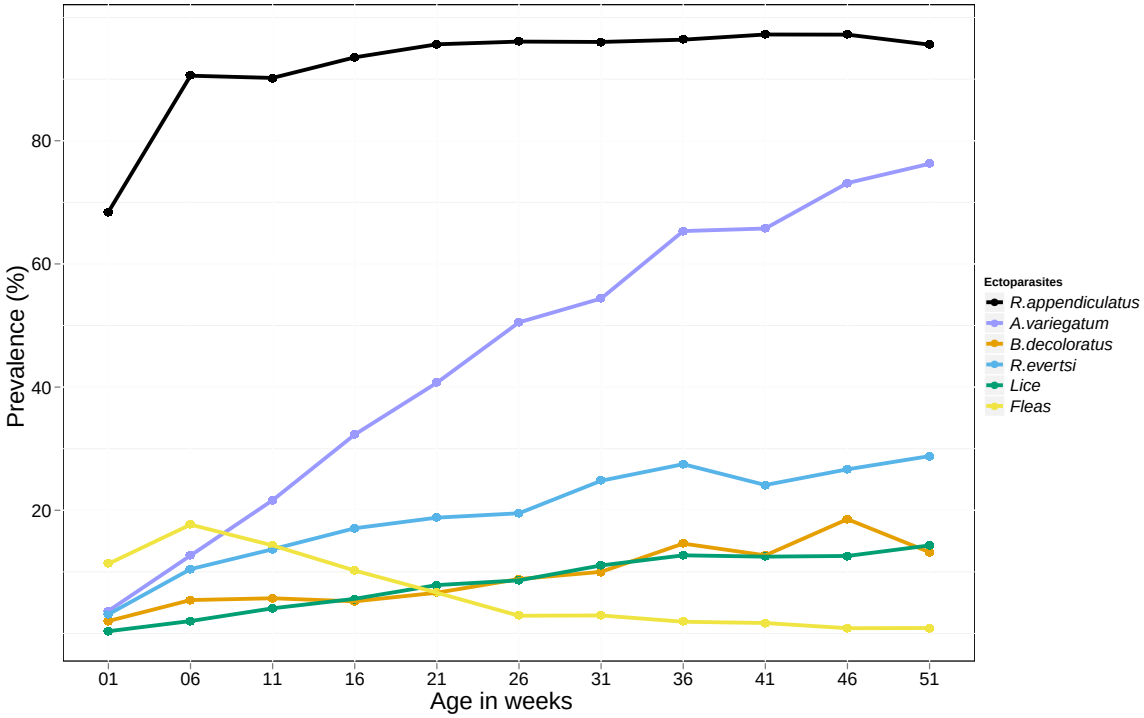


Figure 2.5: Prevalence of ecto-parasites by calf age.

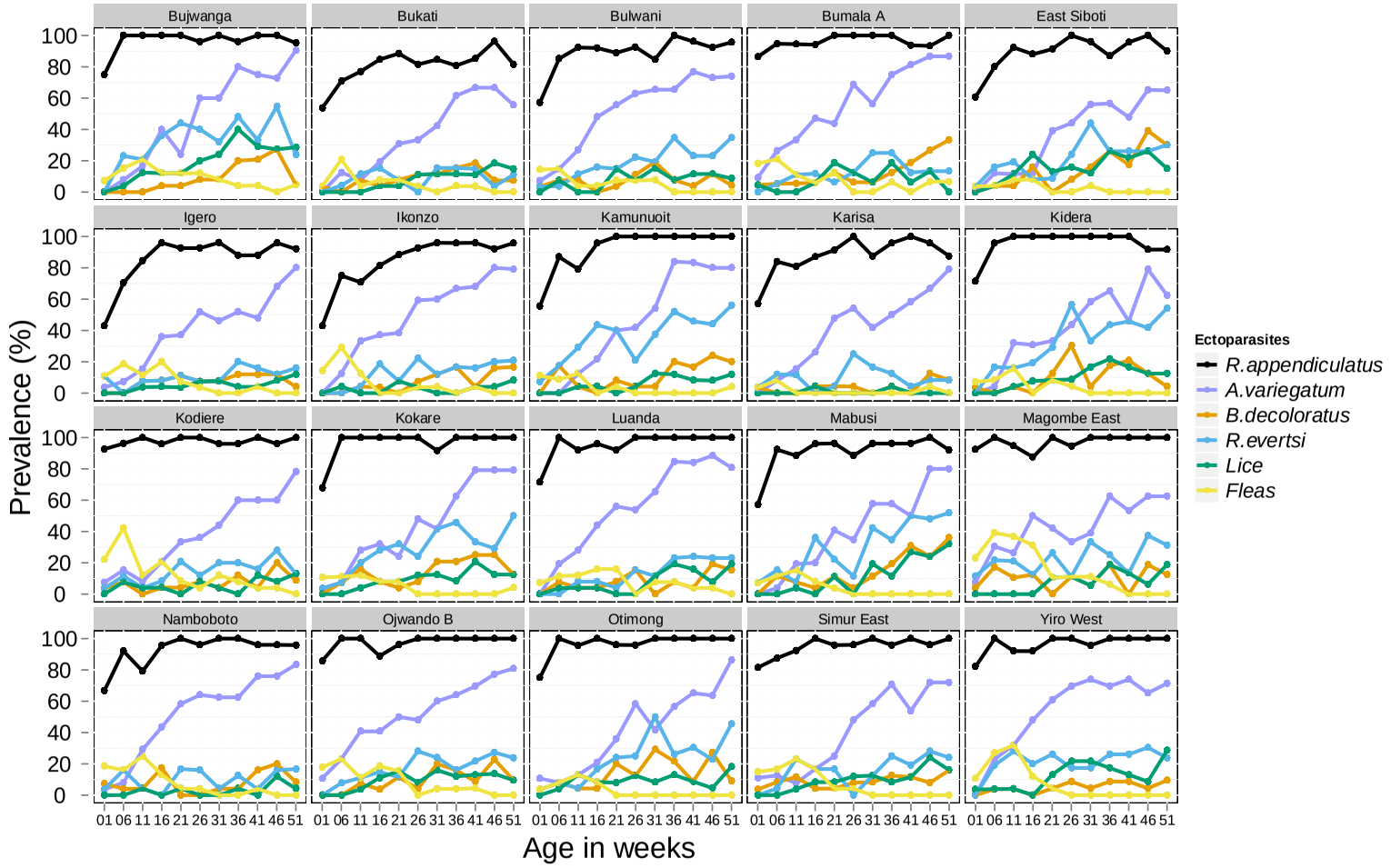


Figure 2.6: Prevalence of different ectoparasites by age and study sublocations.

2.3.3 Helminth infections

The study aimed at collecting 2 faecal samples for each of the 5337 calf visits made. In 85.6% (4571) of the total calf visits at least one faecal sample was successfully obtained, and both samples in 67.5% (3604) of the total visits. Faecal cultures were only prepared if a second sample was available. The different helminth species identified in this study, their location in the host, methods used for their diagnosis, and their prevalences calculated based on the number of samples tested are presented in Table 2.2.

To investigate helminth prevalence patterns by calf age, prevalences at each calf visit time point for each of the helminth species were determined and plotted, see Figure 2.7. Prevalences of *Strongyloides* spp., *T.vitulorum* and *D. viviparus* were highest in the first weeks of life and decreased with age. The rumen flukes (*Calicophoron* spp.) prevalences increased with the age of calves, with 28% of the calves infected by one year. *Fasciola* spp. infections were mainly acquired after 6 months of age, with prevalence rates increasing in the period after 36 weeks of age. The sublocation Luanda had a particularly high prevalence for *Fasciola* spp., while others as Namboboto did not record any cases, Figure 2.8. The common strongyle-type nematodes included *H.placei, T.axei* and *O.radiatum*, which were present in 77%, 41% and 18% respectively of faecal samples cultured for identification of the infective larvae (L3). In addition, *H.placei* accounted for most of the larvae (> 80%) counted following larval culture.

The other species of helminths were detected in under 10% of the samples, Figure 2.7. The prevalences of these helminth infections by sublocation are presented in Figures 2.8.

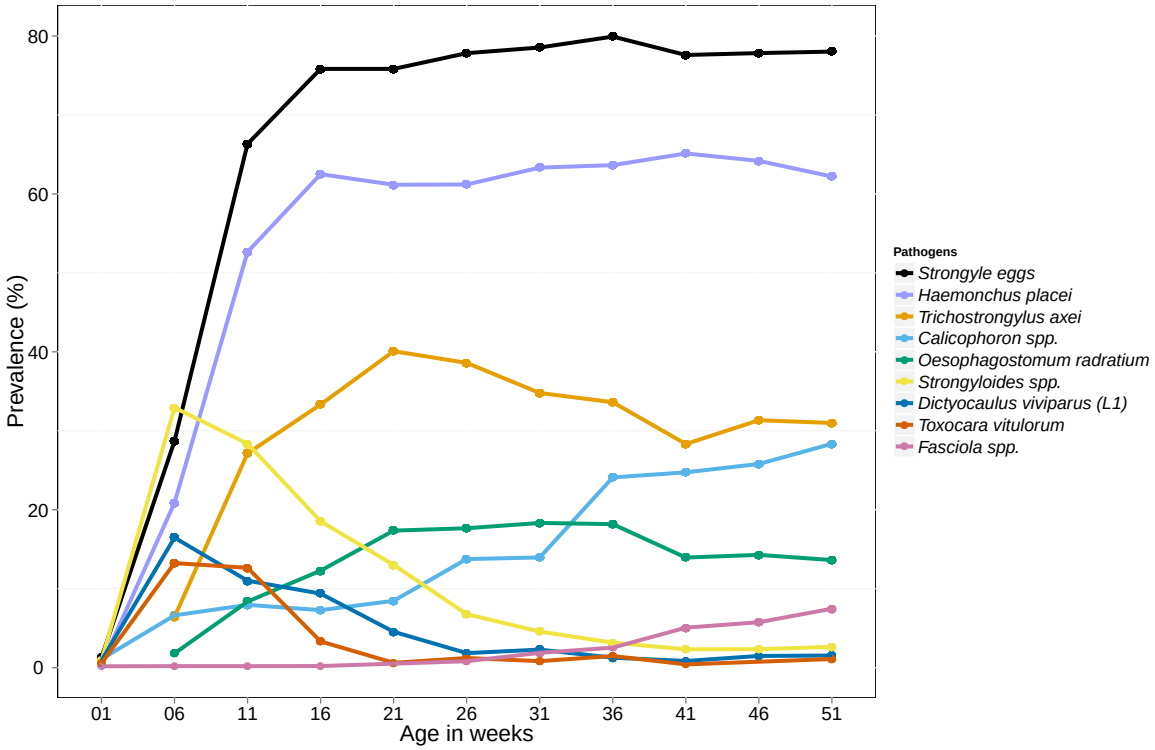


Figure 2.7: Prevalence of helminth infections by age of calves.

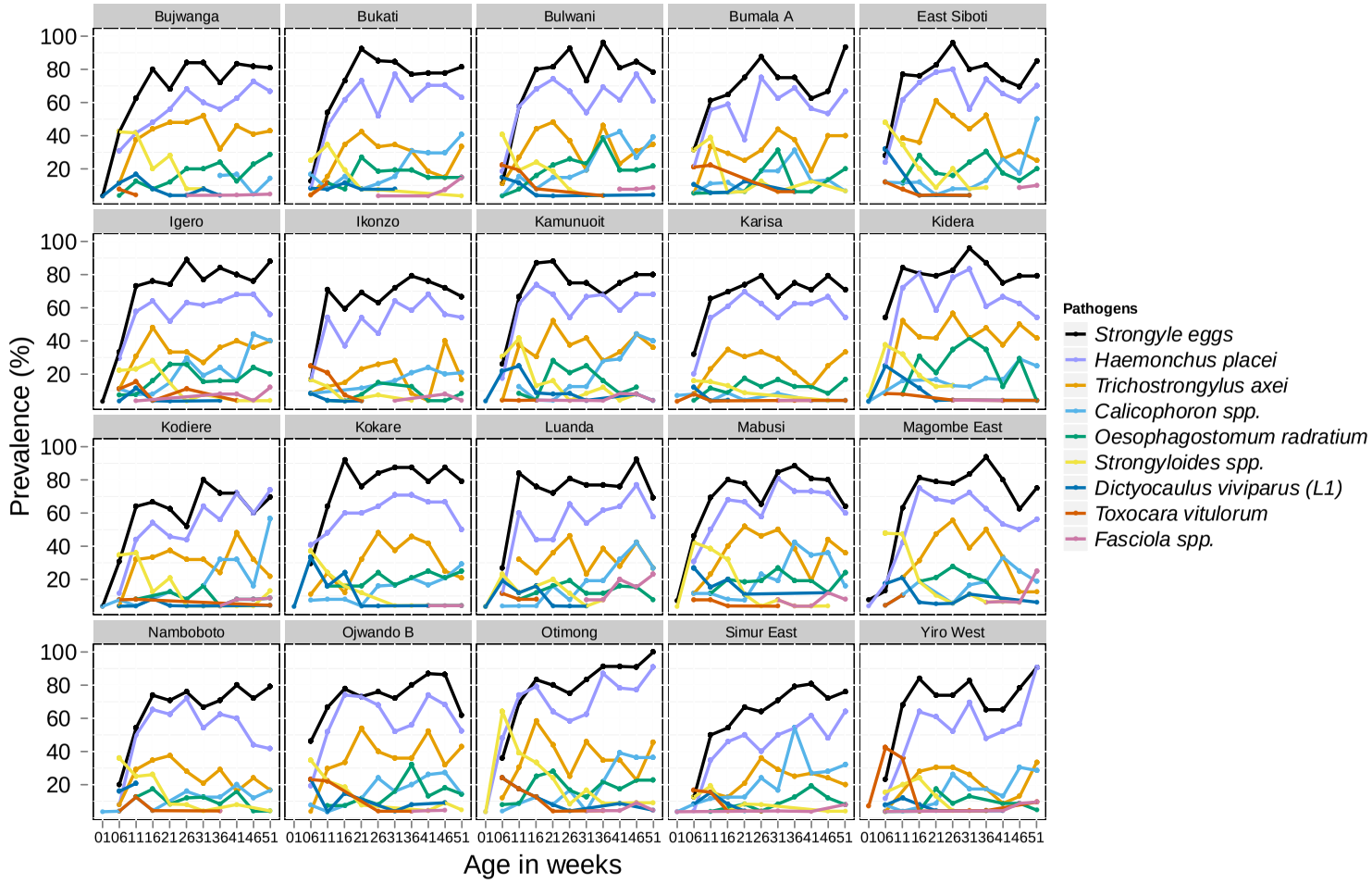


Figure 2.8: Prevalence of helminth infections by sublocation and age of calves. Diagnosis of the helminth species was done using microscopy and larval cultures for speciation of L3's.

2.3.4 Strongyle epg

The strongyle egg count varied greatly with a range of 0 - 18050 epg, with a median of 350 epg for all faecal samples examined. At 6 months, the median count was 400 epg with a range of 1 to 7450. The median epg count by age of calves is presented in Figure 2.9. The burden of strongyle eggs varied between study sublocations with high burdens observed in sublocations such as East Siboti, Kamunoit and Kidera, and lower burdens in Simur East and Magombe East, Figure 2.10. Sublocations with lower burdens were those located in the south of the study area and those with higher burden were in the north of the study area.

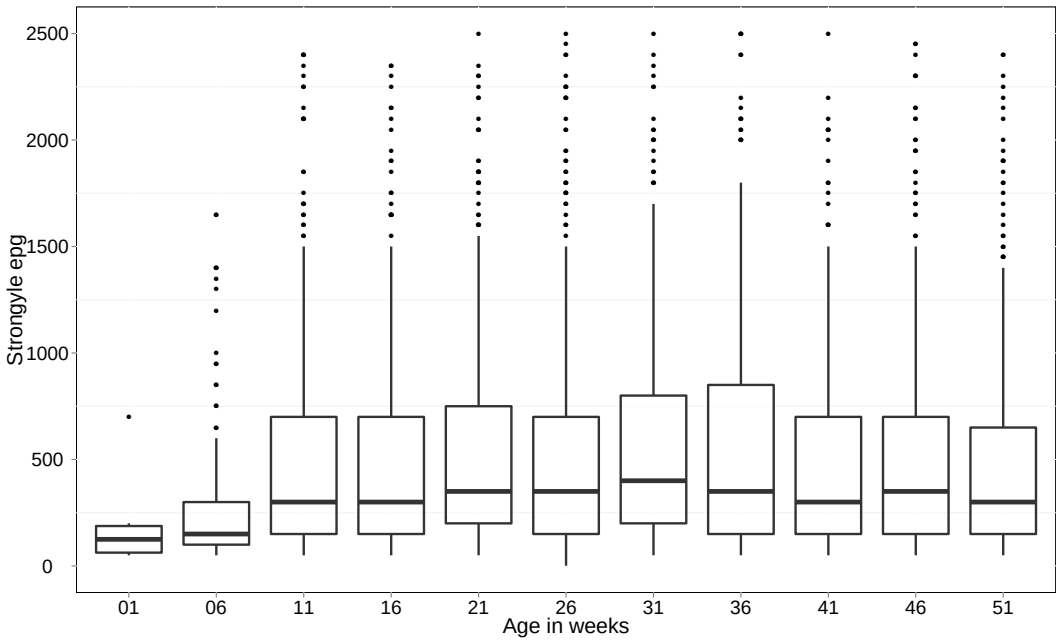


Figure 2.9: Distribution of strongyle egg count by age of calves. Owing to some calves having very high strongyle epg count, the y-axis has been limited to 2500 epg to allow visualisation of differences in the median epg with age of calves. Strongyle epg counts above 5000 were observed from 11 weeks onwards. The horizontal line in the middle of the boxplot represents the median. The upper and lower horizontal lines correspond to the rst and third quartiles. The upper whisker extends from the upper quartile to the highest value that is within 1.5 times inter-quartile range (IQR) while the lower whisker extends from the lower quartile to the lowest value within 1.5 times IQR. Data beyond the end of the whiskers are outliers and plotted as points.

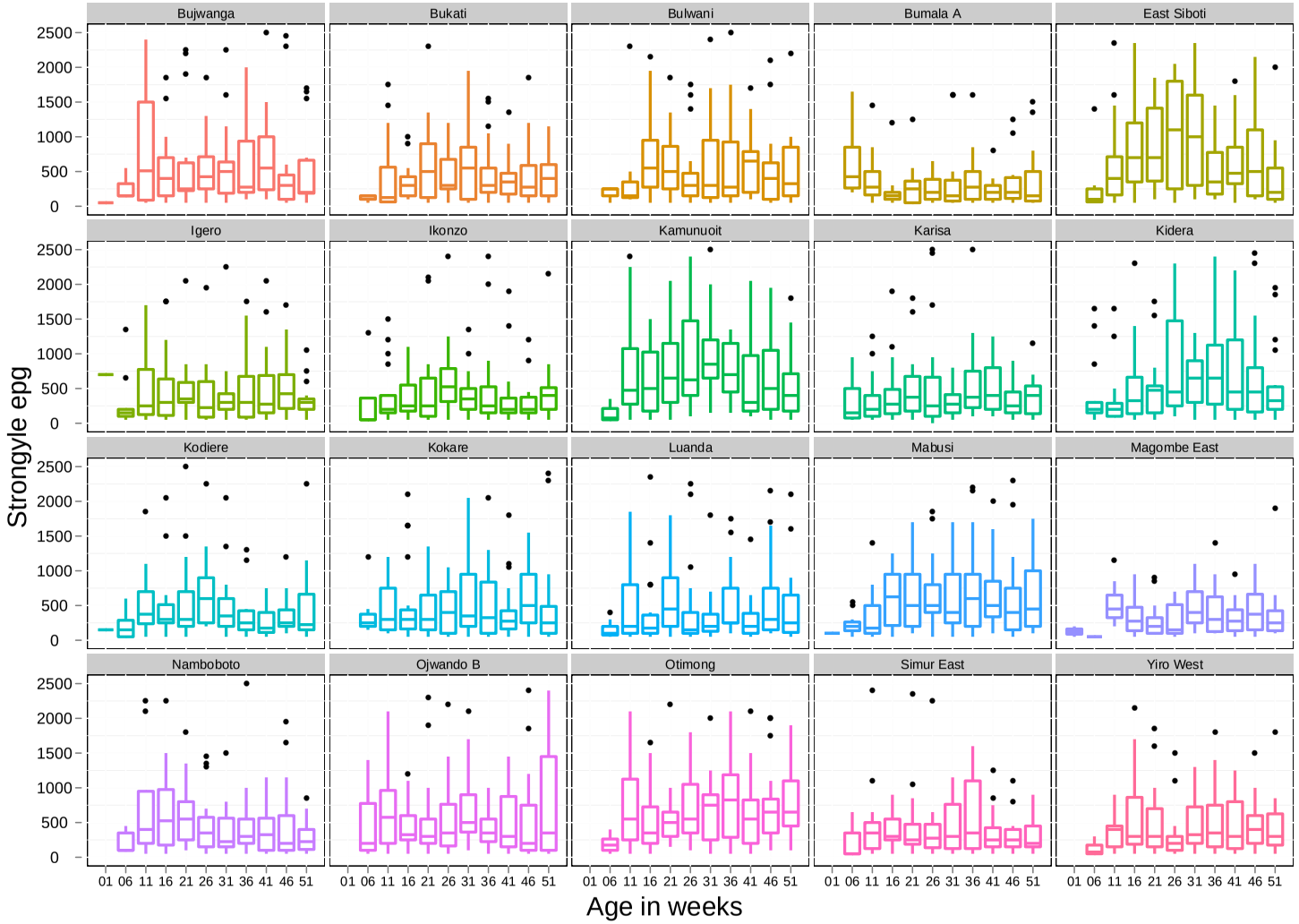


Figure 2.10: Prevalence of strongyle epg count by sublocation and calf age. The boxplots show the median egg count (middle horizontal line), lower and upper quartile egg counts. The points above the whiskers represent outliers falling 1.5 times IQR.

2.3.5 Co-infections

A count number of different infecting pathogens observed per calf at each calf visit was obtained, and plotted as presented in Figure 2.11. This number is conservative as it includes only pathogens diagnosed at the field laboratory, where mainly microscopy techniques were used. For a number of pathogens, it is difficult to distinguish between infecting species morphologically at microscopy, for example between *Theileria* spp. such as *T.parva* and *T.mutans*, or between different *Trypanosoma* spp. Using other more sensitive diagnostic tests such as PCR, ELISA and RLB on samples collected over the one year study period would increase the number of co-infecting pathogens identified. At birth, calves are largely infection free but quickly get infected with a median of 2 pathogens observed by 6 weeks of age. Numbers of co-infecting pathogens increase with age which would correspond to increased exposure. By 6 months of age, the median number of co-infecting pathogens is 4. The range of co-infecting pathogens revealed calves could be infected with as many as 11 different pathogens concurrently.

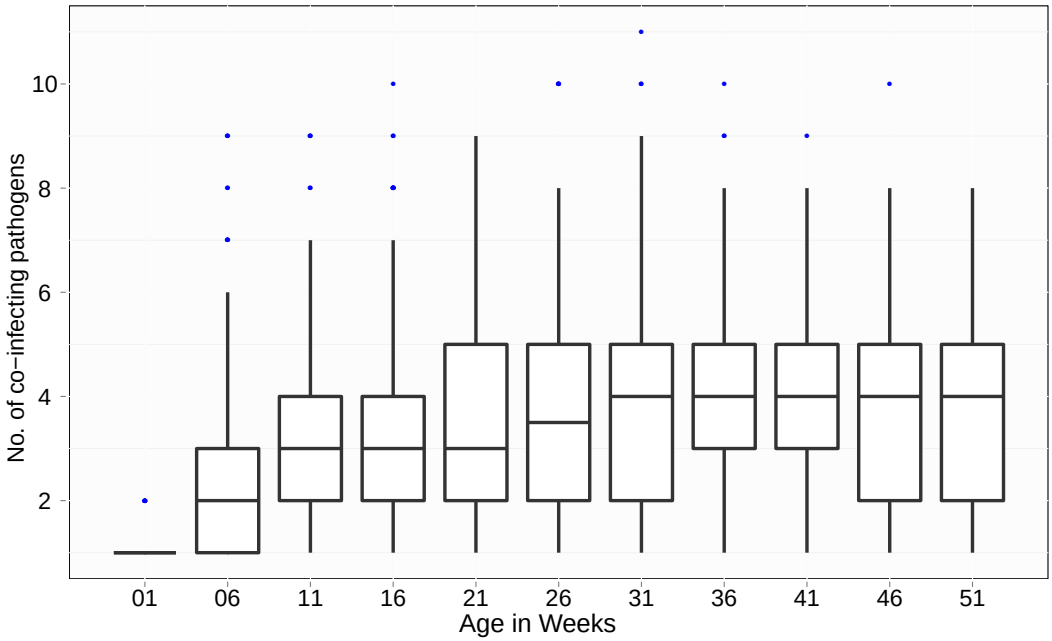


Figure 2.11: Number of co-infections at each calf visit, results of microscopy diagnosis. At recruitment visit, the animals are largely infection free, rising to a median of 2 in 6 weeks. Beyond 26 weeks of age, the median number of infections is 4, and the range up to 11 different pathogens in a single calf at a time. The boxplots show the median egg count (middle horizontal line), lower and upper quartile egg counts. The points above the whiskers represent outliers falling 1.5 times IQR.

2.4 Discussion

In this chapter, I have described the infection data that will be used in the subsequent chapters investigating impact of infections and coinfections on survival and growth rates of zebu cattle under one year. Specifically, this chapter has reported the age-related prevalence and the spatial patterns of different pathogen infections observed in zebu cattle under one year. Most of the pathogen data from the calf follow up visits were obtained using microscopy. As a diagnostic technique, microscopy has the advantage of ease of use under field conditions and is suitable for the diagnosis of clinical cases which will usually have high parasitaemia levels. They however lack the sensitivity required to pick infections in carrier animals, for example those of *Theileria* spp. which have characteristically low levels of detectable lymphocyte and erythrocyte infections. Carrier status is known to persist for long periods, and for epidemiological purposes may play an important role in the transmission of *T.parva* to ticks and its maintenance in the field (Young et al., 1986; Kariuki et al., 1995; Skilton et al., 2002). In addition, identifying the specific *Theileria* species infecting the host is difficult using microscopy: schizonts and piroplasms of different *Theileria* species appear largely morphologically similar.

Ticks and tick-borne diseases

Results from RLB, which detects parasite DNA in samples, showed a high prevalence for *T.mutans* (69.1%) in samples collected at one year, and a much lower prevalence of *T.parva* (12.1%) for the same samples. Taken together with the ELISA results which indicated the animals had high exposures to both parasites (64.2% *T.mutans* and 72.3% *T.parva* seropositivity), this finding raises an interesting question as to the reason *T.parva* is only detectable in a fraction of the calves previously exposed. One possibility would be that RLB is relatively insensitive in picking *T.parva* infections compared to *T.mutans* infections. This however is unlikely and is not supported by results from other field epidemiological studies using RLBs in the investiga-

tion of tick-borne diseases in cattle. For example Salih et al. (2007), using the same oligonucleotide probes as used in this study, reported high prevalences (> 70%) of both parasites pointing to similar detection levels for both *T.parva* and *T.mutans*. Using RLB to study haemoparasite infections in cattle from Uganda, Oura et al. (2004) reported a marked difference in the carrier prevalence of *T.parva* in indigenous cattle (7%) and in that observed in cross-bred cattle (63%). Both cattle breeds had high *T.parva* exposure levels (98% seropositivity).

This result may suggest that indigenous cattle are able to keep levels of *T.parva* in blood low, an ability cross-bred cattle do not seem to possess. In our study, there is no evidence that the exposure to *T.parva* may have decreased with the age of calves. Over 90% of the calves examined at one year were found infested with the tick vector *R.appendiculatus*. Exposure levels would generally be expected to rise with increase in calf age especially after calves are weaned and start grazing out in the field together with adult cattle. *T.mutans* and *T.velifera*, both of which are transmitted by tick vector *A.variegatum*, were carried at higher prevalence at one year compared to *T.parva*, which may be an indication that the indigenous cattle exhibit varying degrees of resistance to specific *Theileria* spp. This finding is in agreement with the result of the same study by Oura et al. (2004) reporting a high prevalence of *T.mutans* and *T.velifera* in the indigenous cattle, higher than levels observed in cross-bred cattle with similar exposure levels.

The prevalence of *R.appendiculatus* over the study period did not show marked fluctuations over time that would be indicative of seasonal variation. Such variations are generally not expected in humid areas including the Lake Victoria basin, mainly because the conditions are favourable for the tick all year round. Seasonality is expected and more marked in highland areas experiencing bimodal rainfall patterns (Norval et al., 1992). Here the animals are infected with *R.appendiculatus* from very early on in life, and remain in continuous contact with the tick. Latif et al. (1991) reported evidence of tick resistance in zebu cattle. Compared to exotic (Friesian) cattle, they observed zebus had a significantly lower *R.appendiculatus* tick attachment

rates, lower proportion of attached ticks feeding to maturity, and lower survival rates for the female ticks. This increased tick resistance among zebu cattle may be one way in which they keep levels of *T.parva* infection low. The high exposure rate evidenced by results of serology tests, and the continuous contact with the *R.appendiculatus* throughout the year indicate the animals in this study population may be in a state of "endemic stability" to theileriosis (Norval et al., 1992). Endemic stability to ECF in Lake Victoria basin among indigenous zebu and Ankole cattle of Uganda has previously been reported (Kivaria et al., 2004; Moll et al., 1986; Deem et al., 1993).

Infection with *Babesia* spp. and *Anaplasma* spp., both at microscopy and using RLB, were low $< 5\%$. The exposure levels as indicated by ELISA tests were ($< 35\%$), and so was the prevalence of the tick (*Boophilus* spp.) which transmits the two pathogens. These findings point to low exposure levels to these two pathogens, unlike *T.parva* and *T.mutans*, with only a small proportion of animals infected by the age of one year. Additionally, there is a well known age-related resistance to *Babesia* and *Anaplasma* infections in cattle (Trueman and Blight, 1978; Kocan et al., 2003). Calves up to 9 months will rarely get clinical babesiosis or anaplasmosis, which is a period longer than that which maternal antibodies are expected to persist. The mechanisms by which the age-related resistance occurs are unclear, although several possible mechanisms have been postulated, including parasite growth inhibition by low molecular weight factors present in calf serum, and differences in humoral and cellular response between calves and adults, see Goff et al. (2002) and reviews by Zintl et al. (2005) and Brown et al. (2006). The relatively lower exposure levels and the age-related resistance to babesiosis and anaplasmosis may explain the observed low morbidity and mortality attributable to either disease in zebu calves. Latif et al. (1995) studying tick-borne diseases in zebu farms in Lake Victoria's Rusinga Island reported prevalence for *B.bigemina* (47%) and *A.marginale* (36%). This was however observed in only some of the study farms, and rarely in calves. Specific farm management practices and environmental conditions are known to be associated with observed prevalences (Gitau et al., 1997; Swai et al., 2005; Gachohi et al., 2010). Other studies in the country have reported higher

prevalences for these two diseases but are not directly comparable to this study either because they were conducted on adult cattle or on cross-bred and exotic breeds, see Deem et al. (1993) and Maloo et al. (2001).

Viral infections

Infectious bovine rhinotracheitis virus (IBRV) has previously been reported widespread in Kenyan cattle, with prevalences between different regions ranging from 24-65% (Jessett and Rampton, 1975). More recent work by McDermott et al. (1997) confirmed that IBRV infections to be common in Kenya and further highlighted the large variation between farms and regions in the transmission dynamics of the disease. Transmission of IBRV is either through respiratory or through reproductive route. The respiratory route would be expected to be more important than venereal transmission in these calves since they are not reproductively active by the time they reach one year. Here, low infection levels for IBRV (5.5%) were observed, which is in agreement with results of other studies of calves under 12 months showing lower prevalence compared to other cattle ages (McDermott et al., 1997). This observation is thought to be due to reduced contact with cattle from other herds, and a shorter exposure period in calves. Jessett and Rampton (1975) observed the highest incidence of IBRV to occur in animals above 2 years of age.

Bluetongue virus (BTV) and Epizootic Haemorrhagic Disease Virus (EHDV) are both members of the genus *Orbivirus* and transmitted by the vector *Culicoides* (Maclachlan, 2011; Savini et al., 2011). These modes of transmission would make infections with these two viruses highly prevalent in areas where conditions are conducive for their vector *Culicoides*. They occur as subclinical diseases in calves and their importance is mainly their association with reproductive disorders in adult cattle (Njiro et al., 2011). There are however arguments for their consideration when they occur as co-infections with other pathogens, due to possible immunosuppression effects and increase in susceptibility to other infections (Handel et al., 2011).

Helminths

Infections with *Haemonchus placei* were the most common nematode infections. Haemonchosis has been reported as the most prevalent nematode disease in zebu cattle around Lake Victoria (Latif et al., 1995), in zebu cattle under nomadic and pastoral systems in Tanzania (Keyyu et al., 2003), among dairy calves in Central parts of Kenya (Waruiru et al., 2000), and in N'Dama cattle in West Africa (Kaufmann and Pfister, 1990; Dwinger et al., 1994), highlighting its importance over wide geographical areas. The haemonchosis disease burden is due to the damage caused by Larval stage 3 (L3) lodging in the gastric glands, from where they moult into L4. The L4 and the adult worm attach in the abomasal mucosa and suck blood leading to anaemia and oedema. An added importance of *Haemonchus placei* and that of other hookworms such as *Oesophagostomum radiatum and Bunostomum trigonocephalum* found present in this study, is the increased pathology while occurring as co-infections with other parasites. Examples include studies in cattle and in humans which have shown that co-infections with hookworms and haemoparasite pathogens associated with destruction of blood cells result in significantly worse anaemia than that attributable to single infections with either pathogen (Dwinger et al., 1994; Brooker et al., 1999).

The distribution and prevalence of helminth species is influenced by a range of factors including host factors such as age and immunity, farm management factors such as antihelmintic use, herd sizes and grazing patterns, environmental conditions as humidity, vegetation cover and rainfall, and parasite factors as their virulence, transmission modes and life-cycles (Hansen and Perry, 1994).

The observed high prevalence of the ascarid *Toxocara vitulorum* soon after birth and the decrease in prevalence with increasing calf age is related to the mode of transmission (infective larvae of *T.vitulorum* are passed from dam to calves through milk) and acquired immunity against *T.vitulorum* following initial infection. Infective larvae of *Strongyloides* spp. can be transmitted in colostrum, explaining the observed pattern of relatively higher prevalence occurring in very young calves compared to older ages. *Dictyocaulus viviparus*,

the cattle lung worm, is spread through ingestion of infective larvae from the pastures. It is not clear why its prevalence is high soon after birth as exposure is expected to be high when calves have been released to pastures, unless if there is a possibility of trans-placental transmission.

The increase in the prevalence of liver flukes (*Fasciola hepatica*) in the period after 6 months of age is likely related to calves accessing infected pastures in marshy areas post-weaning. Flukes require snails as an intermediate host to complete their lifecycle, which may explain why the highest prevalences were observed in Magombe East sublocation which has rice pads and is prone to floods, and Luanda sublocation which neighbours large swampy areas. These provide suitable habitats for the intermediate hosts. Rumen flukes (*Calicophoron* spp.) were more common than *Fasciola hepatica*, and were found in most of the study sublocations. These also require snails as intermediate hosts, and outbreaks in cattle are also known to occur during dry seasons when cattle and snails concentrate around common water sources (Hansen and Perry, 1994).

Different helminth species have different egg-production capabilities. Due to these differences, the strongyle epg count is not always an accurate measure of the burden and damage caused by adult worms present in the gasto-intestinal tract. Additionally, the epg count may be influenced by the level of host immunity, age of host, consistency of faeces, number of adult worms in the gastro-intestinal tract, and the stage of infection in the host (Hansen and Perry, 1994). Despite these limitations, the strongyle epg count remains the easiest and most practical measure of worm burden in live animals. It has been shown to be associated with host performance and fitness (Dwinger et al., 1994; Craig et al., 2008). Results from this study show varying degrees of worm burden, from mild infections to very heavy infections with measures over 10,000 epg of faeces. Such differences in worm burden would be expected to have varying effects on various host outcomes including growth rates and survival.

At any single observation time, calves in this study were infected with about 4 different pathogens. This number would be higher if results from extra tests

such as virus ELISA tests, PCR and RLB tests carried out on samples at one year were to be included. This is good evidence that multiparasitism is common and should not be ignored in epidemiological studies. We have also established parasites known to be highly pathogenic such as *T.parva* and *Haemonchus placei* are highly prevalent in these calves. These data will be used in the subsequent chapters as explanatory variables in determining the effect of each pathogen and its associated combinations on two host outcomes: survival probability and growth rates of the study animals during their first year of life. Subsequently, the risk factors for the pathogens found to be significantly associated with these host outcomes will be determined.

Chapter 3

Mortality in zebu cattle under one year: predictors of infectious-disease mortality

3.1 Introduction

Calf mortality is a significant source of economic losses in the livestock industry. In the smallholder livestock production systems, the survival of female calves is required for herd expansion and breed improvement, while that of male calves is used as a source of income from sales or as draught animals (Gitau et al., 1994). Additional losses are incurred due to waste of investments made on feed and treatment, and reduced saleable milk production in zebu cows which are known to require a suckling calf for effective stimulation of the milk let-down physiological reflex (Coulibaly and Nialibouly, 1998; Sidibé-Anago et al., 2008). Interventions aimed at reducing calf mortality have large benefits on farming enterprises but require data on important causes of mortality and risk factors for each farming system.

Several studies in East Africa have pointed to multifactorial causes of calf mortality within smallholder systems, mainly related to maternal factors including genetics and mothering abilities, farm management practices, and to infectious agents (Gitau et al., 1994; Muraguri et al., 2005; Wymann et al., 2006; Swai et al., 2009). A systematic literature review on causes of morbidity and mortality among smallholder dairy farms in Eastern and South Africa identified tick-borne diseases, diarrhoea and trypanosomiasis as the most

commonly documented causes of mortality (Phiri et al., 2010).

Although these studies have generated useful data on risk factors and mortality rates, most have been cross-sectional and not useful in establishing the sequence of events. Further, the few longitudinal studies conducted have largely focussed on single-pathogen infection systems even in populations known to be commonly coinfected. It is increasingly evident that coinfections, including the numbers and virulence of infecting pathogens, order of infection, infection doses, and interactions between coinfecting pathogens influence the epidemiology of these pathogens, host susceptibility and impacts on infected hosts (Abu-Raddad et al., 2006; Alizon, 2008; Telfer et al., 2010; Claridge et al., 2012).

Among indigenous zebu cattle production system, epidemiological data that can be used to rank risk factors and different infections in order of importance are largely lacking. Better knowledge of impacts of pathogens on survival probabilities of such hosts could potentially improve the design of disease control strategies, and ultimately their effectiveness.

This study aims at identifying and ranking in order of importance, the risk factors and the main aetiological causes of infectious disease mortality in zebu cattle under one year. Specifically, the study a) estimates mortality rates and periods of increased risk for mortality in zebu cattle up to one year of age, b) identifies factors at birth that predict survival of calves and which would be a target for programs aimed at reducing calf mortality, c) infectious and non-infectious risk factors associated with calf mortality, and d) definitive aetiological causes of mortality through a review of post-mortem examination data and results.

3.2 Materials and Methods

3.2.1 Study population

This study followed 548 indigenous zebu calves from 20 randomly selected sub-locations in 4 agro-ecological zones in Western Kenya. The study, con-

ducted between October 2007 and September 2010, focused on smallholder farms in a mixed crop-livestock production system. An average farm is 2 hectares or less in size, grows food crops and keeps approximately 5 cattle. Calves were recruited into the study within a week of birth, and each was then followed throughout its first year of life. Routine monitoring of study calves was done at 5 week intervals, from recruitment time until the calf was one year old or until leaving the study. Detailed description of the study design and protocol are provided in Appendix A.

3.2.2 Data collection

At recruitment and during each of the 5 week routine visits, a complete clinical examination of the study calf was conducted. Blood smears, whole blood and serum samples, faecal samples and other clinically relevant samples were collected for laboratory diagnosis of pathogens and measurement of clinical parameters as total serum proteins and packed cell volume. Live body weight measures (in kgs) and girth measurements (in cms) of study calves were recorded at recruitment, and during each 5 week visit. During each visit, pre-tested questionnaires capturing data on farm characteristics, management practices, herd health, veterinary interventions in the rest of the herd, and cattle entries and exits were administered by 5 well trained animal health assistants. Data on the dam of each study calf including its general body health, udder health, girth measurements and body condition score were also recorded, at each calf visit. This was done until the study calf was weaned off or until leaving the study at one year.

3.2.3 Outcome variable

In this study, the outcome measure of interest was *infectious disease mortality* (ID-mortality): defined as any death of a study calf occurring during the one year observation time and attributed to an infectious disease cause.

3.2.4 Post-mortem analysis

To determine the specific aetiological cause of death for each case, a complete post-mortem (pm) examination was conducted on calves that died or were euthanised during the study time. Standard body system by body system veterinary autopsy routines were followed (King et al., 2006). Blood, lymph node and brain smear samples were collected for parasitological examination for bacterial, rickettsial and protozoan parasites. Bacteriological and helminth analysis were also carried out on faecal samples collected at pm. Tissue samples from the intestines, lung, liver, spleen, kidney, and heart were collected and fixed in 10% formalin for histopathology examination. Where necessary and dependent on suspected aetiologic causes, additional samples were collected and submitted to the Department of Veterinary Tropical Diseases, University of Pretoria, alongside the histopathology samples for further analysis. Results from the laboratory tests, gross and histopathology examinations for each post-mortem case were reviewed by a team of 7 veterinarians and a diagnosis of the main aetiological cause of death for each case determined.

3.2.5 Risk factors for mortality

These were divided into two main groups: a) non-infectious factors comprising of variables related to the farmer, farm management practices, maternal factors, environmental effects and calf factors, and b) infectious factors being the protozoan, helminth and fungal infections identified from samples collected during the recruitment and 5 week routine visits. Bacterial and viral infections were left out of this analysis because their screening was only done in samples collected during clinical episodes and in the last visit at one year. Table 3.1 presents a list of the infectious and non-infectious risk factors for calf-mortality tested in the current study. Detailed descriptions of the data collection and laboratory analysis and pathogens tested for are provided in Appendix A.

Table 3.1: Covariates tested for their relationship with the infectious disease mortality.

Group	Factors
Farm factors	Farmer's age, gender, education level, main occupation, herd size, land size
Management factors	Tick control, worm control, trypanosome control, vaccine use, grazing practices, watering practices, housing
Maternal status	Heart girth measurement, body condition score, suckling, health condition, dam antibody titres against *Theileria parva*, *Theileria mutans*, *Anaplasma marginale*, *Babesia bigemina*
Environmental variables	Normalised difference vegetation index (NDVI), farm altitude (elevation)
Calf factors	Calf sex, birth weight, heterozygosity, European introgression, clinical episodes, total serum protein, packed cell volume, white blood cell counts
Infectious factors	Protozoan: *Theileria parva*, *Theileria mutans*, *Anaplasma marginale*, *Babesia bigemina*, *Trypanosoma* spp., *Coccidia* spp. Helminths: *Calicophoron* spp., *Cooperia* spp., *Dictyocaulus viviparous*, *Fasciola* spp., *Haemonchus placei.*, *Moniezia* spp., *Microfilaria* spp., *Nematodirus* spp., *Oesophagostomum radiatum*, *Toxocara vitulorum*, *Trichostrongylus axei*, *Trichuris* spp., *Strongyloides* eggs, Strongyle eggs Fungi: *Trichophyton* spp.

3.2.6 Statistical analysis

Survival time for each calf was defined as the age at which the calf died due to infectious causes. Animals that died for reasons other than infectious causes, or that were lost or removed from the study before one year for non-compliance were censored. These animals effectively contributed *"at-risk"* time only up to the censoring point. All survivors to one year were censored at the time of leaving the study.

Kaplan-Meier estimates of the survival function were used to determine the overall mortality rates (Kaplan and Meier, 1958). These estimates are a measure of the probability of a calf surviving up to a time t, provided by the product of the probabilities of survival at each risk interval prior time t. The survival probability $S(t)$ at any particular time is given by Equation 3.1.

$$S(t) = \frac{r(t_j) - d(t_j)}{r(t_j)} \tag{3.1}$$

Where (t_j) represents a time interval, $r(t_j)$ is the number at risk at the start of time (t_j), $d(t_j)$ is the number of events (deaths) occurring during (t_j).

To investigate the pattern of risk over the first year of life, instantaneous hazards of failure based on the Kaplan-Meier survival function were used. This measure gives the proportion of the population dying per unit time (Equation 3.2) and describes the instantaneous probability of an event occurring at a point in time given that it did not occur previously (Dohoo et al., 2009, page 482).

$$h(t) = \lim_{\Delta \to 0} \frac{P(t \leq T < t + \Delta t | \geq t)}{\Delta t} \tag{3.2}$$

h(t) is the hazard function defined as the probability (P) that a calf dies in a small interval of time Δt, given that it survived up to the beginning of the small interval Δt, when the size of the time interval approaches zero $\lim_{\Delta \to 0}$. T is the calf's survival time.

An **R** function *epi.instanthaz* in the *epiR* package of **R** (R Development Core Team, 2011) was used to calculate the instantaneous hazard. Kernel-smoother lines, that estimate average values by aggregating neighbouring point estimates, were added to the instantaneous hazard plots to aid in visualisation of changing risk estimates and reveal the underlying shape of the hazard function.

To overcome limitations associated with standard regression analysis for estimating effects of factors associated with mortality, Cox regression models are favoured. These models utilise information from both censored and non-censored observations (Cox, 1972), and have been extended to incorporate frailty terms and time-varying predictors (Therneau and Grambsch, 2000), making it possible to study effects of, for example, infections which are absent at the start of a study and occurring at some point during the study observation time.

To determine the effect of infectious and non-infectious factors on calf mortality, Cox proportional hazard models were used. A frailty term for sublocation was included in the models to adjust for clustering within sublocations. The model used for the analysis is described in Equation 3.3.

$$h_i(t) = h_0(t)e^{\beta X + \epsilon_i} \tag{3.3}$$

It expresses the *hazard* at time t (i.e the probability of calf death at time t) as a function of:

- *Baseline hazard* - $h_0(t)$: which is the value of the hazard when all predictors are 0 or absent. It is an unspecified baseline hazard rate describing the common shape of survival time distribution for all calves.

- *Linear combination of predictors* - βX: an exponential function of a series of explanatory variables $\beta X = \beta_1 X_1 + \beta_2 X_2 + ... + \beta_k X_k$. Their parameters represent the shift in the *log hazard* associated with a unit difference in the corresponding predictor.

- *Error term* - ϵ_i : a random effect accounting for the correlated mea-

surements of animals within the same sublocation (shared frailty for the i^{th} sublocation.)

The hazard rate (HR) was used to determine the effect of covariates, by comparing HRs of groups of calves with different covariate combinations. A covariate with a significant HR < 1 was interpreted as having a protective effect against mortality, and a covariate with HR > 1, as increasing the risk of mortality. For continuous variables, the *hazard ratio* represents a shift in the hazard that is due to a unit change in the predictor whereas for binary predictors, it represents the effect of the factor being present compared to its absence in calves.

To accommodate time-varying predictors in the Cox models, the data were structured such that each calf's observation time consisted of a series of *(start, stop]* "intervals of risk" corresponding to the routine monitoring visits. A calf recruited at 4 days old and with routine visits at age 39, 74, 109 days will have intervals of risk of *(4,39], (39,74]* and *(74,109]*. For each interval, the event of interest (death) is recorded as occurring within this period of risk or not. The survival probability within the intervals of risk is associated with values of covariates as measured at the visit corresponding to *start* time of each interval. The closed bracket on the right is used to indicate that in case of an overlap, eg. event occurring at day 74, the risk computations will involve the former interval and not the later (Therneau and Grambsch, 2000).

Each potential risk factor in Table 3.1 was initially tested in the model as a univariable analysis. Factors with a p-value ≤ 0.2 were then included in the multivariable analysis. Backward selection procedure was carried out until only covariates significant at p-value < 0.05 remained in the model, referred to as the minimum adequate model (MAM). Predictors dropped during backward selection were added back to MAM each at a time to determine if any significantly improved the model fit. Best fit of the models was done by graphical procedures through examination of residual plots. The analysis in this study was carried out using statistical software **R** (R Development Core Team, 2011) and R package **survival** (Therneau, 2012).

3.3 Results

3.3.1 All-cause and infectious-disease (ID) mortality

The 548 animals recruited and followed in the study contributed a total of 175,732 calf days (equivalent to 25,104 calf weeks or 481.1 calf years) of observation. A total of 88 calf deaths were observed. The all-cause mortality rate was estimated at 16.1 CI (13.0-19.2) per 100 calf years at risk, represented by the Kaplan-Meier curves in Figure 3.1. Based on the history before death, 5 of the 88 deaths were considered to be non-infectious and censored in the analysis of infectious disease related deaths (ID-mortality). The non-infectious causes of death included trauma, plant poisoning and starvation. The remaining 83 calves were treated as having died from infectious diseases. The estimated ID-mortality rate was 15.3 CI (12.2-18.3) per 100 calf years at risk.

To determine the temporal risk pattern for ID-mortality during the first year of life, estimates of the instantaneous risk were calculated using the formula shown in Equation 3.2. The results were plotted and Kernel-smoothing lines added to aid visualization and identification of periods of increased risk for ID-mortality. Three periods of increased risk for calf mortality were identified: a) neonatal period, b) period between 4 and 6 months, and c) period towards the last quarter of the year, see Figure 3.2.

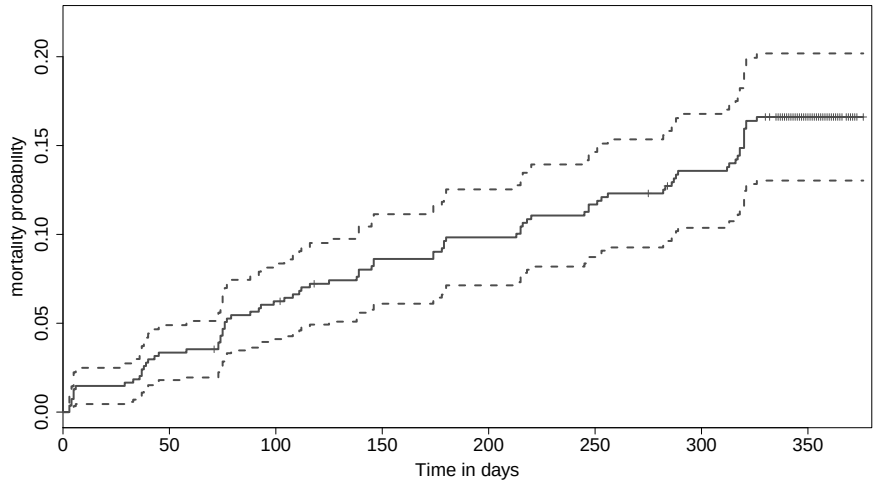


Figure 3.1: Kaplan-Meier cumulative risk curve for calf mortality during the first year of life. The cumulative probability of mortality at one year was estimated at 0.161 CI [0.130 - 0.192].

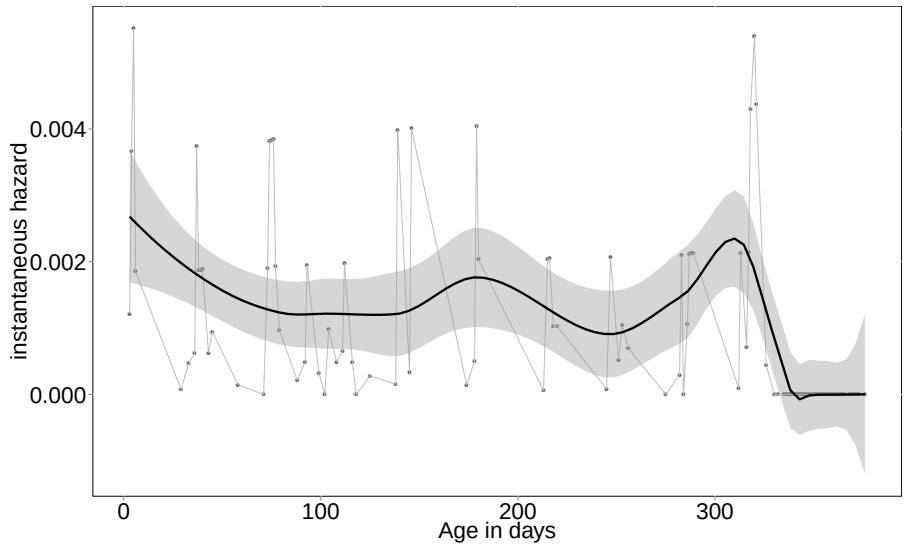


Figure 3.2: Instantaneous hazard estimates with kernel-smoothing for calves under one year. The plot shows three periods of increased risk for mortality: period immediately after birth (neonatal period), period between 4 and 6 months (corresponding to expected time for waning maternal immunity), and period towards one year of age (corresponding to age of weaning).

3.3.2 Spatial pattern in mortality

To explore the spatial patterns in mortality, mortality rates for each study sublocation were determined and plotted as choropleth maps, Figure 3.3. The rates between sublocations ranged from as low as 3.6 to as high as 38.5 per 100 calf years, Table 3.2. The log-rank test for differences in Kaplan-Meier curves of the study sublocations was statistically significant (p-value = 0.01). Mortality rates were higher in the sublocations falling within the southern region of the study area compared to those in the northern region, except for the north most sub-location (East Siboti) which recorded the second highest mortality. Figure 3.4 shows Kaplan-Meier curves for a selected 5 of 20 study sub-locations to demonstrate temporal differences in mortality between sublocations. Results showed for example calves in Bumala A and Magombe East (lying in the South) died at a relatively young age ($<$ 150 days, $<$ 220 days respectively), compared to those in East Siboti (in the North) where death occurred at a relatively older age. This pattern may be related to the aetiological cause of death, with most deaths in Bumala A and Magombe East attributed to East Coast Fever, whereas those in East Siboti were mainly due to haemonchosis. The median survival time for ECF deaths was 93 days CI [73 - 146], while that for deaths due haemonchosis was 271 days CI [125 - NA ($>$ data range)].

Table 3.2: Results of Kaplan-Meier analysis showing the survival probabilities and their 95% confidence intervals for calves by study sublocation.

Sub-location	no. at start	no. of deaths	survival	std.err	lower CI	upper CI
Bujwanga	28	6	0.783	0.0786	0.643	0.953
Bukati	28	1	0.964	0.0351	0.898	1.000
Bulwani	28	5	0.821	0.0724	0.691	0.976
Bumala A	22	6	0.727	0.0950	0.563	0.939
East Siboti	28	8	0.714	0.0854	0.565	0.903
Igero	28	3	0.893	0.0585	0.785	1.000
Ikonzo	28	4	0.857	0.0661	0.737	0.997
Kamunuoit	27	2	0.926	0.0504	0.832	1.000
Karisa	28	3	0.891	0.0592	0.783	1.000
Kidera	28	3	0.891	0.0592	0.783	1.000
Kodiere	27	4	0.852	0.0684	0.728	0.997
Kokare	28	4	0.857	0.0661	0.737	0.997
Luanda	28	2	0.929	0.0487	0.838	1.000
Mabusi	28	2	0.929	0.0487	0.838	1.000
Magombe East	26	10	0.615	0.0954	0.454	0.834
Namboboto	27	3	0.889	0.0605	0.778	1.000
Ojwando B	28	7	0.750	0.0818	0.606	0.929
Otimong	28	6	0.786	0.0775	0.648	0.953
Simur East	27	1	0.963	0.0363	0.894	1.000
Yiro West	28	6	0.780	0.0794	0.639	0.952

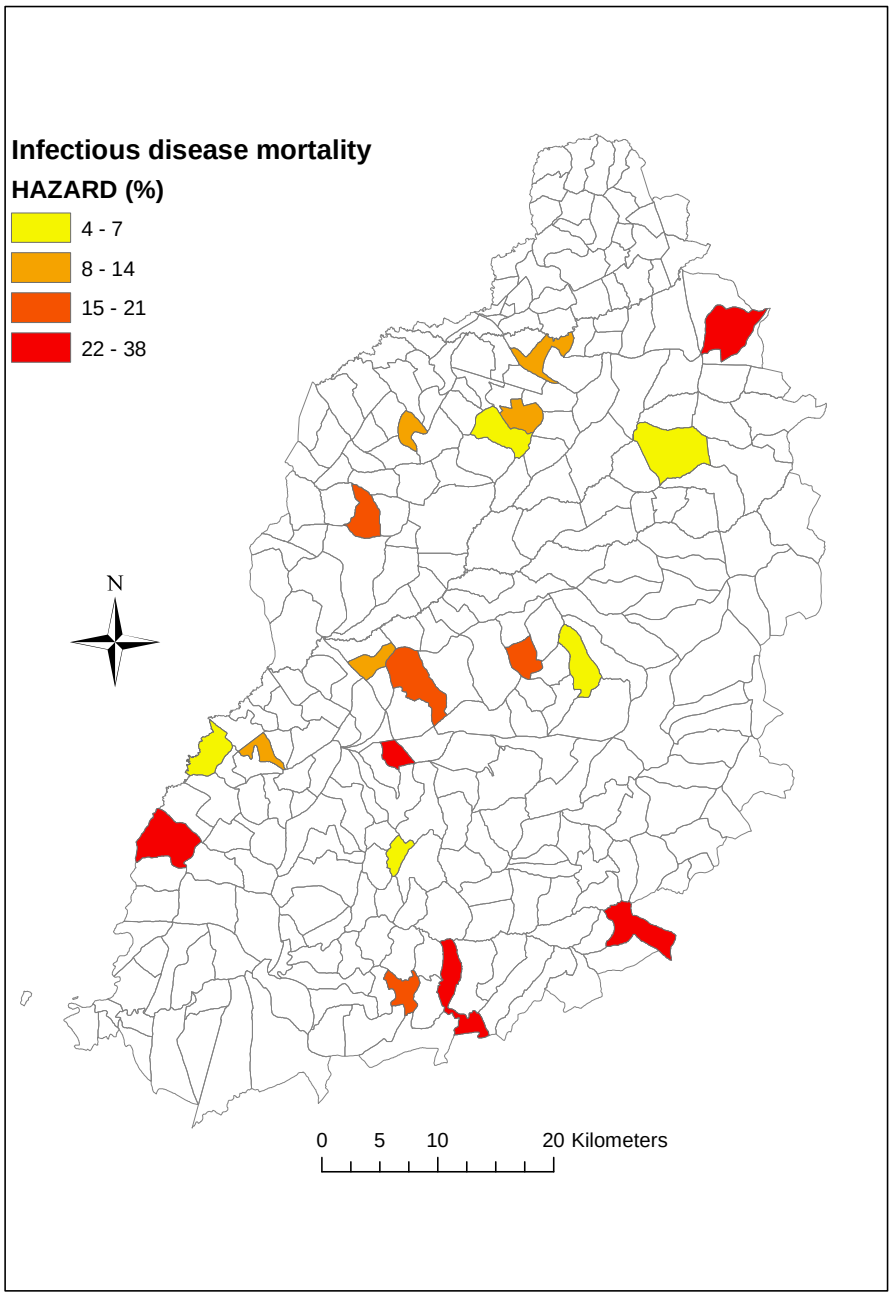


Figure 3.3: Choropleth map showing mortality rates by study sublocation. Higher mortality rates observed in sublocations in the South, and lower rates in sublocations towards North. The variable "Northing" is marginally statistically associated with calf mortality (p-value $= 0.078$). The north most sublocation (East Siboti) has a high mortality and masks the observed association between mortality and northing (p-value $= 0.007$, when East Siboti is omitted).

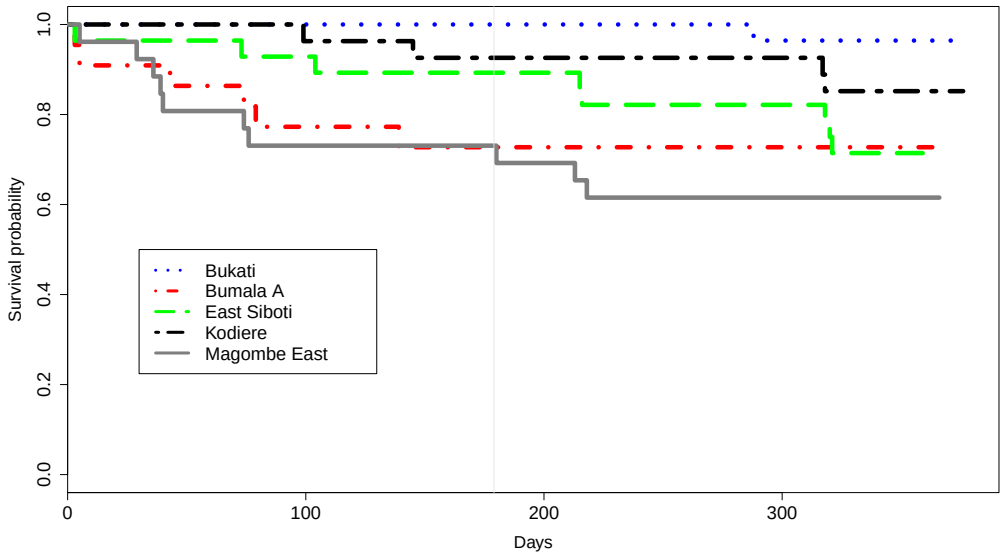


Figure 3.4: Kaplan-Meier curves showing survival probabilities over the one year observation time for a selected 5 of 20 study sublocations demonstrating differences in age-related mortality patterns between sublocations.

3.3.3 Risk factors for mortality

To investigate the risk factors associated with calf mortality, the analysis was carried out to determine the following:

- Risk factors for ID-mortality using data collected at recruitment time (predictors at birth).
- Non-infectious risk factors for mortality over time (included time-varying and time-invariant factors.)
- Infectious risk factors for calf mortality (time-varying predictors).

3.3.3.1 Predictors at birth

Putative factors collected at recruitment time were initially tested as univariable analysis, results shown in Appendix Table C.1. The results of the multivariable analysis with sublocation included as a random effect are presented in Table 3.3. Three variables; watering at homestead which represents farms in which drinking water is provided at the homestead rather than animals being driven a distance away from the homestead, and antibody titres against *T.parva* and *B.bigemina* in the dams were statistically associated with ID-mortality. Watering at homestead was associated with a 50% CI[31, 80] decrease in the hazard for ID-mortality. After controlling for the two other covariates significant at recruitment, the expected survival curve for a calf in a farm providing water at the homestead and for a calf not accessing water at the homestead are provided in Figure 3.5. High antibody titres against *T.parva* and *B.bigemina* in the dams at recruitment were associated with increased ID-mortality hazard by a factor of 1.13 CI[1.04, 1.23] and 1.11 CI[1.03, 1.20] times for every 10 unit increase above the mean for *T.parva* and *B.bigemina* titres respectively.

The standard deviation of the random effect is interpreted as: one standard deviation above the mean corresponds to a relative risk $\exp(0.3309) = 1.40$, a 40% higher risk of death for calves in that sub-location (Therneau, 2011).

Table 3.3: Predictors calf mortality at calf recruitment time.

	coef	exp(coef)	se(coef)	z	p
Fixed effects					
Watering at homestead	-0.69	0.50	0.24	-2.90	0.003
(*T.parva* antibodies/10) - dam	0.13	1.13	0.04	2.86	0.004
(*B.bigemina* antibodies/10) - dam	0.10	1.11	0.04	2.68	0.007
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.3309	0.1096		

Dam antibody titres against *T.parva* and *B.bigemina* have been centered around their means, and divided by 10 to aid interpretation.

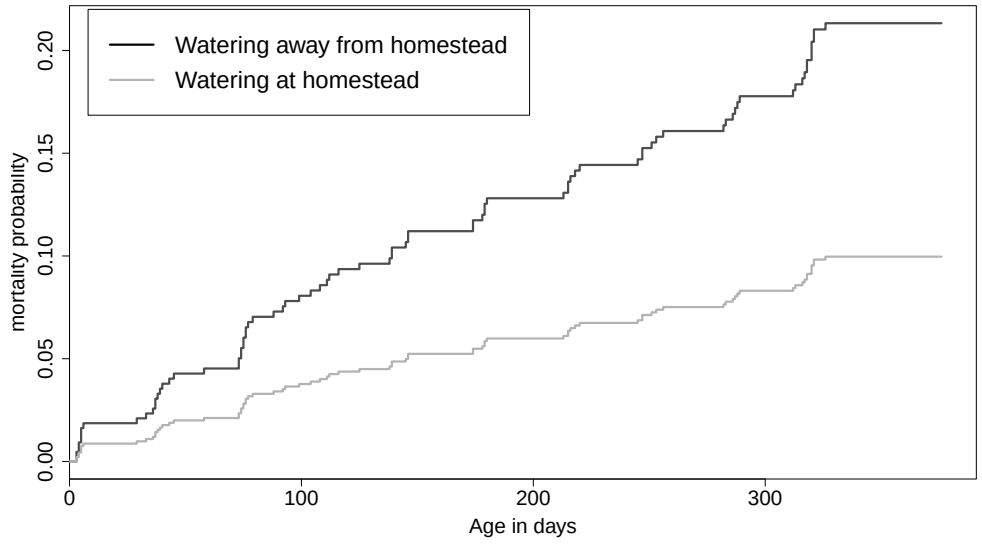


Figure 3.5: Expected mortality curves for calves in farms providing water for the animals at the homestead and those driving animals away from the homestead for water. Providing water at the homestead was associated with decreased hazard for calf mortality by 50% CI[31, 80]. The model controls for other significant covariates in the recruitment model.

3.3.3.2 Non-infectious predictors

The results of the univariable analysis of time-invariant and time-varying predictors are presented in the Appendix Table C.2. Results from multivariable analyses showed two husbandry practices; watering at the homestead and tick control were associated with decreased risk for ID-death. Watering at homestead, which was a significant predictor of ID-mortality at birth, was associated with a decreased risk for ID-mortality by 50% CI [13.2, 71.3] compared to risk in farms where animals were taken away from the homestead to access water. Controlling for ticks was associated with an 80% [65.5, 88.4] decrease in risk for ID-mortality compared to risk in farms that did not control for ticks. An increase of 10 cms in the heart girth size of the dam above the mean was associated with a decrease in the hazard for mortality by 34% CI [5.2, 53.9]. High NDVI values were associated with decreased risk for ID-mortality estimated at 54% CI [16.5, 75.1] decrease in risk for a 10 times difference in mean NDVI values. High antibody titres against *B.bigemina* in the dams were associated with increased hazard for mortality by a factor of 1.12 for every 10 unit increase in the titre. These results are provided in Table 3.4.

In addition, although packed cell volume (PCV), white blood cell counts (WBC), total serum proteins (TSP) and clinical episodes (CE) were all significantly associated with calf mortality, these were not considered risk factors in themselves but as measures of health related to mortality. High values of TSP, PCV and WBC were associated with decreased hazard, whereas the hazard of mortality for calves reported with a clinical episode was increased, see Appendix Table C.3. Their values are likely related to infections but because these were not the subject of investigation in the current study, they were excluded in the multivariable analysis.

Table 3.4: Non-infectious risk factors associated with infectious disease mortality.

Variable	coef	exp(coef)	se(coef)	z	p
Fixed effects					
Watering at homestead	-0.69	0.50	0.28	-2.46	0.014
Tick control - Yes	-1.61	0.20	0.28	-5.77	< 0.001
(Heart girth size*10) - dam	-0.41	0.66	0.18	-2.25	0.024
(Mean NDVI * 10)	-0.78	0.46	0.31	-2.54	0.011
(*B.bigemina* antibodies*10) - dam	0.12	1.12	0.05	2.40	0.016
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.6114	0.3738		

The variables Mean NDVI, heart girth size, and dam antibody titres against *B.bigemina* were centered around their means to aid interpretation of the coefficients. Mean NDVI and heart girth size were also multiplied by a factor of 10 to aid interpretation.
Random effect - one standard deviation above the mean corresponds to a risk ID-mortality that is exp(0.6114) = 1.84 times higher in that sublocation.

3.3.3.3 Infectious risk factors

All pathogens recorded in Table 3.1 were initially evaluated as univariable analysis, results are shown in Appendix Table C.4. Variables with a p-value ≤ 0.2 were offered to the multivariable model and model simplification was done by sequentially removing least significant variables until only variables significant at p-value < 0.05 remained in the model. The minimum adequate model showed that infection with *Trypanosoma* spp., high infection intensity with *Theileria* spp., and high worm burdens were associated with increased risk for ID-mortality. In addition, *T.parva* seropositivity was associated with decreased risk for ID-mortality. The model results are presented in Table 3.5. The effect sizes are shown as the exponentiated coefficients (column 3). Pathogens identified in clinical episode cases only were not included in the multivariable models as these were not routinely tested for.

Table 3.5: Results of minimum adequate model showing the pathogens identified as significantly associated with ID-mortality.

	coef	exp(coef)	se(coef)	z	p
Fixed effects					
Theileria spp. level 1	-0.49	0.61	0.34	-1.43	0.153
Theileria spp. level 2	0.59	1.81	0.58	1.03	0.303
Theileria spp. level 3	3.64	38.07	0.79	4.56	< 0.001
T.parva - seropositivity	-0.80	0.45	0.36	-2.25	0.024
Trypanosoma spp.	1.73	5.61	0.73	2.35	0.019
(Strongyle epg/1000)	0.36	1.44	0.04	8.78	< 0.001
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.0199	0.0004		

Level 1 - One infected cell in more than 10 microscopic fields (low intensity infection).
Level 2 - One or more infected cells for every 10 fields (medium intensity infection).
Level 3 - Multiple infected cells in every microscopic field (high intensity infection).
Random effect - one standard deviation above the mean corresponds to a risk ID-mortality that is exp(0.0199) = 1.02 times higher in that sublocation.

3.3.3.4 Final model: Predictors of ID-mortality

The final Cox regression mixed model included all infectious and non-infectious factors significantly associated with ID-calf mortality, Table 3.6. While holding other covariates constant, high intensity *Theileria* spp. infections (i.e level 3 infections - defined as ≥ 2 infected cells per microscopy field, in multiple fields), infection with *Trypanosoma* spp., and an increase by 1000 for strongyle epg increased the hazard for ID-mortality by a factor of 32 (CI [6, 162]), 6 (CI [1.4, 25]) and 1.4 (CI [1.3, 1.6]) times respectively. The model identified *Theileria* spp. infections, the most lethal of which causes East Coast Fever disease, infection with trypanosomes, and helminth infections as measured by strongyle epg as the three important infections with statistically significant association with ID-mortality in calves. *T.parva* seropositivity was associated with a protective effect, with calves that had seroconverted having a reduced risk of ID-mortality by 63% (CI [24, 82]) compared to animals that did not seroconvert.

Controlling for ticks in the farm and providing drinking water to the animals at the farm were both associated with a protective effect against ID-mortality. Tick control was associated with a 49% (CI [4, 73]) lower mortality hazard compared to farms that did not control for ticks. Watering at homestead reduced the risk of ID-mortality by 61% (CI [26, 80]) compared to farms where watering was not done at the homestead.

The diagnostic plots of scaled Schoenfeld residuals showed that the assumption of proportional hazard was supported by all the variables within the model, except for the variable "watering at homestead" whose effect decreased with age of calf, see Figure 3.6. The results of the formal test for proportional hazard assumption are provided in Appendix Table C.5. The non-proportional hazard for this factor (watering at homestead) was accommodated in the model by stratifying the data based on the two levels for this variable. This assumes each stratum has a different baseline hazard function, while the other covariates are assumed to be constant across strata. The results of this model showed no evidence of non-proportionality in any of the remaining covariates, see Table 3.7. The predictors in the final model (Table

3.6) remained significant even when "watering at homestead" was used as a stratifying variable with little differences in the estimated size of effects of these predictors, see Appendix Table C.6.

A summary schematic diagram showing the predictors, their effect sizes and direction on the outcome ID-mortality is provided in Figure 3.7.

Table 3.6: Results of the minimum adequate survival model with significant variables associated with calf mortality.

Variable	coef	exp(coef)	se(coef)	z	p
Fixed effects					
Tick control	-0.67	0.51	0.32	-2.08	0.038
Watering at homestead	-0.95	0.39	0.33	-2.88	0.004
T.parva - seropositivity	-0.99	0.37	0.37	-2.70	0.007
Theileria spp. level 1	-0.47	0.62	0.35	-1.37	0.172
Theileria spp. level 2	0.70	2.02	0.58	1.21	0.227
Theileria spp. level 3	3.47	32.23	0.82	4.22	< 0.001
Trypanosoma spp.	1.78	5.91	0.74	2.40	0.017
(Strongyle epg /1000)	0.36	1.43	0.04	8.47	< 0.001
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.0199	0.0004		

Level 1 - One infected cell in more than 10 microscopic fields (low intensity infection).
Level 2 - One or more infected cells for every 10 fields (medium intensity infection).
Level 3 - Multiple infected cells in every microscopic field (high intensity infection).
Random effect - one standard deviation above the mean corresponds to a risk ID-mortality that is exp(0.0199) = 1.02 times higher in that sublocation.

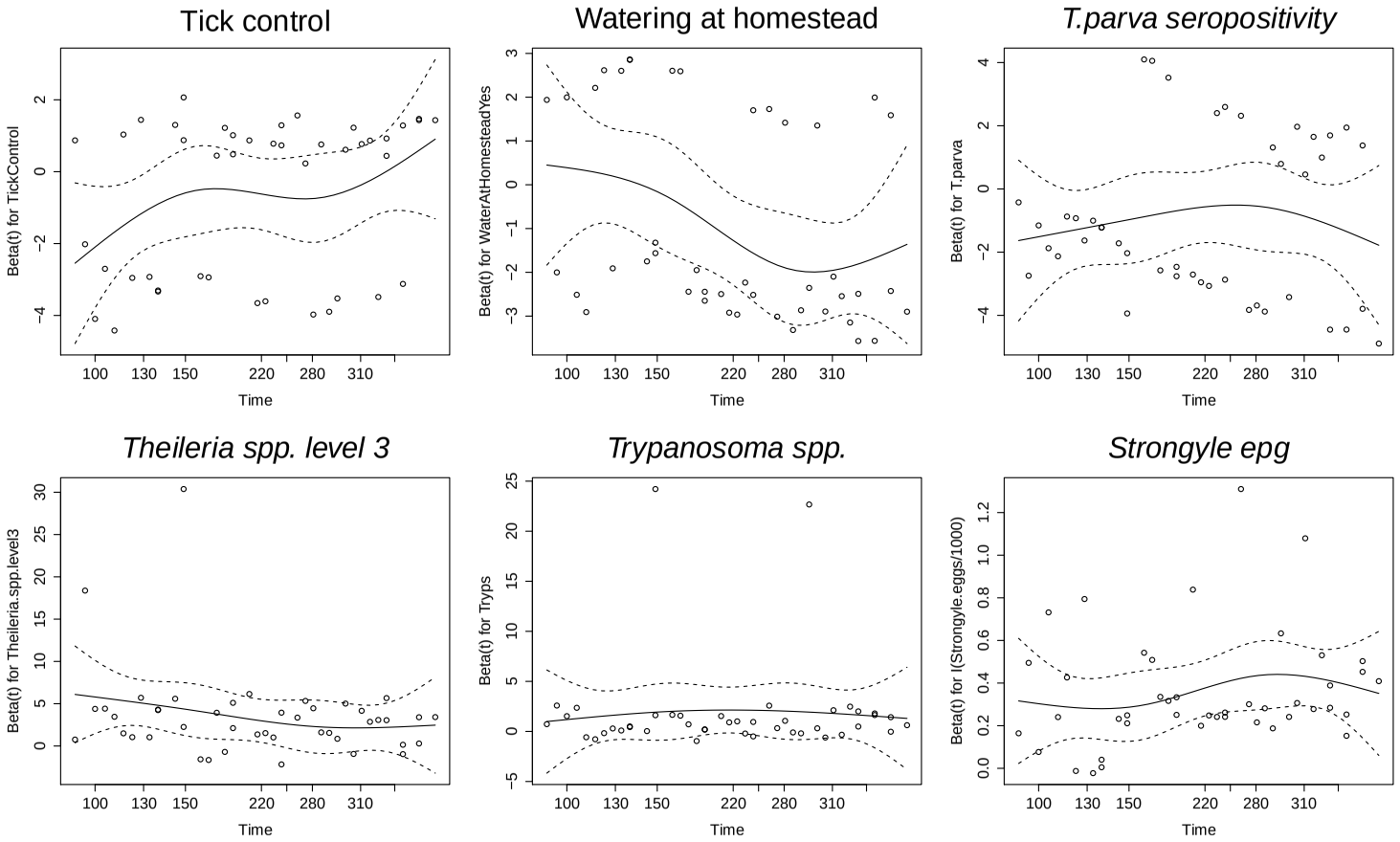


Figure 3.6: Scaled Schoenfeld residuals plotted against transformed time for each of the significant predictors for ID-mortality. The broken lines represent ± 2 standard error around the fit, and the solid line is a smoothing line added to facilitate interpretation. Systematic departures from the horizontal solid line indicate non-proportional hazards. Proportional hazards assumption appears supported by variables *T.parva* seropositivity, *Theileria* spp. level 3, *Trypanosoma spp* and strongyle epg. The effect of "watering at homestead" appears to be decreasing with age. Note variable scales on y axes.

Table 3.7: Results of test for the proportional hazard assumption of Cox regression using covariates identified as significant predictors of ID-mortality. The variable "watering at homestead" has been used to fit a different baseline hazard for each of its levels. The global test statistics shows no evidence of non-proportionality in this model (Therneau and Grambsch, 2000).

	rho	chisq	p
Tick control	0.249	3.007	0.083
T.parva	0.021	0.020	0.887
Theileria spp. level 1	-0.092	0.426	0.514
Theileria spp. level 2	0.199	1.601	0.206
Theileria spp. level 3	-0.221	1.905	0.168
Trypanosoma spp.	-0.009	0.004	0.952
Strongyle.eggs/1000	0.131	0.621	0.431
GLOBAL	NA	9.344	0.229

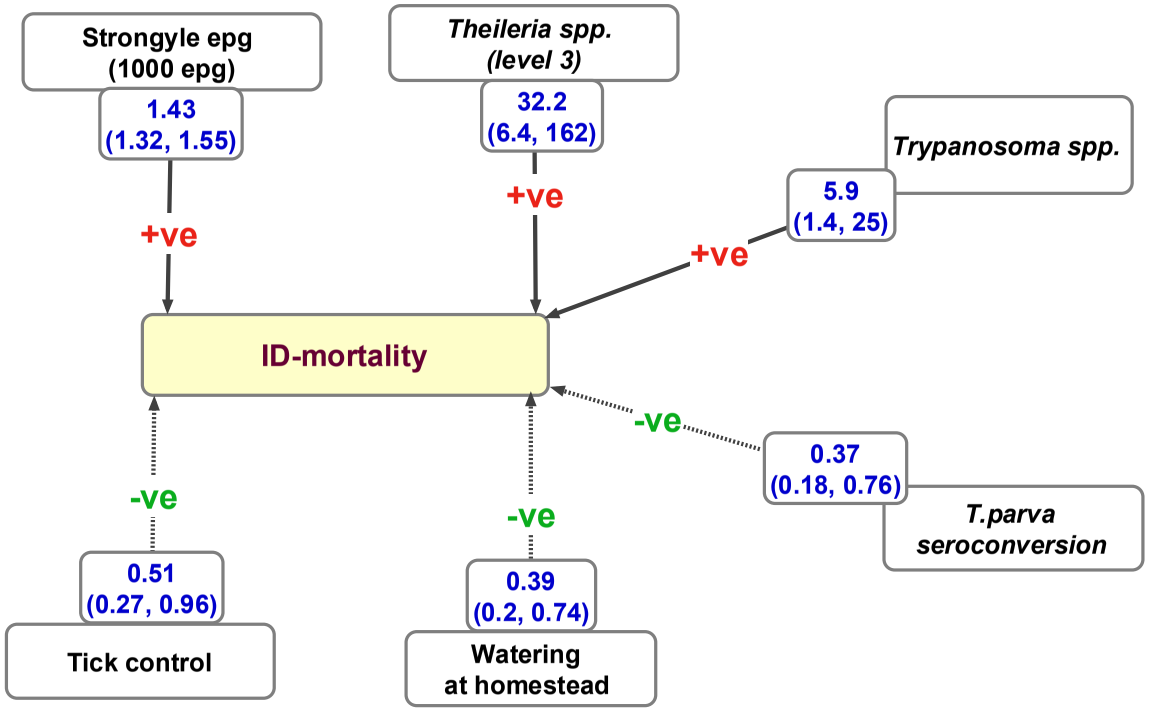


Figure 3.7: Schematic diagram showing the summary results of predictors for infectious disease mortality (ID-mortality), with the size of effect and the 95% confidence intervals. The estimated ID-mortality was 15.3 CI (12.2-18.3) per 100 calf years at risk. The variables with a -ve sign on the lines have a protective effect against ID-mortality. The main pathogens identified to be associated with increased risk for death are infection with *Trypanosoma* spp. mainly being *T.vivax*, high intensity infection with *Theileria* spp. as observed at microscopy, and high worm burden measured by the strongyle eggs per gram of faeces. Seroconversion to *T.parva* was associated with a protective effect.

3.3.4 Cause-specific mortality

The definitive aetiological causes of mortality based on the post-mortem analysis are presented in Figure 3.8. The first main cause of death was East Coast Fever (ECF), accounting for 40% of all deaths due to infectious diseases (6% crude mortality). The second main cause of mortality was haemonchosis, attributed to heavy infection with *Haemonchus placei*, a hookworm that attaches to the abomasal wall sucking whole blood. Haemonchosis was identified as the cause of 12% of the ID-mortality (1.8% crude mortality). Heartwater disease was identified as the third main cause of ID-mortality, accounting for 7.2% of the infectious disease deaths (1.1% crude mortality).

The aetiological causes of death varied between sublocations with deaths due to haemonchosis occurring more in East Siboti (in the North), whereas most ECF deaths were observed in Magombe East and Bumala A (in the south), Figure 3.9.

Although a definitive aetiological cause could not be determined for 29% of the mortality cases, contributing infectious causes for many of these cases were identified. Figure 3.10 shows the definitive causes of mortality and identified contributing infectious causes for each of them. Over half the ECF deaths were complicated by other infections, helminthiasis being the main co-infection. Although trypanosomiasis and anaplasmosis were not identified as definitive causes of calf mortality for any case, they were important co-infecting pathogens for some of the deaths due to haemonchosis and ECF.

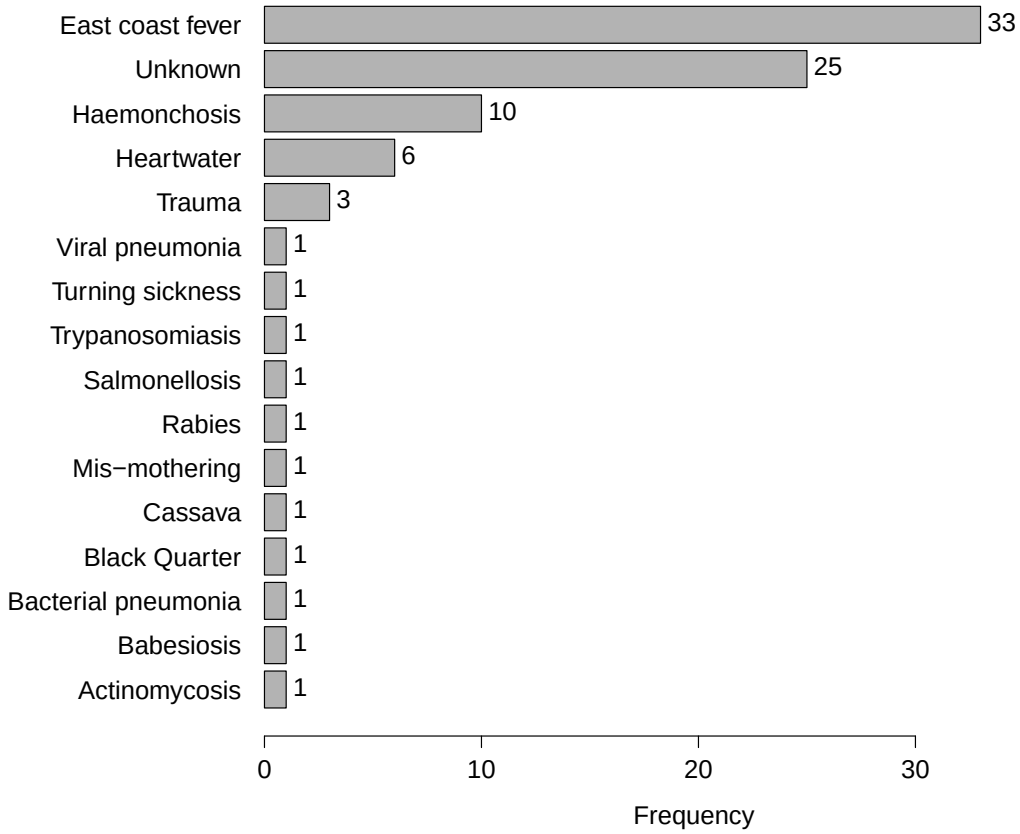


Figure 3.8: Definitive aetiological causes of death. A total of 88 deaths occurred during the study. 5 of these were attributed to non-infectious causes (trauma, mis-mothering and cassava poisoning). East Coast Fever was the main cause of death, followed by haemonchosis and heartwater disease. For 25 deaths, a definitive aetiological cause of death could not be determined.

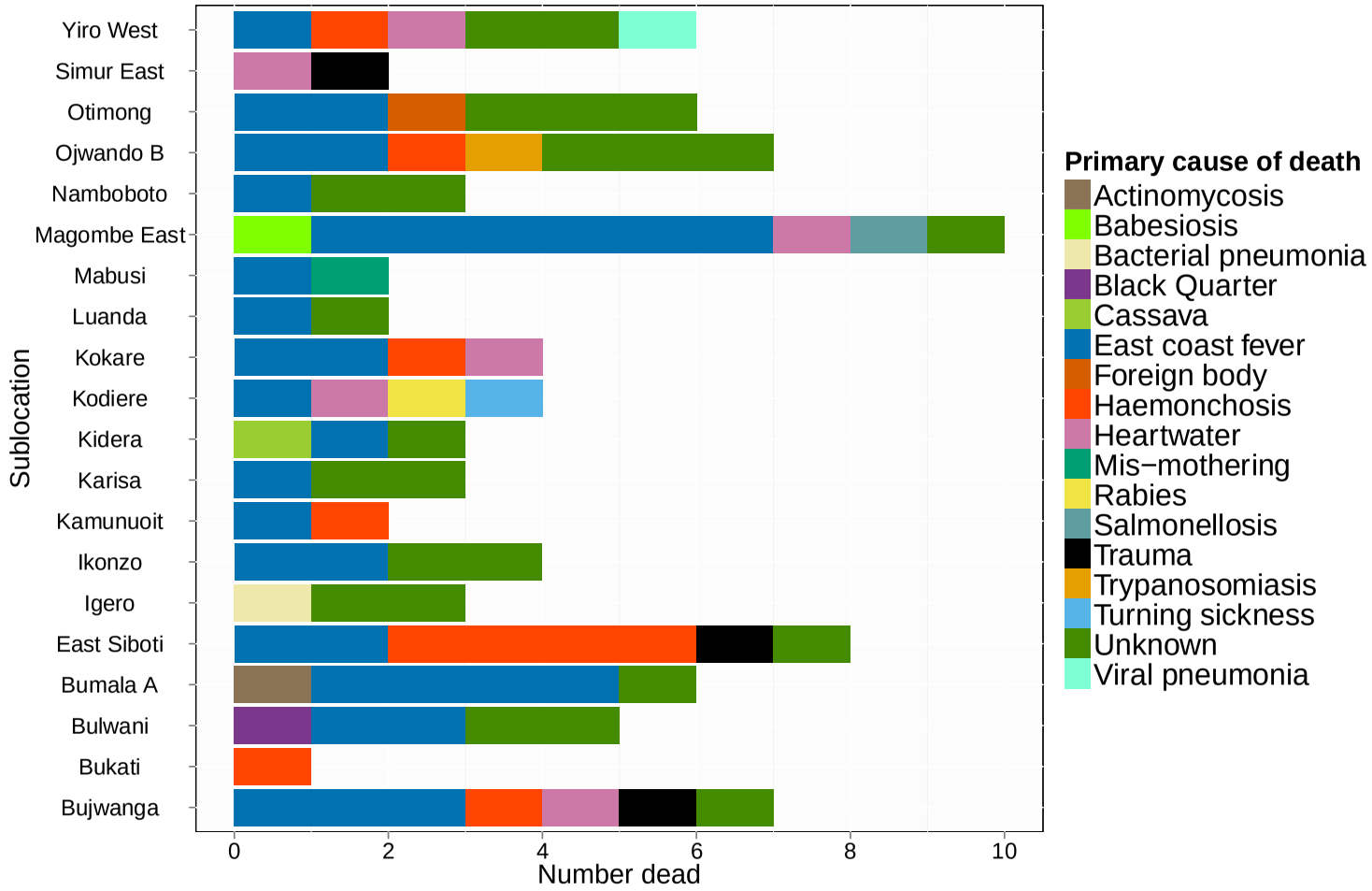


Figure 3.9: Definitive aetiological causes of calf mortality by each sublocation. Magombe East sublocation (located in the south) had the highest mortality with ECF deaths being the most common. The second highest mortality was observed in East Siboti sublocation (located in the north), where haemonchosis was the main cause of death.

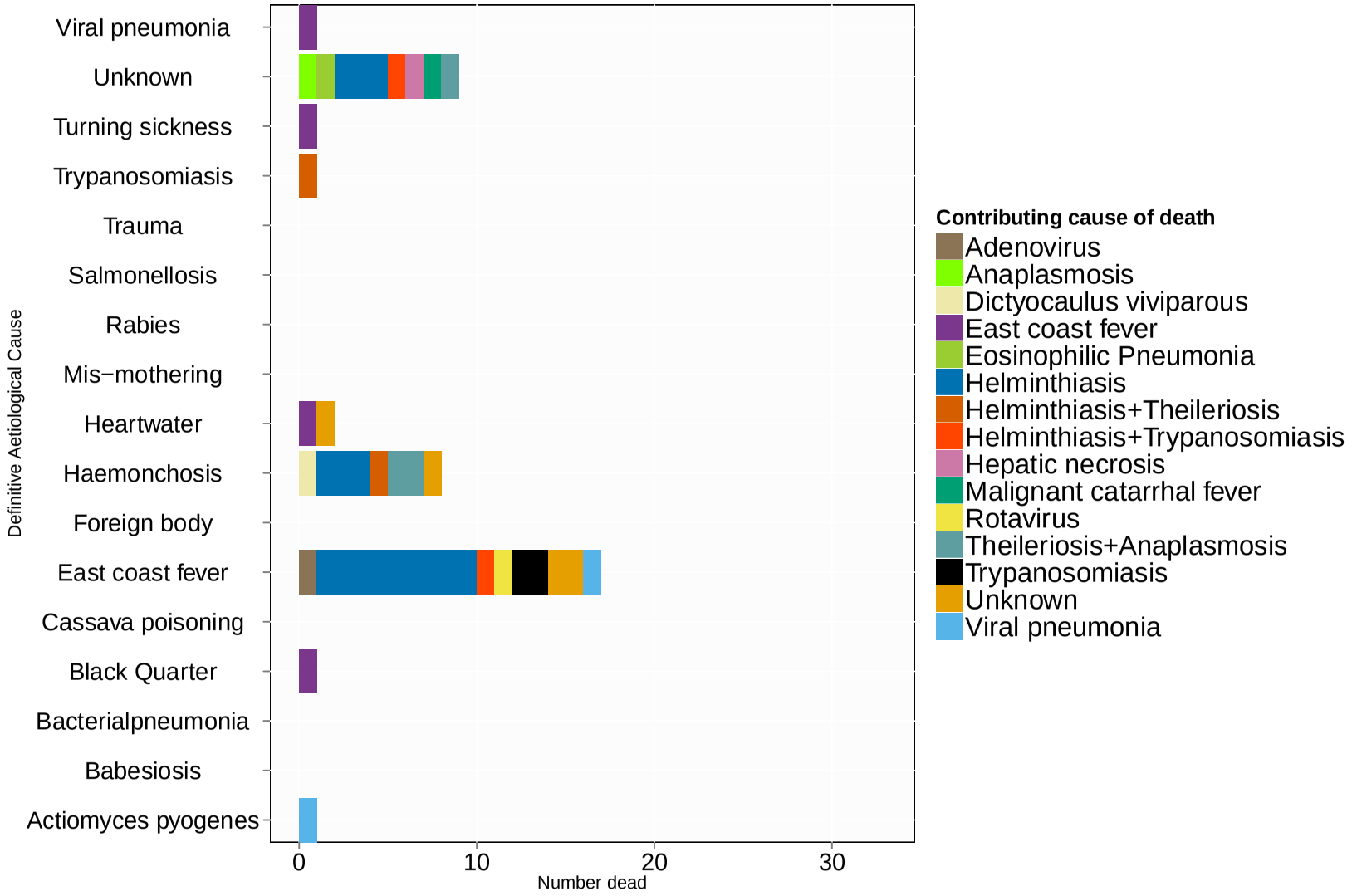


Figure 3.10: Co-infections and diseases contributing to the definitive (primary) aetiological causes of calf mortality.

3.4 Discussion

This study has investigated mortality in indigenous zebu cattle during their first year of life, and identified the main aetiological causes of death and the risk factors associated with infectious disease mortality. The all cause mortality rate was estimated at 16.1 per 100 animal risk years, while mortality related to infectious diseases (ID-mortality) was estimated at 15.3 per 100 animal risk years. Although living in environments of high disease pressure, zebu cattle are considered well adapted to survive in such environments (Hanotte et al., 2010), and mortality rates as observed in this study would cause significant losses. These mortality rates are more than 3 times what is observed in most well managed dairy systems in other countries where the all-cause mortality rates are frequently reported below $< 5\%$ (Heinrichs and Radostits, 2001; Svensson et al., 2006; Gulliksen et al., 2009). Unlike dairy systems which have intensive management and veterinary input, the traditional production systems under which zebu cattle are raised are largely non-interventional with little or no disease control at the farm level.

Similar mortality rates to those observed in this study have been reported among zebu calves in Tanzania (Kanuya et al., 2006; Swai et al., 2009). The review by Otte and Chilonda (2002) focussing on the production parameters among cattle raised under different agro-ecological zones and production systems in Sub-Saharan Africa reported an overall calf mortality risk of 21.7 percent in traditional smallholder mixed production systems. Among exotic and cross-bred animals in Sub-Sahara Africa, the overall mortality rates are usually higher than those observed in zebus, with some studies reporting rates as high as 35% (Gitau et al., 1994; French et al., 2001; Muraguri et al., 2005). From these studies it is evident that although zebu cattle have relatively lower mortality compared to exotics and cross-bred animals, calf mortality is still high and a possible significant impediment to improved livestock production.

In order to establish if there were specific periods when calves were at relatively higher risk of death, this study used the instantaneous hazard

estimates plotted with kernel-smoothing to aid visualisation (Figure 3.2). From this diagram, three periods were identified as having a relatively higher risk for calf ID-mortality: neonatal period, age between 4 and 6 months, and age approaching one year. Most studies on calf mortality identify the neonatal period (first four weeks after birth) as the period with highest risk for calf mortality (Heinrichs and Radostits, 2001; Wudu et al., 2007; Svensson et al., 2006; Gitau et al., 2010). Diarrhoea and pneumonia are frequently reported to be the main causes of death during this period, with inadequate or delayed ingestion of colostrum after birth, unhygienic manual feeding of milk to the calves, poor calf feeding and poor housing being the main risk factors associated with these deaths. In this study, diarrhoea and pneumonia were uncommon and were not identified as causes of death. Zebu calves are allowed to suckle directly from the dams, which reduces the risk of hygiene related illnesses associated with manual feeding of milk to the calves.

The second period of increased risk for calf mortality (period between 4 and 6 months) corresponds to the expected time of waning maternal antibodies. The increased risk may be related to increased susceptibility to infectious pathogens with reduced maternal immunity.

The third period of increased risk was identified as the age approaching one year, which would correspond to the weaning time. Weaning in these study animals was delayed and occurring late into the year, and only half the study animals were weaned by the time they left the study at one year. Following weaning, calves start accessing field pastures used by adult cattle which are likely more contaminated increasing the levels of exposure to helminth infections and other infections through increased contact with animals in the field. This coupled with possible post-weaning stress may increase their susceptibility, exposure and risk for mortality.

Initially the study sought to establish the predictors of ID-mortality at calf recruitment time. High antibody titres against *T.parva* and *B.bigemina* in the dam were associated with increased risk for death. One possibility may be that the antibody titres may be a form of measure of infection pressure in the location the calf is born, with higher titres indicating higher infec-

tion pressure. Their effect was lost in the subsequent models that included infection data.

The husbandry practice of providing drinking water to the animals within the homestead was found to have a protective effect against ID-mortality in the recruitment model, model with only non-infectious factors, and the final model containing both infectious and non-infectious factors. It would be expected that animals that do not require to travel to common watering points for groups of animals from different farms have lower exposure levels to pathogens. This may be one reason why this factor was identified associated with a protective effect against ID-mortality. It would be expected that including pathogen data would reduce the importance of this variable, and its curious its effect remains with pathogen data included in the model. It is unclear what other variation other than that explained by pathogen data, that the variable captures.

Before incorporating infection data into the models, analysis of the non-infectious factors associated with ID-mortality revealed heart girth size, the mean NDVI, watering at homestead, controlling for ticks and dam antibody titres against *B.bigemina* as the significant non-infectious predictors of ID-mortality. The effects of mean NDVI, heart girth size (both protective effects) and antibody titre against *B.bigemina* (increased risk of death) were however lost when infection data was included, indicating these factors may be related to either susceptibility to infections or infection pressure. NDVI measures the health and density of vegetation with high NDVI values indicating healthy vegetation a proxy measure of environmental variables as rainfall and temperature. Since it measures the vegetation health, it may be related with the quality and quantity of feed availability for the animals. High NDVI values may be suggestive of good feed availability to the dam and consequently to a suckling calf, and which would relate to the observed protective effect against calf ID-mortality. The heart girth size in the dam may relate to the condition of the dam and possibly the quality of care extended to the calf mainly through feeding. Absence of tick control and watering at homestead remained significant predictors of calf ID-mortality in the subsequent analysis

including infection factors.

Although the study calves were not themselves being sprayed with acaricides to control for ticks, tick control in the rest of the herd was associated with a 49% lower risk of death when compared to animals in farms where tick control was absent. Tick control in the rest of the herd may reduce the levels of exposure to infected ticks that calves experience, thereby improving their survival probabilities. The frequency with which tick control was done within farms controlled for ticks was itself very low, with only a fraction of farms doing tick control more that two times in the year. This raises a question whether occasional tick control, even though it may not keep the cattle completely tick free, still carries some benefits especially in relation to survival of calves in the herd.

The final Cox survival model identified high intensity infection with *Theileria* spp. observed at microscopy, infection with *Trypanosoma* spp. and high strongyle faecal egg counts to significantly increase the risk of ID-mortality by a factor of 32, 6, and 1.4 (per 1000 epg increase) respectively. The model has used data obtained through microscopy and has not included information such as clinical history (signs before death), gross or histo-pathology findings following post-mortem analysis.

When compared to the results obtained from the independent systematic review of laboratory, gross-pathology and histo-pathology data of all post-mortem cases done by the 7 veterinarians to establish the definitive aetiological causes of death for each case, the findings have good agreement. The review of the post-mortem examination results revealed, in order of importance, the main causes of calf mortality to be East Coast Fever, haemonchosis and heartwater disease. These three infections directly accounted for 60% of the disease-induced mortality. The two main causes of death are as predicted by the model. Larval cultures routinely carried out to identify the species of worms infecting the calves revealed that *Haemonchus placei* accounted for $>$ 80% of all larvae hatched from strongyle eggs. The presence and abundance of *H.placei* worms was confirmed at post-mortem.

Although heartwater disease was identified as a main cause of death through

the review of post-mortem examination results, it would not be possible to predict this since *E.ruminantium*, the causative agent for heartwater disease, is not easily detected in blood. Diagnosis of heartwater is mainly through clinical signs, although deaths may be peracute, and confirmation by demonstration of *E.ruminatum* bodies in brain smears prepared during post-mortems. The model identifies infection with *Trypanosoma* spp. as significantly associated with death. From the pms, trypanosomiasis was identified as the main cause of death for one calf and as a contributing cause of death to other cases.

The diagnosis of the three main infections (*Theileria* spp., *Trypanosoma* spp., and strongyle epg) identified important by the model is done on microscopy which is easily applicable in the field. It is also not labour intensive and requires little time to complete pointing to opportunities of applying simple diagnostic techniques whose results would help significantly reduce calf mortality.

The causes of calf mortality vary between geographical regions and production systems. Within smallholder production systems, some studies report pneumonias, digestive tract disorders (including non-parasitic diarrhoeas, bloat) (Gitau et al., 1994; Wymann et al., 2006; Wudu et al., 2007; Gitau et al., 2010), and tick-borne diseases (TBD) (Maloo et al., 2001; Muraguri et al., 2005; Swai et al., 2009) as the major causes of mortality. Although the current study covered a region within a 45 km radius semicircle from Kenya-Uganda border, differences in mortality rates and patterns between study sublocations were evident. Higher mortality rates were observed in sublocations in the southern region of the study area and occurred in relatively younger animals compared to those in the northern region. These differences corresponded to the aetiological causes of death, with ECF being the main cause of death in the South and haemonchosis in the North. Such spatial heterogeneity within relatively small regions demonstrates the need for evidence based design for the control of disease and reduction of calf mortality.

The importance of the transfer of maternal antibodies into neonate calves

via colostrum is known to be associated with survival chances of neonates (Besser and Gay, 1994). This is especially important in ruminants where very little transfer of such antibody occurs in utero and where the ability of the newborn calf to absorb colostral antibodies is limited to the first few hours of life. It is important to note colostrum uptake was not directly measured in the study, as the calves were recruited 3 to 7 days after birth. Although the quality and amount of colostrum ingested was not established, data on whether the calf suckled immediately after birth was included in the analysis. In addition, antibody titres against the four main tick-bornes (*T.parva, T.mutans, A.marginale, B.bigemina*) in the dam were included in the analysis.

From this study, providing animals with drinking water at the homestead as opposed to walking them a communal watering point, and controlling for ticks within the farm have been identified as the two important husbandry practices that may decrease the risk of calf ID-mortality. When compared to farms where animals do not access drinking water at the homestead, and where tick control is not practised, these two husbandry practices would be estimated to decrease the risk of mortality by 60% and 50% respectively. It is interesting to note however that the protective effect identified is from relatively infrequent tick control, and not the intensive methods used in dairy systems. Tick control would reduce the risk of death due to ECF and heartwater diseases, both of which are tick-borne diseases. An additional method for the control of ECF would be the immunization through the Infection Treatment Method (ITM) which is currently available for use in most of the country. Decisions on treatment of ECF cases can be aided by microscopy results as this study has shown that high infection intensities at microscopy are a strong predictor for calf mortality. Similarly, strongyle epg count in this case was identified as a good predictor for calf death, and can be used for epidemiological purposes as well as decision-making at the farm level.

Chapter 4

Cause-specific mortality among zebu cattle under one year: the role of co-infections

4.1 Introduction

Natural populations under wild or field conditions are constantly exposed to a large diversity of parasites, resulting in widespread parasitism. Individual hosts, including animals and humans, are frequently co-infected with multiple pathogens either concurrently or in sequence (Petney and Andrews, 1998). These multispecies coinfections may result in pathogen-pathogen interactions which may influence the epidemiology of one another (Pedersen and Fenton, 2007; Telfer et al., 2010; Ezenwa and Jolles, 2011) or the consequent effects of infection on host health and performance (Mwangi et al., 2006; Brooker et al., 2007; Craig et al., 2008).

The impact infections have on a host is related to the virulence of the infecting pathogens, measured by the severity of harm on the infected host attributable to the infecting pathogens. Dependent on the mechanism of the pathogen-pathogen interactions, coinfections may cause a) more harm on the host than the combined effect of the component infections, b) harm equal to the combined effect of component infections, or c) less harm than the combined effect of the component infections (Cox, 2001; Alizon and van Baalen, 2008). Knowledge of direction and strength of these pathogen-pathogen interactions, especially among hosts living in areas endemic with many diseases,

represents essential information useful in improving control of parasitism and its impact.

In the last decade, there has been increased attention paid to coinfections, with reported studies in animals (Lello et al., 2004; Craig et al., 2008; Behnke, 2008; Telfer et al., 2008, 2010) and in humans - mainly limited to malaria and helminth infections, see review by Adegnika and Kremsner (2012) or coinfections involving HIV (Harms and Feldmeier, 2002; Abu-Raddad et al., 2006). From these studies and others, it is evident pathogen-pathogen interactions occur and that their effect differs in strength and direction dependent on the mechanisms by which pathogen-pathogen interactions occur. The possible mechanisms by which pathogen-pathogen interactions occur are reviewed in detail by Pedersen and Fenton (2007) and Graham (2008).

Disease-induced mortality will depend on many factors including characteristics of the host, environmental conditions under which the animals are raised, characteristics of infecting pathogens and the pathogen-pathogen interactions in situations where hosts are coinfected. Although most studies on mortality generate useful data on risk factors and mortality rates, the role of coinfections is rarely examined, even in populations where coinfections are known to frequently occur.

Knowledge of pathogen-pathogen interactions is still limited and we do not know which coinfections are important among domestic animals, and how these influence their survival probabilities. If pathogen-pathogen interactions are understood, cost-effective control programs that make use of multispecies approach to the control of morbidity and mortality attributable to infectious diseases can be applied (Drake and Bundy, 2001; Molyneux et al., 2005).

Previous analysis in Chapters 2 and 3 revealed study calves were routinely coinfected, with a median of 4 different pathogens at a time, and that East Coast Fever (ECF), haemonchosis and heartwater disease were the main definitive aetiological causes of death, together accounting for 60% of the observed infectious disease mortalities. By investigating the specific risk factors for deaths due to ECF, haemonchosis and heartwater disease, this study aims at testing for the effect size and direction of coinfections on the

risk of cause-specific calf mortality. Information on synergistic or antagonistic pathogen-pathogen interactions influencing survival probabilities could potentially improve the design of disease control strategies, and ultimately their effectiveness in reducing host mortality.

4.2 Materials and methods

4.2.1 Data collection

Data used in this study comes from the IDEAL cohort of 548 zebu cattle followed during their first year of life, details of the study are provided in Appendix A. This chapter is concerned with mortality cases that were attributed to ECF, haemonchosis, and heartwater disease following the post mortem analysis described in the methods section of Chapter 3. Specifically, the analysis is done to investigate the risk factors for the three main aetiological causes of infectious disease mortality. The risk factors investigated include the non-infectious factors described in Appendix A and B, and the infectious factors described in Chapter 2.

4.2.2 Data analysis

Survival time was defined as the age at which a calf died from the specific aetiological cause under investigation. Each cause-specific mortality was treated as an outcome variable, for example, ECF death - was defined as any death in the study cohort that was directly attributable to ECF as the main aetiological cause of death, based on the review of the gross pathology, histopathology, and laboratory examination results. All other deaths were treated as non-ECF deaths and therefore censored in the analysis of risk factors for mortality due to ECF.

Cox proportional hazard models as described in Equation 4.1 were used.

$$h(t) = h_0(t)e^{\beta X+\epsilon} \tag{4.1}$$

It expresses the *hazard* at time t (i.e the probability of calf death at time t) as a function of:

- *Baseline hazard* - $h_0(t)$: which is the value of the hazard when all predictors are 0 or absent. It is an unspecified baseline hazard rate describing the common shape of survival time distribution for all calves.
- *Linear combination of predictors* - βX: an exponential function of a series of explanatory variables $\beta X = \beta_1 X_1 + \beta_2 X_2 + ... + \beta_k X_k$. Their parameters represent the shift in the *log hazard* associated with a unit difference in the corresponding predictor.
- *Error term* - ϵ : a random effect accounting for the correlated measurements of animals within the same sublocation.

Investigating the effect of infections on the risk of mortality due to specific causes requires that the models are able to incorporate variables whose values change over time. Infection status, presence/absence or a measure of the infection intensity is expected to change over the observation time.

Potential non-infectious and infection risk factors listed in Table 3.1 were tested, each against the specific aetiological causes of death, initially as a univariable analysis. Factors with a p value ≤ 0.2 were incorporated in the multivariable analysis, and backward selection methods used until only factors significant at a p value < 0.05 remained in the model. The dropped variables were then added back to the model each at a time to test if there was significant improvement in model fit.

4.3 Results

The identified main aetiological causes of calf mortality were ECF, haemonchosis and heartwater disease in the order of importance. ECF was identified as the main aetiological cause of death for 40.2% of the infectious disease deaths, haemonchosis 12.2% and heartwater disease 7.3% of the mortalities attributed to infectious diseases. Time to death due each of the three causes

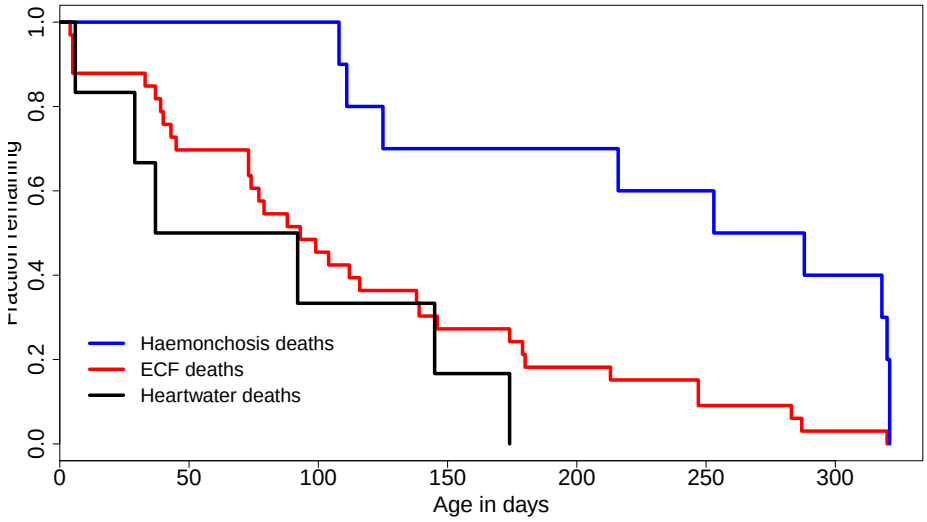


Figure 4.1: Plot of time to death for ECF, haemonchus and heartwater deaths, the three main causes of calf mortality. Deaths due to heartwater disease all occurred in animals below 6 months, whereas those due to haemochosis were recorded more towards one year of age. More than 80% of ECF deaths were observed in calves below 6 months of age, with only a few more observed in older animals.

of death is presented in Figure 4.1. Deaths from heartwater disease occurred in young calves and were recorded only in animals below 6 months of age, whereas deaths due to haemonchosis occurred in older calves, mostly beyond 6 months of age, see Figure 4.1. About 80% of deaths attributable to ECF occurred before calves were 6 months old, with only a few ECF deaths recorded in older calves. Additionally, 5 of the 6 heartwater deaths were confined to the southern region of the study area with only one such death recorded in the north, see Figure 4.2. Deaths attributed to haemonchosis were observed in a number of the study sublocations in low numbers (one death each), while in East Siboti the north most sublocation 4 deaths due to haemonchosis were recorded. ECF deaths were observed across the study region although Magombe East (in the south) and Bumala A recorded higher numbers of ECF deaths (6 and 4 respectively) compared to the other study sublocations.

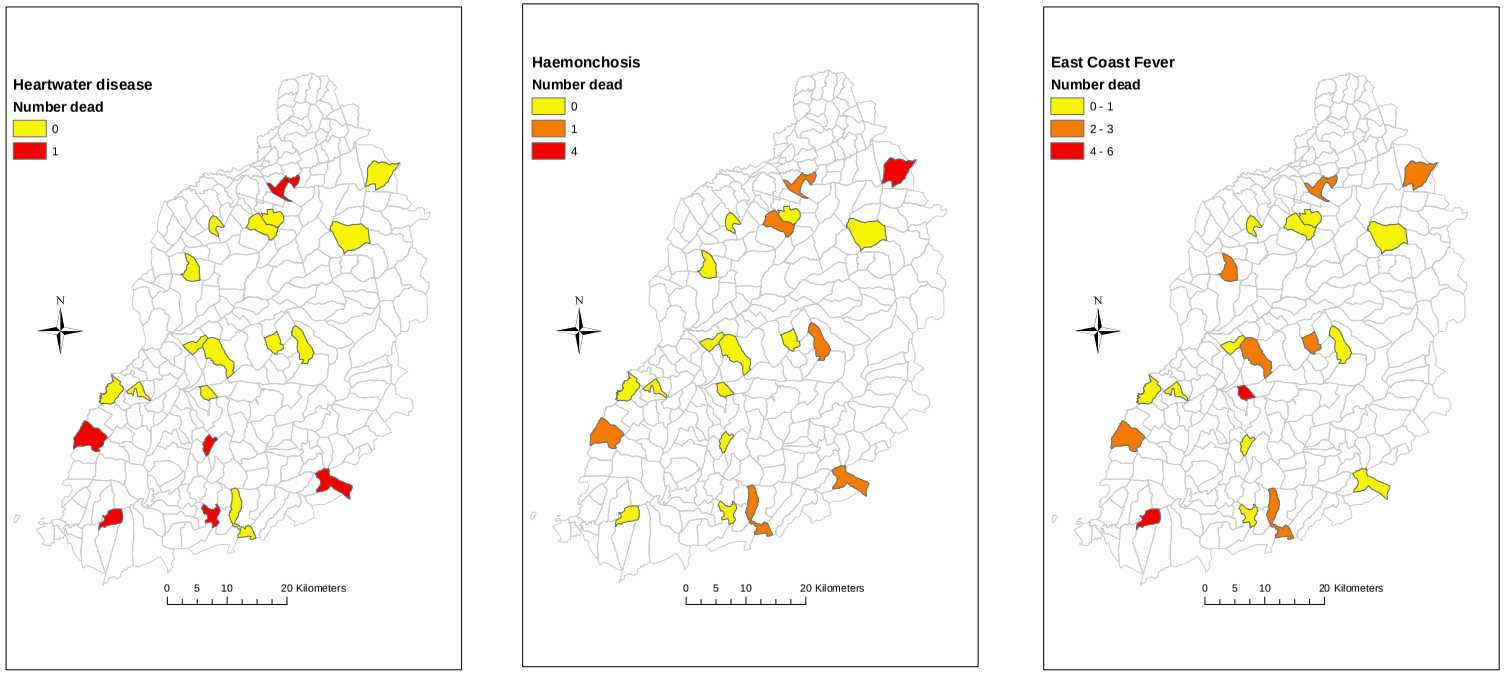


Figure 4.2: Map showing number of deaths per sublocation due to ECF, haemonchosis and heartwater disease. Most deaths due to heartwater were confined within sublocations in the south with only one death in a sublocation in the north. Deaths due to haemonchosis were more widely distributed but East Siboti (north most sublocation) recorded 4 of the 10 deaths. Deaths due to ECF were recorded across the study sublocations, with Magombe East and Bumala A recording high numbers of ECF deaths (6 and 4 respectively).

4.3.1 Predictors for ECF deaths

Results of univariable analysis for non-infectious and infectious factors are in Appendix Tables D.1 and D.2 respectively. Presence of a clinical episode and blood parameters such as packed cell volume, white blood cell count and total serum proteins were significantly associated with ECF-mortality. These variables were however not included in the multivariable analysis since they were considered a consequence of infection. High intensity (level 3) infection with *Theileria* spp. was associated with increased risk for ECF-mortality. This variable was left out in the multivariable analysis since these data had been used as part of the ECF-death case definition. All other variables with a p-value ≤ 0.2 were allowed into the maximum model and model simplification through backwards selection carried out until only factors significantly associated with ECF deaths at the level of $p < 0.05$ remained in the model.

After controlling for other significant covariates in the model, coinfection with *Trypanosoma* spp. was identified to increase the hazard for ECF death by 10.4 times (CI [1.3, 86]). In addition, the hazard for ECF death was increased by presence of strongyle eggs and this was burden dependent. An increase in strongyle eggs of 1000 was associated with a 1.3 times (CI [1.001, 1.7]) increase in the hazard for ECF mortality.

Seropositivity to *T.parva* and *T.mutans* was identified to be associated with a protective effect against ECF-mortality. The risk hazard for ECF-mortality was reduced by 87% (CI [56, 96]) and 71% (CI [17, 90]) in animals that were seropositive for *T.parva* and *T.mutans* respectively, compared to seronegative animals. Controlling for ticks within the farm was identified as the main husbandry practice associated with a protective effect against ECF-mortality. Farms that carried out tick control were associated with lowered hazard for ECF deaths by 74% compared to farms that did not control for ticks in the rest of the herd. The results of the minimum adequate model showing the predictors with significant association with ECF-mortality are provided in Table 4.1. Model diagnostics did not show evidence of violation of the proportional hazards assumption, see results of the test for proportionality in Appendix Table D.3 and the diagnostic plots in Appendix Figure

D.1.

Table 4.1: Results of the minimum adequate model containing the significant predictors for deaths due to East Coast Fever.

Variable	coef	exp(coef)	se(coef)	z	p
Fixed effects					
Tick control	-1.35	0.26	0.53	-2.55	0.010
T.parva - seropositivity	-2.01	0.13	0.60	-3.33	<0.001
T.mutans - seropositivity	-1.23	0.29	0.53	-2.32	0.021
Trypanosoma spp.	2.34	10.37	1.08	2.17	0.030
Strongyle eggs/1000	0.25	1.29	0.13	1.97	0.049
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.0199	0.0004		

4.3.2 Predictors for haemonchosis deaths

The association between non-infectious and infectious factors and haemonchosis deaths was initially tested by running univariable analysis. The results of the univariable screens for non-infectious and infectious factors are in Appendix Tables D.4 and D.5, respectively.

Results from the multivariable model revealed that calves from farms providing supplementary feeding had a 90% (CI [48, 98]) lower hazard for haemonchosis death compared to calves in farms that did not provide supplements. The main supplement given is crop residues which are offered to the calves left at the homestead when adult cattle go grazing in the fields. High altitudes were associated with increased likelihood for haemonchosis deaths, estimated at an increase in hazard by 3.6 times (CI [1.13, 11.6]) for every 100 meters increase in altitude.

High worm burdens as measured by strongyle epg were associated with increased hazard for haemonchosis deaths with an estimated increase of 1.7 times (CI [1.5, 2]) in the hazard for every 1000 strongyle epg count increase. This finding indicates that the risk for haemonchosis death is burden-dependent. Since *H.placei* is a strongyle egg-producing helminth, the variable was omitted from the final model to test if the association of haemonchus deaths with the other covariates remained. The covariates remained significant in the absence of strongyle epg count in the model.

Coinfection with the strongyle nematode *Nematodirus* spp. was associated with a large increase in hazard for haemonchosis death, 44 times (CI [4, 497]). The confidence intervals are very wide since *Nematodirus* spp. was only identified in a few calves (see Table 2.2). The results of the final model containing the significant predictors for haemonchosis deaths are provided in Table 4.2. Model diagnostics did not show evidence of violation of the proportional hazards assumption, see Appendix Table D.6.

Table 4.2: Results showing the final model with predictors of haemonchosis deaths.

	coef	exp(coef)	se(coef)	z	p
Fixed effects					
Use of supplements	-2.29	0.10	0.84	-2.73	0.006
Elevation	1.29	3.63	0.59	2.17	0.030
Nematodirus spp.	3.79	44.41	1.23	3.08	0.002
Strongyle epg/1000	0.53	1.70	0.08	6.54	< 0.001
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.0199	0.0004		

4.3.3 Predictors for heartwater deaths

Heartwater deaths were only observed in calves below 6 months of age. Confirmatory diagnosis of heartwater is only possible at postmortem by examining brain smears for *E.ruminantium* bodies or through the use of PCR diagnosis. No coinfection effect was found associated with heartwater disease.

Higher hazard for death due to heartwater disease was associated with large herd sizes, represented by the tropical livestock units (TLU- measure of total livestock per farm) in this study. For every one TLU log unit increase, the hazard for death due to heartwater disease increased 3.2 times (CI [1.3, 8]). High NDVI values were associated with decreased hazard for heartwater deaths. A 10-unit increase in NDVI value was associated with a 80% (CI [28, 94]) decrease in the relative hazard. Farmer's age was found associated with higher risk for death by a factor of 1.9 (CI [1.01, 3.64]) for every increase in farmer's age by 10 years. The results showing the predictors and their coefficients are provided in Table 4.3. Model diagnostics did not show evidence of violation of the proportional hazards assumption, see Appendix Table D.7.

Table 4.3: Predictors for deaths due to heartwater disease.

	coef	exp(coef)	se(coef)	z	p
Fixed effects					
log(Tropical livestock units)	1.17	3.22	0.46	2.53	0.011
Mean NDVI x 10	-1.61	0.20	0.65	-2.46	0.014
Farmer's age	0.65	1.92	0.33	2.00	0.045
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.02	0.0003		

4.4 Discussion

The findings here show that polyparasitism, which is common in areas endemic with diverse parasites, has important implications on host outcomes, in this study - calf survival. Here I have investigated the risk factors for the three main causes of calf mortality in the study (ECF, haemonchosis and heartwater disease) and tested the role coinfections play in determining the survival probabilities of zebu calves under one year.

East Coast Fever, a disease caused by the protozoan parasite *Theileria parva* and transmitted by the tick *R.appendiculatus*, was identified as the main aetiological cause of death, accounting for 40% of all infectious disease calf mortality. About 80% of these ECF-deaths occurred in calves below 6 months of age and a majority of these deaths being in the sublocations located on the south of the study area. Results of the analysis of risk factors associated with ECF deaths revealed controlling for ticks in a farm was associated with a protective effect. The risk of ECF-death in farms carrying out tick control was 80% lower than in farms not controlling for ticks. Tick control was not done on the study calves and the observed protective effect is a benefit associated with control in the rest of the herd.

Seropositivity to *T.parva* and *T.mutans* was associated with a protective effect against ECF-mortality. This result suggests that animals dying from ECF either die acutely before an immune response that can be detected as a rising titre has occurred, or simply that the animals that do not mount an immune response strong enough to be detected as seroconversion are at a high risk of succumbing to an ECF infection. If ECF death is acute it would be interesting to know why some animals survive first exposure (evidence by seropositivity) and others die on first infection. The intensity of *Theileria* spp. infection, specifically level 3 infection - multiple infected cells in multiple microscopy fields, was identified both in the previous chapter 3 and in the univariable analysis in the current chapter as associated with a high risk for mortality. The risk for death, it appears, is related to the intensity of infection which may be simply the result of a high dose of infection or an

indication of a host unable to control the within host multiplication of the infecting pathogen.

The risk of ECF death was itself significantly increased by high helminth burden (measured as strongyle epg) and by coinfection with *Trypanosoma* spp., evidence of coinfecting pathogens exacerbating the effect of infection with *T.parva*. This is the first time this result has been quantified and demonstrated.

The mechanisms by which *T.parva* and helminth infections interact to result in increased hazards for ECF deaths are unclear, and have not been described before. However, a similar coinfection profile involving *Plasmodium* spp., also a protozoan parasite, and helminth infections (including hookworms) has been a subject of many studies in humans, and in animal models. *Plasmodium* parasites are frequently occurring as coinfections with geohelminths, particularly hookworms with which they are codistributed sharing extensive geographical overlaps in most of Africa (Brooker et al., 2007). Although the literature has conflicting results with reports of synergistic (increasing severity and incidence of malaria) and antagonistic (decreasing malaria cases) interactions (Spiegel et al., 2003; Druilhe et al., 2005; Brutus et al., 2006; Ezeamama et al., 2008) and reviewed by Nacher (2011), most studies point to high helminth burden being associated with increased incidences and severity of malaria cases. More recently, a review by Adegnika and Kremsner (2012) on the epidemiology of malaria and helminth interactions based on studies published in the last decade has concluded a general trend towards a worsening effect on the pathogenesis and incidence of malaria by hookworms and *Schistosoma mansoni*, and a protective effect by *Schistosoma hematobium* and *Ascaris lumbricoides*.

The interactions are thought to occur chiefly through immuno-regulation by helminth infections in two possible ways. First, the immune response becomes skewed to T-helper cell type 2 (Th2), required for fighting extracellular invaders, at the expense of T-helper cell type 1 (Th1) responses which are required for the control of microparasite infections including malaria parasitemia (Hartgers and Yazdanbakhsh, 2006). The second mechanism

is through helminth induced immunomodulation that down-regulates both Th1 and Th2 responses, a strategy thought to be employed by helminths to avoid host immunity and possibly explaining why helminth infections even with known pathogenic species are often asymptomatic (Maizels et al., 2004).

If similar mechanisms are at work with these study calves, a helminth skewed Th2 response and a dampened Th1 response would render a host coinfected with *T.parva* more susceptible to developing disease. Here the risk for ECF death increases with helminth burden (measured by strongyle epg) which from larval cultures and identification of L3 show *Haemonchus placei* to be the main helminth producing strongyle eggs.

These results suggest coinfections with hookworms may be playing a role in reducing the host's ability to fight off *T.parva* infections. It is also possible that hookworms, which attach to the abomasal wall and suck whole blood, may be causing enough damage on their own weakening the calf more and increasing the risk of death with additional pathology from other coinfecting pathogens.

Trypanosomosis was not identified as a major cause of death in these cattle but its presence increased the risk of death from ECF by up to 10 times. Like *T.parva*, infection with *Trypanosoma* spp. is known to lead to immunosuppression. In addition, animals infected with trypanosomes have fever, lowered appetite, considerable weight loss, and anaemia. These effects coupled with immunosuppresion may be lead to increased susceptibility and pathology in the host coinfected with *T.parva*.

Infections with *Haemonchus placei* were themselves identified as the second most important aetiological cause of mortality in zebu calves. A high burden of strongyle eggs was identified as a significant predictor for deaths due to haemonchosis, pointing to their impact being burden-dependent. *H.placei* accounted for more that 80% of all larvae hatched following incubation of the strongyle eggs. Coinfections with *Nematodirus* spp. were also significantly associated with increased hazard for haemonchosis deaths although infections with *Nematodirus* spp. only affected less that one percent of calves.

Farms at high altitudes had a greater risk of death due to haemonchosis and

it may be related to the helminth infection pressure being greater at higher elevations. Farms that reported providing supplements (mainly crop residue) to the animals had significantly lower hazards for haemonchosis deaths than those that did not provide supplements. Calves in such farms are fed mainly while within the homesteads, and as a result will visit grazing pastures less frequently or take longer before starting to access communal grazing fields. These factors reduce the exposure to helminths, and may explain the association between supplement feeding and risk for deaths due to haemonchosis.

Mortality due to heartwater disease occurred in young calves, all 6 calves dying before reaching 6 months of age. Herd size and the age of farmer were statistically associated with increased hazard for heartwater disease deaths, while high NDVI reduced the risk. Large herd sizes would be expected to increase the probability of exposure to infections for the calves. NDVI is a proxy measure of the availability of feed for the animals, and perhaps correlated to the amount of milk the calves receive from their dams but may relate to many other things including suitability of vector and pathogen habitats.

The findings of this study suggest reduction in calf mortality would be attained through improved husbandry practices to reduce levels of exposure to pathogens that calves experience. The results suggest that integrated tick, trypanosomes and worm-control programs would likely have large benefits in not just reducing mortality due to individual diseases, but also excess coinfection exacerbated mortality. In human studies, such integrated control programs have been suggested for example in the control of anaemia-related burden of malaria, which is worsened by high hookworm burden (Brooker et al., 2007).

Chapter 5

Cost of infection and coinfections on growth performance of zebu cattle under one year

5.1 Introduction

In the previous chapters, I have studied infectious disease (ID) mortality in zebu cattle under one year, specifically identifying the main aetiological causes of death and the risk factors for both ID and cause-specific mortality. Taken together with the results on the prevalence of different infectious pathogens identified in the study, it is evident that a large proportion of animals survive infection.

Events that occur early in a host's life, including infection with pathogens, are known to be important determinants of the reproductive and production success of individuals. Parasitic infections seek to exploit host resources for their own reproduction, while hosts have developed a range of adaptations aimed at reducing the likelihood of exposure (e.g physical barriers such as skin, mucosal membranes) or eliminate/confine the pathogen (e.g immune responses).

Results from previous analysis in this thesis show animals that were exposed to *T.parva*, the main parasite associated with most ID deaths, and mounted an immune response (measured as seroconversion) against *T.parva* were strongly protected against death. It may be expected that animals that survive infection suffer trade-off costs on other parameters such as growth

or reproduction as a result of investing energy in defence against infecting pathogens, in addition to the direct effect of the infection itself.

There is debate and good evidence that whereas mounting immune responses against infecting parasites protects the host from adverse parasite effects as mortality or morbidity, there are hidden costs that may ultimately affect other physiological processes including growth and reproduction (Sheldon and Verhulst, 1996; Norris, 2000; Lochmiller and Deerenberg, 2000; Blackwell et al., 2010; Abrams and Miller, 2011). This thinking is anchored on the life history trade-off theory, based on the idea that an organism has a finite energy resource pool from which it allocates energy to various competing demands (Allen and Little, 2011).

This, however, is not the only possible mechanism by which parasites impact negatively on hosts, and it may be difficult to tease out costs due to mounting immune responses and those due to damage on host tissues by replicating parasites. Further complexity may arise from coinfections, which are common in natural populations, with resultant pathogen-pathogen and host-pathogen interactions modifying parasite dynamics, host immune responses and ultimately observed host outcomes (Petney and Andrews, 1998; Cox, 2001; Maizels et al., 2004; Telfer et al., 2010).

Zebu cattle are raised in environments rich with diverse pathogens, and have relatively better resistance to disease compared to higher producing European breeds (Ndungu et al., 2005). Despite this, livestock diseases in zebu cattle remain a major constraint causing mortalities and sub-optimal production, and their control is seen as an important step towards improved production and better livelihoods for farmers (Perry, 2002, 2007; Tomley and Shirley, 2009).

The impact of infections on livestock is largely only measured in terms of mortality, or occasionally in terms of morbidity in cases where infections progress to clinical damage of host tissues with accompanying clinical signs (disease). Rarely is infection identified or its impact quantified in the absence of clinical disease or death, information largely unavailable in smallholder livestock production systems. In addition, we know little about the

consequences of harbouring coinfections on important traits such as growth rates, and whether there are pathogen-pathogen interactions that should be considered in programmes aimed at disease control.

Here I investigate the effects that infections and coinfections have on growth rates of zebu cattle that survive to one year. Specifically, I investigate: a) impact non-infectious factors have on growth rate and, while controlling for the statistically significant non-infectious risk factors, b) effect individual infections have on growth rates, and c) impact of different coinfection profiles on growth rates. This study seeks evidence of costs of surviving parasitic infections by determining the differential impact infections and coinfections have on the outcome "growth rate". Knowledge of environmental factors, infections and coinfection profiles with the greatest impact on growth rates should be an integral part in the designing of programs aimed at disease control, including improved livestock production.

5.2 Materials and Methods

5.2.1 Data collected

Data used in this study came from the Infectious Diseases of East African Livestock (IDEAL) cohort study carried out between October 2007 and September 2010. A total of 548 zebu calves from 20 different sub-locations and 4 agro-ecological zones in Western Kenya were recruited into the IDEAL study at birth. Each study animal was routinely monitored every 5 weeks, starting at recruitment time, until when a year old or lost from the study. During the 5 week routine visits, data on farm management practices and herd health during the inter-visit period were collected. A complete clinical examination was recorded electronically at every 5 week visit; and blood and faecal samples were collected, labelled and linked to respective study animal before transporting them to the laboratory for storage and further processing. Live body weight (to the nearest 0.5 kgs) measurements were recorded at recruitment, and every 5 weeks thereafter until 31 weeks old. A final body weight (using a portable weigh beam) was taken at week 51, before leaving the study. Maternal data including the dam's general health, udder health, girth measurements and body condition score were recorded at every calf visit, until the calf was weaned or left the study. A summary of the type of field visits conducted and the data collected is provided in Figure 5.1. Detailed description of the study design and protocol are provided by Appendix A.

This chapter uses data from a subset of 455 calves of the IDEAL cohort that completed the one year observation time. This subset excludes the 88 animals that died during observation time, and 5 animals that were censored for non-compliance with the study protocol.

5.2.2 Predictor variables

The predictor variables for growth used in this study, broadly classified into a) non-infectious variables, and b) infectious variables, are presented in Table

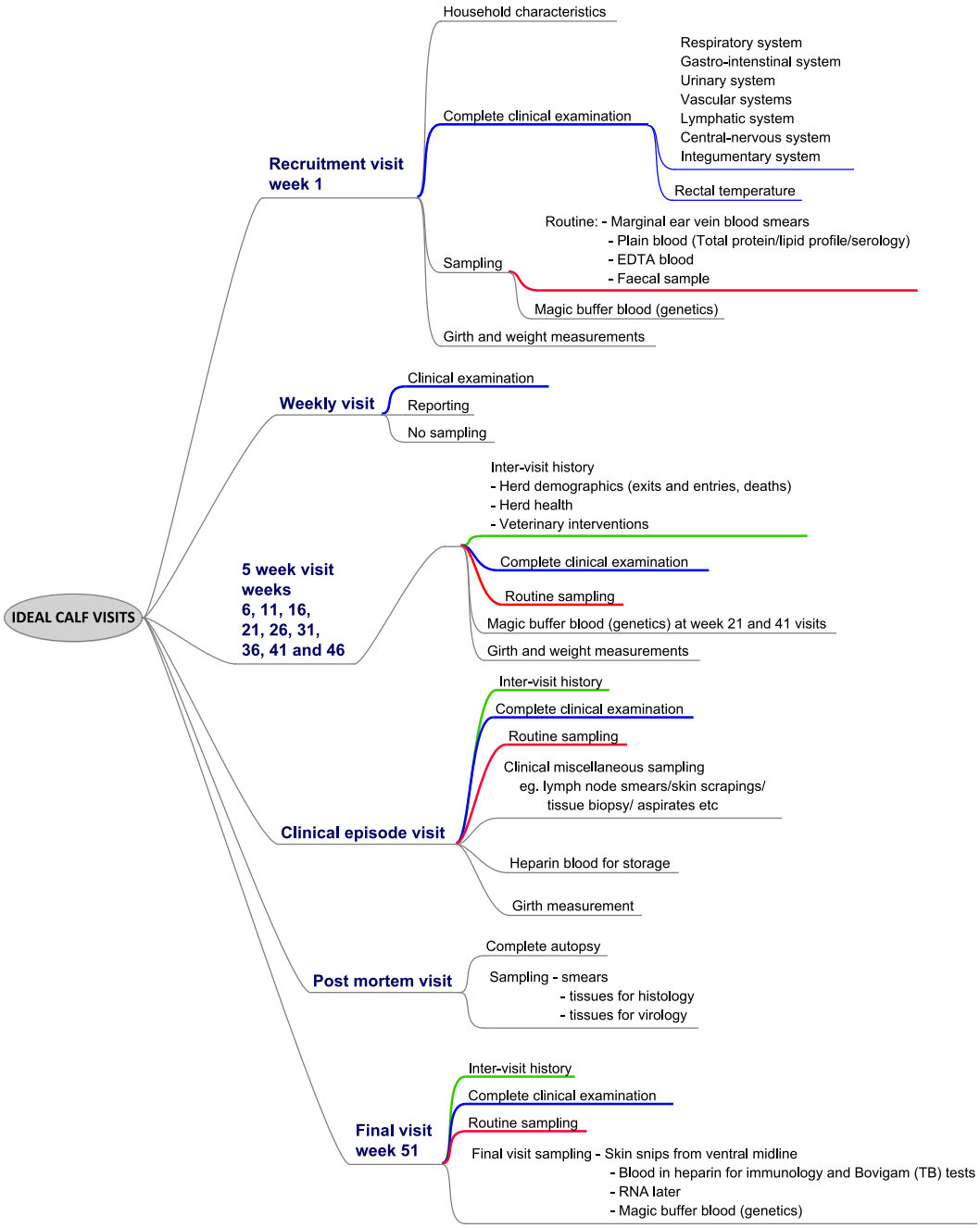


Figure 5.1: A summary of field visits conducted in the IDEAL cohort study, and the corresponding data collected during each visit. Recruitment visit for each study calf was done 3 - 7 days after birth. Weekly visits were done by local animal health assistants (AHAs) and helped to identify clinical episodes that would be missed by the 5-week visits. Clinical episode visits were conducted in response to reports of calf illness with pre-determined clinical signs, received from AHAs or farmers. A complete post-mortem examination was carried out on animals dying during the follow-up time. A final visit was done at one year, before animals left the study. Nodes sharing either blue, green or red colours indicate similar activity across different visit types.

5.1. They comprise factors whose values changed over observation time (time-varying, e.g dam's body condition, infection status) and those whose values did not change (time-invariant, e.g calf sex, heterozygosity). Dependent on the diagnostic methods used, data on the different pathogens was either available for all sampling time points, or only at one year. The details of the diagnostic methods used, and the frequency with which the pathogens were screened for is provided in Chapter 2, Table 2.1 and Figure 2.1 and Appendix A.

Table 5.1: Covariates tested for their relationship with the growth rates.

Variable group	variables
Non-infectious factors	
Farm	Farmer's age, gender, education level, main occupation, herd size, land size
Management	Tick control, worm control, trypanosome control, vaccine use, grazing practices, watering practices, housing, supplementation of feed
Maternal status	Heart girth measurement, body condition score, suckling, health condition, dam serology for *Theileria parva*, *Theileria mutans*, *Anaplasma marginale*, *Babesia bigemina*
Environmental variables	Normalised difference vegetation index (NDVI), Elevation (altitude) of the farm
Calf factors	Calf sex, birth weight, heterozygosity, European introgression
Infectious factors	
Protozoan	*Theileria parva*, *Theileria mutans*, *Anaplasma marginale*, *Babesia bigemina*, *Trypanosoma* spp., *Theileria taurotragi, Theileria sable, Theileria velifera, Babesia bovis, Ehrlichia bovis, Ehrlichia ruminatum, Anaplasma phagocytophilum*, *Coccidia* spp., *Neospora caninum*
Helminths	*Calicophoron* spp., *Cooperia* spp., *Dictyocaulus viviparous*, *Fasciola* spp., *Haemonchus placei*, *Moniezia* spp., *Microfilaria* spp., *Nematodirus* spp., *Oesophagostomum radiatum*, *Toxocara vitulorum*, *Trichostrongylus axei*, *Trichuris* spp., *Strongyloides* eggs, Strongyle egg count
Fungi	*Trichophyton* spp.
Viruses	Blue tongue virus (BTV), Epizootic Haemorrhagic Disease virus (EHDV), Bovine Viral Diarrhoea virus (BVDV), Infectious Bovine Rhinotracheitis (IBR), Bovine parainfluenza virus type 3 (PIV3)

5.2.3 Data analysis

Descriptive growth analysis

Growth data are a form of repeated measures data with an inherent underlying relationship mainly due to:

a) correlation between multiple measures made on the same individual over time

b) correlation between measurements made from individuals from the same herd or geographical location.

Measurements made on the same individual are likely to be more correlated than those made on different individuals. Individuals from the same geographical location or herd share more environmental factors and are likely more correlated with each other than with individuals living further apart or in different herds. Repeated measures raise the problem of pseudo-replication where the degrees of freedom are artificially inflated and require analytical methods that account for these correlations (Huggins and Loesch, 1998; Strathe et al., 2010).

Two possible solutions to overcoming the problem of non-independence in repeated measures data are:

a) Reduce the within-subject series of measurements to one (or several) computed statistics for each subject (Dohoo et al., 2009). This method avoids modelling the within-subject variation. The computed summary statistic is used as the unit of analysis, and its relationship with single or multiple predictors investigated. This method is limited in that it cannot be used to answer any question about within-subject variation. In addition, time-varying predictors are difficult to include in the analysis except if a single value (e.g mean, or change of status) is used.

b) The use of repeated measures/mixed effects models. These models are capable of accounting for the correlation between measurements

collected from same individual or from individuals from same herd or geographical location, and can handle unbalanced data (eg. due to missing data in some of the study subjects). Mixed effects models take into account the sequential structure in the fixed and random effects, and in the correlation structure making it possible to include time-varying predictors instead of their mean values as in a) above. Their effect is therefore modelled against their occurrence time, enabling insights that would otherwise not be gained when ignoring the sequential structure (Willett, 1997; Gröhn et al., 1999).

Both methods have been used in this analysis and their results compared. The next sections describe each of the two methods.

5.2.3.1 Univariate analysis

Here I computed the Average Daily Weight gain (ADWG) as the choice summary statistic (see Equation 5.1), and used it to investigate the size and direction of effects due to infections and their co-infections while controlling for non-infectious factors, see causal diagram Figure 5.2. The analysis is referred to as "univariate" as it avoids modelling the within-subject variation (Dohoo et al., 2009). This term is not to be confused with "univariable" which describes models with a single explanatory variable (Peters, 2008).

$$\text{ADWG} = \frac{\text{Final weight (at 51 weeks) - Recruitment weight (at week of birth)}}{\text{Total observation time (in days)}} \tag{5.1}$$

The following steps were followed in screening the predictor's association with ADWG (Crawley, 2007; Dohoo et al., 2009):

1. Screening predictors based on descriptive statistics:

   - Variables lacking variability were dropped (eg. navel disinfection after birth was done in $< 1\%$ of farms)

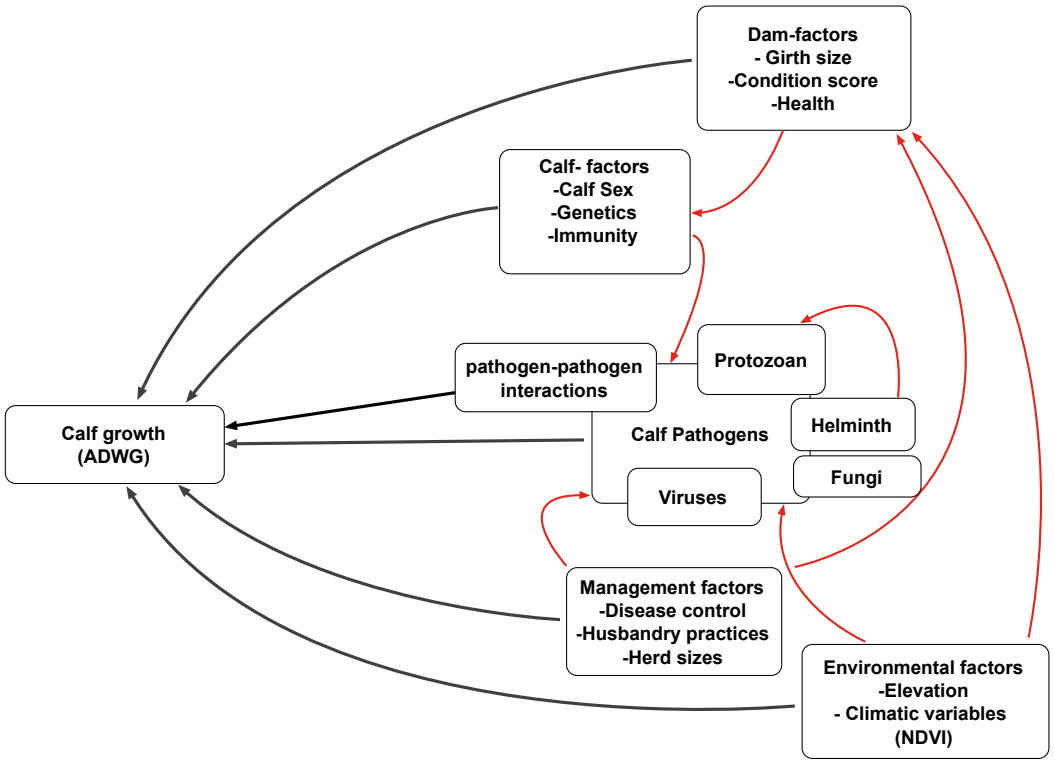


Figure 5.2: Causal diagram showing groups of factors tested for their relationship with growth rate of zebu cattle under a year. The factors may directly act on the calf (black links) to affect "Average Daily Weight Gain (ADWG)", or indirectly by affecting other factors associated with "growth" (red links).

- Categorical variables with many levels, were collapsed to fewer interpretable levels (eg. farmer's main occupation into "salaried" and "non-salaried")
- Variables with large numbers of missing values were removed (eg. $< 50\%$ of the dam ages were known)

2. Correlation analysis:

   - Pairwise correlations among predictors were carried out to check for collinearity problems in the models. A cut-off of > 0.9 correlation coefficients was used. In cases of high correlation between two variables, the variable with more biological meaning is retained as the predictor.

3. Screening based on unconditional associations. Each predictor variable under study was initially run as a univariable analysis. Two main types of variables were analysed:

   (a) Time-invariant predictors - their values remain constant across time, and were used directly in the models.

   (b) Time-varying predictors - their values change over time. For continuous variables, mean values over the observation time were used. For the categorical variables, a single change in status (for example from non-infected to infected occurring at any stage during the follow-up time) was used to differentiate between infection-profiles in the population.

4. Using a liberal p-value ≤ 0.2, all predictor variables from the univariable analysis meeting the cut-off point were selected. A *maximum model* was fitted using the selected predictor variables. Starting with a maximum model avoids overlooking potentially important predictors, but increases the chances of collinearity or including factors in the dataset that do not have biological or logical meaning.

5. Backward elimination was carried out by sequentially removing terms from the maximum model, starting with variables with largest p-values.

The final model, referred to as the *minimum adequate model* contained only significant terms at p-value < 0.05. The terms were then individually added back and model comparisons made to determine if the terms significantly improved model fit.

Model fit comparisons were done using log-likelihood values, Akaike's Information Criterion (AIC) and Bayesian information criterion (BIC). AIC is a likelihood-based measure of model fit which penalises for every extra parameter included in the model (Akaike, 1974). Like AIC, BIC introduces a penalty term for the number of parameters used in the model (Schwarz, 1978). Models with the lowest AIC and lowest BIC have the best "relative fit". To account for the spatial correlation arising from the 2-stage cluster study design and sampling, sub-location was included as a random effect in the final model.

5.2.3.2 Mixed effects models

The summary statistic approach used in Section 5.2.3.1 omits potentially useful information by using only the first and the last observation. The method cannot be used to answer questions concerning within-subject variation (van de Pol and Wright, 2009). Also referred to as multi-level, repeated measures and random coefficients models, mixed models are flexible, utilise available data efficiently and overcome the problem of correlated data by allowing the variance-covariance structure (correlation pattern) to be modelled.

An initial analysis was conducted to establish the growth curve function, non-linear or linear, that best fit growth in zebu cattle under one year.

Non-linear growth models

Biological growth is studied using mathematical models that describe weight-age relationships. Growth functions commonly used include Brody's, Gompertz's, Richard's and Logistic non-linear functions. These functions have been used across a number of species to investigate differences in growth attributable to genotypes, sexes, feed regimes, and environmental conditions (Brown et al., 1976; Forni et al., 2007; Knap et al., 2003; Schinckel and Lange, 1996).

In this study, non-linear functions shown in Table 5.2 were tested to identify the function that best described growth in zebu cattle under one year. The models were fit using package **nlme** (Pinheiro et al., 2012) in R (R Development Core Team, 2011). The fitting of these models in R is described by Pinheiro and Bates (2000, chapter 8) and Crawley (2007, chapter 20). Model comparisons and selection were done using log-likelihoods, AIC's and BIC's. The best non-linear model was picked, and its fit compared with the best linear models.

Table 5.2: Non-linear functions tested for suitability describing growth in zebu calves

Function	Equation
Brody's	$W_t = A - Be^{-kt}$
Gompertz's	$W_t = Ae^{-Be^{-kt}}$
Logistic	$W_t = A/(1 + Be^{-kt})$
Michaelis-Menten's	$W_t = At/(1 + Bt)$

W_t = weight of calf at t age in days
A = weight at maturity, asymptotic limit of weight when age (t) approaches infinity
B = constant of integration
e = base of the natural logarithm
k = maturing rate, relates to how quickly W_t approaches A.

Linear growth model

A two-level analytical approach, described in detail by Singer and Willett (2003, chapter 3), was used. In level one, the growth trajectory of each individual is modelled, and assumed to be influenced by the inherent properties of the study animal. This growth trajectory is determined by individual growth parameters, which include the intercept (starting value), the slope (rate of growth), and the random error. In level two, the individual parameters from level one are assumed to vary as a function of certain measurable characteristics of the individual's environment, background and in this case, infection experiences (Bryk and Raudenbush, 1987).

Level-1 submodel

This is the individual growth model which captures the change in growth that a study subject experiences during its time in the study. To decide on the best model, both visual inspection of the growth trajectories (see Figure 5.3), and information criterion statistics (Akaike information criterion - AIC, Bayesian information criterion - BIC, and log-likelihoods) for linear and non-linear growth models were used to compare the model fits, see Table 5.8. The

linear growth model had the best fit with observed weight (Y) for animal i at time j described as a linear function of its age at that time Age_{ij}, see Equation 5.2.

$$Y_{ij} = \beta_{0i} + \beta_{1i}(Age_{ij}) + \epsilon_{ij} \tag{5.2}$$

β_{0i} represents animal i's weight at birth (when Age=0), β_{1i} is animal i's rate of growth (slope) during the period under study, and ϵ_{ij} (Equation 5.3) which is the residual error calculated for the difference between the observed and predicted weight at time j for animal i, and assumed to be normally distributed *(N)* with a mean 0 and a variance σ^2_ε.

$$\epsilon_{ij} \sim N(0, \sigma^2_\varepsilon) \tag{5.3}$$

Level-2 submodels

Level-2 sub-models use the estimated parameters of level-1 sub-model (*individual growth parameters* - β_{0i} and β_{1i}) to investigate the inter-individual differences in change trajectories as determined by selected predictors, Equation 5.4.

$$\begin{aligned} \beta_{0i} &= \alpha_{00} + \alpha_{01} Predictor_i + \zeta_{0i} \\ \beta_{1i} &= \alpha_{10} + \alpha_{11} Predictor_i + \zeta_{1i} \end{aligned} \tag{5.4}$$

α_{00} and α_{10} are the level-2 intercepts, α_{01} and α_{11} are the level-2 slopes which represent the effect of predictors under investigation. The level-2 residuals ζ_{0i} and ζ_{1i} are assumed bivariate normally distributed with mean 0, and unknown variances σ^2_0 and σ^2_1, and an unknown covariance σ_{01}, the assumptions of which are shown in the Matrix 5.5. These residuals represent the variation in the level-2 outcomes that remain unexplained by the level-2 predictors.

$$\begin{bmatrix} \zeta_{0i} \\ \zeta_{1i} \end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_0^2 & \sigma_{01} \\ \sigma_{10} & \sigma_1^2 \end{bmatrix}\right) \tag{5.5}$$

The level 1 and level 2 (Equations 5.2 and 5.4) are best described by the composite Equation 5.6 which contains both a structural part and an error part.

$$\begin{aligned} Y_{ij} &= (\alpha_{00} + \alpha_{01} Predictor_i + \zeta_{0i}) + (\alpha_{10} + \alpha_{11} Predictor_i + \zeta_{1i})\, Age_{ij} + \epsilon_{ij} \\ &= [\alpha_{00} + \alpha_{10} Age_{ij} + \alpha_{01} Predictor_i + \alpha_{11}(Predictor_i X Age_{ij})] + \\ &\qquad [\zeta_{0i} + \zeta_{1i} Age_{ij} + \epsilon_{ij}] \end{aligned} \tag{5.6}$$

Using a categorical predictor variable (calf sex) for illustration, the structural part of Equation 5.6 estimates four main parameters of interest (corresponding to the fixed effects):

1. $\boldsymbol{\alpha_{00}}$: Estimated mean initial weight of male calf in the population which is the reference category - the reference intercept.

2. $\boldsymbol{\alpha_{01}}$: Estimated differential in the initial weight for female calves - the adjusted intercept value for female calves.

3. $\boldsymbol{\alpha_{10}}$: Estimated mean rate of growth in males calves - the reference slope.

4. $\boldsymbol{\alpha_{11}}$: Estimated differential rate of growth in female calves - the adjusted slope value for female calves.

The statistical significance of these estimated parameters is evaluated to determine if there are significant differences between the starting weights and growth rates among male and female calves. This is easily extended to other covariates of interest. In cases where the predictor variable is continuous, α_{00} would be the estimated initial weight value when the predictor variable x is

zero, and α_{11} would be the differential rate in growth for every unit increase in the level of the predictor variable x.

In certain instances, the estimates of intercept may not be interpretable dependent on the variable under study (e.g estimation of an effect due to an infection event that cannot occur at birth). This is treated in a similar manner as a regular regression where the intercept may fall outside a theoretical possibility without undermining the validity of remaining parameters (Singer and Willett, 2003).

To facilitate interpretation of effects of some of the continuous variables, they can be "centered" on their means (by subtracting a constant eg. the mean value from the predictor) before running the model.

The error part in the composite model Equation 5.6 captures the 3 sources of random variation in longitudinal studies (Diggle et al., 2003):

1. *Random effects:* ($\boldsymbol{\zeta_{0i}}$) Each study subject has intrinsic characteristics different from those of other subjects in the study, giving each individual a specific response profile. These are incorporated in the models by introducing study subjects as random effects, and modelling the within individual variation.

2. *Serial correlation:* ($\boldsymbol{\zeta_{1i}Age_{ij}}$) Weights recorded from the same individual over time may be correlated, with the correlation between a pair of measurements decreasing with increase in separation time. A number of correlation structures for the repeated measure were assumed, and the structure closest to the actual relationship (with the largest log-likelihood among competing models) was selected for use as the base unconditional growth model.

3. *Measurement error:* ($\boldsymbol{\epsilon_{ij}}$) The measurement process as taking of live body weights adds variation in the data.

Correlation structure

In order to account for the hierarchical structure of the data (measurements nested within animals and within sub-locations) within the linear models, additional random effect structures were fitted. These included models with varying intercepts for sub-location only, varying intercepts and slopes for animals only, varying intercept for sub-location and varying intercept and slopes for the animals. Model fit comparisons were made using AIC's, BIC's and log-likelihood tests, see Table 5.7.

The next step was to try to improve the model fit by explicitly modelling the correlation structure between measurements within individual. Several serial correlation structures that model the within group errors and documented by Pinheiro and Bates (2000, Sect.5.3.1) were evaluated. These included:

a) *Compound symmetry* - assumes an equal correlation among all within-group errors for each subject's observations. This may not be realistic as the weight measurements from each subject are collected at different times.

b) *Autoregressive* models which assume higher correlations between observations collected closer in time, and an exponential decay in the correlation as observations get further apart in time.

c) *Autoregressive-moving average* models; these combine the autoregressive, and moving averages which use a number q of the last observations to filter the noise and accurately estimate the mean. Whereas increasing the levels of q may continuously reduce the noise, with few observations per study individual the models may experience convergence problems at higher levels of q.

When the research questions are mainly interested in the *fixed effects*, and not the *variance components*, refining the covariance error structure is a lot of effort for little gain. Although it affects the precision of fixed effects estimates and hence confidence interval construction, it rarely fundamentally

changes parameter estimates (Singer and Willett, 2003, Sect.7.3.7). Notably, variation between profiles may largely account for the sequential correlations, with little further correlation left to explain (Maindonald and Braun, 2006, page 334).

In multi-level models, the fitted values are calculated at each of the levels of variation in the model. Residuals are obtained by subtracting the fitted values from the observed values at each corresponding level. If the model is fitted with the fixed effects only, no adjustment for random effects is done and it evaluates the overall mean. When random effects are involved, the fitted values at each level of the hierarchy are the *best linear unbiased predictors* (BLUPs), calculated as shown in Equation 5.7. In the mixed models the effect size of the predictors is measured using the best linear unbiased predictors (a_i), referred to as *shrinkage* when compared to the analysis of variance estimates given by $(\bar{y} - \mu)$, where μ is the overall mean.

$$a_i = (\bar{y}_i - \mu)\left(\frac{\sigma_a^2}{\sigma_a^2 + \sigma^2/n}\right) \tag{5.7}$$

Where a_i is the fitted value (BLUP), σ^2 is the residual variance, σ_a^2 the between group variance which contains the correlation between repeated measures in each animal, n is the number of subjects. In cases where most of the variation is between subjects σ_a^2, and there is little variation within subjects σ^2/n the fixed effects and BLUPs are similar (Crawley, 2007, page 327).

5.2.3.3 Model simplification

In general, a similar approach as that reported in Section 5.2.3.1 was followed. Model selection was based on the slope (α_{11}) effects which represent the predictors effects on growth rates. Briefly, the steps included:

1. Univariable analysis with all covariates of interest, and select all variables with a p-value ≤ 0.2

2. Fit a maximal model - a model containing all covariates and their interactions of interest from step 1 above.

3. Model simplification by sequentially deleting from the model parameters with the least significant term, starting with the highest order interactions, until only significant variables p-value < 0.05 remain in the model.

4. If the deletion causes an insignificant increase in deviance, leave the term out, otherwise put the term back to the model, until only significant terms are left in the model. This is the *minimum adequate model*, the most parsimonious model. Predictors dropped during the backward selection were added back one at a time to the minimum adequate model, in order to determine if any significantly improved the model fit.

Both *maximum likelihood* (ML) and *restricted or residual maximum likelihood* (REML) estimation methods were used during the analysis. ML was specifically used during the model selection process to compare between models with different fixed effects, whereas REML was used in the final models to reduce the biases of maximum likelihood estimates (Pinheiro and Bates, 2000; Crawley, 2007).

The repeated measures analysis was done using **nlme** package (Pinheiro et al., 2012) and **lme4** package (Bates et al., 2011) in R (R Development Core Team, 2011).

5.2.3.4 Model diagnostics

The model diagnostics for mixed models were done to check on two distributional assumptions (Pinheiro and Bates, 2000):

a) within-calf errors are independent and normally distributed with mean zero and variance σ^2, and independent of the random effects;

b) random effects are normally distributed with mean zero and covariance matrix and independent for different calves.

This was done by visual inspection of the residuals, fitted values and the estimated random effects.

5.3 Results

5.3.1 Univariate analysis

This section presents results from the models using the summary measure ADWG.

Outcome measure

Descriptive statistics of various measures of growth are provided in Table 5.3. The mean body weight at recruitment was 19.2 kgs ± 3.7 SD (range 8 - 29.5), and 65.2 kgs ± 17.72 SD (range 29 - 144) at one year. A big variation in growth rates, up to a 10-fold difference (minimum 0.03kg and maximum 0.34kg daily weight gain) was observed. The percentage body weight gain over one year ranged from as low as 52% to as high as 704%. Variation in observed weights increased with age, see Figure 5.3. The summary statistic chosen for the univariate analysis was the **Average Daily Weight Gain (ADWG)**, calculated as shown in Equation 5.1. This captures the average daily growth rate for each of the 455 calves completing the 51 week observation time.

Table 5.3: Descriptive statistics of summary measures of weights.

Variable	obs	mean	median	s.d.	min.	max.
Average daily weight gain (kgs)	455	0.13	0.12	0.05	0.03	0.34
Recruitment weight (kgs)	455	19.2	19	3.7	8	29.5
Final weight (kgs)	455	65.2	63.5	17.7	29	144
Percent weight gain	455	247	237	99	52	704

The analysis was carried out in steps to answer two questions:

a) can I predict the growth rates based on information available at calf recruitment time?

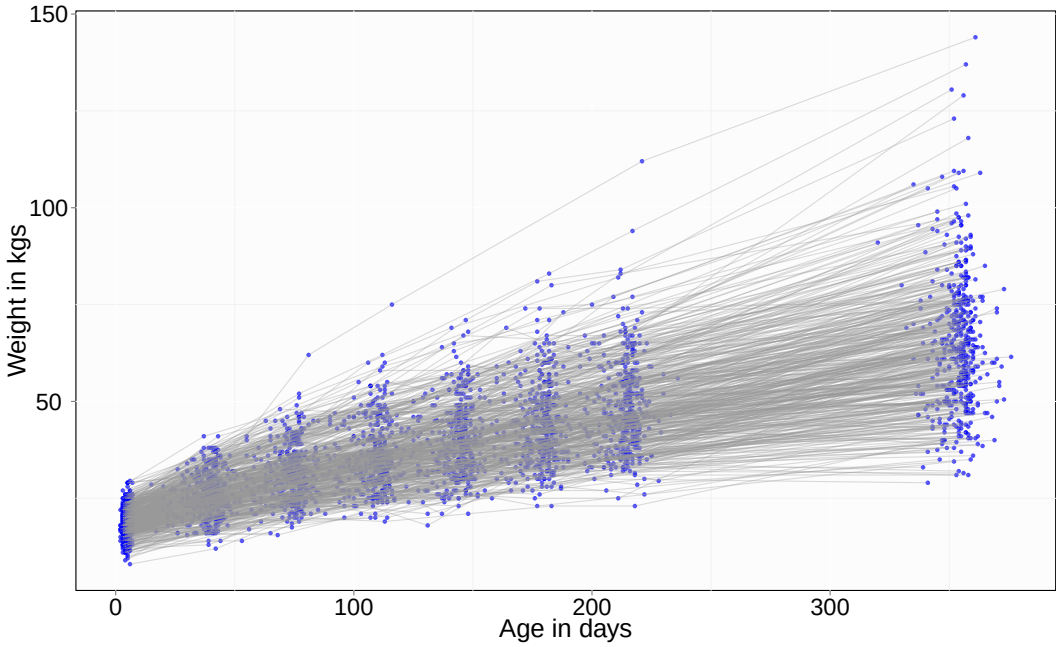


Figure 5.3: Growth trajectories of the 455 calves that completed the one year observation time. The blue dots are individuals weights recorded and the grey lines connect repeated measures for each calf. Routine weight measurements were done from birth up to week 31 of age, and thereafter at the final visit done at week 51 before leaving the study.

b) while controlling for effect of non-infectious factors, what is the impact of infection and co-infection experiences on the ADWG?

To answer the first question, I model the ADWG by time-invariant predictors and initial values of time-varying predictors, i.e. values at calf recruitment time. This initial model referred to as the "recruitment model" determines whether predictions of growth performance can be made from data available at birth. Identification of strong predictors for growth performance at early stage may aid in decision making including selection of breeding animals. Results from the univariable screen for non-infectious factors at recruitment time are provided in Appendix Table E.1. Variables with a p-value of ≤ 0.2 were included in the maximum "recruitment" model and backward elimination used until the final model contained only predictors with a p-value < 0.05.

The variables in the minimum adequate model were replaced with possible

correlates, to determine if these alternative variables improved the model fit, see Appendix Table E.2. In addition, correlation coefficients between the final model variables, and their correlates were calculated to rule out collinearity. None of the predictor pairs had correlations greater than 0.9, the level which would likely create collinearity problems in the model (Dohoo et al., 2009). Figure 5.4 shows the correlation coefficients between significant predictors, their distribution, and a LOESS smoothing curve added on the scatter plots to aid visualisation of the relationship of predictors with ADWG.

From data available at birth, herd size (measured as tropical livestock units), the dam's girth size and its body condition had significant statistical associations with growth rate. In addition, growth rates in male calves were marginally significantly higher than in females. These four variables whose values are known from recruitment time explain up to 15.4% of the observed variation in growth rates, see results in Table 5.4. Sublocation was added as a random effect, and the intra-class correlation (ICC) was 0.097 indicating 9.7% of the variation in growth rate observed is due to differences between sublocations.

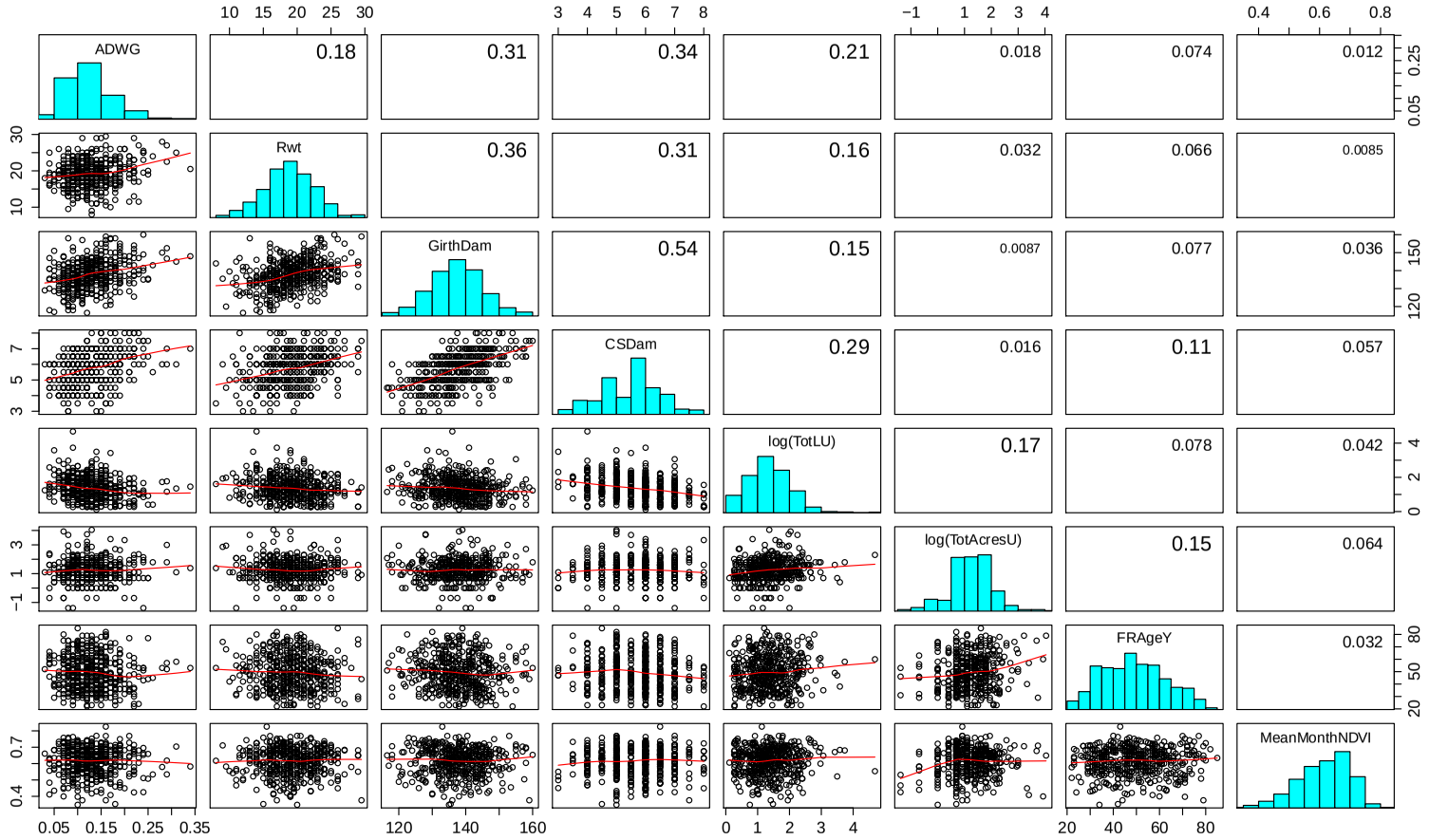


Figure 5.4: Correlates of variables making up the minimum adequate "recruitment" model. Upper panel gives the correlation coefficients between pairs of variables. The diagonal panel contains histograms of the variables in the order from top; average daily weight gain(ADWG) in kgs, recruitment weight (Rwt) in kgs, dam heart girth size (GirthDam) in cms, dam body condition score (CSDam), log(tropical livestock units), log(total land size in acres), farmer's age in years (FRAgeY), and the NDVI value at the month of birth (MeanMonthNDVI). The lower panel has scatter plots with a LOESS smoother added to aid visualisation.

Table 5.4: Minimum adequate "recruitment" model with variables significantly associated with ADWG. The adjusted R-squared was 0.154.

	Estimate	Std. Error	t value	Pr(> |t|)
Fixed effects				
(Intercept)	-0.0725	0.0381	-1.90	0.058
log(Tropical livestock units)	-0.0088	0.0032	-2.67	0.008
Heart girth size - dam	0.0012	0.0003	3.13	0.002
Body condition score - dam	0.0091	0.0025	3.69	< 0.001
Calf sex - female	-0.0087	0.0041	-2.16	0.031
Random effects				
Group	name	Std Dev	Variance	
Sub-location	Intercept	0.0136	0.0002	
Residual error		0.0416	0.0017	

Intra-class correlation 0.097

The next analysis sought to determine the effect size and direction of infections and co-infections on ADWG (question 2). This analysis proceeded in two steps: a) identification of non-infectious factors associated with ADWG, and b) while controlling for significant non-infectious factors, determine the relationship between infectious factors and ADWG.

Values of time-varying quantitative variables (eg. NDVI, girth measurements of dams) were reduced to means based on calf observation time. For qualitative/categorical variables, a change in status (eg. weaning) at whatever time it occurred during the observation period was considered a positive/presence. Results of univariable analysis on these factors are given in Appendix Table E.3, and the maximum and minimum adequate model using means of time-varying predictors in Appendix Table E.4. The girth size of the dam, and its body condition score were the only time-varying non-infectious predictors with a statistically significant (positive) effect on ADWG.

Significant predictors from the "recruitment" model and those from time-varying variables were combined to give the final model for the non-infectious factors, see Appendix Table E.5. Sub-location was included as a random effect in order to account for the correlation between calves from the same geographical location.

The results revealed dam factors (heart girth size and body condition score) and a farm management factor (herd size measured as tropical livestock units) to be significantly associated with ADWG. Calves from dams with large heart girth sizes and high body condition scores had higher growth rates compared to calves with relatively smaller and low body condition score dams. Large herd sizes had a negative relationship with ADWG. In addition, growth rates in female calves were significantly lower than in male calves. This model explained 16.7% of the observed variation in growth. Sublocation was added as a random effect and an intra class correlation coefficient of 0.091 obtained. The model diagnostic plots, shown in Appendix Figure E.1 show the error variance in the residual plots is constant, and a normal distribution of residuals (Q-Q plots) except for the calves with the highest and lowest growth rates.

To determine the relationship between infections and co-infections and ADWG, mean values for quantitative variables (eg. strongyle egg per gram count) were used. For categorical (infected, not infected) data, an animal was considered positive for each specific pathogen if it was ever identified at any point during the observation time. Data from Reverse Line Blots (RLBs) and serology diagnostic tests for viral infections were only available for samples collected at week 51.

The results of univariable screening with infection data and ADWG are presented in Appendix Table E.6. For the multivariable analysis using variables with p-value < 0.2, only variables with data over the one year observation was included. Data obtained only at week 51, for example RLB and viral serology were not included.

The results of the maximum and minimum adequate models for the infection data are presented in Appendix Table E.7. Variables in the minimum adequate models for the infectious and non-infectious factors were combined and multivariable analyses run to give the final model. All factors from the two minimum adequate models remained significant in the final model. A random term for sublocation was added to the final model to account for correlations of observations from animals in the same geographical location.

After including the non-infectious factors and carrying out the multivariable analysis, the final model revealed a significant negative association between *Calicophoron* spp., *Trichophyton* spp., and strongyle epg, see Table 5.5. This final model explains 29.2% of the total variation in growth. When sublocation was added as a random effect, the intra class correlation was 0.088 indicating 8.8% of the observed variation was explained by differences between sublocations. The model diagnostics plots are shown in Figure 5.5.

Table 5.5: Minimum adequate model with infectious and non-infectious predictor variables for ADWG. The model explains 29.3% of the observed variation (Adjusted R-squared).

variable	Estimate	Std. Error	df	t value	p-value
(Intercept)	-0.1246	0.0658	426	-1.90	0.059
log(Tropical livestock units)	-0.0074	0.0029	426	-2.50	0.013
(Mean heart girth size - dam/100)	0.0948	0.0321	426	2.95	0.003
(Elevation/1000)	0.0985	0.0428	426	2.30	0.022
Calf sex - female	-0.0105	0.0037	426	-2.86	0.0045
Body condition score - dam	0.0498	0.0111	426	4.48	< 0.001
Trichophyton spp.	-0.0231	0.0071	426	-3.26	0.001
Calicophoron spp.	-0.0144	0.0044	426	-3.27	0.001
(mean strongyle epg/1000)	-0.0225	0.0031	426	-7.26	< 0.001
Random effects					
Group	name	Std Dev	Variance		
Sub-location	Intercept	0.0119	0.0001		
Residual		0.0382	0.0015		

Intraclass correlation coefficient = 0.088

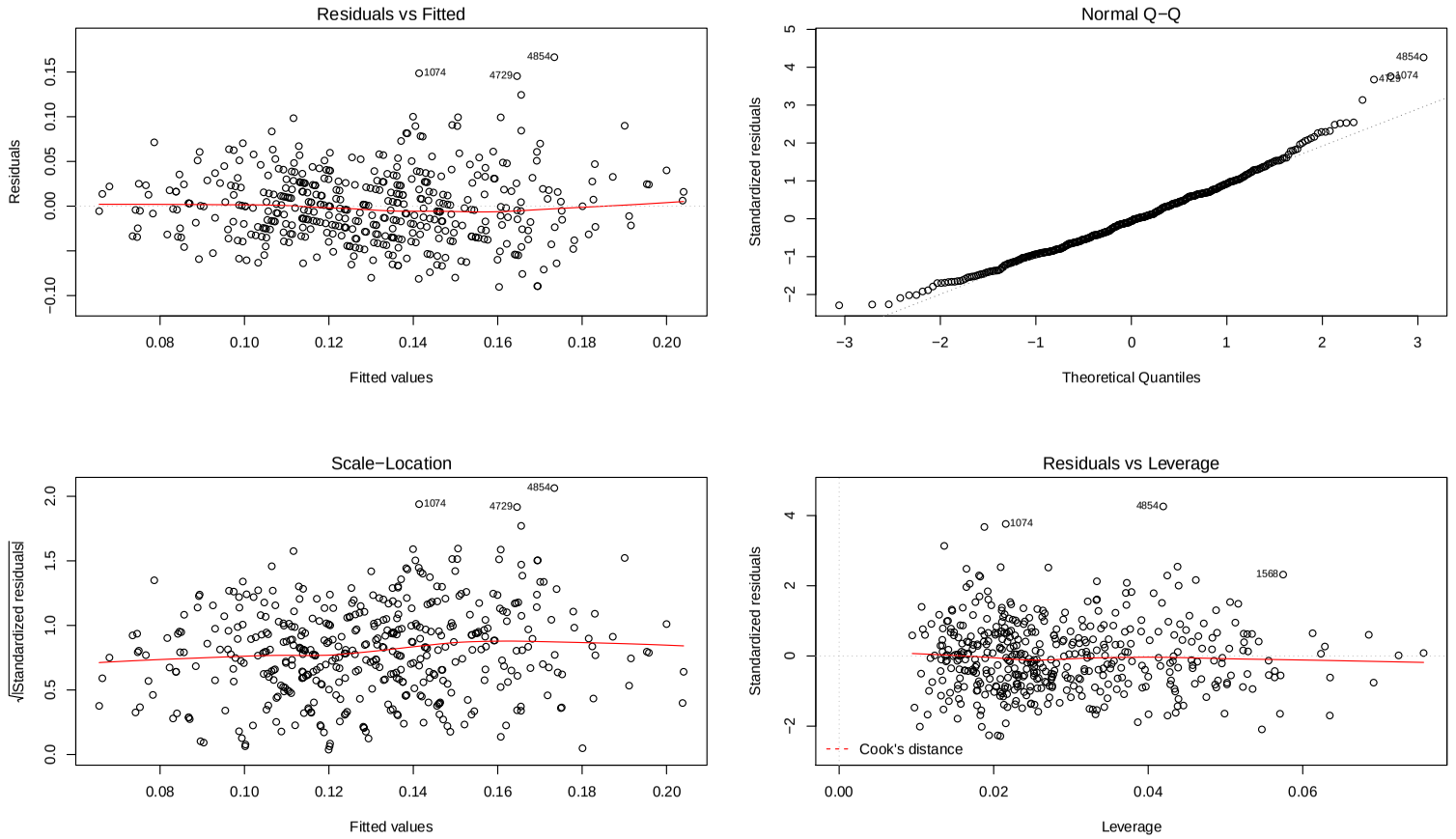


Figure 5.5: Model diagnostics for the final univariate model. The variance was constant (see Residuals vs Fitted plot), and the distribution of daily weight gain residuals was normal (see Normal Q-Q), except for calves with high growth rate. The Scale-location plot does not show any trend in the residuals. The labelled points identify possible outliers - animals with the highest growth rates. The residuals vs leverage plot does not show any outliers with a strong influence in the model, which would be identified by Cook's distance. This model explains 29.2% of observed variation in growth rates.

5.3.2 Multi-level models

5.3.2.1 Unconditional growth curve model

Using the alternative models for assessing change to those described in Section 5.2.3.1, this section reports the results of analysis using the multi-level mixed models. The initial analysis was to determine the growth curve function that best described growth in zebu cattle under one year. Here I begin by identifying the most promising non-linear model. The second step identifies the best linear model. The third step identifies the correlation structure that best describes the relationship between repeated measures within a calf. Fourth, I compare the best linear and best non-linear model to identify the best unconditional growth model to use in the analysis of impact of infections and coinfections on growth rates.

The decision on the growth function to adopt for analysis of factors affecting growth was based on a formal examination of model fit using different growth functions, examination of graphical growth trajectories, and the ease of interpretation of the growth parameters from the models.

Based on information criterion statistics (smallest AIC and BIC) and the largest log-likelihood, the 3 parameter Brody's growth model was selected as the choice non-linear model with best fit among the candidate models, see Table 5.6. The predicted mean growth curve using the Brody's function is shown in Appendix Figure E.2.

A common linear algebraic form was assumed to describe all subject's change trajectories. Linear mixed models with different random effect structures were compared to identify the model with best fit. Three models were fitted a) model with only a varying intercept for each subject, b) model with both a varying intercept and varying slope for each subject, and c) model with a varying intercept and varying slope for each subject and a varying intercept for the sublocation.

A significant improvement in fit was achieved when a varying slope was added to a model with a varying intercept only. However, adding sublocation as a random effect by fitting a varying intercept for the sublocation resulted

in a lower AIC but higher BIC compared to the model with varying intercept and varying slope. In cases where AIC and BIC contradict each other, the differences between the competing models are generally too small and difficult to make a clear-cut decision. The results are shown in Table 5.7.

To improve model fit, different serial correlation structures were included in the model with varying intercept and varying slope and the models compared, see Table 5.8. The best fit linear model (lowest AIC and BIC values, and highest log-likelihood) was identified as the model with a varying intercept and varying slope, and assuming a moving average correlation structure (Model E in Table 5.8). Adding sub-location as a random effect (Model F) did not significantly improve the model fit. Model E had a better fit compared to the selected best (Brody's) non-linear model (model G).

Based on the information criterion statistics, visual examination of growth trajectories (see Figures Figure 5.3 and 5.6) and the ease of interpretation of model parameters, the linear growth model with a varying slope and intercept and assuming a moving average correlation structure (model E) was selected as the unconditional growth model. This was subsequently used in the analysis of infectious and non-infectious factors associated with growth rates in zebu calves.

Table 5.6: Fit of non-linear functions to weight data

Function	Equation	AIC	BIC	Log likelihood
Gompertz's	$W_t = Ae^{-Be^{-kt}}$	25429.4	25453.9	-12710.7
Logistic	$W_t = A/(1 + Be^{-kt})$	25444.1	25468.6	-12718.0
Michaelis-Menten's	$W_t = At/(1 + Bt)$	26774.2	26792.6	-13384.1
Brody's	$W_t = A - Be^{-kt}$	25416.1	25440.7	-12704.1

W_t = weight of calf at t age in days
A = weight at maturity, asymptotic limit of weight when age (t) approaches infinity
B = constant of integration
e = base of the natural logarithm
k = maturing rate, relates to how quickly W_t approaches A.

Table 5.7: Comparison of unconditional linear growth models with different structures of the random effects (weight in kilograms).

	model1	model2	model3
Initial weight	20.770***	20.800***	20.785***
	(0.407)	(0.229)	(0.304)
Daily growth rate	0.129***	0.129***	0.129***
	(0.001)	(0.002)	(0.002)
Log-likelihood	-11785.2	-10581.1	-10578.9
Deviance	23570.4	21162.2	21157.8
AIC	23578.4	21174.2	21171.8
BIC	23602.9	21211.1	21214.8
p-value		< 0.001	0.03
N	3429	3429	3429

Model 1: Weight $\sim$ age + (1 | Animal) - fit varying intercept for each animal
Model 2: Weight $\sim$ age + (age | Animal) - fit varying intercept and slope for each animal
Model 3: Weight $\sim$ age + (1 | Sublocation) + (age | Animal) - fit varying intercept for each sublocation and varying intercept and slope for each animal.
*** p-value < 0.001

Table 5.8: Comparison between linear models with different correlation structures, and between best linear and non-linear growth models. The best model is coloured blue.

Model	df	AIC	BIC	logLik	Test	L.Ratio	p-value
Model A	6	21174.23	21211.07	-10581.12			
Model B	7	21176.23	21219.21	-10581.12	A vs B	0.00	1.00
Model C	8	20727.00	20776.12	-10355.50	B vs C	451.24	< 0.001
Model D	9	20665.75	20721.01	-10323.87	C vs D	63.25	< 0.001
Model E	10	20622.63	20684.03	-10301.31	D vs E	45.12	< 0.001
Model F	11	20622.10	20689.64	-10300.05	E vs F	2.53	0.112
Model G	4	25416.13	25440.68	-12704.07			

Model A. lme(fixed = Weight ~ age, random = (~age |CalfID)).
Model B. lme(fixed = Weight ~ age, random = (~age |CalfID), correlation = corAR1()).
Model C. lme(fixed = Weight ~ age, random = (~age |CalfID), correlation = corARMA(q = 2)).
Model D. lme(fixed = Weight ~ age, random = (~age |CalfID), correlation = corARMA(q = 3)).
Model E. lme(fixed = Weight ~ age, random = (~age |CalfID), correlation = corARMA(q = 4)).
Model F. lme(fixed = Weight ~ age, random = list(~1 | Sublocation, ~age | CalfID), correlation = corARMA(q = 4)).
Model G (Brody's non-linear model). nls(Weight $\sim$ SSasymp(age,A,B,k)) 3-parameter non-linear asymptotic regression model.
corAR1() = autoregressive correlation structure.
corARMA(q) = moving average correlation structure.

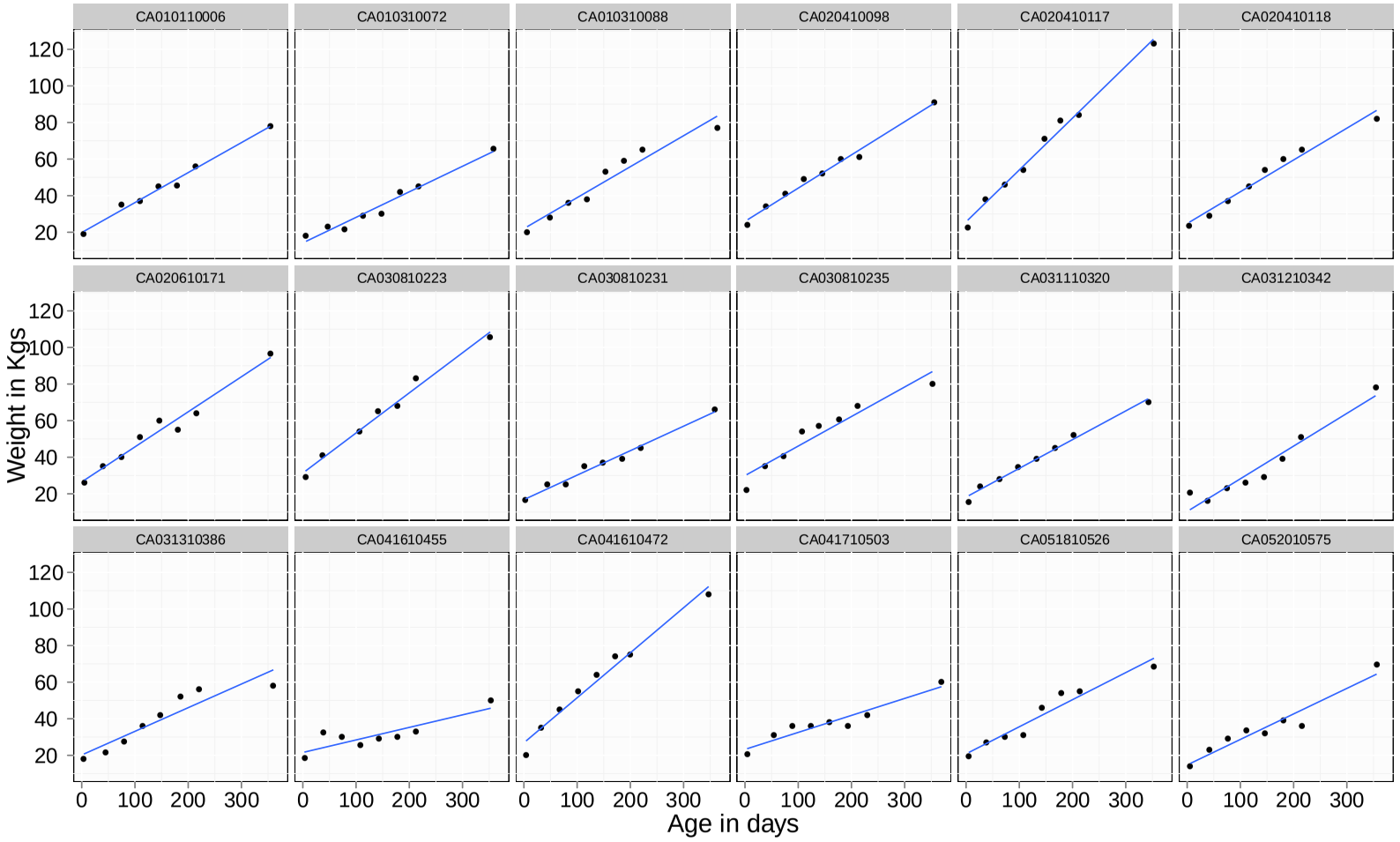


Figure 5.6: Growth trajectories of randomly selected calves from the IDEAL cohort showing differences in the intercepts and slopes (regression line). A linear regression fits each calf's data well and the non linear growths seen on some calves are not consistent in shape.

5.3.2.2 Non-infectious predictors of growth rate

Unlike the previous analysis that only allowed time-varying predictors to be incorporated as means or a single change of status, here values of predictors corresponding to each calf visit were used. A univariable screen of the non-infectious variables was done and their association with the growth rate (slope) presented in the Appendix Table E.8. Factors from this initial screening with a p-value ≤ 0.2 were offered to the multivariable analysis and model simplification through backward selection (slopes) carried out until only factors significant at $p < 0.05$ remained in the model.

Large herd sizes were negatively associated with growth rate. Calves from large dams (large heart girth sizes in dams) with a large birth weight (high recruitment weights in calves) had a relatively higher growth rate. Additionally, high NDVI values and high altitude of the farm were associated with higher growth rates. Calves from farms where the farmer was salaried had a higher growth rate compared to farms where the farmer was not salaried. The results of the minimum adequate model for non infectious factors associated with growth rate are provided in Table 5.9.

Table 5.9: Minimum adequate model for non-infectious factors associated with growth rate (slope) in kg/day.

	Value	Std.Error	DF	t-value	p-value
Age in days	0.1354	0.0031	2297	44.31	<0.001
(Tropical livestock units/10)	-0.0079	0.0036	2297	-2.18	0.03
(Heart girth size - 135)/10	0.0137	0.0018	2297	7.61	<0.001
Occupation - salaried	0.0140	0.0066	2297	2.12	0.034
(Elevation - 1240)/100	0.0081	0.0039	2297	2.10	0.036
(Recruitment weight - 19.2)	0.0014	0.0006	2297	2.27	0.024
(Mean NDVI - 0.62)*10	0.0158	0.0058	2297	2.71	0.007

Dam heart girth size (in cms) , elevation (in meters), recruitment weight (in kgs) and mean NDVI have been centered around their means to aid interpretation.

5.3.2.3 Final model: predictors of growth rate

Initially all the infectious factors identified from the routine visits were run as univariable analysis to determine their effect on growth rate, see Appendix Table E.9. From this analysis, infections with *Theileria* spp. (detected through microscopy), and exposure (serology results) to *T.parva, T.mutans, A.marginale* and *B.bigemina* were all associated with decreased growth rate. Additionally, interactions between *T.parva* and *T.mutans*, and between *T.parva* and *A.marginale* were found to be significantly associated with growth rate.

Compared to uninfected animals, animals that tested positive for coccidia oocysts and those infected with the fungus *Trichophyton* spp. had a decreased growth rate. Infections with the helminth species *D.viviparous, H.placei, O.radiatum, T.axei*, *Fasciola* spp., *Moniezia* spp., *Trichuria* spp., *Strongyloides* spp. and strongyle epg were all negatively associated with growth rate.

All infectious factors with a p-value <0.2 were offered to a model containing the significant non-infectious factors in Table 5.9. Model simplification through backward selection based on the slopes was done until only significant factors (p-value <0.05) remained in the model. The dropped terms were then added back to the model one at a time, and model fit re-examined until a minimum adequate model was identified. The results of the final model are presented in Table 5.10, and the model diagnostic plots in Figure 5.7.

The growth rate was estimated at 134.7 grams/day (equivalent to 49.2 kg weight gain in a year). Several non-infectious factors were identified to be statistically associated with growth rate. Calves in farms providing drinking water to the livestock within the homestead had significantly higher growth rates, estimated at 4.2 kg (in a year) more gain than calves in farms accessing drinking water a distance away from the homestead.

Calves in farms where the farmer had a salaried income had higher growth rates and the model estimates that a calf from such a farm would have gained 8.3 kg more in a year compared to farms where the farmer wasn't salaried.

Table 5.10: Minimum adequate mixed model results showing the significant infectious and non-infectious associated with growth rate (kg/day) in zebu calves under one year. Dam heart girth size and farm altitude (elevation) were centered around their mean values to facilitate interpretation.

	Estimate	lowerCI	upperCI	DF	t-value	p-value
Intercept						
Initial weight estimate	20.8551	19.3443	22.3659	1055	27.09	< 0.001
(Heart girth size - 135)/10	-0.4644	-1.4028	0.4740	1055	-0.97	0.332
Occupation - salaried	-1.2303	-3.6853	1.2247	427	-0.99	0.325
(Elevation - 1240)/100	0.8716	-0.4557	2.1988	427	1.29	0.198
Watering at homestead	0.8052	-0.8011	2.4116	427	0.99	0.325
Calf sex - female	-0.0584	-1.6541	1.5373	427	-0.07	0.943
T.parva seropositive	1.1094	-0.3547	2.5735	1055	1.49	0.137
T.mutans seropositive	-0.3396	-1.6373	0.9581	1055	-0.51	0.608
A.marginale seropositive	2.4094	0.2477	4.5711	1055	2.19	0.029
Trichophyton spp.	1.0961	-2.2540	4.4462	1055	0.64	0.521
(Strongyle epg/1000)	0.2871	-0.1473	0.7215	1055	1.30	0.195
Growth rate (slope)						
Age in days	0.1347	0.1238	0.1456	1055	24.28	< 0.001
Age:(Heart girth size - 135)/10	0.0159	0.0102	0.0216	1055	5.49	< 0.001
Age:Occupation - salaried	0.0227	0.0075	0.0378	1055	2.94	0.003
Age:(Elevation - 1240)/100	0.0115	0.0031	0.0200	1055	2.68	0.007
Age:Watering at homestead	0.0116	0.0014	0.0219	1055	2.23	0.026
Age:Calf sex - female	-0.0130	-0.0231	-0.0029	1055	-2.52	0.012
Age:*T.parva* seropositive	-0.0184	-0.0284	-0.0084	1055	-3.62	< 0.001
Age:*T.mutans* seropositive	-0.0021	-0.0120	0.0079	1055	-0.41	0.683
Age:*A.marginale* seropositive	-0.0021	-0.0144	0.0102	1055	-0.33	0.741
Age:*Trichophyton* spp.	-0.0254	-0.0468	-0.0039	1055	-2.32	0.021
Age:(Strongyle epg/1000)	-0.0044	-0.0069	-0.0018	1055	-3.38	< 0.001
Age:*T.parva:T.mutans*	0.0115	0.0037	0.0193	1055	2.90	0.004
Age:*T.parva:A.marginale*	-0.0113	-0.0193	-0.0033	1055	-2.76	0.006
Random effects						
Groups	Name	Variance	Std.Dev.			
Calf	(Intercept)	36.7253	6.0601			
Calf	Age	0.0016	0.0406			
Residual		9.7398	3.1209			

Intra-class correlation = 0.79

Female calves are estimated to gain 4.7 kg less compared to male calves in a year. Large heart girth size in the dams was associated with higher growth rates, a 10 cm deviation from the mean girth of the population was estimated to result in an extra 5.8 kg gain in a year from the average. An increase in the altitude of the farm by 100 meters was associated with a 4.2 kg higher gain in weight in a year.

Controlling for the effects of non-infectious factors, infections with helminths (strongyle epg), with fungi *Trichophyton* spp., and with *T.parva* had a significant negative association with growth rate. Additionally, there was evidence of coinfection interactions of different sizes and direction; antagonistic interactions between *T.parva* and *T.mutans*, and synergistic interactions between *T.parva* and *A.marginale*.

Calves that were ever seropositive for *T.parva* were estimated to on average gain 6.7 kg less compared to animals that did not seroconvert in the one year observation time. This is the equivalent of 13.7% decrease in average growth rate associated with *T.parva* ever seropositivity. The model estimate for the effect of infection with *A.marginale*, while controlling for all other significant predictors, was marginal decrease in growth (0.7 kg difference in weight gained over one year compared to uninfected animals). However, animals coinfected with *T.parva* and *A.marginale* had an estimated growth rate lower than the combined negative effects of each infection, a synergistic interaction. Calves coinfected with the two were estimated to have gained 11.6 kg less in one year compared to uninfected animals, equivalent to 23.6% less average growth rates for uninfected animals.

Coinfections between *T.parva* and *T.mutans* were antagonistic with the effect on growth rates of the more pathogenic *T.parva* infections moderated in the presence of *T.mutans*. Whereas the weight gain of *T.parva* seropositive calves was estimated to reduce by 6.7 kg over a year, animals seropositive for both *T.parva* and *T.mutans* were estimated to have a weight gain only 3.3 kg less that of uninfected animals. This is equivalent to a 6.7% decrease in average growth rate associated with *T.parva*-*T.mutans* coinfections, approximately half (13.7%) that estimated for a *T.parva*-only infection.

Infection with *Trichophyton* spp. was associated with reduced weight gain estimated at 9.3 kg less the average weight gain in a year, equivalent to 18.9% decrease in average growth rate. An increase in strongyle epg by a count of 1000 eggs was associated with a decrease of 3.3% in the average growth rate, estimated at 1.6 kg less total gain in a year.

A schematic diagram (Figure 5.8) shows the relationship between the daily weight gain (slope in the models) and the various significant factors identified in the study. Particularly, there is evidence of co-infection interactions of different effect sizes and direction. Adding sublocation as a random effect did not significantly improve the model fit.

The intra-class correlation coefficient was used to determine the fraction of the total residual variation that was accounted for by differences between calves, also interpreted as the correlation among observations within the same calf (Weir, 2005). The calculated ICC was 0.79 indicating 79% of the residual variation in growth rates was accounted for by between calf differences, the remaining being error.

The final model was re-run using base unconditional growth models assuming simpler correlation structures (moving average with fewer q parameters, and auto-regressive structure). This did not alter the model coefficients either in direction or strength, suggesting that variation between profiles of infection experiences largely accounted for the sequential correlations.

An alternative mixed model package (lme4) in R that assumes a compound symmetry correlation structure was used to fit covariates in the final model above, as confirmatory analysis. The p-values for this model were based on 10,000 MCMC samples generated from the posterior distribution of the fitted mixed model parameters. The model results are provided in Appendix Table E.10, and Appendix Figure E.3 showing the posterior density of the parameters. The results from this MCMC sampling method were similar with those of the previous models.

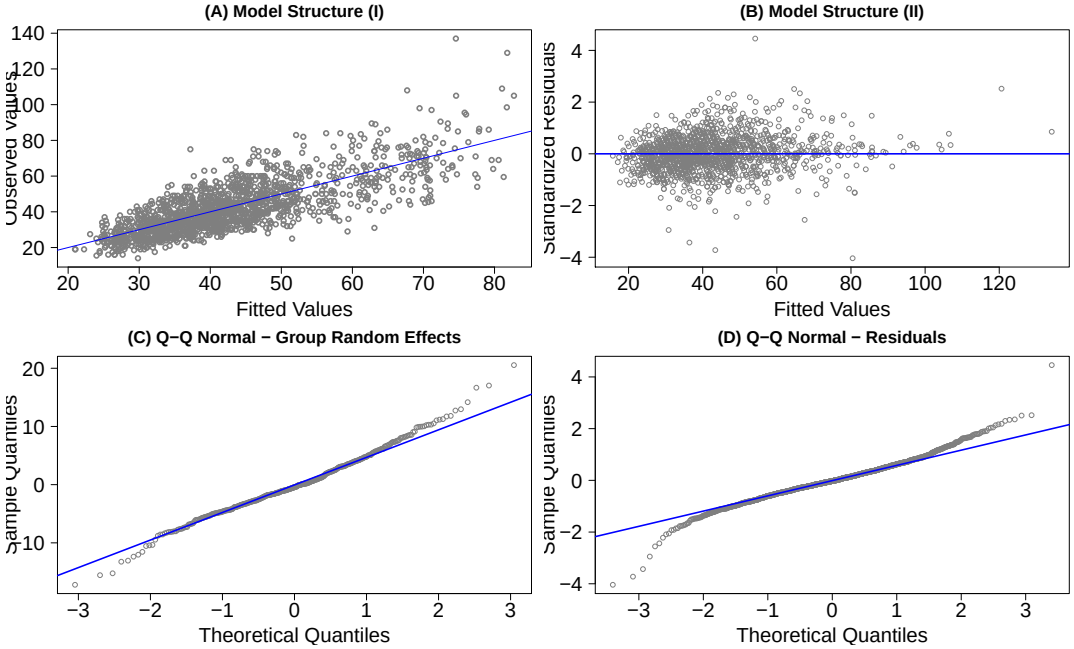


Figure 5.7: Diagnostic plots for the minimum adequate mixed model, Table 5.10. Plot (A) shows the observed values and the fitted values conditional only on fixed effects. It indicates the explanatory power of the model. Plot (B) of fitted values and Pearson's residues conditional on both fixed and random effects. Plots (C) and (D) are qq-plots that check if the estimated random effects and Pearson's residues respectively are normally distributed with constant variance. The within-group error residues are constant and the normality assumptions are not violated.

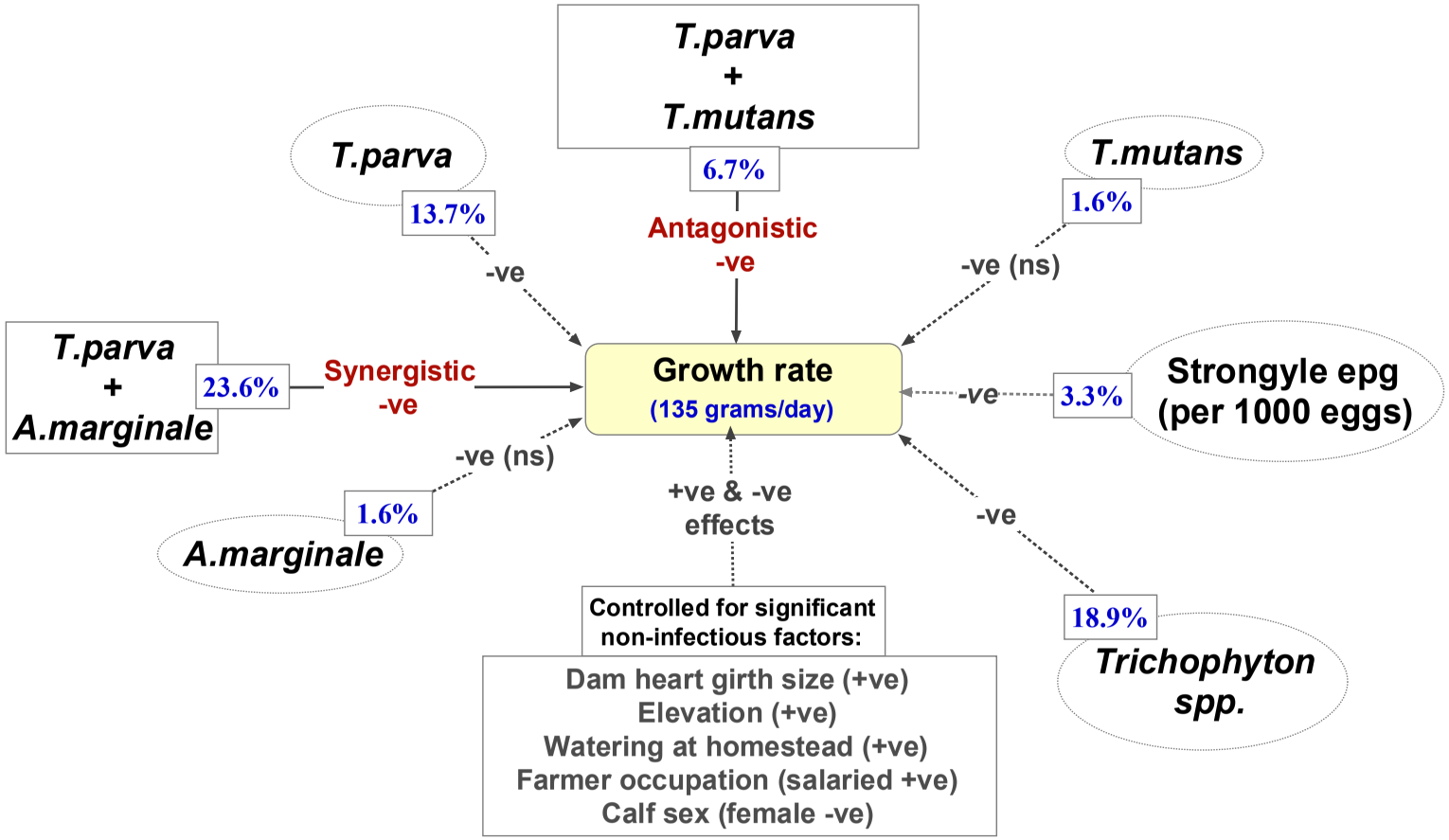


Figure 5.8: Schematic diagram showing associations between average daily weight gain and different infections and co-infections. Negative associations with ADWG have the sign (-ve), and positive (+ve). All single infections have a negative effect on ADWG. The size of the effect expressed as a percentage of the average growth rate in uninfected animals is shown in blue. Co-infections of *T.parva* and *A.marginale* have a significant negative effect (synergistic) on ADWG, above the sum of their individual effects. Animals co-infected with *T.parva* and *T.mutans* have a significant positive interaction (antagonistic), with average growth rates in coinfected animals higher than in animals infected with *Theileria parva* only. The model controls for the non-infectious factors. (ns) = non-significant effect.

5.4 Discussion

This study has investigated factors that determine growth rates in zebu cattle under one year of age, specifically determining the impact of infections and their co-infections on growth rates.

In this study growth during the first year of life was best described as linear, with an approximately constant growth rate estimated at 134.7 grams/day. This rate was slightly higher than that reported by Latif et al. (1995) (120 grams/day) among zebu calves in Lake Victoria's Rusinga Island, Western Kenya. The range of growth rates was however very wide starting from as low as 30 grams/day to as high as 340 grams/day. Although these growth rates were much lower compared to those observed in smallholder farms in parts of Central Kenya (240-290 grams/day) mainly keeping improved breeds (Gitau et al., 2001), some of the fastest growing animals in the current study gained more than 300 grams per day.

This finding of some indigenous zebu cattle growing at rates higher than improved breeds in smallholder settings, if it has a genetic basis, points to possible opportunities for improved livestock production through within-breed selection. Despite the fact that when compared to selection between breeds or cross-breeding, within-breed is considered a slow method for genetic improvement, it is more permanent and cumulative in areas of high disease pressures where other breeds easily succumb to disease (FAO, 2007). The smallholder farms in Central Kenya predominantly keep improved breeds as opposed to those in Western Kenya keeping zebus, but do not themselves meet the recommended target growth rates for dairy farms of 400-500 grams/day (Gitau et al., 2001; Heinrichs and Radostits, 2001).

The initial analysis was carried out to identify factors with information available at calf recruitment time ("recruitment" model) that were predictors for average daily weight gain (Table 5.4). In cases where information available at calf recruitment time is a good predictor for calf growth, it may be useful in early decision making on selection of animals for use in breeding programs. From this analysis, dam factors (heart girth size and body condition score),

herd size (total livestock units) and calf sex were all significantly associated with average daily weight gain, explaining 15.4% of the observed variation in growth. Calves from dams with large heart girth sizes and body condition scores had relatively higher growth rates, possibly relating to better genetics, or the quality of care given to the calf, or both. The association between large herd sizes and decreased growth rates may indicate greater probability of infection with increased animal-animal contacts associated with large herds. Large herd sizes have previously been associated with lower growth rates and thought related to the little attention given to individual calves when in a large herd (Gulliksen et al., 2009). From this information, a calf from a large dam with a high body condition score and reared in a relatively small herd would grow faster and likely attain breeding weight sooner.

The model investigating both time-varying and time-invariant non-infectious factors (Table 5.9) identified herd size, heart girth size in the dam (both of which were predictors in the "recruitment" model), farm elevation, recruitment weight, farmer occupation, and NDVI to be the non-infectious predictors significantly associated with growth rates. All except herd sizes were positively associated with growth. NDVI is a measure of the vegetation health and density and is used as a proxy measure of environmental conditions. In this case, it is associated with increased growth as would be expected if high NDVI values indicate greater availability of feed for the animals. This variable's effect is not significant when infection data is included which may point to a connection between infections and NDVI.

Animals with large recruitment weights were predicted to grow faster, although this effect was not observed when infection data was included (Table 5.10). Large recruitment weights may be associated with genetics; univariable analysis identified substantial European introgression to be associated with higher growth rates. This effect of European introgression was however lost in models including the dam heart girth size and calf recruitment weight which would be expected to capture some of the variation due to genetics.

The final model (Table 5.10) allowing for the infectious factors revealed watering practice, farmer occupation, heart girth size, farm elevation (alti-

tude), and calf sex to be the important non-infectious factors significantly associated with growth rates. Herd size and NDVI were lost as predictors in the model containing infection data, and instead watering at homestead was identified as significantly associated with higher growth rates. Providing drinking water at the homestead rather than walking animals a distance away from the homestead to access water may be a correlate of lower infection risk. Watering animals at the homestead was associated with an estimated 8.6% higher growth rate. Growth rates in farms where farmers were salaried were on average 16.8% higher than in farms where farmers were not salaried. A salary income may be an indicator of a type of farm with relatively better overall husbandry practices, although this connection was not been directly made in the current analysis.

The heart girth size of the dam remained significantly positively associated with growth rate with infection effects included. Its effect size on growth is estimated at 11.8% for every 10 cm increase above the mean girth size. Calves from large dams have relatively higher growth rates and, as mentioned earlier, it may relate to both genetics and quality of care which may include amount of milk available to the calf. It was not possible to collect data on milk production in these dams, information that may help tease out an effect due to genetics and that due to better feeding.

The study area lay between an altitude of 1114 and 1446 meters above sea level. Growth rates in the higher altitude farms were higher than in the lower altitude, with the estimated effect size of 8.5% increase in average growth rate for every 100 meters rise in altitude. Altitude is associated with climatic conditions and its correlate in the model was the "northing" indicating a possible differential disease pressure. Previous analysis in this thesis showed existence of a spatial gradient in the force of infection with *T.parva*, with the disease pressure being higher in sublocations located in the southern region (lower altitudes). One would expect by including *T.parva* effect in the model, if it is a proxy measure for *T.parva* infection pressure, the effect of altitude would disappear. In this case, it remains significant in the model meaning it captures more variation other than due to infection alone.

While controlling for the important non-infectious factors, this study has identified gastro-intestinal worm burden (strongyle epg), a dermatophyte (*Trichophyton* spp.) and tick-borne disease *T.parva* with its coinfections with *T.mutans* and *A.marginale* to be the important infections associated with greatest negative impact on growth rates.

The effect of strongyle eggs is estimated to be 3.3% decrease in growth rate for every 1000 increase in eggs per gram of faeces. Some study calves had high helminth burden reaching up to 12,000 epg, meaning the decrease in growth rate due to worms could well be over 30% in heavily infected calves. By colonising the gastro-intestinal tract, helminth infections lead to inefficient feed utilization, and in certain cases such as during infections with hookworms, result in pathological lesions on gastro-intestinal walls to which they attach. Here the negative effects of individual worm infections (for example *H.placei, T.axei* and *O.radiatum* were all found to negatively affect growth) in the model are masked when strongyle egg counts are introduced as a variable, suggesting strongyle epg could be a good practical composite measure of worm burden in these calves.

Infection with the dermatophyte *Trichophyton* spp., although identified in only 7.8% of the calves, was associated with an 18.9% decrease in growth rate. Fungal infections will usually not cause clinical disease or be associated with weight loss but their effect is enhanced in immuno-suppressed hosts, a good example being in humans with AIDS. This association is however yet to be confirmed in animals (Blanco and Garcia, 2008). In this study, the animals identified infected with *Trichophyton* spp. were systemically affected and with stunted growth. This fungal infection may be the result of bad health as opposed to being the cause, with poor growth being the result of other underlying conditions.

Regarding tick borne diseases, seropositivity for *T.parva*, the protozoan parasite causing ECF disease, was associated with the greatest impact on growth estimated at 13.7% decrease in growth rate. No other tick-borne infection had a significant effect on growth rate in its own right. However, the results did reveal evidence of a coinfection interaction between *T.parva*

and *A.marginale*. The growth rate in animals seropositive with the two pathogens was decreased by 23.6%, a percentage greater than the effect of individual pathogens added together (15.3%), suggesting a synergistic interaction (harming the host more).

A.marginale is an intracellular rickettsia, transmitted by the *Boophilus* tick species, primarily infecting erythrocytes (Kocan et al., 2010). The parasitised red blood cells are phagocytosed by bovine reticuloendothelial cells resulting in anaemia and icterus without haemoglobinuria. Severe cases lead to death, and the survivors remain "carriers" with life-long immunity. The risk of clinical anaplasmosis in calves is low due to maternal immunity and the risk of clinical disease in subsequent infections following initial infections is diminished due to the life-long acquired immunity.

The results revealed evidence of a coinfection interaction between *T.parva* and *A.marginale*. The process by which interactions between *T.parva* and *A.marginale* occur are unclear and have not been investigated fully. McHardy and Kiara (1995) observed about 50% of clinical cases of ECF among improved breeds in Kiambu district, Kenya were complicated by *A.marginale* infections. Experimental studies from their work showed super-infection with *T.parva* resulted in a relapse of severe clinical anaplasmosis and severe anaemia even though *A.marginale* parasitaemia remained low.

Although these two pathogens have one of their life-stages in the erythrocytes, "crowding" effect interactions would be unlikely as the pathogenic phase of *T.parva* is more pronounced during the schizont phase which involves the lymphocytes. The two parasites are further apart on the evolutionary tree and do not share epitopes that would allow for cross-protection.

It may be possible that the effect observed is mediated through the immune system and related to an immune-suppression associated with the destruction of lymphocytes infected with *T.parva*. In the same study, McHardy and Kiara (1995) showed that calves super-infected with *T.parva* when the carrier state of *Anaplasma* had stabilised had mild anaplasmosis disease with moderate fall in PCV suggesting that ECF may interfere with the immunity to anaplasmosis. Experimental studies looking at immunological changes ac-

companied by these different infection profiles may help our understanding of the mechanisms by which *T.parva* and *A.marginale* may be interacting.

The second important *T.parva* co-infection was with *T.mutans*. Unlike interactions with *A.marginale*, having a *T.parva*-*T.mutans* coinfection was identified to be advantageous on host growth rate. Animals that were ever seropositive for *T.mutans* had an estimated decrease in growth rate of 6.7%, which was less than half the sum of the *T.parva* and *T.mutans* pathogen effects (15.3%), an antagonistic interaction.

It is worth noting that coinfection here means animals that were ever seropositive with *T.parva* and with *T.mutans*, and it is possible that animals that got infected at some point may not have the parasites at a future date. This data is based on serology results, and data based on presence or absence of parasites in blood, for example RLB across all time points would be useful to confirm results.

This result suggests the presence of *T.mutans*, considered a benign pathogen (Brocklesby et al., 1972; Coetzer and Tustin, 2004, page 480), may be reducing the negative impact exerted on host growth rate by the more pathogenic *T.parva* infection. The mechanisms by which these two *Theileria* species may be interacting are unclear and have not been reported before. Two possible mechanisms can however be postulated and investigated through experimental work: a) competitive interactions, and b) interactions through host protective immunity.

Competitive interactions would occur if the presence of *T.mutans* negatively affected *T.parva* parasite densities, thereby reducing the large impact *T.parva* has on host growth. The two *Theileria* species share near similar life-cycles and utilise similar host cells for their survival and transmission success. Infection in cattle follows inoculation of *Theileria* sporozoites through tick saliva, approximately 4 days after an infected tick starts feeding. The *T.parva* sporozoites immediately invade target cells (lymphocytes) in the nearest lymphoid tissue where they undergo asexual replication by transforming infected cells into an uncontrolled proliferation with each daughter cell infected with *T.parva* schizonts (Dobbelaere et al., 1988; Dobbelaere and

Heussler, 1999). This proliferation stage is the one associated with the observed ECF clinical signs and mortality, as proliferating lymphocytes invade all lymphoid tissue and infiltrate into non-lymphoid tissue (Morrison and McKeever, 2006).

T.mutans multiplication is thought to happen at the piroplasm stage within erythrocytes. Although the two parasites have their main multiplication in different cells types, the two parasites will be found both in lymphocytes and in erythrocytes. If competitive interactions were to occur, stages of *T.mutans* in the lymphocytes would have the greatest impact in reducing densities of the more pathogenic *T.parva*. It may be possible that the presence of *T.mutans* has an effect of controlling *T.parva* densities through competition for cell resources, and this is speculative and can be investigated. Such competitive interactions between parasites modifying densities of competing parasites have been demonstrated in other studies (Lello et al., 2004; Conlan et al., 2009).

The second possible mechanism by which these parasites could interact is through the host-immune system. This would be true if immune responses elicited following *T.mutans* infection offers some level of protection against subsequent *T.parva* infections. Currently, there is little evidence in immunology literature supporting this theory and to a large extent solid cross-protection against *Theileria* is thought to work only among homologous parasites.

Immunity against *T.mutans* however is largely unstudied, perhaps because *T.mutans* is benign and has attracted little interest among researchers. The protective immunity against *T.parva* is thought to occur in two ways: a) humoral immunity against the sporozoites injected by infected ticks, and b) cell-mediated immune responses against macroschizont infected cells which are thought to express surface antigens that can be targeted by effector killer T-cells. Effects of humoral responses are thought to be limited mainly due to the thousands of sporozoites a single infected tick injects in a host, and the rapidity with which the sporozoites enter target lymphoid cells. Studies have shown that endocytosis is complete in under 10 minutes, effectively limiting

the time injected sporozoites are accessible to the immune system (Urquhart, 1980; Ivan Morrison, 1984).

However, *in vitro* studies have demonstrated that antibodies against *T.parva* (Muguga strain) neutralized infectivity not just against homologous sporozoites but against other *T.parva* strains as well (Musoke et al., 1984). This finding is important even though it is still unclear how important these humoral responses are in reducing sporozoites infectivity *in vivo*, and whether the observed cross-protection between *T.parva* strains extend to other species such as *T.mutans* or vice-versa. This would offer a possible explanation to the observed beneficial effects of a *T.parva* co-infection with *T.mutans*. Experimental work looking at the immune responses with different combinations of the infections with these two *Theileria* species may help improve our understanding of these interactions.

The two *Theileria* spp. are transmitted by different ticks: *T.parva* by *R.appendiculatus* and *T.mutans* by *A.variegatum*. Experimental work by Purnell and Branagan (1970) demonstrated transmission success occurred only with *Amblyomma* species and not with *R.appendiculatus*. These two ticks share large geographical overlaps, supported by results of sero-surveillance studies done in different regions showing near similar prevalence rates for both *T.mutans* and *T.parva* (Deem et al., 1993; Swai et al., 2005, 2009; Gachohi et al., 2010). This widespread co-occurrence of the two *Theileria* species may indicate that although *T.parva* is still associated with great losses in livestock, the effect is moderated to an extent by co-occurrence with *T.mutans*.

In this chapter, two approaches on data analysis were used; the first using a summary measure ADWG obtained using the difference in live weight at recruitment time and at end of follow-up period for each study calf, and the second approach using mixed effects models that take account of all data collected.

The first approach is fairly straight forward and easy to implement but has the limitation that it does not utilise all the available data, compromising its power to detect effects. This is evidenced by the results of the minimum

adequate models using the two methods. Whereas the two identify fairly the same non-infectious predictors, the main difference was in the model's ability to detect effects of time-varying predictors, in this case infections and their coinfections. Using a summary measure (ADWG) method assumes effects of infection events is the same regardless of when the animal was infected. Secondly, continuous variables are incorporated in the models as mean values (over the study animal's observation time), in which case the model cannot pick time-dependent effects.

The repeated measures (mixed effects) approach, is more difficult to implement but has the advantage of utilising data collected over the year as opposed to the start and finish points only. Using this approach, time-varying predictors as infection events are allowed into the model flexibly such that their effects are modelled to only occur from the time of infection onwards. If correctly implemented these models are more robust and safeguard against inaccurate and misleading results (Pollitt et al., 2012).

The summary measure approach was not able to detect the effects associated with tick-borne diseases, and which accounted for a large percent decrease in growth rates. Unlike data on helminth burden which is available from very early in life, seroconversion with any of the four tick-borne infections occurred relatively later in the year, which the summary measure method was unable to detect. It however, like the mixed effect models, was able to detect the negative effects associated with strongyle epg and *Trichopyton* spp. Although workings of mixed models are relatively challenging to understand and implement, they present the more flexible and appropriate analysis of longitudinal data (Paterson, 2003; Telfer et al., 2008). Some of the results may be hard to interpret when they appear to show reverse causation in time, for example *A.marginale* effect on the intercept. This is however a logical impossibility as infection with *A.marginale* is not possible at first week of life, and the intercept effect may not be interpreted. It may however be tested directly by determining whether the risk of seroconversion increases with recruitment weight. This effect was tested and it was not significant.

The results obtained here identify simple farm management practices that would help improve the growth rates of zebu calves. Although it is not entirely clear how providing drinking water to the animals from within the homestead works, this simple husbandry practice is estimated to be associated with preventing an estimated 8.6% reduction in growth rate compared to farms where animals walk a distance away from the homestead to access water. Secondly, since dam heart girth sizes were identified as good predictors for growth rate in calves, farmers or breeders can improve their decision making in the selection of animals to keep for breeding based on the relative dam sizes.

The two main infections, with high prevalences and strongly associated with decreased growth rate are helminths and *T.parva* infections. Although animals are infected with many different species of worms, this study has identified strongyle epg as a good composite measure quantifying the effect helminths have on the host. Data on strongyle epg is relatively easy and inexpensive to obtain, requiring a microscope which is easily adaptable for field conditions. A herd's helminth burden can be estimated and a decision on helminth control made based on the results. Here, the results show helminth control would increase growth rates by up to 30% for animals heavily infected.

Tick control would be expected not only to reduce the direct effects exerted by feeding ticks [the tick *A.variegatum* has been associated with decreased growth rates (Stachurski et al., 1993)] but also on the impact of pathogens they transmit. Specifically, it would be expected the beneficial effect would be not just reducing impact of *T.parva* but of the more harmful *T.parva*-*A.marginale* coinfections. The finding of *T.mutans* reducing the impact of the more pathogenic *T.parva* in cases of co-infections with the two parasites may be relationship that can be exploited to reduce impacts of *T.parva* infections. Such relationships have been used to control for *Anaplasmosis* where the more benign *A.centrale* has been used as a vaccine for the more pathogenic *A.marginale* (Kocan et al., 2010).

This study has identified *T.parva* and its coinfection with *A.marginale*, and heavy helminth infection based on strongyle epg count to be associated with

the greatest impact in growth rates. It demonstrates that although animals may survive a *T.parva* infection, the main cause of death among zebu calves as demonstrated in earlier chapters of this thesis, *T.parva* infection causes further losses in reduced growth rates, and that this effect is made worse in the presence of *A.marginale*. This information points to evidence that by reducing the prevalence of one pathogen, the benefit is likely greater beyond that estimated by just removing effect of individual pathogens. There is need to better understand the mechanisms by which *T.parva* interacts with *A.marginale* and with *T.mutans*, possibly through experimental work, as these provide opportunities for improved design of disease control strategies and increased livestock production.

Chapter 6

Risk factors for seroconversion to tick-borne diseases, trypanosomes and helminth worm burden

6.1 Introduction

In the previous analysis on mortality and its risk factors, and the factors determining growth rates in the zebu cattle under one year, different pathogen infections were identified to increase mortality rates and reduce growth rates. Specifically, serology results showing exposure to *T.parva* and its coinfections with *T.mutans*, *A.marginale*, and *Trypanosoma* spp., and high worm-burdens were particularly important, causing the greatest impact on growth and survival probabilities of calves to one year.

Infection with *T.parva* which causes East Coast Fever (ECF) disease, and high helminth burden as measured by strongyle eggs per gram (epg) of faeces, had a negative effect on growth rates and increased the hazard for calf mortality before reaching one year. Moreover, the study revealed that the risk for death due to ECF, the main aetiological cause of death accounting for 40% of the infectious disease mortality (ID-mortality), was itself significantly increased by helminth infections and that this was burden dependent. The risk of death to ECF was also increased 10 times in animals found coinfected with *Trypanosoma* spp.

A high infection intensity with *Theileria* spp. as identified through microscopy was associated with increased hazard for ID-mortality. However,

seroconversion (an indication of exposure and an immune response to infection) to *T.parva* was associated with decreased hazard for ID-mortality. Although animals that seroconverted to *T.parva* had higher probability of surviving to one year, the growth rates in *T.parva* seropositive animals were significantly lower compared to animals that remained seronegative to one year. Further, growth rates in animals that were coinfected with *T.parva* and *A.marginale* were significantly reduced compared to growth rates in animals infected with *T.parva* only. A *T.parva* coinfection with *T.mutans* was however found advantageous to the host, with statistically higher growth rates observed in animals coinfected with the two compared to those infected with *T.parva* only.

This chapter investigates the risk factors associated with these selected infections having the greatest impact on calf growth and survival. Specifically, it investigates the non-infectious and infectious factors associated with increased risk for seropositivity to *T.parva, T.mutans* and *A.marginale*, for infection with trypanosomes, and high strongyle egg counts.

6.2 Materials and methods

6.2.1 Seroconversion to tick-borne infections

Serum from blood samples collected from the jugular vein of the study animals at the recruitment and 5 week routine visits were tested for antibodies against *T.parva, T.mutans* and *A.marginale*. Indirect enzyme-linked immunosorbent assays (ELISA) for *T.parva* (Katende et al., 1998), *T.mutans* and *A.marginale* (Morzaria et al., 1999) were used.

Results from the ELISA tests were initially recorded as percentage positivity (PP) values based on a known positive sample, derived from Equation 6.1 . To determine the positive and negative samples, cut-off points (positive/negative threshold) provided in the references for ELISA tests were used. The cut-off points used for *T.parva*, *T.mutans*, *A.marginale* and *B.bigemina* were 20, 20, 15 and 15 respectively. The tests sensitivity and specificity

for the four tick-bornes *T. parva, T. mutans* and *A. marginale* are (94%, 94-99%),(99%, 99%) and (90%, 90%) respectively (Katende et al., 1998; Morzaria et al., 1999).

$$\frac{\text{Optical density of test sample}}{\text{Optical density of strong positive}} \times 100 \tag{6.1}$$

A seroconversion event was only declared if the 2nd and 3rd of 3 consecutive results were above the cutoff point, and were at least 5 points higher than the 1st result. This conditional seroconversion rule was developed in order to avoid false positives from maternally derived antibodies in the period after birth, and to capture a rising titre which would be indicative of exposure and activation of an immune response.

The outcome variable for each of the tick borne diseases investigated was its corresponding **sero-status** at each calf observation time. A sero-positive status was only declared if the conditions of the seroconversion rule described above were met.

6.2.2 Infection with *Trypanosoma* spp.

The screening for trypanosome infections was done on blood smears stained with Giemsa and from blood in EDTA bottles collected during the recruitment and 5 week routine calf visits. In addition to examining the Giemsa stained blood smears, the Haematocrit Centrifugation Technique (Woo, 1970), and dark ground microscopy (Murray et al., 1977) were used to improve detection rates and ability to differentiate between different *Trypanosoma* spp. The results from these techniques were available for every time point and have been used in the investigation of risk factors associated with infection with *Trypanosoma* species.

The outcome variable for infection with trypanosomes was **presence or absence of trypanosome infection** at every sampling point. Identification of trypanosomes by any of the above diagnostic methods was considered positive, and the absence of trypanosomes from all the above diagnostic methods

was considered negative. A variable "time to infection with trypanosome" was created which captured the age of the calf at which each positive case was identified.

6.2.3 Strongyle epg

The study aimed at collecting two faecal samples, directly from the rectum of the animal, during the recruitment visit, in every 5 week routine visit, and at one year before the calf left the study. The first of the 2 samples was processed and using the McMaster counting technique, coccidia oocytes, nematode and cestode eggs were identified and quantified as number of eggs per gram (epg) of faeces (Hansen and Perry, 1994). Nematode worms produce "strongyle-type" eggs which look similar morphologically and are difficult to differentiate at microscopy. To determine the helminth species producing these strongyle eggs, faecal cultures were run on the second faecal sample collected and larvae stage 3 (L3) identified. The strongyle epg count is used as a composite measure of nematode infections and their intensity.

The outcome measure used in this analysis was the **strongyle epg count**, recorded as a count measure for every faecal sample tested.

6.2.4 Statistical analysis

Tick-borne diseases and trypanosome infections

To determine factors associated with the risk of seroconversion for each of the three tick-borne diseases and infection with trypanosomes, Cox proportional hazard models described in Chapter 4, Equation 4.2 were used. These models were extended to allow for the inclusion of time-varying predictors, making it possible to estimate the effect infection events have on the outcome measure under study. In addition, frailty effects were included to account for the unobserved heterogeneity in risk among individuals from different geographical location, in this case study sublocations.

Strongyle egg counts

In modelling response variables that are counts, a Poisson probability distribution is assumed, conditional that the mean and the variance of the response variable are equal. Parasite count is often aggregated in certain individuals resulting in data with a variance exceeding the mean. Using Poisson distributions in such cases will lead to inaccurate estimates of variance terms, small standard errors and inflated probabilities of Type 1 error (Quinn and Keough, 2004; Wilson et al., 2004). The overdispersion may be caused by factors not measured in the study. A number of solutions aimed at improving model fit in over-dispersed data exist. One solution is to calculate an adjustment factor based on the estimate of the dispersion parameter and applying this on the standard errors (Gardner et al., 1995). The second solution is to assume a negative binomial distribution, and add a random term that captures the between-subject variation that remains unexplained (Gardner et al., 1995). A third solution is to use quasi-Poisson models which estimate the dispersion parameter k from the data instead of fixing it at 1 as in Poisson models (Quinn and Keough, 2004). The coefficient estimates for quasi-Poisson are the same as for standard Poisson models but adjusted for over-dispersion.

A fourth solution and which is preferred in this study is to assume a Poisson log-normal distribution for the strongyle egg count but add a random term for every individual observation to account for overdispersion that remains unaccounted for by the fixed effects and by the Poisson random variation around the mean strongyle epg per calf (Elston et al., 2001; Korsten et al., 2009). This method was preferred since it allows the fitting of random effects assigning observed overdispersion to various heterogeneity sources including individual calf heterogeneity and that associated with sublocations they are raised in. The model used by Elston et al. (2001) was adapted here, see Equation 6.2.

$$log(\mu_{ij}) = \alpha_j + \beta x_j + e_i + \epsilon_{ij} \tag{6.2}$$

where μ_{ij} is the strongyle egg counts for calf i observed at time j modelled via a log link, to have linear dependency on explanatory variables x

at time j. α_j is the baseline strongyle count when x=0. The error terms are assumed to have normal distributions with mean zero and variances α_e^2 and α_ϵ^2 for the calves and individual observations within calves respectively, $e_i \sim N(0, \alpha_e^2), \epsilon_{ij} \sim N(0, \alpha_\epsilon^2)$. The risk factors associated with strongyle egg counts are estimated by the fixed effects.

6.3 Results

6.3.1 Tick-borne diseases

Overall, 548 study animals were followed for a total of 481.1 calf years. During the one year observation period, the sero-prevalence was 73.3% CI(69.1 - 77), 75.1% CI(70.9 - 78.6) and 35.5% CI(31.1 - 39.7) for *T.parva, T.mutans* and *A.marginale* respectively. Seroconversion to *T.mutans* occurred in calves of younger age compared to those seroconverting to *T.parva*. The computed median survival time, time by which 50% of the animals have seroconverted, was 145 days CI (129 - 160) for *T.mutans* and 214 days CI (191 - 221) for *T.parva*, see Figure 6.1.

Time to *T.parva* seroconversion was statistically different between study sublocations (p-value < 0.001, log-rank test). The median age to seroconversion ranged from 143 days in Magombe East to 323 days in Karisa sublocation, see the map shown in Figure 6.2. Differences between sublocations were not observed for *T.mutans* and *A.marginale* (p-value > 0.05, log-rank test).

6.3.1.1 Risk factors for *T.parva* seropositivity

Results of univariable screening of non-infectious and infectious factors on *T.parva* seroconversion are provided in Appendix Tables F.1 and F.2. From the results of multivariable analysis, several factors were identified to have a significant association with the risk for *T.parva* seroconversion. The probability of seroconverting to *T.parva* was higher in suckling calves compared

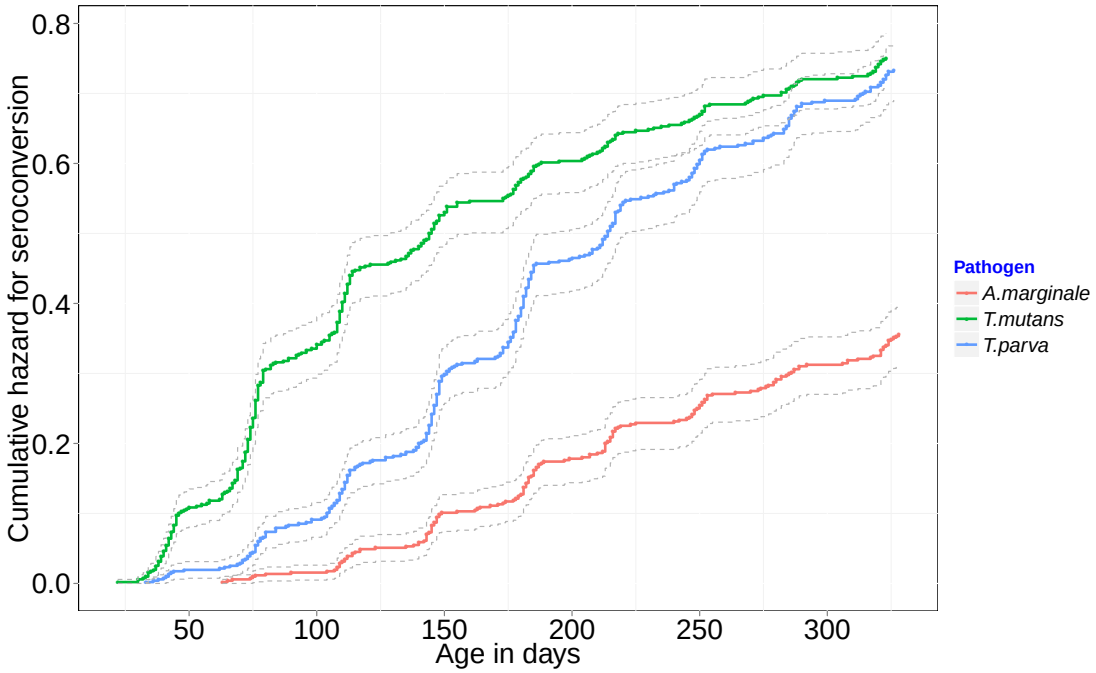


Figure 6.1: Cumulative hazard curves for sero-conversion to *T.parva*, *T.mutans* and *A.marginale*, the 3 major tick-borne diseases identified to have greatest effect on growth rates and survival probability to one year. Animals seroconverted to *T.mutans* relatively earlier than to *T.parva* but had similar levels by one year. Seroconversion to *A.marginale* was relatively at an older age compared to *T.parva* and *T.mutans*.

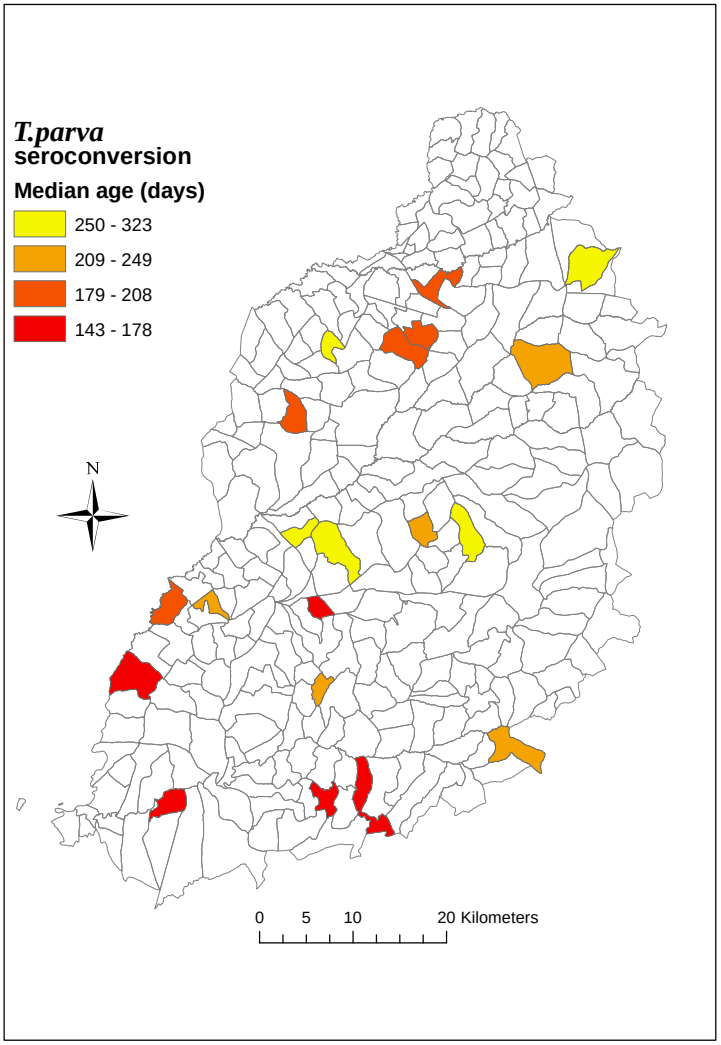


Figure 6.2: The median age to *T.parva* seroconversion showing differences between sublocations. Survival curves for the study sublocations were different statistically ($p < 0.001$, log-rank test). The latitude Northing was statistically associated with seroconversion to *T.parva*, the risk decreased with increasing latitude value.

to weaned calves, in calves with clinically healthy dams compared to sick dams, and in calves from dams with high antibody titres against *A.marginale*. Smaller herd sizes, provision of drinking water within the homestead, providing feed supplements and low NDVI values were all statistically associated with decreased probability for seroconversion to *T.parva*. The model identified infection with *Microfilaria* spp., *T.vitulorum*, *T.mutans* and the presence of eggs from *Strongyloides* spp. at the start of a risk period to be associated with an increase in the risk for *T.parva* seroconversion. However, infection with *H.placei* worms was associated with a reduced risk for *T.parva* seroconversion. The results showing the coefficients for these statistically significant factors are presented in Table 6.1.

Table 6.1: Results of multivariable analysis showing factors associated with seroconversion to *T.parva*.

Variable	coef	exp(coef)	lower CI	upper CI	p
Fixed effects					
Health of dam - sick	-1.023	0.359	0.149	0.878	0.024
Calf suckling - yes	0.317	1.372	1.139	1.651	< 0.001
A.marginale antibody - dam	-0.012	0.988	0.982	0.994	< 0.001
log(Total livestock units)	0.091	1.095	1.003	1.199	0.045
Watering at homestead	-0.250	0.779	0.690	0.881	< 0.001
Use of supplements	-0.310	0.733	0.639	0.841	< 0.001
Mean NDVI	-1.882	0.152	0.029	0.802	0.025
Haemonchus placei	-0.184	0.832	0.745	0.929	0.001
Microfilaria spp.	1.189	3.280	1.202	9.023	0.021
Strongyloides spp.	0.431	1.539	1.211	1.955	< 0.001
Toxocara vitulorum	0.649	1.913	1.301	2.813	< 0.001
T.mutans	0.178	1.195	1.056	1.350	0.004
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.2346	0.0551		

6.3.1.2 Risk factors for *T.mutans* seropositivity

Results of univariable screening of potential risk factors for *T.mutans* seroconversion are in Appendix Tables F.3 and F.4, for the infectious and non-infectious risk factors respectively. Results of the minimum adequate model are presented in Table 6.2. The relative risk of seroconverting to *T.mutans* were higher in calves from farms that reported receiving veterinary support, and from dams with high antibody titre against *B.bigemina*. Calves with a large recruitment weight and that had high total serum proteins had higher probability of seroconverting to *T.mutans*. Animals with substantial European introgression genes were less likely to seronconvert compared to the pure indigenous calves. *Cooperia* spp., infection with *Theileria* spp. (on microscopy) and presence of *Strongyloides* spp. eggs were all associated with increased hazard for seroconversion to *T.mutans*.

Table 6.2: Results of multivariable models showing factors associated with seroconversion to *T.mutans*.

Variable	coef	exp(coef)	lowerCI	upperCI	p
Fixed effects					
Veterinary support - yes	0.251	1.286	1.119	1.477	< 0.001
B.bigemina antibody - dam	0.003	1.003	1.000	1.005	0.014
Recruitment weight	0.018	1.018	1.005	1.032	0.008
Moderate introgression	0.072	1.075	0.927	1.246	0.340
Substantial introgression	-0.464	0.629	0.481	0.821	< 0.001
Heart girth size - dam	0.007	1.007	1.000	1.014	0.0376
Cooperia spp.	0.485	1.625	1.069	2.469	0.023
Theileria spp. level 1	0.157	1.170	1.050	1.305	0.005
Theileria spp. level 2	0.310	1.363	1.093	1.700	0.006
Theileria spp. level 3	0.234	1.264	0.404	3.956	0.688
Strongyloides spp.	0.291	1.338	1.123	1.593	0.001
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.2013	0.0405		

6.3.1.3 Risk factors for *A.marginale* seropositivity

Appendix Tables F.5 and F.6 show results of univariable screens for potential infectious and non-infectious factors associated with seroconversion to *A.marginale*. From the minimum adequate model, a number of factors related to farm-management practices including vaccine use in farms, accessing veterinary support, and provision of feed supplements were associated with increased hazard for seroconversion to *A.marginale*. Female calves were also more likely to seroconvert than male calves, but high farmer education level, large dam girth sizes, and farmer's reported knowledge of diseases occurring at the farm were associated with decreased likelihood for *A.marginale* seroconversion. Infection with *T.vitulorum* and *T.mutans* were found to be associated with the likelihood for seroconverting to *A.marginale*. Results of factors identified as having significant statistical association with seroconversion to *A.marginale* are shown in Table 6.3.

Table 6.3: Results of multivariable models showing factors associated with seroconversion to *A.marginale* seroconversion.

Variable	coef	exp(coef)	lowerCI	upperCI	p
Fixed effects					
Education-Primary school	-0.2519	0.7773	0.616	0.981	0.0338
Education-Secondary school	-0.4873	0.6143	0.465	0.812	< 0.001
Supplements use	0.2695	1.3093	1.010	1.698	0.0420
Vaccine use	0.3982	1.4892	1.243	1.785	< 0.001
Veterinary support	0.4577	1.5804	1.128	2.214	0.0078
Knowledge of diseases	-0.4719	0.6238	0.470	0.828	0.0011
Dam girth size	-0.0192	0.9809	0.969	0.993	0.0020
Calf sex - female	0.2396	1.2708	1.074	1.503	0.0051
T.vitulorum	0.8814	2.4144	1.305	4.468	0.0050
T.mutans	0.5280	1.6955	1.373	2.094	< 0.001
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.421	0.177		

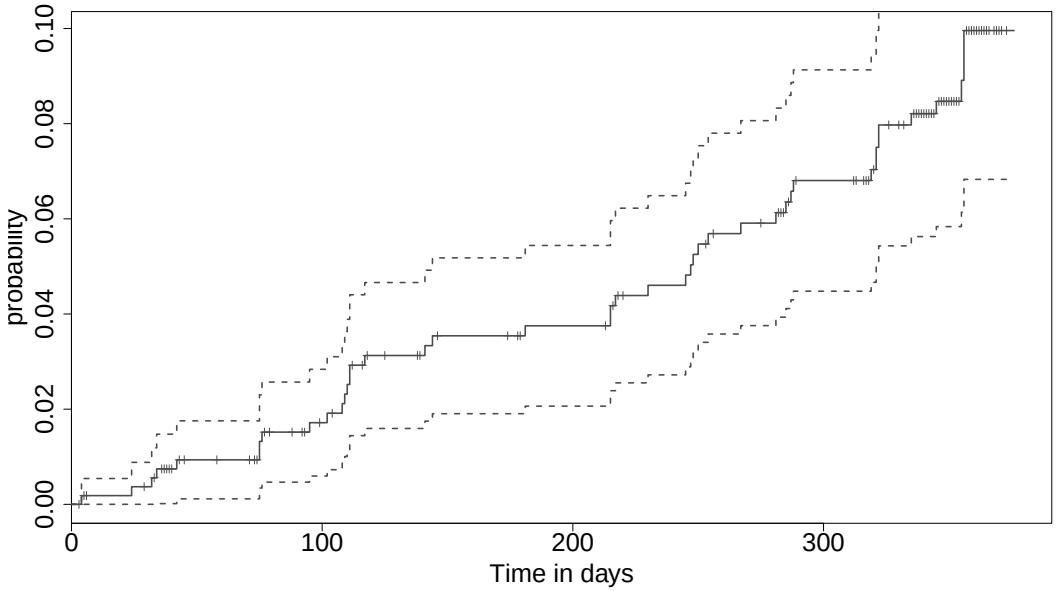


Figure 6.3: Cumulative hazard curves for infection with *Trypanosoma* spp.

6.3.2 Risk factors for infection with *Trypanosoma* spp.

Results of univariable analysis for non-infectious and infectious factors associated with risk for trypanosome infection are listed in Appendix Tables F.7 and F.8 respectively. Providing drinking water for the animals within the homestead was associated with reduced risk for trypanosome infections. Calves from farms that reported using anti-protozoan drugs in the rest of the herd had a greater risk for infection with trypanosomes. Calves that had not been weaned had a lower hazard for trypanosome infection. High NDVI values were associated with increased hazard for trypanosome infections. Seroconversion to *B.bigemina* was associated with increased risk for trypanosome infection. The results of all statistically significant factors associated with trypanosome infections are provided in Table 6.4.

Table 6.4: Risk factors for infection with *Trypanosoma* spp.

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Variable	coef	exp(coef)	lowerCI	upperCI	p
Fixed effects					
Calf suckling - yes	-0.693	0.500	0.270	0.935	0.028
Watering at homestead	-1.111	0.329	0.182	0.577	< 0.001
Mean NDVI x 10	1.844	6.321	2.315	16.036	< 0.001
T.mutans antibodies - dam	-0.039	0.962	0.937	0.986	0.003
Protozoal control	1.553	4.727	1.749	13.969	0.003
B.bigemina - seropositivity	0.678	1.970	1.064	3.722	0.033
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	1.59	2.52		

6.3.3 Strongyle egg counts

Over the observation time, the strongyle egg count recorded varied greatly between calves, ranging from 0 to 18,050 eggs per gram of faeces (mean 756, median 350). Strongyle epg count increased with age of calf but the median epg count did not vary greatly after 11 weeks of age, see Figure 6.4. Differences between sublocations were observed pointing to a North-South strongyle egg count gradient. Higher strongyle egg counts were observed in the sublocations falling in the north of the study area compared to those in the south, see Figure 6.5. The latitude (Northing) value of study farms had a strong statistical association with strongyle egg counts recorded in the corresponding calves (p-value < 0.001). The distribution of mean strongyle egg counts per calf was skewed to the right, Figure 6.6. Random effect terms for sublocation and calf were added into the models investigating risk factors for strongyle egg infection in order to account for the observed heterogeneity in strongyle egg counts between different sublocations and repeated measures per study calf. In addition, a random term for each calf observation was included in order to account for the overdispersion (Elston et al., 2001).

The results of univariable screens for the non-infectious and infectious factors associated with strongyle egg counts are presented in Appendix Tables F.9 and F.10 respectively. After model simplification through backward selection methods, a minimum adequate model containing only factors with

significant statistical association (95% significance level) with strongyle egg counts was obtained, results in Table 6.5. Calves from farms that provided housing for their animals had a significantly lower strongyle epg count compared to those in farms where animals were not housed. High NDVI values were associated with low egg counts, whereas female calves had lower egg counts compared to male calves. Calves with a high recruitment weight had lower egg counts. Although growth rate was not included in the final model since high worm burden decreases growth rates, a low average daily weight gain was found to be significantly associated with increased strongyle egg counts.

Low values of total serum proteins and packed cell volume were associated with high strongyle egg counts, indicating their potential as an indicative diagnostic measure of helminth burden. Seropositivity to *T.parva* and infection with *Trichuris* spp. was associated with high strongyle egg counts.

Table 6.5: Results of multivariable model for the explanatory variables with significant association with strongyle egg counts.

	Estimate	Std.Error	lowerCI	upperCI	p value
Fixed effects					
Housing - stallshed	-0.189	0.069	-0.324	-0.054	0.006
Mean NDVI	-2.419	0.923	-4.228	-0.609	0.009
Calf sex - female	-0.239	0.065	-0.366	-0.111	< 0.001
Recruitment weight	-0.031	0.009	-0.049	-0.013	< 0.001
T.parva seropositivity	0.158	0.042	0.075	0.242	< 0.001
Trichuris spp.	0.312	0.141	0.036	0.589	0.027
Random effects					
VisitID	0.9562	0.9779			
CalfID	0.3547	0.5956			
Sublocation	0.0272	0.1649			

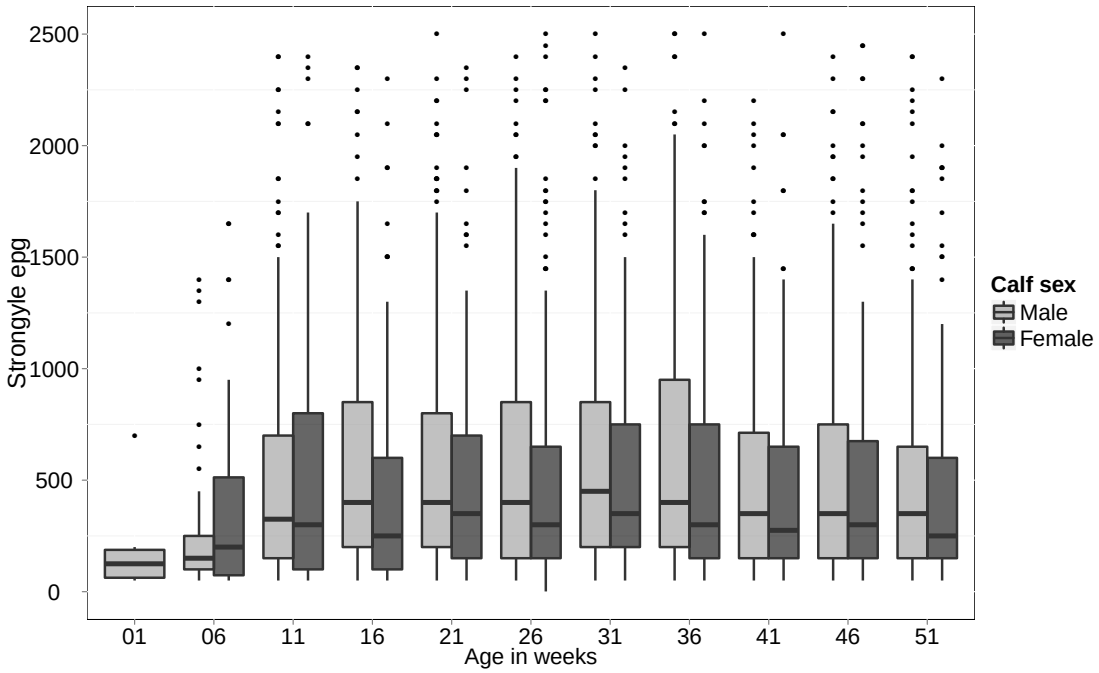

Figure 6.4: Distribution of strongyle egg count by age and calf sex. Calves were rapidly infected after birth up to about 11 weeks of age. The median epg count did not vary much with age beyond 11 weeks but male calves had a higher strongyle egg count compared to female calves. The boxplot shows the median, upper and lower quartile marks. Outlier points are those greater than 1.5 times the inter-quartile range.

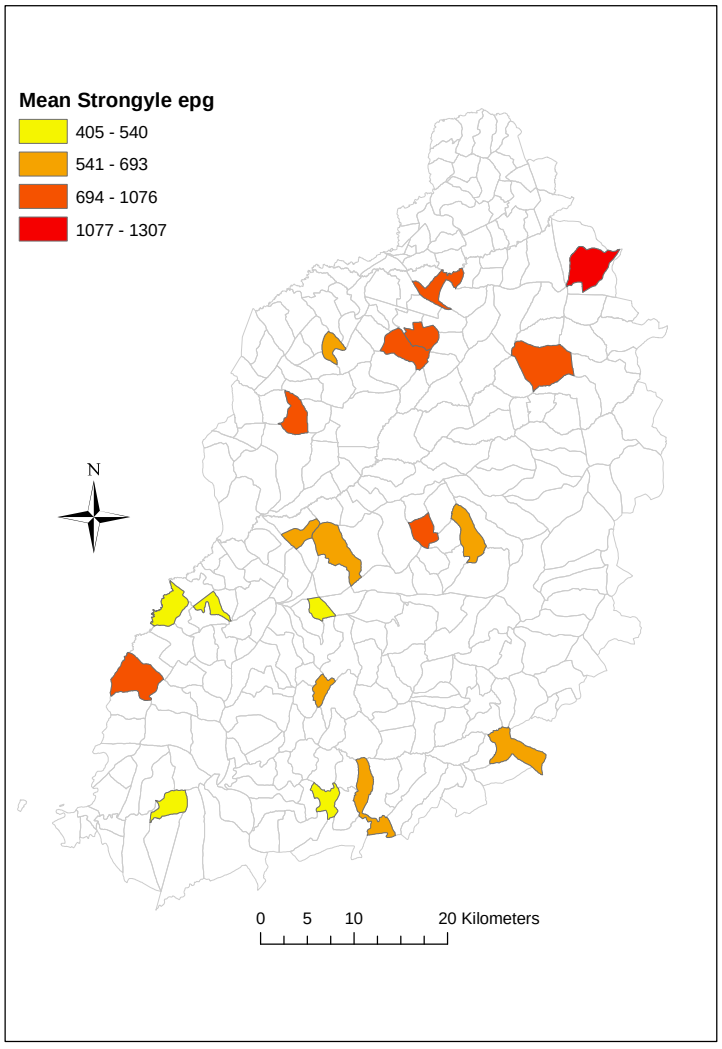

Figure 6.5: Map of mean strongyle egg counts by sublocation. Calves in the sublocations in the north had relatively higher worm burdens than those in the south (Northing (latitude), p-value < 0.001) pointing to a possible strongyle egg burden gradient in the study region.

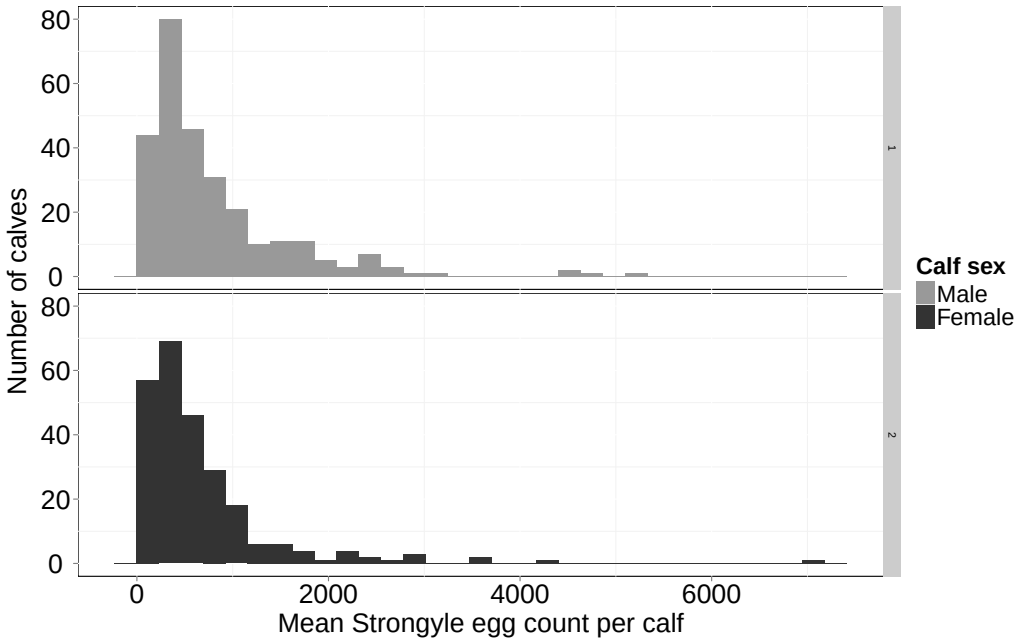

Figure 6.6: Frequency distribution of strongyle egg counts per calf by calf sex

6.3.4 Shared risk factors

The risk factors identified for each of the selected pathogens are summarised in Table 6.6. The risk factors were grouped into a) factors related to farmer's knowledge and husbandry practices, b) environmental factors that may indicate infection pressure or conditions conducive for pathogens or the host, c) dam and calf factors and d) coinfecting pathogens. Farms that reported having access to veterinary support had an increased likelihood for seroconversion to *T.mutans* and *A.marginale*. Farms that provided supplements had a decreased risk for *T.parva* seroconversion but an increased risk for *A.marginale* seroconversion even though the latter was only marginally significant.

Providing water at the homestead which would be related to decreased exposure to infections, was associated with a significant decrease in the risk for *T.parva* seroconversion and trypanosome infections. Providing housing, also a husbandry practice that would reduce contamination of pastures within a homestead and calf exposure, was associated with decreased strongyle epg count.

High NDVI values were associated with increased risk for trypanosome infections but decreased risk for *T.parva* seroconversion and low strongyle epg counts. High dam antibody titres against *A.marginale* and *B.bigemina* were associated with increased risk for *T.parva* seroconversion and *T.mutans* respectively.

Calves with a high recruitment weight had a higher risk of seroconversion and lower strongyle epg counts. Large dam sizes were associated with increased risk for *T.mutans* seroconversion but decreased risk for seroconversion to *A.marginale*. Female calves were at a higher risk for *A.marginale* but were associated with lower strongyle epg count. Calves suckling was associated with increased hazard for seroconversion to *T.parva* and a decreased hazard for infection with trypanosomes.

The survival analysis results showed seroconversion to *T.mutans* was associated with an increased hazard for seroconversion to *T.parva* and to

A.marginale. The presence of *Haemonchus placei*, the most prevalent strongyle egg producing helminth, was associated with decreased probability for seroconversion to *T.parva*. Seroconversion to *T.parva* was itself associated with increased counts of strongyle epg.

Presence of *Strongyloides* spp. of worms was associated with increased likelihood for seroconversion to *T.parva* and to *T.mutans* seroconversion. Coinfection with *T.vitulorum* was associated with increased probability for seroconversion to *T.parva* and *A.marginale*.

Table 6.6: Summary table showing the risk factors seropositivity to *T.parva, T.mutans,A.marginale*, infection with *Trypanosoma* spp., and strongyle epg count.

Variable	*T.parva*	*T.mutans*	*A.marginale*	Strongyle epg	*Trypanosoma* spp.
Farmer knowledge					
Education-primary school			0.777(0.616,0.981)*		
Education-secondary school			0.614 (0.465,0.812)***		
Knowledge of diseases			0.624(0.470,0.828)**		
Veterinary support		1.286(1.119-1.477)***	1.580(1.128,2.214)**		
Husbandry practice					
Vaccine use			1.489(1.243,1.785)***		
Supplements use	0.733(0.639,0.841)***		1.309(1.010,1.698)*		
Protozoal control					4.727(1.749,13.969)**
log(Total livestock units)	1.095(1.003,1.199)*				
Watering - at homestead	0.779(0.689,0.881)***				0.329(0.183,0.577)***
Housing - stall-shed				-0.189(-0.324,-0.054)**	
Environment factors					
Mean NDVI	0.152(0.029,0.802)*			-2.419(- 4.228,-0.609)**	6.321(2.315,16.036)***
Dam - *A.marginale*	0.988(0.982,0.994)***				
Dam *B.bigemina*		1.003(1.000-1.005)*			
Dam *T.mutans*					0.962(0.937,0.989)**
Dam and calf factors					
Dam health - sick	0.359 (0.146,0.878)*				
Calf Suckling - yes	1.372(1.139,1.651)***				0.500(0.270,0.935)*
Moderate introgression		1.075(0.927,1.246)			
Substantial introgression		0.629(0.481,0.821)***			
Dam girth size		1.007(1.000,1.014)*	0.981(0.969,0.993)**		
Recruitment weight		1.018(1.005-1.032)**		-0.031(-0.049, -0.013)***	
Calf sex			1.271(1.074,1.503)**	-0.239(-0.365,-0.112)***	
Coinfections					
T.mutans	1.195(1.056,1.350)**		1.696(1.373,2.094)***		
T.parva				0.158(0.0751,0.2415) ***	
B.bigemina					1.970(1.064,3.722)*
H.placei	0.832(0.745,0.929)**				
Microfilaria spp.	3.280(1.202,0.023)*				
Strongyloides spp.	1.539(1.211,1.955)***	1.338(1.123,1.593)**			
T.vitulorum	1.913(1.301,2.813)***		2.414(1.305,4.468)**		
Trichuris spp.				0.312(0.0356,0.5889)*	

Blank - not significant, * p-value < 0.05, ** p-value < 0.01,*** p-value < 0.001

All results except for strongyle epg count represent a hazard ratio. Variables with coefficients < 1 are associated with a protective effect, whereas variables with coefficients > 1 are associated with increased risk for seropositivity or infection with trypanosomes. Negative coefficients for strongyle epg indicate decreased counts, while positive coefficients indicate increased strongyle counts. The shared risk factors are presented in **bold**, red color associated with increased risk, and blue color associated with decreased risk. The relationship between *T.parva* and *H.placei* have been highlighted since these two form the most common infections with largest impact of survival and growth rates.

6.4 Discussion

This study has been concerned with establishing the non-infectious and infectious risk factors associated with pathogens identified to have the greatest impact on calf growth and survival probability to one year, either as single infections or as coinfections. Specifically, focus has been on risk factors for seroconversion to *T.parva, T.mutans* and *A.marginale*, risk factors for infection with *Trypanosoma* spp., and risk factors for high strongyle faecal egg counts. The current study has identified both the specific and shared risk factors for these selected pathogens.

Infections with *T.parva* and high strongyle egg counts were the single most important infections associated with both increased risk for calf mortality and lowered growth rates. At microscopy, differentiation of important *Theileria* spp. is difficult since piroplasms and schizonts from *T.parva* and *T.mutans* are morphologically similar. Serology tests, which were carried out on samples collected at recruitment and routine monitoring calf visits, allowed for speciation of infecting tick-borne pathogens by detecting immune responses mounted by the host against specific pathogens. In this study, the serology data was used to determine exposure to pathogens. Results from serological tests may be used to not only indicate exposure to pathogens but also provide information on important epidemiological parameters and their variation between hosts, parasites, time and space. One such measure is the force of infection which is related to the average age of infection.

In this population, time to *T.parva* seroconversion varied between the study sublocations with an observed north-south gradient of increasing *T.parva* infection pressure. Animals in sublocations located in the south of the study seroconverted to *T.parva* at a relatively young age compared to those in the north. Differential infection pressures as observed between sublocations may be driven by geographical variation in prevalence of the parasites and density of the tick-vectors (Norval et al., 1992; Swai et al., 2007). To account for the unmeasured factors leading to the observed differences between sublocations, a random term for sublocation was added in the analysis.

The risk of seroconversion to *T.parva* was significantly increased in farms reporting husbandry practices that may be associated with increased risk of exposure to pathogens. Specifically, calves from farms where animals were driven a distance away from the homestead to access drinking water were significantly more likely to seroconvert to *T.parva* compared to those accessing drinking water at the homestead. It would be expected that sharing communal watering holes with other animals would increase contact between animals, and the risk of infestation with infective ticks from pastures around the watering holes or in paths and pastures shared between these animals. This factor was also associated with increased risk for infection with trypanosomes, perhaps related to tsetse exposure. In view of results from previous chapters in this thesis showing that infection with trypanosomes increased the hazard for ECF deaths by up to 10 times, this husbandry practice is an important common risk factor. It is curious however why the factor was not identified to significantly increase risk for seroconversion with the other tick-borne pathogens which would be expected and it may be that the factor captures variation in more than just levels of exposure to pathogens.

The second husbandry practice that may be related to exposure to pathogens is whether housing for animals was provided at the homestead at night or animals were left free within the homestead. This factor was found to be associated with strongyle epg counts with calves from farms that offered a form of enclosure for the animals having a significantly lower strongyle epg count compared to those left to roam freely within the homestead. Housing animals may reduce the contamination levels of grasses around the homestead, which are frequently accessed by calves before weaning or starting to graze with adult cattle in the fields.

Large herd sizes, as measured by total livestock units in a farm, were associated with increased risk for *T.parva* seroconversion possibly indicating increased contact between animals and consequent exposure. Like the variable "watering at homestead", herd size was not identified to be associated with risk for other pathogens investigated, suggesting *T.parva* epidemiology may be more sensitive to differences in the level of exposures.

Higher odds for seroconversion among animals accessing pastures compared to zero-grazed animals, and other factors thought related to exposure to pathogens including geographical location, season, frequency of tick control, age and herd sizes have been reported in studies investigating risk factors for tick-borne diseases (Gitau et al., 1997; Swai et al., 2005, 2007; Salih et al., 2007; Gachohi et al., 2010; Simuunza et al., 2011).

This study has used normalised difference vegetation index (NDVI) as a proxy measure of enviromental conditions. It captures the health of the vegetation with high NDVI values relating to greener and denser vegetation and is a proxy measure of variables as moisture and temperature. It captures the temporal and spatial variation in these environmental variables. High NDVI values were associated with increased likelihood for infection with trypanosomes, which may be interpreted as an indication of conducive environments for tsetse flies, the arthropod vectors for trypanosomes. However, high NDVI values were also associated with a decreased hazard for seroconversion to *T.parva* and with lower strongyle egg counts. This observation is counter-intuitive as high NDVI values have been associated with increased exposure to ticks and probability for *T.parva* seroconversion (Gachohi et al., 2010) and higher worm burdens in humans (Pullan and Brooker, 2008; Brooker et al., 2012). However, it is possible to have environments that are conducive for pathogens and their vectors all year round such as those where there is little variation in rainfall and temperatures over time (Hansen and Perry, 1994). In such cases, NDVI may not be identified to be associated with increases in exposure to parasites and vectors. High NDVI values may not only relate to suitability of environments for pathogens and vectors, but may be also reflect on feed availability to the animals.

The complex nature of host-pathogen interactions and pathogen-pathogen interactions in coinfected hosts may modify the outcomes studied in this chapter. For instance, faecal egg counts in animals with strong Th2 immune responses (which target helminths) may be reduced, even in animals heavily infected with adult worms (Markus and Fincham, 2007). Further, in chronic helminth infections, Th2 response is associated with Treg activity with pro-

duction of anti-inflammatory cytokines associated with the downregulation of host's inflammatory responses, and dampening Th1 responses required for the control of microparasite infections (Maizels et al., 2004; Moreau and Chauvin, 2010).

Where such mechanisms are at work, observations such as low strongyle egg counts may not always be accurate measures of the levels of adult worm burdens, and there may be interference with immune responses to other parasites including results of immunodiagnostic tests. A good example is the recent finding that cattle coinfected with *Fasciola hepatica* and *Mycoplasma bovis* have significantly reduced skin test reaction for *M.bovis* test (Claridge et al., 2012). This is thought to be associated with Th2 responses stimulation by *F.hepatica* and inhibition of Th1 responses, reducing the efficacy of diagnostic skin tests for bovine tuberculosis. Whether such mechanisms are at work in the present study, and whether the observed association between NDVI and low strongyle epg counts and reduced risk for seroconversion to *T.parva* is through good feed availability and strong immune responses in hosts is unknown. Markus and Fincham (2007) have argued the importance of including immunological measures of responses to worm antigens in addition to faecal parasitological examinations while investigating effects helminths might have on other co-occurring diseases.

This study reveals the presence of *H.placei* infection was associated with a decreased risk for seroconversion to *T.parva*. The mechanisms by which *H.placei* relates to decreased hazard for seroconversion to *T.parva* is unclear. It is however possible it may be immune mediated as described above through strong Th2 responses against *H.placei* and affecting responses to *T.parva* infections. This however remains an unanswered question open for investigation.

A second important coinfection effect identified here was the finding that seroconversion to *T.parva* was itself associated with increased counts of strongyle epg. The two infections were found to have negative effects against survival probability to one year, and growth rates. If one pathogen enhances the susceptibility to the other, it would be expected the burden due to these

diseases would be increased.

Clinically unhealthy dams were associated with up to a 65% decrease in the probability of seroconversion to *T.parva*. Additionally, suckling calves were found more likely to seroconvert compared to weaned calves. These two results point to a possible relationship between the condition of the calf and seroconversion. Sick dams would be expected to offer less milk to the calves while weaning is stressful to the animals, both of which would affect the condition of the calf and possibly its immune responses.

This study cannot establish cause-and-effect with the coinfection risk factors. The results point to an association between seroconversion to *T.parva* in animals that seroconverted to *T.mutans*, or were coinfected with *Microfilaria* spp., *Strongyloides* spp., and *T.vitulorum*. High strongyle egg counts were also observed in animals that seroconverted to *T.parva*, and in animals detected with the whip-worm *Trichuris* spp. Further exploration and analysis to understand the mechanisms for the observed associations between these coinfecting pathogens have not been explored in the current study, and further work to better understand these interactions is recommended.

Calves from farms that reported knowledge of prevalent diseases, and those where the farmer had education level beyond primary school had decreased probabilities for *A.marginale* seroconversion. Good farmer education and better knowledge of diseases observed in the farm may be correlated to farm practices that would influence exposure to diseases although it is not clear why these are only significant for *A.marginale* infections. Studies in humans for instance show that education level and socioeconomic status are associated with both malaria and helminth prevalences. These factors are thought to determine access to proper antimalarial treatment, bednets (which reduce risk for malarial infections) and are related to hygiene and water contact behaviour which will relate to exposure to helminth infections (Varandas et al., 2000; Mwangi et al., 2006).

Taken together, the results of this study identify factors that increase the probability of exposure to pathogens, the environmental conditions, and those that determine the body condition of the animal (possibly associated

with immune responses) to have the greatest association with seroconversion to tick-borne diseases, infection with trypanosomes and high strongyle epg counts. The parasite-parasite associations observed here are only preliminary analysis and caution should be taken interpreting them. They however raise interesting questions for further research especially taking into consideration immunological measures.

To control for these important infections and therefore reduce calf mortality and effect of pathogens on growth rates, improving husbandry through practices that reduce exposure levels of calves to pathogens is one practical step. Providing a form of enclosure for the animals and drinking water within the homestead are simple practices that may protect calf health. In addition, ensuring the animals are well fed and in good nutritional status has advantages in their ability to fight infections. The benefits of these simple practical steps would not only reduce effect of single infections but of coinfections found to multiply impact of pathogen infections on calf mortality and growth rates.

Chapter 7

General discussion

This thesis has been concerned with establishing the burden of infectious diseases in zebu cattle under one year, specifically determining the impact infections and coinfections have on two host outcome measures: a) survival probability to one year, and b) growth rates during the first year of life. As opposed to focusing on single-pathogen systems, this study has used a holistic approach in determining disease impacts by considering coinfections and possible pathogen-pathogen interactions that may occur, and which may modify both the epidemiologies of infectious diseases and the impact they have on host outcomes.

By considering multiple infections, this study has provided a comprehensive quantitative assessment of the entire infectious disease burden of zebu cattle under one year. Secondly, it has presented evidence that it is not enough to study single infections as though they work independent of other coinfecting pathogens, but pathogen-pathogen interactions should be considered as they can play an important role in determining host survival and development.

This discussion chapter is divided into 3 sections. Section A provides a summary of what I think are important findings from the work carried out in this thesis. Section B deals primarily with the question of what practical information can be gained from the work so far, and can be used in the control of infectious diseases and their impacts in the study population. Section C

deals with what I think are interesting scientific questions raised by this work, and what the future research direction should be. The last part gives concluding statements.

Section A: Summary of important findings

A schematic diagram showing the summary results of this thesis work is presented in Figure 7.1. Here I begin by providing a list of what I think are important findings from this thesis work:

(a) Coinfections are common in this system; study calves were frequently coinfected, a median of 4 different pathogen species were identified infecting an individual at a time.

(b) The environment in which the study calves were raised in is rich with a large diversity of pathogens; over 35 different pathogen species were identified in the study.

(c) The smallholder mixed crop livestock production system studied here is largely low input; disease control practices at the farm level were rarely done. Over the one year observation time 4%, 14%, 27% and 70% of the farms used vaccines, controlled for tsetse and trypanosomes, controlled for worms and controlled for ticks in their herds respectively. Among those controlling for tsetse and trypanosomes, worms and ticks, the frequency of control was 10 times less than what is recommended for effective control in areas of high disease intensity (Pegram et al., 1993).

(d) Infectious disease (ID) mortality rates in the population are estimated at 15.3 for every 100 calf years at risk. *Theileria* spp. high intensity infections, infection with *Trypanosoma* spp., and high helminth burden (measured by strongyle eggs per gram of faeces) are associated with increased risk for ID-mortality by a factor of 32 (CI [6, 162]), 6 (CI [1.4, 25]) and 1.4 (CI [1.3, 1.6]) per 1000 epg increase, respectively. *T.parva* seropositivity is associated with a protective effect estimated to reduce

risk of ID-mortality by 63% (CI [24, 82]) compared to risk in seronegative animals.

(e) East Coast Fever is the single most important cause of death, accounting for 40% of all infectious disease mortality. Haemonchosis and heartwater disease also cause high mortality rates, estimated at 12% and 7% respectively.

(f) The risk of ECF death is itself increased by up to 10 times (CI [1.3, 86]) in animals coinfected with *Trypanosoma* spp. In addition, strongyle epg increases the risk of ECF death but this is burden dependent. ECF death hazard increases by 1.3 times (CI [1.001, 1.6]) with every increase in strongyle epg by a count of 1000 eggs. This evidence of coinfection effects demonstrates the importance of considering coinfections while investigating impacts infections have on host outcomes.

(g) Seroconversion to *T.parva* and *T.mutans* were associated with decreased risk for ECF deaths, HR = 0.13 (CI [0.04, 0.44]) and HR = 0.29 (CI [0.10, 0.83]) respectively.

(h) Deaths due to haemonchosis are burden dependent; the risk for haemonchosis death increases by a factor of 1.7 (CI [1.5, 2]) for every increase in strongyle epg count by 1000 eggs.

(i) Growth in zebu cattle during the first year of life is linear; the estimated average daily weight gain is 134.7 grams per day. There is a large variation in daily weight gain (up to 10 times differences) ranging from 30 grams/day to 340 grams/day. Some of the fastest growing animals average a daily weight gain greater than has been reported among improved breeds in smallholder production systems. If this has a genetic basis, it points to potential opportunities for improved livestock production through within-breed selection. Otherwise may point to potential gains through improved management.

(j) Infections with *T.parva* and *Trichophyton* spp. were associated with large negative impacts on the growth rates, estimated at 13.7% and 18.9%

respectively. Helminth infections have a strong negative effect on growth rates but this is burden dependent, estimated at 3.3% decrease per 1000 increase in strongyle epg count.

(k) There is evidence of pathogen-pathogen interactions modifying infection effects on growth rates. Coinfections between *T.parva* and *A.marginale* resulted in a substantial reduction in growth rates (24%), which was 1.5 times greater than would be expected if the effects of these two species had independent effects on growth rates. However, coinfections between *T.parva* and *T.mutans* were associated with a reduction in growth rate (7%), less than half the size of that which would be expected if their effects on growth rates were independent (15%).

(l) Dam factors are important both in the survival probability of the calves, and in their growth rates. Bigger, healthier dams (heart girth size, general health) were associated with a protective effect against ECF deaths, and with faster growth rates. Additionally, having a sick dam was associated with a 64% decrease in the probability of seroconversion to *T.parva* perhaps indicating poor feeding in calves with sick dams and subsequent decreased energy to mount strong immune responses detectable as a rising titre. *T.parva* seroconversion was itself associated with a protective effect against ECF deaths.

(m) Husbandry practices that would reduce the risk of exposure to pathogens (e.g restricting animal movement by providing water within the homestead, housing animals, and tick control) were associated with reduced risk for mortality, and with higher growth rates. These husbandry practices were identified as shared risk factors for the risk of infection (and seroconversion) with the pathogens identified to have the greatest effect on both calf survival and growth.

(n) Environmental effects (measured using NDVI values of the areas the calves were raised in) were associated with decreased risk for death due to heartwater disease, and higher growth rates although this effect was masked by infections. NDVI was however associated with increased risk

of infection with *Trypanosome* spp., but decreased risk for *T.parva* seroconversion and with low strongyle epg counts.

(o) Preliminary analyses showed prior seroconversion to *T.mutans* was associated with increased likelihood for seroconversion to *T.parva*, and that presence of *H.placei*, the most prevalent strongyle-egg producing helminth, was associated with decreased probability for seroconversion to *T.parva*. Seroconversion to *T.parva* was itself associated with increased counts of strongyle epg. These relationships, although only the results of preliminary analysis, point to possible pathogen-pathogen interactions that may be dictating the epidemiologies of coinfecting pathogens and ultimately the observed host outcomes.

In settings, such as the current study, which are rich with a diversity of potentially harmful pathogen species, the control of infectious diseases would be best guided through identification of priority infections (those with greatest impact), and the knowledge of relationships coinfecting pathogens have on each other within an infected host.

To a large extent, this information has been lacking making it difficult to make evidence-based decisions on how to invest the often limited funds assigned for disease control. This is especially true for Sub-Saharan Africa, rich with endemic diseases, but with scanty epidemiological data and insufficiently funded veterinary services (Perry et al., 2001; Perry and Grace, 2009).

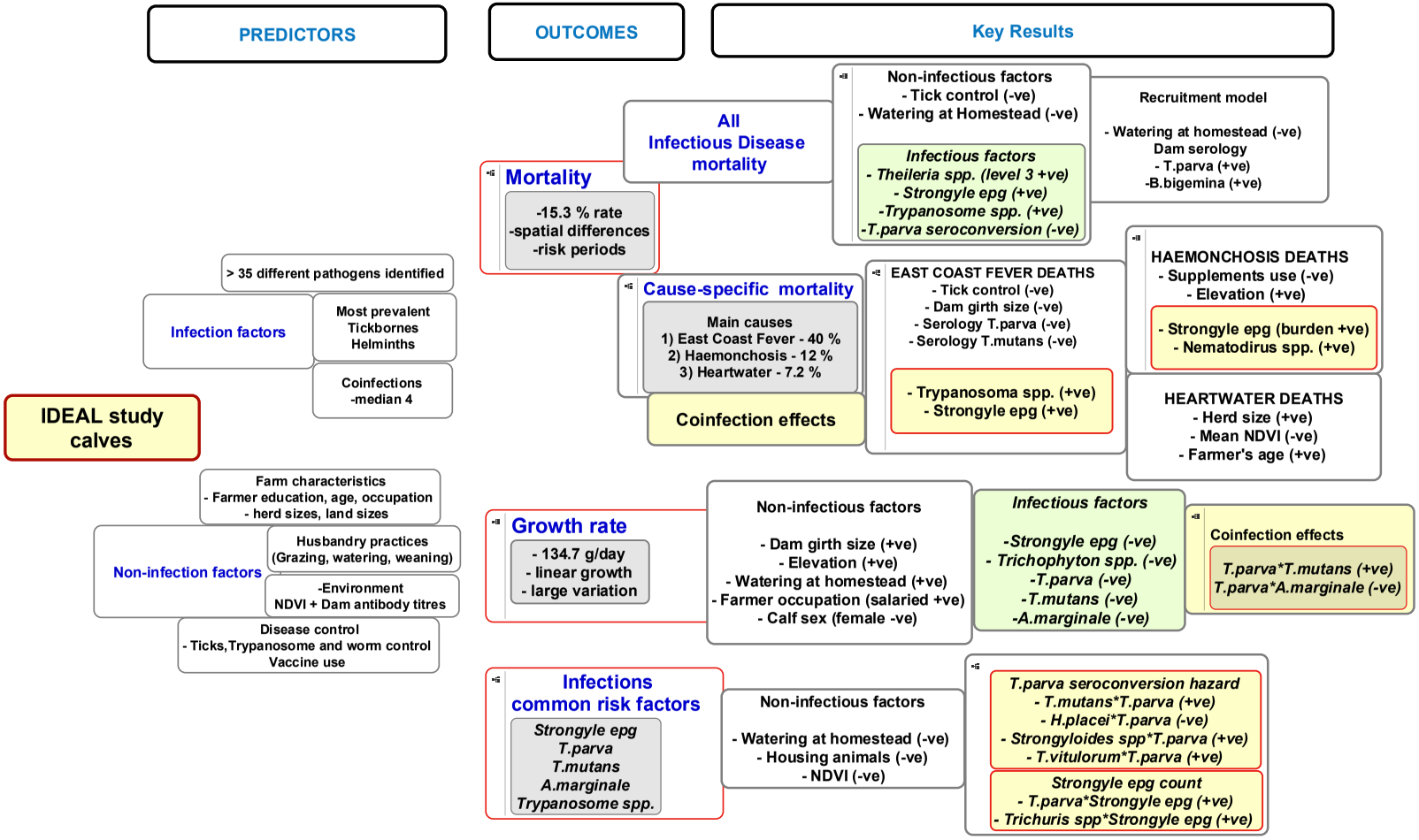

Figure 7.1: Summary diagram showing the main host outcomes studied (infectious disease mortality, cause-specific mortality and growth rates), and the factors associated with each outcome. Significant infectious factors are in green coloured boxes, and significant co-infections in the yellow coloured boxes. Non-infectious factors are in plain boxes. Alongside each predictor variable is a sign (+ve or -ve) marking its relationship with the corresponding outcome. Risk factors for infections with greatest impact on survival and growth rates are provided.

Section B: practical information

To guide this discussion, I would like to start by discussing the first two of three questions considered as essential contributions of epidemiology in disease control (Perry et al., 2001), and attempt to base the answers on the results of this thesis work.

1. Which diseases come first? (priority setting)
2. In controlling disease, which strategy should be adopted? (decision-making)
3. How can optimum delivery and adoption of selected interventions be best achieved? (disease control implementation)

The third question on the optimum delivery and adoption is important but would require more considerations beyond the scope of this thesis.

Which diseases come first?

The Law of the Vital Few (Pareto principle - stating the greater part of the effects comes from the smaller part of causes) has been demonstrated in epidemiology and considered useful in helping target interventions (Woolhouse et al., 1997, 2005). Where possible, epidemiology studies should help identify diseases or factors with the greatest contribution to disease spread or impact on infected hosts. If these are known and targeted, the expected result is greatest gain against mortality and other disease-related losses attained in most efficient ways. Priority setting requires a good understanding, preferably from quantitative data, of the "vital few" in settings with "trivial many" infections (Deleeuw et al., 1995; Perry and Grace, 2009).

To-date, most information on diseases and their ranking according to their order of importance has been largely non-quantitative, and dependent on expert-opinion or farmer opinion (Perry, 2002; Rushton and Heffernan, 2002; Bett et al., 2009). Whereas methods such as participatory epidemiological

techniques or interviews with experts are useful in filling in areas where surveillance is not carried out, data obtained from interviewing farmers is context dependent and often overestimates diseases with visible signs while underestimating those without dramatic signs (Perry and Grace, 2009). Where little empirical data exists, expert opinion is equally problematic and likely based on guesses (Perry and Grace, 2009).

By carrying out a quantitative assessment of infections and their impacts, this thesis work identifies **two main** infections, *T.parva* and strongyle epg, that qualify as "vital few" whose control would specifically have the largest reduction in calf mortality and losses in growth rates. The prevalence rates for *T.parva* and *H.placei*, the most prevalent strongyle egg-producing helminth, are both high - above 70%. At such high prevalences coinfections with the two, whether occurring by chance due to co-distribution or through increased susceptibility as a result of infection with one pathogen before the other, would be common. This is true also for coinfections between any of these two prevalent infections, and any other pathogen in the system.

Each of these two pathogens have a significant negative effect on survival probabilities and growth rates. Their interactions are associated with increased hazard for dying from ECF, and the two infections are responsible for more than half the ID-mortalities. Coinfection with *Trypanosoma* spp. significantly increased the risk of ECF death, making it an important **third** target pathogen for intervention. PCR analysis of these *Trypanosoma* spp. showed most were *T.vivax*.

In relation to growth, the data in this thesis identifies infection with *A.marginale* to be strongly negatively associated with growth when occurring as a coinfection with *T.parva*, and would consider *A.marginale* as a **fourth** priority infection. By targeting *T.parva* and *A.marginale*, the beneficial effects would be greater than a simple addition of the individual negative effects *T.parva* and *A.marginale* have on the host.

Although this study only considers effects on survival probabilities and growth rates, the overall impact of these pathogens is likely greater than investigated here affecting other functionalities including reproduction. In ad-

dition, there are costs incurred in treatment and prevention of these pathogens, waste of feed resources resulting in increased production costs, or condemnation of meat in heavily worm infected animals during slaughter. An additional useful measure to the information provided in this thesis work would be the economic value associated with the avoidable losses following control of these four infections identified as the "vital few" (Mclnerney, 1996; Perry and Randolph, 1999).

Which strategy should be adopted in controlling disease?

Strategies for disease control are mainly based on any of the following; a) reduction in exposure to pathogens, b) development of resistance either naturally or through use of vaccines, or c) control through treatment using chemicals or drugs. The question on the best strategy for control should be in line with control for *T.parva* and its important coinfections *A.marginale* and *Trypanosoma* spp., and on helminth control with holistic view of integrated *T.parva* and helminth control.

The control of tick-borne diseases has mainly been aimed at reducing contact with the tick-vector, through dipping of animals with acaricides. The method was initiated in the early 1900's, mainly to allow introduction of exotic and crossbred animals which could not survive without protection (Norval et al., 1992; Dolan, 1999). For most countries, dipping of cattle was funded by governments with accompanying laws that made it compulsory. This was the case until the early 1990's when through the structural adjustment programmes, governments pulled out the government-sponsored dipping services, henceforth requiring that tick-control and clinical services are demand-driven and provided by the private sector. Whereas this worked for some of the highland areas with commercial smallholder dairy farms, for most other places there was a breakdown in tick-control services.

Western Kenya, with environments conducive for ticks all year round and not fully commercial, had a near complete breakdown in tick-control. As observed during this study, dipping services in the study area are non-existent, and although small-scale farmers have the option of hand spraying, results from this study show that a large proportion of the farms did not regularly

control for ticks.

To effectively control for ticks in areas of high infestation as Western Kenya, treatment with acaricides would need to be provided as often as twice a week (Pegram et al., 1993). In this study, about quarter of the study farms did not carry out any tick control over the one year observation time, and among those that controlled the frequency of control was low - less than 10% of the farms sprayed for ticks at least twice in the year.

Tick-control through acaricide spray is clearly not an easy option as a strategy to reduce *T.parva* in this setting. At its current level of use, a huge effort in extension messages accompanied by economic justification of this method would be needed in order to raise levels of control to what would be considered effective against ticks. The choice of zebus as the preferred breed of cattle to keep in the region is deliberate, for the very reason that they require less control against ticks compared to the improved breeds (Rege et al., 2001; Amimo et al., 2011).

In addition, areas as the current study region where the seroprevalence rates for *T.parva* are high ($\geq 70\%$), are thought to be in a state of "endemic stability". This is described as a state where the host, agent and vector achieve ecological balance, characterised by high levels of challenge with infected ticks and concurrent low incidences of clinical disease (Norval et al., 1992). Intensive tick control, even if it were possible, would likely destabilise this equilibrium resulting in clinical outbreaks as soon as the control is relaxed (Coleman et al., 2001). Decisions about interventions are complex and should be guided by various considerations including economic analyses, development of resistance in ticks and environmental concerns.

Given the above factors, the question remains whether tick-control can play a role in controlling for *T.parva* in this setting. Although the area would be considered endemically stable, a mortality rate of 16% - almost half of which is directly attributable to *T.parva* presents an opportunity to significantly reduce calf mortality. This study has provided evidence that although tick-control is infrequently done, while controlling for other important variables in the models used, controlling for ticks in the rest of the herd was associated

with a reduction in the probability of ID-mortality and ECF death by an estimated 53% and 74% respectively. This finding is interesting since tick control was never done on the study calves but in the rest of the herd, suggesting possible benefits resulting from lower exposure to the calf from the rest of the herd.

Calves from farms which had restricted movement for the animals, represented by providing drinking water at the homestead as opposed to walking animals a distance away in search of water, had a lower probability of ID-mortality. This husbandry practice was also associated with decreased risk for seroconversion to *T.parva* and for infection with *Trypanosoma* spp., as well as associated with higher growth rates. It is most likely a measure of the exposure levels animals are subjected to, and an easily achievable practice.

It would be desirable to have animals exposed to *T.parva* but only at low doses in order to acquire immunity, and it would appear these practices, restricted movement and occasional tick control, may have provided a good environment protecting animals against ECF mortality. The challenge would be determining how frequent the optimal "occasional" tick control should be. This study shows a protective effect associated with tick control 1 - 5 times a year, but this is in animals that are likely to be exposed constantly.

A more appealing strategy and one which would help achieve acquired immunity in a more controlled way is ECF immunization through the infection and treatment method (ITM). The method involves inoculation of animals with *T.parva* parasites followed by administration of long-acting antibiotics (mainly Tetracyclines which are more affordable compared to theilericidal drugs). The infected animal develops a mild reaction to the parasite infection and develops immunity against future infections.

This method has however been faced with 2 main problems: a) immunisation with one strain of *T.parva* does not always protect against all strains, leading to development of unacceptable clinical reactions if infected with these different strains (Urquhart, 1980), b) occasional occurrence of a few piroplasms in the blood of artificially immunised cattle due to inadequate suppression by the drug has raised the possibility of introducing new strains

of *T.parva* into new areas where the vaccine is deployed (Skilton et al., 2002; McKeever, 2007; Geysen, 2008). The first problem has been solved through use of a vaccine cocktail with *T.parva* Muguga, Kiambu 5 and Serengeti strains with good success. The second problem can be solved through characterisation of strains across areas and designing vaccines based on available strains.

Live immunization may provide perhaps the best option yet for the control of *T.parva*. It has the extra advantage that animals require a much lower frequency of acaricide use, which reduces the cost incurred on control, as well as environmental concerns and the problem of resistance of ticks to drugs. In this study immunity, as measured by *T.parva* seroconversion, has been associated with both decreased hazard for ID-mortality and ECF mortality (74% and 87% respectively).

The most common strongyle egg-producing helminth in this system was identified as *H.placei*, a hookworm that attaches to the abomasal wall sucking whole blood and that is usually associated with anaemia and death in severe cases. Over 80% of the larvae hatched from the incubated strongyle eggs collected in the study were found to be *H.placei*. Haemonchosis was identified as the main cause of death for 12.2% of the ID-mortality, making it the second biggest cause of calf death after ECF. In terms of helminth control, the main methods include the use of antihelmintic drugs, and where possible management practices that reduce pasture infection (Rushton and Heffernan, 2002).

In this study, the most important husbandry practice associated with decreased strongyle epg count was housing animals at the homestead. The likely connection between this factor and the decreased strongyle epg is decrease in contamination levels of pastures within the homestead compounds where animals are confined in some form of structure. Young calves are often left at the homestead when other animals in the herd are driven to the fields, and they utilise pastures within or around the homesteads. This is a practical and easy husbandry practice to employ and when combined with strategic deworming, helminth burden may be kept low.

The environment in Lake Victoria basin is conducive for helminths all year round and there may be little variation in terms of seasonality and infection intensities to base treatment decisions on (Hansen and Perry, 1994). However, I find in this study a strong correlation between the strongyle epg count and packed cell volume. This presents a good opportunity for a semi-quantitative measure of anaemia levels by examining mucus membranes. The FAMACHA® system that grades the level of anaemia based on the appearance of mucus membranes may be useful in this system to help decisions on when to treat (Reynecke et al., 2011; Marcotty et al., 2008).

By controlling for *T.parva*, and based on the finding that *T.parva*-*A.marginale* and *T.parva*-strongyle epg interactions cause significant decreases in growth rates and increase in hazard for ECF deaths, it would be expected that the benefits would be greater than just removing the negative effects of *T.parva*.

Taken together, strategies that involve reducing exposure to pathogens (husbandry practices as restricting movement of calves, housing calves) as well as increasing resistance against *T.parva* through vaccination, accompanied by control of heavy worm infections would most likely reduce mortality rates and losses on growth rates. Such integrated protozoan-helminth infection control programs have been suggested in humans, based on the findings that anaemia related to malaria is greatly exacerbated by heavy hookworm infections (Brooker et al., 1999, 2007; Mwangi et al., 2006). Additional clinical field trials specifically designed to test the effect of controlling for these "vital few" as individual infections or in combinations as integrated programs would determine with certainty the usefulness of these suggested approaches.

Beyond this, the third question by Perry et al. (2001) on how best to achieve optimum delivery and adoption of selected interventions would need to be addressed. In the context of this study system, it would involve delivery and administration of vaccines, diagnosis and determination of intensity of helminth infections and subsequent treatment, and identification of actors (public or private) that would be charged to ensure the interventions are delivered. The questions of "return on investments", mainly whether these interventions are attractive economically to the farmers, would determine

their adoption. Adoption in areas where livestock are kept for commercial reasons more than subsistence is much higher. It is however possible that with improved survival rates and better performance of cattle following control of "vital diseases", livestock may increasingly play a role of providing regular income through milk sales.

Section C: Interesting scientific questions and future directions

During this study I have made a number of observations of what I think form interesting questions that may require further investigation. The study has focused on the impact infections have, both as individual infections and as coinfections, on survival and growth.

The diagnosis of *T.parva* and *T.mutans* in this study has been based on serology (ELISA) and RLB methods. Results from serology tests indicate host exposure to the pathogen and an immune response directed to the pathogen, recognizable as a rising titre. This test was carried out on serum samples collected during the entire follow up period. RLB's test detect the presence of parasite DNA, and were used on blood samples collected at one year.

The seroprevalences for *T.mutans* and *T.parva* were 64.2% and 72.3% respectively, whereas the RLB results showed a high prevalence for *T.mutans* (69.1%) but a much lower prevalence for *T.parva* (12.1%) for the same animals at the one year time-point. It would be interesting to know why *T.parva* specifically, and not *T.mutans*, is only detectable in a fraction of calves previously exposed. Data from Oura et al. (2004) showed a similar observation but only in indigenous cattle and not cross-bred cattle. Both breeds of cattle in their study had high exposure levels (98%) at serology, but *T.parva* was only found in 7% of indigenous cattle using RLBs as opposed to 63% in cross-bred cattle using the same method. It may be worth investigating whether indigenous zebu cattle have differential clearance of *T.parva*, not present in cross-bred cattle and not occurring for *T.mutans* infections.

A number of important coinfection profiles have been identified in this

study. One key one is the coinfection between *T.parva* and strongyle epg which was associated with increased hazard for ECF death. To my knowledge this is the first quantitative investigation into this relationship in cattle. Similar relationships involving *Plasmodium* spp. and intestinal helminth worms in humans and focusing on various outcomes including anaemia levels, severity of clinical malaria, incidences of malaria, birth outcomes, and immune responses have been the subject of study, increasingly in the last decade (Druilhe et al., 2005; Brooker et al., 2007; Boel et al., 2010; Yatich et al., 2010; Hartgers and Yazdanbakhsh, 2006; Knowles, 2011).

The results from these many studies and others have sometimes given conflicting results, but the evidence points at these two infections having interactions strong enough to significantly affect host outcomes, see review by Adegnika and Kremsner (2012). We know little about how *T.parva*, a protozoan parasite like *Plasmodium* spp., interacts with the most common helminth *H.placei*. The two are largely co-distributed and if their coinfections have impacts on host survival and other outcomes not studied here, this information would add to epidemiological knowledge and eventual control of the infections and their impact in the population.

An interesting additional observation is the decreased probability of seroconversion to *T.parva* in the presence of *H.placei*. Besides being an indicator of exposure, seroconversion may be thought of as an indicator of a successful mounting of an immune response following infection. There is now evidence that helminth infections modulate the immune responses in an infected host, in a way that would affect its response to other parasites (Maizels et al., 2004). Specifically, helminth infections induce strong Th2 immune responses and lead to dampening of Th1 responses required for the control of microparasites.

If this is true for these calves, it may mean *H.placei* is modulating the immune responses and affecting seroconversion to *T.parva*. A good practical example is the recent finding that cattle coinfected with *Fasciola hepatica* and *Mycoplasma bovis* have significantly reduced skin test reaction for *M.bovis* test, leading to what is thought to be a possible substantial underestimation

of TB prevalence and spread in parts of the UK prevalent with *F.hepatica* (Claridge et al., 2012). One of the suggested control options for *T.parva* is immunization, and if immune responses following infection are greatly masked by helminth infections, efficacy of vaccines may be affected and this needs investigation.

Finally, the coinfections between *T.parva* and *A.marginale*, and between *T.parva* and *T.mutans* suggest interesting questions. McHardy and Kiara (1995) had noted an interaction between *T.parva* and *A.marginale*, specifically observing that super-infection with *T.parva* of animals with anaplasmosis resulted in clinical anaplasmosis, with some animals going into terminal decline similar to that observed in chronic ECF cases. Here I observe a synergistic interaction where the negative effects on weight in coinfected animals were significantly greater than would be if *T.parva* and *A.marginale* were acting independent of each other. The mechanisms by which these apparent interactions occur are unclear and should be investigated. The immunization against ECF by infection and treatment method could potentially precipitate anaplasmosis in carriers and this should be ruled out. In terms of practical veterinary care of ECF cases especially in areas with high prevalence of the two infections, it may be advantageous to treat all ECF cases as though concurrently infected with anaplasmosis.

Effects of infection with *T.parva* on growth rate were found to be modulated in animals coinfected with *T.mutans*. Little attention has been given to *T.mutans*, and understandably so since it is considered a benign pathogen. However, it should raise interest now in light of its association with *T.parva*. If the mechanisms of their interactions are clear, it may be possible to design control programs that take advantage of this apparent modulation of *T.parva* effects by *T.mutans*. At the moment, it is likely the interactions between these two parasites is immune mediated as they use different tick-vectors and utilise different host cells for their reproduction. Experimental work may help shed light into the mechanisms of these interactions.

Conclusion

From this study it is clear that there are benefits of epidemiological studies taking into considerations multiple pathogen infections as opposed to single-pathogen focus studies. There is evidence of pathogen-pathogen interactions that are strong enough to significantly alter host outcomes in a manner different than if the coinfecting pathogens were each acting independently. The study has identified important coinfection effects, whose mechanism of interactions are unclear and open for further investigation. It has also identified the top most important infections associated with greatest losses in growth rates and in calf mortality during the first year of life. There is enough information from this to start programs aimed at reducing calf mortality and losses in growth, and room to keep improving on this knowledge by following up on the many unclear mechanisms underlying the host-pathogen and pathogen-pathogen interactions.

Appendices

Appendix A

The Infectious Diseases of East African Livestock (IDEAL) Project: Descriptive epidemiological report of a longitudinal calf cohort study in Western Kenya

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The Infectious Diseases of East African Livestock (IDEAL) Project: Descriptive epidemiological report of a longitudinal calf cohort study in Western Kenya

Barend Mark de Clare Bronsvoort[1,*], Samuel Thumbi[3], Jane Poole[2], Henry Kiara[2], Olga Tosas-Auguet[3], Ian Graham Handel[1], Amy Jennings[1], Ilana Conradie[5], Mary Ndila[2,4] Phil Toye[2], Olivier Hanotte[2,4], Koos Coetzer[5], Mark Woolhouse[3]

1 The Roslin Institute, Easter Bush, University of Edinburgh,Roslin, Midlothian, EH25 9RG, Edinburgh, UK

2 International Livestock Research Institute, P.O. Box 30709, Nairobi 00100, Kenya

3 Institute of Evolutionary Biology, University of Edinburgh, Kings Buildings

4 University of Nottingham, University Park, Nottingham, NG7 2RD, UK

5 Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private bag X04, Onderstepoort, South Africa

∗ **E-mail: Corresponding mark.bronsvoort@roslin.ed.ac.uk**

Abstract

There is a widely recognised lack of baseline epidemiological data on the dynamics and impacts of infectious cattle diseases in east Africa. The Infectious Diseases of East African Livestock (IDEAL) project is a multi-year epidemiological study of cattle health in western Kenya implemented with the aim of providing baseline epidemiological data, investigating the impact of different infections on key responses such as growth, mortality and morbidity, the additive and/or multiplicative effects of co-infections and the influence of management and genetic factors.

A longitudinal cohort study of new born calves was conducted in the region of western Kenya between 2007-2009. Individual calves were randomly selected from a list of 3-7 day old calves reported by sub-location chiefs each week. A team of veterinarians and trained animal health assistants (AHAs) carried out recruitment, routine 5 weekly, clinical and postmortem visits, collecting data using hand held computing devices (Palm pilots) and paper questionnaires. Blood and tissue samples were also collected for laboratory based diagnostics carried out post-visit.

The study followed 548 calves over the first 51 weeks of life visiting calves at 5 weekly intervals and when they were reported clinically ill. The key findings were a high mortality rate of 16% due to all causes with at least 13% due to infectious diseases. Only 307 clinical episodes were observed at routine visits, with a further 216 reported by farmers. Mortality was mainly due to east coast fever and haemonchosis. Over 50 different pathogens were detected in this population with evidence of exposure to a further 6 viruses and bacteria. The high levels of infection with key pathogens such as *Theileria parva* and their co-infections and clinical outcomes offer the potential to improve our understanding of their impact and potential to develop novel control methods.

Author Summary

Introduction

It is estimated that by 2050 the global human population will have reached 9 billion requiring a doubling of food production on current 2010 levels and increasing competition for food. Much of this growth will have to come from the resource limited and economically stagnant regions currently unable to deliver

such increases. There is therefore an urgent need to improve food production in these regions. Part of this increase will come from livestock, which accounts for 25% of the GDP for sub-Saharan Africa (SSA) as a whole. In addition to providing food through milk and meat, they also provide hides, draught power, manure for fertiliser, building and fuel, capital reserves and cultural services and in many marginal regions are the only useful way of utilising poor quality grazing land. Livestock are key to poor peoples' livelihoods and offer an important route out of poverty.

Constraints on livestock production are varied and include disease, nutrition, management, access to markets and natural catastrophes. However, of these the single most important constraint is considered to be infectious diseases. SSA harbours 12 of the 15 former O.I.E. list A diseases considered most contagious including African swine fever, Rift Valley fever and African horse sickness. In addition many less contagious but arguably more important diseases such as east coast fever, trypanosomosis, brucellosis and leptospirosis are widespread. This limits production directly but also ensures that international markets are also closed to animals and their products from affected regions. However, rinderpest is a clear example where a regional approach has produced a highly successful eradication programme and the world is now rinderpest free. This points to the need for targeted research to understand the full spectrum of disease problems in a farming system and how an integrated control package might release the genetic potential of the existing livestock while maintaining genetic resilience to environmental or emerging disease threats.

Previous work in infectious disease epidemiology has focused on single disease studies eg. Zhang [1], Bronsvoort [2] and Gachohi [3] or a few closely related diseases eg. [4] but, in reality, organisms are normally infected with a number of more or less pathogenic organisms at any one time. There is increasing scientific interest in how pathogens interact, within both individuals [5,6] and populations [7]. Examples include studies of viruses, bacteria, protozoa and helminth infections in both humans and livestock [6–15]. These interactions can be positive or negative and involve mechanisms such as: common risk factors and transmission routes (including shared vectors); non-specific immune responses; cross-reactive acquired immune responses; increased susceptibility of immuno-suppressed or immuno-compromised hosts; non-specific effects of genetic polymorphisms and nutritional deficiencies; the demographic and behavioural impacts of infectious diseases and of intervention measures. There may also be consequences of variations in the timing and ordering of exposure, infection and disease caused by different pathogens, including responses to vaccinations [16, 17].

Animal health research in this region has traditionally focussed on specific infections, particularly tick-borne and tsetse-borne diseases, not necessarily because they are the major diseases of cattle kept by the poor in these environments, but because they are known historically to be serious constraints to commercial systems using improved breeds. Livestock in the tropics are routinely exposed to a wide variety of pathogens [18] whose direct and indirect impacts on animal health are unlikely to be independent of one another. Local breeds have been reared in these heavy disease challenge settings for many centuries which has resulted in selection for broad disease resistance likely at the expense of higher production [19]. Yet there have been no integrated studies of the co-distribution, co-incidence and overall impact of the major infectious diseases of livestock in the tropics. There is a need for detailed knowledge of the burden of infectious diseases impose on livestock as a prerequisite to informed decision making, resource allocation, prioritisation of research and selection of interventions. However, there is growing evidence that disease impacts cannot be fully understood by reference to single infections in isolation [20]. Instead, a holistic approach is required which considers both direct and indirect interactions between pathogens and the effects of these on the epidemiologies of infectious diseases of cattle and of the disease burdens they impose and, ultimately, of their impacts on human welfare [11, 21].

The IDEAL project is a multi-disciplinary study which addresses two major issues: 1) the widely recognised lack of baseline epidemiological data on the dynamics and impacts of infectious diseases of cattle in the tropics; and 2) improving understanding of interactions between multiple infections and their sequelae by testing two specific hypotheses: i) that the negative impacts of different infections are

not independent; ii) that positive traits (e.g. resistance to infection, higher growth rates, low morbidity) cluster in certain individuals. In order to test these hypotheses we designed a longitudinal epidemiological field study to follow a random sample of new born indigenous short horn zebu calves, with known genotype, through the first 12 months of life and to monitor them closely to identify when and what pathogens they were exposed to and the impact these had individually and in combination.

This paper describes the study design and reports on the basic descriptive epidemiology of the sampled population. In particular we provide baseline data on the farm demographics and characterise the small holder African Shorthorn Zebu farming system of western Kenya which may be representative of the wider Lake Victoria basin. We also report the overall infectious disease related mortality rates and incidence of clinical episodes, the range of pathogens and exposures observed and the proportion of the cohort affected by each. Further more detailed analyses of all of these will follow in more specific analyses.

Materials and Methods

Study setting

There has been intensive work to define the distribution of different agricultural production systems in East Africa (eg. [22, 23]). This study focused on a specific production system, sedentary mixed crop-livestock smallholdings. This system encompasses >50% of poor people (defined as income below US$15 per month [24]) resident in East Africa [25], covers extensive areas of Kenya and beyond, and is of increasing importance as populations grow.

The study site was an area of western Kenya approximately 45 x 90km covering some or all of Busia (95.9%), Teso (96.3%), Siaya (55.5%), Butere/Mumias (26.9%) and Bungoma (20.4%) districts. Each district is further divided into sublocations (SL) which are the smallest administrative unit in Kenya for which data was available on cattle numbers. A SL is typically about 10 km across, contains 80 to 900 households per km^2. Land plots are typically 1-5 ha in size, with 60% of households owning 2-3 breeding cattle grazed communally. The study site included 280 SL (excluding 2 that were in Busia and Mumias towns) across 5 agro-ecological zones (AEZ). The areas of Kakamega, Vihiga, Lugari and Mt Elgon districts were not included as they were considered less representative of smallholder livestock farmers in East Africa (e.g. Mt Elgon slope, large-scale dairy farming more prominent) and due to logistic restrictions (i.e. the diagnostic laboratory was in Busia town, to which samples were transported daily).

Study design and recruitment

A stratified 2-stage random cluster sample of calves was drawn. The 1st stage cluster sample (by sub-location) was selected by random sampling with replacement within each AEZ stratum. A total of 20 SLs were selected (table 1 and figure 1). A sample size of 28 calves per SL was chosen to achieve the desired minimum sample size of 500 calves and to allow for some losses (table 2). A reporting system was established in each of the 20 selected sub-locations using a reporting pathway from Farmer → Sub-location-chief → Sub-chief → IDEAL Office. Each recruitment day the animal heath assistants (AHA) collated the eligible calf births for the sub-location and randomly selected 1-2 calves. In order to be eligible the calf had to meet a set of specific selection criteria which were (1) the calf had to be between 3 and 7 days old at recruitment; (2) it was not as a result of artificial insemination; and (3) the dam was not managed under zero-grazing conditions. These criteria were set to give a reasonable window to capture calves being born without being too old and to avoid recruitment of exotic breeds rather than indigenous cattle. The sub-locations were visited on a rolling 5 week cycle to ensure there was an even distribution of calves across space and season. Calves were recruited in a 5 week cycle with 4/20 sub-locations being visited each week. Only one calf per dam was recruited and a farmer could only have one calf at a time in the study. Recruitment was conditional on the farmer allowing access to the calf and willingness to

report clinical episodes to the project and not "self treat". A flat rate of compensation was agreed with the local veterinary office for this. Owners were asked to call the IDEAL team if a calf was observed to be ill between visits and one of the project veterinary surgeons would examine the calf and treat if considered to be seriously ill or a welfare issue. Calves were censored after any visit where a treatment was begun.

Upon recruitment a household questionnaire was completed by interview with the owner/head of the household. The questionnaire included questions about the farm size, crops, water sources, and other livestock. The dam was examined and a form completed and if it or the calf failed any of the eligibility criteria, the calf was excluded. The calf was then examined and a recruitment form and routine visit form completed. The calf was examined for congenital deformities and excluded if any were found.

Data collection and training of data collectors

Data collection took place at the farm or small holding. A team comprising a veterinary surgeon/senior AHA and two AHAs went to each animal and followed a standard protocol for the physical examination and collection of compulsory samples. If the dam was also being visited there was an additional protocol for dam examinations. The AHAs were also trained in data collection and all questionnaires and data collection tools were piloted over about 9 months during the set-up phase of the project in Western Kenya. Data were collected via a hand held Palm OS® Personal-Digital Assistant (PDAs) and simultaneously on a paper questionnaire form. Barcodes were used to identify and link samples to individual animals. At the diagnostic field laboratory in Busia, data were downloaded from the hand held device to a database and cross-checked against the paper records and any discrepancies resolved with the AHA who collected the data.

Routine clinical examination of calves

The clinical examination consisted of a systematic physical examination of the calf. This included observation of the animal at rest, posture, alertness, rectal temperature, weight, girth, FAMACHA score [26], mucus membrane colour, skin elasticity, presence and species of ticks and other ectoparasites and full palpation of the body checking for lesions and discharges. In addition to the physical examination of the calf a short questionnaire was used to update other activities on the farm such as any animal purchases or sales, treatment of the other livestock or cases of illness in other livestock.

Routine samples were collected at either recruitment (7D), 5 weekly (5W), and 51 weeks (Y) visits. A marginal ear vein sample was used to make a thick and a thin blood smear to screen for haemoparasites and for manual differential cell counts following shipment to Pretoria University. A jugular vein sample was collected into plain tubes for total serum protein estimation using a refractometer (model RHC-200ATC, Westover Scientific) and storage for antibody screening for a range of haemoparasites, bacteria and viruses and 0.5ml was added to RNAlater® (Ambion®) and stored at 4^0C. An EDTA sample with 'magic buffer' was collected for genomic analysis (7D). An EDTA sample for: (a) DNA extraction for pathogens; (b) direct microscopy on thick and thin smears for haemoparasities and (d) routine haematology including WBC, RBC, PCV, MCV, HGB, MCH, MCHC using a Sysmex pocH-100iV Diff automated blood analyser (Sysmex® Europe GMBH) was also collected. A further EDTA sample was stored at -80^0C until DNA extraction and shipping to Pretoria University for screening (Y or last visit before death) for a large range of blood borne parasites using the reverse line blot (RLB) [27]. A heparinised blood sample was collected for *Mycobacterium bovis* screening using the "Bovigam" ELISA (Prionics®, Celtic Diagnostics Ltd., Ireland) (Y only). In addition samples were collected for white blood cell stimulation, however, this was discontinued early in the study because of logistical constraints. Faecal samples were collected via rectal palpation for screening for helminths using standard techniques [28]. Samples were divided and one part put in a plastic bag and stored overnight at 4^0C for screening by McMasters technique for strongyle eggs, by the direct Baermans technique for *Dictyocaulus vivperia* larvae, by ZiehlNeelsen

stained smear for *Cryptosporidium spp.* and *M. avium paratuberculosis* and by sedimentation for fluke species eggs. The second part was stored in a pot at room temperature overnight and then prepared for larval culture to speciate strongle eggs. Samples with >2000 coccidia oocyts were also cultured to type the species of coccidia present. Three superficial skin snips were taken from the ventral abdomen and incubated directly in RPMI to screen for *Onchocerca spp.* microfilaria [29]. Results from diagnostic tests done in the field laboratory in Busia were entered directly in a separate laboratory database.

Clinical episodes and post mortem examinations in calves

In addition to routine clinical examinations and in order to capture as many clinical episodes as possible local AHAs working for the Kenyan Department of Veterinary Services in the SLs made weekly visits to each calf. These weekly visits involved a limited clinical examination focusing on identifying any acute disease and in particular any pyrexic or traumatic episodes. In the event that they identified pyrexia, enlarged lymph nodes or respiratory distress, they contacted an IDEAL project veterinary surgeon and an extra non-routine visit was made. The main triggers for a visit were a temperature of >40.5, generalised lymph node enlargement, anorexia, diarrhoea, generalised skin conditions, non-weight bearing lameness, coughing or respiratory distress. However each case report was considered and was visited depending on history and if there was believed to be a compromise in welfare. A full clinical examination was carried out and additional samples were collected based on the clinical syndrome observed. These included swabs of any discharges for bacteriological culture and typing, viral swabs and heparin blood samples for virological culture, and needle aspirates from enlarged lymph nodes for microscopy. If calves were in a severely diseased state the project veterinarian used their professional judgement and a set of criteria agreed with the ethics committee at UoE/ILRI and the animal was euthanised if necessary.

In the event that an animal died or was euthanised a full gross post mortem examination was carried out following standard veterinary approaches working through the body systems. A standard set of tissues was collected from each animal, including lung, liver, duodenum, ileum and lymph nodes, with additional samples specific to the suspected aetiology where appropriate. In the event of a history of sudden death a marginal ear vein blood smear was made and stained with methylene blue and checked for the presence of anthrax bacilli prior to further examination. In the event of a positive smear no post mortem was performed and the carcass buried. If there were neurological signs and/or a history consistent with rabies the head was removed and sent for testing at the Central Veterinary Laboratories at Kabete, Kenya and the remainder of the carcass incinerated. For those animals with neurological signs and no history of possible bites, a brain smear was prepared using the standard approach for identification of *E. ruminantium* the cause of heartwater disease.

Examination of the Dams

In addition to the above the calf's dam was examined at each visit. At recruitment a full clinical examination was done (including manual palpation of the udder for evidence of mastitis), the girth measured and the animal was condition scored using a standard 10 point score [30]. Two plain and 3 EDTA vacuutainers of blood were collected for possible use later. At each 5 weekly visit up to the visit after the calf was weaned the dam was re-examined, the girth was measured, the animal was condition scored and the udder examined.

In the initial phase of the study we attempted to collect milk samples from dams at each visit. These are low production animals and have very small udders and teats compared to a holstein for example. In the majority of cases we were unable to collect samples as the calf would have suckled before we arrived and/or the owner had milked the dam. Similarly the AHAs were initially trained to use the California milk test [31] but again it proved very difficult to get enough milk to test. Both these activities were suspended after the first 3 months in December 2007.

Laboratory analysis

A full list of pathogens that the project attempted to identify that we believed likely to be present in this setting is given in table 3 and includes 100 different pathogens. The various techniques used and the time points at which they were done are also provided for reference. In some cases there is overlap as some techniques will only differentiate to genus level while others will allow species specific identification.

In addition, the project screened stored sera from calves at 51 weeks or from their last visit prior to death for evidence of exposure to a number of other diseases believed likely to be important in this region. Further, plasma and DNA were analysed at a number of external laboratories (table 4).

Whole blood samples in EDTA were stored in "magic buffer" and were genotyped using the Illumina 50K bovine SNP chip (Illumina Inc.®).

Database and sample tracking

The project managed data in a set of linked Access databases (Microsoft Corp.). All reports of calf births and recruitment visits were managed in the reporting database. After animals were recruited the main household questionnaire and the routine clinical visits, clinical episodes and post mortems were recorded using palm pilots running Satellite Forms (SatelliteForms.net). These were connected to the field database and daily downloaded. Every animal was tagged with a bar coded ear tag and visit sheets for each individual were kept. At every visit, the bar code was scanned to minimise recording errors. The field database generated a list of samples and then tests that were to be carried out on them in the local Busia laboratory and this was synchronised each evening so the laboratory staff knew what testing to do each day. The laboratory database linked all the barcoded samples in the field database to the respective calf, to the test results, to where the samples and any daughter samples generated from the original field sample were stored and when they were moved to the ILRI lab in Nairobi or to other laboratories outside Kenya. At the end of the field work the field and laboratory databases were merged and moved to a multiuser MySQL database that could be accessed and updated remotely giving all staff access to the data for analysis. All samples eventually were moved to ILRI Nairobi and were appended to the ILRI laboratory information management system for sample management and tracking. Samples where possible were stored in duplicate and only one of the duplicates moved at a time to reduce the risk of losing complete sample sets. At ILRI duplicates are stored in separate buildings in either -20^0C or -80^0 freezers or in vapour phase in large liquid nitrogen biobank chambers as appropriate.

Tropical Livestock Units

Tropical Livestock Unit (TLU) is a standardising measure used to quantify different types and sizes of livestock. It gives a reference unit that captures the total number of livestock units present in a farm, with 1 TLU being the equivalent to an animal of 250 kg liveweight. One TLU is equivalent to 1 cow, 10 goat or sheep, 5 pigs, 100 chicken, and 0.7 camels [32,33]. This unit has been used for different purposes, including calculating insurable livestock units in the index-based livestock insurance programmes in northern arid areas of Kenya. The different species and sizes of livestock kept in the farms were converted in to TLUs to serve as a proxy indicator for livestock wealth of each household. The conversion factors used here are those reported by Njuki *et al.* [34].

Analysis

The R software version 2.9.1 (http://cran.r-project.org/) was used to generate the descriptive statistics and graphics of the farm characteristics and frequencies of pathogens.

Survival time for each calf was defined as the age at which the study calf died due to infectious causes. Animals that died for reasons other than infectious causes, or that were lost or removed from the

study before one year for non-compliance were censored. These contributed at-risk time only up to the censoring point. All survivors to one year were censored at the time of leaving the study. Kaplan-Meier estimates of the survival function were used to determine the overall mortality rates [35].

Results

Cohort Characteristics

A total of 548 calves were recruited and followed for up to 51 weeks or until they died over the 3 year period of the field work. The spatial distribution of the selected SL is given in figure 1 and the number of calves recruited as a proportion of the breeding dams in each SL is given in table 2. The cattle densities in each SL ranged from 220/km^2 to 2439/km^2 and the SLs ranged in size from 4.38 km^2 to 22.5 km^2. The average herd size across all SL ranged from 2.2 breeding cows in Karisa a more hilly area compared to 6.2 animals in Kokare. The life line for each calf is illustrated in figure 2 and highlights the drop out of calves from death and euthanasia and the pattern of clinical episodes. In addition there were 2 periods where sampling and particularly recruitment were suspended. The first was following the political unrest in 2008 and work in the field was suspended for 6 weeks. This resulted in a small number of calves missing visits for one or two 5 weekly visits. The second was over an extended holiday period in 2009/2010.

Farm Characteristics

A total of 548 owners/household heads were interviewed. Data on the owner's age, gender, education and training level attained, and main occupation are summarised in figure 3 and table 5. Of the 548 owners, 69% were men and 31% women. The mean age in years for male owners was 50.7 (range 22 - 85) and that for females 49.0 (range 20 - 78). Differences in ages between male and female farmers were statistically insignificant ($p = 0.1679$, df= 352, 2-sample t-test). Approximately 15% of the farmers had no formal education, and none had attained university education. A small percentage (21%) had gained technical skills allowing them to work in the informal markets with the common ones being masonry, tailoring and carpentry. The majority (86.2%) of the interviewed owners reported farming as their only source of income, with the rest reporting teaching, civil service, pension and business as their main sources of income with farming offering supplementary income.

The average farm size was only 1.98 $\pm$ (0.1 SE) hectares (range 0.1 to 23.1 ha), with majority (96.1%) being owned. Such land is continuously sub divided, to give adult sons an inheritance and ownership rights. This practice results in families owning small pieces of land that are sometimes not economically viable for agriculture. The rest (3.9%) rented the land they farmed on. All the farms selected for the study kept cattle and also planted food crops, with each farm having a median 5 (range 1 to 131) cattle. The indigenous zebu cattle were the predominant breed kept, with only a small percentage (3.1%) keeping zebu crosses as well. Farmers kept more than one species of livestock an attribute identified as a strategy for spreading risk of losses [36, 37]. Different livestock species serve different purposes within the farm enterprise. The general herd structure is given in table 6, with adult females comprising 41.4% of all cattle kept, and adult males 9.8%.

Husbandry and management practices

Almost 60% of the farms provided housing for livestock. This was usually in the form of an open yard/kraal surrounded by a fence made of untreated wood or bushes with no roof. The remaining 40% of farms provided no housing and the animals were left free or tethered within the homestead during the night. Among those providing housing, 83.1% housed calves separate from the dams/bulls. Calves were not allowed to graze with adults (in 94.4% of the farms) until after weaning. This was mainly to prevent calves suckling dams while out in the field. Calves were allowed to suckle as the farmer milked,

with some farmers reporting that milk-let-down in their zebus only happened when stimulated by calves. Other farmers obtained their share first and left the rest for the calf to suckle.

During the dry season, 49.1% of the farms reported providing drinking water for the cattle within the homestead. The rest drove their animals to a water source. These proportions did not differ significantly between the dry and the wet seasons. Distances to the watering points were below 1 km for 73.8% and 75.8% of the farms in the dry and wet seasons respectively, with the rest travelling more than 1 km to access drinking water. Table 7 shows data on the housing, distances to watering points, frequency of watering, and quality of water both in the dry and wet seasons.

Cattle trading and breeding practices

Almost all the cattle purchases and sales (98.9%) were done through cattle markets (table 8). The rest (1.1%) of the farms reported trading animals directly with neighbouring farms. A total of 24 different cattle markets were reported serving the 20 SLs, spanning four administrative districts. However, a quarter (6/24) of these markets served 71.2% of all the farmers in the study, an indication that farmers preferred trading in big markets, where they are likely to get more competitive prices.

There were no reports of organised breeding programmes, and farmers did not keep any written breeding records. The choice of breeding bulls was mostly based on availability of a bull, and if more than one then the farmer decided on personal preferences. Only 11.4% and 8.2% of the farms kept own-bred or purchased breeding bulls respectively (see table 8). Most farmers (76.2%) borrowed breeding bulls whenever their cows needed service. Based on this, only a few bulls are available to serve animals, raising the chances of widespread inbreeding. A few farmers (3.4%) indicated they did not make any direct breeding decisions and depended on their cows being served while grazing in the same communal areas or at watering points. This number is likely to be much higher than reported as animals mix freely and frequently at watering points and communal grazing fields.

Access to Veterinary services

During the farmer interview at the recruitment visit, most farmers (84.7%) reported accessing some form of veterinary services, mainly provided by private animal health workers, and to a lesser extent by government animal health workers, and veterinary drug suppliers (see table 9). A few farmers indicated they did not use the services of an animal health worker, and instead treated their sick animals themselves. Approximately 90% of farmers reported using tick control with most (89.9%) using whole body spraying with acaricides at the farm. Only a few farmers reported accessing communal cattle dips. Most of the cattle dips in the study sub-locations are abandoned and not in use. Interestingly only just over 50% of farmers reported using any form of anthelminthic treatment and only 18% reported using any form of tsetse control. A moderate proportion of farmers reported using vaccination (52%) although most (76.7%) did not know what vaccine they had given their animals or what they were protected against and their use seems to be largely driven by need rather than a regular programme of control.

There was a notable difference between the proportion of farmers who reported carrying out disease control measures during the initial visit, and the actual proportion of farmers who reported using any preventive measures during the one year follow up period. This highlights the need for caution in interpreting responses especially from cross-sectional data (see table 10).

Morbidity and Mortality

The 548 recruited calves contributed a total of 175,732 calf days of life to the study. Figure 2 shows the temporal pattern of deaths and clinical episodes over the 3 years of the study. A total of 88 calves died before reaching 51 weeks of age giving an overall/all causes mortality rate of 16.4 (13.2-19.5) per 100 calves in their first year of life (table 11). Unfortunately due to logistical reasons *post mortems*

were not carried out on 6 of these calves. Of these 88 deaths, 10 were of unknown cause (including 6 where no *post mortem* was conducted), 8 were due to non infectious causes and the remainder were due infectious causes although in a further 10 cases the specific pathogen causing death could not be identified. This gives a minimum mortality rate due to infectious causes of 13.3% (10.4-16.2) per 100 calves in the first year of life. Fifteen calves were euthanised and were considered to have died from the primary pathology reported on *post mortem*. The distribution of times of deaths by AEZ is given in the Kaplan-Meier plot (figure 4) showing that AEZ5 which is UM3 in figure 1 and includes Magombe East, followed by AEZ1 (LM1) which include Bumala A had much higher death rates than other AEZs. The reasons are not yet clear and are the subject of ongoing analyses. Deaths were also attributed to a secondary or contributing cause of death when this was appropriate. The contributing causes associated with the common infectious causes of death are summarised in tables 12, 13 and 14. For both ECF an haemonchosis deaths co-infection with gut helminths were considered to have contributed to the death. It is interesting to note that in an area generally considered to have high tsetse challenge there seemed to be little clinical trypanosomiasis.

A further 307 clinical episodes were observed by the AHAs on their routine 5 weekly visits and 216 clinical episodes were reported during non routine visits in response to reported illness. The details of all the clinical signs and patterns is currently under analysis but the overall distribution of clinical episodes by age is given in figure 5. This suggests a bimodal pattern with a large peak around 16 weeks at the time when maternal antibodies might be expected to be waning. There is a second smaller peak later around 41 weeks when many calves are weaned.

Pathogens and exposures

Figure 6 shows the list of pathogen/test combinations experienced by the calf by the time of publication crudely stratified into endoparasites, haemoparasites, bacteria and viruses. Some of the common pathogens such as *Theileria spp.* appear several times as a number of techniques were used to identify them. In addition, some assays do not distinguish species such as microscopy. More detailed analysis of these co-infections is on going. What this figure shows very clearly is that this population of calves is infected with over 50 different pathogens and has been exposed to at least a further 6 bacteria and viruses. However, there are relatively few pathogens that were found in the majority of calves and the main pathogens are helminths and protozoan haemoparasites. What is of particular interest is that, given such high incidences of these key pathogens such as *T. parva*, *A. marginale*, *B. bigemina* and *H. placei*, why more of these calves did not die. One of the main objectives of the continuing analyses of this dataset is to unravel the coinfections and relate these to the calf genotype and key outcomes such as growth rate, morbidity and mortality. It is also interesting that there are very few bacterial diagnoses and these appear to have only sporadic occurence and rarely appear as a contribution to death. One reason may be lack of time to have been exposed to these and we plan to look in more detail at the dam serology but of the 2 pathogen exposures looked at in the dams, *Brucella spp.* and *Leptospira hardjo* the seroprevalences were extremely low, 0.036 (0.022-0.050 adjusted 95% CI) and 0.068 (0.035-0.101 95% adjusted CI) respectively. Also there was little clinical evidence of some of the major viral diseases such as foot-and-mouth disease.

This population of calves is the first to have a comprehensive investigation of the pathogen burden and exposures of any animal population. The analyses of the biobanked samples will continue and it is expected that there will be further pathogens added to the list.

Discussion

The IDEAL project is the first attempt to describe the entire disease burden of any naturally occurring population. Funding was only available to follow calves for the first 12 months of life. The use of a

longitudinal design, though enormously logistically challenging in this environment, allowed us to generate a unique dataset to study the effects of co-infections in the SHZ breed in this small holder setting. This may be applicable across a large sector of the Great Lakes basin where very similar breeds and husbandry are in operation.

When designing the project a number of different approaches were considered. They included stratification by management system, wealth/herd size, livestock distribution, location, ethnicity, etc. However, the lack of available data on several of these factors led to the decision to stratify by agro-ecological zone only. Random cluster sampling will have ensured that reasonable representation was provided for the various levels of each of the un-stratified factors, i.e. the total sample size will include farmers with varying herd sizes and management systems. The proportion of sub-locations sampled in each AEZ is in proportion of each AEZ in the total survey area (based on numbers of sub-locations). The study was constrained by logistics to an area of 45 km radius from Busia town in order to make repeated visits possible. Initially other options were reviewed but following piloting of sampling in the field it became clear that given the road conditions and number of animals that would have to be sampled per day at the peak of sampling in year 2 this was the most practical approach.

Owners were paid a retainer for the year to allow access to the animals and therefore compliance was very high. There were a small number of instances of animals being stolen and of owners treating the calves with anthelminthics without consulting the project vet. Where these were identified animals were censored and their data from the visit following treatment discarded.

The descriptive analysis from the recruitment interview indicate that livestock production in this system is characterised by low-input, with as few as 30% of the farms carrying out any form of disease control during the follow-up time. Even for those farms that reported carrying out disease control measures, the frequency of these per year was below what would be effective. This level of management would likely be insufficient to support the use of improved "exotic" breeds which are kept in the region but which we intentionally excluded from this study. Western Kenya accounts for only 4% of Kenya's total exotic dairy herd [38]. This is despite major breed improvements programs instituted to support smallholder farmers in the region through increased livestock productivity [38, 39].

Livestock disease and vector control are required for increased livestock productivity, and prevention of losses through disease-related morbidity, mortality and loss of markets for livestock products. The observed lack of disease control has implications on some of the strategies envisaged to rapidly improve livestock-dependent livelihoods. It also highlights the need to provide support not just for the imported exotic breeds but also for the indigenous breeds in order to minimise the losses and maximise productivity. The consistent use of disease control practices has contributed to the relative success of the smallholder dairy sector in the Kenyan highlands [40]. The benefits of such controls, carried out at community level, have also been demonstrated in other settings [41]. Failure to consider these disease issues is recognised as a factor that could seriously reduce rural growth [42].

The mortality rates in this indigenous calf population were higher than anticipated at the design stage. There are few reports that we could find from similar systems but other reports from the region suggest a range of mortalities. In a Tanzanian smallholder dairy system mortality rates of 35% were reported [43] within the first year with 42% reported as of unknown cause and 19% due to redwater (babesiosis). Swai *et al.* [44] reported mortality rates of 12% in small holder dairy systems in Zimbabwe with 56% ascribed to tick borne disease particularly east coast fever. Gitau *et al.* [45] reported 7% mortality in calves up to 6 months of age from the same area of Western Kenya. A more recent large study of calf mortality in Mali [46] reported an overall calf mortality of 17% but when this was broken down by system the more intensive systems had high mortality rates of 19% and 25% compared to 10% in the traditional pasturalist systems. Interestingly they report gastrointestinal disorders as causing 28% of their overall mortality followed by perinatal problems (16%) and accidents (14%). Direct comparisons are very difficult to make with many of these studies as the design, breeds, environment etc are not the same. However, it is useful to get an overall impression of how these animals are performing in this system. The mortality

rate in the IDEAL cohort appears high given it is an indigenous breed that might be expected to have had time to adapt to the conditions. There are likely to be many contributing causes including possible inexperience in raising cattle compared to traditional cattle owning groups such as the Maasai or Fulani and the co-infection combinations present in the region.

The identification of pathogens at all time points in the study is on going. We adopted a very pragmatic approach using the best field techniques available as the method of diagnosis but for many pathogens this is not sufficient. For example speciation of theileria parasites requires more detailed analysis such as RLB [47]. It must be noted that detection of pathogens is limited by the sensitivity of the assay, the presence of the pathogen at the time of sampling and its location in the tissue which is sampled. This presents many challenges in trying to produce a definitive list of pathogens at every time point for each calf. For this preliminary presentation of the pathogens we have simply summed across all visits to estimate the proportion of calves with each pathogen (or pathogen/test combination). This ignores the dynamics of the order of exposure but this is to be reported in a number of other papers. The list of pathogens is extensive but unsurprisingly there are actually only a few very high prevalence pathogens. Theses are mainly gut helminths and tick borne haemoparasites, in particular *T. parva*.

Many countries in sub-Saharan Africa have had to make structural adjustments to their veterinary infrastructure and the services they provide which leaves farmers and herdsmen without the support needed to introduce exotic genetic stock. Further, Rege *et al.* [48] argue that breeding strategies in the context of smallholder farms should be based on improving food security, income and overall livelihoods of the livestock keepers and not about genetic improvement of livestock. Focus should be on providing the most appropriate genotypes in a local context. However, identifying these appropriate genotypes is itself complex. Mwachara *et al.* [49] identify the need to involve the livestock keepers in designing the breeding programmes to take into account the full array of contributions to livelihoods that these animals make and so identify genetic characteristics related to these functions. Whereas most programs have concentrated on cross-breeding, there exists a lot of potential and advantages for improvements based on within-breed selection.

The IDEAL project is providing unique data on total livestock disease burden in the region, which will allow for ranking of infectious diseases in order of importance. Such data are important for prioritising interventions. The absence of such data and lack of metrics to assess the impact of livestock diseases leads to inefficient resource allocation [50]. In addition, the project will provide data on performance on key traits as growth rates, clinical tolerance and resistance, and survival providing a basis for identifying desirable traits that may be taken up while designing within-breed improvement programs. Within-breed selection may not yield faster results in achieving increased productivity per animal compared to cross-breeding methods, but retains the adaptive characteristics which are increasingly important with changing climates. The findings of positive associations between knowledge of diseases and access to veterinary support with whether farmers carry out disease control practices supports the idea that increased extension services would have significant positive effect on livestock productivity.

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Figure Legends

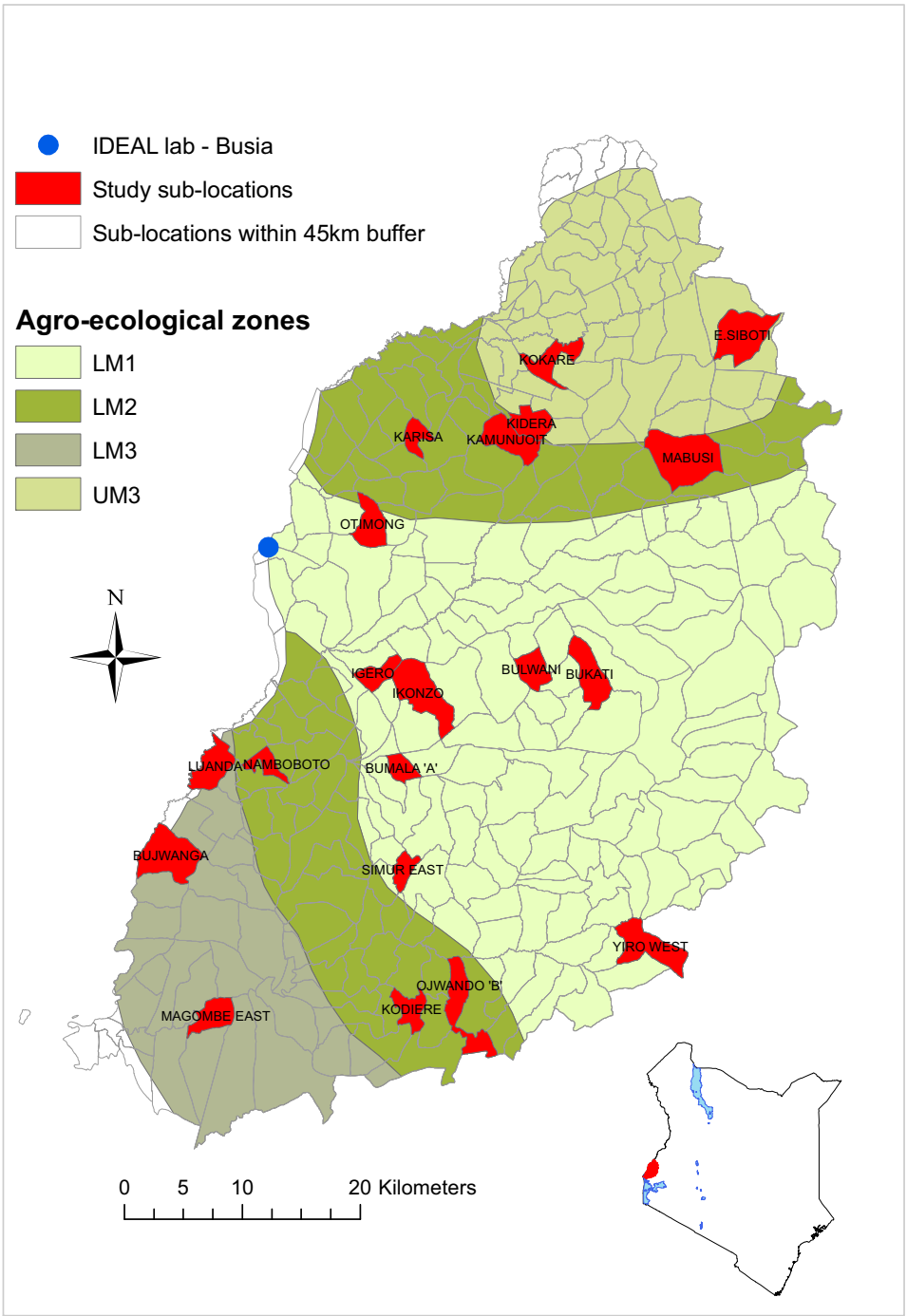

Figure 1. Map of western Kenya region and sublocations

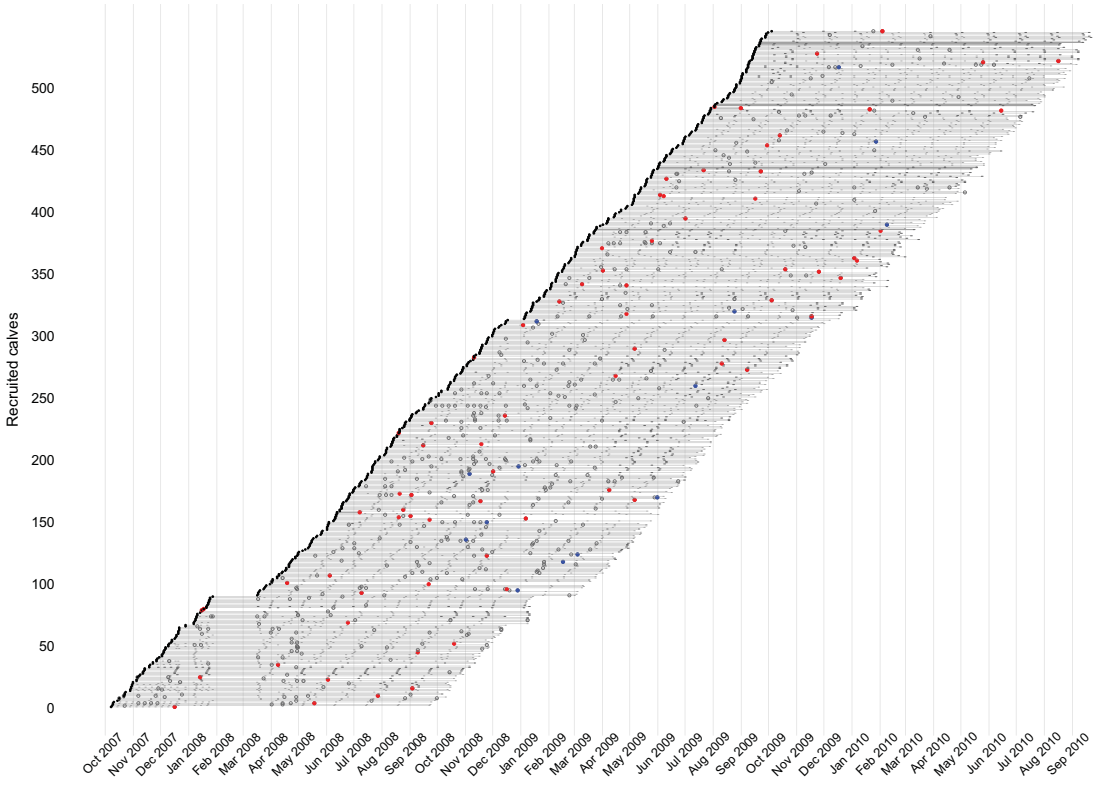

Figure 2. Life lines for each calf. Black dot=recruitment date, grey bar=weekly visit, grey circle=clinical episode; red dot= died and blue dot = euthanised

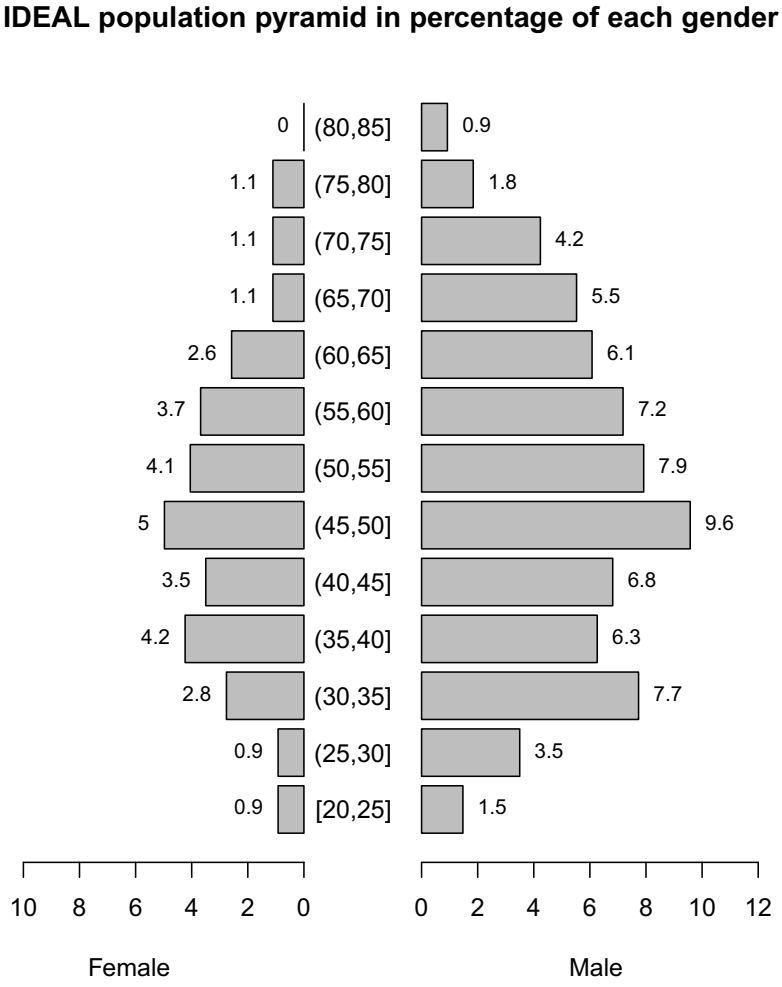

Figure 3. Population pyramid showing the age structure for male and female household heads. Each bar value represents the percent number of farmers in that age group.

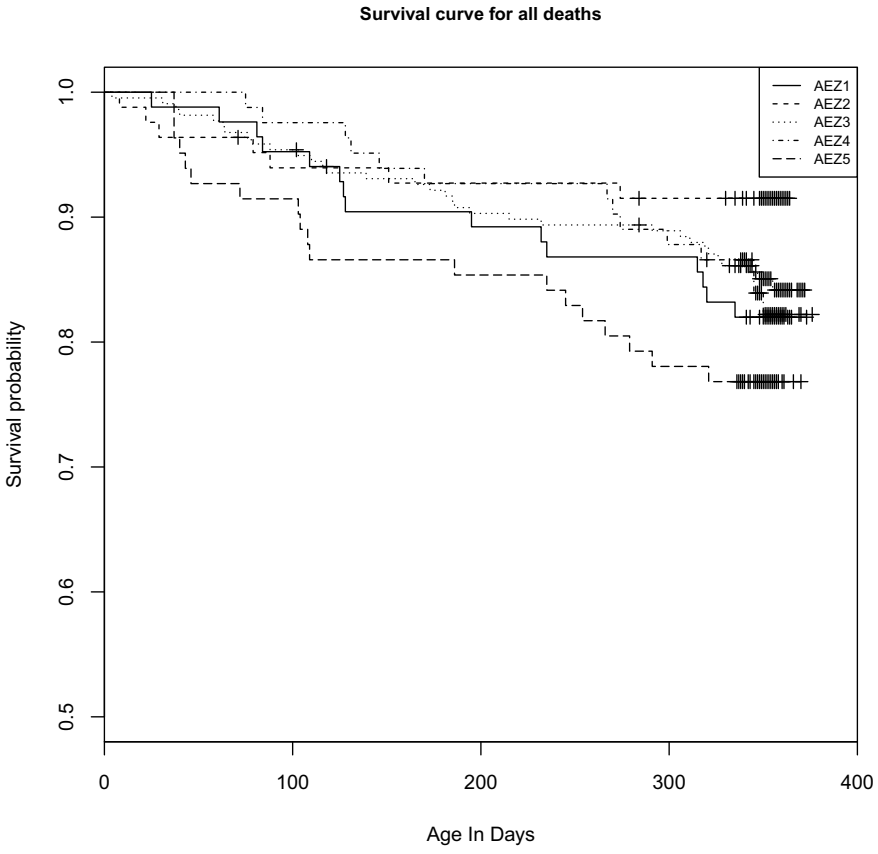

Figure 4. **Kalpan-Meier survival curves for deaths due to all causes**

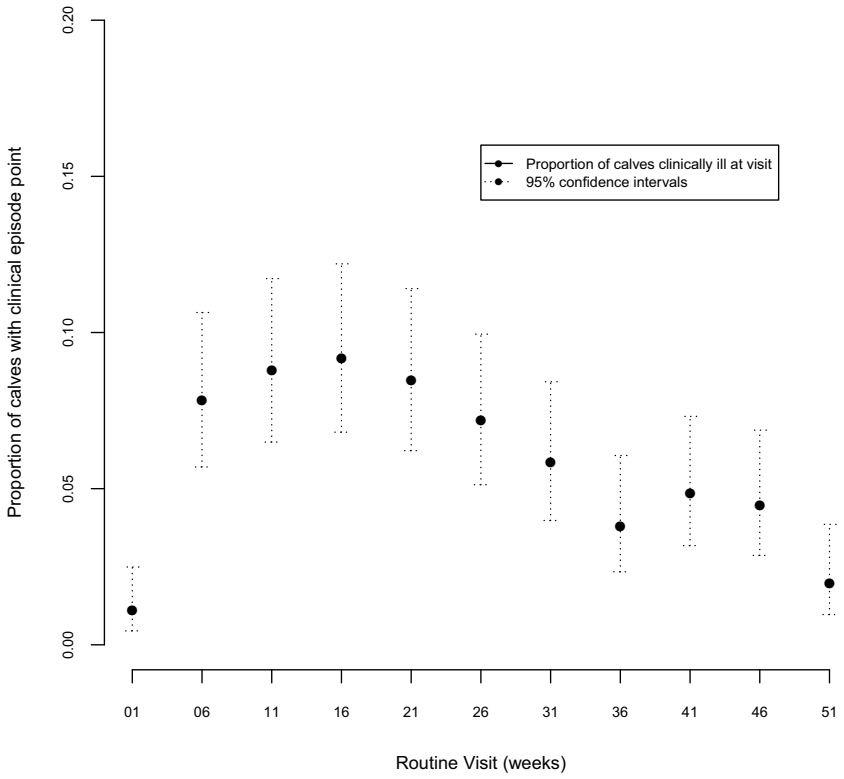

Figure 5. **Distribution of the proportion of calves classed as having a clinical episode by visit number.**

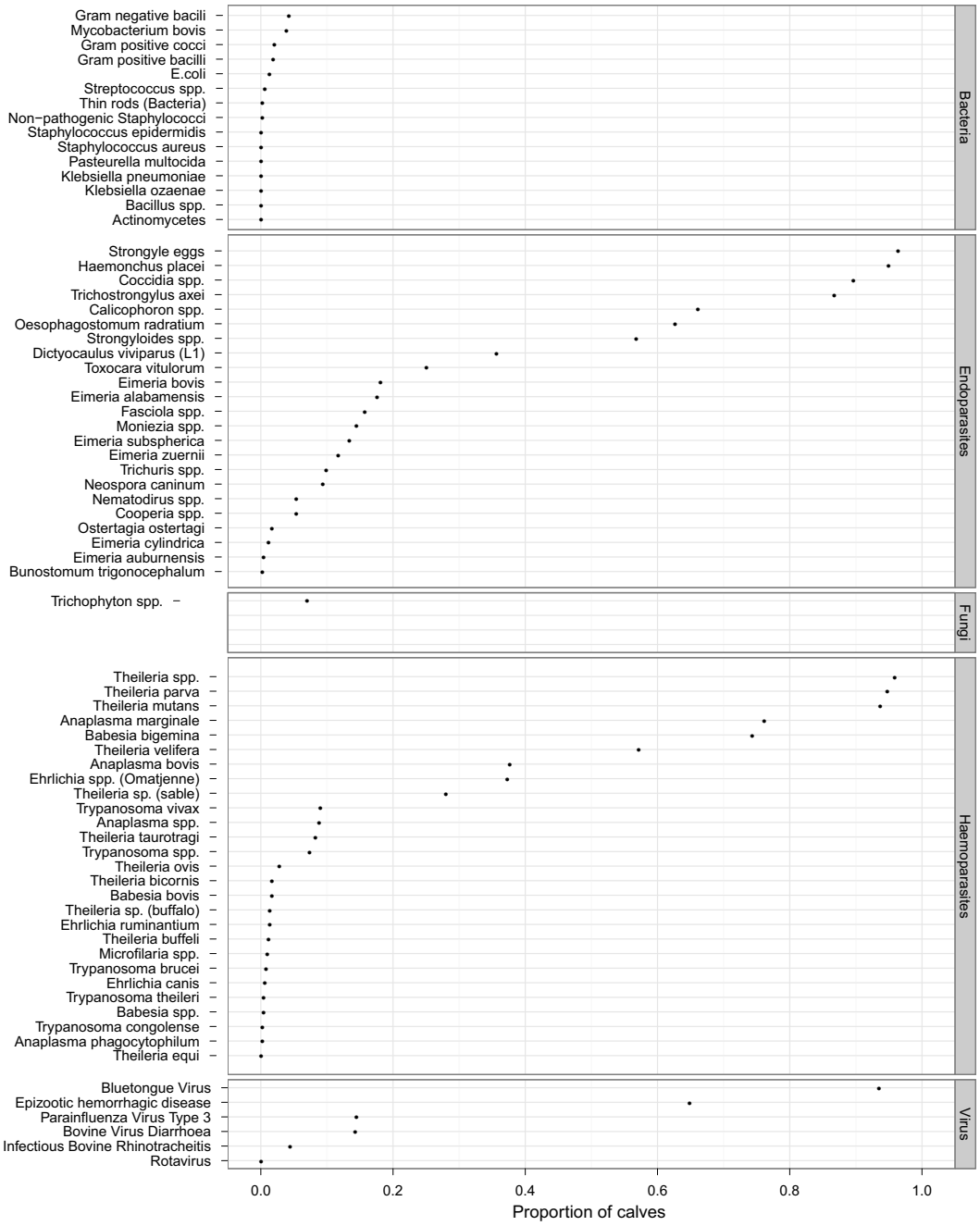

Figure 6. Proportion of animals positive at any time for a given pathogen/test combination.

Tables

Table 1. Distribution of sublocations (SL) across agroecological zones (AEZs) in Western Kenya and number selected for the IDEAL study

AEZ	No. SL/AEZ	Proportion/AEZ	No. SL selected
LM1	114	0.40	8
LM2	86	0.30	6
LM3	28	0.10	3
LM4	4	0.01	0
UM3	53	0.19	3
Total	285		20

Table 2. Selected sublocations with census/demographic characteristics

AEZ	Sub-Location	No. Households	Area(km^2)	Cattle density	Average herd size
UM3	East Siboti	1245	15.80	2439	3.4
	Kokare	325	8.29	937	6.1
	Kidera	314	7.36	728	4.8
LM1	Yiro West	1361	13.70	1187	3.9
	Simur East	415	4.32	425	3.8
	Igero	532	5.60	681	3.6
	Bumala A	724	4.38	222	2.3
	Ikonzo	1421	16.40	598	2.8
	Bulwani	478	6.87	578	3.2
	Bukati	993	11.20	1259	2.5
	Otimong	506	8.66	869	4.1
LM2 middle	Mabusi	1575	22.50	1575	3.1
	Kamunuoit	556	11.00	957	4.0
	Karisa	292	4.63	247	2.2
LM2 South	Ojwando B	832	12.60	1095	4.6
	Kodiere	630	6.38	849	4.7
	Namboboto	351	4.46	220	2.7
LM3	Luanda	726	9.76	730	4.7
	Bujwanga	1025	16.70	792	4.2
	Magombe East	578	7.67	852	5.4

Table 3. Pathogens screened for during the study

Pathogen	Test	Visits tested	Pathogen	Test	Visits tested
Actinomyces sp.	RB	CE	*Hepatozoon spp.* catch-all	RLB	Y
Actinomycetes	RB	CE	*Hyalomma spp.*	CL	7D, 5W, Y
Amblyomma variegatum	CL	7D, 5W, Y	*Hypoderma bovis*	CL	7D, 5W, Y
Anaplasma bovis	RLB	Y	*Klebsiella ozaenae*	RB	CE
Anaplasma centrale	RLB	Y	*Klebsiella pneumoniae*	RB	CE
Anaplasma marginale	RLB	Y	*Listeria spp.*	RB	CE
Anaplasma ovis	RLB	Y			
Anaplasma phagocytophilum	RLB	Y	Lumpy skin disease	PCR	CE
Arcanobacterium pyogenes	RB	CE	*Micrococcus spp.*	RB	CE
Babesia bicornis	RLB	Y	*Moniezia spp.*	FM,FC	7D, 5W, Y
Babesia bigemina	RLB	Y	*M. avium paratuberculosis*	ZN	Y
Babesia bovis	RLB	Y	*Nematodirus spp.*	FM,FC	7D, 5W, Y
Babesia caballi	RLB	Y	*Non-pathogenic Staphylococci*	RB	CE
Babesia canis	RLB	Y	*Oesophagostomum radratium*	FM,FC	7D, 5W, Y
Babesia divergens	RLB	Y	*Onchocerca spp.*	SNP,MIC	Y
Babesia felis	RLB	Y	*Ostertagia ostertagi*	FM+FC	7D, 5W, Y
Babesia gibsoni Japan	RLB	Y	*Pasteurella multocida*	RB	CE
Babesia microti	RLB	Y	*Rickettsia spp.* catch-all	RLB	Y
Babesia motasi	RLB	Y	*Rickettsia spp. (DnS14) raoultii*	RLB	Y
Babesia odocoilei	RLB	Y	*Riphicephalus appendiculatus*	CL	7D, 5W, Y
Babesia ovis	RLB	Y	Rotavirus	ELISA	CE
Babesia rossi	RLB	Y	*Salmonella spp.*	RB	CE
Babesia vogeli	RLB	Y	*Sarcocystis spp.*	HIS	PM
Bacillus anthracis	RB	PM	*Staphylococcus aureus*	RB	CE
Bluetongue virus	PCR	Y, CE	*Staphylococcus epidermicus*	RB	CE
Bacillus spp.	RB	CE	*Staphylococcus epidermidis*	RB	CE
Boophilus spp.	CL	7D, 5W, Y	*Staphylococcus spp.*	RB	CE
Borrelia afzelii	RLB	Y	*Streptococcus bovis*	RB	CE
Borrelia burgdorferi s. lato	RLB	Y	*Streptococcus spp.*	RB	CE
Borrelia burgdorferi s. stricto	RLB	Y	*Theileria annae*	RLB	Y
Borrelia garinii	RLB	Y	*Theileria annulata*	RLB	Y
Borrelia valaisiana	RLB	Y	*Theileria bicornis*	RLB	Y
Bunostomum trigonocephalum	FM	7D, 5W, Y	*Theileria buffeli*	RLB	Y
Bovine Viral Diarrrhoea Virus	ELISA - ag	Y	*Theileria cervi*	RLB	Y
Calicophoron spp.	FM,FC	7D, 5W, Y	*Theileria equi*	RLB	Y
Chabertia ovina	FM,FC	7D, 5W, Y	*Theileria equi*-like	RLB	Y
Clostridium spp.	RB	CE	*Theileria lestoquardi*	RLB	Y
Coccidia spp.	FM,FC	7D, 5W, Y	*Theileria mutans*	RLB	Y
Coccobacillary	RB	CE	*Theileria orientalis* 1	RLB	Y
Cooperia spp.	FM,FC	7D, 5W, Y	*Theileria parva*	RLB,PCR	Y
Corynebacterium spp.	RB	CE	*Theileria spp.* (buffalo)	RLB	Y
Cryptosporidium spp.	ZN,MIC	7D, 5W, Y	*Theileria spp.* (duiker)	RLB	Y
Dermatophilus congolensis	RB	CE	*Theileria spp.* (kudu)	RLB	Y
Dictyocaulus viviparus (L1)	FB	7D, 5W, Y	*Theileria spp.* (sable)	RLB	Y
E.coli	RB	CE	*Theileria spp.*	MIC, (RLB)	7D, 5W, Y, CE
Ehrlichia chaffeensis	RLB	Y	*Theileria taurotragi*	RLB	Y
Ehrlichia ruminantium	RLB,MIC,PCR	Y, CE	*Theileria velifera*	RLB	Y
Ehrlichia spp. (Omatjenne)	RLB	Y	*Toxocara vitulorum*	FM,FC	7D, 5W, Y
Eimeria alabamensis	FM,MIC	7D, 5W, Y	*Trichophyton spp.*	MIC	CE
Eimeria auburnensis	FM,MIC	7D, 5W, Y	*Trichostrongylus axei*	FM,FC	7D, 5W, Y
Eimeria bovis	FM,MIC	7D, 5W, Y	*Trichuris spp.*	FM,FC	7D, 5W, Y
Eimeria cylindrica	FM,MIC	7D, 5W, Y	*Trypanosoma brucei*	HCT,DG,PCR	7D, 5W, Y
Eimeria ellipsoidalis	FM,MIC	7D, 5W, Y	*Trypanosoma congolense*	HCT,DG,PCR	7D, 5W, Y
Eimeria subspherica	FM,MIC	7D, 5W, Y	*Trypanosoma spp.*	HCT,DG,PCR	7D, 5W, Y
Eimeria zuernii	FM,MIC	7D, 5W, Y	*Trypanosoma theileri*	HCT,DG,PCR	7D, 5W, Y
Epizootic haemorrhagic disease	PCR	Y, CE	*Trypanosoma vivax*	HCT,DG,PCR	7D, 5W, Y
Fasciola spp.	FS,MIC	7D, 5W, Y	*Weksella zoohelcum*	RB	CE
Haemonchus placei	FM,FC	7D, 5W, Y			

RB=routine bacteriology; CE=clinical episode; CL=clinical examination; RLB=reverse line blot; 7D=recruitment visit; 5W=routine 5 weekly visit; Y=final visit at 51 weeks; FM=faecal examination by McMaster's technique; FC=faecal culture; MIC=routine microscopy; SNP=skin snip and culture; ZN=ZiehlNeelsen stain; DG=dark ground microscopy; HCT=haematocrit; PCR=polymerase chain reaction

Table 4. Serological screening tests to pathogens

Pathogen	Ab/Ag based	Test name	Manufacturer	Visits tested
M. bovis	Ab	Bovigam ELISA	Prionics	Y
Respiratory Syncitial Virus	Ab	ELISA	Svanova	Y
Bluetongue Virus	Ab	ELISA	PI	Y
T. parva	Ab	ELISA	ILRI in house	7D, 5W, Y
T. mutans	Ab	ELISA	ILRI in house	7D, 5W, Y
A. marginale	Ab	ELISA	ILRI in house	7D, 5W, Y
B. bigemina	Ab	ELISA	ILRI in house	7D, 5W, Y
Parainfluenza 3 Virus	Ab	ELISA	Svanova	Y
Bovine Viral Diarrhoea Virus	Ab	ELISA	Svanova	Y
Bovine Viral Diarrhoea Virus	Ag	ELISA	Svanova	Y
Epizootic Haemorrhagic Disease Virus	Ab	ELISA	PI in house	Y
Akabane Disease Virus	Ab	ELISA	PU in house	Y
Palyam group	Ab	ELISA	PU in house	Y
Infectious Bovine Rhinotracheitis Virus	Ab	ELISA	Svanova	Y
Neospora caninum	Ab	ELISA	Svanova	Y
Brucella spp.	Ab	ELISA	IDEXX	Dam 7D
Leptospira hardjo	Ab	ELISA	Linnodee	Dam 7D

PI is the Pirbright Institute (formerly the Institute for Animal Health). Ab=antibody; Ag=antigen

Table 5. Descriptive statistics for Farmer's demographic variables

	N*	Frequency	Percent**
Sex of house head	548		
Male		370	69
Female		178	31
Education level of house head	544		
None		81	14.9
Primary education		337	61.9
Secondary education		126	23.2
University education		0	0
Technical Training	541		
No		415	76.7
Yes		126	23.3
Main Occupation	544		
Farmer		469	86.2
Teacher		6	1.1
Civil servant		11	2
Business		22	4.1
Retired with Pension		14	2.6
Other		22	4

*Not all the farmers responded to the questions in the questionnaires and N notes the number of respondents to the particular question

**The proportions are calculated using the number of respondents to the question

Table 6. Land sizes, livestock species kept and the herd structure

	N	Percent	mean	median	s.d.	min.	max.
Land size owned (hectares)							
	517	94.3	1.98	1.37	2.28	0.1	23.1
Livestock Numbers							
All Cattle	548	100	6.5	5	7.6	1	131
Indigenous cattle	548	100	6.5	5	7.6	1	131
Cross breds	17	3.1	1.4	1	1	1	5
Goats	209	38.1	3.5	3	3.8	1	33
Sheep	112	20.4	3.9	2.5	5.3	1	48
Pigs	150	27.3	2.2	1	2.2	1	13
Chickens	485	88.5	14.3	10	12.7	1	120
Dogs	297	54.2	2.04	2	1.4	1	9
Tropical Livestock Units	546	99.6	5.8	4.1	6.71	0.48	114.3
Herd Structure (Indigenous)	548	Frequency	Mean/farm	Percent			
Adult females		1463	2.7	41.4			
Adult males		345	0.6	9.8			
Female calves		465	0.8	13.2			
Male calves		446	0.8	12.6			
Weaning females		399	0.7	11.3			
Weaning males		417	0.8	11.8			
Total		3535	6.5	100.0			

Table 7. Description of housing, and watering practices in the dry and wet seasons

	N	Dry season		Wet season	
Housing	545	Freq	Percent	Freq	Percent
Kraal/yard		321	58.9	322.0	59.3
None		224	41.1	223.0	40.7
Access to water	547				
Distance to furthest watering point					
At Homestead		91	16.6	100.0	18.3
<1km		313	57.2	314.0	57.5
1-5km		141	25.8	131.0	24
6-10km		2	0.4	1.0	0.2
Frequency of watering					
Freely available		11	2	13.0	2.4
Once a day		149	27.2	446.0	81.5
Twice a day		367	67.1	87.0	15.9
Thrice a day		20	3.7	1.0	0.2
Water Quality					
Good,clear		533	97.4	508.0	92.9
Muddy		14	2.6	39.0	7.1

Table 8. Location of trading markets and sources of breeding bulls

	N	Freq	Percent
Location of purchasing point	504		
Within Sublocation		75	14.9
Neighbouring Sublocation		396	78.6
Other		33	6.5
Purchasing point	539		
Market		533	98.9
Neighbouring farm		6	1.1
Breeding practices	552*		
Own Bull (Bred)		63	11.4
Own Bull (Bought)		45	8.2
Bull Donated		2	0.4
Bull Borrowed		422	76.4
Communal Area Bull		19	3.4
Other		1	0.2
Total		552	100

*some farmers used more than one bull source

Table 9. Description of access to veterinary services and disease control practices in the farm as reported during the calf recruitment visit.

		Frequency	Percent
Access to Veterinary services	544		
YES		461	84.7
NO		83	15.3
Type of Veterinary support type	473		
Private animal health worker		264	55.8
Government animal health worker		176	37.2
Veterinary drug supplier		23	4.9
Farmer		10	2.1
Tick-control	548		
Yes		498	90.9
No		50	9.1
Application method	520		
Spraying whole body		462	88.8
Spraying legs only		9	1.7
Pour on		6	1.2
Hand Dressing		25	4.8
Dipping		8	1.5
Other(Traditional,manual removal)		10	2
			100
Worm control	548		
Yes		309	56.4
No		239	43.6
Application method	319		
Drench		265	83.1
Bollet		47	14.7
Others(injectables/unknown)		2	0.6
Traditional		5	1.6
Trypanosome control	548		
Yes		98	17.9
No		450	82.1
Method used	101		
Spraying whole body		51	50.5
Chemotherapy		32	31.7
Pour-on		10	9.9
Other(Dipping/head dressing/unknown)		8	7.9
Use of Vaccines	546		
YES		284	52
NO		262	48
Frequency of use	277		
Routinely		9	2.9
When need arises		269	97.1
Vaccine type used	299		
Unknown		230	76.7
Anthrax		8	2.7
Black quarter		11	3.7
Contagious Bovine Pleural Pneumonia		1	0.3
Foot and Mouth Disease		25	8.3
Lumpy Skin Disease		18	6
Other		6	1.7

Table 10. Table comparing the proportion of farms reporting using each disease control measure at initial visit alongside actual proportion of farms that carried out the measures during the follow up period (n=548).

Type of control	Initial visit %	Actual practice %
Tick control		
Yes	90.9	69.9
No	9.1	30.1
Worm control		
Yes	56.4	26.8
No	43.6	73.2
Tsetse and trypanosome control		
Yes	17.9	14.1
No	82.1	85.9
Vaccine use		
Yes	52	96.4
No	48	3.6

Table 11. Counts of primary cause of deaths attributed by expert committee

Cause Of Death	No. calves
East coast fever	32
Unknown	20
Haemonchosis	9
Heartwater	6
Trauma	3
Actiomyces pyogenes	1
Babesiosis	1
Bacterialpneumonia	1
Black Quarter	1
Cassava	1
Foreign body	1
Mis-mothering	1
Rabies	1
Salmonellosis	1
Trypanosomiasis	1
Turning sickness	1
Viral pneumonia	1
No post mortem carried out	6
Total	88

Table 12. Contributing causes attributed by expert committee in those cases assigned East Coast Fever as a primary cause of death (24 of 35 cases of ECF were attributed a contributing cause)

Contributing Cause	Count Of Deaths
Haemonchosis	5
Helminthisasis	3
Trypanosomiasis	2
Adenovirus	1
Poor nutrition	1
Rotavirus	1
Theileriosis	1

Table 13. Contributing causes attributed by expert committee in those cases assigned haemonchosis as a primary cause of death (7 of 9 cases of haemonchosis were attributed a contributing cause)

Contributing Cause	Count Of Deaths
Helminthisasis	4
Theileriosis	2
Dictyocaulus viviparous	1

Table 14. Contributing causes attributed by expert committee in those cases assigned heartwater as a primary cause of death (2 of 6 cases of heartwater were attributed a contributing cause)

Contributing Cause	Count Of Deaths
East coast fever	1
Lead Poisoning	1

Appendix B

Description of non-infectious factors

Introduction

This thesis uses data from the longitudinal cohort study following 548 zebu cattle in Western Kenya from birth to when one year old. This cohort study has been fully described in Appendix A. This section describes non-infectious factors which have been used in the analysis chapters determining the effects of infections and coinfections on the survival probability and growth performance of zebu cattle under one year. Specifically it provides information of farm management practices, environmental factors, dam factors and calf level factors as used in this thesis.

A list of the non-infectious factors, with a short description for each is provided in Table B.1. The proportion of farms with each level of the non-infectious factors (categorical) is provided in Table B.2. The continuous variables as Normalised Difference Vegetation Index (NDVI), and dam variables are presented.

Table B.1: List and short description of the non-infectious variables used in the thesis

Variable name	Variable type	Brief description
Farm level factors	Continuous	Age of the farmer in years.
Farmer's age		
Farmer's gender	Categorical	Sex of the farmer (Male/Female).
Education	Categorical	Farmer's education level. Three levels: a) no formal education, b) primary education and c) secondary education.
Training	Categorical	Whether farmer has any technical training (Yes/No).
Occupation	Categorical	Main occupation of the farmer. Two levels: a) no salaried income (farmer), b) salaried income (employed).
Total acres owned	Continuous	Size of land owned in acres.
Livestock units owned	Continuous	Herd size presented as the number of tropical livestock units owned.
Housing	Categorical	Whether cattle are provided with any form of housing/enclosure within the homestead. Two levels a) no housing, and b) housing provided (stall-shed).
Milking prior calving	Categorical	Whether the dam was milked prior calving (Yes/No).
Milking post calving	Categorical	Whether the dam was milked immediately after calving (Yes/No).
Graze with adults	Categorical	Whether calves are fed/grazed with adults (Yes/No).
Watering at homestead	Categorical	Whether animals are provided with drinking water at the homestead or have to walk a distance away from the farm to access water. Two levels: a) watering at homestead (Yes), b) watering away from homestead (No).
Water quality	Categorical	Quality of water accessed by animals. Two levels: a) clear, b) muddy
Use supplements	Categorical	Whether calves receive any nutritional supplements like crop residues (Yes/No).
Use Vaccines	Categorical	Whether any vaccines were used in the farm over the study time (Yes/No).
Veterinary support	Categorical	Whether the farm receives any veterinary support from veterinarians or animal health assistants.
Knowledge of diseases	Categorical	Whether farmer has knowledge of diseases prevalent within the farm (Yes/No).
Tick control	Categorical	Whether the farm controlled for ticks in the rest of the herd during the study time (Yes/No).
Worm control	Categorical	Whether the farm controlled for worms in the rest of the herd during the study time (Yes/No).
Trypanosome control	Categorical	Whether the farms controlled for trypanosomes in the rest of the herd during the study time (Yes/No).
Antibiotics use	Categorical	Whether antibiotics were used on the rest of the farm during the study time (Yes/No).

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Variable name	Variable type	Brief description
Dam level factors		
Heart girth size	Continuous	Measure of the heart girth size of the dam in cms.
Body condition score	Continuous	Measure of the body condition of the dam using a standard 10 point score.
Dam health	Categorical	Subjective score of dam's health. Two levels a) healthy, b) sick.
Blind teats	Categorical	Whether any of the dam teats is blind (Yes/No).
Antibody titres	Continuous	Antibody titres against 4 tick borne diseases (*T.parva,T.mutans,A.marginale* and *B.bigemina* in the dam from serum samples collected at recruitment visit. These are presented as a percent positivity (PP) values based on a known positive sample.
Environmental factors		
NDVI	Continuous	Normalised difference vegetation index - measure of the vegetation health and density.
Elevation	Continuous	Altitude at which each study farm is located (in meters).
Calf level factors		
Calf sex	Categorical	Sex of the calf (Male/Female).
Introgression	Categorical	Genetics: level of European introgression. Three categories, a) pure indigenous zebu, b) animals with moderate European introgression, c) animals with substantial European introgression.
Recruitment weight	Continuous	Weight of calf at recruitment visit (in kgs).
Heterozygosity	Continuous	Degree of relatedness calculated based on European introgression, presented as a proportion.

Table B.2: Results of the categorical non-infectious factors showing the proportion of farms with each level of the variable.

Variable	Category	Proportion (%)
Farmer's gender	Females	31
	Males	69
Education	No formal Education	15
	Primary	62
	Secondary	23
Training	Technical training	23
	No technical training	77
Occupation	Salaried	14
	Non-salaried	86
Housing	None	41
	Stall-shed	59
Water quality	Clear	97
	Muddy	3
Milking prior calving	Yes	9
	No	91
Milking post calving	Yes	77
	No	23
Graze with adults	Yes	6
	No	94
Watering at homestead	Yes	52
	No	48
Veterinary support	Yes	85
	No	15
Knowledge of diseases	Yes	78
	No	22
Use supplements	Yes	82
	No	18
Use vaccines	Yes	3
	No	97
Tick control	Yes	70
	No	30
Worm control	Yes	27
	No	73
Trypanosome control	Yes	14
	No	86
Antibiotics use	Yes	41
	No	59
Introgression	Pure indigenous zebu	81
	moderate European introgression	14
	Substantial introgression	5

Environmental variables - Normalised difference vegetation index

Normalised Difference Vegetation Index (NDVI) is a measure of the vegetation cover calculated from the visible and near-infrared light reflected by vegetation, from satellite imagery. High NDVI values are associated with healthy and dense vegetation and low NDVI values are associated with decreasing amounts of green vegetation. NDVI values may be used as a proxy measure of environmental variables such as rainfall and temperature, especially useful in areas without weather station as the IDEAL study area. The NDVI value ranges from 0 to 1, with 0 being barren rock and increasing values towards 1 indicate increasing values of green vegetation.

A 30m by 30m pixel size of the satellite imagery around the homestead were each calf was recruited was used. Two satellites images per month were obtained, and NDVI values extracted (processed by Geographical Information System (GIS) group at the International Livestock Research Institute). An average NDVI value per calf per month was obtained, and further a mean value of NDVI over the calf observation was obtained. The mean value for the mean NDVI over the one year was 0.62, with a range of 0.40 to 0.73. The NDVI measures across the study period is shown in Figure B.1. The altitudes at which the study farms were located ranged from 1,114 to 1,446 meters above sea level.

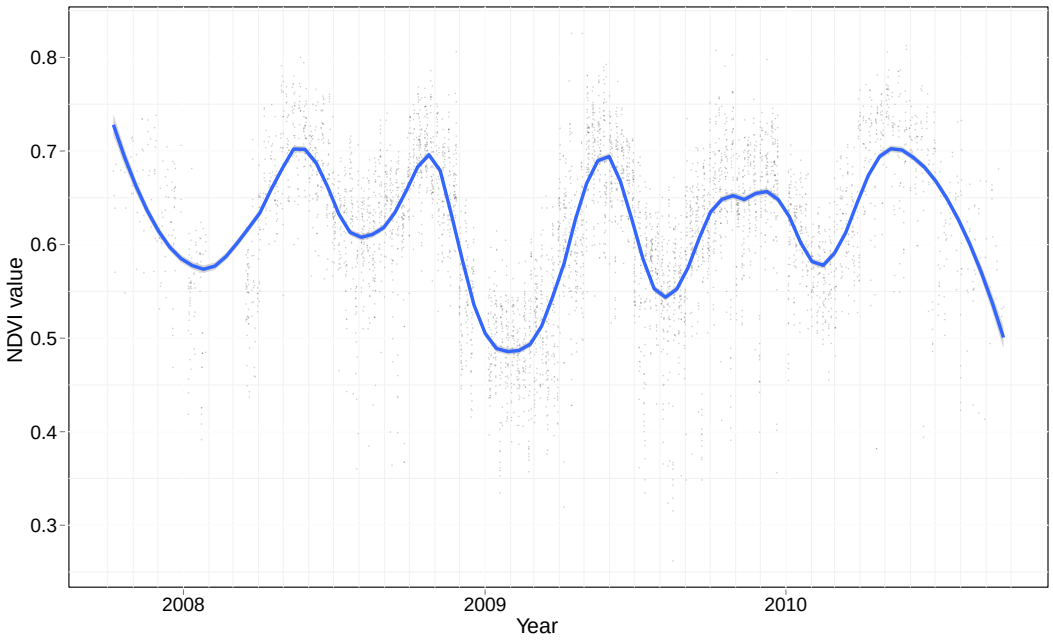

Figure B.1: Plot showing the NDVI values per for all the calves in the study at each time during the study period. A smoother line has been added to aid in visualisation of the trends with time. The NDVI values fluctuate around a narrow range of about 0.3.

Dam factors

During the recruitment visit, blood was collected from the dam and screened for tick-borne infections (*T.parva, T.mutans, A.marginale* and *B.bigemina*) through ELISA tests. Histograms showing the antibody titres and the cut off points at which they would be considered seropositive indicated in the red line, see Figure B.2.

During each calf visit until weaning, data on the dam was collected. This including a subjective score of its health (whether healthy or sick), a heart girth measurement in cms, a body condition score based on a 1-10 scale (with 10 being a dam in very good body condition), and its udder health. At recruitment time, the average girth size for the dams was 138cm (range

114cm - 160cm) while the dam body condition score average at recruitment was 6 (range 3 - 8).

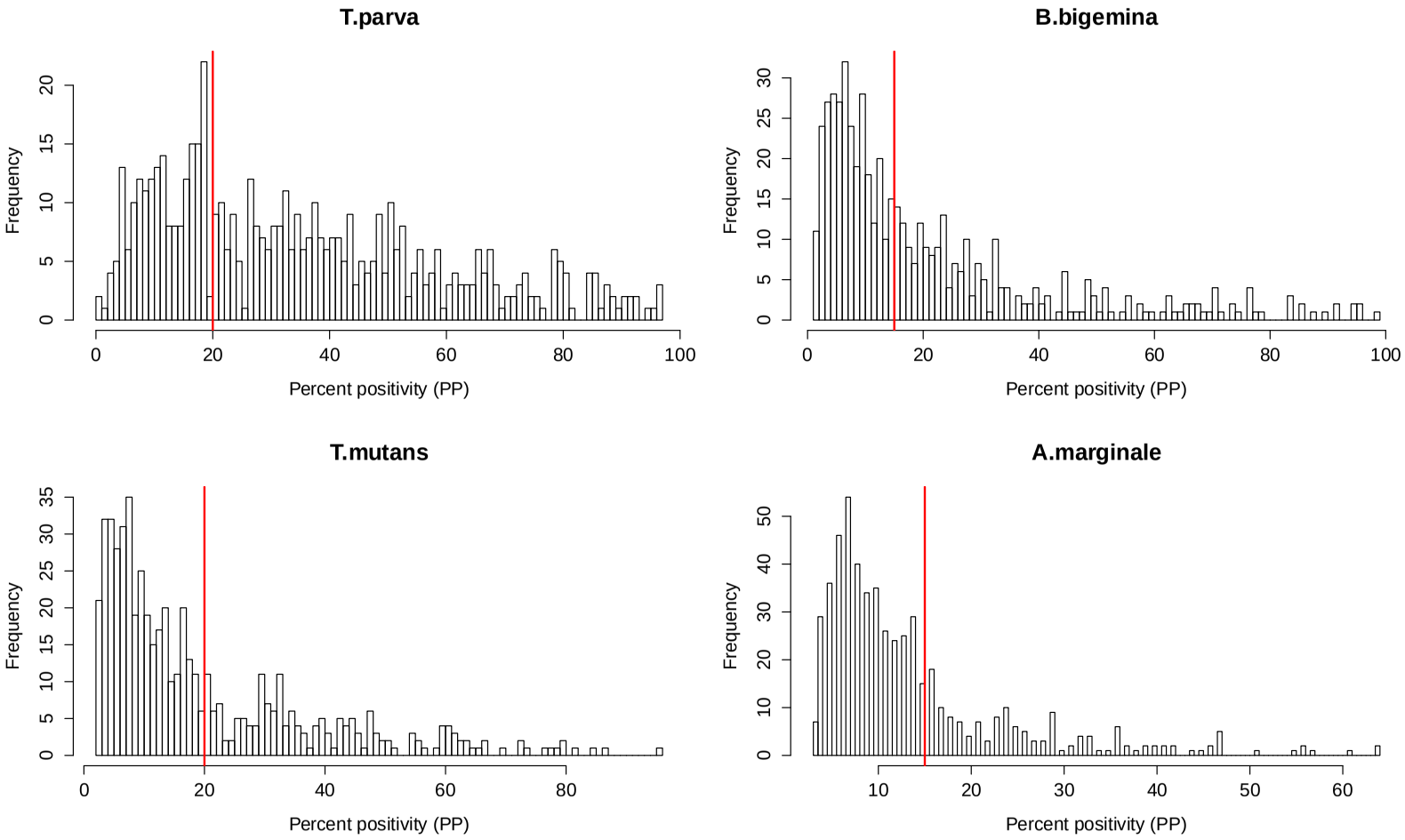

Figure B.2: Histograms showing the distribution of antibody titres in the dam from samples collected at recruitment visit.

Appendix C

Mortality in zebu cattle under one year: predictors of Infectious-Disease mortality

Table C.1: Results of univariable survival analysis of potential predictors of calf ID-mortality at recruitment time.

	coef	exp(coef)	se(coef)	z	p
Farmer's sex	0.2146	1.2393	0.2436	0.8807	0.3785
Farmer's age	0.0163	1.0165	0.0079	2.0613	0.0393
Education - primary school	-0.3790	0.6845	0.2894	-1.3095	0.1904
Education - secondary school	-0.1597	0.8524	0.3286	-0.4859	0.6270
Occupation - salaried	0.1297	1.1385	0.3014	0.4305	0.6668
log(Total acres owned)	-0.0061	0.9939	0.1410	-0.0435	0.9653
log(Tropical livestock units)	0.3797	1.4619	0.1482	2.5630	0.0104
Housing calves - yes	-0.2466	0.7814	0.4401	-0.5604	0.5752
Use supplements - yes	-0.3833	0.6816	0.2510	-1.5268	0.1268
Vaccine use - yes	0.1235	1.1315	0.2166	0.5702	0.5685
Veterinary support - yes	0.2167	1.2420	0.3232	0.6706	0.5025
Tick control - yes	-0.4269	0.6525	0.0868	-4.9215	<0.001
Worm control - yes	-0.3793	0.6843	0.2044	-1.8562	0.0634
Trypanosome control - yes	-0.2573	0.7731	0.2483	-1.0366	0.2999
Milk prior calving	-0.9981	0.3686	0.5880	-1.6976	0.0896
Milk post calving	-0.3598	0.6978	0.2381	-1.5112	0.1307
Knowledge diseases	0.1550	1.1676	0.2708	0.5723	0.5671
Housing stall-shed	-0.2563	0.7739	0.4392	-0.5837	0.5594
Graze with adults	-0.5081	0.6016	0.5881	-0.8640	0.3876
Watering at homestead	-0.7852	0.4560	0.2288	-3.4314	0.0006
Distance to water - <1km	-0.3373	0.7137	0.2348	-1.4363	0.1509
Moderate introgression	-0.1726	0.8415	0.3381	-0.5104	0.6098
Substantial introgression	0.2007	1.2223	0.4629	0.4336	0.6645
European introgression	1.4987	4.4757	1.9401	0.7725	0.4398
Heterozygosity	-8.0219	0.0003	5.4717	-1.4661	0.1426
Calf sex	-0.2360	0.7898	0.2186	-1.0796	0.2803
Recruitment weight	-0.0465	0.9546	0.0293	-1.5859	0.1128
T.parva antibodies - calf	0.0037	1.0037	0.0038	0.9774	0.3284
T.mutans antibodies - calf	-0.0003	0.9997	0.0056	-0.0559	0.9554
A.marginale antibodies - calf	-0.0036	0.9964	0.0062	-0.5808	0.5614
B.bigemina antibodies - calf	0.0052	1.0052	0.0038	1.3790	0.1679
Total serum proteins	-0.1318	0.8765	0.0838	-1.5726	0.1158
White blood cell count	-0.0483	0.9528	0.0355	-1.3616	0.1733
Packed cell volume	0.0184	1.0185	0.0219	0.8391	0.4014
Heart girth size - dam	-0.0260	0.9744	0.0141	-1.8384	0.0660
Body condition score - dam	-0.0965	0.9080	0.1067	-0.9040	0.3660
T.parva antibodies - dam	0.0121	1.0122	0.0042	2.8981	0.0038
T.mutans antibodies - dam	0.0021	1.0021	0.0058	0.3554	0.7223
A.marginale antibodies - dam	-0.0022	0.9978	0.0102	-0.2130	0.8313
B.babesia antibodies - dam	0.0123	1.0123	0.0037	3.3306	< 0.001
mean Monthly NDVI	-1.3177	0.2678	1.5951	-0.8261	0.4088
Elevation	0.0005	1.0005	0.0018	0.2907	0.7713

Table C.2: Results of univariable survival analysis for non-infectious risk factors for ID-mortality. The model used accomodates both time-invariant and time-varying predictors.

	coef	exp(coef)	se(coef)	z	p
Farmers sex - Males	0.2056	1.2283	0.2437	0.8438	0.3988
Farmer's age	0.0139	1.0140	0.0083	1.6758	0.0938
Education - primary school	-0.3960	0.6730	0.2895	-1.3678	0.1714
Education - secondary school	-0.1728	0.8413	0.3286	-0.5258	0.5990
Occupation - salaried	0.1148	1.1216	0.3014	0.3808	0.7033
log(Tropical livestock units)	0.3914	1.4790	0.1485	2.6348	0.0084
log(Total acres owned)	-0.0064	0.9936	0.1399	-0.0460	0.9633
Watering at homestead	-0.7892	0.4542	0.2288	-3.4491	< 0.001
Distance to water - <1km	-0.3514	0.7037	0.2348	-1.4964	0.1345
Housing calves - yes	-0.2391	0.7874	0.4401	-0.5432	0.5870
Suckling - yes	-0.8473	0.4286	0.3931	-2.1555	0.0311
Graze with adults - yes	-0.5114	0.5996	0.5881	-0.8697	0.3845
Milk prior calving	-0.9883	0.3722	0.5880	-1.6809	0.0928
Milk post calving	-0.3225	0.7243	0.2385	-1.3521	0.1763
Use supplements - yes	-0.3868	0.6792	0.2510	-1.5407	0.1234
Vaccine use - yes	0.1214	1.1291	0.2167	0.5604	0.5752
Veterinary support - yes	0.2117	1.2357	0.3232	0.6550	0.5125
Knowledge of diseases	0.1552	1.1679	0.2708	0.5731	0.5666
Housing - stall-shed	-0.2951	0.7445	0.2159	-1.3666	0.1717
Tick control - yes	-1.1423	0.3191	0.2164	-5.2795	< 0.001
Trypanosomes control- yes	-0.3759	0.6867	0.3523	-1.0671	0.2859
Worm control - yes	-0.4214	0.6561	0.2709	-1.5555	0.1198
Antibiotics use - yes	-0.5061	0.6029	0.2348	-2.1551	0.0312
Mean NDVI	-5.1083	0.0060	2.3009	-2.2202	0.0264
Elevation	0.0004	1.0004	0.0018	0.2346	0.8145
Heart girth size - dam	-0.0487	0.9524	0.0171	-2.8537	0.0043
Body condition score - dam	-0.3474	0.7066	0.1247	-2.7865	0.0053
Health of dam - sick	1.3200	3.7435	1.0109	1.3058	0.1916
Calf sex	-0.2435	0.7839	0.2186	-1.1137	0.2654
Moderate introgression	-0.1823	0.8333	0.3381	-0.5392	0.5897
Substantial introgression	0.1739	1.1899	0.4631	0.3755	0.7073
European introgression	1.3728	3.9464	1.9600	0.7004	0.4837
Heterozygosity	-8.1879	0.0003	5.4257	-1.5091	0.1313
T.parva antibodies - dam	0.0132	1.0133	0.0042	3.1277	0.0018
T.mutans antibodies - dam	0.0019	1.0019	0.0059	0.3210	0.7482
A.marginale antibodies - dam	-0.0032	0.9968	0.0106	-0.2996	0.7645
B.babesia antibodies - dam	0.0122	1.0122	0.0037	3.2456	0.0012

Table C.3: Results of univariable survival analysis for correlates of ID-mortality not considered as risk factors in themselves.

	coef	exp(coef)	se(coef)	z	p
Clinical episodes	2.5405	12.6855	0.2338	10.8648	< 0.0001
Total serum proteins	-1.1739	0.3092	0.1296	-9.0552	< 0.0001
White blood cells count	-0.1771	0.8377	0.0395	-4.4882	< 0.0001
Packed cell volume	-0.1943	0.8234	0.0194	-10.0006	< 0.0001

Table C.4: Results of univariable survival analysis for infection predictors of ID-mortality. The infection data is in both binary (presence/absence at start of risk period) and as a quantitative measure (eg. strongyle epg) where applicable.

	coef	exp(coef)	se(coef)	z	p
Anaplasma spp.	0.2754	1.3171	1.0078	0.2733	0.7846
Theileria spp.	0.1776	1.1944	0.2622	0.6774	0.4982
Trypanosoma spp.	1.4528	4.2749	0.5911	2.4576	0.0140
Trypanosoma vivax	1.7109	5.5339	0.7217	2.3706	0.0178
T.parva - seropositivity	-1.5019	0.2227	0.2819	-5.3281	<0.001
T.mutans - seropositivity	-0.7059	0.4937	0.2770	-2.5486	0.0108
A.marginale - seropositivity	-0.4196	0.6573	0.3105	-1.3513	0.1766
B.bigemina - seropositivity	-0.1455	0.8646	0.3379	-0.4307	0.6667
Calicophoron spp.	0.0035	1.0036	0.3499	0.0101	0.9919
Coccidia spp.	-0.0597	0.9421	0.2609	-0.2287	0.8191
Cooperia spp.	1.4661	4.3323	0.7336	1.9986	0.0456
Dictyocaulus viviparus	0.2572	1.2933	0.4400	0.5846	0.5588
Fasciola spp.	0.4266	1.5321	0.7300	0.5844	0.5589
Haemonchus placei	0.1711	1.1866	0.2557	0.6693	0.5033
Moniezia spp.	4.2058	67.0774	1.0691	3.9342	< 0.001
Nematodirus spp.	0.8086	2.2448	1.0144	0.7971	0.4254
Oesophagostomum radiatum	0.0565	1.0581	0.3622	0.1560	0.8760
Toxocara vitulorum	-0.1455	0.8646	0.6038	-0.2409	0.8096
Trichophyton spp.	1.3232	3.7553	0.7234	1.8292	0.0674
Trichostrongylus axei	0.1013	1.1066	0.2681	0.3779	0.7055
Trichuris spp.	0.2298	1.2583	1.0081	0.2279	0.8197
Theileria spp. level 1	0.0433	1.0443	0.2703	0.1603	0.8726
Theileria spp. level 2	0.9699	2.6377	0.4633	2.0935	0.0363
Theileria spp. level 3	2.4197	11.2430	0.7329	3.3014	< 0.001
Strongyle epg/1000	0.3481	1.4164	0.0384	9.0604	< 0.001
log(*Strongyloides* spp.)	0.1147	1.1216	0.1784	0.6430	0.5203
log(*Coccidia* spp.)	0.1614	1.1751	0.1556	1.0373	0.2996
log(*Calicophoron* spp.)	0.4294	1.5363	0.2945	1.4580	0.1448

Table C.5: Results of test for the proportional hazard assumption of cox regression using covariates identified as significant predictors of ID-mortality. The variable "watering at household" is identified to violate the proportional hazard assumption. The global test statistics show marginally significant non-proportionality in the final model.

	rho	chisq	p
Tick control	0.2785	3.54637	0.0597
T.parva	0.0277	0.03554	0.8505
Watering at homestead	-0.3578	6.15102	0.0131
Theileria spp. level 1	-0.0982	0.46894	0.4935
Theileria spp. level 2	0.1728	1.17381	0.2786
Theileria spp. level 3	-0.2370	2.25237	0.1334
Trypanosoma spp.	0.0138	0.00807	0.9284
Strongyle.eggs/1000	0.1782	1.26071	0.2615
GLOBAL	NA	15.95848	0.0430

Table C.6: Results of the final model with predictors of ID-mortality using "watering at homestead" as the *strata* factor. The model fits a different baseline hazard for each level of the variable "watering at homestead". This model accounts for non-proportional hazard associated with the variable "watering at homestead" whose effect decreases with age of calf.

	coef	exp(coef)	se(coef)	z	p
Fixed effects					
Tick control	-0.7143	0.4895	0.3257	-2.1933	0.0283
T.parva - seropositivity	-1.1153	0.3278	0.3766	-2.9617	0.0031
Theileria spp level 1	-0.5746	0.5629	0.3494	-1.6446	0.1000
Theileria spp level 2	0.5972	1.8170	0.5941	1.0052	0.3148
Theileria spp level 3	3.5195	33.7683	0.8749	4.0227	< 0.001
Trypanosoma spp.	2.0125	7.4822	0.7565	2.6602	0.0078
Strongyle eggs/1000	0.3725	1.4513	0.0462	8.0556	< 0.001
Random effects					
Group	Variable	Std Dev	Variance		
Sub-location	Intercept	0.0199	0.0004		

Level 1 - One infected cell in more than 10 microscopic fields (low intensity infection).
Level 2 - One or more infected cells for every 10 fields (medium intensity infection).
Level 3 - Multiple infected cells in every microscopic field (high intensity infection).
Random effect - one standard deviation above the mean corresponds to a risk ID-mortality that is exp(0.0.0199) = 1.02 times higher in that sublocation.

Appendix D

Cause-specific mortality among zebu cattle under one year: the role of co-infections

Table D.1: Results of survival analysis univariable screening for non-infectious predictors of ECF-mortality.

	coef	exp(coef)	se(coef)	z	p
Education-Primary school	-0.7768	0.4599	0.4083	-1.9025	0.0571
Education-Secondary school	-0.8946	0.4088	0.5271	-1.6972	0.0897
log(Tropical livestock units)	0.3380	1.4021	0.2435	1.3879	0.1652
Occupation - salaried	0.0996	1.1048	0.4857	0.2052	0.8374
log(Total acres owned)	-0.2248	0.7987	0.2250	-0.9993	0.3176
Farmer's age	0.0255	1.0258	0.0125	2.0401	0.0413
Farmer's sex	-0.1139	0.8923	0.3693	-0.3085	0.7577
Housing calves - yes	0.7745	2.1696	0.6010	1.2886	0.1975
Suckling - yes	-1.7598	0.1721	0.7833	-2.2466	0.0247
Watering at homestead	-0.4263	0.6529	0.3522	-1.2102	0.2262
Distance to water < 1km	-0.3485	0.7058	0.3789	-0.9198	0.3577
Grazing with adults	-0.6189	0.5385	1.0165	-0.6088	0.5426
Milk prior calving	-1.1469	0.3176	1.0155	-1.1294	0.2587
Milk post calving	0.3042	1.3555	0.4517	0.6734	0.5007
Vaccine use - yes	-0.0187	0.9814	0.3484	-0.0538	0.9571
Supplements use - yes	-0.6869	0.5031	0.3788	-1.8133	0.0698
Housing stall-shed	-0.5649	0.5684	0.3497	-1.6154	0.1062
Knowledge of diseases - yes	-0.1040	0.9013	0.4062	-0.2559	0.7980
Veterinary support - yes	-0.1940	0.8236	0.4514	-0.4298	0.6673
Worm control - yes	-0.9958	0.3694	0.5335	-1.8666	0.0620
Antibiotics use - yes	-1.1673	0.3112	0.4514	-2.5863	0.0097
Trypanosome control - yes	-0.5356	0.5853	0.6056	-0.8845	0.3764
Tick control - yes	-1.6809	0.1862	0.3695	-4.5491	< 0.001
Mean NDVI	-7.9858	0.0003	3.4122	-2.3404	0.0193
Elevation	-0.0033	0.9967	0.0031	-1.0591	0.2895
Heart girth size - dam	-0.0409	0.9599	0.0275	-1.4892	0.1364
Body condition score - dam	-0.4115	0.6626	0.2035	-2.0224	0.0431
Health of dam - sick	2.3967	10.9872	1.0295	2.3280	0.0199
A.marginale antibodies - dam	-0.0124	0.9877	0.0185	-0.6716	0.5019
T.mutans antibodies - dam	0.0011	1.0011	0.0095	0.1136	0.9096
B.bigemina antibodies - dam	0.0121	1.0122	0.0059	2.0510	0.0403
T.parva antibodies - dam	0.0156	1.0157	0.0066	2.3563	0.0185
Calf sex	-0.3361	0.7145	0.3563	-0.9435	0.3454
Recruitment weight	-0.0482	0.9530	0.0474	-1.0160	0.3096
Moderate introgression	-0.4393	0.6445	0.6086	-0.7218	0.4704
Substantial introgression	0.2359	1.2660	0.7329	0.3219	0.7475
Heterozygosity	-7.0960	0.0008	8.8901	-0.7982	0.4248
Clinical episode	2.5873	13.2933	0.3688	7.0156	< 0.001
Total serum proteins	-1.0589	0.3468	0.1983	-5.3404	< 0.001
White blood cell count	-0.2774	0.7577	0.0651	-4.2609	< 0.001
Packed cell volume	-0.2052	0.8145	0.0295	-6.9581	< 0.001

Table D.2: Results of survival analysis univariable screening for infectious predictors of ECF-mortality.

	coef	exp(coef)	se(coef)	z	p
T.parva - seropositivity	-1.8999	0.1496	0.5264	-3.6094	0.0003
T.mutans - seropositivity	-1.5190	0.2189	0.4836	-3.1412	0.0017
A.marginale - seropositivity	-1.4427	0.2363	0.7500	-1.9236	0.0544
B.bigemina - seropositivity	-0.3867	0.6793	0.6325	-0.6114	0.5410
Anaplasma spp.	1.4318	4.1862	1.0182	1.4062	0.1597
Theileria spp.	-0.1798	0.8354	0.4076	-0.4412	0.6591
Trypanosoma spp.	2.0561	7.8153	0.7369	2.7901	0.0053
Trypanosoma vivax	1.9136	6.7775	1.0279	1.8617	0.0626
Calicophoron spp.	0.6950	2.0038	0.5078	1.3689	0.1710
Coccidia spp.	0.0360	1.0366	0.4212	0.0854	0.9319
Dictyocaulus viviparus	-0.2211	0.8016	0.7441	-0.2972	0.7663
Fasciola spp.	1.4627	4.3176	1.0572	1.3835	0.1665
Haemonchus placei	0.8480	2.3349	0.4421	1.9182	0.0551
Toxocara vitulorum	-0.6830	0.5051	1.0289	-0.6638	0.5068
Trichostrongylus axei	-0.2038	0.8156	0.4780	-0.4263	0.6699
Strongyle eggs	1.1736	3.2336	0.5038	2.3295	0.0198
Trichophyton spp.	1.4201	4.1375	1.0284	1.3809	0.1673
Theileria spp level 1	-0.5009	0.6060	0.4461	-1.1228	0.2615
Theileria spp level 2	0.7814	2.1845	0.7721	1.0121	0.3115
Theileria spp level 3	3.0052	20.1897	0.7546	3.9824	0.0001
Calicophoron spp./1000	-0.1871	0.8294	1.6334	-0.1145	0.9088
Coccidia spp./1000	-0.0033	0.9967	0.0446	-0.0737	0.9412
Strongyle eggs/1000	0.2826	1.3265	0.0816	3.4647	0.0005

Table D.3: Results of test for the proportional hazard assumption of Cox regression using covariates identified as significant predictors of ECF-mortality. The global test statistics shows no evidence of non-proportionality in this model.

	rho	chisq	p
Tick control	0.1999	0.645	0.422
T.parva - seropositivity	0.3152	1.186	0.276
T.mutans - seropositivity	0.4120	2.289	0.130
Trypanosoma spp.	0.3058	1.576	0.209
Strongyle eggs/1000	-0.0401	0.015	0.903
GLOBAL	NA	6.755	0.240

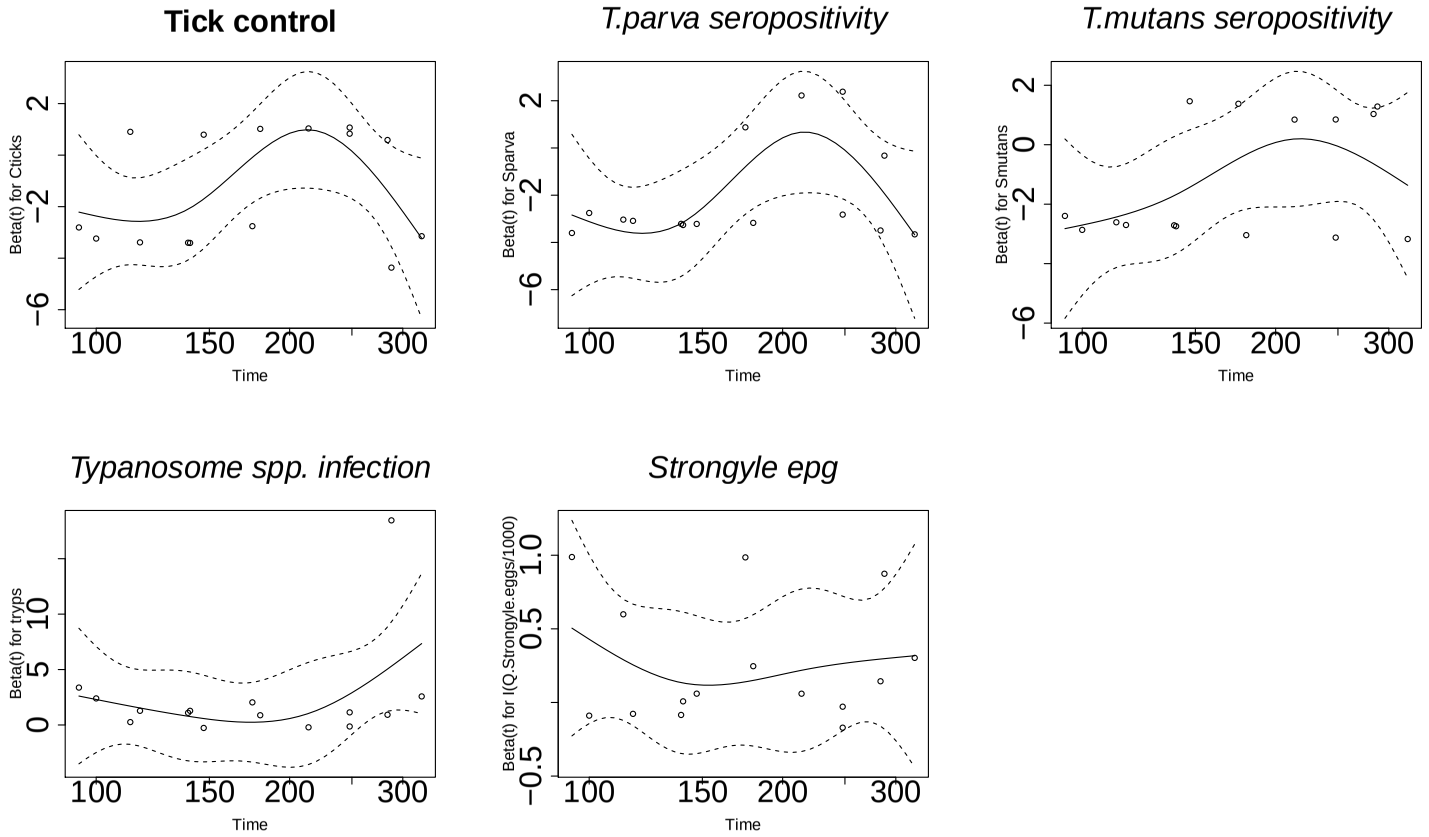

Figure D.1: Scaled Schoenfeld residuals plotted against transformed time for each of the significant predictors for ECF-mortality. The broken lines represent ± 2 standard error around the fit, and the solid line is smoothing line added to facilitate interpretation. There is no evidence of systematic departures from the horizontal line which would indicate non-proportional hazards.

Table D.4: Results of survival analysis univariable screening for non-infectious predictors of haemonchosis deaths.

	coef	exp(coef)	se(coef)	z	p
Education-Primary school	0.1379	1.1478	1.0955	0.1258	0.8999
Education-Secondary school	0.8968	2.4517	1.1180	0.8021	0.4225
log(Tropical livestock units)	0.1323	1.1415	0.4611	0.2870	0.7741
Occupation - salaried	-0.3539	0.7020	1.0541	-0.3357	0.7371
log(Total acres owned)	0.4868	1.6270	0.3856	1.2623	0.2068
Farmer's age	-0.0142	0.9859	0.0236	-0.6015	0.5475
Farmer's sex	0.5991	1.8206	0.7906	0.7579	0.4485
Housing calves - yes	0.4616	1.5866	1.1547	0.3998	0.6893
Suckling - yes	-1.6464	0.1927	0.8143	-2.0220	0.0432
Watering at homestead	-1.0102	0.3642	0.6901	-1.4637	0.1433
Distance to water < 1km	-0.7883	0.4546	0.6455	-1.2212	0.2220
Grazing with adults	0.5629	1.7558	1.0541	0.5340	0.5933
Milk post calving	0.1379	1.1479	0.7906	0.1744	0.8615
Vaccine use - yes	0.3493	1.4180	0.6455	0.5411	0.5884
Supplements use - yes	-1.1164	0.3275	0.6455	-1.7295	0.0837
Housing stall-shed	-0.8008	0.4490	0.6455	-1.2406	0.2148
Knowledge of diseases - yes	0.1481	1.1597	0.7906	0.1874	0.8514
Veterinary support - yes	-0.2836	0.7531	0.7906	-0.3587	0.7198
Worm control - yes	0.0947	1.0994	0.6901	0.1373	0.8908
Antibiotics use - yes	-0.5421	0.5815	0.6901	-0.7856	0.4321
Tick control - yes	0.3259	1.3853	0.7906	0.4122	0.6802
Mean NDVI	-8.7281	0.0002	6.4139	-1.3608	0.1736
Elevation	0.0101	1.0101	0.0042	2.3822	0.0172
Heart girth size - dam	0.0155	1.0156	0.0538	0.2872	0.7740
Body condition score - dam	-0.4456	0.6404	0.4227	-1.0541	0.2918
A.marginale antibodies - dam	0.0208	1.0210	0.0232	0.8971	0.3696
T.mutans antibodies - dam	0.0160	1.0162	0.0142	1.1302	0.2584
B.bigemina antibodies - dam	0.0153	1.0154	0.0105	1.4568	0.1452
T.parva antibodies - dam	0.0209	1.0212	0.0119	1.7630	0.0779
Calf sex	-0.3213	0.7252	0.6455	-0.4978	0.6187
Recruitment weight	0.0590	1.0608	0.0862	0.6845	0.4937
Moderate introgression	1.0864	2.9636	0.7071	1.5364	0.1244
Substantial introgression	1.0352	2.8157	1.0802	0.9584	0.3379
Heterozygosity	2.1225	8.3517	18.3775	0.1155	0.9081
Clinical episode	3.7554	42.7518	0.6969	5.3887	< 0.001
Total serum proteins	-3.9585	0.0191	0.6302	-6.2816	< 0.001
White blood cell count	-0.4878	0.6140	0.1237	-3.9443	< 0.001
Packed cell volume	-0.9162	0.4000	0.2207	-4.1506	< 0.001

Table D.5: Results of survival analysis univariable screening for infectious predictors of haemonchosis deaths.

	coef	exp(coef)	se(coef)	z	p
T.parva - seropositivity	-1.3808	0.2514	0.6457	-2.1385	0.0325
T.mutans - seropositivity	-0.2330	0.7921	0.6901	-0.3377	0.7356
A.marginale - seropositivity	-0.2141	0.8072	0.6901	-0.3103	0.7563
B.bigemina - seropositivity	-0.9822	0.3745	1.0541	-0.9318	0.3514
Calicophoron spp.	0.1193	1.1267	0.8113	0.1471	0.8831
Dictyocaulus viviparus	0.9629	2.6193	1.1103	0.8672	0.3858
Nematodirus spp.	2.6178	13.7054	1.0865	2.4094	0.0160
Oesophagostomum radratium	1.4429	4.2329	0.6502	2.2190	0.0265
Strongyloides spp.	0.0391	1.0398	1.1272	0.0347	0.9723
Trichostrongylus axei	0.7575	2.1330	0.6334	1.1959	0.2317
Calicophoron spp./1000	1.6499	5.2065	0.7856	2.1003	0.0357
Strongyle eggs/1000	0.4858	1.6254	0.0706	6.8799	< 0.001

Due to the small number of haemonchosis deaths, the survival models could not make estimates of hazard risk for many of the pathogens identified in the study. This table shows results with only pathogens where the model was able to estimate effects.

Table D.6: Results of test for the proportional hazard assumption of Cox regression using covariates identified as significant predictors of haemonchus deaths. The global test statistics shows no evidence of non-proportionality in this model.

	rho	chisq	p
Use of supplements - yes	0.299	0.637	0.4249
Elevation/100	0.252	0.344	0.5578
Nematodirus spp.	-0.565	3.368	0.0665
Strongyle epg/1000	-0.456	1.427	0.2322
GLOBAL	NA	6.293	0.1783

Table D.7: Results of test for the proportional hazard assumption of Cox regression using covariates identified as significant predictors of heartwater deaths. The global test statistics shows no evidence of non-proportionality in this model.

	rho	chisq	p
log(Total livestock units)	-0.112	0.0409	0.840
Mean NDVI x 10	0.384	1.3977	0.237
Farmer's age/10	0.755	2.3251	0.127
GLOBAL	NA	4.1511	0.246

Appendix E

Cost of infection and coinfections on growth performance of zebu cattle under one year

Table E.1: Results of "recruitment" model univariable screen for non-infectious factors associated with growth rate. The model uses data obtained at recruitment time only.

	Estimate	Std. Error	t value	Pr(> |t|)
Farm level factors				
Farmer's age	-0.0003	0.0002	-1.57	0.1178
Farmer's gender	0.0041	0.0048	0.85	0.3964
Education - Primary school	-0.0029	0.0065	-0.45	0.6531
Education - Secondary school	0.0015	0.0075	0.20	0.8390
Training - Technical training	-0.0015	0.0053	-0.29	0.7744
Occupation - salaried	0.0087	0.0065	1.33	0.1847
log(Total acres owned)	0.0011	0.0030	0.37	0.7126
log(Livestock units owned)	-0.0151	0.0033	-4.65	< 0.001
Housing stall - shed	-0.0048	0.0047	-1.04	0.3011
Graze with adults - Yes	0.0083	0.0094	0.88	0.3785
Distance to water - at homestead	0.0066	0.0053	1.25	0.2124
Water quality - Muddy	0.0002	0.0160	0.01	0.9899
Use supplements - yes	-0.0017	0.0059	-0.29	0.7746
Use Vaccines - yes	-0.0025	0.0045	-0.56	0.5748
Vet Support - yes	-0.0068	0.0061	-1.11	0.2677
Milking prior calving	-0.0054	0.0076	-0.71	0.4793
Milking post calving	0.0056	0.0054	1.04	0.2969
Calf level factors				
Calf sex - female	-0.0071	0.0044	-1.60	0.1097
Moderate introgression	0.0064	0.0065	0.99	0.3249
Substantial introgression	0.0257	0.0106	2.43	0.0156
Introgression - pure	0.0113	0.0057	1.96	0.0503
Recruitment weight	0.0021	0.0006	3.37	< 0.001
Heterozygosity	0.3131	0.1224	2.56	0.0108
Antibodies - *T.parva*	-0.0000	0.0001	-0.46	0.6447
Antibodies - *A.marginale*	0.0001	0.0001	0.55	0.5839
Antibodies - *B.bigemina*	-0.0001	0.0001	-1.48	0.1402
Antibodies - *T.mutans*	-0.0000	0.0001	-0.21	0.8362
Total serum proteins	-0.0008	0.0018	-0.48	0.6347
White blood count	0.0001	0.0001	0.64	0.5245
Packed cell volume	-0.0002	0.0005	-0.45	0.6545
Dam level factors				
Heart girth size - dam	0.0019	0.0003	7.01	< 0.001
Body condition score - dam	0.0161	0.0021	7.77	< 0.001
Blind teats - Yes	-0.0017	0.0096	-0.18	0.8561
Antibodies - *T.parva*	-0.0000	0.0001	-0.08	0.9357
Antibodies - *A.marginale*	-0.0002	0.0002	-0.89	0.3717
Antibodies - *B.bigemina*	-0.0002	0.0001	-1.56	0.1203
Antibodies - *T.mutans*	-0.0001	0.0001	-0.54	0.5866
Environmental factors				
NDVI (month of birth)	-0.0067	0.0266	-0.25	0.8021
Elevation	0.0001	0.0000	3.03	0.0026

Table E.2: Correlates of variables associated with ADWG in the minimum adequate "recruitment" model, and variance explained (R-squared) when minimum adequate model variables (in blue) are replaced with known correlates (in black).

	Estimate	Std. Error	t value	Pr(> |t|)	R-squared
(Intercept)	-0.1579	0.0572	-2.76	0.0062	
log(Livestock units owned)	-0.0088	0.0032	-2.73	0.0066	0.154
log(Total acres owned)	0.0012	0.0028	0.45	0.6520	0.135
Farmer's age	-0.0001	0.0002	-0.63	0.5274	0.134
Dam Girth	0.0012	0.0003	3.84	0.0001	0.154
Recruitment weight	0.0008	0.0006	1.31	0.1916	0.133
Condition Score Dam	0.0091	0.0025	3.61	0.0003	0.154
Recruitment weight	0.0004	0.0006	0.59	0.5545	0.129
Moderate introgression	0.0008	0.0061	0.13	0.8969	0.129
Substantial introgression	0.0100	0.0103	0.97	0.3344	0.129
Calf sex - female	-0.0087	0.0041	-2.10	0.0360	0.154

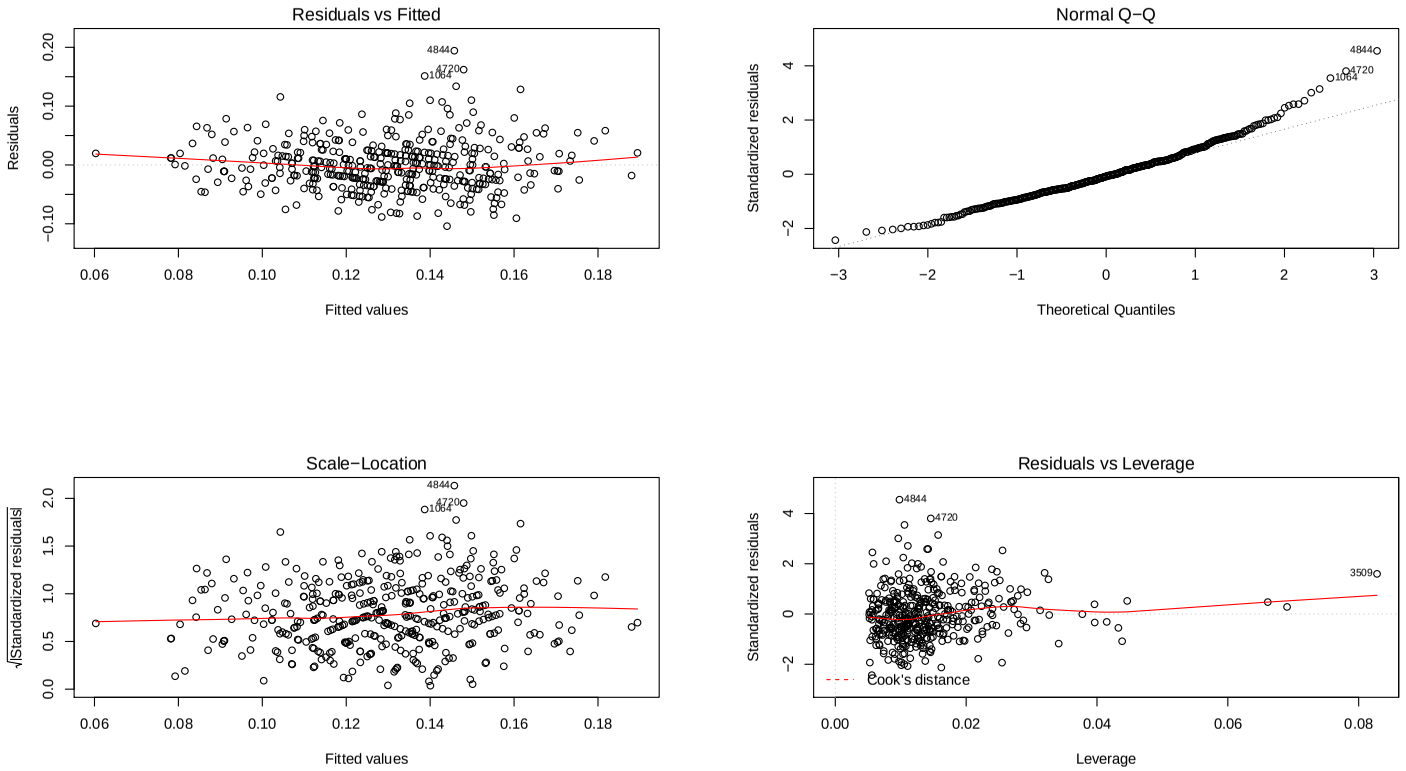

Figure E.1: Model diagnostics for the final recruitment model for growth. The distribution of average daily weight gain is not perfectly normal (see Normal Q-Q), particularly for calves with high growth rate. The variance was constant (see Residuals vs Fitted plot). The Scale-location plot does not show any trend in the residuals. The labelled points identify possible outliers - animals with the highest growth rates. The residuals vs leverage plot does not show any outliers with a strong influence in the model, which would be identified by Cook's distance.The fitted model explains 16.7% of the variation in growth rates.

Table E.3: Results of univariable analysis of time varying predictors, using the summary measure data analysis approach.

	Estimate	Std. Error	t value	Pr(> |t|)
(Intercept)	0.1279	0.0044	29.21	< 0.001
Disease control				
Tick control	0.0024	0.0051	0.47	0.6403
Worm control	-0.0109	0.0049	-2.22	0.0270
Trypanosome control	-0.0004	0.0063	-0.06	0.9536
Vaccine use	0.0149	0.0108	1.38	0.1678
Antibiotics use	0.0027	0.0045	0.59	0.5545
Dam factors				
Weaned -Yes	-0.0048	0.0044	-1.08	0.2803
Mean body condition score - dam	0.0195	0.0025	7.87	< 0.001
Mean heart girth size - dam	0.0022	0.0003	7.27	< 0.001
Blind udder quarter - dam	-0.0008	0.0072	-0.11	0.9086
Environmental factors				
Mean NDVI	0.0511	0.0569	0.90	0.3692

Table E.4: Results of maximum and minimum models for the time-varying non-infectious factors

	Estimate	Std. Error	t value	Pr(> |t|)
maximum model				
(Intercept)	-0.0915	0.0450	-2.03	0.0426
Worms control	-0.0056	0.0046	-1.22	0.2248
Vaccine use	0.0057	0.0102	0.56	0.5759
Mean body condition score - dam	0.0129	0.0032	3.98	0.0001
Mean heart girth size - dam	0.0011	0.0004	2.89	0.0041
minimum model				
(Intercept)	-0.0985	0.0447	-2.20	0.0280
Mean body condition score - dam	0.0133	0.0032	4.12	< 0.001
Mean heart girth size - dam	0.0012	0.0004	2.97	0.0032

Table E.5: Minimum adequate model for the non-infectious factors' relationships with ADWG. The footnotes indicate the changes in amount of variation explained by the model when variables are replaced with their correlates.

	Estimate	Std. Error	t value	Pr(> |t|)
Fixed effects				
(Intercept)	-0.1131	0.0418	-2.71	0.0071
log(Tropical livestock units)	-0.0092	0.0032	-2.91	0.0038
Mean heart girth size - dam	0.0015	0.0003	4.47	< 0.001
Body condition score - dam	0.0093	0.0024	3.89	< 0.001
Calf sex - female	-0.0087	0.0041	-2.14	0.0332
Random effects				
Group	name	Std Dev	Variance	
Sub-location	Intercept	0.0131581	0.0001731	
Residual		0.0414025	0.0017142	

-Above model - R-squared 16.7%
-Replacing condition score at birth with mean condition score R-squared 15.7%
-Replacing mean Dam Girth with Girth at birth R-squared 15.4%
-Removing Calf sex reduces the R-squared slightly to 16.0%
-Intraclass correlation coefficient = 0.091 (sublocation)

Table E.6: Results of univariable analysis with infection data and ADWG. The data used captures either presence of the infection at one year or history of infection with the pathogen over the one year observation time.

	Estimate	Std. Error	t value	Pr(> |t|)
Serology				
Viral and infections				
Blue tongue virus	0.0124	0.0099	1.25	0.2114
Epizootic Hemorrhagic disease virus	0.0094	0.0047	2.01	0.0448
Infectious Bovine Rhinotracheitis	-0.0017	0.0095	-0.18	0.8590
Bovine viral diarrhoea virus	0.0048	0.0059	0.82	0.4145
Parainfluenza virus type 3	0.0019	0.0058	0.32	0.7472
Protozoa				
Neospora caninum	-0.0034	0.0064	-0.52	0.6024
TBD seroconversion				
Theileria parva	-0.0085	0.0049	-1.73	0.0835
Theileria mutans	0.0013	0.0049	0.26	0.7932
Babesia bigemina	0.0022	0.0055	0.40	0.6882
Anaplasma marginale	0.0003	0.0048	0.07	0.9445
Reverse line blots				
Anaplasma bovis	-0.0025	0.0046	-0.54	0.5879
Ehrlichia omatjenne	0.0048	0.0045	1.06	0.2893
Theileria sable	0.0069	0.0048	1.42	0.1573
Theileria mutans	0.0030	0.0048	0.63	0.5316
Theileria parva	0.0025	0.0068	0.37	0.7142
Theileria taurotragi	0.0251	0.0085	2.95	0.0034
Theileria velifera	-0.0027	0.0046	-0.58	0.5619
Hemoparasites (microscopy)				
Anaplasma spp.	-0.0156	0.0073	-2.14	0.0325
Babesia spp.	-0.0299	0.0274	-1.09	0.2759
Trypanosoma spp.	-0.0048	0.0084	-0.57	0.5720
Helminths - Presence Absence				
Calicophoron spp.	-0.0151	0.0050	-3.03	0.0026
Coccidia spp.	-0.0178	0.0106	-1.68	0.0930
Cooperia spp.	-0.0070	0.0096	-0.74	0.4622
Dictyocaulus viviparus	-0.0039	0.0046	-0.83	0.4044
Fasciola spp.	0.0056	0.0058	0.96	0.3366
Haemonchus placei	-0.0103	0.0336	-0.31	0.7585
Microfilaria spp.	-0.0031	0.0195	-0.16	0.8741
Moniezia spp.	0.0154	0.0238	0.65	0.5172
Nematodirus spp.	-0.0169	0.0103	-1.64	0.1026
Oesophagostomum radiatum	-0.0096	0.0047	-2.04	0.0424
Ostertagia ostertagi	-0.0008	0.0160	-0.05	0.9579
Strongyloides spp.	-0.0063	0.0045	-1.39	0.1639
Toxocara vitulorum	-0.0066	0.0052	-1.27	0.2065
Trichophyton spp.	-0.0301	0.0084	-3.56	0.0004
Trichostrongylus axei	-0.0068	0.0083	-0.82	0.4142
Trichuris spp.	-0.0092	0.0072	-1.28	0.2018
mean Strongyle epg	-0.00002	0.0000	-6.99	< 0.001

Table E.7: Results from the maximum model and minimum adequate (univariate) model showing associations between infections from routine visits and average daily weight gain.

	Estimate	Std. Error	t value	Pr(> |t|)
Maximum model				
(Intercept)	0.1634	0.0064	25.45	< 0.001
Anaplasma spp.	-0.0124	0.0069	-1.81	0.0716
Serology - *T.parva*	-0.0044	0.0048	-0.92	0.3579
Calicophoron spp	-0.0138	0.0048	-2.87	0.0043
Coccidia spp	0.0024	0.0045	0.54	0.5902
Nematodirus spp.	-0.0123	0.0098	-1.26	0.2101
Oesophagostomum radiatum	-0.0025	0.0046	-0.55	0.5833
Strongyloides spp.	-0.0011	0.0043	-0.26	0.7947
Trichophyton spp.	-0.0218	0.0081	-2.68	0.0077
(Mean strongyle epg/1000)	-0.0228	0.0036	-6.41	< 0.001
Minimum adequate model				
(Intercept)	0.1581	0.0047	33.74	< 0.001
Calicophoron spp.	-0.0146	0.0047	-3.09	0.0021
Trichophyton spp.	-0.0230	0.0081	-2.86	0.0045
(Mean strongyle epg/1000)	-0.0238	0.0034	-6.96	< 0.001

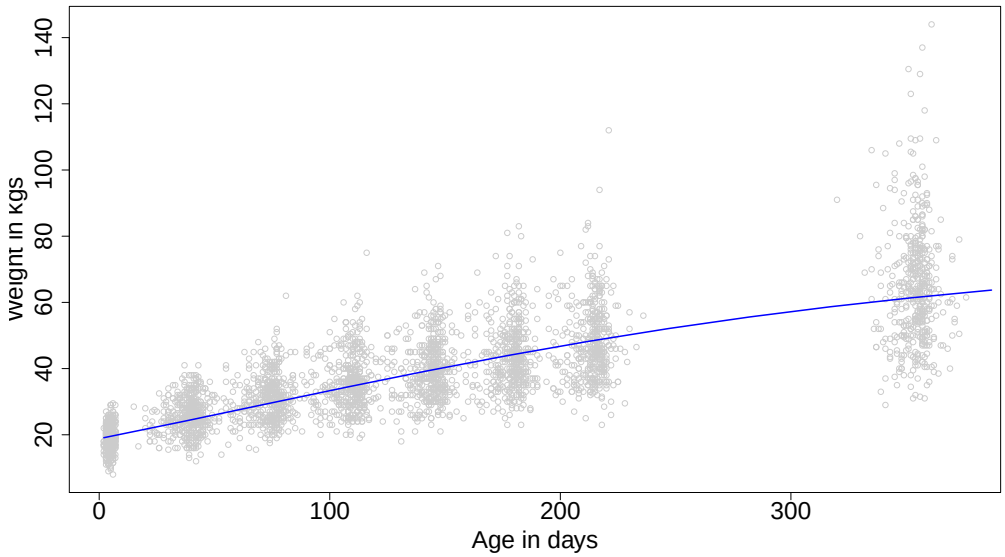

Figure E.2: Plot showing predicted mean growth curve using Brody's growth model.

Table E.8: Results of univariable analysis showing the relationship between growth rate (slope) and non-infectious factors, using mixed models.

	Value	Std.Error	DF	t-value	p-value
Farmers sex - Males	0.0051	0.0048	2972	1.0659	0.2865
Farmer's age	-0.0003	0.0002	2946	-1.8030	0.0715
Primary school	0.0005	0.0065	2952	0.0695	0.9446
Secondary school	0.0044	0.0075	2952	0.5833	0.5597
Occupation - salaried	0.0098	0.0065	2953	1.4951	0.1350
Tropical livestock Units	-0.0011	0.0003	2966	-3.0823	0.0021
Total acres	-0.0001	0.0004	2803	-0.1730	0.8627
Watering at homestead	0.0110	0.0045	2902	2.4352	0.0149
Distance to water	0.0090	0.0053	2958	1.7084	0.0877
Housing calves	0.0047	0.0074	1716	0.6399	0.5223
Suckling	0.0267	0.0050	2966	5.3261	< 0.001
Graze with adults	0.0080	0.0094	2928	0.8462	0.3975
Milk prior calving	-0.0051	0.0076	2941	-0.6751	0.4997
Milk post calving	0.0058	0.0054	2966	1.0776	0.2813
Use supplements	-0.0013	0.0059	2954	-0.2262	0.8211
Vaccine use	0.0177	0.0108	2972	1.6367	0.1018
Veterinary support	-0.0074	0.0061	2952	-1.2208	0.2222
Knowledge of diseases	-0.0050	0.0054	2953	-0.9220	0.3566
Housing stall-shed	-0.0062	0.0046	2952	-1.3578	0.1746
Tick control -Yes	0.0026	0.0051	2972	0.5011	0.6163
Trypanosomes control- Yes	0.0010	0.0063	2972	0.1559	0.8762
Worm control - Yes	-0.0096	0.0049	2972	-1.9525	0.0510
Antibiotics use -Yes	0.0026	0.0045	2972	0.5707	0.5682
Moderate introgression	0.0083	0.0065	2964	1.2868	0.1983
Substantial introgression	0.0277	0.0106	2964	2.6225	0.0088
Heterozygosity	0.3310	0.1225	2905	2.7022	0.0069
Calf sex	-0.0064	0.0044	2972	-1.4506	0.1470
Recruitment weight	0.0024	0.0006	2972	4.1339	<0.001
Heart girth size - dam	0.0013	0.0002	2319	6.9765	<0.001
Body condition score - dam	0.0056	0.0012	2328	4.8449	<0.001
Dam *T.parva* antibodies	0.0000	0.0001	2972	0.1408	0.8880
Dam *T.mutans* antibodies	-0.0001	0.0001	2972	-0.5677	0.5703
Dam *A.marginale* antibodies	-0.0002	0.0002	2972	-0.9169	0.3592
Dam *B.bigemina* antibodies	-0.0002	0.0001	2972	-1.5663	0.1174
mean Monthly NDVI	-0.0196	0.0108	2971	-1.8071	0.0709
Mean NDVI	0.0509	0.0570	2972	0.8930	0.3719
Elevation	0.0001	0.0000	2972	2.7680	0.0057
Rhipicephalus appendiculatus	-0.0125	0.0031	2971	-4.0364	<0.001
Amblyomma variegatium	-0.0226	0.0017	2971	-13.5362	<0.001
Boophilus microplus	-0.0039	0.0031	2971	-1.2597	0.2079
Rhipicephalus evertsi	-0.0149	0.0022	2971	-6.6807	<0.001
Lice	-0.0250	0.0034	2971	-7.3473	<0.001
Fleas	0.0040	0.0042	2970	0.9593	0.3375

Table E.9: Results of mixed models univariable analysis of infectious factors and their interactions associated with growth rate (slope).

	Value	Std.Error	DF	t-value	p-value
Anaplasma spp. - microscopy	-0.0107	0.0087	2971	-1.2332	0.2176
Babesia spp. - microscopy	-0.0986	0.0545	2971	-1.8081	0.0707
Theileria spp. - microscopy	-0.0119	0.0017	2971	-7.1119	<0.001
Trypanosoma spp. - microscopy	-0.0137	0.0074	2971	-1.8340	0.0668
Trypanosoma vivax - microscopy	-0.0155	0.0102	2971	-1.5146	0.1300
Trypanosoma theileri - microscopy	0.0082	0.0321	2971	0.2564	0.7977
T.parva - serology	-0.0171	0.0013	2972	-13.0000	<0.001
T.mutans - serology	-0.0099	0.0018	2972	-5.6048	<0.001
A.marginale - serology	-0.0072	0.0017	2972	-4.2699	<0.001
B.bigemina - serology	-0.0095	0.0020	2972	-4.7441	<0.001
T.parva:T.mutans	0.0066	0.0025	2970	2.6208	0.0088
T.parva:A.marginale	-0.0063	0.0027	2970	-2.3652	0.0181
T.parva:B.bigemina	0.0005	0.0033	2970	0.1523	0.8790
T.mutans:A.marginale	0.0017	0.0038	2970	0.4440	0.6571
B.bigemina:A.marginale	0.0030	0.0036	2970	0.8363	0.4031
Calicophoron spp.	-0.0141	0.0024	2971	-5.9688	<0.001
Coccidia spp.	-0.0094	0.0019	2971	-4.9724	<0.001
Cooperia spp.	0.0055	0.0211	2971	0.2626	0.7929
Dictyocaulus viviparus	-0.0114	0.0049	2971	-2.3389	0.0194
Fasciola spp.	-0.0153	0.0070	2971	-2.1812	0.0293
Haemonchus placei	-0.0148	0.0016	2971	-9.0233	<0.001
Microfilaria spp.	0.0065	0.0209	2971	0.3110	0.7558
Moniezia spp.	-0.8659	0.4184	2971	-2.0695	0.0386
Nematodirus spp.	0.0304	0.0357	2971	0.8514	0.3946
Oesophagostomum radiatum	-0.0140	0.0032	2971	-4.4257	<0.001
Ostertagia ostertagi	-0.0185	0.0405	2971	-0.4577	0.6472
Strongyle epg/1000	-0.0046	0.0012	1565	-4.0208	<0.001
Strongyloides spp.	-0.0105	0.0030	2971	-3.5214	<0.001
Toxocara vitulorum	0.0025	0.0063	2971	0.3961	0.6920
Trichophyton spp.	-0.0291	0.0059	2971	-4.9206	<0.001
Trichostrongylus axei	-0.0151	0.0021	2971	-7.2579	<0.001
Trichuris spp.	-0.0202	0.0084	2971	-2.3955	0.0167

Table E.10: Results of the final minimum adequate model using MCMC sampling. The Estimate and MCMCmean columns (column 1 and 2) show the model estimates and the mean estimate across the MCMC samples. Columns 3 and 4 show upper and lower 95% highest posterior density intervals. The last column the p-values based on t-distribution.

	Estimate	MCMCmean	HPD95lower	HPD95upper	Pr(> |t|)
Intercept					
(Intercept)	20.9328	21.0214	19.8048	22.2744	< 0.001
Age in days	0.1340	0.1374	0.1256	0.1497	< 0.001
(Heart girth size - 135)/10	-0.4857	1.2263	0.4848	2.0273	0.2795
Occupation - salaried	-1.3728	-1.4122	-3.1672	0.4149	0.2431
(Elevation - 1240)/100	1.0051	1.0067	-0.0675	2.0476	0.1111
Watering at homestead	0.8835	0.7452	-0.4067	1.7735	0.2477
Calf sex - female	-0.1148	-0.2229	-1.3370	0.8157	0.8797
T.parva	1.3103	1.2436	-0.2875	2.8284	0.0705
T.mutans	-0.3719	-0.2254	-1.5192	1.0613	0.5604
A.marginale	2.3397	2.4580	-0.0077	4.7681	0.0309
Trichophyton spp.	0.8943	-1.5346	-4.8520	1.4766	0.5849
(Strongyle epg/1000)	0.2589	0.0138	-0.4972	0.5169	0.2472
Growth rate (slope)					
Age:(Heart girth size - 135)/10	0.0159	0.0105	0.0045	0.0160	< 0.001
Age:Occupation - salaried	0.0234	0.0241	0.0071	0.0403	0.0014
Age:(Elevation - 1240)/100	0.0108	0.0115	0.0027	0.0209	0.0077
Age:Watering at homestead	0.0113	0.0110	-0.0006	0.0221	0.0214
Age:Calf sex - female	-0.0129	-0.0131	-0.0245	-0.0025	0.0081
Age:T.parva	-0.0194	-0.0211	-0.0315	-0.0100	0.0001
Age:T.mutans	-0.0020	-0.0051	-0.0163	0.0058	0.6865
Age:A.marginale	-0.0013	-0.0027	-0.0160	0.0116	0.8298
Age: *Trichophyton* spp.	-0.0252	-0.0183	-0.0404	0.0021	0.0130
Age:(Strongyle epg/1000)	-0.0042	-0.0042	-0.0070	-0.0012	0.0011
Age:*T.parva:T.mutans*	0.0123	0.0131	0.0038	0.0221	0.0016
Age:*T.parva:A.marginale*	-0.0114	-0.0090	-0.0188	0.0007	0.0050

The function `pvals.fnc()` from the `R` package `languageR` was used to calculate the p-values reported here. The degrees of freedom used for the t-distribution is an upper bound: number of observations minus number of fixed-effects parameters. The p-values from this are anti-conservative for small samples. For large degrees of freedom the t-distribution converges to a standard normal distribution (Baayen et al., 2008).

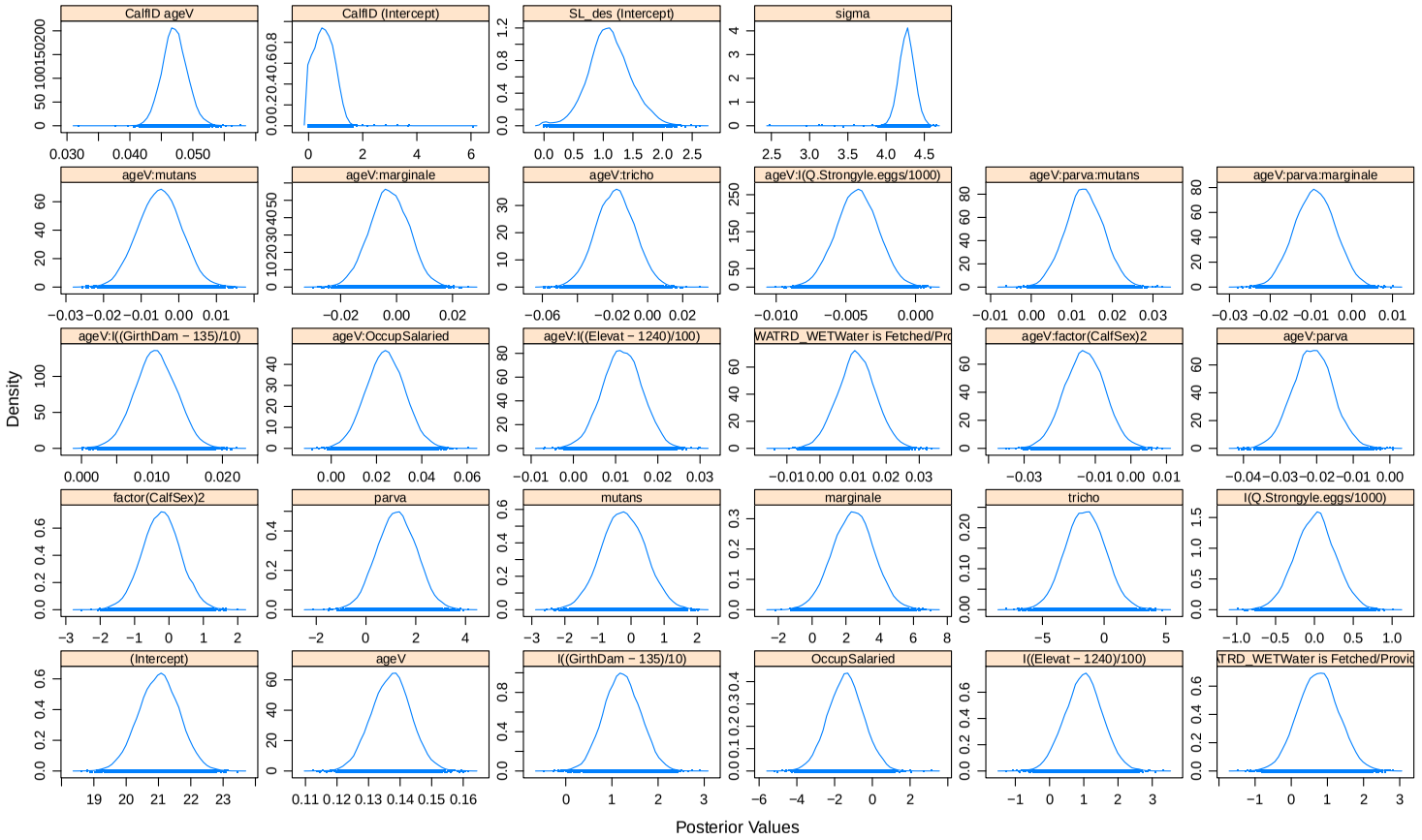

Figure E.3: Posterior distribution densities for the final variables with significant statistical associations with average daily weight gain. The posterior density of the fixed effects is symmetric and with a Gaussian distribution. Variables: ageV - age in days, mutans - *T.mutans*, marginale - *A.marginale*, tricho - *Trichophyton* spp., Q.Strongyle.eggs - strongyle epg, parva - *T.parva*, GirthDam - dam heart girth size in cms, OccupSalaried - occupation salaried, Elevat - elevation, WATRD_WETWater is fetched - watering at homestead, CalfSex - calf sex. The colon (:) is used to indicate interaction between age in days and the variables (slope).

Appendix F

Risk factors for seroconversion to tick-borne diseases, trypanosomes and helminth worm burden

Table F.1: Results of univariable analysis of non-infectious risk factors for *T.parva* seroconversion.

	coef	exp(coef)	se(coef)	z	p
Education-primary school	-0.0685	0.9338	0.0644	-1.0639	0.2874
Education-secondary school	0.0332	1.0338	0.0723	0.4599	0.6456
Occupation-Salaried	0.0776	1.0807	0.0643	1.2067	0.2275
log(Total livestock units)	0.2380	1.2687	0.0305	7.7960	<0.001
log(Total acres owned)	0.0929	1.0974	0.0292	3.1862	0.0014
Farmer's age	0.0019	1.0019	0.0016	1.1733	0.2407
Farmer's sex - Male	0.0156	1.0157	0.0473	0.3302	0.7412
Housing calves	-0.0707	0.9318	0.0771	-0.9166	0.3593
Grazing with adults	-0.1466	0.8637	0.0971	-1.5100	0.1310
Calf suckling - yes	0.1709	1.1864	0.0598	2.8595	0.0042
Watering - at homestead	-0.2966	0.7433	0.0443	-6.6887	<0.001
Supplements use	-0.3747	0.6875	0.0524	-7.1545	<0.001
Milked prior calving	-0.0543	0.9471	0.0764	-0.7115	0.4768
Milked post calving	-0.1412	0.8683	0.0517	-2.7326	0.0063
Vaccine use	0.1636	1.1777	0.0440	3.7160	<0.001
Veterinary support	0.1859	1.2044	0.0646	2.8800	0.0040
Knowledge of diseases	0.1219	1.1296	0.0543	2.2455	0.0247
Housing - stall-shed	-0.0748	0.9280	0.0445	-1.6820	0.0926
Mean dam condition score	-0.0294	0.9710	0.0262	-1.1214	0.2621
Mean dam girth size	0.0064	1.0065	0.0032	2.0156	0.0438
Mean NDVI	-1.3407	0.2617	0.5488	-2.4429	0.0146
Elevation	-0.0019	0.9981	0.0004	-4.8615	<0.001
T.parva antibodies - dam	0.0019	1.0019	0.0009	2.0346	0.0419
A.marginale antibodies - dam	-0.0045	0.9956	0.0021	-2.1625	0.0306
T.mutans antibodies - dam	0.0010	1.0010	0.0012	0.8386	0.4017
Heart girth size - dam	0.0047	1.0047	0.0036	1.3012	0.1932
Body condition score - dam	-0.0135	0.9866	0.0260	-0.5172	0.6050
Mean month NDVI	-0.2594	0.7715	0.2640	-0.9826	0.3258
Dam health - sick	-0.5681	0.5666	0.4099	-1.3860	0.1658
Calf sex	0.0401	1.0409	0.0439	0.9128	0.3613
Recruitment weight	-0.0004	0.9996	0.0059	-0.0669	0.9466
Moderate introgression	-0.0489	0.9523	0.0647	-0.7559	0.4497
Substantial introgression	0.0105	1.0105	0.1020	0.1026	0.9182
Heterozygosity	-3.2619	0.0383	1.1459	-2.8464	0.0044
Tick control	-0.0760	0.9268	0.0493	-1.5415	0.1232
Trypanosome control	0.1232	1.1312	0.0594	2.0745	0.0380
Worm control	0.0678	1.0702	0.0474	1.4293	0.1529
Antibiotics use	0.1547	1.1673	0.0440	3.5188	0.0004
Vaccine Use	-0.2088	0.8116	0.1216	-1.7169	0.0860
Protozoal control	0.2682	1.3075	0.1476	1.8164	0.0693
Traditional methods use	0.0265	1.0268	0.1618	0.1637	0.8699
Clinical episode	0.3502	1.4193	0.0948	3.6956	0.0002
Total serum protein	0.0790	1.0823	0.0357	2.2152	0.0267
White Cell count	-0.0023	0.9977	0.0021	-1.1127	0.2659
Packed cell volume	-0.0130	0.9871	0.0047	-2.7330	0.0063
R.appendiculatus	0.2417	1.2734	0.1259	1.9197	0.0549
A.variegatum	0.0454	1.0464	0.0466	0.9740	0.3301
B.decoloratus	0.0332	1.0338	0.0644	0.5161	0.6058
R.evertsi	0.1807	1.1981	0.0492	3.6692	<0.001
Lice	0.0994	1.1045	0.0630	1.5760	0.1150
Fleas	0.2919	1.3389	0.1313	2.2226	0.0262

Table F.2: Results of univariable analysis of infectious risk factors for *T.parva* seroconversion.

	coef	exp(coef)	se(coef)	z	p
Anaplasma spp.	-0.1282	0.8797	0.2146	-0.5974	0.5503
Babesia spp.	0.7177	2.0498	0.7096	1.0115	0.3118
Theileria spp.	0.0373	1.0380	0.0514	0.7246	0.4687
Trypanosoma spp.	-0.0114	0.9887	0.1736	-0.0654	0.9478
Trypanosoma theileri	0.3522	1.4221	1.0015	0.3516	0.7251
Trypanosoma vivax	0.1437	1.1546	0.2377	0.6046	0.5454
Calicophoroni spp.	-0.0179	0.9822	0.0544	-0.3299	0.7415
Coccidia spp.	-0.0585	0.9431	0.0470	-1.2443	0.2134
Cooperia spp.	0.5000	1.6487	0.2924	1.7099	0.0873
Dictyocaulus viviparus	0.3144	1.3694	0.1364	2.3053	0.0211
Fasciola spp.	0.0531	1.0545	0.1142	0.4646	0.6422
Haemonchus placei	-0.1237	0.8836	0.0451	-2.7445	0.0061
Microfilaria spp.	1.1717	3.2274	0.3796	3.0863	0.0020
Moniezia spp.	0.8625	2.3690	0.4098	2.1046	0.0353
Nematodirus spp.	-0.0929	0.9113	0.2687	-0.3457	0.7296
Strongyle eggs	-0.1206	0.8864	0.0517	-2.3328	0.0197
Strongyloides spp.	0.3612	1.4351	0.0941	3.8396	0.0001
Toxocara vitulorum	0.6810	1.9758	0.1797	3.7894	0.0002
Trichophyton spp.	0.1048	1.1105	0.2792	0.3754	0.7073
Trichostrongylus axei	-0.0109	0.9892	0.0467	-0.2332	0.8156
Trichuris spp.	0.1264	1.1347	0.1872	0.6751	0.4996
Theileria spp. level 1	0.0197	1.0199	0.0518	0.3808	0.7034
Theileria spp. level 2	0.1498	1.1616	0.1047	1.4317	0.1522
Theileria spp. level 3	0.6575	1.9299	0.3810	1.7256	0.0844
T.mutans serology	0.1555	1.1682	0.0488	3.1832	0.0015
B.bigemina serology	-0.0559	0.9457	0.0597	-0.9357	0.3495
A.marginale serology	0.0284	1.0288	0.0497	0.5709	0.5681
Strongyle eggs/1000	0.0235	1.0238	0.0205	1.1462	0.2517

Table F.3: Results of univariable analysis of non-infectious risk factors for *T.mutans* seroconversion.

	coef	exp(coef)	se(coef)	z	p
Education-primary school	-0.0794	0.9237	0.0560	-1.4173	0.1564
Education-secondary school	-0.0940	0.9103	0.0643	-1.4607	0.1441
Occupation-Salaried	-0.0830	0.9203	0.0598	-1.3886	0.1650
log(Total livestock units)	0.0035	1.0035	0.0287	0.1208	0.9038
log(Total acres owned)	-0.0033	0.9967	0.0260	-0.1280	0.8982
Farmer's age	0.0014	1.0014	0.0014	1.0229	0.3064
Farmer's sex - Male	-0.0468	0.9542	0.0414	-1.1324	0.2575
Housing calves	0.1539	1.1663	0.0625	2.4634	0.0138
Grazing with adults	0.0996	1.1047	0.0776	1.2827	0.1996
Calf suckling - yes	0.0364	1.0370	0.0567	0.6415	0.5212
Watering - at homestead	0.0485	1.0497	0.0393	1.2335	0.2174
Supplements use	-0.0217	0.9785	0.0508	-0.4273	0.6692
Milked prior calving	0.1699	1.1852	0.0622	2.7320	0.0063
Milked post calving	0.0105	1.0105	0.0474	0.2214	0.8248
Vaccine use	-0.0068	0.9932	0.0388	-0.1752	0.8609
Veterinary support	0.1815	1.1990	0.0569	3.1899	0.0014
Knowledge of diseases	0.0971	1.1020	0.0477	2.0346	0.0419
Housing - stall-shed	0.0327	1.0332	0.0398	0.8212	0.4116
Mean dam condition score	0.0162	1.0164	0.0231	0.7032	0.4819
Mean dam girth size	0.0051	1.0051	0.0028	1.8076	0.0707
Mean NDVI	0.4787	1.6139	0.4978	0.9615	0.3363
Elevation	-0.0002	0.9998	0.0003	-0.7312	0.4647
T.parva antibody - dam	0.0021	1.0021	0.0008	2.6408	0.0083
A.marginale antibody - dam	-0.0019	0.9981	0.0018	-1.0814	0.2795
B.bigemina antibody - dam	0.0022	1.0022	0.0008	2.6327	0.0085
T.mutans antibody - dam	-0.0017	0.9983	0.0011	-1.5512	0.1209
Heart girth size- dam	0.0050	1.0051	0.0031	1.6300	0.1031
Body condition score - dam	0.0184	1.0186	0.0222	0.8289	0.4072
Dam health - sick	-0.3108	0.7328	0.3346	-0.9289	0.3529
Calf sex	0.0155	1.0156	0.0388	0.4002	0.6890
Recruitment weight	0.0089	1.0089	0.0053	1.6886	0.0913
Moderate introgression	0.0167	1.0168	0.0557	0.2988	0.7651
Substantial introgression	-0.3474	0.7065	0.1059	-3.2802	0.0010
Introgression - pure	0.0665	1.0688	0.0508	1.3092	0.1905
Heterozygosity	-3.8417	0.0215	1.0009	-3.8382	0.0001
Tick control	0.0154	1.0155	0.0445	0.3458	0.7295
Trypanosome control	0.0126	1.0126	0.0545	0.2302	0.8180
Worm control	-0.0687	0.9336	0.0433	-1.5876	0.1124
Antibiotics use	0.0212	1.0214	0.0391	0.5418	0.5879
Vaccine use	-0.0322	0.9683	0.0991	-0.3245	0.7455
Protozoal control	-0.5206	0.5941	0.1900	-2.7397	0.0061
Traditional methods use	-0.5502	0.5768	0.1837	-2.9956	0.0027
Clinical episode	0.2000	1.2214	0.0838	2.3861	0.0170
Total serum protein	0.1277	1.1362	0.0318	4.0210	0.0001
White Cell count	-0.0029	0.9971	0.0021	-1.3451	0.1786
Packed cell volume	0.0017	1.0017	0.0041	0.4053	0.6852
R.appendiculatus	0.2123	1.2365	0.1059	2.0054	0.0449
A.variegatum	0.0522	1.0536	0.0412	1.2677	0.2049
B.decoloratus	-0.0169	0.9833	0.0604	-0.2792	0.7801
R.evertsi	0.0345	1.0351	0.0459	0.7526	0.4517
Lice	0.1318	1.1409	0.0568	2.3186	0.0204
Fleas	0.1442	1.1551	0.1031	1.3984	0.1620

Table F.4: Results of univariable analysis of infectious risk factors for *T.mutans* seroconversion.

	coef	exp(coef)	se(coef)	z	p
Anaplasma spp.	-0.0660	0.9361	0.1937	-0.3408	0.7333
Babesia spp.	-0.2334	0.7918	1.0014	-0.2331	0.8157
Theileria spp.	0.2097	1.2333	0.0460	4.5542	<0.001
Trypanosoma spp.	-0.3255	0.7222	0.1841	-1.7676	0.0771
Trypanosoma theileri	-0.1223	0.8849	1.0009	-0.1222	0.9027
Trypanosoma vivax	-0.3755	0.6870	0.2686	-1.3981	0.1621
Calicophoroni spp.	-0.0160	0.9841	0.0504	-0.3177	0.7507
Coccidia spp.	0.0089	1.0089	0.0413	0.2142	0.8304
Cooperia spp.	0.5744	1.7760	0.1992	2.8835	0.0039
Dictyocaulus viviparus	0.1696	1.1849	0.1075	1.5780	0.1146
Haemonchus placei	-0.0716	0.9309	0.0399	-1.7916	0.0732
Microfilaria spp.	0.0664	1.0686	0.5783	0.1147	0.9087
Moniezia spp.	0.2710	1.3113	0.5013	0.5406	0.5888
Nematodirus spp.	-0.0368	0.9639	0.2370	-0.1551	0.8768
Oesophagostomum radiatum	-0.0866	0.9171	0.0559	-1.5475	0.1217
Ostertagia ostertagi	0.6559	1.9269	0.3805	1.7239	0.0847
Strongyle eggs	-0.0531	0.9483	0.0458	-1.1586	0.2466
Strongyloides spp.	0.2513	1.2857	0.0738	3.4031	0.0007
Toxocara vitulorum	0.3412	1.4066	0.1420	2.4033	0.0162
Trichophyton spp.	0.0861	1.0899	0.2199	0.3916	0.6953
Trichostrongylus axei	-0.0425	0.9584	0.0415	-1.0231	0.3062
Trichuris spp.	0.0997	1.1048	0.1680	0.5936	0.5528
Theileria spp. level 1	0.2006	1.2221	0.0463	4.3292	<0.001
Theileria spp. level 2	0.2971	1.3459	0.0944	3.1483	0.0016
Theileria spp. level 3	-0.0938	0.9104	0.5018	-0.1870	0.8516
A.marginale serology	0.1040	1.1096	0.0458	2.2715	0.0231
T.parva serology	-0.0129	0.9872	0.0427	-0.3023	0.7624
B.bigemina serology	0.0367	1.0374	0.0547	0.6705	0.5025
Strongyle eggs/1000	-0.0366	0.9641	0.0192	-1.9019	0.0572

Table F.5: Results of univariable analysis of non-infectious risk factors for *A.marginale* seroconversion.

	coef	exp(coef)	se(coef)	z	p
Education-primary school	-0.2449	0.7827	0.0926	-2.6444	0.0082
Education-secondary school	-0.3020	0.7393	0.1089	-2.7742	0.0055
Occupation-Salaried	0.0370	1.0377	0.1011	0.3663	0.7141
log(Total livestock units)	0.1711	1.1866	0.0478	3.5794	0.0003
log(Total acres owned)	0.0850	1.0887	0.0449	1.8907	0.0587
Farmer's age	0.0057	1.0057	0.0024	2.3356	0.0195
Farmer's sex - Male	-0.1549	0.8565	0.0710	-2.1823	0.0291
Housing calves	-0.1965	0.8216	0.1176	-1.6705	0.0948
Grazing with adults	0.0873	1.0913	0.1345	0.6493	0.5161
Calf suckling-yes	0.0185	1.0186	0.0861	0.2143	0.8303
Watering - at homestead	-0.0028	0.9972	0.0698	-0.0408	0.9674
Supplements use	0.2109	1.2348	0.0965	2.1865	0.0288
Milked prior calving	0.2907	1.3373	0.1030	2.8221	0.0048
Milked post calving	-0.3816	0.6828	0.0757	-5.0430	<0.001
Vaccine use	0.3609	1.4346	0.0689	5.2356	<0.001
Veterinary support	0.3334	1.3957	0.1055	3.1614	0.0016
Knowledge of diseases	0.1470	1.1584	0.0845	1.7412	0.0817
Housing - stall-shed	0.1289	1.1376	0.0701	1.8403	0.0657
Mean NDVI	1.5713	4.8130	0.9049	1.7364	0.0825
Elevation	-0.0022	0.9978	0.0006	-3.5860	0.0003
T.parva antibody - dam	0.0026	1.0026	0.0014	1.8541	0.0637
A.marginale	0.0076	1.0076	0.0028	2.7224	0.0065
B.bigemina	0.0041	1.0041	0.0014	2.8727	0.0041
T.mutans	-0.0025	0.9975	0.0020	-1.2495	0.2115
Heart girth size - dam	-0.0216	0.9786	0.0058	-3.7438	0.0002
Body condition score - dam	-0.1442	0.8657	0.0412	-3.4991	0.0005
Dam health - sick	-0.2989	0.7416	0.5799	-0.5154	0.6062
Calf sex	0.1882	1.2071	0.0681	2.7634	0.0057
Recruitment weight	-0.0225	0.9778	0.0092	-2.4543	0.0141
Moderate introgression	-0.1244	0.8830	0.1023	-1.2157	0.2241
Substantial introgression	-0.4062	0.6661	0.1894	-2.1450	0.0320
Total serum protein	0.2828	1.3268	0.0547	5.1660	<0.001
White Cell count	0.0019	1.0019	0.0019	1.0132	0.3110
Packed cell volume	-0.0226	0.9777	0.0074	-3.0574	0.0022
Worm control	0.0275	1.0279	0.0737	0.3732	0.7090
Antibiotics use	0.0153	1.0154	0.0684	0.2238	0.8229
Trypanosome control	0.0147	1.0148	0.0949	0.1552	0.8767
Tick control	0.3707	1.4487	0.0859	4.3156	<0.001
Vaccine use	-0.0261	0.9743	0.1726	-0.1510	0.8800
R.appendiculatus	0.1041	1.1097	0.1886	0.5518	0.5811
A.variegatum	0.1407	1.1511	0.0733	1.9190	0.0550
B.decoloratus	0.0175	1.0176	0.0986	0.1772	0.8594
R.evertsi	0.1753	1.1916	0.0757	2.3168	0.0205
Lice	0.2276	1.2556	0.0912	2.4966	0.0125
Fleas	0.4840	1.6225	0.2021	2.3951	0.0166

Table F.6: Results of univariable analysis of infectious risk factors for *A.marginale* seroconversion.

	coef	exp(coef)	se(coef)	z	p
Anaplasma spp.	0.0592	1.0610	0.3039	0.1949	0.8455
Babesia spp.	-12.0054	0.0000	653.9875	-0.0184	0.9854
Theileria spp.	0.2657	1.3043	0.0852	3.1169	0.0018
Trypanosoma spp.	-0.0344	0.9662	0.2619	-0.1314	0.8954
Trypanosoma vivax	0.0986	1.1036	0.3583	0.2752	0.7832
Calicophoroni spp.	-0.0196	0.9806	0.0824	-0.2382	0.8117
Coccidia spp.	-0.1080	0.8976	0.0732	-1.4758	0.1400
Cooperia spp.	0.5515	1.7359	0.5064	1.0892	0.2761
Dictyocaulus viviparus	0.1700	1.1853	0.2401	0.7078	0.4791
Fasciola spp.	0.0829	1.0864	0.1670	0.4964	0.6196
Haemonchus placei	-0.0876	0.9162	0.0700	-1.2515	0.2108
Microfilaria spp.	0.6880	1.9898	0.7092	0.9701	0.3320
Moniezia spp.	1.3078	3.6982	0.5029	2.6008	0.0093
Nematodirus spp.	0.0951	1.0998	0.3804	0.2501	0.8025
Oesophagostomum radiatum	0.0365	1.0372	0.0930	0.3929	0.6944
Ostertagia ostertagi	0.9562	2.6018	1.0088	0.9479	0.3432
Strongyle eggs	-0.0486	0.9526	0.0815	-0.5959	0.5512
Strongyloides spp.	0.3221	1.3800	0.1587	2.0299	0.0424
Toxocara vitulorum	0.8022	2.2305	0.2951	2.7181	0.0066
Trichophyton spp.	-0.7692	0.4634	0.7089	-1.0851	0.2779
Trichostrongylus axei	-0.1092	0.8966	0.0734	-1.4865	0.1371
Trichuris spp.	-0.0979	0.9067	0.3183	-0.3076	0.7584
Theileria spp. level 1	0.2613	1.2986	0.0854	3.0581	0.0022
Theileria spp. level 2	0.2016	1.2234	0.1723	1.1705	0.2418
Theileria spp. level 3	-0.2408	0.7860	1.0033	-0.2400	0.8103
T.parva serology	0.1928	1.2126	0.0756	2.5507	0.0108
T.mutans serology	0.6350	1.8870	0.0857	7.4070	<0.001
B.bigemina serology	0.2975	1.3465	0.0820	3.6260	0.0003
Strongyle eggs/1000	0.0060	1.0060	0.0326	0.1841	0.8539

Table F.7: Results of univariable analysis of non-infectious risk factors for infection with *Trypanosoma* spp.

	coef	exp(coef)	se(coef)	z	p
Education-Primary school	-0.7283	0.4827	0.3114	-2.3387	0.0194
Education-Secondary school	-0.6779	0.5077	0.3790	-1.7889	0.0736
Occupation-Salaried	-0.5486	0.5777	0.4668	-1.1753	0.2399
log(Total livestock units)	0.3583	1.4308	0.1740	2.0588	0.0395
log(Total acres owned)	0.0402	1.0410	0.1666	0.2412	0.8094
Farmer's age	0.0175	1.0177	0.0096	1.8220	0.0684
Farmer's sex - Male	0.2508	1.2850	0.2742	0.9147	0.3603
Housing calves	-0.0730	0.9296	0.4131	-0.1768	0.8597
Grazing with adults	-0.6358	0.5295	0.7188	-0.8845	0.3764
Calf suckling-yes	-0.6037	0.5468	0.2994	-2.0160	0.0438
Watering - at household	-1.0551	0.3482	0.2728	-3.8683	< 0.001
Distance to water - at household	0.6944	2.0025	0.3579	1.9404	0.0523
Supplements use	-0.1980	0.8203	0.3011	-0.6577	0.5107
Milked prior calving	-1.0916	0.3357	0.7194	-1.5173	0.1292
Milked post calving	-0.2093	0.8111	0.2903	-0.7211	0.4709
Vaccine use	0.4368	1.5478	0.2496	1.7504	0.0800
Veterinary support	0.3236	1.3821	0.3764	0.8597	0.3900
Knowledge of diseases	0.5209	1.6835	0.3424	1.5212	0.1282
Housing - stall-shed	0.7938	2.2117	0.2866	2.7701	0.0056
Body condition score - dam	-0.0024	0.9976	0.1310	-0.0182	0.9854
Heart girth size - dam	-0.0348	0.9658	0.0189	-1.8415	0.0655
Mean NDVI x 10	1.4607	4.3091	0.3759	3.8857	< 0.001
Elevation	-0.0140	0.9861	0.0026	-5.3888	< 0.001
T.parva antibody - dam	0.0044	1.0044	0.0049	0.9031	0.3665
A.marginale antibody - dam	0.0056	1.0056	0.0103	0.5413	0.5883
B.bigemina antibody - dam	0.0014	1.0014	0.0052	0.2715	0.7860
T.mutans antibody - dam	-0.0409	0.9599	0.0115	-3.5550	< 0.001
Calf sex - female	0.2443	1.2767	0.2414	1.0119	0.3116
Recruitment weight	-0.0946	0.9097	0.0325	-2.9074	0.0036
Moderate introgression	-1.3237	0.2662	0.5905	-2.2415	0.0250
Substantial introgression	-1.3881	0.2496	1.0077	-1.3775	0.1684
Heterozygosity x 10	-1.3070	0.2706	0.5342	-2.4467	0.0144
Tick control	0.0494	1.0506	0.2794	0.1767	0.8598
Trypanosome control	-0.0025	0.9975	0.3420	-0.0073	0.9942
Worm control	-0.1857	0.8306	0.2795	-0.6643	0.5065
Antibiotics use	-0.2735	0.7607	0.2505	-1.0917	0.2750
Vaccine Use	0.4266	1.5321	0.5152	0.8281	0.4076
Protozoal control	2.0975	8.1456	0.3990	5.2574	< 0.001
Traditional methods use	0.0394	1.0401	0.7223	0.0545	0.9565
Clinical episode	1.0866	2.9642	0.3420	3.1769	0.0015
Total serum protein	-0.1214	0.8856	0.1391	-0.8728	0.3828
White cell count	0.0036	1.0036	0.0043	0.8499	0.3954
Packed cell volume	-0.1327	0.8758	0.0216	-6.1321	< 0.001
R.appendiculatus	0.3233	1.3816	0.5094	0.6346	0.5257
A.variegatum	0.3522	1.4221	0.2404	1.4649	0.1430
B.decoloratus	0.9044	2.4705	0.2973	3.0425	0.0023
R.evertsi	0.4570	1.5794	0.2689	1.6998	0.0892
Lice	0.5521	1.7370	0.3198	1.7266	0.0842
Fleas	0.6909	1.9955	0.3761	1.8370	0.0662

Table F.8: Results of univariable analysis of infectious risk factors for infection with *Trypanosoma* spp.

	coef	exp(coef)	se(coef)	z	p
Anaplasma spp.	0.4150	1.5144	1.0073	0.4120	0.6804
Theileria spp.	0.2136	1.2381	0.2495	0.8563	0.3919
Calicophoron spp.	-0.8363	0.4333	0.4643	-1.8011	0.0717
Coccidia spp.	-0.3772	0.6858	0.2916	-1.2935	0.1958
Dictyocaulus viviparus	0.4864	1.6264	0.4644	1.0474	0.2949
Fasciola spp.	-0.4440	0.6414	1.0073	-0.4408	0.6593
Haemonchus placei	0.0850	1.0887	0.2401	0.3541	0.7233
Nematodirus spp.	1.7717	5.8808	0.7176	2.4689	0.0136
Oesophagostomum radiatum	-0.2436	0.7838	0.3984	-0.6115	0.5409
Strongyle eggs	0.2482	1.2817	0.2615	0.9491	0.3426
Strongyloides spp.	0.5206	1.6830	0.3289	1.5828	0.1135
Toxocara vitulorum	-0.8120	0.4440	1.0073	-0.8061	0.4202
Trichophyton spp.	0.6929	1.9996	1.0073	0.6879	0.4915
Trichostrongylus axei	0.3912	1.4787	0.2499	1.5656	0.1174
Theileria spp. level 1	0.0944	1.0990	0.2491	0.3790	0.7047
Theileria spp.level 2	-0.1873	0.8292	0.7330	-0.2555	0.7983
Theileria spp. level 3	2.0281	7.6000	1.0186	1.9910	0.0465
T.parva - serology	0.5523	1.7372	0.2414	2.2875	0.0222
T.mutans -serology	-0.0704	0.9320	0.2408	-0.2923	0.7701
B.bigemina - serology	0.9201	2.5096	0.2919	3.1522	0.0016
A.marginale - serology	0.5014	1.6510	0.2794	1.7944	0.0728
log(*Calicophoron* spp.)	0.1435	1.1544	0.4103	0.3499	0.7264
Strongyle.eggs/1000	-0.2372	0.7889	0.1976	-1.2001	0.2301

Table F.9: Univariable screening of non-infectious factors associated with strongyle epg

	Estimate	Std.Error	Z value	p value	lowerCI	upperCI
Farmer's age	-0.0023	0.0024	-0.9557	0.3392	-0.0071	0.0025
Farmer's sex - Male	0.1441	0.0724	1.9907	0.0465	0.0022	0.2860
Education-primary school	0.0099	0.0989	0.1006	0.9199	-0.1840	0.2039
Education-secondary school	0.0008	0.1129	0.0075	0.9940	-0.2205	0.2222
log(Total acres owned)	0.0414	0.0445	0.9312	0.3517	-0.0457	0.1285
log(Total livestock units)	0.0367	0.0502	0.7311	0.4647	-0.0618	0.1352
Occupation-Salaried	0.0684	0.0981	0.6973	0.4856	-0.1238	0.2606
Housing calves	-0.3054	0.1159	-2.6362	0.0084	-0.5325	-0.0784
Housing - stall-shed	-0.2359	0.0674	-3.4998	0.0005	-0.3680	-0.1038
Grazing with adults	-0.2061	0.1463	-1.4091	0.1588	-0.4928	0.0806
Supplements use	0.0632	0.0870	0.7256	0.4681	-0.1074	0.2337
Calf suckling	-0.0326	0.0547	-0.5956	0.5514	-0.1397	0.0746
Watering - at homestead	-0.0528	0.0681	-0.7759	0.4378	-0.1863	0.0806
Distance to water - at household	-0.0963	0.0781	-1.2320	0.2179	-0.2494	0.0569
Veterinary support	0.1537	0.0929	1.6538	0.0982	-0.0285	0.3358
Knowledge of diseases	0.0755	0.0807	0.9347	0.3500	-0.0828	0.2337
Vaccine use	0.0163	0.0671	0.2428	0.8082	-0.1153	0.1479
Mean NDVI	-2.1977	0.8006	-2.7449	0.0061	-3.7669	-0.6285
Elevation	0.0008	0.0006	1.5321	0.1255	-0.0002	0.0019
T.parva antibodies - dam	0.0036	0.0014	2.5703	0.0102	0.0009	0.0064
A.marginale antibodies - dam	0.0029	0.0031	0.9530	0.3406	-0.0031	0.0089
B.bigemina antibodies - dam	0.0028	0.0015	1.9116	0.0559	-0.0001	0.0057
T.mutans antibodies - dam	0.0058	0.0018	3.1916	0.0014	0.0023	0.0094
mean Heart girth size - dam	-0.0138	0.0049	-2.8276	0.0047	-0.0233	-0.0042
mean Body condition score - dam	-0.1482	0.0389	-3.8098	0.0001	-0.2245	-0.0720
Heart girth size - dam	-0.0070	0.0042	-1.6571	0.0975	-0.0152	0.0013
Body condition score - dam	-0.1025	0.0267	-3.8382	0.0001	-0.1549	-0.0502
Calf sex	-0.1888	0.0664	-2.8435	0.0045	-0.3189	-0.0587
Recruitment weight	-0.0194	0.0091	-2.1249	0.0336	-0.0373	-0.0015
Moderate introgression	0.1601	0.0963	1.6619	0.0965	-0.0287	0.3488
Substancial introgression	-0.1095	0.1591	-0.6884	0.4912	-0.4214	0.2023
Heterozygosity	-0.7876	1.7261	-0.4563	0.6482	-4.1708	2.5955
Clinical episode	0.2506	0.0763	3.2853	0.0010	0.1011	0.4000
Total serum protein	-0.3374	0.0300	-11.2452	0.0000	-0.3962	-0.2786
Packed cell volume	-0.0552	0.0036	-15.5147	0.0000	-0.0622	-0.0482
Worm control	0.0389	0.0747	0.5209	0.6024	-0.1075	0.1853
Antibiotics use	0.0102	0.0678	0.1510	0.8800	-0.1226	0.1431
Trypanosome control	0.0494	0.0954	0.5181	0.6044	-0.1375	0.2363
Tick control	0.0711	0.0756	0.9407	0.3469	-0.0770	0.2192
Vaccine use	-0.2753	0.1712	-1.6077	0.1079	-0.6108	0.0603
R.appendiculatus	0.1513	0.0895	1.6900	0.0910	-0.0242	0.3268
A.variegatum	0.0695	0.0377	1.8448	0.0651	-0.0043	0.1434
B.decoloratus	0.1426	0.0603	2.3672	0.0179	0.0245	0.2607
R.evertsi	0.1021	0.0454	2.2514	0.0244	0.0132	0.1910
Lice	0.1888	0.0641	2.9435	0.0032	0.0631	0.3144
Fleas	-0.1423	0.0860	-1.6549	0.0979	-0.3109	0.0262

Table F.10: Univariable screening of infectious factors associated with Strongyle epg

	Estimate	Std.Error	Z value	p value	lowerCI	upperCI
Anaplasma spp.	-0.1067	0.1821	-0.5861	0.5578	-0.4635	0.2501
Babesia spp.	0.3386	1.0444	0.3242	0.7458	-1.7085	2.3856
Theileria spp.	0.1154	0.0400	2.8867	0.0039	0.0371	0.1938
Trypanosoma spp.	0.1075	0.1648	0.6524	0.5141	-0.2154	0.4304
Trypanosoma theileri	1.4699	1.0595	1.3873	0.1653	-0.6067	3.5466
Trypanosoma vivax	-0.1377	0.2300	-0.5988	0.5493	-0.5884	0.3130
Coccidia spp.	0.0571	0.0375	1.5220	0.1280	-0.0164	0.1306
Cooperia spp.	-0.6578	0.1892	-3.4775	0.0005	-1.0286	-0.2871
Dictyocaulus viviparus	0.0244	0.0835	0.2921	0.7702	-0.1393	0.1881
Fasciola spp.	-0.0196	0.1112	-0.1763	0.8601	-0.2376	0.1984
Haemonchus placei	0.1320	0.0430	3.0678	0.0022	0.0477	0.2163
Microfilaria spp.	-0.3932	0.5223	-0.7529	0.4515	-1.4170	0.6305
Moniezia spp.	0.2407	0.4327	0.5563	0.5780	-0.6073	1.0887
Nematodirus spp.	0.1757	0.2039	0.8619	0.3887	-0.2238	0.5752
Oesophagostomum radiatum	0.3713	0.0464	7.9958	0.0000	0.2803	0.4623
Ostertagia ostertagi	-1.0089	0.4701	-2.1460	0.0319	-1.9303	-0.0874
Strongyloides spp.	-0.1273	0.0593	-2.1482	0.0317	-0.2435	-0.0112
Toxocara vitulorum	-0.0215	0.1070	-0.2013	0.8405	-0.2312	0.1881
Trichophyton spp.	0.2666	0.1945	1.3710	0.1704	-0.1145	0.6478
Trichostrongylus axei	0.3952	0.0365	10.8299	0.0000	0.3237	0.4667
Trichuris spp.	0.3037	0.1397	2.1746	0.0297	0.0300	0.5774
T.parva serology	0.1615	0.0427	3.7856	0.0002	0.0779	0.2451
T.mutans serology	0.0453	0.0488	0.9270	0.3539	-0.0504	0.1410
A.marginale serology	0.0493	0.0569	0.8655	0.3868	-0.0623	0.1608
B.bigemina serology	-0.0247	0.0686	-0.3606	0.7184	-0.1591	0.1097

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