CHAPTER 5

ANAEMIA IN EAST AFRICAN SHORT-HORN ZEBU CALVES:
THE DISTRIBUTION OF PATHOGENS IN THE POPULATION

1. INTRODUCTION

Animals that live in a tropical environment are exposed to a variety of pathogens, including tick-borne pathogens, tsetse-borne pathogens and intestinal parasites. The clinical significance of pathogens on a population basis is in part determined by the exposure rate of animals to these pathogens. If the prevalence of pathogens is significantly high, the risk of exposure is high and the potential that infectious loads are high enough to cause disease is increased.

In this chapter measuring the pathogen burden of calves in the study area during the first year of life is attempted. The frequency distributions of pathogens were calculated to determine which pathogens were the most prevalent in the study area. The variation in the pathogen burden with age was investigated through the prevalence and cumulative incidences of pathogens. These two indices were used to highlight periods of high infection risk.

The frequency of co-infections of pathogens was also investigated in this chapter, both at the visit-level (specific age groups) and the calf-level. Particular reference was made to pathogens that are known to cause anaemia, such as strongyle-type worms and *Trypanosoma* spp.

2. MATERIALS AND METHODS

* General methodology is discussed in Chapter 2.

2.1 Frequency distribution of pathogens

The samples from 5-weekly routine visits and the additional clinical visits from October 2007 to September 2010 from calves of the IDEAL project were used to describe the distribution of pathogens in the population at a calf level, as well as at a visit level. Results from the following diagnostic tests were used for this analysis: thin and thick peripheral blood smears, DG and HCT for trypanosomes, McMasters test, faecal flotation, direct Baermann test,

faecal sedimentation, faecal larval culture, and serology for tick-borne pathogens. Where diagnostic tests, e.g. RLBT, were only done on the 51-week samples, the prevalences of these pathogens were calculated only at this time-point. The pathogen variables were distinguished by the test used for diagnosis, e.g. serology or microscopy. At the calf level, a calf was considered positive if the relevant pathogen was detected at least once during its follow-up period.

2.2 Prevalence and cumulative incidences of pathogens

The point prevalence of a pathogen is the number of positive animals as a percentage of the animals sampled at that visit (age) (Dodge 2003). Comparing the point prevalences of pathogens at sequential time-points (age group) allows one to measure the variation in prevalence of pathogens with age. Infections of short duration are also highlighted since animals that have cleared themselves of infection will not be included at subsequent time points unless they are re-infected. Separate histograms were used to illustrate the point prevalences of intestinal parasites (helminths and coccidia) and the blood-borne pathogens (tick-borne pathogens and *Trypanosoma* spp.).

The incidence of a pathogen is the number of new infections during a specified time period as a percentage of the total number of calves at risk at the beginning of that time period. The cumulative incidence is the sum of the incidences of each subsequent time-point from the beginning of the study period. The cumulative incidence is in essence a measure of the total number of calves that become infected with specific pathogens over the follow-up period (Dodge 2003) and can be used to calculate the probability of infection at specific time-points. The cumulative incidences of intestinal parasites and tick-borne pathogens are illustrated with Kaplan-Meier graphs.

Serology results were used to calculate the cumulative incidence of seroconversion to the tick-borne pathogens (*Theileria parva, Theileria mutans, Anaplasma marginale* and *Babesia bigemina*). The cut-off values in percentage positivity (PP) values for specific pathogens were as follows:

1. PP value >20 for *T. parva* and *T. mutans* (Katende *et al.* 1998); and
2. PP value >15 for *A. marginale* and *B. bigemina* (Katende *et al.* 1990).

Calves were classed as seroconverted under the following criteria:

1. In calves under 10 weeks of age, if there was a rise in titre above the cut-off for the specific pathogen;

2. If antibody PP levels in calves that did not seroconvert before 10 weeks of age increased to levels above specific cut-offs for at least two consecutive visits; or

3. If, at the last visit of each calf that had not seroconverted previously, the PP value was above the cut-off.

2.3 Prevalence of co-infections

The level of co-infections of pathogens was investigated with the use of histograms which illustrate the frequency distribution of the number of co-infecting pathogens per visit as well as the number of infections per calf. The number of co-infections per visit is the number of infections that were detected at one visit. The number of infections per calf is the total number of pathogens the calf was infected with during its follow-up period, be that at the same time or at subsequent follow-up visits. Results from routine 5-weekly and clinical visits were used to calculate the number of co-infecting pathogens.

The frequency distribution of co-infections between pathogens that are known to cause anaemia were additionally analysed and illustrated by the histograms. The pathogens known to cause anaemia that were considered include*: T. mutans; B. bigemina; A. marginale; Trypanosoma* spp*.;* strongyle-type nematodes; and specifically *Haemonchus bedfordi; coccidia and Fasciola gigantica*.

2.4 Data analysis

Data analysis was done using R 2.8.1 (Ihaka & Gentleman 1996). Cumulative incidences and Kaplan-Meier plots were calculated with survival analysis and life tables using the Survival package (version 2.36-9) in R. All other graphs were drawn in gg-plots (Wickham 2009).

3. RESULTS

3.1 The frequency distribution of pathogens in the calf population

The frequency distributions of pathogens at calf and at visit level are shown in Table 5. The most common pathogen detected in the population was *Theileria* spp. with 97.63% of calves positive for piroplasms on microscopy at some time point during their follow-up period. A high number of calves were exposed to both *T. parva* and *T. mutans*, as reflected by seroconversion rates of over 70% for both pathogens. Just over 35% of calves seroconverted to *A. marginale* and fewer than 10% seroconverted to *B. bigemina*. There were even lower numbers of calves positive for these two pathogens on microscopy (fewer

Table 5 The frequency distribution of pathogens per calf and per visit

Pathogen		Number of calves positive for factor n=548		Number of visits positive for factor n=5602	
		n	%	n	%
Tick-borne pathogens					
Theileria spp.	Piroplasma (mcr)[1]	535	97.63	3260	58.19
T. parva	seroconverted	396	72.26	-	-
T. mutans	seroconverted	396	72.26	-	-
Anaplasma spp.	Mcr[1]	53	9.67	56	1.0
A. bovis	Positive[3]	207	38.12	-	-
A. marginale	seroconverted	197	35.95	-	-
Babesia spp.	Mcr[1]	4	0.73	4	0.07
B. bigemina	seroconverted	132	24.09	-	-
Trypanosoma spp.					
Trypanosoma spp.	Mcr[1]	42	7.66	68	1.21
T. vivax	Pcr[2] at final visit	49	8.94	-	-
Intestinal pathogens					
Coccidia	Positive[3]	477	87.04	1442	25.74
	Av OPG > 1000[4]	112	20.44	-	-
Strongyle-type nematodes	Positive[3]	531	96.90	3555	63.46
	Av EPG >1 000[5]	117	21.35	-	-
	EPG > 1000[6]	-	-	725	12.94
Haemonchus bedfordi	Positive[3]	525	95.80	2857	51.0
Strongyloides spp.	Positive[3]	319	58.21	604	10.78
Fasciola gigantica	Positive[3]	88	16.06	119	2.12

[1] Mcr: microscopy. This indicates that microscopy was used to diagnose the pathogen.
[2] Pcr: polymerase chain reaction. This indicates that PCR were used to diagnose the pathogen.
[3] Positive. This indicates, at the calf-level, whether the calf was infected at least once, and at the visit-level, whether the pathogen was detected.
[4] Av OPG>1000. The average oocysts per gram faeces per calf was over 1000.
[5] Av EPG>1000. The average eggs per gram faeces per calf was over 1000.
[6] EPG>1000. This indicates that the eggs per gram faeces per visit was over 1000.

than 10% for *Anaplasma* spp. and less than 1% for *Babesia* spp.). Calves were only tested at their last visit for *Anaplasma bovis* using the RLBT with 38.12% of calves testing positive.

The PCR for *Trypanosoma* spp. was done only at visits where calves had tested positive by microscopy as well as at the last visit of each calf. The prevalence of *T. vivax* (PCR) was therefore only calculated on a calf level. More calves were positive for *T. vivax* on PCR at

the last visit than calves positive for *Trypanosoma* spp. on microscopy and it can be assumed that the *T. vivax* is the main trypanosome in the population.

The second most common pathogen type detected was strongyle-type nematodes with almost 97% of calves infected at least once. Over 20% of calves had an average EPG over 1000 and at almost 13% of visits the EPG was over 1000. Specifically, *Haemonchus* spp. were detected at least once in over 95% of calves. Coccidia was the second most common intestinal pathogen type and the third most common pathogen overall with over 87% of calves infected at least once. A high number of calves (20.44%) had an average OPG over 1000. Although almost 60% of calves were infected with *Strongyloides* spp. at least once, *Strongyloides* spp. were only detected at 10.8% of visits. A relatively low number of calves (16.1%) were affected by *Fasciola gigantica*.

3.2 The prevalence and cumulative incidences of pathogens

Intestinal parasites
The point prevalences of intestinal parasites, specifically strongyle worms, *Strongyloides* spp. worms, coccidia and *Fasciola gigantica* are illustrated in Figure 5.1. The cumulative incidences of intestinal parasites are illustrated in Figure 5.2.

Figure 5.1 The point prevalences of intestinal parasites
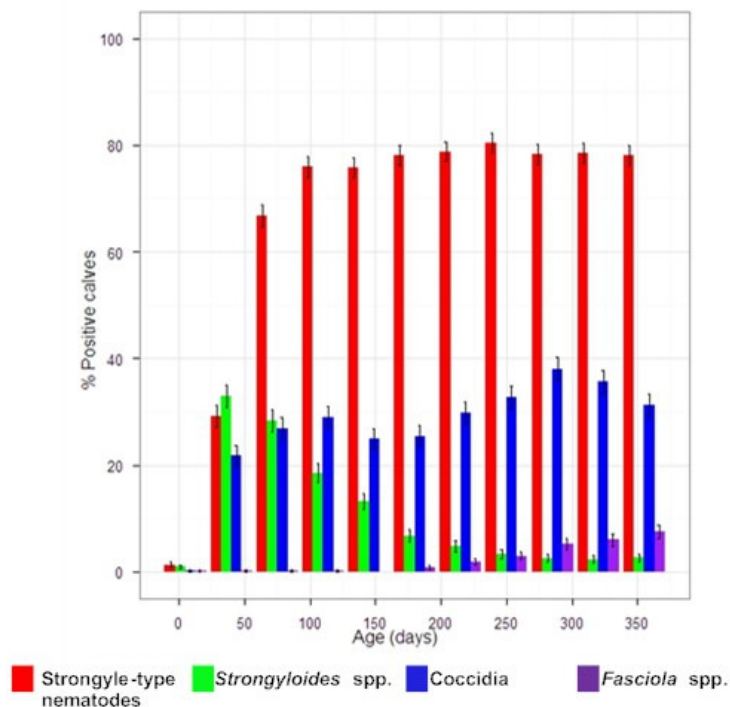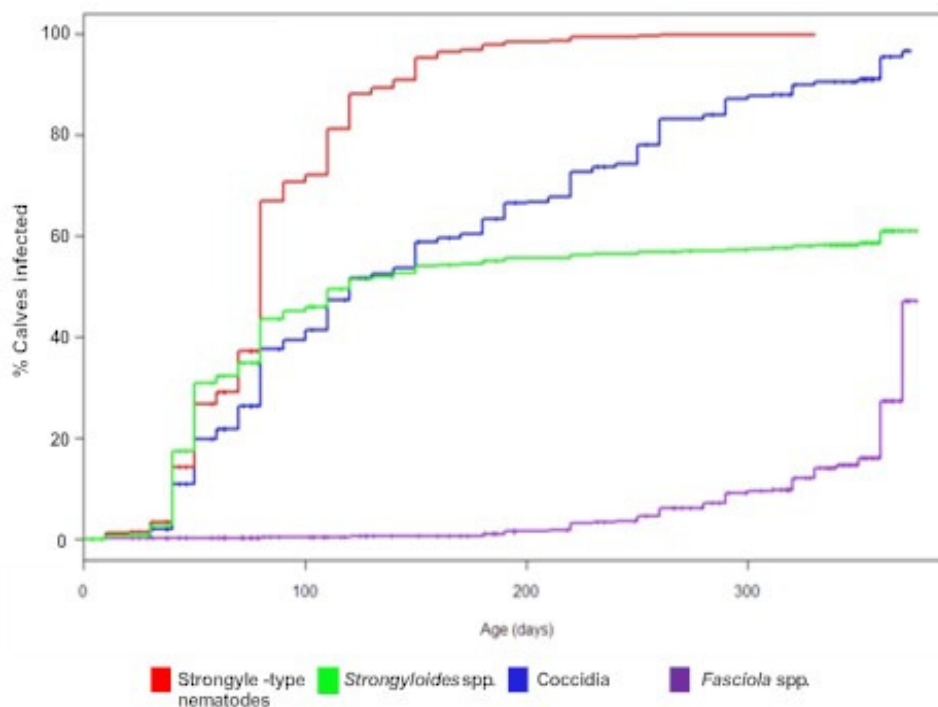
Figure 5.2 Cumulative incidences of intestinal parasites

Very few calves were infected with any helminth species at one week of age. Strongyle worms were the most prevalent of the helminth species at most time-points. The prevalence of strongyle worms increased with age with a prevalence that remained between 67% and 80% from 16 weeks (100 days) age. A relatively high number of new cases occurred between 6 and 11 weeks, with a 68% probability of becoming infected by 80 days. The probability of becoming infected by 150 days was 95%. The probability of becoming infected by 350 days was 99.8%.

Strongyloides spp, had the highest prevalence of the intestinal parasites at week 6 (33%), but the prevalence decreased with age and very few calves were infected with these worms by 51 weeks of age (2.6%). The probability of becoming infected by the end of the follow-up period was 61%. The highest number of new cases occurred between 40 and 80 days (218 new cases). Few calves acquired new *Strongyloides* infections after 180 days of age, with only 20 new cases between 180 days and the end of the follow-up period.

Coccidia, as the second most common intestinal parasites, had a prevalence above 20% from 6 weeks and older. The highest prevalence was at 41 weeks (38%). The probability of becoming infected by the end of the follow-up period was 97%. The number of new infections increased steadily over the study period. The total number of new infections by

100 days was 220, between 100 and 200 days a total of 129 new infections, and between 200 and 350 days a total of 119 new infections.

Fasciola gigantica showed an increase in prevalence with age with the highest prevalence of 7.5% at 51 weeks. Compared to other intestinal pathogens, the number of cases at any time point was relatively low, except when compared with *Strongyloides* that had a lower prevalence than *F. gigantica* after 250 days.

Blood-borne pathogens (microscopy)

The point prevalences of blood-borne pathogens identified by microscopy on the 5-week visits are illustrated in Figure 5.3.1. No distinction to species level was made on microscopy. *Theileria* spp. (piroplasms and/or schizonts) were the predominant parasites observed, with prevalence fluctuating around 80% of calves infected from 250 days and over. Figure 5.3.2 illustrates the blood-borne pathogens other than *Theileria* spp., as identified on microscopy. The number of calves infected with either *Anaplasma* spp., *Babesia* spp. or *Trypanosoma* spp. was considerably lower than the number infected with *Theileria* spp. *Anaplasma* spp. were identified in calves at all time points, although a higher number of older calves were positive. It was the most prevalent blood-borne pathogen, after *Theileria* spp., in neonatal calves. Trypanosomes were also indentified in calves of all age groups, with more calves infected in older age groups. Based on microscopy, *Trypanosoma* spp. was the most prevalent blood-borne pathogen at 51 weeks, after *Theileria* spp., with 2.4% of calves infected. Only three individual cases of *Babesia* spp. were detected on microscopy.

Figure 5.3.1 Point prevalences of blood-borne pathogens identified by microscopy
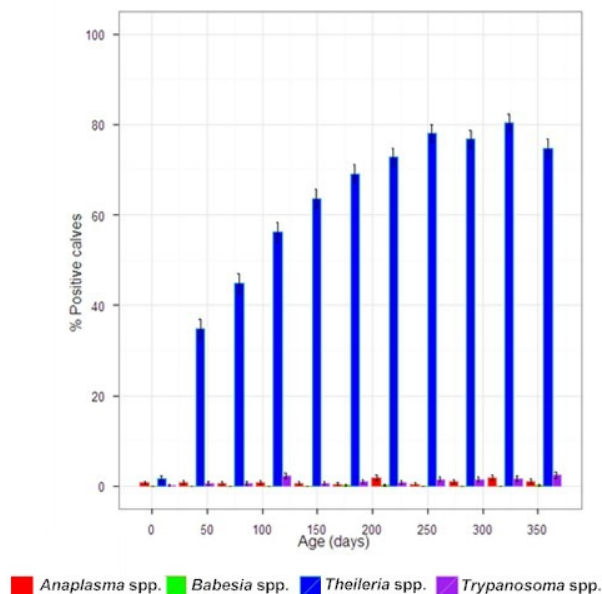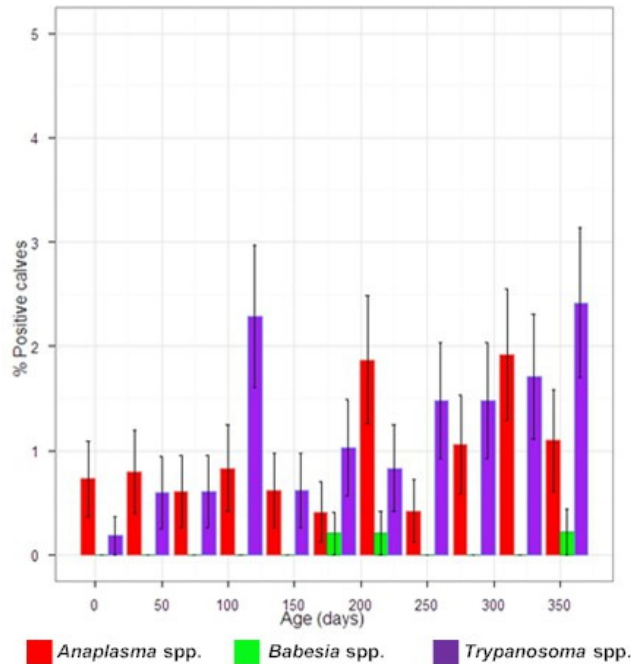

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**Figure 5.3.2** Point prevalences of blood-borne pathogens other than *Theileria* spp. identified by microscopy

Seroconversion is a measure of exposure to pathogens. The cumulative incidences of seroconversion to the tick-borne pathogens *Theileria mutans, Theileria parva, Anaplasma marginale* and *Babesia bigemina* are illustrated in Figure 5.4.

Of the 548 recruited calves, 396 (72.3%) seroconverted to *T. parva*, 395 (72.1%) seroconverted to *T. mutans*, 197 (35.9%) seroconverted to *A. marginale* and 132 (24.1%) seroconverted to *B. bigemina*. The probability of seroconversion to *T. parva, T. mutans, A. marginale* and *B. bigemina* by 51 weeks of age was 89.8%, 87.6%, 40.2% and 26.9%, respectively.

There appeared to be a high risk period of seroconversion to *T. mutans* at 11 weeks, as indicated by the high probability (44.5%) of seroconversion, with a total of 138 new cases reported for this period. The number of calves that seroconverted to *T. parva* peaked a bit later, at 21 weeks, with a total of 71 new cases reported for this period. The number of new cases of seroconversion to *A. marginale* was consistent over time and no specific high risk-period could be identified. The picture for *B. bigemina* was similar, except for a slightly higher number of cases seroconverting at week 51.

Figure 5.4 Cumulative incidence of seroconversion of tick-borne disease
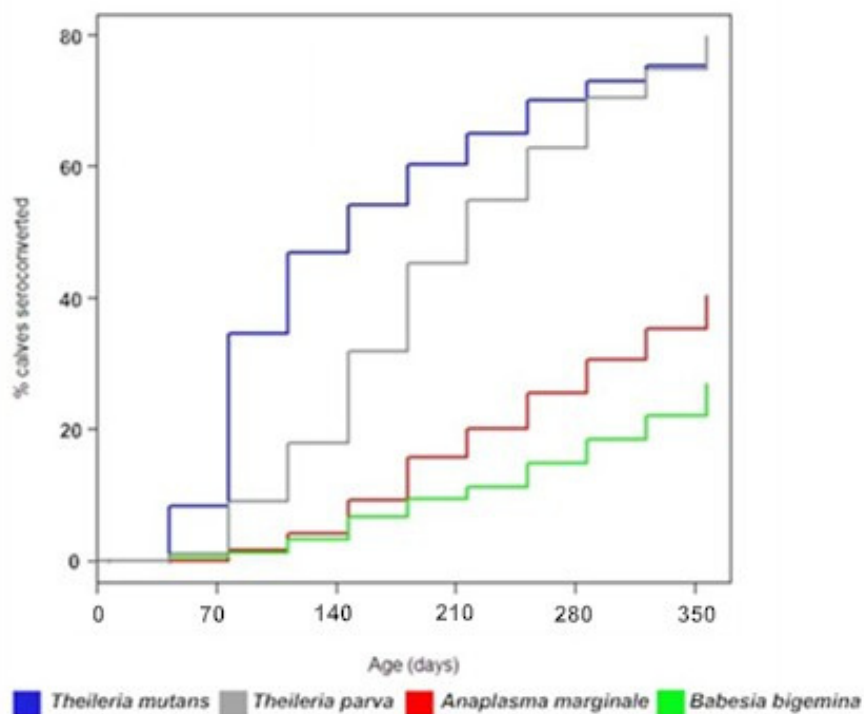

3.3 The level of co-infections of different pathogens in the study population

Co-infections per visit

The distribution of number of pathogens per visit detected is illustrated in Figure 5.5. No pathogens were detected at 15.4% (851) visits, the majority (508 visits) being calf recruitment visits. The maximum number of pathogens detected per single visit was 11 (two observations). At least four pathogens were detected in the majority (19.1%) of visits.

No pathogens known to cause anaemia were detected at 31.4% (1 742) visits, the majority of which were the calf recruitment visits. At over 16% of the visits at least one pathogen known to cause anaemia was detected. At 44% of visits at least two pathogens known to cause anaemia were detected. At least four pathogens known to cause anaemia were detected at 85 (1.5%) visits.

Figure 5.5 The frequency distribution of co-infections per visit
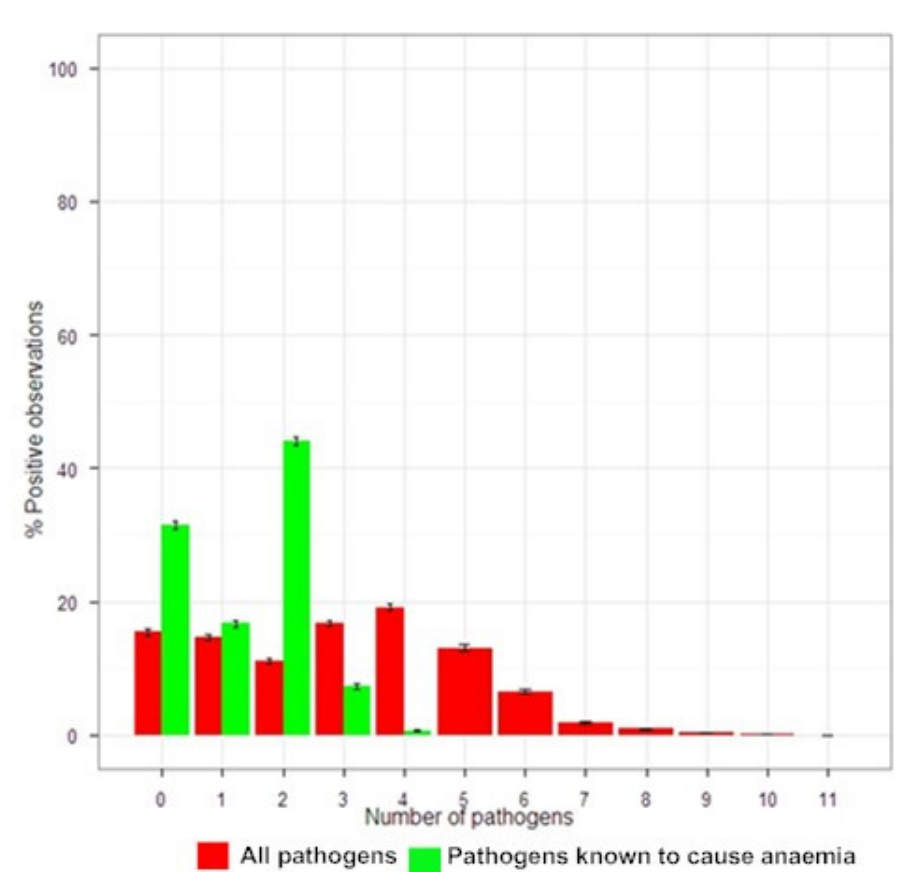
Co-infections per calf

The distribution of the number of pathogens per calf detected is illustrated in Figure 5.6. In seven calves, no pathogens were detected. These calves were only visited once because they died before the 6-week visit. More than 90% of calves were exposed to at least seven pathogens, and over 95% of calves were exposed to at least two pathogens known to cause anaemia in the first 51 weeks of their lives. Over 37% were exposed to at least three pathogens known to cause anaemia, with six calves exposed to six of these pathogens over the first year of life. In only 16 calves were none of these anaemia-causing pathogens detected.

Figure 5.6 The frequency distribution of co-infections per calf
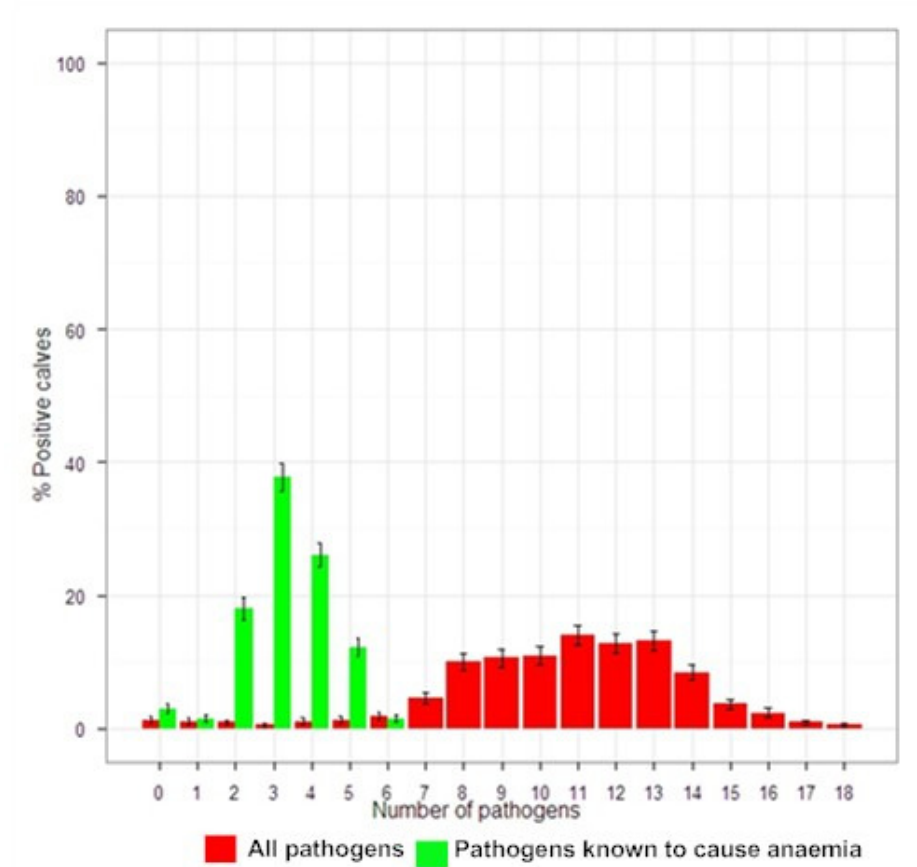
4. DISCUSSION

Few pathogens were detected at recruitment visits. The reasons might be that the time between birth and recruitment was too short for exposure to have occurred. Also, for the calves that were exposed before the recruitment visit, it is possible that the incubation period before pathogens could be detected exceeded the period between exposure and sampling. Calves would not yet have grazed, which is the main route of infection for intestinal pathogens, as evidenced by the significant increase in prevalence of these parasites by the second visit. The pathogens that were detected at recruitment could be due to trans-placental infection, such as *Anaplasma* spp. (Aubry & Geale 2010), or through colostrum or milk, such as some *Strongyloides* species (Kaufmann 1996).

No distinction was made between *Theileria* species on microscopy and one can not rule out the presence of species other than *T. parva* and *T. mutans*. The high percentage of calves infected with *Theileria* spp. (mcr) is reflected in the high number of calves that seroconverted

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to both *T. parva* and *T. mutans*. With over 70% of calves that were exposed to both these pathogens, co-infections with both were probably common. Both *T. parva* and *T. mutans* should be considered as very important pathogens in the calf population. The antibody prevalence rates for *T. parva* and *T. mutans* is an indication of endemic stability of these two pathogens in the study area (Okello-Onen *et al.* 1995; Perry & Young 1995). In a situation of endemic stability one would expect to see few clinical cases and these would be mainly seen in young calves (Perry & Young 1995).

Relatively few calves seroconverted to *A. marginale* and even fewer to *B. bigemina*. The discrepancy in the number of seroconversions to *A. marginale* and *B. bigemina* and the number of calves positive on microscopy is due to the difference in sensitivity between the two methods. Microscopy has a detection level of parasitaemias with a minimum of 1 parasite per 10^7 red blood cells (OIE 2005). Microscopy is mostly useful in detecting acute infections. In latent carrier animals the parasitaemia is often too low to detect via microscopy (Potgieter & Stoltsz 2004). Serology is an indirect measure of exposure and is useful in detecting carrier animals (Aubry & Geale 2010). It would seem that there are significant numbers of carrier animals of both *B. bigemina* and *A. marginale* in the population.

Interestingly, over 38% of calves were infected by *A. bovis* at their last visit. This is not a well-studied parasite but generally thought to be benign (Oura *et al.* 2004). It would be interesting to investigate its clinical significance in the study.

A relatively low number of calves tested positive for *Trypanosoma* spp., compared to other tick-borne diseases. The low number of visits where *Trypanosome* spp. were detected might be due to the fluctuating nature of parasitaemias (Connor & Van den Bossche 2004). The five-week intervals between visits probably caused an underestimate of the prevalence of this pathogen group. Trypanosomes were detected in calves at all time-points, with a few calves even testing positive at recruitment. Congenital transmission of *T. vivax* has been described, but is not common (Uilenberg 1998). It would be interesting to test these calves' dams for the presence of either the parasite or antibody against trypanosomes. Although *Trypanosoma* spp. did not appear to be common pathogens of very young calves, their prevalence increased with age and they are potentially of more clinical importance in adult animals in the study area. *Trypanosoma vivax,* which appeared to be the most prevalent trypanosome in the population, is generally considered less pathogenic than *T. congolense* (Uilenberg 1998). The clinical importance of *T. vivax* depends on the susceptibility of the animal, however, and will be investigated in the next chapter.

Intestinal parasites were some of the most common pathogens at all time-points and should be considered as important pathogens in the calf population. The probability of infection with strongyle-type nematodes by 51 weeks of life was almost 100%. Although strongyles are not considered to be as pathogenic in cattle as in sheep, the clinical significance of infection and the probability of causing clinical signs, such as anaemia, are dependent on the infective dose as well as the animal's susceptibility to infection (Kaufmann 1996). A high number of calves had significantly high EPGs at each of their visits, even from relatively young ages. Even infections with low EPG can potentially become clinically significant and should not be discounted. These infections seldom occur singly, and tend to occur in conjunction with multiple pathogens, some of which share the same niche in the host, namely the intestines (e.g. coccidia). The impact of these co-infections on the clinical course of infection will be investigated in Chapter 7.

Strongyloides worms were the predominant helminth at week 6. Strongyloides worms can infect calves before birth by crossing the placenta and are also passed to new-born calves in colostrum (Kaufmann 1996). Infection can also be through skin penetration (Kaufmann 1996). A relatively significant number of animals were infected by Strongyloides spp., but calves appeared to self-cure the infection and by 51 weeks very few calves were still infected. This could be due to premunity that develops in calves that were infected at early ages, or strongyle species which have high fecundity rates that out-compete the Strongyloides spp. for host resources.

When one considers the high rainfall and tropical conditions in the study area, the small number of calves infected with Fasciola spp. was perhaps a little surprising. The presence of Fasciola spp. infection was diagnosed through testing faecal samples for eggs by the sedimentation technique. The eggs of Fasciola spp. only appear in the faeces 10 weeks post-infection (Kaufmann 1996). This would explain in part why calves only tested positive late in the follow-up period. Fasciola gigantica infections did not appear to be significant in young calves but are likely to be more prevalent and of clinical relevance in older animals.

Co-infections

It is evident that the calves were suffering from the burden of multiple pathogens rather than single infections. Calves were infected with multiple pathogens at the majority of visits but also suffered from consecutive infections in following visits. Pathogens known to cause anaemia represented a significant number of these pathogens. The number of co-infections detected was limited by the number of tests done on samples from each visit. It is likely that

the infectious burdens of these calves were even higher, given that many more pathogens would probably have been detected if these calves were subjected to more tests.

5. CONCLUSIONS

It is clear that the calves in this study suffered from high burdens of multiple infectious agents from early calfhood. Many of the most common pathogens that infect these calves are known to cause anaemia. The pathogens with the most significant prevalences and cumulative incidence are the intestinal parasites, strongyle-type worms and coccidia, and the two tick-borne pathogens, *T. parva* and *T. mutans*. The calves had a high probability of getting infected by all of these pathogens by the first six month of life. At a population level, calves living with such an infectious burden should either have an innate resistance to these pathogens or tolerance to the clinical effects of disease for the population to survive into adulthood. It is probable that many of these infections are subclinical and many animals become latent carriers.

Despite the high infectious burden of calves in this study, the majority of calves survived to at least 51 weeks of age, which illustrates the resilience of the East African short-horn Zebu breed. The clinical significance of these infections is further investigated in the following two chapters.