CHAPTER 6

ANAEMIA IN EAST AFRICAN SHORT-HORN ZEBU CALVES: THE PATHOGENIC CAUSES OF ANAEMIA

1. INTRODUCTION

There are several pathogenic causes of anaemia, and the clinical course in many of infectious diseases has been well studied (Irvin 1983; Murray & Dexter 1988). Apart from anaemia, other haematological parameters are also affected during infection, be that directly by the pathogen and its by-products or by the host's response to infection. Fewer studies have investigated the effects of infection on the complete haematological profile of calves.

It is known that cattle breeds differ in their susceptibility to infection by pathogens and parasites (Perry & Young 1995) and their ability to control the development of clinical signs of infection (Murray *et al*. 1982). East African short-horn Zebu cattle are considered to be less trypanotolerant than West African cattle breeds (Murray *et al*. 1982), yet are considered to be more resistant against certain tick-born diseases, e.g. East Coast fever (Perry & Young 1995).

In this chapter the effect of certain pathogens on the haematological profile of the East African short-horn Zebu calves under field conditions was investigated. The aim was to describe the haematological outcome of infection with these pathogens and each pathogen's contribution towards the occurrence of anaemia in the study population. Pathogens investigated included the tick-borne parasites *Theileria parva, Theileria mutans, Anaplasma marginale* and *Babesia bigemina, Trypanosoma* spp. and specifically *T. vivax*, and intestinal parasites *Strongyloides* spp., strongyle-type nematodes, coccidia and *Fasciola gigantica.*

2. MATERIALS AND METHODS

* General methodology is discussed in Chapter 2

2.1 The haematological profile of calves infected with specific pathogens

The changes in the haematological profile over time due to infection were investigated for each pathogen. The haematological parameters investigated include: packed cell volume

(PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white cell counts (WCC), absolute lymphocyte counts (Lymph), absolute eosinophil counts (Eos), absolute monocyte counts (Mono), absolute neutrophil counts (Neut), platelet counts (Plt), platelet distribution width (PDW) and mean platelet volume (MPV). Pathogen infections were investigated in their singularity and co-infections with other pathogens were not considered. Co-infections are investigated in Chapter 7.

As the majority of calves became infected with *T. parva* and *T. mutans* during their follow-up period, no comparison was made between infected and non-infected calves. The calves were thus grouped into three groups based on their age of first seroconversion, namely Group 1 (1-16 weeks); Group2 (16-31 weeks); and Group 3 (>31 weeks). The changes in the haematological profile of the calves were compared between these various groups. Grouping of calves according to age of seroconversion to *A. marginale* and *B. bigemina* was based on the same age groups. A further group (Group 4) was included for calves that did not seroconvert during their follow-up period.

A distinction was made between *Trypanosoma* spp. diagnosed by microscopy (*Trypanosoma* spp. (mcr)) and *T. vivax*, as diagnosed by PCR since molecular diagnostics was not done at each visit. Calves infected with *Trypanosoma* spp. (mcr) were classed as either infected or not infected. No distinction was made between the *Trypanosoma* species based on microscopy. All calves that survived to 51 weeks were screened with species-specific PCR for *Trypanosoma* spp. The haematology in calves that tested positive for *Trypanosoma vivax* was investigated for this time-point only.

Roundworm eggs were identified as either strongyle-type nematodes or *Strongyloides* spp. The mean strongyle EPG per calf over its entire follow-up period was then calculated. The strongyle-infected calves were then grouped based on their mean EPG as high (mean EPG > 1000) or low (mean EPG ≤ 1000). Similarly coccidia infected calves were also grouped based on their mean OPG as high (mean OPG > 1000) or low (mean OPG ≤ 1000).

The haematological parameters in calves infected with *Strongyloides* spp. were only investigated at week 6 due to the low number of calves infected at other time points. Calves were either grouped as *Strongyloides* spp. infected or non-infected.

Calves infected with *Fasciola gigantica* were only investigated at week 51 due to the low numbers of calves infected at younger ages. The haematological parameters in *Fasciola gigantica* infected calves were compared to non-infected calves.

2.2 Data analysis

The significance of the difference in the means of haematological parameters at different time-points was calculated, be that between infected and non-infected calves, or between groups of infected calves, using either the Student's t-test, or where assumptions of normality were not met, the Mann-Whitney Test (see Chapter 2.4). A p-value <0.05 was considered as significant. All graphs were drawn in gg-plots (Wickham 2009) in R.

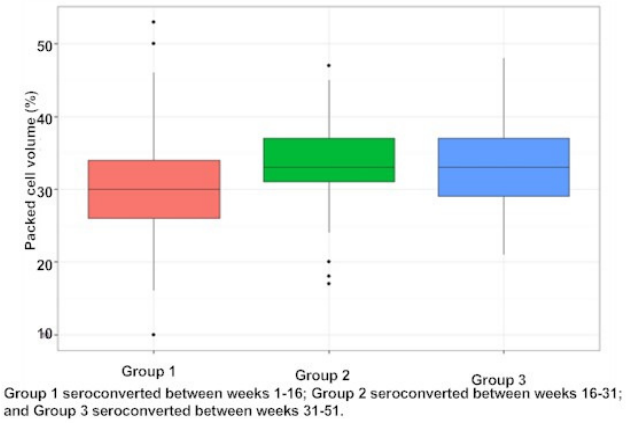
3. RESULTS

3.1 Infectious causes of anaemia: Blood-borne pathogens

Theileria mutans

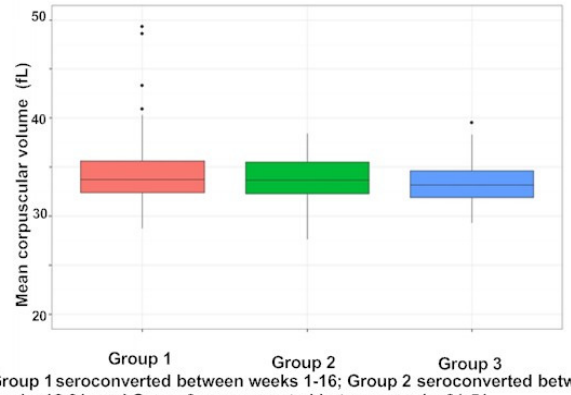
Calves that seroconverted to *T. mutans* by 16 weeks of age (Group 1, n=216) had a significantly lower PCV from week 6 to week 16 than calves that seroconverted later ($p<0.05$). There was no significant difference between the PCV of calves that seroconverted between 21 to 31 weeks (Group 2, n=79) and calves that seroconverted between 36 and 51 weeks (Group 3, n=54) ($p>0.05$). Group 1 had a significantly higher MCV than Group 3 from week 16 to 31 ($p<0.05$). Group 2 had a significantly higher MCV than Group 1 and 3 between weeks 26 to 31. The distribution of PCV and MCV of each group for Week 16 and Week 51 are illustrated in Figure 6.1.1-6.1.4. There were no significant differences in MCHC between the groups ($p>0.05$).

Figure 6.1.1 The distribution of packed cell volume at Week 16 in calves of different ages of seroconversion to *T. mutans*



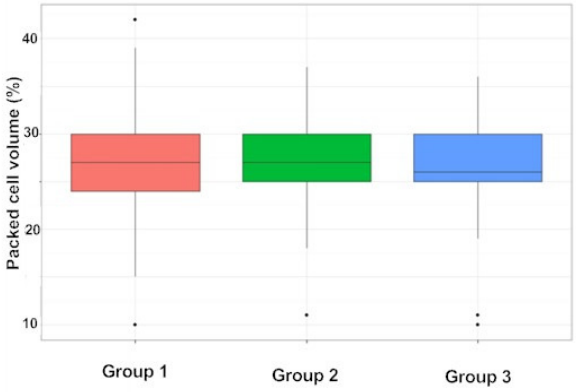
Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

Figure 6.1.2 The distribution of mean corpuscular volume at Week 16 in calves of different ages of seroconversion to *T. mutans*



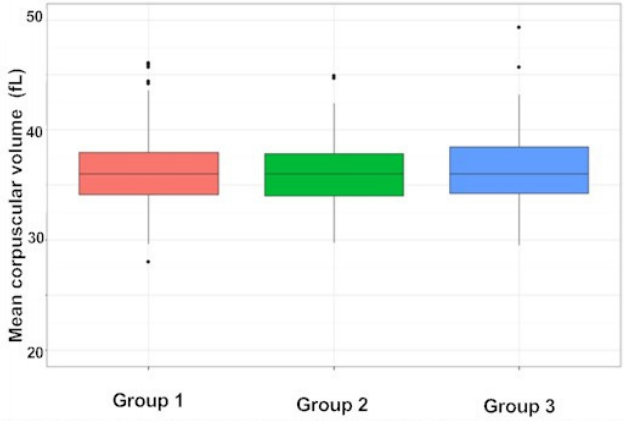
Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

Figure 6.1.3 The distribution of packed cell volume at Week 51 in calves of different ages of seroconversion to *T. mutans*



Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

Figure 6.1.4 The distribution of mean corpuscular volume at Week 51 in calves of different ages of seroconversion to *T. mutans*



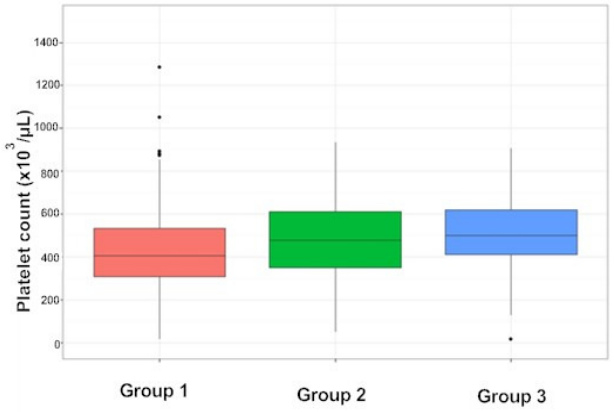
Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

The platelet counts were lower following seroconversion to *T. mutans* for all groups. There was a significant difference in platelet counts between calves in Group 1 and 2 at week 11 and 16, between Group 1 and 3 from week 11-31, and between Group 2 and 3 from week 21-31 ($p<0.05$). There was no significant difference between the three groups by week 36 onward when calves in Group 3 also seroconverted.

Figures 6.1.5-6.1.7 illustrate the distribution of platelet counts at week 16, week 31 and week 51 of the calves that have seroconverted to *T. mutans*. The median platelet count at week 16 of Group 1 was 405x10^3/µL, of Group 2 479x10^3/µL and Group 3 502x10^3/µL. The median platelet count at week 31 of Group 1 was 410x10^3/µL, of Group 2 456x10^3/uL and Group 3 559x10^3/µL. By week 51, the mean platelet counts of all three groups were below 400 x10^3/µL. No significant differences were found in MPV and PDW between any group at any time-point ($p>0.05$).

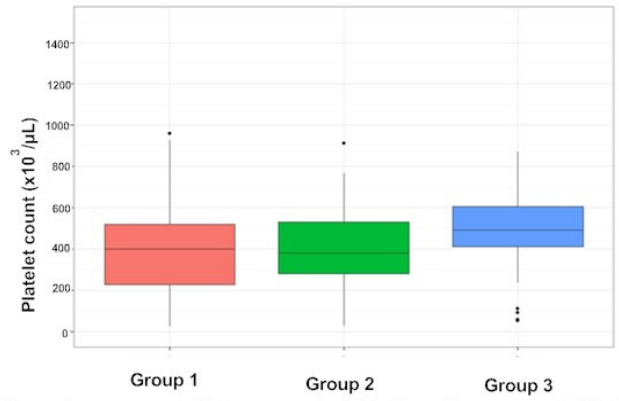
There were no significant differences in any white cell parameter between the *T. mutans*-seroconversion groups.

Figure 6.1.5 The distribution of platelet counts at Week 16 in calves of different ages of seroconversion to *T. mutans*



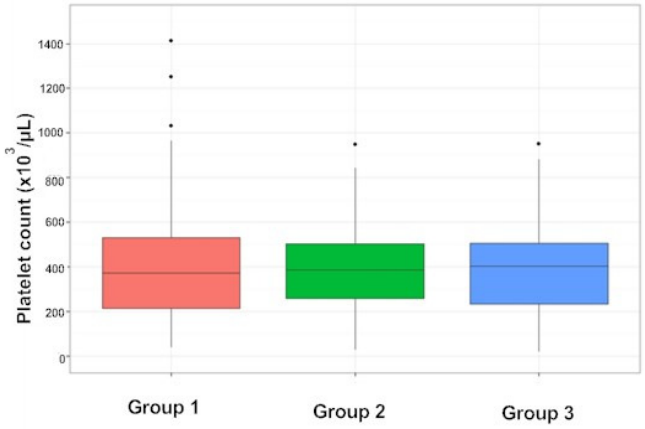
Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

Figure 6.1.6 The distribution of platelet counts at Week 31 in calves of different ages of seroconversion to *T. mutans*



Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

Figure 6.1.7 The distribution of platelet counts at Week 51 in calves of different ages of seroconversion to *T. mutans*



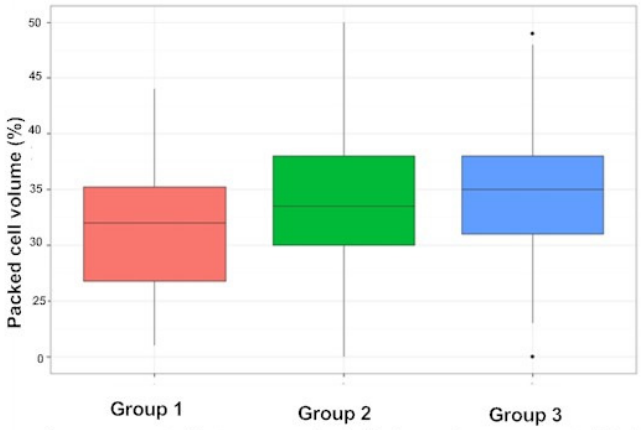
Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

Theileria parva

The PCV of Group 1 (n=92) was significantly lower at week 6-11 than Group 2 (n=184) and lower than Group 3 (n=120) from week 6-11 ($p<0.05$). There were no other significant differences in PCV between groups at other time points. No significant differences in MCV or MCHC were measured at any time point in calves that seroconverted to *T. parva*. The distribution of PCV in the three groups at week 11 is illustrated in Figure 6.2.1.

Calves from Group1 had significantly lower WCC and Lymph than both Group 2 and 3 from week 6-16 ($p<0.05$). Group 1 also had significantly lower Eos, Mono and Neut than both Group2 and 3 at week 6. No significant differences were found in any white cell parameter

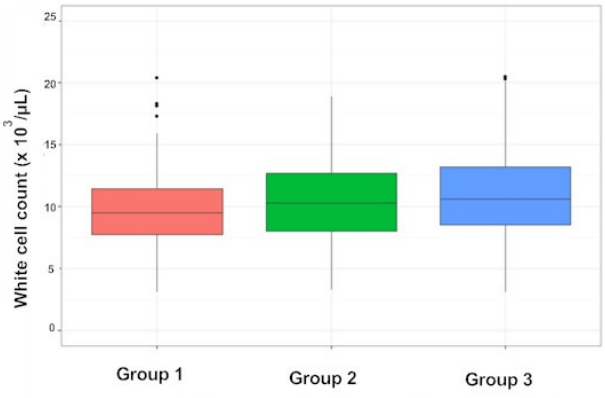
Figure 6.2.1 The distribution of packed cell volume at Week 11 in calves of different ages of seroconversion to *T. parva*



Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

between Group 2 and Group 3. The distribution of WCC at week 11 in calves in different groups that seroconverted to *T. parva* is illustrated in Figure 6.2.2.

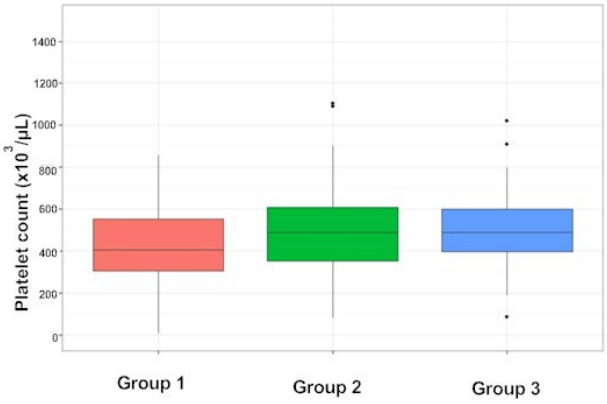
Figure 6.2.2 The distribution of white cell counts at Week 11 in calves of different ages of seroconversion to *T. parva*



Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

Calves from Group 1 also had significantly lower platelet counts than Group 2 at week 11 and 31, and lower than Group 3 at week 6, 11 and 31. No differences in MPV and PDW were found between any groups at any time point. The distribution of Plt at week 11 in calves in different groups that seroconverted to *T. parva* is illustrated in Figure 6.2.3.

Figure 6.2.3 The distribution of platelet counts at Week 11 in calves of different ages of seroconversion to *T. parva*

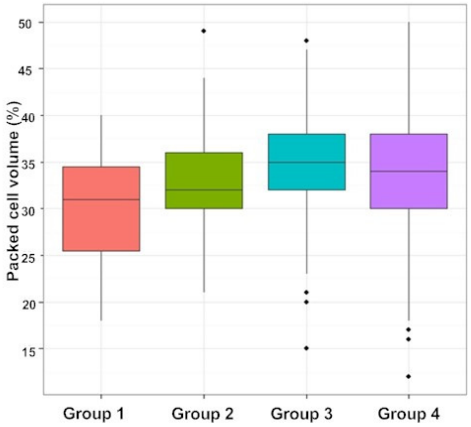


Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; and Group 3 seroconverted between weeks 31-51.

Anaplasma marginale

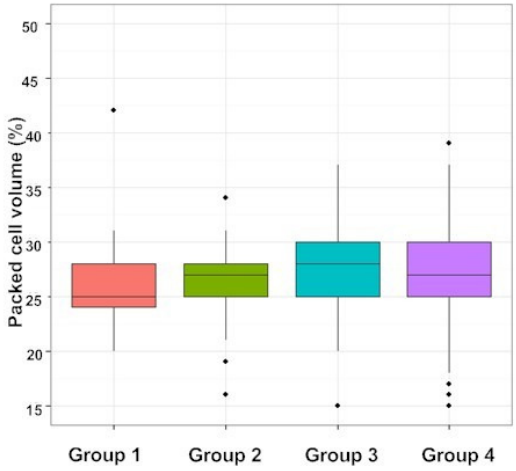
The PCV of Group 1 (n=22) was significantly lower than Group 3 (n=96) at week 6-16, and the PCV of Group 2 (n=79) was significantly lower than Group 3 at week 11-31 ($p<0.05$). There was a significant difference in PCV between Group1 and 4 (n=351) at week 11 ($p<0.05$). No other significant differences in PCV were found at other time points. The mean PCV at week 11 for Groups 1, 2, 3 and 4 was 29.8%, 33.1%, 34.5% and 33.8% respectively (Figure 6.3.1). At week 26 the mean PCV for Groups 1, 2, 3 and 4 was 28.2%, 27.7%, 29.6% and 28.1% respectively. At week 51 the mean PCV for Groups 1, 2, 3 and 4 was 26.6%, 25.9%, 27.4% and 27.1% (Figure 6.3.2). No significant differences were found in other haematological parameters at any time-point ($p>0.05$).

Figure 6.3.1 The distribution of packed cell volume at week 11 in calves of different ages of seroconversion to *A. marginale*



Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; Group 3 seroconverted between weeks 31-51; and Group 4 did not seroconvert by week 51.

Figure 6.3.2 The distribution of packed cell volume at week 51 in calves of different ages of seroconversion to *A. marginale*



Group 1 seroconverted between weeks 1-16; Group 2 seroconverted between weeks 16-31; Group 3 seroconverted between weeks 31-51; and Group 4 did not seroconvert by week 51.

Babesia bigemina

No significant differences were found in any haematological parameter between groups of calves that seroconverted to *B. bigemina* (Group 1: n=14; Group 2: n=40; Group 3: n=75) or between seroconverted and non-seroconverted (Group 4: n= 416) calves.

Trypanosoma spp. (mcr)

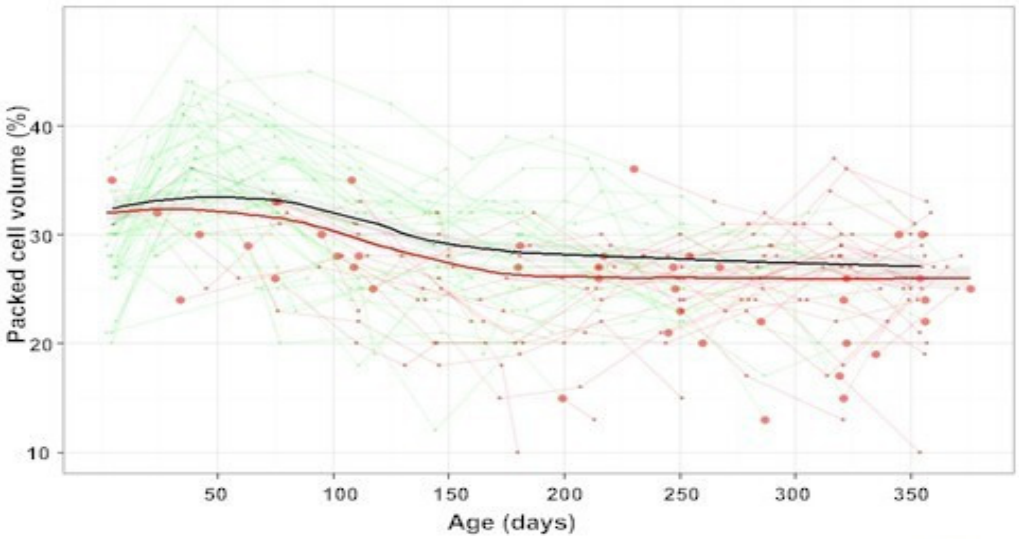
The PCV profile of *Trypanosoma*-infected calves, diagnosed by microscopy (n=42), is illustrated in Figure 6.4.1. The *Trypanosoma*-infected calves had a significantly lower PCV than the total population from week 26 to 36 ($p<0.05$). The calves that were infected at an early age (before 100 days) had a considerably lower PCV during early calf-hood. Towards 350 days there was significant variation in PCV with many of the individual infected calves recovering their PCV to similar levels as uninfected calves. Several calves positive for *Trypanosoma* spp. developed severe anaemia (PCV <15%) at various time points.

There was no significant difference in MCV and MCHC between the *Trypanosoma*- positive and negative calves at any time point.

The variation in WCC between individual positive calves was marked, with several calves displaying quite low WCC (< 5 x 10^3/µL) and several calves that developed relatively high WCC (> 20 x 10^3/µL). In contrast to the total WCC, calves infected with *Trypanosoma* spp. developed a lymphocytosis compared to the negative calves, which became significant from

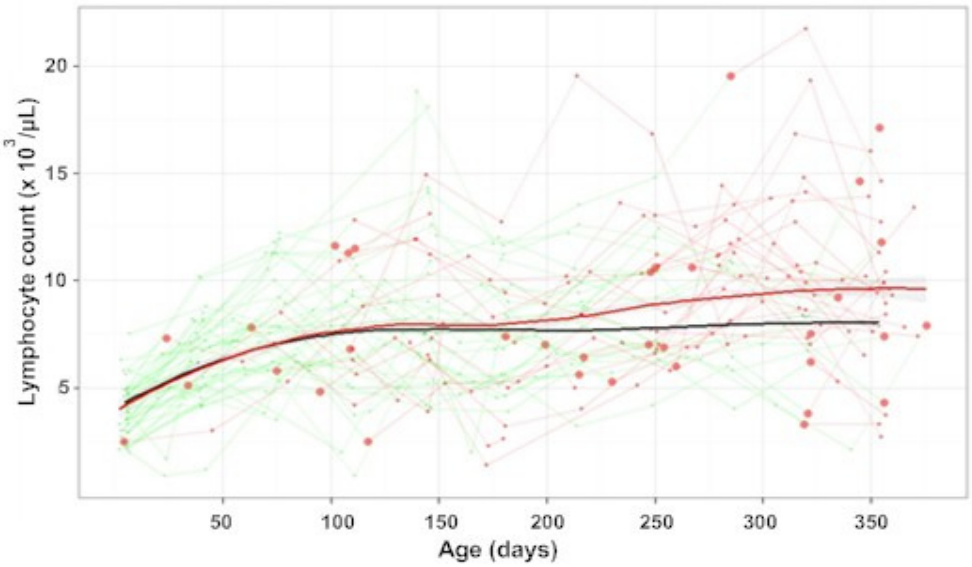
week 31 to week 51 ($p<0.05$). The Lymph profile of *Trypanosoma*-infected calves is depicted in Figure 6.4.2.

Figure 6.4.1 The packed cell volume profile in *Trypanosoma* spp. (mcr) infected calves



• The point of first infection for each calf . The profile line of each calf is green coloured (–) prior to this • point (negative/uninfected), after which the colouring changes to red (–) (positive/infected). The smoothed red line (—) is the mean of the infected calves over time. The mean for the total population is indicated in black (—).

Figure 6.4.2 The lymphocyte count profile in *Trypanosoma* spp. (mcr) infected calves



Figures 6.4.1-6.4.2 indicate the profile of individual calves infected with *Trypanosoma* spp. for the Lymph. The point of first infection for each calf is indicated by (•). The profile line of each calf is green coloured (—) prior to this • point negative/uninfected), after which the colouring changes to red (—) (positive/infected). The smoothed red line (—) is the mean of the infected calves over time. The mean for the total population is indicated in black (—).

No significant difference between positive and negative calves in any other white blood cell parameter was found at any time point.

The most significant difference in the profiles of *Trypanosoma*-infected calves compared to negative calves was the platelet counts, with most calves developing a very low platelet count from the point of infection (indicated with a red dot on the graph, Figure 6.4.3). The difference in mean platelet count between infected and non-infected calves was significant from week 21 to week 51 ($p<0.05$). Several infected calves appeared to recover from thrombocytopenia with several calves displaying a relatively high Plt at 350 days. There was no significant difference in MPV and PDW at any time point between the infected calves and negative calves.

Figure 6.4.3 The platelet count profile in *Trypanosoma* spp. (mcr) infected calves

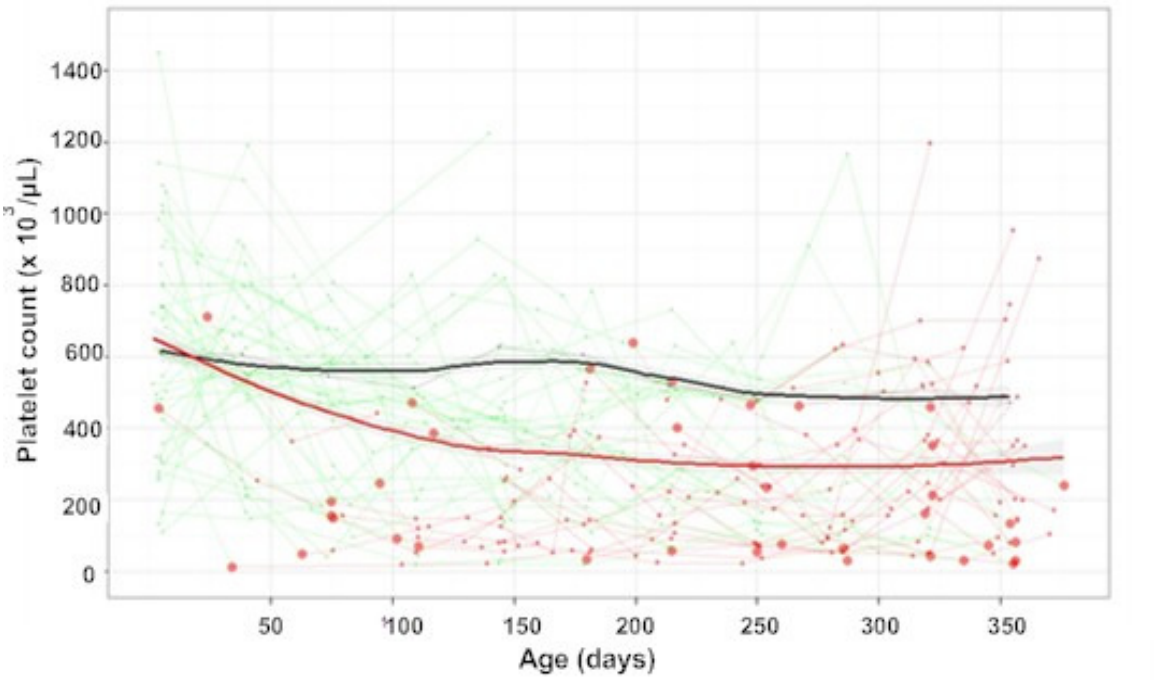


Figure 6.4.3 indicates the profile of individual calves infected with *Trypanosoma* spp. for the PLT. The point of first infection for each calf is indicated by (●). The profile line of each calf is green coloured (—) prior to this ● point negative/uninfected), after which the colouring changes to red (—) (positive/infected). The smoothed red line (—) is the mean of the infected calves over time. The mean for the total population is indicated in black (—).

Trypanosoma vivax (week 51)

Forty-nine calves were positive for *T. vivax* by PCR at week 51. The *T. vivax* positive calves had a significantly higher Lymph (p=0.023) and a significantly lower Plt (p=0.004) than negative calves. No significant differences were found between *T. vivax* positive and negative calves for any other haematological parameter, including PCV. Boxplots (Figures 6.5.1-6.5.2) are used to illustrate the difference in Lymph and Plt between calves that tested positive and negative, respectively, for *T. vivax* at the final visit.

Figure 6.5.1 The distribution of absolute lymphocyte counts in *T. vivax* calves at week 51

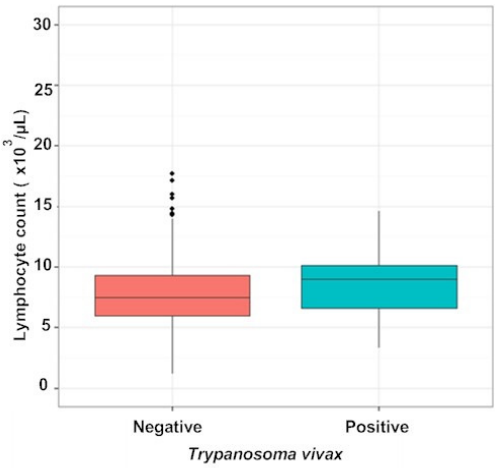
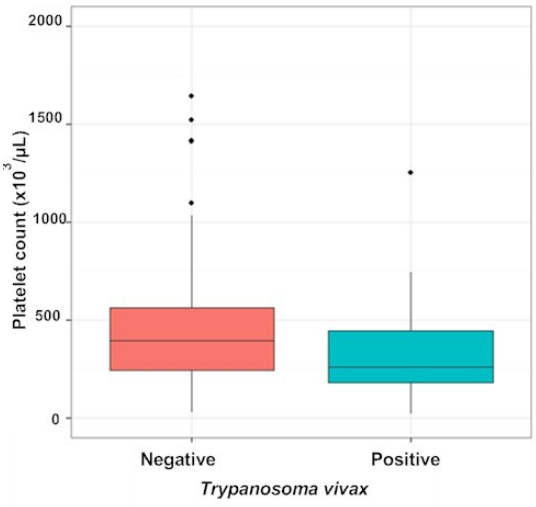


Figure 6.5.2 The distribution of platelet counts in *T. vivax* calves at week 51



3.2 Infectious causes of anaemia: Intestinal parasites

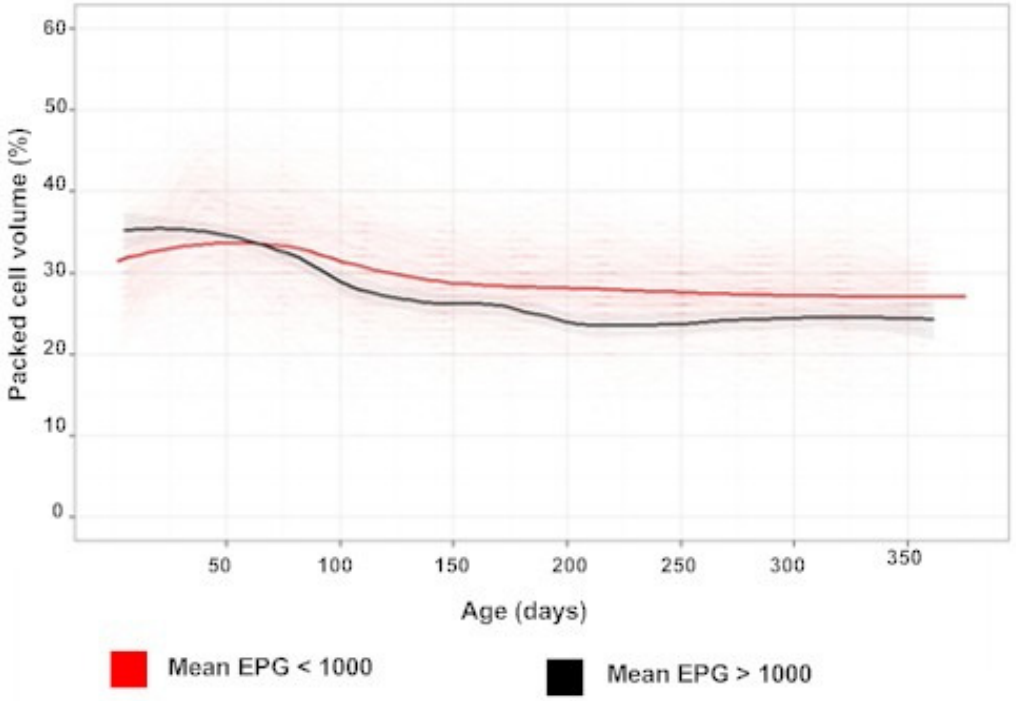
Strongyle-type nematodes

Calves with a high mean strongyle EPG had a significantly lower PCV ($p<0.05$) than calves with a low mean EPG from 100 days and older (Figure 6.6). The apparently higher PCV at week 1 in calves with a high mean EPG was due to high variance in PCV in calves at this time-point. The MCV in the calves became progressively higher in calves with a high mean EPG, with a significant difference from 250 days. There was no significant difference in MCHC between the two groups.

There were also differences in the WCC, Lymph and TSP between the calf groups, with the calves with a high mean EPG displaying a lower WCC, Lymph and TSP values than the calves with a low mean EPG. The differences were significant from 100 days to the end of the follow-up period. No differences were found in Eos, Mono and Neut between the two groups.

The calves suffering from a high mean EPG developed a mild thrombocytosis compared to the calves with lower mean EPG.

Figure 6.6 The packed cell volume in calves infected with strongyle-type nematodes with a high mean EPG compared to calves with a low mean EPG



Strongyloides spp.

There were no significant difference in any parameter measured between *Strongyloides*-infected calves and non-infected calves, as measured at the 6-week visit ($p>0.05$).

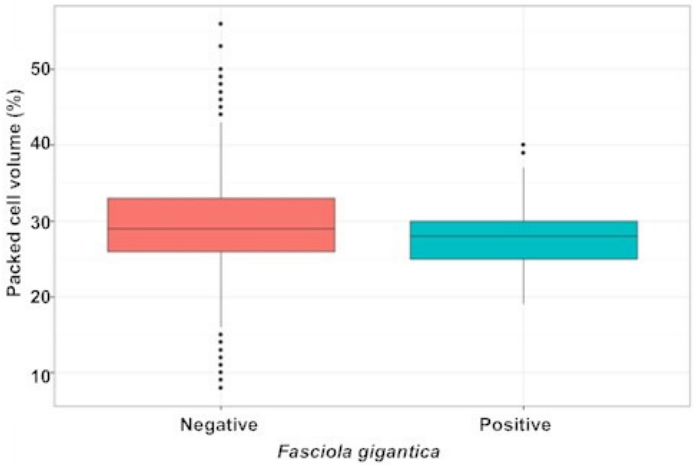
Coccidia

There were also no differences in any parameter measured between coccidia-infected calves with a high and low mean OPG at any time point ($p>0.05$).

Fasciola gigantica

There was a significant difference in PCV (%) ($p = 0.0002$) between calves infected with *F. gigantica* (n=34) compared to non-infected calves, as measured at 51 weeks of age (see Figure 6.7). There were no differences between the two groups in either MCV or MCHC. No difference could also be measured for any white blood cell parameter or platelet counts.

Figure 6.7 The distribution of packed cell volume in *Fasciola gigantica* infected and non-infected calves at the 51-week visit



4. DISCUSSION

4.1 Infectious causes of anaemia: Blood-borne parasites

Theileria mutans

Although usually considered as benign, *T. mutans* has been described as a cause of anaemia in cattle in Kenya (Young, Purnell, Payne, Brown & Kanhal 1978; Moll *et al*. 1986; Kariuki 1990). The calves in this study developed a lower PCV when they were exposed to *T. mutans* infection during early calfhood, compared to calves that were exposed at a later age. Anaemia is thought to be caused by the invasion of the parasite during intraerythrocytic

proliferation which results in erythrocyte destruction (Young *et al.* 1978, Fandamu *et al*. 2007). The prevalence of *T. mutans* in the study population is relatively high. Considering that there appears to be a high risk of exposure to *T. mutans* around 11 weeks of age and calves that get infected around this age tend to develop an anaemia compared to the rest of the calves, *T. mutans* is potentially a significant cause of anaemia during early calf-hood in the study population.

Theileria parva

In contrast to *Trypanosoma* spp., it appears that Zebu cattle are more resistant than taurine breeds to the clinical effects of East Coast fever (ECF) (Irvin 1983). The clinical course of the disease is, however, dependent on the infectious dose, the pathogen strain, and the animals' history of previous exposure (Koch *et al.* 1990). Anaemia is not consistently seen in cases of *T. parva* (Mbassa *et al*. 1994), but has been described in literature (Irvin 1983), and can occur as part of a pancytopenia syndrome (Maxie, Dolan, Jura, Tabel & Flowers 1982; Mbassa *et al*. 1994). No compensatory erythropoeisis occurs in clinical cases of ECF (Hill & Matson 1970; Maxie *et al*. 1982), which results in a normocytic normochromic anaemia (Fandamu *et al*. 2007). Hill & Matson (1970) reported reticulocytosis in subacute cases of experimentally infected animals. These animals recovered after infection. Field cases have been thought to be due to co-infection with other TBD such as *Anaplasma* spp*., Babesia* spp*.* or *T. mutans* (Moll *et al*. 1986).

In this study the age at infection with *T. parva* appeared to be of importance in determining whether a calf develops anaemia. Calves that were infected at a very early age had lower PCV, WCC and Plt counts than calves that were infected at a later age. A pancytopenia would suggest suppression of precursor cells in the bone marrow (Irvin 1983). The apparent pancytopenia was only temporary, however, for the haematological parameters did not differ between groups as the calves grew older. The haematological profile of the total calf population indicated that these calves are in a physiological state of cellular regeneration during early calfhood. It would seem that infection with *T. parva* during this period causes a slightly reduced rate of cellular regeneration, from which the calves in general were able to recover. The severity of infection would of course depend on the infectious load and strain of the parasite (Lawrence *et al*. 2004a). Although *T. parva* should be considered a very important pathogen in the study population, it was not an important cause of anaemia.

Anaplasma marginale & Babesia bigemina

Similar to *T. mutans* and *T. parva*, calves that seroconverted to *A. marginale* before 16 weeks of age displayed lower PCV around this period than calves that seroconverted later.

By 51 weeks there were no significant differences between calves that seroconverted to *A. marginale* at different ages. The PCV was not significantly lower in calves exposed to *B. bigemina* compared to unexposed calves. These two pathogens did not appear to be significant causes of anaemia in the population.

The relatively low prevalence and seroconversion rates for *A. marginale* and *B. bigemina* suggest a state of endemic instability and one would expect that infection with either pathogen would more likely result in clinical disease (Bock *et al*. 2004). One reason for the low incidence of clinical anaplasmosis and babesiosis might be the limited follow-up period of 51 weeks. Typically, clinical anaplasmosis usually develops only in adult animals that have not been exposed during calfhood (Gale, Dimmock, Gartsidet & Leatch 1996a). This is in part due to the age-related resistance against the disease in young animals. This is also true for babesiosis, where innate resistance lasts up to 9 months of age (Mahoney *et al*. 1973; De Vos *et al.* 2004). A low infective dose would not explain the low incidence of clinical disease since the outcome of infection and severity of disease does not depend on the infective dose in either parasite (Gale *et al.* 1996a; Allred 2007). The infected animals are likely to have become latent carriers, which is life-long in the case of *A. marginale* (Potgieter & Stoltsz 2004). There is still a risk of developing clinical disease when these carriers become immunocompromised to the extent that they can no longer contain these latent infections. Carrier animals have been reported to develop clinical anaplasmosis when immunosuppressed due to concurrent trypanosomosis (Magona & Mayende 2002).

Trypanosoma spp.
Trypanotolerance is defined as an ability to control parasitaemia as well as the ability to control the progressive development of anaemia (Murray & Dexter 1988). Zebu cattle, although indigenous, are not as tolerant to *Trypanosoma* spp. infection as N'dama or West African short-horn breeds, and do develop anaemia after infection (Murray & Dexter 1988). Calves appear to be more tolerant than adult animals due to a better erythropoietic response (Murray & Dexter 1988).

Trypanosoma vivax is thought to be less pathogenic than *T. congolense* and most animals develop a less severe anaemia with *T. vivax* infection than wtih *T. congolense* infection (Uilenberg 1998). However, a syndrome marked by acute death accompanied by massive non-specific haemorrhage has been described in *T. vivax* (Murray & Dexter 1988).

The Zebu calves in this study developed anaemia and showed only a slow recovery or partial recovery of anaemia after infection, with many calves surviving with persistent low-

grade anaemia. Compared to *Trypanosoma* spp. negative calves, there was no difference in the MCV and MCHC. Reticulocyte counts would need to be done to confirm this. This would suggest that the erythropoietic response by the bone-marrow is not increased, which would result in a progressive anaemia. The majority of infected calves did not develop a terminal anaemia, however, which suggests some level of trypanotolerance in this breed. The relatively low number of calves [42 (mcr) and 49 (PCR)] that were infected with *Trypanosoma* spp. implies that this parasite is not an important cause of anaemia in the population.

Thrombocytopenia is consistently found in *Trypanosoma* spp. infection in all species (Murray & Dexter 1988). Davis, Robbins, Weller & Braude (1974) found a direct response relationship between the degree of thrombocytopenia and the height of the parasitaemia. This response occurs irrespective of the *Trypanosoma* strain or species. The causes of thrombocytopenia in trypanosomosis are multifactorial. The first cause is parasite by-products that cause initial damage to these cells, while immunological reactions, such as antigen-antibody complexes and auto-antibodies to platelets maintain the thrombocytopenia (Murray & Dexter 1988). Fibrin deposits that form due to disseminated intravascular coagulation (DIC) further damage thrombocytes. The formation of platelet aggregations has been histologically shown in *T. vivax* infections (Murray & Dexter 1988) and *T. rhodesiense* (Davies *et al*. 1974), which indicates a consumptive loss of thrombocytes. These platelet aggregations are thought to be due to antibodies directed against platelets (Assoku & Gardiner 1989). Clumping of platelets can artificially cause a low PLT since such clumps would not be counted as platelets using an automated cell analyser.

There was a marked thrombocytopenia in calves infected with *Trypanosoma* spp. It is interesting that the PCV in some trypanosome-infected calves recovered after initial anaemia, yet many were unable to recover from the thrombocytopenia. This implies that there are continuing subclinical disease processes despite apparent recovery from clinical anaemia. The clinical significance of the thrombocytopenia would depend on the pathogenesis. The formation of platelet aggregations due to subclinical trypanosomosis might predispose calves affected by other infectious agents to succumb to terminal DIC and accelerated the time to death. DIC has been implicated in terminal disease in several infectious agents, including ECF (Maxie *et al*. 1982). Thrombocytopenia due to cellular destruction might also result in bleeding tendencies if severe enough (Assoku & Gardiner 1989) and would present clinically as multifocal petechiation and ecchymosis (Duncan *et al*. 1994).

4.2 Infectious causes of anaemia: Intestinal parasites

Strongyloides spp.

The prevalence of *Strongyloides* worms was only significant in very young calves. This parasite did not contribute significantly to the level of anaemia in the population at any time-point.

Strongyle-type nematodes

After 6 weeks strongyle worms became the predominant intestinal parasite with the majority of calves becoming infected by the end of their follow-up period. Infection occurs through ingestion of infective larvae and calves are increasingly exposed as they start grazing. Strongyles cause anaemia in animals through blood loss. *Haemonchus* spp., a strongyle-type nematode, is considered as one of the most pathogenic parasites of ruminants (Kaufmann1996) and is consistently reported as the most prevalent helminth species in cattle in Kenya (Moll *et al.* 1984; Latif *et al*. 1995; Waruiru *et al*. 2001; Waruiru *et al.* 2002). The pathogenesis is that of a haemorrhagic anaemia (Kaufmann *et al.* 1992) and presents as a hypochromic macrocytic anaemia. During extremely high parasite burdens the animal will die due to severe blood loss. In chronic cases animals develop a steady drop in PCV and serum albumin which results in emaciation of the animal. If the animal survives, the compensatory erythropoiesis will eventually deplete iron reserves (Kaufmann *et al*. 1992). This hypoferonaemia then presents as a normochromic microcytic anaemia (Duncan *et al.* 1994).

The development of anaemia appears to be dose-dependent, as only calves with a strongyle-associated EPG > 1000 developed anaemia. The increased MCV levels in the calves with a high strongyle EPG indicate a regenerative response. If the animals were treated with anthelmintics they were likely to have recovered and their PCV to return to pre-infection levels. Strongyle worms should be considered as a potentially important cause of anaemia in the population and due to the chronic nature of infection it is likely a very erosive disease.

Coccidia

Almost all calves were infected with coccidia at some point in their follow-up period. It did not appear to be an important cause of anaemia in the study, but the clinical significance of its presence would depend on the particular species involved (Kaufmann 1996) and might prove interesting to investigate further.

Fasciola gigantica

Fasciola gigantica infection is usually associated with chronic hepatic fibrosis and hyperplastic cholangitis in cattle (Kaufmann 1996). Cattle are less susceptible to infection than sheep, but in cases of high infectious load of metacercariae calves can develop acute symptoms such as anaemia, weight loss and hypoalbuminaemia. Anaemia develops due to blood consumption by adult flukes, depletion of iron stores from chronic bloodloss and haemolysis due to chronic inflammation and parasite by-products (Valero, Gironès, Garcia-Bodelón, Periago, Chico-Calero, Khoubbane, Fresno & Mas-Coma 2008). Fasciolosis appeared to become of more relevance in older calves. This was also reported to be the case in Uganda where adult cattle had higher prevalence of *F. gigantica* than calves (Magona & Mayende 2002). Although fasciolosis caused anaemia in the calves in this study, it is not an important cause of anaemia during the first year of life but may become more important in adult animals. The economic importance of fasciolosis lies in its erosive effect on the production of livestock through reduced weight gain or weight loss (Kaufmann 1996).

5. CONCLUSIONS

Strongyle-type nematodes were shown to be an important infectious cause of regenerative anaemia in the calf cohort while *T. mutans, T. parva, Trypanosoma* spp. and *Fasciola gigantica* caused non-regenerative anaemia in the calf cohort. The clinical outcome of strongyle infections, although a highly prevalent pathogen, depended on the infectious load of the pathogen and only caused a significant change in PCV at high doses.

In Chapter 4 the haematological profile of the East African short-horn Zebu calves was discussed. It was shown that these calves have a physiological rise in red cell parameters during early calfhood. It was speculated that this period of red cell generation buffered the effects of infection during early calf-hood and that it possibly formed part of their innate ability to control infection from developing into overt disease. There was a relative decrease, or a reduced physiological increase of PCV of calves that seroconverted to *T. mutans, T. parva* and *A. marginale* before 16 weeks at the time of seroconversion which was not found in calves that seroconverted at later ages. The immune system in calves is not completely mature by 16 weeks, and calves are still vulnerable to infection, despite their innate resistance to infection. These calves recovered, however, and PCV levels between early seroconverters and late seroconverters did not differ at later time points.

Other pathogens known to cause anaemia in cattle, e.g. *A. marginale* and *B. bigemina*, were shown not to contribute significantly to the level of anaemia in the calf population. This was possibly due to low prevalences, age-related resistance against infection, or the limited time-frame of the follow-up period. Although these latent infections appeared to be benign, they do pose a risk to these calves and can develop into clinical disease if the animal becomes immunocompromised (Potgieter & Stoltsz 2004).

Several pathogens did not cause acute decreases in PCV at infection, but due to the chronic nature of infection, were responsible for persistent low-grade anaemia in infected calves. The clinical significance of such infections, e.g. chronic strongyle infection and *Trypanosoma* spp., lies in the erosive effect on the production of the calf. There was also evidence of ongoing subclinical disease processes in animals that apparently recovered from certain overt clinical diseases, such as trypanosomosis. Similar to calves that suffer from low-grade progressive disease, calves with unapparent subclinical conditions are likely to suffer from ill-thrift and slow weight gain and are possibly more susceptible to super-infections with other pathogens or the effects of malnutrition.

In field conditions, where calves are more likely to be burdened by multi-pathogen infections, it is more relevant to consider the combined impact of pathogens on the clinical course of infection. The additive effects of multi-pathogen infections, even at low infectious loads or subclinical disease, might result in clinically significant changes in the haematological parameters of the infected calves. The effect on the haematological profile in multi-pathogen infections will be further investigated in Chapter 7.