CHAPTER 7

ANAEMIA IN EAST AFRICAN SHORT-HORN ZEBU CALVES: THE IMPACT OF CO-INFECTIONS ON THE HAEMATOLOGICAL PROFILE OF CALVES

1. INTRODUCTION

Under field conditions an animal is more likely to suffer from the burden of multiple infectious causes of disease rather than single infections (Petney & Andrews 1998). In Chapter 5 it was shown that this is true for the East African short-horn Zebu calves in western Kenya. These calves are infected with multiple pathogens from early calfhood, either at the same time point or in succession over their lifetime. Co-infections complicate the clinical presentation of disease as each pathogen is likely to contribute to the clinical outcome of infection in the host. Pathogens often share and compete for the same resources in the host. Interactions between such pathogens can alter the onset, duration and clinical course of disease, as well as the host's susceptibility or tolerance to other pathogens (Cox 1987; 2001).

It is therefore more relevant to study an infectious disease in context of the complete multi-pathogen burden the animal is suffering from (Moll *et al.* 1984). In Chapter 6 the impact of single pathogenic infections on the haematological profile of East African short-horn Zebu calves, in particular the PCV, was investigated. It was shown that several pathogens alter the haematological profile of the calves and that several of these pathogens cause anaemia in the population. Co-infections with other pathogens were not considered.

This chapter aims to investigate the impact of co-infections of the most prevalent pathogens in the study area on the haematological response of the population. This was achieved by modelling the infectious status of pathogens against specific haematological responses. The presence of significant interactions between pathogens was also investigated by the models. Finally, the predicted outcomes of these models were then used to illustrate the cumulative effect of co-infections between different pathogen pairs on each haematological parameter of the calves.

2. MATERIALS AND METHODS

* General methodology is discussed in Chapter 2

2.1 Univariate analysis: The impact of single pathogens

Univariate analysis of single pathogen infections was used as a preliminary screening tool for potential inclusion in the subsequent multivariable analysis. The haematological parameters, analysed as response variables, include packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white cell counts (WCC), absolute lymphocyte counts (Lymph) and platelet counts (Plt).

Pathogens considered for analysis as independent variables included the tick-borne parasites *Theileria* spp., *Theileria mutans, Theileria parva, Anaplasma* spp., *Anaplasma marginale* and *Babesia bigemina; Trypanosoma* spp. and specifically *T. vivax;* and the intestinal parasites strongyle-type nematodes, strongyloides-type nematodes, coccidia and *Fasciola gigantica*.

Theileria spp. and *Anaplasma* spp. were diagnosed by microscopy and no species differentiation was made. There were only four visits where *Babesia* spp. was detected by microscopy and thus *Babesia* spp. was not included as a covariate.

Theileria parva, T. mutans, A. marginale and *B. bigemina* diagnosis was based on seroconversion. The time-point of seroconversion was firstly used as a covariate in the analysis to capture the acute response around the time of infection. Secondly, to capture the long-term impact of infection on the haematology, an indicator for seroconverted status was included as a covariate and for this purpose a calf was considered as positive (seroconverted) for all visits post seroconversion.

Trypanosoma-positive calves were deemed to be those calves that were diagnosed positive bymicroscopy (mcr) and no distinction between species was made. *Trypanosoma vivax*-positive calves were diagnosed by microscopy and speciation confirmed by PCR.

Strongyle worm species were considered as a categorical covariate at three levels, namely negative, positive with a high EPG of strongyle-type eggs (EPG> 1000) or positive with a low EPG (EPG<1000). The same classification was used for *Strongyloides* spp., as either negative, or positive with a high EPG (>1000) or low EPG (<1000) of strongyloides-type eggs. Similarly coccidia were analysed as a three-level categorical covariate as either

negative, or based on the OPG, as positive with either high OPG (>1000) or low OPG (<1000). *Fasciola gigantica* was only categorised as either present or absent.

Generalized mixed effect models were used for univariate models, and included single pathogens as fixed effects, with calf identification and sublocation as random effects. The significance of terms was determined by the *p*-value <0.1. A less restrictive *p*-value was chosen to increase the sensitivity of the univariable models a screening tool for identifying independent variables that will be used in further multivariable model building.

2.2 Multivariable analysis: The impact of interaction between co-infecting pathogens
Generalized additive mixed effect (GAMM) models were used in multivariable analysis, due to the non-linear distribution of the haematological parameters with age, as discussed in Chapter 2. Random effects included calf identification and sublocation, and fixed effects included age and pathogens, including interactions between age and pathogens and two-way interactions between pathogens.

Multivariable modelling started by including all covariates (pathogens) in the model that had a significant outcome based on univariate analysis. Two-way interaction terms were included in a step-up process and tested for both significance and model fit (reduction in residuals and AIC). If the interaction term was found significant ($p<0.05$), the term was left in the model and the next interaction term included. When found insignificant, the interaction terms were excluded. All covariates excluded based on univariate analysis were fitted into the multivariable model to check for significant interaction terms. The final minimal model thus only included significant terms of pathogens and age, where applicable, and interaction terms as fixed effects and calf identification and sublocation as random effects.

The estimated coefficient of each covariate included in the minimal model is interpreted as an increase in the response variable equal to the coefficient when the covariate (pathogen) is positive (two-level categorical covariates), or an increase equal to the value of the coefficient for an increase in one unit of the covariate (for three-level categorical covariates). A negative value of a coefficient should be interpreted as a decrease in the response variable, calculated in the same way as for positive coefficients.

To improve model fit, log transformation of PIt data was necessary. The model parameters reported represent the log-transformed data. The coefficient is interpreted as a percentage increase in the response variable equal to the value of the coefficient when positive, or a percentage decrease in the response variable when the coefficient is negative.

2.3 Pathogen interactions

Interaction between the co-infecting pathogens is said to occur when the outcome of infection, e.g. a reduction in PCV, during co-infections differs from the sum of the individual outcomes of the single infections. A positive interaction occurs when the outcome of infection is increased compared to the sum of the individual outcomes, e.g. a more severe reduction in PCV than what is expected to occur due to both pathogens combined. A negative interaction occurs when the outcome of infection is reduced compared to the sum of the individual outcomes, e.g. the reduction in PCV is less severe than what is expected from the combined effect of the individual pathogens.

For the purpose of this study, only two-way interactions between pathogens were considered.

2.4 Predicted outcomes of interactions between co-infecting pathogens

The predicted mean value of the response variables for each model was calculated for uninfected calves, calves positive for each single covariate, as well as two-way combinations between pathogen pairs. The model-predicted mean value was calculated from the sum of the intercept and the coefficients of the selected pathogens included in the minimal model. The 95% confidence intervals (95%CI) for the predicted mean values were calculated in the same way, using the standard error (SE) of each coefficient. To calculate the predicted mean Plt (pPlt), the model-predicted log interval (mean ±95%CI) was first calculated and then back transformed through exponentiation (10^x) by the calculated value (x).

3. RESULTS

3.1 Univariate analysis

The covariates found to significantly predict the various response variables in univariate analysis are listed in Table 7.1. These covariates were further used in model-building in multivariable models.

Table 7.1 The significance of *p*-values (p<0.1) obtained from univariate analysis of single covariates

Covariate		Response variables					
		PCV[6]	MCV[7]	MCHC[8]	WCC[9]	Lymph[10]	Plt[11]
Blood-borne pathogens							
Theileria spp. (mcr)[2]	Presence/absence	****	NS	*	NS	NS	****
T. parva	Positive	NS	*	**	**	****	****
	At seroconversion	NS	NS	NS	****	****	*
T. mutans	Positive	****	***	NS	NS	****	****
	At seroconversion	**	NS	NS	NS	NS	**
Anaplasma spp. (mcr)[2]	Presence/absence	NS	NS	NS	NS	*	NS
A. marginale	Positive	NS	*	NS	***	****	****
	At seroconversion	NS	NS	NS	NS	NS	**
B. bigemina	Positive	NS	****	NS	**	****	****
	At seroconversion	NS	NS	NS	NS	*	**
Trypanosoma spp. (mcr)[2]	Presence/abscence	****	NS	NS	NS	NS	****
T. vivax (PCR)[3]	Positive	****	NS	NS	NS	*	****
Intestinal pathogens							
Coccidia	Presence/absence	****	*	NS	**	*	NS
	OPG[4] > 1000	NS	NS	NS	NS	NS	NS
Strongyle-type species	Presence/absence	****	NS	*	*	****	****
	EPG[5] > 1000[7]	****	****	*	****	****	NS
Strongyloides spp.	Presence/absence	NS	NS	NS	NS	****	****
	EPG[5] > 1000	NS	NS	NS	NS	****	****
Fasciola gigantica	Presence/absence	NS	***	NS	NS	*	NS

**** p<0.0001, ***p<0.001, **p<0.01, *p<0.05, NS: non significant (p>0.0.5)
[1] s(): use smoothing function
[2] mcr: microscopy. This indicates the test used to diagnose the pathogen.
[3] PCR: polymerase chain reaction. This indicates the test used to diagnose the pathogen.
[4] OPG>1000: Oocysts per gram faeces per >1000.
[5] EPG>1000: Eggs per gram faeces >1000.
[6] PCV: packed cell volume
[7] MCV: mean corpuscular volume
[8] MCHC: mean corpuscular haemoglobin concentration
[9] WCC: white cell count
[10] Lymph: absolute lymphocyte count
[11] Plt: platelet count

3.2 Generalized additive mixed effect models

Packed cell volume

The coefficient estimates of the GAMM model for PCV are tabulated in Table 7.2. The effect of age on PCV was significant but nonlinear and was included in the model as a covariate with a smoothing function (estimated degrees of freedom (edf) = 7.698 and p<0.0001).

Pathogens that caused a significant decrease in PCV include *Theileria* spp. (mcr); *T. mutans; Trypanosoma* spp. (mcr); and strongyle worms (p<0.05). Strongyle species had a more severe effect on PCV when EPG was high. The only pathogen that caused a significant increase in PCV was coccidia (p<0.05). *Theileria mutans* caused a slightly more severe decrease in PCV at the time of seroconversion than at later time-points after seroconversion.

Table 7.2 Generalized mixed-effect model analysis of packed cell volume (n=3917)

Covariate	**Coeff** [1]	**SE** [2]	***p*** [3]
Theileria spp. (mcr)[4]	-2.147	0.321	<0.0001
T. mutans (at seroconversion)	-1.172	0.29	<0.0001
T. mutans (seroconverted)	-0.892	0.324	0.006
Trypanosoma spp. (mcr) [4]	-6.089	1.027	<0.0001
Strongyle-type species (EPG<1000) [5]	-0.76	0.258	0.003
Strongyle-type species (EPG>1000) [5]	-3.148	0.306	<0.0001
Coccidia (positive)	0.459	0.139	<0.001
T. mutans : *Theileria* spp.	0.709	0.326	0.03
T. parva (at seroconversion) : *Theileria* spp.	-0.889	0.279	0.001
Theileria spp. : age	0.007	0.002	<0.0001
Trypanosoma spp. : *Theileria* spp.	4.542	1.212	<0.001
Anaplasma marginale : *Strongyloides* spp. (EPG>1000)[5]	-5.006	2.376	0.035

[1] Coefficient
[2] Standard error of coefficient
[3] *p*-value indicate significance of coefficient
[4] mcr (microscopy)
[5] Eggs per gram faeces >1000

The impact of *Theileria* spp. on PCV became less severe as the calf age increased. Interactions were found between *T. mutans* and *Theileria* spp., as well as *T. parva* and *Theileria* spp. The decrease in PCV was 1.7 times more severe in calves when positive for both *Theileria* spp. (mcr) and *T. mutans* (seroconverted) than when only positive for *T. mutans* on serology only. *Theileria parva* had a significant impact on PCV only at the time of seroconversion, and only when seroconverted calves were also positive for *Theileria* spp. on microscopy.

A negative interaction was found between *Trypanosoma* spp. and *Theileria* spp. Although both pathogens caused a decrease in PCV by themselves, the total decrease in PCV during co-infections with *Trypanosoma* spp. and *Theileria* spp. (–3.696) was less than single infections with *Trypanosoma* spp. (-6.089).

An interaction between *A. marginale* and *Strongyloides* spp. was also found. Although neither pathogen had a significant impact on PCV by themselves, co-infection with these two pathogens caused a significant decrease (-5.006) in PCV.

Mean corpuscular volume

The effect of age on MCV was significant but nonlinear and was included in the model as a covariate with a smoothing function (edf = 8.067 and $p<0.0001$). The coefficient estimates of the model are depicted in Table 7.3. Pathogens that caused a significant increase in MCV include *B. bigemina, T. mutans, T. parva* and strongyle-type nematodes ($p<0.05$). The only pathogen that caused a statistically significant decrease in MCV was *A. marginale* ($p<0.05$). The impact of *A. marginale* and *T. parva* was very small, however, and dependent of the age of the calf.

A positive interaction was found between strongyle-type nematodes and *Fasciola* spp. The increase in MCV was 3.89 times higher in co-infection with *Fasciola* spp. than infection with strongyle-type nematodes alone. The interaction depended on the infectious load of the strongyles (EPG).

Negative interactions were found between *T. parva* and *Fasciola* spp. and *B. bigemina* and coccidia. *Fasciola* spp. had no significant impact on MCV by itself, but in *T. parva*-positive calves, *Fasciola* spp. infection caused a decrease in MCV of 1.178. The increase in MCV was 3.7 times less in co-infections with coccidia than in only *B. bigemina*-positive calves.

Table 7.3 Generalized additive mixed-effect model analysis of mean corpuscular volume (n=3904)

Covariate	**Coeff** [1]	**SE** [2]	***p*** [3]
Theileria mutans	0.479	0.117	<0.0001
Babesia bigemina	1.078	0.182	<0.0001
Anaplasma marginale : age	-0.001	0.0005	0.004
Theileria parva : age	0.001	0.0005	0.04
B. bigemina : Coccidia	-0.79	0.189	<0.0001
Strongyles (EPG>1000)[4]	0.591	0.127	<0.001
T. parva : *Fasciola* spp.	-1.178	0.246	<0.001
Strongyles (EPG>1000) [4] : *Fasciola* spp.	1.708	0.444	<0.001

[1] Coefficient
[2] Standard error of coefficient
[3] *p*-value indicate significance of coefficient
[4] EPG: eggs per gram faeces

Mean corpuscular haemoglobin concentration

The effect of age on MCHC was significant but nonlinear and was included in the model as a covariate with a smoothing function (edf = 8.138 and $p<0.0001$). Only two pathogens had an impact on MCHC, namely *T. parva* and *A. marginale.* There was an interaction between both pathogens and the age of the calf. The model coefficient estimates are depicted in Table 7.4. Very little of the variance of the model was explained by the presence of pathogens ($R^2=0.089$)

Table 7.4 Generalized additive mixed-effect model analysis of mean corpuscular haemoglobin concentration (n=5516)

Covariate	**Coeff** [1]	**SE** [2]	***p*** [3]
Theileria parva : age	-0.001	0,0002	0.017
Anaplasma marginale : age	0.001	0.0002	0.035

[1] Coefficient
[2] Standard error of coefficient
[3] *p*-value indicate significance of coefficient

White cell count

The effect of age on WCC was significant but nonlinear and was included in the model as a covariate with a smoothing function (edf = 1 and $p<0.001$). The coefficient estimates of the model are tabulated in Table 7.5.

Pathogens that had a negative impact on WCC include strongyle-type nematodes (EPG>1000) and *T. parva* (at the time of seroconversion). Pathogens that had a positive impact on WCC include *Trypanosoma* spp., *T. parva* (after seroconversion), and *B. bigemina*. The impact of *T. parva* (after seroconversion) and *B. bigemina* depended on the age of the calf.

Two negative interactions were found, between strongyle-type nematodes and coccidia, and between *Trypanosoma* spp. and coccidia. The decrease in WCC during co-infections with coccidia was four times less than in single infections with strongyles. By themselves *Trypanosoma* spp. caused an increase in WCC, but with co-infection with coccidia, a decrease in WCC was found.

Table 7.5 Generalized additive mixed-effect model analysis of mean white cell count (n=3906)

Covariate	**Coeff** [1]	**SE** [2]	***p*** [3]
Strongyle-type nematodes (EPG>1000) [4]	-0.931	0.156	<0.0001
Trypanosoma spp. (mcr)[5]	1.393	0.468	0.003
Theileria parva (at seroconversion)	-0.724	0.163	<0.0001
Theileria parva (after seroconversion) : age	0.002	0.0006	0.012
Babesia bigemina ; age	0.002	0.0007	0.02
Strongyle-type nematodes (EPG>1000) [4] : Coccidia	0.703	0.208	<0.001
Trypanosoma spp. : Coccidia	-2.154	0.865	0.013

[1] Coefficient
[2] Standard error of coefficient
[3] p-value indicate significance of coefficient
[4] Eggs per gram faeces > 1000
[5] Microscopy

Absolute lymphocyte count

The effect of age on Lymph was significant but nonlinear and was included in the model as a covariate with a smoothing function (edf = 6.016 and $p<0.0001$). The coefficient estimates of the model are tabulated in Table 7.6.

The only pathogens that caused a significant increase in Lymph were *B. bigemina* and *Trypanosoma* spp. ($p<0.05$). The impact *Trypanosoma* spp. had on Lymph was dependent on the age of the calf. Both strongyles and *T. parva* (at time of seroconversion) caused a decrease in Lymph ($p<0.05$).

Interaction between *T. parva* (after seroconversion) and strongyle-type nematodes (EPG>1000) resulted in a decrease in Lymph 1.47 times lower than in a strongyle (EPG>1000) infection alone. *Anaplasma marginale* caused an increase in Lymph only when calves were co-infected with coccidia (OPG>1000).

Table 7.6 Generalized additive mixed-effect model analysis of lymphocyte count (n=3856)

Covariate	**Coeff** [1]	**SE** [2]	***p*** [3]
Babesia bigemina : age	0.001	0.0005	0.0145
Theileria parva (at seroconversion)	-0.467	0.116	<0.0001
Trypanosoma spp. (mcr) [4] : age	0.003	0.001	0.016
Strongyle-type nematodes	-0.206	0.101	0.04
A. marginale : coccidia (OPG>1000) [5]	0.923	0.339	0.007
T. parva : Strongyles (EPG>1000) [6]	-0.193	0.074	0.009

[1] Coefficient
[2] Standard error of coefficient
[3] *p*-value indicate significance of coefficient
[4] Microscopy
[5] Oocysts per gram faeces > 1000
[6] Eggs per gram faeces > 1000

Platelet counts

The platelet counts were log transformed to allow for a better fit of the final model by normalizing the distribution of the residuals. The coefficient estimates of the model are listed in Table 7.7.

Table 7.7 Generalized additive mixed-effect model analysis of log-transformed platelet counts (n=3856)

Covariate	Coeff [1]	SE [2]	*p* [3]
Anaplasma marginale	-0.405	0.077	<0.0001
Babesia bigemina	-0.092	0.04	0.021
Theileria spp.	-0.096	0.033	<0.0001
T. mutans	-0.117	0.03	<0.001
T. parva	-0.307	0.076	<0.0001
Trypanosoma spp. (mcr) [4]	-1.647	0.326	<0.0001
T. vivax	-0.37	0.094	<0.0001
Strongyle spp.	-0.114	0.04	<0.01
Strongyloides spp.	0.122	0.036	<0.001
Theileria spp. : *A. marginale*	0.227	0.059	<0.001
T. parva : *Strongyle* spp.	0.197	0.075	0.009
T. parva : *Fasciola* spp.	0.142	0.066	0.03
T. mutans : *Strongyloides* spp.	-0.188	0.058	0.001
T. mutans : *Trypanosoma* spp.	0.416	0.167	0.013
Trypanosoma spp.: *Strongyle* spp.	0.854	0.325	0.009

[1] Coefficient
[2] Standard error of coefficient
[3] *p*-value indicate significance of coefficient
[4] Microscopy

The only pathogen that caused a significant increase in Plt was *Strongyloides* spp. Pathogens that caused a significant decrease in Plt included *A. marginale*, *B. bigemina, Theileria* spp., *T. mutans, T. parva, Trypanosoma* spp. (mcr), *T. vivax,* and *Strongyle* spp. *Trypanosoma* spp. caused the most severe decrease in Plt.

The decrease in Plt was 1.48 times more in *A. marginale*-positive calves than calves positive for both *A. marginale* and *Theileria* spp. The decrease in strongyle-positive animals was twice as low when also positive for *T. parva. Theileria parva* also interacted with *Fasciola* spp. On its own, *Fasciola* spp. had no significant impact on Plt, but in *T. parva*-positive calves caused a decrease of 16%.

There was also an interaction between *Trypanosoma* spp. and strongyl*e*s. Co-infection with these two pathogens resulted in a decrease in Plt almost eight times more than in strongyle infection alone. An interaction between *Trypanosoma* spp. and *T. mutans* was also detected. The decrease was ten times more than in *T. mutans*-positive calves that were not infected with *Trypanosoma* spp. as well.

3.3 Model-predicted mean of the response variables

Predicted packed cell volume

The model-predicted mean PCV (pPCV) at 150 days of age was calculated to illustrate the impact of the cumulative effect of pathogens on the PCV of the calves (Table 7.8). The pathogen-group that caused the most severe decrease in pPCV was *Trypanosoma* spp. (mcr). Strongyle-type helminths caused the second most severe decrease in pPCV, but the decrease was only clinically significant at a high EPG. Other pathogens that caused a decrease in mean pPCV were *T. mutans* and *Theileria* spp. but these were not clinically significant unless they occurred as part of multi-pathogen infections. Coccidia caused a slightly higher pPCV than in uninfected calves, but this was not clinically significant.

There was a cumulative decrease in pPCV in co-infections between *Trypanosoma* spp. and both strongyle-type helminths and *T. mutans*. The overall lowest mean pPCV was found in co-infections with *Trypanosoma* spp. and strongyle-type species (EPG>1000) (mean pPCV=21.38% at 150 days). Co-infection with coccidia only marginally improved the pPCV. The mean pPCV in concomitant infections between *Trypanosoma* spp. and strongyle-type species, *T. mutans*, *Theileria* spp. and coccidia is illustrated in Figure 7.1.

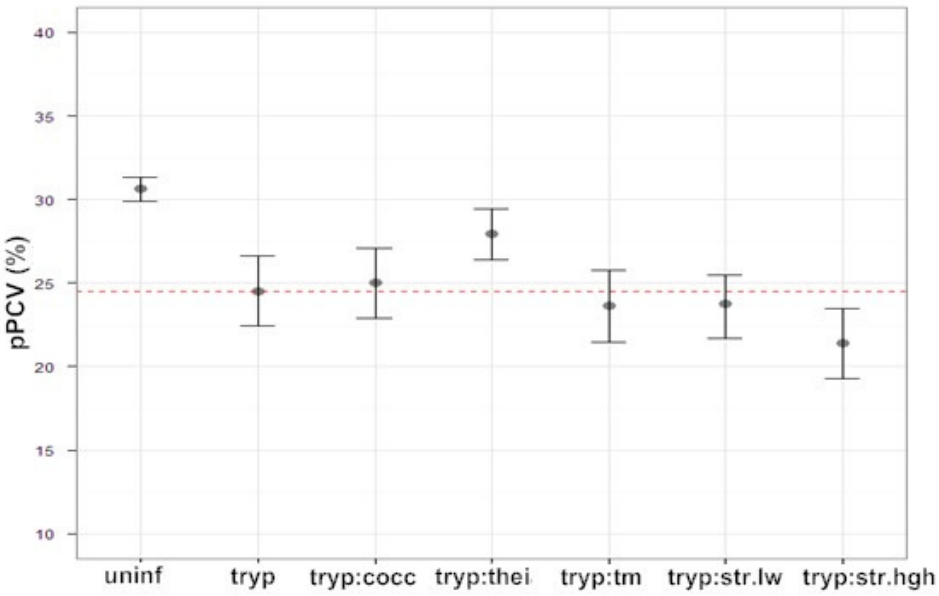
The decrease in pPCV in *T. mutans*-positive calves was slightly more significant when the calf was also positive for *Theileria* spp. on microscopy. The impact of *Theileria* spp. on pPCV was dependent on the age of the calf, and this was also reflected in the impact of *T. mutans* on pPCV (Figure 7.2). At 300 days of age, the pPCV of *T. mutans*-positive calves (28.05 (27.34-28.76) %) was not markedly different than in uninfected calves (pPCV=28.38 (27.51-29.25) %).

Table 7.8 The GAMM-predicted mean packed cell volume (%) and 95% confidence intervals in co-infections with pathogen pairs at 150 days

Covariate	Uninfected	*Theileria* spp,	*T. mutans*	*T. parva*	*Trypanosoma* spp.	Strongyle–type species (EPG<1000)	Strongyle-type species (EPG>1000) [1]	Coccidia
Uninfected	30.62 (29.91-31.32)	-	-	-	-	-	-	-
Theileria spp. (mcr)[1]	29.47 (28.77-30.17)	29.29 (28.58-30.00)	28.58 (27.73-29.44)	**27.92 (26.39-29.46)**	28.71 (28.20-29.22)	26.32 (25.73-26.92)	29.93 (29.24-30.62)	
Theileria mutans		29.73 (28.94-30.52)	29.73 (28.94-30.52)	**23.64 (21.49-25.78)**	28.97 (28.32-29.61)	26.58 (25.85-27.30)	30.19 (29.41-30.97)	
Theileria parva (at seroconversion)			30.62 (29.91-31.32)■	**24.53 (22.43-26.63)**	29.86 (29.33-30.39)	27.47 (26.86-28.08)	31.08 (30.38-31.77)	
Trypanosoma spp. (mcr) [1]				**24.53 (22.43-26.63)**	**23.77 (21.71-25.83)**	**21.38 (19.30-23.46)**	**24.99 (22.89-27.09)**	
Strongyle-type species (EPG<1000) [2]					29.86 (29.33-30.39)	NA	30.32 (29.76-30.87)	
Strongyle-type species (EPG>1000) [2]						27.47 (26.86-28.08)	27.93 (27.30-28.55)	
Coccidia							31.08 (30.38-31.77)	

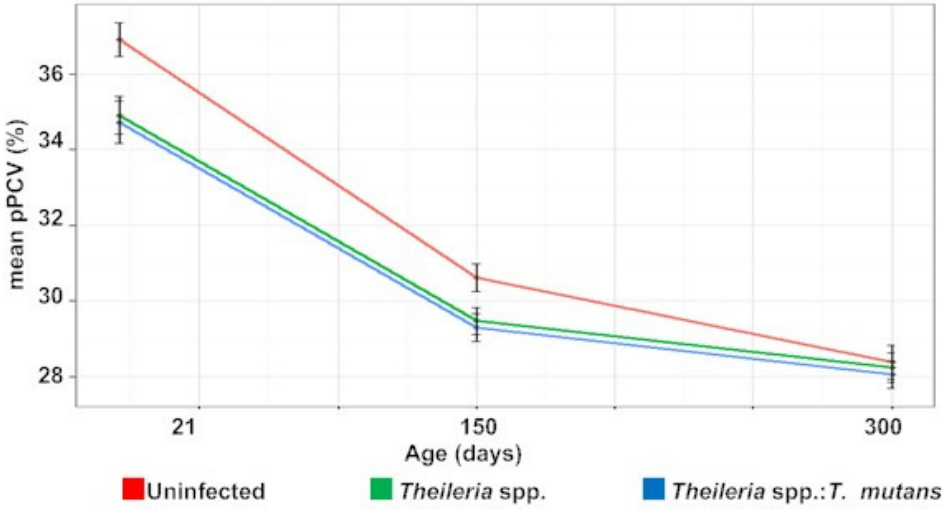
■ pPCV not significantly different than pPCV of uninfected calves.
■ Co-infections with *Trypanosoma* spp. (mcr).
[1] mcr: microscopy
[2] EPG: eggs per gram faeces.

Figure 7.1 The distribution of the GAMM-predicted mean packed cell volume in co-infections associated with *Trypanosoma* spp.-positive calves at 150 days of age



pPCV- model-predicted packed cell volume; a:b denotes co-infection between pathogen a and b; uninf- uninfected; tryp- *Trypanosoma* spp.; cocc- Coccidia; thei- *Theileria* spp.; tm- *Theileria mutans*; str.lw- *Strongyle* spp. (EPG<1000); str.hgh- *Strongyle* spp. (EPG>1000); ----- mean pPCV of *Trypanosoma* spp. -positive calves.

Figure 7.2 The distribution of the GAMM-predicted mean packed cell volume in *Theileria mutans*-positive calves at 150 days of age



pPCV- model-predicted mean packed cell volume; •*Theileria* spp.: *T. mutans*-calves are positive for both *Theileria mutans* on serology and *Theileria* spp. on microscopy.

Predicted mean corpuscular volume

The model-predicted mean MCV (pMCV) for co-infections with pathogen pairs was calculated to illustrate what the impact of the cumulative effect and interaction between pathogen was on MCV.

Overall there was very little difference between pMCV in uninfected calves and calves infected with various pathogens. Both *T. mutans* (34.19 (33.91-34.84) fL at 150 days) and strongyle-type nematodes (34.14 (33.82-34.45) fL at 150 days) caused an increase in pMCV compared to uninfected calves (33.76 (33.48-34.03) fL at 150 days). The lowest pMCV (32.51 fL at 150 days) was found in calves positive for both *T. parva* and *Fasciola* spp. The highest pMCV (36.15 fL at 150 days) was found in calves infected with both strongyle-type worms and *Fasciola* spp.

Mean corpuscular haemoglobin concentration

The model-predicted mean MCHC (pMCHC) at three time points was calculated to illustrate the impact of the cumulative effect of pathogens on the MCHC in calves. The pMCHC of uninfected calves at 21 days of age was 31.86 (31.75-31.97) g/dL. *Theileria parva*- and *A. marginale*-positive calves had a pMCHC 31.85 (31.74-31.96) g/dL and 31.87 (31.76-31.98) g/dL respectively at 21 days of age. In calves positive for both pathogens at 21 days the pMCHC was 31.86 (31.75-31.98) g/dL.

At 150 days of age uninfected calves had a pMCHC 31.92 (31.8-32.04) g/dL; *T. parva*-positive calves 31.84 (31.71-31.66) g/dL, and *A. marginale*-positive calves 31.99 (31.86-32.13) g/dL. Calves positive for both pathogens had a pMCHC of 31.91 (31.77-32.05) g/dL.

At 300 days of age there was still very little difference between uninfected and infected calves. The pMCHC of uninfected calves at this age was 32.79 (32.63-32.94) g/dL. The pMCHC at 300 days of age in *T. parva*-positive calves was 32.62 (32.49-32.76) g/dL, in *A. marginale*-positive calves 32.94 (32.75-33.13) g/dL, and in calves positive for both pathogens 32.77 (32.62-32.93) g/dL.

White cell count

The model-predicted mean WCC (pWCC) at 150 days of age was calculated to illustrate the impact of the cumulative effect and interaction between pathogens on WCC. The results are tabulated in Table 7.9.

The pathogen with the most significant impact on pWCC was *Trypanosoma* spp. This pathogen caused an increase of 1.39 x 10^3/µL WCC in pWCC which was not dependent on age. The highest pWCC was in co-infection between *B. bigemina* and *Trypanosoma* spp. (pWCC = 13.28 x10^3/µL at 350 days). Co-infections between *Trypanosoma* spp. and either strongyle-type nematodes or coccidia resulted in a lower pWCC than infections with only *Trypanosoma* spp. The pWCC in calves infected with both *Trypanosoma* spp. and coccidia was even lower than in uninfected calves. Strongyles caused a decrease in pWCC but only when the EPG>1000. With co-infections with coccidia the difference in pWCC between strongyle-infected and uninfected calves became even less. This decrease was, however, clinically insignificant.

Table 7.9 The GAMM-predicted mean white cell count (x10^3/µL) and 95% confidence intervals at age 150 days for co-infections with pathogen pairs

Covariate	Uninfected	*B. bigemina*	*T. parva*	*Tryapnosoma* spp.	Strongyle-type nematodes (EPG>1000)	Coccidia
Uninfected	10.77 (10.54-11.00)	-		-	-	-
Babesia bigemina		11.03 (10.72-11.34)	11.26 (10.95-11.56)	12.42 (11.46-13.39)	10.1 (9.69-10.51)	11.03 (10.72-11.34)
Theileria parva			11.00 (10.76-11.24)	12.39 (11.45-13.33)	10.07 (9.70-10.43)	11.00 (10.76-11.24)
Trypanosoma spp.				12.16 (11.23-13.1)	11.23 (10.26-12.21)	10.01 (8.52-11.5)
Strongyle-type nematodes (EPG>1000)					9.84 (9.48-10.2)	10.54 (10.15-10.93)
Coccidia						10.77 (10.54-11.00)■

■ pWCC not significantly different than pWCC of uninfected calves

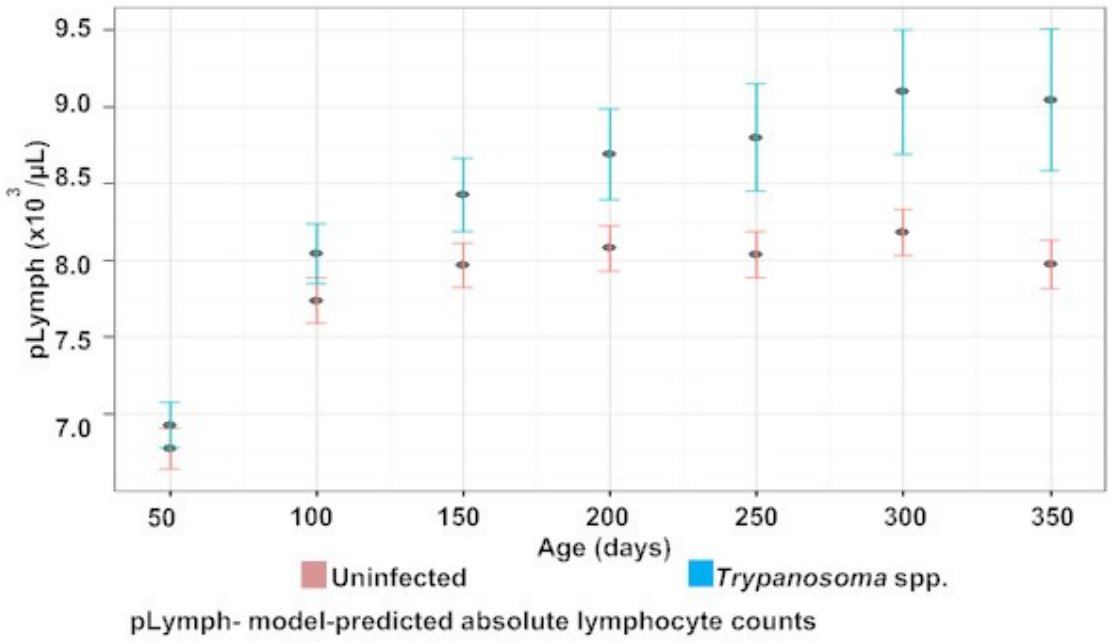
The impact of *B. bigemina* and *T. parva* on pWCC was age-dependent and the predicted mean value increased as the calves grew older. Infection with these two pathogens therefore increased the pWCC in co-infections with other pathogens as well.

Predicted absolute lymphocyte counts

The model-predicted mean absolute lymphocyte counts (pLymph) at 150 days of age associated with co-infection with different pathogen pairs was calculated to illustrate the impact of co-infections on Lymph of calves (Table 7.10).

The highest pLymph (8.89 x10^3/µL) at 150 days was in calves positive for both *A. marginale* and coccidia. The lowest pLymph (7.55 x10^3/µL) at 150 days was in infections with strongyles with a high EPG. The pLymph for *Trypanosoma* spp. (mcr) increased with age (Fig. 7.3). At 350 days of age the pLymph was 9.044 (8.14-9.95 x10^3/µL), which is 1.13 times higher than in uninfected calves.

Figure 7.3 The GAMM-predicted mean absolute lymphocyte counts for *Trypanosoma* spp. (mcr) positive calves at different ages



pLymph- model-predicted absolute lymphocyte counts

Table 7.10 The GAMM-predicted mean absolute lymphocyte counts (x10^3/µL) and 95% confidence intervals at 150 days for co-infections with pathogen pairs

Covariate	Uninfected	*A. marginale*	*B. bigemina*	*T. parva*	*Trypansoma* spp.	Strongyle-type nematodes.	Strongyles (EPG>1000)	Coccidia (OPG>1000)
Uninfected	7.97 (7.68-8.25)	-	-	-	-	-	-	-
Anaplasma marginale		7.97 (7.68-8.25)■	8.15 (7.83-8.47)	7.97 (7.68-8.25)	8.43 (7.96-8.9)	7.76 (7.55-7.97)	7.55 (7.26-7.84)	8.89 (8,17-9.61)
Babesia bigemina			8.15 (7.83-8.47)	8.15 (7.83-8.47)	8.61 (8,12-9.11)	7.95 (7.7-8.2)	7.74 (7.42-8.06)	8.15 (7.83-8.47)
Theileria parva				7.97 (7.68-8.25)■	8.43 (7.96-8.9)	7.76 (7.55-7.97)	7.36 (7.12-7.61)	7.97 (7.68-8.25)■
Trypanosoma spp. (mcr)					8.43 (7.96-8.9)	8.22 (7.79-7.97)	8.014 (7.55-8.48)	8.43 (7.96-8.9)
Strongyle-type nematodes						7.76 (7.55-7.97)	NA	7.76 (7.55-7.97)
Strongyle-type nematodes. (EPG>1000)[1]							7.55 (7.26-7.84)	7.55 (7.26-7.84)
Coccidia (OPG>1000) [2]								7.97 (7.68-8.25)■

■ pLymph not significantly different than pLymph of uninfected calves
[1] EPG: eggs per gram faeces
[2] OPG: oocyts per gram faeces

Predicted platelet counts

The model-predicted mean platelet counts (pPlt) associated with co-infection with different pathogen pairs was calculated to illustrate the impact of co-infections of pathogens on the Plt of calves (Table 7.11).

Table 7.11 The back-transformed GAMM-predicted mean platelet counts (x10^3/µL) and 95% confidence intervals for co-infections with pathogen pairs

Covariate	Uninfected	*A. marginale*	*B. bigemina*	*Theileria* spp.	*T. mutans*	*T. parva*	*Trypanosoma* spp.	*T. vivax*	Strongyle-type nematodes	*Strongyloides* spp.
Uninfected	1657 (1368-2006)	-	-	-	-	-	-	-	-	-
A. marginale		651 (444-957)	527 (346-805)	882 (625-1245)	648 (447-943)	417 (272-641)	**15 (3-67)**	278 (160-484)	502 (356-707)	862 (586-1269)
B. bigemina			1341 (1032-1742)	1076 (827-1400)	1025 (780-1348)	661 (458-954)	**30 (6-134)**	572 (351-934)	1032 (834-1278)	1775 (1376-2291)
Theileria spp.				1329 (1094-1616)	1015 (821-1257)	655 (474-907)	**30 (7-130)**	567 (356-904)	1023 (907-1155)	1760 (1455-2128)
Theileria mutans					1266 (1018-1575)	625 (451-864)	**74 (16-347)**	540 (337-868)	975 (837-1136)	1088 (827-1430)
Theileria parva						817 (589-1133)	**18 (4-81)**	349 (204-595)	990 (848-1157)	1082 (768-1523)
Trypanosoma spp. (mcr)[1]							**37 (8-162**	**16 (4-68)**	**205 (113-375)**	**49 (11-216)**
Trypanosoma vivax								707 (445-1125)	544 (351-845)	936 (591-1482)
Strongyle-type nematodes									1275 (1126-1444)	1688 (1421-2006)
Strongyloides spp.										2193 (1832-2625)

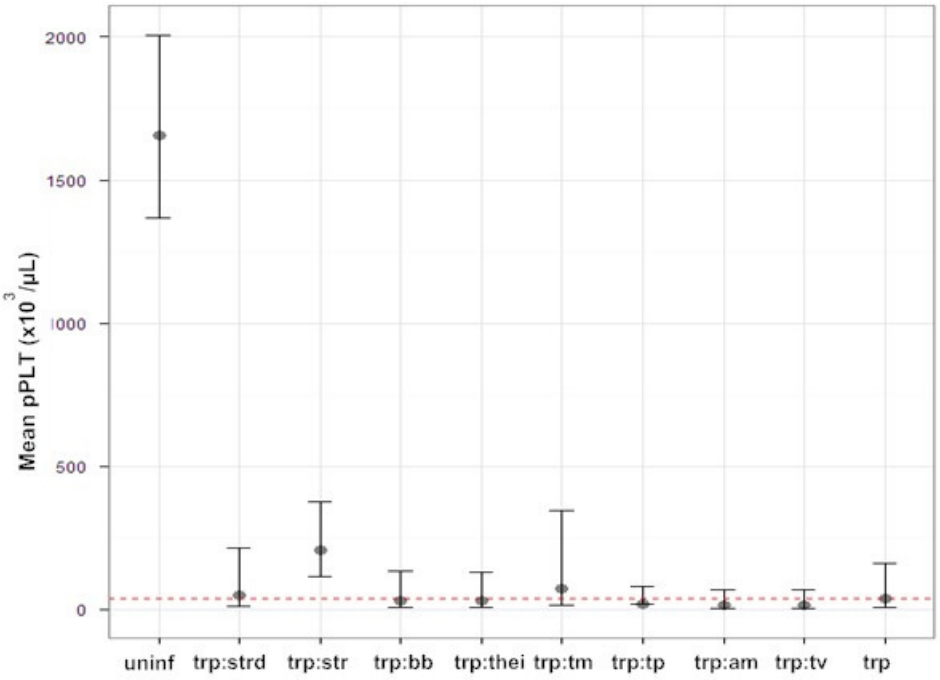
[1] mcr: Microscopy; ■ *Trypanosoma* spp.-associated co-infections

The pathogen that had the most significant impact on pPlt was *Trypanosoma* spp. (mcr). The mean pPlt for *Trypanosoma*-infected calves was less than 3% of the mean in uninfected calves. The mean pPlt in calves that were positive for both *Trypanosoma* spp. (mcr) and *T. vivax* (PCR) was half (16 x10^3/µL) of the pPlt in calves only positive on microscopy. The distribution of pPlt for various co-infections associated with *Trypanosoma* spp. is illustrated in Figure 7.4.

Tick-borne diseases all caused a significant drop in pPlt, although not as severe as the trypanosomes. *Anaplasma marginale* had the lowest pPlt of the tick-borne pathogens, with a mean pPlt of 651x10^3/µL. The lowest pPlt for tick-borne disease occurred in concomitant infections with *A. marginale* and *T. parva*. Co-infections between *T. parva* and the other tick-borne parasites also resulted in decreased pPlt. The distribution of pPlt for the various co-infections with tick-borne parasites is illustrated in Figure 7.5.

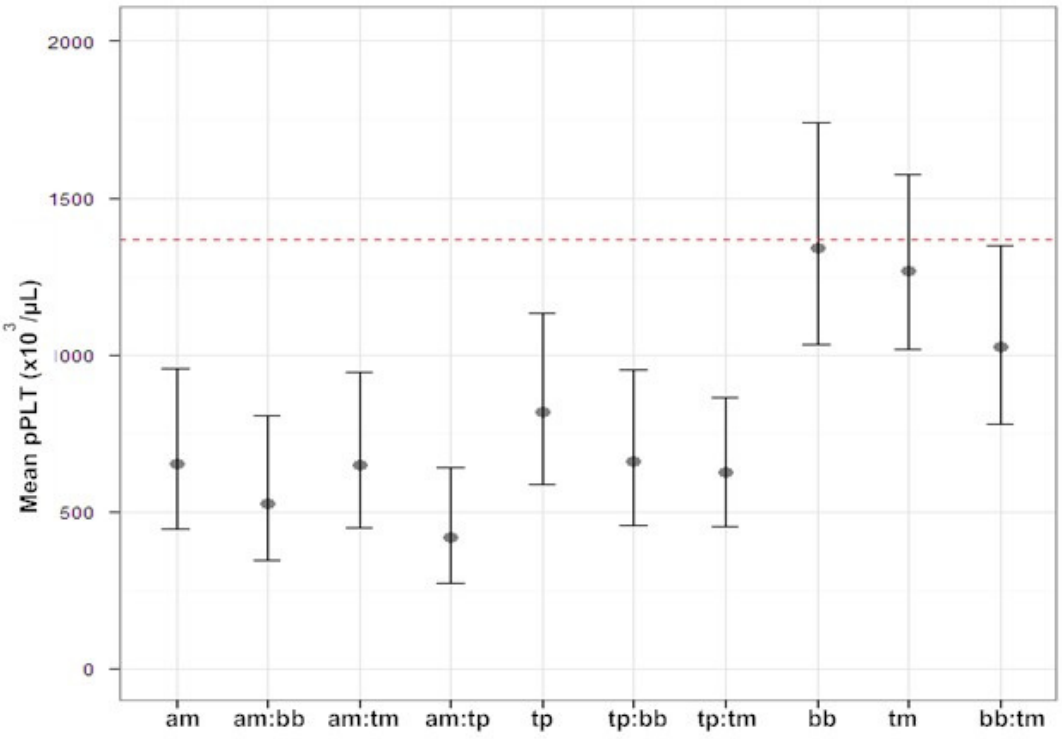
The only pathogen that caused an increase in pPlt was *Strongyloides* spp.

Figure 7.4 The GAMM-predicted mean platelet counts for co-infections associated with *Trypanosoma* spp. infections



pPLT- model-predicted platelet count; a:b denotes co-infection between pathogen a and b; uninf- uninfected; trp- *Trypanosoma* spp.; strd- Strongyloides-type worms; str- Strongyle-type worms; bb- *Babesia bigemina*; thei- *Theileria* spp.; tm- *T. mutans*; tp- *T. parva*; am- *Anaplasma marginale*; tv- *T. vivax*; ---- mean pPLT of *Trypanosoma* spp.-positive calves.

Figure 7.5 The GAMM-predicted mean platelet counts for co-infections associated with tick-borne pathogens



pPLT- model-predicted platelet count; a:b denotes co-infection between pathogen a and b;
am- *Anaplasma marginale;* bb- *Babesia bigemina*; tm- *T. mutans*; tp- *T. parva*;
---- mean pPLT of uninfected calves.

4. DISCUSSION

Packed cell volume

Trypanosoma spp., followed by strongyle-type nematodes, should be considered as the two major pathogenic causes of anaemia in the population. Although the pPCV in all co-infections involving *Trypanosoma* spp. were well below the pPCV of uninfected calves, interactions with other pathogens, apart from strongyle-type nematodes, reduced the final pPCV only marginally compared to single infections with *Trypanosoma* spp. The pathogenicity of *Trypanosoma* spp. partially depends on the intensity of the parasitaemia. The parasitic wave during trypanosome infections coincides with the drop in PCV (Murray & Dexter 1988). Due to its relatively low sensitivity compared to molecular diagnostic tests, microscopy is more likely to detect the peak of such parasitic waves (Uilenberg 1998). This

would explain why *Trypanosoma* spp. (mcr) caused a reduced PCV whereas this was not the case with *T. vivax*, which was diagnosed by PCR.

Interaction between strongyle-type nematodes and other pathogens, apart from coccidia, also resulted in a reduced PCV compared to single infections with strongyles. The pathogenicity of strongyle-type nematodes depended on its infectious load, with a more significant decrease in PCV as the parasitic load (EPG) increased. Co-infection with strongyle-type nematodes at high EPG and *Trypanosoma* spp. caused the most severe decrease in PCV compared to uninfected animals. The cumulative impact of these two pathogens can potentially result in anaemias severe enough to cause mortality.

The pathogenicity of coccidia is dependent on the species involved. Clinical coccidiosis in calves is most commonly caused by *Eimeria zuerni* and *E. bovis* (Kaufmann 1996). Although coccidiosis can result in haemorrhagic diarrhoea, it is not consistently found (Kaufmann 1996). The slight increase in PCV due to coccidia in this study was not clinically significant and was possibly due to a level of dehydration due to diarrhoea.

The total decrease in PCV caused by *T. mutans* was of clinical significance, particularly in young calves. This decrease in PCV became more significant if the calf also had piroplasms on bloodsmears as indicated by a positive *Theileria* spp. status. It is possible for a calf to be positive for antibodies against *T. mutans* in the absence of a parasitaemia. This would imply that the calf has either cleared the infection or it has become a latent carrier. In latent carriers the parasitaemia is often too low to be detected by microscopy (Young *et al*. 1990c). The detection of parasitaemia on microscopy would thus indicate a higher parasitic load which is reflected in the lower PCV. Speciation of *Theileria* spp. was, however, not done on microscopy. One can therefore, not rule out that co-infection between *T. mutans* and other *Theileria* species was the reason for the additional decrease in PCV. By one year of age the decrease in PCV caused by *T. mutans* was not clinically significant. This age-related tolerance is likely related to the development of immunity in the calf.

Mean corpuscular volume

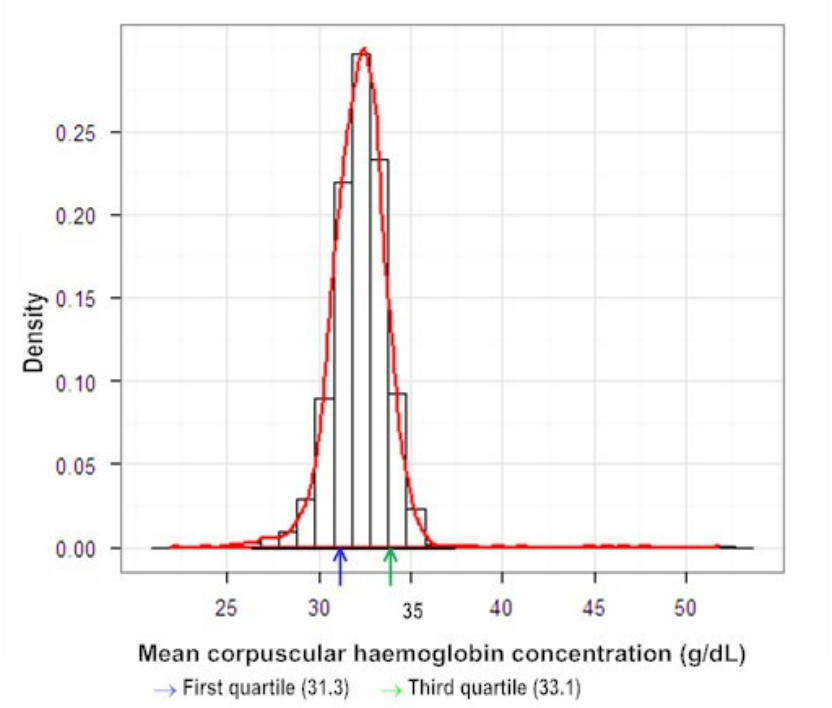
Both strongyle-type nematodes, at EPG>1000, and *T. mutans* infections resulted in an increase in MCV. This is expected in light of the decrease in PCV that is caused by both pathogens. An increase in MCV indicates that the infected calf elicits a regenerative red blood cell response to compensate for the developing anaemia. Interaction between strongyle-type nematodes and *T. mutans* both resulted in an increase in MCV equal to the sum of the response of infection with each pathogen.

Trypanosoma spp. did not have a significant effect on pMCV. The pMCV in concomitant infections between this pathogen and either strongyle-type nematodes or *T. mutans* was not high enough to compensate for the decrease in pPCV that was seen in such infections. This would imply that that the calf will not be able to compensate for the anaemia caused by *Trypanosoma* spp. and in time possibly develop a severe progressive anaemia. Reticulocyte counts are a more accurate measure of red cell regeneration, however, and will need to be done to confirm this statement.

Mean corpuscular haemoglobin concentration

Only two pathogens had a statistically significant impact on MCHC, namely *A. marginale* and *T. parva*. The impact on MCHC was not clinically significant in either of these two pathogens. The range of MCHC of all observations over the age range of 51 weeks is very limited. The frequency distribution of MCHC for all observations is illustrated in Figure 7.6. It is possible that the number of reticulocytes during anaemia caused by the pathogens is too low to significantly decrease the MCHC.

Figure 7.6 The frequency distribution of mean corpuscular haemoglobin concentration of all observations (n=5516)



White cell count and absolute lymphocyte counts

The pathogen with the most significant impact on WCC was *Trypanosoma* spp. Acute trypanosomosis typically causes a leucopenia which coincides with the first parasitic wave. This leucopenia is followed by a leucocytosis (Murray & Dexter 1988). The long interval between observation points probably does not reflect the acute change in WCC in trypanosome-infected calves in this study.

Theileria parva and *B. bigemina* caused an increase in WCC, which was dependent on age of the calf. There was possibly a confounding effect between these two pathogens and age, since the likelihood of seroconversion increased with age. The WCC can be used as an indicator of prognosis in cases of ECF (Irvin 1983). Chronic ECF is usually associated with a leucopenia (Maxie *et al*. 1982; Irvin 1983). Animals that maintain their WCC are more likely to recover from ECF (Irvin 1983).

The pWCC of uninfected calves also increased with age. This is probably due to challenge by pathogens other than what is accounted for in the model.

Strongyle-type nematodes interacted with blood-borne pathogens by decreasing the WCC in co-infections. This interaction was only significant in infections of high intensity (EPG>1000). Helminth infections are typically associated with polarization of the host's immune reaction towards the Th2 (T-helper lymphocytes) –type response as indicated by increased peripheral eosinophil levels and mast cells, as well as parasite-specific IgE levels (Maizels & Yazdanbakhsh 2003). Cytokines associated with Th2-cell response down-regulate the Th1-cell response which is associated with a potentially pathogenic cell-mediated inflammatory response, thus ensuring the host's own survival and ultimately the parasites' survival. Bacteria, viruses and certain protozoa are also associated with Th-1 responses (Jankovic *et al*. 2001; Fenton, Lamb & Graham 2008). This suppression of Th-1 cell response by the Th2-response is dependent on high parasite intensities (Maizels & Yazdankakhsh 2003) with a stimulatory effect seen in low infection burdens (Kamal & El Sayed Khalifa 2006). This modulation of the immunity can possibly explain the reduced WCC responses against co-infections that are associated with strongyle-type nematodes. To confirm this one will have to investigate further including cytokine and antibody levels (Bradley & Jackson 2008).

Platelet count

Trypanosoma spp. caused the most significant decrease in platelet counts. The pPlt of *Trypanosoma* spp. was 40 times lower than in uninfected calves. Co-infections with other pathogens decreased the pPlt even more, although not considerably. Platelet counts as low

as those seen in infections associated with *Trypanosoma* spp. cause coagulopathies, and clinically present as generalized petechiation and ecchymosis. This was confirmed by post mortem examinations of calves that died during the course of this study (data not shown here).

All four tick-borne infections caused a reduction in Plt, particularly *A. marginale* and *T. parva*. Co-infections between all pairs of tick-borne diseases resulted in a cumulative decrease of platelets. The pathogenesis of the reduced Plt in all four infections is multifactorial, and includes a combination of factors form reduced platelet production, increased consumption to immune-mediated platelet destruction (Pantanowitz 2003). Splenomegaly is a common symptom of many tick-borne diseases, and also contributes to low Plt due to sequestration of platelets and destruction by macorphages in the spleen (Pantanowitz 2003). There is thus a cumulative reduction in Plt during co-infections with tick-borne diseases.

5. CONCLUSIONS

Traditionally studies on infectious diseases have focused on single pathogens, often based on experimental conditions. Animals in the field are exposed to a variety of pathogens that occur in the animal's environment (Petney & Andrews 1998), particularly in a tropical environment such as western Kenya. It is therefore impossible under field conditions to study a disease in isolation without reference to other causes of disease (Moll *et al.* 1984).

It is evident that co-infections between pathogens complicate the clinical picture of disease (Cox 2001). The animal might present with clinical signs that can not be explained by the disease that was diagnosed. For example, classical ECF is not associated with anaemia, but concomitant infections with strongyle worms or trypanosomes can result in clinical anaemia. If diagnosis are based solely on clinical signs, co-infections could be overlooked if two or more pathogens cause the same presenting signs in a host.Co-infections could lead to a missed diagnosis when, in the case of concomitant infections with pathogens that present with similar clinical signs, and the diagnosis was solely based on clinical presentation. Such a patient might not respond to treatment as expected and this should prompt the investigator to consider further diagnostic procedures.

Co-infection between pathogens also affects the prognosis of a disease state (Cox 2001). Each concomitant pathogen contributes to the clinical outcome of infection (Petney & Andrews 1998). Even if the contribution of the pathogen in itself was clinically insignificant, the cumulative effect of the various co-infecting pathogens could potentially shift the host

from a state of apparent health into a state of clinical disease. *Theileria mutans* caused a statistically significant, yet clinically insignificant decrease in PCV. Such a small decrease in PCV would not present as overt clinical symptoms, e.g. pallor of mucous membranes, but such subclinical disease processes would erode the overall health status of the calf.

Immunosupression caused by certain pathogens, such as *Trypanosoma* spp. or *T. parva* (Holmes *et al* 1974; Askonas 1984; Moll *et al*. 1986), or immune-modulation, as seen in helminth infections at times (Maizels & Yazdankakhsh 2003), could undermine the host's response to other pathogens which in turn increase the host's susceptibility to infection or impede its ability to resolve such an infection. Premune latent carrier animals have been reported to develop clinical anaplasmosis after superinfection due to *Trypanosoma vivax* and *T. congolense* (Magona & Mayende 2002).

Not all pathogens caused clinical disease in the calf population. Clinical anaplasmosis and babesiosis in cattle are classically associated with anaemia (De Vos *et al*. 2004; Potgieter & Stoltsz 2004). However, neither *A. marginale* nor *B. bigemina* appear to be significant causes of anaemia in the study population. This implies that the majority of calves infected with either of these pathogens are probably latent carriers. There is evidence that both pathogens cause subclinical disease processes, however, particularly in association with multi-pathogen infections. The significant thrombocytopenia that was found in *A. marginale*-positive calves may be an example of such a process.

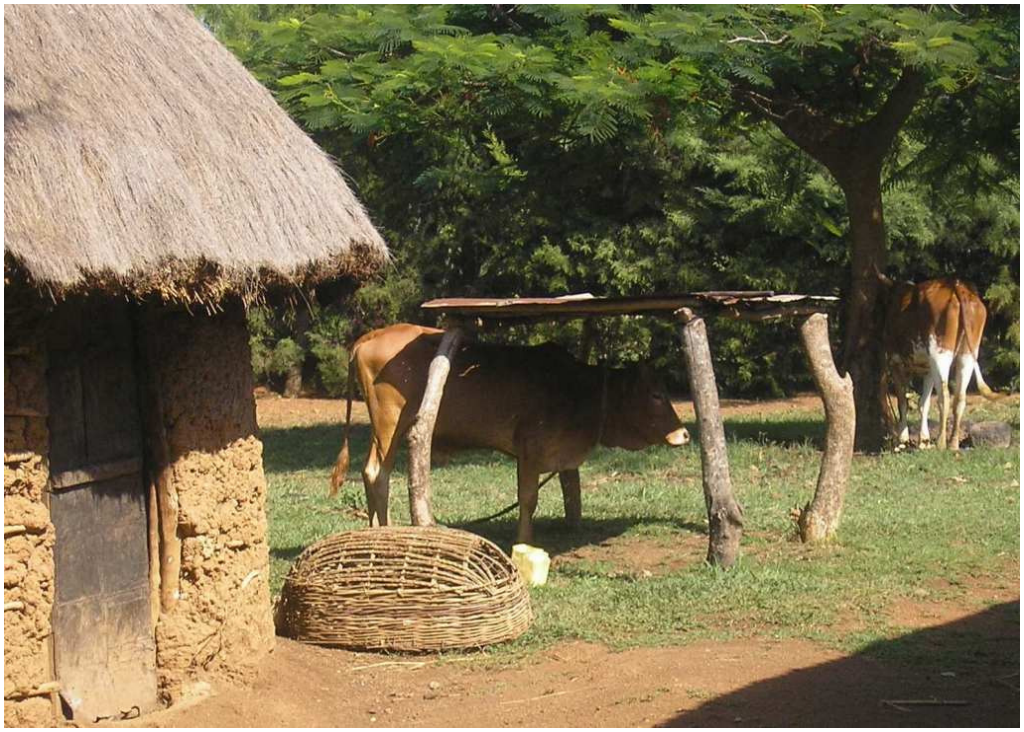
The impact many pathogens and concomitant infections had on the haematological parameters was dependent, at least partially, on the infectious load of the pathogen, e.g. strongyle-type nematodes and trypanosomes. This is known to be true for *T. parva* as well (Koch *et al*. 1990). The diagnosis of tick-borne diseases in this study was based on serology, which is a measure of exposure to the pathogen. The antibody response does not necessarily correlate to the infectious load of the pathogen. The pathogen load and possibly parasite strain of both of these parasites are likely to affect the clinical presentation of infection. Using quantitative antigen-based diagnostic tests for tick-borne parasites, in particular *T. parva* and *T. mutans*, would therefore have added some dimension to this study. Both pathogens had a high prevalence in the population and ECF is considered as a major disease in the area.

Interactions between pathogens are not limited to pairs of pathogens, however, and to get a complete understanding of disease processes one would have to consider the whole pathogenic community of the host. Many other infectious agents, such as bacteria and

viruses, circulating in the calf population were tested for (results beyond the scope of this study) and probably many more present for that were not tested for, that could have impacted on the haematological parameters of the calves. In addition there are several pathogen-related factors that dictate the pathogenicity of the infection, including infectious load, pathogen strain, and virulence types within strains. Several inherent characteristics of the host, such as breed and age, determine the susceptibility of the animal to infection, whereas other acquired attributes such as maternal antibody, premunity due to prior infection, or nutritional state could alter the animals' ability to respond to infection.

It is evident that interactions between concomitant pathogens complicates the clinical picture in infected calves and should be taken into consideration in any study that investigates disease under the field conditions.

Small-holding homestead with East African short-horn cattle



CHAPTER 8

GENERAL CONCLUSIONS

Livestock production in Western Kenya is constrained by harsh environmental conditions as well as the impact of infectious disease (Uilenberg 1995). Livestock-dependent and mixed crop-livestock small-holder farmers prefer to keep indigenous livestock breeds (Minjauw & McLeod 2003), due to their adaptability to the environmental constraints and disease tolerance. It is evident from this study that calves are exposed to significantly high infectious burdens from early calfhood. The economically most important diseases in the area include the tick-borne diseases, e.g. ECF, anaplasmosis, and babesiosis, trypanosomosis and helminthosis.

Anaemia was shown to be an important syndrome in cattle in Western Kenya. The FAMACHA© scoring test is a field test that is used to diagnose anaemia in animals (Anon. 2002b). It meets the criteria of being cheap, simple and easy to use, and with proper training, farmers should be able to perform the test themselves. Although the test is not necessarily useful in the diagnosis of specific infectious disease, it does serve as a useful early screening tool to identify animals that require intervention. There is a real need for robust pen-side diagnostic tests that are affordable and suitable for use under the challenging field conditions in rural Africa. In human medicine several point of care rapid tests have been developed to diagnose anaemia, mainly based on haemoglobin levels in patients, such as Hemocue (Hemocue AB®, Sweden) and WHO haemoglobin colour scale (Stott & Lewis 1995). The costs and availability of such tests constrain the uptake of this technology in veterinary medicine (Magona, Walubengo, Anderson, Olaho-Mukani, Jonsson & Eisler 2004), yet these tests can potentially be very valuable as pen-side tests in animal health in settings where access to diagnostic laboratories is limited.

After pen-side diagnostics, the next level in an animal health care system is local field laboratories. The Sysmex© automated analyser was also validated for use in the field laboratory in Busia, Western Kenya, and was proved to be useful in monitoring the clinical profile of calves throughout the study. Automated analyzers are easy to use and results are available within minutes, although some technical training is required for interpretation of the results and maintainance of the analyzer. Such technology is ideal for veterinarians working in remote areas, where maintenance of a cold chain for sample storage and transport to regional laboratories, mostly located in larger towns, is not practical.

Any diagnostic test or analyser first needs to be validated for the specific population on which it will be used. For this purpose, baseline values need to be available in order to accurately interpret the diagnostic test. In this study it was shown that the East African short-horn calf has a unique haematological profile. Despite the amount of research done on the infectious diseases of these indigenous cattle, for example ECF and trypanosomosis, data on the baseline physiological parameters, such as haematological reference values, on indigenous breeds in the literature is scant. Many studies used extrapolated data, from other breeds, particularly more exotic breeds, e.g. PCV cut-offs as a measure of anaemia (Dargie *et al*. 1979; Fanduma *et al*. 2007) and this practice may prove to be inappropriate. The age-related changes in red cell parameters of the Zebu calves in this study, particularly during the first 12 weeks of life, do not correlate with what is described in literature for exotic breeds. There is a need to determine breed-specific baseline reference levels for indigenous livestock breeds.

Helminths, particularly strongyle-type nematodes, and trypanosomes were the two most important infectious causes of anaemia in the population. Although almost all calves became infected with strongyles during the first year of life, clinical anaemia only developed in cases where the helminth burden was quite high.

These calves also had a high rate of seroconversion to tick-borne pathogens. Despite the high prevalence of tick-borne pathogens, relatively few infections, apart from ECF, actually resulted in clinical disease in the study population. East Coast fever was found to be a significant disease in the study area, but was not an important cause of anaemia. Neither anaplasmosis nor babesiosis, both diseases typically associated with the development of anaemia, resulted in a significant decrease in PCV. This might be due to the calves' innate age-related resistance to these two diseases or possibly breed-related tolerance to the diseases (De Vos *et al*. 2004; Potgieter & Stoltsz 2004). Indigenous cattle, such as the East African short-horn Zebu, that are raised in areas where tick-borne disease is endemic, over many generations develop a level of tolerance to endemic pathogens (Norval *et al.*1992; Perry & Young 1995; De Vos *et al.* 2004; Potgieter & Stoltsz 2004).

Although many infections never developed into overt clinical disease, there was evidence of ongoing subclinical disease processes in many infected calves. Even in infections with pathogens considered to be benign, such as *T. mutans*, calves developed mild thrombocytopenias. Over the long term, these subclinical disease processes will have an erosive effect on the overall health status of the population, as is illustrated by the progressive decline in PCV as the calf population ages.

Co-infections between pathogens were also shown the have a significant impact on the haematological profile of infected calves. In many cases the cumulative effect of or interactions between concomitant pathogens affected the severity of clinical signs, such as anaemia, and in turn affected the prognosis of such calves. Considering the high prevalence of concomitant infections, in particular pathogens that cause anaemia, any individual, be that a researcher, a clinician or a farmer, who is investigating a disease condition in livestock under field conditions, should not lose sight of the fact that the clinical presentation of the animal is likely to be complicated by several super-infecting pathogens. Diagnostic approaches should be thorough and treatment and vaccination regimens should be thoughtfully planned.

The majority of the economically important pathogens that burden cattle in Western Kenya are either preventable or treatable. Therefore, the production losses of livestock incurred through these pathogens should, at least in theory, be manageable. One constraint in disease control in the developing world is the development of drug resistance against many anthelminthic (Gray *et al*. 2012) and trypanocides (Connor & Van Den Bossche 2004), two of the most important causes of anaemia in the population in this study. Strategic treatment has been proposed in commercial farming systems to curb the development of drug resistance. In this system only animals with high parasite loads or clinical signs are treated and by doing so the high costs of pharmacological treatments are reduced.

Another option to lessen the burden of livestock diseases is farm with breeds that are more resistant to infectious agents. Herein lays the value of indigenous breeds. The East African short-horn Zebu breed is known to be adapted to the infectious burdens it faces under field conditions (Perry & Young 1995). They survive under conditions where other breeds, such as improved European cattle breeds, do not.

The last recruited IDEAL calf