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The response of Boer goats to natural and deliberate gastrointestinal nematode infection

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Abstract

Gastrointestinal nematode infection is one of the major diseases affecting sheep and goats but most of the studies have focused on sheep. As a highly productive meat breed of goats, it is important to study the response of Boer breed to gastrointestinal nematode infection. The aim of this study was to investigate the interaction between Boer goats and their gastrointestinal nematodes by evaluating the phenotypic (faecal egg counts (FEC), IgA, packed cell volume (PCV), peripheral eosinophil counts and bodyweight) and parasitological (nematode number, length and index) variables following natural and deliberate co-infections that were dominated by *Teladorsagia circumcincta* and *Haemonchus contortus*, respectively.

This study also aimed to estimate the repeatability, which provides an upper limit on heritability, and heritability of phenotypic variables among Boer goats, in particular the FEC as this is an important marker of resistance to gastrointestinal nematode infection. The study on natural infection was conducted in a farm in England with a semi-intensive grazing system whereas a deliberate infection study was conducted in a farm in Malaysia with an intensive management system.

The FEC was confirmed to follow a zero-inflated Poisson distribution after comparing the observed and predicted zeroes in a Poisson regression. Mixed model repeated measures analysis that was conducted in a Bayesian framework was then used to analyse the phenotypic data. The findings from the studies of the phenotypic variables showed that the repeatability was moderate for FEC and PCV but relatively high for IgA activity and peripheral eosinophil counts and decreased significantly as the interval between sampling increased. The repeated measures models showed that FEC variation among Boer goats was affected by eosinophilia through time with the presence of an interaction with IgA responses, but that FEC was not heritable among Boer goats.

The bodyweight of Boer goats was shown to be highly heritable despite them being infected with gastrointestinal nematodes. Additionally, the unaffected PCV levels and the increase in bodyweight over the course of infection suggest that Boer goats may be relatively resilient to gastrointestinal nematode infection.

Multiple linear regression analyses of the mean phenotypic variables and the parasitological variables measured at necropsy suggested that *H. contortus* and *Trichostrongylus colubriformis* affected each other during co-infection. Shorter *T. colubriformis* was associated with greater *H. contortus* index and number whereas longer

T. colubriformis was associated with a reduction in *H. contortus* number. Additionally, increased in IgA activity was associated with increased *H. contortus* length but a reduction in *T. colubriformis* index during co-infection.

Together the findings show that eosinophils and IgA do play a role in the response of goats to gastrointestinal nematode infection, but the effects are less pronounced than in sheep. Moreover, the finding of a very low heritability of FEC is in contrast to findings in sheep. Most of the findings also suggest that the Boer breed is relatively resilient to infection but further work is needed to confirm whether these finding might be due to low infection doses.

In conclusion, this study has expanded knowledge of the host-parasite relationship in goats as well as demonstrating interspecific nematode interactions between *H. contortus* and *T. colubriformis,* which commonly co-infect goats under natural conditions. However, future studies that overcome the limitations in the present study are needed to confirm the resistance status of Boer goats against these nematode species.

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Abbreviations

±	Plus or minus
^{o}C	Degree Celcius
µg	Microgram
µl	Microliter
µm	Micrometre
BWT	Bodyweight
CI	Credible interval
COWP	Copper oxide wire particle
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EO	Peripheral eosinophil counts
epg	Eggs per gram
FEC	Faecal egg counts
F_H_N	Female *H. contortus* number
F_H_L	Female *H. contortus* length
F_T_N	Female *T. colubriformis* number
F_T_L	Female *T. colubriformis* length
g	Gram
HC_L	*H. contortus* length
IgA	Immunoglobulin A
L1	First stage larvae
L2	Second stage larvae
L3	Infective stage larvae
L4	Fourth stage larvae
L5	Fifth stage larvae
Log_HC_M	Log *H. contortus* index
Log_HC_N	Log *H. contortus* number
Log_TC_M	Log T. colubriformis index
Log_TC_N	Log T. colubriformis number
kg	Kilogram
M	Molar
ml	Millilitre
OD	Optical density
p-value	Significance probability
PBS	Phosphate-buffered saline
PBS-T	PBS pH 7.4 containing 0.05 % of Tween 20
PCV	Packed cell volume
R^2	R-squared value
rcf	Relative centrifugal force
SCOPS	Sustainable control of parasites in sheep
SEM	Standard error of mean
SD	Standard deviation
TB	Types of birth
TC_L	*T. colubriformis length*
TMB	3,3',5,5'-Tetramethylbenzidine

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Author's Declaration

I declare that the thesis is my original work except for citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at the University of Glasgow or any other institution.

Basripuzi Nurul Hayyan binti Hassan Basri, June 2019.

List of Publications and Presentations

Some of the contents of this thesis have been published and presented as follows:

1. Basripuzi, N. H., Salisi, M. S., Isa, N. M. M., Busin, V., Cairns, C., Jenvey, C., and Stear, M. J. (2018). Boer goats appear to lack a functional IgA and eosinophil response against natural nematode infection. Veterinary Parasitology, 264, 18-25.

2. Typical right skewed distributions of faecal egg counts among Boer goats following natural and deliberate infection of gastrointestinal nematodes. 29th Veterinary Association Malaysia Congress. One Health: Bridging Veterinary and Allied Sciences Towards Nation's Well Being, 6th – 8th October 2017, Shah Alam, Selangor, Malaysia, pp. 18.

3. Comparison of faecal egg counts distribution in Boer goats between natural infection in England and deliberate infection in Malaysia. 26th International Conference of the World Association for the Advancement of Veterinary Parasitology. Combating Zoonoses: Strength in East-West Partnership., 4th – 8th September 2017, Kuala Lumpur, Malaysia, pp. 258.

I dedicate this thesis to my supervisor, Professor Louise Matthews
and all novice users of MCMCglmm R package

Chapter 1: General Introduction

1.1 Preface

My curiosity about goats and their parasites began more than 10 years ago when I did my industrial training in a Boer breeder farm. As a diploma student, I did not learn much about medication, but I wondered why the farmer was so concerned about treating parasite infection in his farm. All animals were given anthelmintics, including the healthy ones, and I assumed this was a typical herd health programme. A few years later when I visited many goat and sheep farms during the Ruminant Rotation in my clinical years in the Vet School, I realized that almost all farms commonly used more than one class of anthelmintic. There was a possibility that the goats that were drenched with oral anthelmintic, for example a benzimidazole class to treat gastrointestinal nematode infection, were also injected with ivermectin, a macrocylic lactone class to treat ectoparasite infection. As a broad spectrum anthelmintic, ivermectin can be used to treat both kinds of infection, thus the use of benzimidazole was unnecessary. The indiscriminate use of anthelmintics can promote the development of anthelmintic resistance among small ruminants and this was the topic of my undergraduate final year project.

I pursued my studies through an MVSc and extended my interests to the exploration of ethnoveterinary medicines as an alternative to chemical anthelmintics and examined the possibility of gastrointestinal nematode infection occurring through the use of manure on grasses grown as animal feed. Through these studies I realized that sheep have been the focus of most research on resistance and immunity to gastrointestinal nematode infection, and that goats had been largely overlooked. This triggered my interest in exploring host-parasite interactions in goats using the same methods that were used to study gastrointestinal nematode resistance among sheep.

Goats are important for global food security due to their contribution to the supply of meat and milk. Based on the Food and Agriculture Organization of the United Nations (FAO, 2013) database in the years 2000 and 2013, the world's goat population has been increasing rapidly, by up to 33.8% between 2000 and 2013, in comparison with the sheep population that increased by only 10.7% in the same period. During that period, Asia held first place with 59.4% of the world's goat population (Skapetas and Bampidis; 2016). Consequently, it is increasingly important to assess the resistance of goats to gastrointestinal nematode infection and establish criteria for inclusion in selective breeding programs.

1.2 General overview

Gastrointestinal nematode infection is a common worldwide threat to small ruminants that affects their production, health and welfare (van Dijk et al., 2008). In Great Britain, gastrointestinal nematode infection has become the most costly disease for sheep, with an estimated annual loss of £84 million (Nieuwhof and Bishop, 2005). McLeod (2004) reported that gastrointestinal nematode infection in Indonesia cost 13 million USD in 1999 and nematode infection caused the largest production loss in the small ruminant industry in Southeast Asia and India. Similarly, annual production loss and costs to control of gastrointestinal nematodes in the sheep industry in Australia is estimated at AUD 436 million (Lane et al., 2015).

Gastrointestinal nematode infection is mainly controlled by chemical anthelmintics but effective control is restricted by the development of anthelmintic resistance (Waller, 2002; Sargison et al., 2007). Therefore, there is an urgent need to reduce reliance on chemical anthelmintics and to explore effective and sustainable non-chemical control approaches. These include rotational and integrated grazing system (Mahieu 2013), dietary supplementation (Hoste et al., 2016), consumption of tanniferous plants with anthelmintic properties (Villalba et al., 2013), biological control by nematophagous fungi (Vilela et al., 2018) and the use of copper oxide wire particles (Maina et al., 2017). Additionally, genetic variation for resistance against gastrointestinal nematodes exists both within and between breeds of sheep and goats (Stear et al., 2009). Thus, genetic variation can be exploited in selective breeding programs to improve resistance against infection (Bishop 2012; Zvinorova at al., 2016, Aguerre et al., 2018) as discussed in section 1.5.5.2.

Gastrointestinal nematodes elicit similar concerns in terms of pathology and economic importance in goats and sheep because both species are infected with the same major nematode species (Table 1.1).

Table 1-1. Main nematode species found in the digestive tract of sheep and goats (adapted from Hoste et al., 2010).

Digestive tract	Sheep	Goat
Abomasum	*Haemonchus contortus* *Teladorsagia circumcincta* *Trichostrongylus axei*	*H. contortus* *T. circumcincta* *T. axei*
Small intestine	*Trichostrongylus vitrinus* *Trichostrongylus colubriformis* *Cooperia curticei* *Nematodirus battus* *Nematodirus fillicolis* *Nematodirus spathiger*	*Trichostrongylus capricola* *T. colubriformis* *N. battus* *N fillicolis* *N. spathiger*
Large intestine	*Oesophagotomum venulosum* *Oesophagotomum columbianum* *Chabertia ovina*	*O. columbianum* *O. venulosum* *C. ovina*

However, the majority of research on host-parasite interactions, control approaches and evidence for resistance to gastrointestinal nematodes has focused on sheep (Baker and Gray, 2004; Bishop and Morris, 2007). The bias towards sheep may be due to the relatively larger population of sheep than goats in developed countries where research funds are more widely available (Hoste et al., 2010). Consequently, despite goats and sheep being infected with the same gastrointestinal nematode species, there are fewer studies in goat breeds in comparison to sheep (Hoste and Chartier, 1993; Baker and Gray, 2004) (Table 1-2).

An effective immune response is important for the development of resistance against gastrointestinal nematode infection in small ruminants. Previous studies have shown that sheep and goats respond differently to gastrointestinal nematode infection. Strong parasite-specific IgA responses are associated with decreased faecal egg counts (FEC) in sheep breeds (Strain et al., 2002, Amarante et al., 2005) but high parasite-specific IgA responses were associated with high FEC in goats that were selectively bred for improved nematode resistance (de la Chevrotière et al., 2012; McBean et al., 2016). This difference highlights the need for further exploration of the host-parasite interaction in goats in order to understand their immune response against gastrointestinal nematode infection and determine their resistant status.

Resistance to gastrointestinal nematodes and genetic variation among small ruminants has been extensively explored (Stear et al., 1996; Stear et al., 2009; Bishop 2012; Zvinorova et al., 2016) and in sheep many studies have shown it to be under genetic control (Stear et al. 1997). Regardless of species, research into gastrointestinal parasitism in domestic livestock that has the advantage of frequent availability of pedigree data and ability to standardize or at least control for many non-genetic sources of variation in the parasitism rates (Stear and Murray, 1994) has provided many insights.

In cool temperate regions such as the United Kingdom, *Teladorsagia circumcincta* is considered the most prevalent nematode and can kill the host in extreme cases (Stear et al., 2009, de Cisneros et al. 2014). In warm tropical or sub-tropical regions like Malaysia, annual mortalities exceeding 20% of the flock can be expected in infections associated with *Haemonchus contortus* (Waller, 2004). Infection by both of these parasites is associated with hyperplasia in the abomasal mucosal epithelium. Haemonchosis causes generalised lesions in the superficial layers whereas teladorsagiosis causes formation of nodules affecting the full mucosal thickness that impairs digestive function (Scott et al., 1999). Clinical signs of infection depend on the presence of gastrointestinal nematode and the intensity of infection. In sheep, clinical signs range from subclinical weight loss to severe pathologies such as diarrhoea, anaemia and protein loss.

Table 1-2. Small ruminant breeds with reported resistance traits against gastrointestinal parasites (adapted from Zvinorova et al., 2016).

Species	Breed		Type of infection	Parasite	References
	Resistant	Susceptible			
Goat	Sabi	Dorper	N	Hc	Matika *et al*. (2003)
	Small East African	Galla	N	Hc, S, O spp.	Baker *et al*. (1994; 1998)
	Jamunapari	Barbari	N	Hc	Rout *et al*. (2011)
	Creole	-	N	Hc, Tc	Mandonnet et al. (2001)
	Creole	-	A	Hc	Bambou et al. (2009)
	Creole	-	N	Hc	de la Chevrotiere et al. (2012)
	West African	-	N	Mixed	Behnke et al. (2011)
Sheep	Gulf Coast Native	-	N	Hc	Peña *et al*. (2004)
	F1 and F2 Suffolk X Gulf Coast Native	-	N	Hc	Y. Li et al. (2001); Miller et al. (2006)
	INRA 401	-	A	Hc, Tc	Gruner et al. (2004)
	Merino	-	A	Hc, Tc	Andronicos et al. (2010)
	Gulf Coast Native	Suffolk	N	Hc, Tc	Miller et al. (1998); Shakya et al. (2009)
	Red Masai	Blackheaded Somali, Dorper, Romney Marsh	A/N	Hc	Mugambi et al. (1997)
	Barbados Black Belly	INRA 401	A	T	Gruner et al. (2003)
	Santa Ines	Ile de France, Suffolk		Hc, Oc	Amarante et al. (2004)
	Texel	Suffolk	N	Td, Ne	Sayers et al. (2005); Good et al. (2006)
	Florida native, Florida native X Rambouillet	Rambouillet	N	Hc	Amarante et al. (1999)
	Dorper X Katahdin	Hampshire	A/N	Mixed	Burke and Miller (2002)
	Lohi	Thalli, Kachhi	A/N	Hc	Saddiqi et al. (2010a)
	Caribbean Hair, Katahdin	Crossbred-Dorper	A	Hc	Vanimisetti et al. (2004)

N = natural infection; A = artificial challenges, Hc = *H. contortus*, Tc = *T. colubriformis*; O = *Oesophagostomum* spp; S= *Strongyloides* spp.

The most common criterion to assess resistance against gastrointestinal nematode infection is the FEC which provides an indication of nematode burden. In the case of haematophagous gastrointestinal nematode infection such as *H. contortus,* packed cell volume (PCV) is usually measured because a low proportion of red blood cells in the circulating blood is an indicator of anaemia.

The alternatives or additional parameters include (a) measurement of resistance by total nematode counts, nematode length and fecundity; (b) measurement of immune response

by eosinophilia and immunoglobulins; (c) measurement of infection consequences such as anaemia (by PCV and/or FAffa MAlan CHArt (FAMACHA) score), gastrin, pepsinogen or fructosamine concentrations; and (d) measurement of resilience by bodyweight or growth rate and requirement of treatment frequency (Bishop, 2012).

To date, however, most of these measures have been used to investigate gastrointestinal infection and resistance in sheep, and goats have been much less thoroughly studied. This is true of the Boer breed which is a common breed that is reared for meat, but the resistance status of this breed against gastrointestinal nematodes is largely unknown. According to Baker and Gray (2004), a study in Philippines provided some preliminary evidence that Boer goats may be somewhat resistant to gastrointestinal nematode. For these reasons, this thesis will focus on the response to infection and estimation of resistance to the major gastrointestinal nematode infecting small ruminants namely *T. circumcincta* and *H. contortus* in Boer goats, as well as the impacts of co-infection with *H. contortus* and *T. colubriformis*.

1.3 Gastrointestinal nematode life cycle

1.3.1 Free living stages

In general, *H. contortus, T. circumcincta, T. colubriformis* and *Oesophagostomum* sp. have similar life cycles (see Figure 1-1 for the life cycle of *H. contortus*). The eggs are shed in the faeces of infected animals and hatch into pre-infective first-stage larvae (L1) that moult into second-stage larvae (L2) on pasture. Both L1 and L2 are free-living and feed on microorganisms in the faeces. The L2 moult into infective stage larvae known as L3 which are surrounded by a protective sheath (Goater et al., 2014). Development from L1 to L3 can be as rapid as 6 days (Sam-Mohan, 1995; Basripuzi et al., 2013) under optimal temperatures but is slower during cold weather.

The L3 stage is a non-feeding free-living stage that requires stored energy reserves particularly lipids in order to survive (Medica and Sukhdeo, 1997). The L3 survive longer than L1 and L2, which may be due to their resistance to desiccation (Amaradasa et al., 2010), having a protective sheath and the ability to migrate to more favourable microenvironments (O'Connor et al., 2006). In addition, under unfavourable conditions such as heavy rainfall, the L3 may remain dormant in the faeces for weeks. Large numbers of L3 will emerge when the conditions become favourable again. Once ingested by the host, the L3 lose their protective sheath during the passage through the gastrointestinal tract and have a histotropic phase prior to their transition to the fourth stage larvae (L4) and pre-adult stages (L5). If conditions in the host are unfavourable in the host, the L4 of

trichostrongylids (*H. contortus, T. circumcincta, T. colubriformis*) will undergo a period of arrested development or hypobiosis and resume their activity and development when conditions become favourable again. The L5 will finally moult into the adult stage, mate and produce eggs (Roeber et al., 2013a).

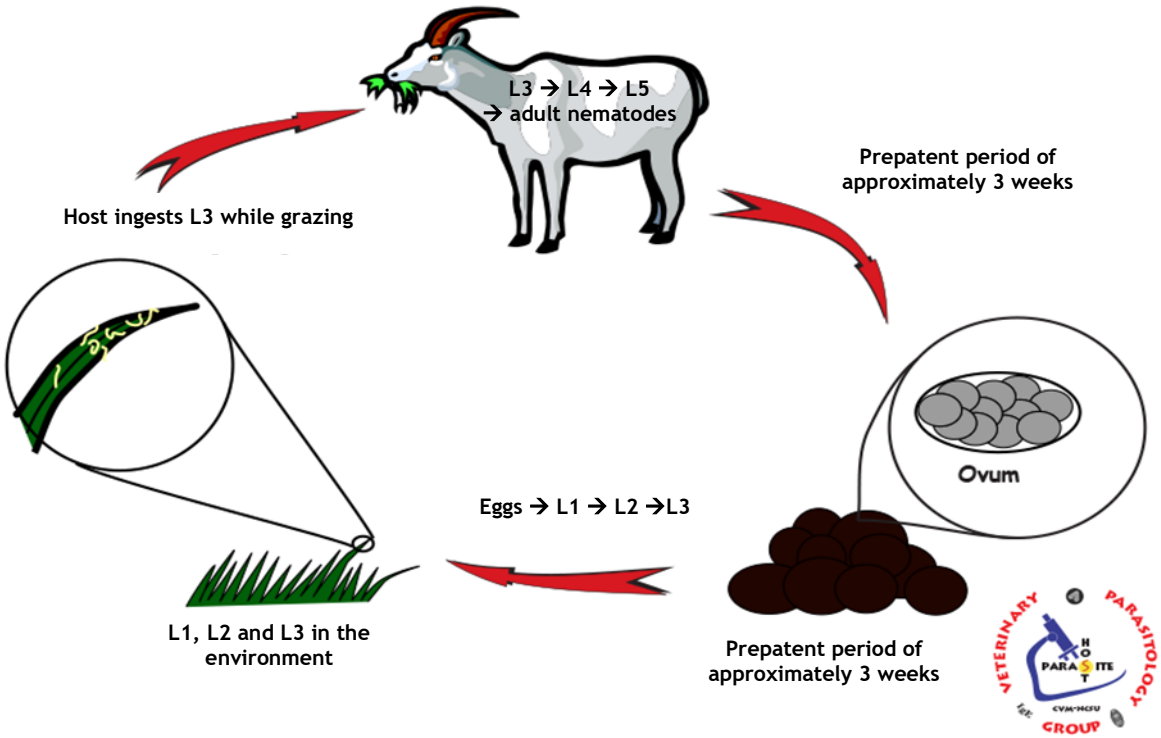


Figure 1-1. Life cycle of *Haemonchus contortus* in goats (Adapted from Veterinary Parasitology Group of North Carolina State University website; https://parasitology.cvm.ncsu.edu/life_cycles/nematodes/haemonchus.html, 16/3/2019).

The key features that differentiate the free-living stages of *H. contortus, T. circumcincta* and *T. colubriformis* are the response to environmental influences (O'Connor et al.,2006). Cool, moist weather and sub-freezing winters such as in the UK are optimal survival conditions for *T. circumcincta* L3 whereas the L3 of *H. contortus* require warm and moist weather such as that in Malaysia. *T. colubriformis* however can survive in both cool and warm, moist weather.

1.3.2 *Haemonchus contortus*

H. contortus is also known as 'barber's pole worm' due to the blood-filled gut and the white egg-filled uterus in the female wrapping around each other to resemble a barber's pole (Taylor et al, 2007). It is also easily identified due to its large size which can be up to 30 mm in length (Bowman, 2003).

Once L3 are ingested, they penetrate the abomasal glands and moult into L4. The presence of L3 and L4 lead to abomasal gland hyperplasia, inflammatory cell infiltration, and the substitution of parietal cells secreting hydrochloric acid with young non-secreting cells. Subsequently, the abomasal pH increases and the transformation of pepsinogen to pepsin decreases, reduces protein digestion, increases mucosal permeability, and increases the loss of abomasal endogenous proteins. The minimum time for *H. contortus* to reach the pre-patent period in goats is as little as 15 days post infection (Rahman and Collins, 1990) and the completion of its life cycle is approximately 17 to 21 days post infection (Machen et al., 1998).

The adult *H. contortus* reside in the abomasal lumen and feed on blood (Taylor et al, 2007). The blood loss per nematode per day was estimated to range between 0.003 and 0.05 ml (Dargie and Allonby, 1975; Muñoz-Guzmán et al., 2006). The haematophagous activity of *H. contortus* often leads to significant losses in small ruminant industry due to morbidity and mortality of infected animals (Rahman and Hamid, 2007). In addition, female *H. contortus* is a prolific egg layer with production of up to 5000 eggs per day per nematode (Le Jambre, 1995). The pathogenicity of *H. contortus* is thought to be derived from a combination of this short generation interval and also high fecundity (Emery et al., 2016). Furthermore, *H. contortus* secretes a calreticulin which binds to clotting factors, thus inhibits clotting and allows blood to continually flow from the site where it feed on blood (Suchitra and Joshi, 2005). Therefore, heavy infections of *H. contortus* will lead to severe anaemia, hypoproteinemia, hyperglobinemia and submandibular edema which is often referred to as “bottle jaw”.

In sheep with a heavy *H. contortus* burden, progressive anaemia will rapidly lead to death if blood loss exceeds the infected animal’s hematopoietic capacity (Bowman, 2003). Chronic infection by *H. contortus* is characterised by progressive weight loss and weakness while acute infection often results in melaena (dark coloured faeces containing digested blood), lethargy and submandibular oedema as a result of hypoproteinaemia (Taylor et al., 2007; Besier et al., 2016a).

Although *H. contortus* infection generally occurs concurrently with the other gastrointestinal nematodes, it consistently dominates the FEC and often generating up to 90% of contamination on pastures in the tropics or subtropics (Krecek and Waller, 2006). *H. contortus* has been reported as the predominant gastrointestinal nematode in both private (56% prevalent) and government (78% prevalent) small ruminant farms in Peninsular Malaysia in comparison to the other species such as *Trichostrongylus* spp. and *Oesophagostomum* spp. which have lesser prevalence (Khadijah et al., 2006a; 2006b). Consequently, *H. contortus* is considered the most important gastrointestinal nematode

in small ruminant farms in Malaysia as well as in other tropical countries (Waller and Chandrawathani, 2005). However, it is worth noting that high fecundity nematode species (e.g *H. contortus*) as reflected from high FEC does not necessarily mean the species dominates the infection.

1.3.3 *Teladorsagia circumcincta*

T. circumcincta or 'brown stomach worm' also inhabits the abomasum. Adults are slender with a short oral cavity and are reddish brown in colour (Taylor et al. 2007). The average nematode size varies considerably among sheep. Females range in size from 0.6 to 1.2 cm (Stear et al. 1997) with males typically about 20% smaller (Taylor et al. 2007). The number of eggs in the uterus of mature females can range from less than 10 to more than 60 (Stear et al. 1995) and is heavily dependent on the host immune response (Stear and Bishop 1999). The number of eggs produced per day by an adult female nematode is much lower than for *H. contortus* and has been estimated as ranging from 0 to approximately 350 (Stear and Bishop 1999), with longer females laying more eggs.

The interaction between sheep and *T. circumcincta* is one of the best understood host-parasite interactions with detailed information available on the epidemiology, immunology and genetics underpinning the infection dynamics (Stear et al., 2007). Comprehensive studies have revealed the development of acquired immunity and mechanisms involved in the regulation of nematode burden, length and fecundity in sheep (Stear et al., 1995a; Stear et al., 1996). The genetic variation in resistance to *T. circumcincta* infection captured via the measurement of heritability and identification of resistance genes in sheep have also been described (Stear et al. 2008).

An important pathogenic effect of *T. circumcincta* is due to the immune response (Stear et al., 2003) whereby mast cell degranulation leads to parietal cells reducing hydrochloric acid production. Therefore, pepsinogen fails to convert to pepsin resulting in elevated plasma pepsinogen levels and reduced protein digestion (Roeber et al., 2013b). The significant damage by *T. circumcincta* to the abomasal mucosa leads to protein deficiency and increase in nutrient loss in the gut (Coltman et al., 1999). The typical features and clinical signs of *T. circumcincta* infection range from increased mucus production, hyperplasia, decreased acid production, gastrinemia, pepsinogenemia to diarrhoea, dehydration and death (Stear et al., 2003; McNeilly et al., 2009). Diarrhoea, poor weight gain or weight loss are observed in moderate infection. In sheep with a heavy burden of *T. circumcincta*, infected animals may develop diarrhoea, anaemia, or hypoproteinemia, whereas death can result from severe infection (Zajac, 2006). The severity of infection can also depend on concurrent infections and the nutritional state of the host as well as

the ability of the host to mount protective immune responses against *T. circumcincta* (Stear et al., 2003).

T. circumcincta with multi-drug resistance to anthelminthic treatments is now the predominant gastrointestinal nematode surviving treatment in sheep farms in England, Wales and Scotland where faecal egg count reduction tests (FECRT) have been conducted (Bartley et al., 2004; Taylor et al., 2007). The widespread anthelmintic resistance among *T. circumcincta* populations lead to the loss of production in sheep industry. Thus this particular gastrointestinal nematode species is considered to be the most important gastrointestinal nematode of small ruminants in the United Kingdom.

1.3.4 *Trichostrongylus colubriformis*

T. colubriformis is also known as 'black scour worm' because the infection is characterised by dark coloured diarrhoea (Le Jambre et al., 2007). This hair-like gastrointestinal nematode resides in the duodenum and anterior small intestine of infected hosts. Following ingestion, the L3 of *T. colubriformis* will penetrate intestinal mucosa, form sub-epithelial tunnels and moult into L4. The tunnels will rupture as the L4 mature into L5 (Urquhart et al., 1996).

T. colubriformis causes haemorrhaging and loss of plasma protein into the intestinal lumen, resulting in hypoalbuminaemia and hypoproteinaemia. Inappetence, poor growth rates accompanied by soft faeces are the common clinical signs in low infection, while rapid weight loss is also observed in heavy infection. Mortality can occur, particularly in malnourished animals and those that ingest a high number of L3 over a short period (Taylor et al., 2007). Nevertheless, infection with this genus is often asymptomatic, except when it occurs with heavy burden of 10,000 or more nematodes.

Commonly, the FEC of *T. colubriformis* is low and rarely exceeds 5000 eggs per gram (epg) because of its small size and the faeces of infected animals are greatly diluted with water (Bowman, 2003). Additionally, *Trichostrongylus* spp. infection often occurs concurrently with the other gastrointestinal nematode infections and mixed infections are more likely to cause parasitic gastroenteritis (Kassai, 1999).

1.3.5 *Oesophagostomum* spp.

Oesophagostomum spp. is also called the 'nodular worm' due to the formation of nodules which encapsulate individual L3 that penetrate the intestinal mucosa of infected animals (Sutherland and Scott, 2010a). These inflammatory nodules become caseated and calcified thus interrupting intestinal motility which subsequently cause intussusception, which is the inversion of one portion of the intestine within another (Bowman, 2003).

Acute infection is characterised by severe, dark-green diarrhoea that is associated with emaciation, rapid weight loss and death in young animals. In chronic infection, *Oesophagostomum* spp. infection often leads to intermittent diarrhoea, inappetence, emaciation, and anaemia (Taylor et al., 2007). *Oesophagostomum* spp. also contributes to parasitic gastroenteritis with the other gastrointestinal nematode of small ruminants (Elsheikha and Khan, 2011).

1.3.6 Co-infection with gastrointestinal nematode species

H. contortus and *Trichostrongylus* spp. have been reported to be the common species of nematodes that naturally co-infect small ruminants. A study using polymerase chain reaction (PCR), a molecular identification technique, has shown that natural co-infection with *H. contortus* and *Trichostrongylus* spp. is more common in goats than other livestock including cattle, deer and swine (Tan et al., 2014). By DNA sequencing from faecal samples of goats, the same study also revealed that *H. contortus* has a higher infection rate than *Trichostrongylus* spp. in which the number of positive samples detected with *H. contortus* is higher than the number of positive samples detected with *T. colubriformis*.

In sheep, interaction between *H. contortus* and *T. colubriformis* has been explored by deliberate infection (Lello et al., 2018). The study revealed that the blood feeder *H. contortus* in the abomasum is negatively affected by the immune response induced by the mucosal browsing nematode *T. colubriformis*. In contrast, *T. colubriformis* gained advantages for its survival and establishment in the intestines due to the suppression of host's immune response by *H. contortus*. Interestingly, a study of natural infection in rabbits also has similar findings between the blood feeding nematode *Graphidium strigosum* that resides in the stomach with the intestinal mucosal browser *Trichostrongylus retortaeformis* (Lello et al., 2004). These findings suggest that co-infection with different nematode species that occupy different niches in the gastrointestinal tract, especially blood feeding nematodes and the mucosal browsing nematodes, can have similar effects in different host species.

1.4 Immune response to gastrointestinal nematode infection in small ruminants.

The immune mechanisms underlying resistance to gastrointestinal nematodes in sheep have been extensively reviewed (Williams, 2011; Venturina et al., 2013; McRae et al., 2015; Hendawy, 2018). The same mechanisms could be functional in the resistant goats although currently the evidence is different from that in sheep. Indeed, a high IgA response was associated with high FEC in Scottish Cashmere goats that were selectively bred for nematode resistance (McBean et al., 2016). Moreover, goats lack eosinophil receptors for IgA against nematode infection (Basripuzi et al., 2018). Nevertheless, we review the information available on immune responses in sheep and where available, in goats.

The immune response to gastrointestinal infection is a combination of both innate and acquired immune responses, which either kill the nematodes or prevent reproduction. The advantage of studying the immune response is that it can provide markers for resistance to incorporate into breeding programs to limit gastrointestinal nematode burden in small ruminants (Sweeney et al., 2016; Hendawy, 2018). There are a number of factors that affect the development of immunity to gastrointestinal nematodes including the genotype (Beraldi et al., 2008), age (Smith et al., 1985), and different reproduction stages such as during parturition and lactation (Barger, 1993). In the following sections we focus on the mechanisms by which the innate and acquired immunity act.

1.4.1 Innate immune responses

Innate immunity refers to the combination of non-specific protective responses related to initial infection that helps to defend against a range of pathogens (Sweeney et al., 2016). In the case of gastrointestinal nematode infection, the innate immune system acts via a physical barrier to protect against gastrointestinal nematode, senses the presence of gastrointestinal nematode and then activates the acquired immune response (McRae et al., 2015).

The first line of defence against gastrointestinal nematode infection is formed by mucin that is secreted by goblet cells as it creates a protective mucus layer on the intestinal epithelium (Grencis et al., 2014). The L3 have to pass through this physical barrier in order to reach the epithelium. For instance, a mucin known as Muc5ac has been demonstrated to play a role in the expulsion of a nematode, *Trichuris muris* in mice (Hasnain et al., 2011). In addition, galectins that are secreted by the epithelial cells

interact with mucins, increase mucus viscosity and impair gastrointestinal nematode movement (Meeusen et al., 2005).

The pattern recognition receptors (PRR), expressed by mucosal surface cells and immune cells such as macrophages and dendritic cells, are some of the first molecules to detect gastrointestinal nematode. PRR identify pathogen-associated molecular patterns and damage-associated molecular patterns that are able to initiate the inflammatory response. Additionally, PRR also induce cytokines and other immune signals to stimulate the acquired immune system (McRae et al., 2015). During PRR activity, dendritic cells link the innate and acquired immune systems by activating T cell responses while macrophages engulf foreign pathogens (Garza, 2014).

T. circumcincta and *T. colubriformis* spend most of their life in the host causing damage to the abomasal mucosa and the intestinal mucosa respectively, while *H. contortus* feeds on blood in the abomasum (Williams, 2011). Destruction of the tight junctions between epithelial cells causes an increase in mucus secretion and a decrease in acid production. As pH increases, the autocatalytic conversion of pepsinogen to pepsin is prevented, thus leading to pepsinogenaemia as well as hypergastrinaemia which causes inappetance. These occur concurrently with the reduction in albumin and fructosamine concentrations due to the loss of protein into the intestinal lumen (Stear et al., 2009).

Following damage to the gastrointestinal tract caused by the gastrointestinal nematode, the interleukins known as IL-33 and IL-25 as well as thymic stromal lymphopoietin are released. These interleukins activate the innate lymphoid cells known as ILC to produce type 2 cytokines including IL-13, IL-5, and IL-9 that are released by T helper 2 (Th2) cells (Grencis et al., 2014). Cytokines are important as they coordinate and modulate immune responses against gastrointestinal nematode infection (Garza, 2014). Once the interaction between epithelial cells, ILCs and antigen presenting cells (APCs) such as intestinal macrophages and dendritic cells has been activated, the innate immune response will be generated to combat the primary infection. Then, the acquired immune response will be triggered to control the secondary infections (Hendawy, 2018).

1.4.2 Acquired immune responses

The acquired immune response is the capability of the host to recognize specific pathogens by using both cellular (cytokine production) and humoral (antibody production) pathways to eliminate the pathogens or impair their ability to reproduce (Sweeney et al., 2016).

1.4.2.1 Cellular pathway

The first part of the pathway is for the naïve APCs such as macrophages and dendritic cells to engulf gastrointestinal nematode antigens and break them down into peptides at the site of infection. The peptides are then bound to major histocompatibility complex (MHC) class II receptors and presented on the APC surface for the recognition by CD4+ T cells to generate the acquired immune response (Williams, 2011; Sweeney et al., 2016). Then, the naïve CD4+ T cells will differentiate into several lineages of T helper cells such as Th1, Th2, Th17 and Treg cells with their particular cytokines (Zhu et al., 2010). A Th2 response is the predominant response in resistant sheep that are infected with nematodes such as *T. circumcincta* and is associated with the expression of IL-4, IL-5 and IL-13 cytokines (Venturina et al., 2013). IL-4 initiates a Th2 response and concurrently suppresses the development of Th1 and Th17 cells (Sweeney et al., 2016).

Long-term uncontrolled activation of Th2 cells may cause severe inflammatory reactions and immunopathology (Mulcahy et al., 2004). However, in the absence of an adequate Th2 response, the activation of Th17 cells reflect the inability to control L3 colonization, adult *T. circumcincta* infection and their egg production in susceptible Blackface lambs (Gossner et al., 2012). The relatively resistant Scottish Blackface and Texel sheep display a balanced expression of Th1, Th2 and Treg immune response in comparison to the more susceptible Suffolk breed (Sweeney et al., 2016). This suggests that a balanced immune response is important for controlling gastrointestinal nematode effectively and at the same time can avoid a damaging inflammatory response.

The cytokines IL-5 and IL-13 that are also synthesized by Th2 cells will cause the proliferation, maturation and recruitment of eosinophils from the bone marrow via the blood to the tissues which is the trademark of gastrointestinal nematode infection (Stear et al., 2002, 2003; Craig et al., 2014). Although eosinophilia can also lead to the hypersensitivity reaction (Garza, 2014), the majority of eosinophil interactions occur in the mucosal layer which minimizes tissue damage caused by toxic eosinophil mediators (Balic et al., 2006). The relationship between peripheral eosinophils and tissue eosinophils has been shown to be relatively weak thus peripheral eosinophils may not explicitly explain the kinetics of eosinophils recruitment into tissues (Henderson and Stear, 2006). However, the investigation or measurement of tissue eosinophilia in live animals is deemed difficult in comparison to peripheral eosinophilia.

IL-4, IL-5 and IL-13 promote IgE synthesis, stimulate tissue healing fibrosis and enhance gastrointestinal nematode expulsion by increasing mucus production, mucosal permeability and muscle contraction (Meeusen et al., 2005; Allen and Wynn, 2011; Yang

et al., 2013). Th2 cells then stimulate mast cell proliferation and maturation and also activate the humoral response by signalling B cells to synthesize parasite-specific antibodies such as IgA and IgE (Sweeney et al., 2016).

1.4.2.2 Humoral pathway

Acquired immunity to nematodes is mainly driven by the humoral pathway where parasite-specific antibodies such as IgE, IgA and IgG play important roles in gastrointestinal nematode infection. Following exposure to L3 antigen, IgE is activated through the classical type 1 hypersensitivity reaction that is mediated by mast cell proliferation and degranulation (Venturina et al., 2013). Mast cells are granular leucocytes that contain specific mediators such as histamine and serotonin that are released following degranulation of mast cells once IgE binds to its receptors (Abraham and St.John, 2012; Hendawy, 2018).

Degranulation increases the permeability of mucous membrane at the infection sites, permits transmission of antibodies and other mediators to the lumen, triggers mucus secretion, stimulates enteric nerves and contraction of smooth muscles that can lead to gastrointestinal nematode expulsion (Dawicki and Marshall, 2007; Abraham and St.John, 2012; McRae et al., 2015).

IgE elevation has been observed in *H. contortus* (Kooyman et al., 2000), *T. colubriformis* (Shaw et al., 1998) and *T. circumcincta* (Pettit et al., 2005) infections in sheep. The ability of sheep to control gastrointestinal nematode numbers is strongly associated with parasite-specific IgE antibody and mast cell degranulation (Murphy et al., 2010) while nematode length is strongly associated with parasite-specific IgA antibody (Henderson and Stear, 2006).

Once L3 have successfully established in the gastrointestinal tract mucosa and developed into L4; parasite-specific IgA antibody will come into action (Sweeney et al., 2016). IgA is secreted by plasma cells which are stimulated to differentiate from B-cells by dendritic cells that present antigens (Miller, 1996). IgA controls the length, maturation and therefore the parasite fecundity and number of nematode eggs released into the environment (Strain et al., 2002; Stear et al., 2004; Martínez-Valladares et al., 2005; Lacroux et al., 2006; Henderson and Stear, 2006).

Parasite-specific IgG that recognises certain carbohydrate larval antigens is also released to combat gastrointestinal nematode infection. Although the specific role of IgG1 and IgG2 is not clear, elevation of IgG1 levels have been observed in deliberately infected

Romney sheep with *T. colubriformis* (Douch et al., 1994). IgG1 may function with bradykinin to facilitate gastrointestinal nematode expulsion in sheep (Williams, 2012).

In resistant animals, the constant high level of immunoglobulins as well as local and circulating eosinophils correspond with the establishment and maintenance of Th2 immune response. In contrast, in susceptible animals an initial increase followed by a gradual decrease of immunoglobulins, local and circulating eosinophils suggest the development of the Th1 response (Garza, 2014). Susceptibility to gastrointestinal nematode infection in sheep is generally associated with a Th1 response through elevated levels of IL-12 and IFNγ as well as Th17 response via up-regulation of pro-inflammatory cytokines IL-6, IL-21, IL-23A and IL-17A. These cytokines inhibit the Th2 response and lower IgA levels, reducing the number of globular leucocytes and the number of mast cells and eosinophils. Consequently, these events result in high FEC and high number of adult gastrointestinal nematode in the susceptible sheep (Venturina et al., 2013; Arsenopoulos et al., 2017) The acquired immune responses in resistant and susceptible host are illustrated in Figure 1-2.

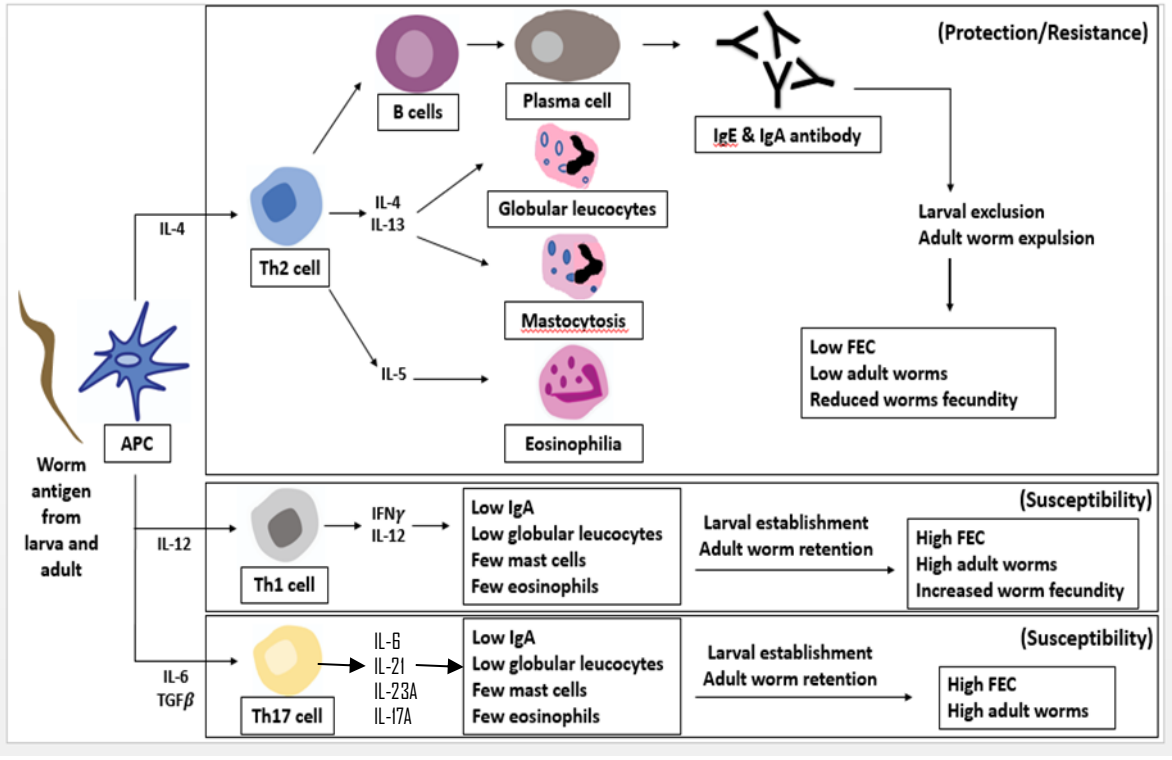


Figure 1-2. Acquired immune response in resistant and susceptible host (adapted from Venturina et al., 2013).

1.5 Control of gastrointestinal nematodes

1.5.1 Anthelmintics

Anthelmintic drugs are the preferred method to control gastrointestinal nematode infection in small ruminants, and non-chemical alternatives have met with limited success (Besier, 2008). The available anthelmintics against gastrointestinal nematode are limited to three old classes which are the narrow spectrum benzimidazoles and broad-spectrum classes of imidazothiazoles and macrocyclic lactones (McKellar and Jackson, 2004), as well as three newer classes of broad-spectrum anthelminthics namely the aminoacetonitrile derivatives (AADs), spiroindols and cyclodepsipeptides (Epe and Kaminsky, 2013).

The broad spectrum anthelmintics have been frequently used during the past five decades because of their efficacy, good tolerability and low cost. However, treatment failures are observed (Lanusse et al., 2014) and may be due to several factors: (a) inadequate integration between management strategies and chemotherapy; (b) incorrect use of anthelmintic drugs due to insufficient knowledge of their pharmacological features; and (c) insufficient understanding of the relationship between pharmacological properties that could lead to modifications on the pharmacokinetic behaviour resulting in decreased antiparasite efficacy of the chosen drug. Although all these factors may reduce efficacy of anthelminthic treatment, the major source of treatment failure is anthelmintic resistance which is discussed in the next section.

Anthelmintic products could be sustained by limiting the exposure of nematode populations to drugs by leaving a proportion of the nematode populations “in refugia” (Fitzpatrick, 2013). This could be achieved by leaving some animals untreated (Besier, 2012) thus maintaining nematodes in the host population where only a few animals are heavily infected but the rest of the animals possess low nematode burden (Stear et al., 2007). Then, targeted selective treatment towards animals that have been identified with heavy infection, susceptible to infection (non-resistant and/or non-resilient) or those that most contaminate pasture could be implemented to ensure continual source of nematodes in refugia (Kenyon et al., 2009). A practical system has been developed by Bath and van Wyk (2009) to guide farmers for targeted selective treatment of internal parasites in small ruminants.

1.5.1.1 Anthelmintic resistance

Control of gastrointestinal nematode infection in small ruminants all over the world relies almost exclusively on anthelmintics. However, its effectiveness is limited by the development of anthelmintic resistance. According to Prichard et al. (1980), resistance is the ability of individuals within a population to better tolerate doses of a compound than in a normal population of the same species and in addition it is heritable. Resistance develops when parasites survive anthelmintic treatments and pass on resistance genes to their offspring thereby increasing the frequency of resistance genes in populations undergoing treatment (Sangster et al., 2018). Thus, anthelmintic resistance is propagated by the inherited ability of surviving parasites to continuously pass the genes on to their next generation (Sangster, 2001).

The prevalence and importance of anthelmintic resistance continues to increase globally throughout the livestock industries since the first report of anthelmintic resistance in *H. contortus* population in sheep about 50 years ago (Sangster et al., 2018). According to the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines (Coles et al., 1992), anthelmintic resistance is said to be *present* when FEC reduction percentage for a particular anthelmintic drug is less than 95% and the 95% lower confidence limit is less than 90%; and *suspected* if only one of these two criteria is met.

In recent years, multiple drug resistance to old anthelmintic groups has been reported in many parts of the world (Nabukenya et al., 2014; Keegan et al., 2015; Crook et al., 2016; Herrera-Manzanilla et al., 2017). Additionally, treatment failures have been reported for new broad spectrum anthelmintics in sheep (George et al., 2012; Sager et al., 2012) and goats (Bailey, 2015).

Kaplan and Vidyashankar (2012) briefly review anthelmintic resistance status in small ruminants reported in the United States, Brazil, Africa, New Zealand, Australia and Europe. Anthelmintic resistance in Asian countries is not as serious as in the large-scale small ruminant farming countries such as Australia, South America and Africa, with the exception of Malaysia and Fiji where anthelmintic resistance is one of the major threats (Waller, 2002).

Anthelmintic resistance has been suspected in Malaysia since 1980s due to unofficial reports of anthelmintic failures (Sani et al., 2004). Benzimidazole resistance in small ruminant industry in Malaysia had been detected in a nationwide surveys using FECRT since the early 1990s (Dorny et al., 1994). Further studies also revealed the presence of anthelmintic resistance to other classes of anthelmintics, namely macrocyclic lactones,

imidazothiazoles and salicylanilides (Rahman, 1994; Pandey and Sivaraj, 1994; Sivaraj et al., 1994; Chandrawathani et al., 1999, 2003, 2004; Khadijah et al., 2006a, 2006b, 2008; Nor-Azlina et al., 2011; Basripuzi et al., 2012; Abubakar et al., 2015; Khadijah et al., 2018).

In the UK, similar patterns are seen, with the prevalence and levels of benzimidazole resistance increasing in *Teladorsagia* spp. and *Trichostrongylus* spp. populations (Learmount et al., 2016).

Leathwick and Besier (2014) identified the high-risk management practices that lead to anthelmintic resistance as (a) excessive frequency of treatment, (b) underdosing with anthelmintics, (c) treatment on low contamination pasture, (d) treatment of ewes immediately pre or post lambing and (e) monocultures of young stock.

Rotational drenching strategies are suggested as a way of minimising the development of anthelmintic resistance (Sutherland and Scott, 2010a). The use of varying classes of anthelmintics in rotational drenching is a form of counter selection where nematodes that have developed resistance to one anthelmintic class are assumed to be more susceptible to another class of anthelmintic. In the absence of the selection pressure exerted by a particular anthelmintic class, the resistant nematodes will be less fit and less able to survive anthelmintic treatment by another drug type than their susceptible counterparts.

This problem of anthelminthic resistance has also driven interest in reducing reliance on anthelmintics by exploring non-chemical approaches to the control of gastrointestinal nematode infection in small ruminants.

In the UK, this has led to the development of schemes for the sustainable control of parasites in sheep (SCOPS) which has resulted in a significant reduction in benzimidazole usage and may potentially delay the development of anthelmintic resistance (Abbott et al., 2012). The SCOPS guidelines incorporate all available evidence for best practice with the objective to slow the development of anthelmintic resistance on UK sheep farms but this problem is still unresolved.

1.5.2 Non-conventional anthelmintic treatments

Certain tanniferous plants against gastrointestinal nematode species that affect different stages of their life cycle are observed to have anthelminthic effects (Hoste et al., 2012). Hoste et al. (2006) summarised the results of in vivo experiments with various tanniferous plants in sheep, goats and deer that showed reductions in gastrointestinal nematode

numbers, fecundity and FEC. There are three main mechanisms associated with the impact of the consumption of tanniferous plants: a) reduction of L3 establishment, b) reduction in the excretion of eggs by the adult female nematodes, and c) reduction in the development of nematode eggs to L3 (Hoste et al., 2012).

However, high concentrations of condensed tannins are known to reduce the intake and digestibility of feed which consequently affects animal production (Kearney et al., 2016). Moreover, tanniferous plants are highly astringent, may result in a pucker tongue, causing the mucous membranes of the oral cavity to dry, and also interfering with digestion by disrupting the microorganism and enzyme environment (Engel, 2003). These factors limit the feasibility of tanniferous plants as an alternative to chemical anthelmintics even though their anthelmintic properties are able to control gastrointestinal nematode infection.

Copper oxide wire particle (COWP) has been shown to have anthelmintic effects against gastrointestinal nematodes, particularly *H. contortus* (Besier et al, 2016b). After ingestion, the COWP capsules will remain in the abomasum and release the particles for at least 32 days. They move throughout the gastrointestinal tract, increasing the concentrations of copper in the abomasal digesta, creating an unfavourable environment for *H. contortus* that causes the expulsion of the adult nematodes (Burke et al., 2010; Galindo-Barboza et al., 2011). However, Vatta et al. (2012) has demonstrated the lack of efficacy of COWP beyond 28 days in goats.

1.5.3 Grazing management and housing system

Grazing management is one of the strategies to control gastrointestinal nematode infection in ruminants used in temperate, sub-tropical and tropical countries (Waller, 2006). Mahieu et al. (2008) summarised the grazing management recommendations from several studies in which (a) the animals are allowed to graze in the same paddock for not more than one week, (b) pasture irrigation is done at least three days after the paddock has been emptied and (c) the pasture is rested for at least four weeks before the animals graze in the same paddock again.

The timing of grazing can also be adjusted to reduce gastrointestinal nematode infection. Goats that grazed in the morning were found to have a significantly higher nematode burden than those that grazed in the afternoon (Nor-Azlina et al., 2011) which is thought to be due to differences in the L3 population. A study in Indonesia found that the L3 population was significantly greater on grasses cut at 7.00 a.m than grasses cut at 9.30 a.m., 12.00 p.m., 2.30 p.m and 5.00 p.m. (Kusumamihardja, 1982).

Mixed or alternate grazing of different species (eg. sheep/cattle), because different host species will be susceptible to different nematode species, or alternate grazing of different ages (eg. young/adult) as immunity response increases with age have also been suggested as a useful strategy to control gastrointestinal nematode infection (Sutherland and Scott, 2010b).

In addition, the housing system has also been found to be one of the risk factors associated with gastrointestinal nematode infection (Ratanapob et al., 2012). In Malaysia, grazing management is less applicable due to limited land resources for farming purposes. Hence, most of the small ruminant farms are managed under intensive or semi-intensive systems that limit grazing activity. Instead, the animals are fed with cut grasses which may reduce exposure to gastrointestinal nematode infection (Basripuzi et al., 2013).

1.5.4 Feed supplementation

According to Torres-Acosta et al. (2012), supplementary feeding means extra feed provided by the farmers in addition to that consumed during grazing or browsing. A supplement consists of one or more ingredients with certain quantities of protein, energy and other macro- and micro-nutrients designed to increase productivity.

In lactating ewes, an increased intake of metabolizable protein supplementation resulted in a lowered FEC and an increased concentration of abomasal globule leucocytes as well as immature nematodes (Houdijk et al., 2000). In addition, non-protein nitrogen incorporated in urea molasses blocks as a feed supplementation has been shown to enhance the resilience of grazing sheep to gastrointestinal nematode infection (Knox et al., 2006). Lambs given a dietary supplementation of soya bean meal had a lower FEC, higher PCV and higher concentrations of total plasma protein and plasma albumin than lambs given the basal diet although their mean nematode burdens were similar (Wallace et al., 1995).

Retama-Flores et al. (2011) found that maize-supplementation reduced fodder intake of grass and *Leucaena leucocephala* with the outcome that the lambs had a 30% lower FEC than the non-supplemented lambs. Less fodder is consumed by the lambs when more supplement is offered; therefore, the supplemented animals are presumed to ingest fewer L3 than the non-supplemented ones, reducing exposure to gastrointestinal nematode infection and consequently lowering FEC. However, supplemented animals may excrete more faeces in comparison to the non-supplemented animals (McDonald et al., 2002). Consequently, the supplemented animals may have lower FEC because of the dilution of gastrointestinal nematode eggs in the faeces. Hence, low FEC among

supplemented animals could be misinterpreted as the result of reduced gastrointestinal nematode infection (Tarazona, 1986).

Overall, the advantages of supplementary feeding include reduced pathophysiological impact of gastrointestinal nematode, improvement in productivity, reduction of natural infections, dilution of FEC in the faeces, change in the pattern of consumption in the field, possible direct anthelmintic effects of the supplements and improved resilience to gastrointestinal nematode infection (Torres-Acosta et al., 2012). Interestingly, lambs supplemented with additional protein produce more IgA and have shorter adult *H. contortus* than lambs that received the basal diet (Strain and Stear, 2001), which indicates that IgA activity against gastrointestinal nematodes is influenced by the quality of feed.

Although there have been relatively few studies in goats, studies in Alpine dairy goats show that goats supplemented with a high protein diet have a low FEC and high eosinophil counts suggesting that resistance to gastrointestinal nematode was enhanced by protein supplementation. Furthermore, milk production and milk composition parameters among high producing goats were also enhanced with a high protein diet (Etter et al., 2000). Thus, feed supplementation may be an effective option for the control of gastrointestinal nematode infection in goats as well as sheep (Hoste and Torres-Acosta, 2011). However, continuous consumption of supplementary concentrates with low amount of roughages can disrupt microbial activity in the rumen thus leads to the impairment of digestion.

1.5.5 Selective breeding for gastrointestinal nematode resistance

1.5.5.1 Gastrointestinal nematode resistance in sheep and goats

Investigations into the genetic variation in resistance to gastrointestinal nematode in ruminants are driven by the occurrence of widespread anthelmintic resistance and the realisation that anthelmintics residues may accumulate in the tissues of treated animals. These factors have prompted interest in control approaches that are less dependent on anthelmintics such as selective breeding for host resistance to gastrointestinal nematode infection (Woolaston and Baker, 1996; Smith et al., 1999).

The term ‘resistance’ here refers to the failure of the parasite to establish an infection, failure to complete its development when infection is established or failure to both establish and develop infection. In these situations, the parasite is either controlled or eliminated by the host after the parasite gains entry into the body (Stear and Wakelin, 1998). Characteristics of resistance include relatively lower number of nematodes as

manifested by low FEC, reduced fecundity of females, reduced size of adult nematodes, increased proportion of inhibited larvae, or combination of above (McRae et al., 2014). In contrast, resilience is the maintenance of growth and production of the host during gastrointestinal nematode infection (Simpson et al., 2009). A resilient animal able to withstand the effects of prolonged nematode challenge on pasture thus maintain acceptable productivity with minimal anthelmintic treatment (Morris et al., 2010).

Variation in resistance to gastrointestinal infection in small ruminants has been found both between and within breeds. Most of the small ruminant breeds that are identified as being resistant are indigenous breeds such as Alpine goats (France), Small East African goats (Kenya), Red Masai sheep (Kenya) and Barbados Blackbelly sheep (Caribbean) which are found to be more resistant than the imported breeds reared in the same countries. These breeds of sheep and goats presumably have been under natural selection to gastrointestinal nematode in their native populations for many centuries without anthelmintic treatment (Baker and Gray, 2004). For instance, local Pakistani goat breeds, namely Beetal and Teddy, were found to be more resistant to *H. contortus* infections than the imported Angora breed based on measurement of bodyweight, FEC, PCV and FAMACHA scores (Babar et al., 2015). In addition, the Gulf Coast Native lambs were found to have increased production of IgG, IgA and IgE production compared to Suffolk lambs during *H. contortus* infection (Shakya et al., 2011).

The evidence for between and within-breed variation in gastrointestinal nematode resistance in goats remains much scarcer (see Section 1.5.5.2 below for a summary of the studies in goats) but nevertheless some selective breeding schemes in goats as well as sheep do exist.

1.5.5.2 Breeding programs for gastrointestinal nematode resistance

Selective breeding for gastrointestinal nematode resistance in sheep and goats is increasingly important. Selective breeding and systematic production measurements have been implemented in Merino, Romney and Scottish Blackface sheep as well in as Cashmere and Guadeloupe goats (Sani and Gray, 2004). Geographically, commercial breeding schemes that make use of estimated breeding values for resistance against gastrointestinal nematode have been established in Australia, New Zealand and the UK (Jackson and Miller, 2006).

In theory, genetic parameters such as heritability and correlations between traits are specific to a given environment and a population. In practice, however, these genetic parameter estimates for the production traits such as growth and reproduction have been

found to be relatively robust across breeds and different environments and are therefore expected to be similar in different environments for gastrointestinal nematode resistance (Woolaston and Baker, 1996).

It is beneficial to know the possible consequences for production traits before starting a selective breeding programme. If there is a genetic correlation between two traits, selection for one trait will change the other trait (Stear et al., 1999). For example, the genetic correlation between FEC and bodyweight in Scottish Blackface sheep is large and negative of nearly -1.0, indicating that the most important genes for growth rate are those that have the ability to control gastrointestinal nematode infection (Bishop et al., 1996).

In order to design an optimal breeding programme, it is necessary to quantify the phenotypic variation and the heritability of the trait being selected. This is because the rate of genetic improvement is proportional to the heritability (Woolaston and Baker, 1996). The largest source of variation is additive genetic variation which is the sum of the average effects of genes and is estimated from the similarity among relatives. Heritability is the ratio of additive genetic variation to the total variation excluding fixed effects. Since FEC heritability in sheep is similar to the growth rate heritability in beef cattle and milk production heritability in dairy cattle, the selective breeding of sheep for reduced FEC is justified (Stear et al., 2009). The most important genes for the growth rate of Scottish Blackface sheep are those that have the ability to control gastrointestinal nematode infection based on the strong negative genetic correlation between FEC and bodyweight (Bishop et al., 1996). FEC has now been used as a marker for selective breeding in the UK, Australia and New Zealand.

These selective breeding programmes are based on multiple studies which have demonstrated the heritability of FEC in sheep. For example, heritability estimates for FEC in Scottish Blackface lambs increased from 0.06 ± 0.11 in one-month-old lambs to 0.21 ± 0.11 when they were four-month-old, and later to 0.33 ± 0.15 in the same lambs aged six months (Bishop et al., 1996; Smith et al., 1999). Very similar to Scottish Blackface sheep, the heritability estimates of FEC in Merino sheep in Australia and Romney sheep in New Zealand are 0.33 ± 0.18 and 0.34 ± 0.09, respectively (Bisset et al., 1992).

In a study conducted in Kenya, Red Maasai ewes were found to be more resistant to nematode infection than Dorper ewes while Small East African does were more resistant than Galla does as indicated by their lower FEC and higher PCV (Baker et al., 1994). In the same study, the FEC heritability estimates of Red Masai lambs increased from 0.09 ± 0.03 at six months old to 0.18 ± 0.08 when they were eight months of age. The findings

indicate that FEC and PCV are feasible markers for nematode resistance in breeding selection of both sheep and goats.

In Scottish Blackface sheep, the heritability estimates were 0 for the number of L4, 0.08 ± 0.09 for the number of L5 and 0.14 ± 0.10 for the number of adult nematodes in the mixed infection predominated by *T. circumcincta*. Therefore, there was no evidence that nematode burden was under genetic control. In contrast, the estimated heritability for nematode length was high at 0.62 ± 0.20. The results indicate that Scottish Blackface sheep appear to be able to control egg production and nematode growth but not nematode establishment (Stear et al., 1997). One explanation is that the sheep immune system was unable to respond to the relevant nematode antigen, but a weak response cannot be ruled out against nematode number. Western blotting with L3, L4 or adult nematode preparations showed extensive heterogeneity in antigen recognition. None of the sheep was able to recognise all antigens and only a few nematode molecules were recognised by antibody from all sheep (Stear et al., 1999b). The heritability estimates of a trait can be maintained if the number of genes contributing to the trait is sufficiently high (Burger et al., 1989).

Table 1-3 shows the heritability and repeatability estimates of FEC in different breeds of sheep and goats. The repeatability is the proportion of the total phenotypic variance due to variation among animals and provides the upper limit for heritability (Falconer and Mackay, 1996). The repeatability provides a measure of quality control which indicates the similarity between measurements taken at different times during infection, provides guidelines for the optimal time of sampling as well as the optimal number of samples to identify relatively resistant and susceptible animals. The repeatability is particularly suitable for studying responses to infection because it is a ratio of variances and as such it is not affected by changes in the mean that occur in the response to infection.

Table 1-3. Heritability and repeatability estimates of FEC in sheep and goat breeds.

Breeds	Heritability	Repeatability	References
Sheep			
Parendale sheep	0.27 ± 0.07	0.38 ± 0.08	Watson et al., 1995
Scottish Blackface sheep	0.23 ± 0.05	0.25 ± 0.04	Bishop and Stear, 2001
Romney sheep	0.37 ± 0.06	0.46 ± 0.03	Morris *et al.*, 1998
Uruguayan Merino sheep: -Lambs -Periparturient ewes	 0.25 ± 0.03 0.08 ± 0.03	 0.34 ± 0.02 0.18 ± 0.03	Goldberg et al., 2012
Goats			
Creole goats	0.10 ± 0.02	0.17 ± 0.02	Mandonnet et al., 2006
Saanen goats: -Mid lactation -Late lactation	 0.09 ± 0.05 0.06 ± 0.04	 0.09 ± 0.06 0.15 ± 0.04	Morris et al., 1997

1.6 Markers of resistance to gastrointestinal nematodes

The intensity of infection expresses itself through a complex interaction among many traits which include those associated with nematode number, length and fecundity (Stear et al., 2003), those associated with host immune responses such as IgA and eosinophil responses (Henderson and Stear, 2006) and those associated with nematode impact on the host such as reduction in weight gain (Miller and Horohov, 2006). The phenotypic markers utilised to assess the resistance status of sheep breeds with their advantages and limitations are shown in Table 1-4. Some of these parameters are relatively straightforward to measure and hence commonly assessed and others much less common in practice.

1.6.1 Faecal egg count (FEC)

FEC is the most frequently used parameter to assess resistance because of its applicability across multiple gastrointestinal nematode genera and because it is a direct measure of infected animals' involvement in pasture contamination (Woolaston and Baker, 1996a; Kemper et al., 2010). Its variability among animals and moderate heritability make it the most feasible and widely use parameter of gastrointestinal nematode resistance (Bishop and Morris, 2007). McRae et al. (2015) validated FEC as a reliable method to identify resistant animals, demonstrating that selected Scottish Blackface lambs exhibited consistently lower FEC than their higher FEC counterparts throughout the course of infection with *T. circumcincta*.

Selective breeding for increased resistance commonly relies on the assessment of FEC in live animals or nematode counts at necropsy in dead animals (Baker and Gray, 2004). In the UK, FEC following natural infection is used as a guideline in selective breeding of sheep for gastrointestinal nematode resistance (Stear et al., 2006). FEC has a moderate heritability of around 0.2 to 0.3, a little lower than the heritability of other important traits in small ruminants such as body weight (0.4), fleece weight (0.4), average fibre diameter (0.5) but higher than that of reproduction rate (0.1) (Woolaston and Baker, 1996).

Resistance to gastrointestinal nematode infection corresponding to low FEC could be due to lower nematode burden, reduced size of adult gastrointestinal nematode, reduced female fecundity, increased proportion of inhibited larvae, or a combination of these elements (Balic et al., 2000; Stear et al., 2003). Selection for nematode resistance based on low FEC was conducted when the average flock FEC increased to more than 500 epg for Rylington Merino sheep (Karlsson and Greeff, 2006) and reached the target value of

1000 to 1500 epg for Romney sheep (Bisset et al., 1992) to ensure all animals received adequate infection challenge. Increased adult female *T. circumcincta* length was associated with an increased number of eggs per female nematode, and with an increased number of FEC in both natural and deliberate infection of Scottish Blackface sheep (Stear and Bishop, 1999).

Faecal egg count has also been shown to be associated with undesirable outcomes. For example, a highly positive genetic correlation was found between FEC and FAMACHA© eye score ($r=0.66 \pm 0.27$) indicating that lambs with high anaemia score would also have high FEC (Cloete et al., 2016). FEC was also reported to have a high negative correlation with daily weight gain in Scottish Blackface lambs (between 0.63 ± 0.32 and -0.90 ± 0.28), suggesting that resistant to gastrointestinal nematode infection is an important determinant of growth rate (Bishop et al., 1996).

Following deliberate infection, the variation in FEC is probably due to the variation in the ability among infected animals to produce protective responses against gastrointestinal nematode infection (Stear et al., 1995b). In natural infection, however, the FEC variation may be due to the differences in infection intensity, with L3 arising by chance or from differences in grazing habit as well as variation in protective responses among animals. However, the heritability of FEC following natural infection is similar to the heritability of FEC following deliberate infection, which suggests that chance events are not very important and most of the variation is in genetically determined immune responses or genetically determined differences in grazing behaviour.

A key feature is that FEC are typically overdispersed among hosts in a population. This means that a small number of animals are responsible for a disproportionate amount of the pasture contamination (Stear et al., 2007). Selective breeding therefore has an additional epidemiological advantage in which culling of the high FEC producers will reduce the number of nematode eggs on pasture and lower infection rates (Bishop et al., 1996). Mathematical models have been developed that capture these features and have shown additional benefits to selective breeding due to the reduction in pasture contamination (de Cisneros et al., 2014).

Table 1-4. Phenotypic traits used to assess the resistance status of sheep breeds with their advantages and drawbacks (adapted from Saddiqi et al., 2012).

Phenotypic traits	References	Advantages	Drawbacks
Faecal egg counts	Stear et al., 1995a; Mugambi et al., 1996; Bishop et al., 1996; Miller et al., 1998; Matika et al., 2003; Amarante et al., 2005; Good et al., 2006; Kemper et al., 2009; Saddiqi et al., 2010a, 2010b.	A parameter that is repeatable, heritable and reliable for screening/evaluation of resistance/resilience status of animals. Demonstrates either positive or negative correlations with the other phenotypic traits such as body weight and IgA.	Time consuming and labour extensive for low fecundity gastrointestinal nematodes. Identification of eggs in mixed infections is difficult due to similar morphology.
Nematode count	Gray et al., 1992; Stear et al., 1995a, 2000; Mugambi et al., 1996; Aumont et al., 2003; Gruner et al., 2003; Amarante et al., 2004; Good et al., 2006; Miller et al., 2006; González et al., 2008; Saddiqi et al., 2010a, 2010b; Kemper et al., 2010.	Provide powerful confirmation of FEC outcomes and to minimize the uncertainty relating to gastrointestinal nematode identification. Helpful for the study of abomasal histology.	Animals must be slaughtered to count the nematodes. Hence, it is expensive in terms of labour and cost.
Fecundity and nematode length	Stear et al., 2000; Aumont, Gruner and Hostache, 2003; Gruner et al., 2003; Davies et al., 2005; Kemper et al., 2010; Saddiqi et al., 2010b.	Correlated with the other parasitological and phenotypic parameters. Helpful in understanding the mechanisms of host resistance against gastrointestinal nematode.	Study can be performed at necropsy; thus, it is costly and not routinely practiced.
Periparturient rise in faecal egg counts	Courtney et al., 1984; Zajac and Sullivan, 1988; Woolaston, 1992; Wanyangu et al., 1997; Rocha et al., 2004.	Simple, inexpensive, easy to be implemented in livestock farms.	Not adequate for the declaration of resistance status on its own. Not applicable for sires and lambs selection.
Packed cell volume	Gray et al., 1992; Mugambi et al., 1996; Wanyangu et al., 1997; Miller et al., 1998, 2006; Notter et al., 2003; Bricarello et al., 2004; Burke and Miller, 2004; Mugambi et al., 2005; González et al., 2008; Chiejina et al., 2010; Saddiqi et al., 2010a, 2010b.	Useful indicator in the case of haematophagous gastrointestinal nematode. Routinely practiced along with FEC, mostly in studies for the resilience status of animals.	Labour expensive and time consuming.
Blood eosinophils	Mugambi *et al.*, 1996; Wanyangu *et al.*, 1997; Amarante *et al.*, 1999; Aumont et al., 2003; Rocha, Amarante et al., 2004; Davies et al., 2005; Alba-Hurtado *et al.*, 2010.	A valuable parameter for gastrointestinal nematode infections. Correlated with the other parameters.	Labour intensive, expensive and not routinely practice.
Total plasma protein level	Mugambi *et al.*, 1996; Li et al., 2001; Amarante *et al.*, 2004; Bricarello *et al.*, 2004; Rocha et al., 2004; González *et al.*, 2008; Saddiqi *et al.*, 2010a, 2010b.	Useful to determine protein loss and confirmation of parasitological and haematological findings, serves as an important constituent of meat, wool and enzymes.	Expensive, labour intensive and not routinely practice.
Immunoglobulins	Strain et al., 2002; Amarante et al., 2005; Davies et al., 2005; Henderson and Stear, 2006b.	Useful for the mechanism involved in resistance and for confirmatory tests of the other parameters of resistance.	Not regularly practiced in poor and developing countries due to expensive and lack of laboratory facilities.

Phenotypic traits	References	Advantages	Drawbacks
Abomasal histology	Bisset et al., 1996; Douch et al., 1996; Amarante et al., 1999, 2005; Bricarello et al., 2004; Saddiqi et al., 2010.	Valuable for understanding the local immune mechanism involved in the case of abomasal gastrointestinal nematode and to correlate with parasitological and haematological parameters.	Expensive, laborious and only applicable at necropsy thus not routinely practiced by researchers.
Anthelmintic treatments	Miller et al., 1998; Amarante et al., 1999, 2004; Matika et al., 2003; Burke and Miller, 2004; Vanimisetti et al., 2004; Rocha et al., 2004; Saddiqi et al., 2010.	Simple and serves as a supplementary parameter that can be considered in routine practice to reduce mortality.	Impractical if the number of animals or infection level is low.
Pasture infectivity	Kahn et al., 2003; Amarante et al., 2004; Rocha et al., 2004.	To determine pasture contamination as an indirect indication of host resistance status.	Not routinely practiced by researchers.
Live weight	Barger, 1984; Stear *et al.*, 1995a; Li et al., 2001; Haile *et al.*, 2002; Kahn *et al.*, 2003; Notter et al., 2003; Burke and Miller, 2004; Mugambi et al., 2005; Chiejina *et al.*, 2010; Saddiqi *et al.*, 2010a, 2010b.	Routinely practiced in livestock farms (either weekly and/or monthly) and serves as an indicator of economic losses in terms of meat production.	Different breeds have different growth rates thus live weight is ineffective to determine resistant; but it is a valuable parameter to determine within breed variability in natural resistance to gastrointestinal nematode.
Wool growth	Barger, 1984; Kahn et al., 2003.	Valuable parameter in wool production industries.	Not routinely practiced particularly in developing and poor countries.
FAMACHA system	Burke and Miller, 2004; Alba-Hurtado et al., 2010.	Cheap, feasible for routine practice, useful in minimizing anthelmintic resistance based on justifiable use of dewormers	Only useful in haematophagous gastrointestinal nematode infections.
Histamine concentration	Douch et al., 1984; Zajac et al., 1990.	Valuable parameter to confirm the findings from IgE levels and abomasal histology.	Only applicable at necropsy, expensive and not routinely practiced.

1.6.2 Protective immune response

Immunological responses against gastrointestinal nematode infection are dependent on the activation of Th2 cells but the effective components vary depending on nematode genera and on infection stages within the same genera (Saddiqi et al., 2012). Antibodies IgE, IgA and IgG are produced during gastrointestinal nematode infection, but the protective immune responses are most strongly associated with IgE activity against L3 and IgA activity against L4 (Strain et al., 2002; Murphy et al., 2010; Stear et al., 2011). Anti-parasite antibody is regarded as the method with the best potential to measure resistance against gastrointestinal nematode infection due to the ease of appropriate sample collection and the availability of assay techniques for large sample size (Shaw et al, 2012).

1.6.2.1 Immunoglobulin A

IgA antibody has been shown to have significant relationship with the reduction in FEC, nematode length and nematode numbers in sheep (Strain et al., 2002; Beraldi et al., 2008; Gossner et al., 2012). Resistant animals produce more parasite-specific IgA molecules than their susceptible counterparts (Stear et al., 1999a). The quantity and specificity of parasite-specific IgA regulates nematode length and fecundity in deliberate infections (Stear et al., 1995a; Stear et al., 1996; McCririe et al., 1997). Strain and Stear (2001) also demonstrated significant association between reduction in female nematode length with an increase in anti-L3 IgA ($p<0.05$) and anti-L4 IgA ($p<0.05$). However, IgA activity against developing L4 appears to be the major mechanism regulating the growth and development of *T. circumcincta* in sheep (Stear at al., 1995a; Strain et al., 2002). In contrast, there is a little genetic variation in the number of inhibited L4 which suggests that arresting larval development is not an important mechanism of resistance to natural gastrointestinal nematode infection in lambs (Stear et al., 1997).

The strong relationship between resistance measurements and IgA activity to *T. circumcincta* infection suggests that IgA is a more useful parameter of resistance than FEC (Stear et al., 1999b). Moreover, FEC may not be the best marker to use in selective breeding schemes for *T. circumcincta* infections because of density-dependent constraints on fecundity which can result in heavily infected animals produce fewer eggs (Bishop and Stear, 2000). Thus, IgA which affects nematode size and fecundity is a plausible alternative to FEC (Stear et al., 1999), and indeed, nematode length has been shown to have a much higher heritability (0.62 ± 0·20) than FEC (0.23 ± 0.05) or nematode number (0·14 ± 0·10) particularly in Scottish Blackface sheep.

Plasma IgA can be routinely measured in live animals but mucosal IgA which is the local IgA at the site of infection is usually measured in post-mortem samples. Mucosal IgA showed negative correlations with nematode length ($r=-0.66$; $p<0.05$) and nematode burden ($r=-0.37$; $p<0.05$) in resistant versus susceptible Canaria breed sheep (Hernandez et al., 2016). In addition, mucosal IgA anti-L3 was found to be negatively correlated with FEC and nematode burden in Santa Ines (-0.32; -0.41) and Ile de France (-0.53; -0.40) sheep respectively (Amarante et al., 2005). Increased mucosal IgA activity was associated with increased plasma IgA activity (de Cisneros et al., 2014). Mair et al. (2015) demonstrated a negative correlation between plasma IgA and nematode length ($r=0.17 \pm 0.027$) in Scottish Blackface lambs which was consistent with the previous studies (Stear et al. 1999b; Strain et al. 2002). Therefore, plasma IgA activity can be used as a proxy for mucosal IgA activity.

IgA plays an important role in identifying resistant and susceptible animals particularly against *T. circumcincta* infection (Stear et al., 1997). However, some sheep with normal IgA responses still have reasonably fecund nematodes; this observation suggests that not all sheep have the ability to recognize the specific parasite molecules (Stear et al., 1996).

The IgA response in sheep has been well-studied and as discussed above is associated with improved resistance. In contrast, a significant positive correlation of FEC with IgA ($r=0.06$; $p<0.001$) was found in Scottish Cashmere goats that were selected for gastrointestinal nematode resistance (McBean et al., 2016). In Creole goats, FEC had weak negative correlations with *H. contortus* anti-L3 IgA ($r= -0.04$; $p>0.05$) which suggest that IgA response may only reflect the size of *H. contortus* burden as measured by FEC rather than the efficiency of protective immune response (de la Chevrotière et al., 2012). These findings were in contrast to the findings in sheep which suggests that the antibody response against gastrointestinal nematode is not as protective in goats as it is in sheep. This could be due to the lack of IgA receptors on eosinophils in goats that impair their protective response against gastrointestinal nematodes (Basripuzi et al., 2018).

1.6.2.2 Immunoglobulin E

The IgE response regulates L3 establishment and hence the number of adult gastrointestinal nematode in the infected host (Stear et al., 1995a). Lambs with low FEC were found to have significantly higher IgE activity ($p<0.01$) than lambs with high FEC (Huntley et al., 2001). The heritability of IgE against L3 and L4 of *T. circumcincta* was found to be relatively high at 0.39 and 0.50 respectively (Murphy et al., 2010).

In a natural infection in Texel sheep dominated by *T. circumcincta*, IgE activity against tropomyosin was associated with decreased FEC and with reduced bodyweight among Texel sheep (Murphy et al., 2010). However, IgE also has its undesirable effects. Once IgE binds to the larval molecules, mast cell degranulation will be induced, and breaking the tight junctions between the epithelial cells, which subsequently lead to protein deficiency as observed in sheep infected with *T. circumcincta* (Stear et al., 2007). This mechanism can lead to reduction in bodyweight.

In Creole goats, IgE anti-L3 was observed to have a negative genetic correlation with FEC ($r=-0.32 \pm 0.08$) but a positive genetic correlation with bodyweight ($r=0.44 \pm 0.13$) in natural mixed infection predominated by *H. contortus* (de la Chevrotière et al., 2012). However in Scottish Cashmere goats, a positive correlation existed between FEC and *T. circumcincta* IgE anti-L3 (McBean et al., 2016), suggesting that the role of IgE in goats may be breed and species dependent.

These findings indicate that IgE can have both desirable effect and undesirable effects on gastrointestinal nematode infection in sheep and goats and that these outcomes may be breed dependent. IgE is not as desirable as IgA as a marker for resistance against gastrointestinal nematode infection in sheep due to its association with hypersensitivity reaction.

1.6.2.3 Immunoglobulin G

The specific role for IgG against gastrointestinal nematode infection is less clear than IgA and IgE but there is some evidence for its response to infection in sheep (Sweeney et al., 2016). A significant negative correlation ($r= 0.83$; $p <0.05$) has been observed between IgG and FEC in resistant Blackbelly sheep infected with *H. contortus* (Muñoz-Guzmán et al., 2006). Additionally, resistant breeds such as Merino (Gill et al., 1993) and Texel (Sayers et al., 2007) showed higher levels of IgG1 and IgA than their susceptible counterparts. Larval-specific IgA and IgG2 were also found earlier in sheep of resistant lines in comparison to susceptible lines in secondary T. colubriformis challenge (Pernthaner et al., 2006). High levels of specific IgA and IgG against T. colubriformis were also observed in Santa Ines sheep suggesting prevention of larval establishment into adult stage (Cardia et al., 2011). However, IgG1 has been shown with a lesser response than IgA against *H. contortus* infection based on the comparison between the numbers of IgG1- and IgA-containing cells in the abomasum of infected sheep (Schallig, 2000).

1.6.3 Eosinophilia

Eosinophilia, an increase in the number of eosinophils in the blood, is characteristic of parasitic infection in which potent mediators may be involved in killing L3 during an infection (Sayers and Sweeney, 2005). The role of eosinophils against gastrointestinal nematode infection has mainly been based on relationship of eosinophilia with resistance status and histological observations of eosinophils in close proximity to gastrointestinal nematode larvae (Balic et al., 2000).

Electron microscopy from a study in an infected Merino sheep (Balic et al., 2006) has shown clear evidence of *H. contortus* L3 that are surrounded by eosinophils and collapsed due to vacuolation and degradation of their internal structures. Interestingly, even though no significant relationship was detected between tissue eosinophilia and number of adult *T. circumcincta* in Scottish Blackface sheep, tissue eosinophilia accounted for 40% of the variation in adult female *T. circumcincta* length among the same sheep (Henderson and Stear, 2006). These findings suggest that different immune mechanisms may have evolved in sheep against *H. contortus* and *T. circumcincta*, or that different breeds of sheep may have different protective immune responses towards gastrointestinal nematode infection, particularly in the action of eosinophils. In addition, spectrum of responses may be up or down-regulated depending on the intensity of nematode infection.

Several studies have found increased levels of peripheral and local eosinophils in resistant breeds of sheep, including Crioula Lanada, Barbados Blackbelly and Gulf Coast Native breeds (Bricarello et al., 2004; Muñoz-Guzmán et al., 2006; Terefe et al., 2009; Shakya et al., 2011). Peripheral eosinophil counts were also higher in Scottish Blackface sheep with lower FEC, but only in lambs at least 3 months of age following natural, predominantly *T. circumcincta* infection. These findings suggest that peripheral eosinophil levels are a useful measure of resistance to gastrointestinal nematode infection, but only in older sheep that have been constantly exposed to infection (Stear et al., 2002). In contrast to these findings in sheep, there was no significant difference observed in peripheral eosinophil counts between resistant and susceptible Creole goats throughout deliberate infection with *H. contortus* (Bambou et al., 2009).

Muñoz-Guzmán et al. (2006) found that FEC in resistant Blackbelly sheep had a significant positive correlation with eosinophil counts in the fundic abomasal region ($r=0.42$; $p<0.05$), pyloric abomasal region ($r=0.19$; $p<0.05$) and abomasal lymph nodes ($r=0.30$; $p<0.05$) and that the eosinophil counts were also significantly higher than in the susceptible Columbia sheep. Higher densities of eosinophils have been detected in the gastrointestinal mucosa of resistant Merino lambs infected with *H. contortus* in comparison to the random-bred

lambs (Gill et al., 2000). These findings suggest that local eosinophils play an important role in the protection against gastrointestinal nematode in resistant sheep.

However, the relationship between peripheral and local eosinophilia is relatively weak in Scottish Blackface sheep, with only a proportion of circulating eosinophils moving into the abomasal mucosa following deliberate infection of *T. circumcincta* (Henderson and Stear, 2006). This finding suggests that peripheral eosinophil counts may not be reflective of eosinophil levels at the site of infection, which is located at the abomasal mucosa. .

1.6.4 Packed cell volume (PCV)

Packed cell volume (PCV) is a measurement of red blood cell proportion in the whole blood (also contains plasma and white blood cells), which is expressed as percentage volume. PCV is a useful parameter of gastrointestinal nematode resistance for breeding selection when haematophagous parasites such as *H. contortus* dominate the infection (Gauly and Erhardt, 2001). Low PCV is commonly related to high FEC due to the blood sucking activity of adult gastrointestinal nematode in the abomasa of infected animals (Baker et al., 2003). Consequently, the effects of haematophagous parasites such as *H. contortus* on animal health are generally monitored by PCV (Saddiqi et al., 2012). A number of resistant sheep breeds including St. Croix, Red Maasai, Florida Native, Gulf Coast Native and Crioula Lanada have been shown to have higher PCV than susceptible breeds when infected with *H. contortus* (Zajac et al., 1990; Mugambi et al., 1996; Amarante et al., 1999; Li et al., 2001; Bricarello et al., 2004). PCV is not a useful measure however for non-haematophagous parasites such as *T. circumcincta*.

1.6.5 Number and length of gastrointestinal nematodes

Nematode length is an important measure because length is strongly correlated with fecundity which then influences FEC. Nematode length in turn depends on the IgA response and the density-dependent effect of the nematode burden in the infected host (Bishop and Stear, 2000). The number and fecundity of the nematodes regulate the egg deposition onto pasture and consequently the number of available L3 to be ingested by the host (Stear et al., 1995a; 1999b).

In *T. circumcincta*, there is a strong phenotypic correlation between nematode length and the number of eggs *in utero* indicating that nematode length is associated with fecundity ($r=0.7$; $p<0.001$) (Stear et al., 1997). Female *T. circumcincta* length and therefore the numbers of eggs *in utero* is the major factor influencing FEC for this gastrointestinal nematode species (Stear et al., 1995a; 1999b). In *H. contortus,* nematode

length has also been shown to be positively correlated (r=0.847) with the numbers of eggs *in utero*. In the same study, increased eosinophils, mast cells and globule leukocytes were found to have significant associations ($p<0.01$) with a reduction of female *H. contortus* length and with *in utero* egg numbers (Lacroux et al., 2006).

The average length of adult female *T. circumcincta* and the number of eggs *in utero* have also been found to have high heritabilities of 0.62 ± 0.20 and 0.55 ± 0.19 respectively, showing that length is under strong genetic control. The high heritability of nematode length suggested that genetic variation in the host accounted for almost twice as much of the variation in mean nematode length as all other factors combined (Stear et al., 1999a). The pathogenicity of a gastrointestinal nematode infection is more closely related to the length of gastrointestinal nematode, rather than the numbers of infecting gastrointestinal nematodes, as suggested by Stear et al. (1999a). Nematode length or size is a plausible criterion of pathogenicity because larger gastrointestinal nematodes require more nutrients, release more secretory or excretory products, and thus cause more damage to the host than the smaller gastrointestinal nematode.

Nematode number might also be assumed to be important in pathogenicity. However, evidence from studies in sheep shows a strong genetic correlation (-0.8) between bodyweight and FEC, indicating that the genes that control FEC are those that also control bodyweight (Bishop et al., 1996). Nematode length, but not number, has been shown to be under strong genetic control in lambs (Stear et al. 1997). Studies also show that plasma pepsinogen were more strongly associated with variation in the mean length of the adult female nematodes than with variation in the number of nematodes present (Stear et al., 1999c). Together these results indicate that pathogenicity is more associated with length than number. However, a potential role of number is also made difficult to disentangle, due to the density-dependent effect on fecundity in which female *T. circumcincta* fecundity declines when the nematode burden increases (Bishop and Stear, 2000).

These findings in sheep demonstrate that (a) gastrointestinal nematode length increases as fecundity increases; (b) both gastrointestinal nematode length and fecundity decrease as gastrointestinal nematode numbers increase; and (c) lambs are able to control gastrointestinal nematode length more effectively than gastrointestinal nematode numbers (Stear et al., 1995a; 1999a; 1999b). Nevertheless, mature sheep may have a greater ability to control gastrointestinal nematode numbers and fecundity than lambs due to the acquisition of effective immune responses, possibly immediate hypersensitivity reactions against incoming third-stage larvae (Stear et al., 1999c).

1.7 Key questions and thesis objectives

The relatively few studies in goats have shown that they respond differently to gastrointestinal nematode infection compared to sheep. The findings also suggest that the immune response of goats is not protective against nematode infection. However, goats have received much less attention than sheep regarding their interaction with nematodes, even though this small ruminant species has become an important source of meat-based protein due to its increasing population worldwide. Therefore, this study was designed to fill in the knowledge gaps underlying the interaction between goats and its common nematodes in temperate and tropical countries namely *T. circumcincta* and *H. contortus* respectively.

Specifically, this PhD study investigated the immune responses (as represented by IgA activity and peripheral eosinophil counts) of Boer goats, an important meat breed goat and their impact on FEC, PCV and bodyweight as the consequences of gastrointestinal nematode infection. This study also used a deliberate infection study design to explore the potential interaction between *H. contortus* and *T. colubriformis* which are commonly found together during natural infection.

The objectives of this thesis are:

1. To determine the distribution of FEC among Boer goats in natural and deliberate co-infections that were dominated by *T. circumcincta* and *H. contortus*, respectively.

2. To identify the significant explanatory variables for FEC, body weight and PCV among Boer goats during natural and deliberate co-infection.

3. To estimate the heritability and repeatability of FEC, IgA, peripheral eosinophil counts and PCV in Boer goats following natural and deliberate co-infection.

4. To estimate the heritability of bodyweight among naturally infected Boer goats.

5. To determine the relationships between phenotypic variables (FEC, IgA and peripheral eosinophil counts) during the course of infection and nematode variables (number, length and index) at necropsy.

6. To examine whether there are observable interactions between *H. contortus* and *T. colubriformis* following deliberate infection in Boer goats.

Chapter 2: General Materials and Methods

The parasitology methods described in this chapter from Section 2.1 to 2.4 were based on protocols in the Manual of Veterinary Parasitological Laboratory Techniques provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986).

2.1 Modified McMaster technique

The Modified McMaster technique is a quantitative technique to demonstrate and count nematode eggs in the faeces of herbivores. The eggs are floated in a known volume of faecal suspension and counted microscopically on a McMaster slide (Lyndall-Murphy, 1993). The modified version of the original McMaster technique to count nematode eggs (Gordon and Whitlock 1939) allows a minimum of 50 epg to be detected. The faecal samples were kept at 4°C and processed for McMaster technique within one to two days after collection.

Faecal pellets weighing 3 g were collected from the rectum of each goat and were broken up in 42 ml of water by homogenizing. The faecal suspension was then filtered through a fine mesh sieve (aperture 250 µm). The filtrate was then collected, agitated and poured into a 15 ml test tube before centrifugation at 2500 rpm for 5 minutes. The supernatant was poured off and discarded. The tube was agitated until the sediment formed a homogenous sludge at the bottom of the tube. The tube was then filled with 45 ml of saturated sodium chloride (NaCl) solution. Then, the suspension was mixed thoroughly by inverting the tube six times. A Pasteur pipette was used to withdraw an aliquot from the suspension to fill one McMaster chamber which takes 0.15ml. After further mixing, a second aliquot was withdrawn to fill another chamber. All eggs under the two separated grids of a McMaster slide were counted microscopically.

Counting eggs from two chambers each containing 0.15ml of the 45ml suspension from 3g of faeces means that the procedure is counting the number of eggs in

$$\frac{2 \times 0.15 \text{ ml NaCl} \times 3 \text{ g}}{45 \text{ ml NaCl}} = 1/50 \text{ of a gram of faeces}$$

Therefore, the number of eggs per gram of faeces (epg) was obtained by multiplying the total number of eggs in the two grids by 50. In this study, each faecal sample was counted on two McMaster slides with two chambers each to improve the likelihood of detection. Thus a minimum 25 epg could be detected (Beraldi et al. 2008).

2.2 Faecal culture

Unlike nematode eggs, which are typically similar in size and shape, L3, the infective larvae stage, are morphologically distinct and readily differentiated (Lyndall-Murphy, 1993). Faecal culture therefore allows the FEC to be broken down by nematode genus and the predominant infecting nematodes to be identified.

The faecal culture method works by providing a suitable environment for the nematode eggs to hatch and develop into third stage larve. Three to five faecal pellets were taken from the fresh faecal sample of each animal to be pooled for faecal culture. The faeces were broken up finely using either mortar and pestle or gloved hand depending on the faecal consistency to give a crumbly mixture. The faecal mixture of 10 to 20 g was then packed in a container and moistened with distilled water. A moistened filter paper or gauze was used to cover the packed faeces and checked daily for moisture content. Distilled water was sprinkled on the filter paper or gauze when it appeared dry. The faecal culture was incubated at room temperature for 14 to 16 days to harvest the L3 of gastrointestinal nematodes associated with temperate environments such as *T. circumcincta* and *Trichostrongylus* spp., and for 7 days to harvest the L3 of gastrointestinal nematodes associated with a tropical environment such as *H. contortus, Trichostrongylus* spp. *and Oesophagostomum* spp.

On the final day of incubation, the filter paper or gauze was removed. Next, the container was filled with lukewarm distilled water. The container was covered with a petri dish and inverted. Afterwards, the petri dish was filled with lukewarm distilled water and allowed to stand for 30 minutes for larval migration from the faecal culture. Distilled water containing third stage larvae, L3 in the petri dish was pipetted into a universal bottle and stored at 4 ºC.

2.3 Enumeration and identification of L3

A pipette was used to transfer 0.25 ml larval suspension into a cavity block. The L3 were killed and stained by adding a drop of Lugol's iodine to the larval suspension. The killed L3 appeared to be straightened. Free living nematodes appeared more yellow under staining whereas infective stage larvae were less stained. A drop of stained larval suspension was placed on a microscope slide, covered with a coverslip and examined under x10 objective of a compound microscope. The L3 were then enumerated and differentiated by observing the morphology of the head and sheath tail based on the manual provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986).

2.4 Total worm counts, identification, sexing and measurement of nematode length.

The abomasum from each slaughtered goat was ligated with a string to separate it from the omasum and duodenum. Scissors were used to open the abomasum along the greater curvature to empty the abomasal contents into a gradated bucket. The emptied abomasum was washed thoroughly between the folds of mucosal membrane into the bucket. The total volume of abomasal contents and washings was made up to 1 L with tap water. A ladle was used to mix the bucket contents thoroughly.

A total of 100 ml of the bucket contents was transferred into a glass jar. The jar was filled with tap water and fitted with a secure sieve-like lid. The jar was tilted slightly in an inverted position and vigorously shaken until most of the fluid contents were removed. This process was repeated until the contents appeared clear with the final volume of approximately 100 ml. The final contents of the jar were then transferred into a 120 ml specimen bottle. The nematodes were preserved by adding 5 ml of 75 % ethanol to the specimen bottle.

The contents in the specimen bottle were thoroughly mixed and poured into a petri dish in small volumes until finished. The nematode were counted, identified, individually sexed and measured under a stereo microscope. The total worm count for each goat was determined by assuming a dilution factor of 1:10 (Lyndall-Murphy, 1993).

The same process was used to count and identify nematodes in the small and large intestines. The only difference was that the ligation separated the small intestine from duodenum and large intestine. The contents of abomasum, small intestine and large intestine were processed separately.

2.5 Peripheral eosinophil counts

The method to count peripheral eosinophils followed Stear et al. (2002). Carpentiers eosinophil counting solution was prepared by adding 2 ml of 2% aqueous solution of Eosin Y, 3 ml of 40% formaldehyde saturated with calcium carbonate and 95 ml of distilled water. A 10 µl sample of blood was added to 90 µl Carpentiers solution and the eosinophils were counted in a haemocytometer. The preserved blood was stored at room temperature. Each cell counted represented 5.6 cells/µl of whole blood.

2.6 Packed cell volume estimation

Blood collected in an EDTA tube was transferred using capillary action into a glass capillary tube. The unfilled end of the tube was sealed with Cristaseal (Hawskley and Sons Ltd) by holding the tube at a 90^{o} angle to the Cristaseal tray. The tube was pushed to the bottom of the tray and twisted before removal from the tray. The tube was placed in a numbered slot with sealed end pointing outwards and touching the rim gasket. The rotor lid was screwed down firmly and the lever was placed in the locked position before operating the centrifuge. The PCV was determined by centrifuging the blood in a capillary tube at 700 relative centrifugal force (rcf) for five minutes and read with a rotoreader.

2.7 Storage of plasma and buffy coat

Blood collected in an EDTA tube was centrifuged at 2500 rpm at 20^{o} C for 20 minutes and was separated into three layers; namely plasma, buffy coat and erythrocytes. Clear plasma in the topmost layer was pipetted into a 7 ml bijou bottle. The buffy coat, which appeared as a thin whitish layer in the middle that contained leucocytes and platelets, was pipetted into a 1.5 ml eppendorf tube. Erythrocytes in the lowest layer were discarded. The plasma and buffy coat were stored at -20^{o} C. The plasma was used for ELISA IgA and the buffy coat was kept for future research.

2.8 Preparation of nematode antigen

The initial stock of *T. circumcincta* L3 was a gift from Dr. David Bartley, Moredun Research Institute, Edinburgh. These larvae were used to infect twin lambs reared at Cochno Farm, Glasgow in order to harvest sufficient L3 from the faeces to prepare nematode antigen. The pooled faeces of the lambs were subjected to larval culture three times a week for two months (see section 2.2).

As no monospecies stock of L3 was available in Malaysia, the pooled faeces of deliberately infected Boer goats were subjected to larval culture to obtain L3 stock as the source of nematode antigen for the ELISA assay. The final ratio of L3 stock of *Haemonchus contortus: Trichostrongylus* sp*: Oesophagostomum* sp. was 14:2:1.

Preparation of L3 nematode antigen for ELISA assay in this study was performed using the same method as for fourth-stage larvae (L4) described by Strain et al. (2002). The solutions used were prepared as described in Appendix A. Approximately 50,000 infective stage larvae, L3 were centrifuged in 50 ml Falcon tube at 1000 rcf for 10 minutes. The larvae were left to settle for 10 minutes before removal of supernatant. Then, the larvae

were resuspended with 50 ml Phosphate Buffered Saline (PBS), centrifuged at 1000 rcf for 15 minutes and supernatant was discarded. Next, 25 ml of PBS containing an antibiotic solution was used to wash the larvae. The solution was then centrifuged at 1000 rcf for 10 minutes and supernatant was removed. Afterwards, 50 ml Tris-Poisons solution was used to wash the larvae, centrifuged at 1000 rcf for 15 minutes and supernatant was discarded. The pelleted larvae were then kept at -20° C. Later, the frozen pelleted larvae were resuspended in an equal volume of Tris-Poisons containing sodium deoxycholate solution. The suspension was then homogenized on ice and centrifuged at 2000 rcf or 20 minutes at 4° C. Consequently, the homogenate was filtered through 0.2 µm syringe filter and centrifuged again at 2000 rcf for 20 minutes at 4° C. Finally, the supernatant was divided into aliquots in 1.5 ml Eppendorf tubes and kept at -20° C for subsequent use. The protein concentration of parasite antigen was estimated with bicinchonic acid and adjusted to 5 µg/ml in 0.06 M bicarbonate buffer (pH 9.6) before use.

2.9 ELISA IgA

The activity of plasma IgA responses were measured by an indirect ELISA as described by Abuargob and Stear (2014). To detect responses in goats the protocol was optimised as described in the following paragraph.

Each well of a flat-bottomed microtiter plate was coated with 100 µl of parasite antigen prepared at 5 µg/ml in 0.06 M bicarbonate buffer at pH 9.6. The plate was incubated overnight at 4° C. Then, the antigen was discarded and 200 µl of blocking buffer containing 4% of dried skimmed milk powder was added to each well. The plate was incubated at 37° C for 2 hours. The plate was washed five times in PBS pH 7.4 containing 0.05 % of Tween 20 (PBS-T). Subsequently, 50 µl of non-diluted plasma sample was added in triplicate and incubated at 37° C for 30 minutes. After five washes in PBS-T, 100 µl of mouse anti-bovine/ovine monoclonal IgA (diluted 1:100 in PBS-T) was added to each well and incubated at 37° C for 30 minutes. Next, the plate was washed five times with PBS-T before adding 100 µl of goat anti-mouse immunoglobulins conjugated to horseradish peroxidase (diluted 1: 2000 with PBS-T) and incubated at 37° C for a further 30 min. After a final four washes with PBS-T and two washes with distilled water, the wells were developed by adding 100 µl of the mixture of 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide (1 : 1). The plate was incubated at room temperature for 10 minutes. The reaction was stopped with 100 µl of 2 M sulphuric acid. The buffer and solutions were prepared as described in Appendix A. The optical density (OD) of each well was read at 450 nm with an ELISA plate reader. A positive control was prepared by pooling plasma samples from six different goats which had the highest IgA optical densities in the preliminary studies. In this experiment, foetal bovine serum was used as negative

control. The positive and negative controls were also run in triplicate. The IgA OD index for each sample was estimated by the following formula:

$$\text{IgA OD index} = \frac{\text{Sample mean – Negative control mean}}{\text{Positive control mean – Negative control mean}}$$

The reagents and preparation of ELISA buffers and solutions is provided in Appendix A.

2.10 Statistical methods

2.10.1 Introducing the animal model

In this and the following sections, an overview is presented of the most important statistical method used in this thesis, namely the animal model. Specific details of its use to analyse specific data sets are also presented in the methods sections of Chapter 3 and Chapter 4.

The animal model (Wilson et al., 2010) has been frequently used to analyse datasets that comprise of observations on individuals when pedigree data are available. The animal model is a form of mixed effects model that disentangles phenotypic variance into genetic and environmental sources. The animal model is also used to estimate the key parameters such as the heritability of a trait or the genetic correlations between traits. This approach requires the information of relatedness or kinship among individuals in a population (Wilson et al., 2010). To put the animal model in context, in this section, the animal model is introduced by beginning with a simple linear model, its development into a generalized linear model then a generalized linear mixed model and ultimately to the animal model.

2.10.2 Linear models and linear mixed models

2.10.2.1 Linear models describe a continuous response variable as a function of one or more predictor variables. If we assume that the data refer to a set of observations on individuals, the simple linear model is expressed as

$$y = X\beta + \varepsilon \tag{2.1}$$

where β is a vector of regression coefficients and *X* is matrix of observations where each row of the matrix *X* contains the set of measured predictors for each individual.

Expressed instead for each individual, i, the linear model has the following form:

$$y_i = X_i\beta + \varepsilon_i \tag{2.2}$$

where X_i is a row vector of measured predictors for individual *i*.

2.10.2.1 Linear mixed models are an extension of linear models to include random effects associated with groups of observations (Paterson and Lello, 2003) and also assume normally distributed errors (Bolker, 2015). If p observations are made on the ith individual to give a vector of observations $y_i = (y_{i1,\dots}\, y_{ij,\dots}\, y_{ip,})$, and there are *k* predictor variables, a linear mixed model will take the form:

$$y_i = X_i\beta + Z_i b_i + \varepsilon_i\,, \tag{2.3}$$

where y_i is a column vector of length *p* as the outcome variable; X_i is a $p \times k$ matrix of the k predictor variables; β is a $k \times 1$ column vector of the fixed effects regression coefficients; Z is the $p \times r$ design matrix for the r random effects; b is a $r \times 1$ vector of the random effects (the random complement to the fixed β); and ε is a $p \times 1$ column vector of the residuals (Institute for Digital Research and Education, UCLA, 2017). The random effects influence the variance of the trait while the fixed effects are unknown constants that affect the mean of a distribution.

2.10.3 Generalized linear models (GLM)

Generalized linear models can be used when data are not normal (Paterson and Lello 2003). This is the case, for example, in most parasitological studies, where count data which is characterized by aggregation and high proportion of zeroes typically violate the assumptions of normality (Sebatjane et al., 2019). Although response variables can be transformed to meet the linear model assumptions, it is often difficult to find a transformation that simultaneously linearizes the mean and gives constant variance (O'hara and Kotze, 2010).

Therefore, these issues lead to the need for generalized linear models (GLMs) which are the extension of the general linear model framework to include non-normal response variables like FEC.

GLMs generally take the form:

$$\begin{aligned} E[y_i] &= \mu_i \\ g(\mu_i) &= X_i\beta, \end{aligned} \tag{2.4}$$

where $g(.)$ is a link function used to relate a vector of predictor variable, X_i associated with the response variable y_i to the expected value of y_i, μ_i (Paterson and Lello, 2003).

A GLM is made up of (a) a linear predictor that describes how the different predictor variables affect the expected value of the response variable, (b) a probability distribution called as 'family' or 'error structure' that is assumed to describe the response variable and (c) a link function that describes the mathematical relationship between the expected value of the response variable and the linear predictor (Beckerman et al., 2017). In the statistical software R, the family and its default link function of GLMs are specified as shown in Table 2-1.

Table 2-1. List of family and link function of generalized linear models specified in R (adapted from Kabacoff 2011).

Family	Default link function
binomial	(link = "logit")
gaussian	(link = "identity")
gamma	(link = "inverse")
inverse.gaussian	(link = "1/mu^2")
poisson	(link = "log")
quasi	(link = "identity", variance = "constant")
quasibinomial	(link = "logit")
quasipoisson	(link = "log")

2.10.4 Generalized linear mixed models (GLMM)

Although GLMs can deal with various non-normal error distributions, they are not suitable for all kinds of parasitological data because the data violate the GLM assumption that the data are independent (Paterson and Lello, 2003), for example when there are multiple observations on an individual.

These data can be analysed by generalized linear mixed models (GLMMs) that combine the properties of (a) linear mixed models by incorporating random effects and (b) generalized linear models that handle non-normal data by using specified distributions and link functions. These properties make GLMMs the best tool to analyse non-normal data that involve random effects (Bolker et al., 2008).

The random effects can be added to generalized linear models (equation 2.4) to give the corresponding generalized linear mixed model, GLMM, given by the following:

$$E[y_i|b] = \mu_i \qquad (2.5)$$
$$g(\mu_i) = X_i\beta + Z_i b.$$

2.10.5 The animal model

The animal model in its simplest form is a type of linear mixed effects model where the additive genetic value of individual animals is treated as a random effect. The term 'animal model' arises merely because the model is defined at the individual animal level (Kruuk, 2004). In the simplest case of a phenotypic trait (y) in an individual (i), the animal model can be written as:

$$y_i = \mu + a_i + e_i \quad (2.6)$$

where μ is the mean of the population, a_i stands for the breeding value and e_i is a residual term (Kruuk, 2004), which is a simplified version of the general mixed model (2.5). The main assumptions of an animal model are (a) the observed trait is normally distributed but this assumption can be dropped by using generalized models (Sorensen and Gianola 2002), (b) the breeding value a_i is normally distributed and correlated among related individuals, with the pedigree determining the structure of the correlation between individuals by taking their kinship into account and (c) the residual e_i is normally distributed, uncorrelated and independent of the breeding value (de Villemereuil, 2012).

The animal model decomposes the phenotypic variance into genetic and environmental sources and can be used to estimate the repeatability and heritability of a trait. Greater phenotypic similarity between more closely related individuals allows inference about the genetic effects underlying the phenotypic variance. Hence, the animal model requires information on the relatedness between individuals in a population where an individual's breeding value, a_i, is included as the predictor variable which is also treated as a random effect for a phenotypic trait of interest (Wilson *et al.*, 2010).

The variance estimates provided by the animal model are for a base population from which all other individuals in the population are assumed to have descended, in practice the first generation of animals in the data (Kruuk, 2004). Because the animal model accounts for the flow of genetic information across generations, it is not affected by issues of small population size, inbreeding or assortative mating (Kruuk, 2004). A population pedigree determines how the breeding values should covary among individuals. The closer the relatedness between two individuals and the higher the additive genetic variance V_A underlying the trait, the greater the expected covariance between the individuals.

The expected additive genetic covariance for a pair of individuals i and j is equal to $2\theta_{ij}V_A$ where θ_{ij} is a parameter known as the coefficient of coancestry. Doubling the θ_{ij} produces the relatedness or kinship values, which are 0.5 for parent-offspring pairs and full-

siblings, 0.25 for half-siblings and 0.125 for first cousin (Wilson *et al.*, 2010) (see Table 2-3). Table 2-2 shows an example of a pedigree which determines the kinship between animals shown in Table 2-3. The animals with identifier A1, A2, A3 and A4 in Table 2-2 are known as the founders because they are the earliest generation and for which the parentage information is unknown or missing.

Table 2-2. Example of a pedigree data frame (adapted from de Villemereuil, 2012)

No.	Animal	Mother	Father
1	A1	NA	NA
2	A2	NA	NA
3	A3	NA	NA
4	A4	NA	NA
5	A5	A2	A4
6	A6	A1	A3
7	A7	A1	A3
8	A8	A1	A3
9	A9	A2	A4
10	A10	A2	A4

Table 2-3. Kinship values for pair of individuals based on the pedigree data frame shown in Table 2-2 (adapted from de Villemereuil, 2012)

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
A1	1	0	0	0	0	0.5	0.5	0.5	0	0
A2	0	1	0	0	0.5	0	0	0	0.5	0.5
A3	0	0	1	0	0	0.5	0.5	0.5	0	0
A4	0	0	0	1	0.5	0	0	0	0.5	0.5
A5	0	0.5	0	0.5	1	0	0	0	0.5	0.5
A6	0.5	0	0.5	0	0	1	0.5	0.5	0	0
A7	0.5	0	0.5	0	0	0.5	1	0.5	0	0
A8	0.5	0	0.5	0	0	0.5	0.5	1	0	0
A9	0	0.5	0	0.5	0.5	0	0	0	1	0.5
A10	0	0.5	0	0.5	0.5	0	0	0	0.5	1

The matrix given in Table 2-3 is the additive genetic variance-covariance matrix A and is included in the animal model to estimate the associated variance component, V_A. Note that the off-diagonal elements between the founders are zero because they are assumed to be unrelated. The diagonal elements are 1 for all individuals because they are perfectly related to themselves.

The breeding values, a_i and the residuals, e_i in the simple animal model shown in (2.6) are assumed to be normally distributed as follows:

$$\begin{pmatrix} a_1 \\ \dots \\ a_n \end{pmatrix} \sim N(0, \boldsymbol{A}V_A); \quad \begin{pmatrix} e_1 \\ \dots \\ e_n \end{pmatrix} \sim N(0, \boldsymbol{I}V_R) \tag{2.7}$$

The additive genetic variance, V_A is derived from the breeding values, a_i; and the residual variance, V_R is derived from residuals, e_i that are normally distributed

2.10.5.1 Heritability estimates

The estimates of additive genetic variance, V_A and residual variance, V_R are key outcomes from an animal model. These two variance components sum to give total phenotypic variance, V_P. These variances are used to estimate the heritability, which is a measure of how much variability in a trait is likely to be transmitted from generation to generation. The heritability, h^2 ,is given by:

$$h^2 = \frac{V_A}{V_P} = \frac{V_A}{V_A + V_R} \tag{2.8}$$

generating a value that lies between 0 and 1 (de Villemereuil, 2012). The heritability is the proportion of the phenotypic variance due to additive genetic effects after accounting statistically for differences in the effects such as type of birth, date of birth, sex and year (Stear et al., 1997).

2.10.5.2 Repeated measures and repeatability

When multiple measurements are available for individual animals, rather than using a single average value for each animal, all available measurements can be exploited in the mixed model framework underlying the animal model. Repeated measures can readily be incorporated within the animal model framework by including a further random effect i.e. estimation of a further component of variance, which defines the permanent environment common to all observations on the same individual other than those that are due to the additive genetic effects (Kruuk, 2004).

For instance, an individual that experiences a particular environment during its early growth may develop persistent effects that last throughout the adulthood (Kruuk et al., 1999; Lummaa and Clutton-Brock, 2002; Marshall et al., 2017). The repeated measurements will divide the permanent environmental effects between individuals from the residual error variance (Kruuk, 2004). The repeatability of a phenotypic trait can be considered as the heritability plus the variance due to permanent environmental effects.

The repeatability of a trait can be estimated as the proportion of a phenotypic variance explained by the individual identity. This is achieved by including ID and additive genetic variance as random effects as well as residual variance, generating estimates for the respective variance components denoted V_{ID}, V_A (the additive genetic variance as above) and V_R. The repeatability of a phenotypic trait (Wilson et al. 2010) can be estimated by:

$$Repeatability = \frac{V_A + V_{ID}}{V_A + V_{ID} + V_R} \quad (2.9a)$$

In the case that there is no 'animal' random effect specified, the repeatability would be given by

$$Repeatability = \frac{V_{ID}}{V_{ID} + V_R} \quad (2.9b)$$

2.10.5.3 Non-Gaussian traits

In the same way that standard linear mixed models can be extended to accommodate non-Gaussian data, the animal model can also be used when data are non-Gaussian by using appropriate link functions. The Bayesian approach offered by the MCMCglmm package in R is viewed as particularly advantageous for non-Gaussian distribution (Wilson et al., 2010).

2.10.6 Implementing the animal model

There are multiple packages that can be used to implement the animal model including ASReml, ASReml-R, WOMBAT and MCMCglmm packages (Wilson et al., 2010). In this study, the MCMCglmm package (Hadfield, 2010) available in R, which takes a Bayesian approach, was used.

2.10.6.1 MCMCglmm R package

Generalizing linear mixed models to non-Gaussian data can be difficult to implement because of the challenges involved in integrating over the random effects. Markov Chain Monte Carlo (MCMC) provides a robust alternative approach for estimating the random effects (Hadfield, 2010). The R package MCMCglmm fits generalized linear mixed models (GLMMs) in a Bayesian framework using MCMC methods (Houslay and Wilson, 2017).

The MCMCglmm package supports a range of distributions including zero-inflated Poisson distribution to explore the animal model as shown in Table 2-4.

Table 2-4. Types of distribution that can be fitted using MCMCglmm R package.

Types of distribution	R code for family argument
Gaussian	"gaussian"
Poisson	"poisson"
Categorical	"categorical"
Multinomial	"multinomial"
Ordinal	"ordinal"
Exponential	"exponential"
Censored gaussian	"cengaussian"
Censored gaussian	"cenpoisson"
Censored exponential	"cenexponential"
Zero-inflated poisson	"zipoisson"
Zero-truncated poisson	"ztpoisson"
Hurdle poisson	"hupoisson
Zero-altered poisson	"zapoisson"
Zero-inflated binomial	"zibinomial"

Although MCMC can be challenging, its implementation via the package MCMCglmm is simple and straightforward for a novice user (Van Ravenzwaaij et al., 2018). This relative ease of use comes from the fact that the syntax for defining models is similar to that for standard models in R (as demonstrated in Chapters 3 and 4). In comparison to the other packages that implement MCMC methods such as WinBUGS (Spiegelhalter et al., 2003), MLwiN (Rasbash et al., 2005), glmmBUGS (Brown and Zhou, 2009) and JAGS (Plummer, 2003) which require more coding expertise, MCMCglmm can be used by novice users, is relatively quick to run and also supports several types of variance structures for random effects and residuals. These include the interactions with continuous and categorical variables as well as more complicated variance structures such as through a pedigree or a phylogeny (Hadfield, 2010).

2.10.6.2 Markov Chain Monte Carlo

Markov Chain Monte Carlo (MCMC) is a combination of two processes namely Monte Carlo and Markov chain. Monte Carlo means using random sampling methods to generate numerical results e.g. a simple case would be to draw a large number of random samples from a normal distribution and calculate the sample mean. The Markov chain component generates the random samples by a special sequential process where each random sample is used as a stepping stone to produce the next random sample, producing a chain of samples. In a Markov chain, each new random sample depends on the one before it but is independent on any samples before the previous one (Van Ravenzwaaij et al., 2018). MCMC generates random samples for fixed and random effects (Bolker et al., 2008). In this way, MCMC generates samples from distributions which can then allow us to calculate

information about those distributions, such as means, variances, and credible intervals (the Bayesian equivalent of a confidence interval).

Bayesian inference is conducted by using information provided by the observed data about a parameter, to update a prior state of beliefs about the parameter in order to become a posterior state of beliefs about the parameter. MCMC is designed to converge on the posterior probability distribution of the parameters that combines information on the likelihood of the parameter given the data with the prior belief about the parameter (Bolker et al., 2008; Van Ravenzwaaij et al., 2018).

2.10.6.3 Specifying the prior distribution

Markov Chain Monte Carlo (MCMC) is a method that can find the posterior distribution of the parameter of interest by taking Bayesian approach. The posterior comes from combining of the prior distribution with the likelihood of obtaining the observed data. The outcome in a Bayesian approach is a probability distribution (the posterior distribution) over the of parameter of interest. All inferences are then based on the posterior distribution.

A prior distribution of a parameter, for example the effect of IgA on growth, is the probability distribution that represents the uncertainty about this parameter before the data are examined. In the present study, the default prior in MCMCglmm were chosen because they are only weakly informative (Hadfield, 2017). The default prior is appropriate in this case as there was no pre-existing data or prior expectation to follow from previous study.

A standard choice for the prior distribution for variance components in MCMCglmm is an inverse-Gamma distribution. In the MCMCglmm package, this distribution is defined by two parameters, 'V' and 'nu' where nu is the 'degree of belief' parameter. The default values are to set V = 1 and nu = 0.002. When nu is small, the prior is considered weakly informative. This prior would be specified in MCMCglmm as follows:

```
prior <- list (R = list(V=1, nu=0.002), G = list (G1 = list(V=1, nu=0.002)))
```

The R argument is the prior on the residual variance while the G argument is for the random effect variances. The list of priors in G depends on the number of random effects, for example, the prior for a model with three random effects would be written:

```
prior <- list (R = list(V=1, nu=0.002),
               G = list (G1 = list(V=1, nu=0.002),
                         G2 = list(V=1, nu=0.002),
                         G3 = list(V=1, nu=0.002)))
```

The standard approach for priors on fixed effects is to use the defaults in MCMCglmm, which assumes a very wide normal distribution (de Villemeuil, 2012) resulting in a weakly informative prior. The default prior for fixed effects is a normal distribution with mean = 0 and a very large variance (10^{-6}, 10^{6}) which is considered as an uninformative prior (Hadfield, 2017). The example of an uninformative prior with a large variance are shown in Figure 2-1 and Figure 2-2 with a narrower x-axis scale which demonstrates that the prior is very flat (i.e. very uninformative) in the centre range where we expect our parameter estimates to fall. In this thesis, the default priors in MCMCglmm were chosen because they are only weakly informative (Hadfield, 2017). The use of default priors are appropriate as there was no pre-existing data or prior expectation to use from previous studies.

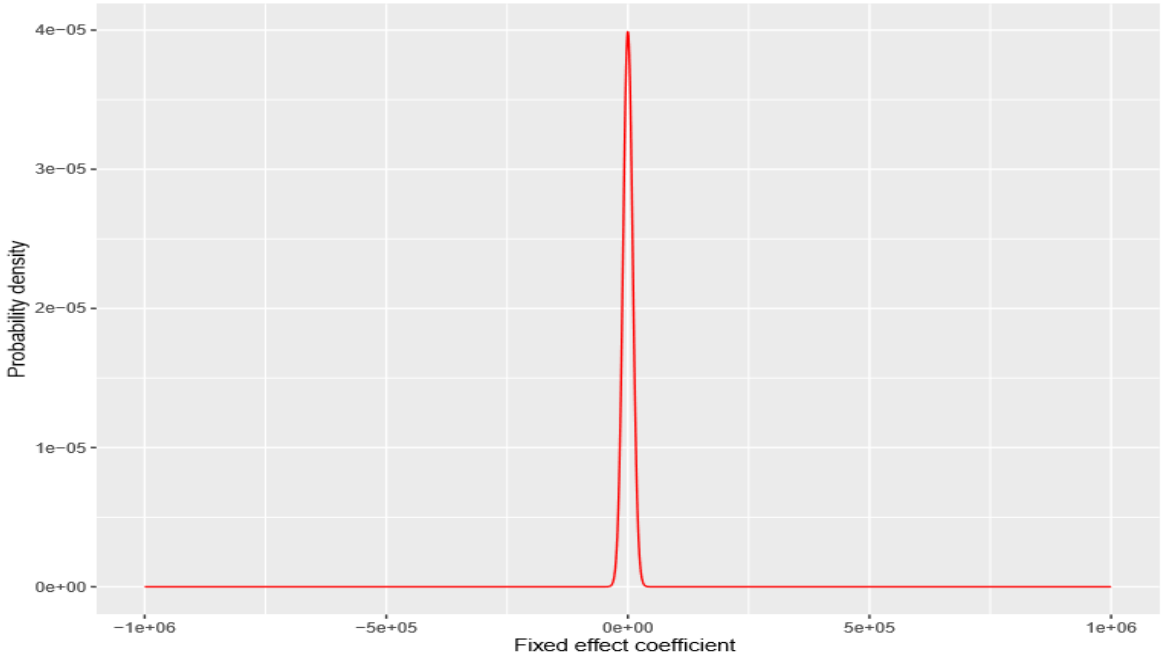


Figure 2-1. Uninformative prior with mean = 0 and a large variance of 10^{6} shown with a large x-axis scale.

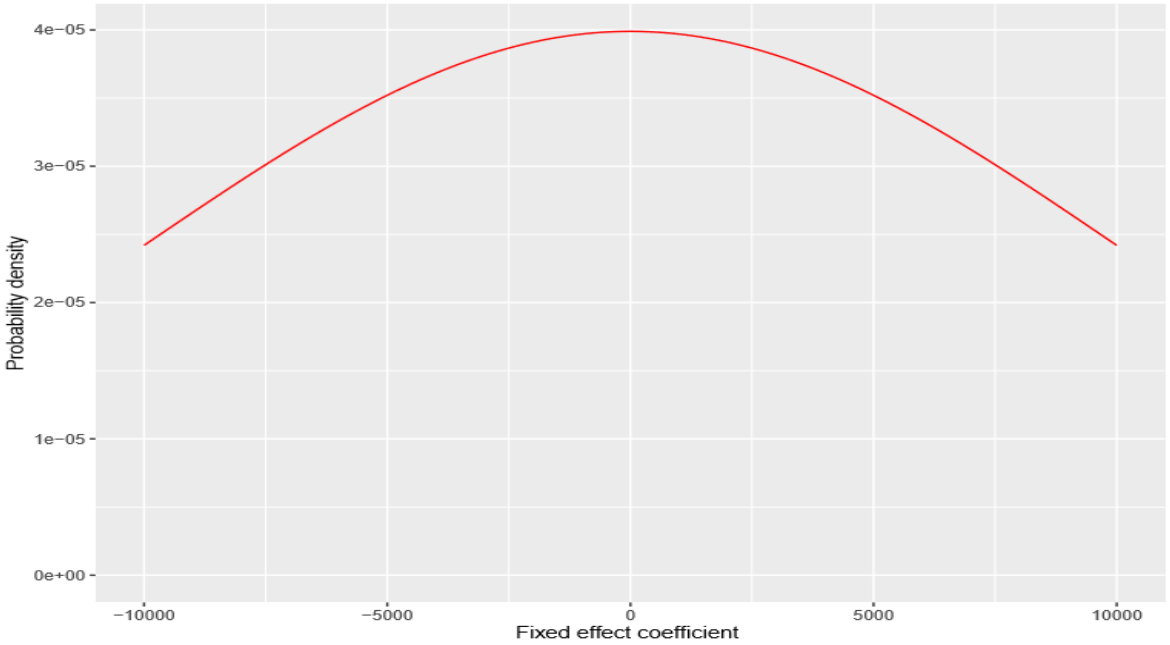


Figure 2-2. Uninformative prior with mean = 0 and a large variance 10^6 illustrated on a smaller x-axis scale.

2.10.6.4 MCMC diagnostics

The model output has two components which are model$Sol containing the fixed effects and model$VCV for the residual variance and random effects. All these estimates need to be monitored for convergence and autocorrelation. One way to observe convergence and mixing behaviour of the MCMC algorithm chains is by a simple graphical tool called the 'trace plot' of the posterior distribution. Examples of trace plots for fixed and random effects are shown in Figure 2-3 (left) and Figure 2-4 (left), respectively.

Figure 2-3 (left), shows the series of values of 9000 samples of the posterior distribution of model intercept (or mean μ) as the MCMC algorithm explores parameter space while the plot on the right (the density plot) shows the same data as distribution. Figure 2-4 shows corresponding plots for the estimates additive genetic variance (animal) and residual variance, denoted 'units' in MCMCglmm.

In a trace plot, waves or trends in the patterns indicate autocorrelations between successive samples from the posterior whereas ideally what should be seen is a 'fuzzy caterpillar' indicating that there is no trend in the trace and therefore little or no autocorrelation between samples (Sorensen and Gianola, 2002).

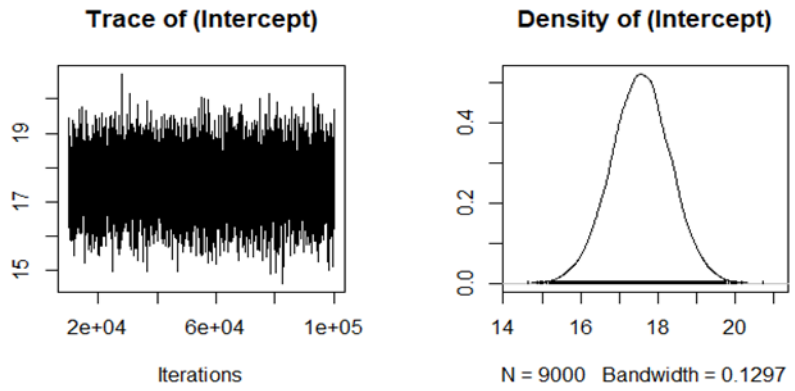


Figure 2-3. Trace plot (left) and density plot (right) of the posterior distribution of fixed effect (intercept).

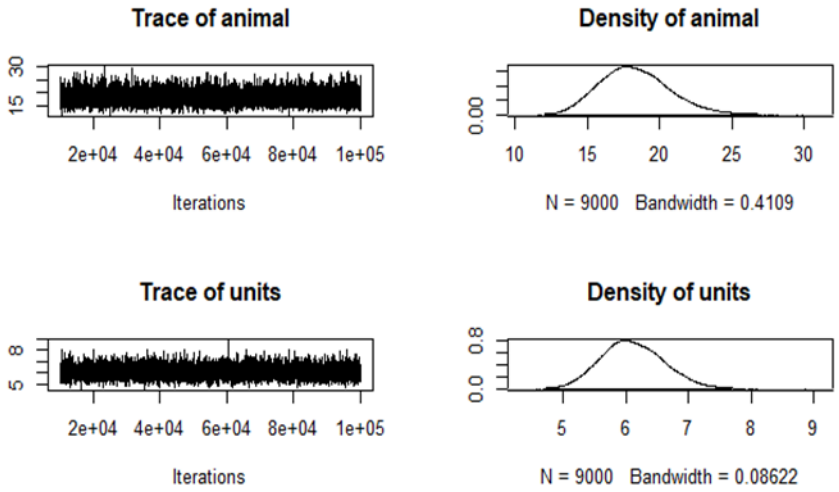


Figure 2-4. Trace plot (left) and density plot (right) of the posterior distribution of the animal random effect variance (top) and residual variance (bottom).

Another method for monitoring autocorrelation is by calculation of autocorrelation among samples at different Lags (see example shown in Table 2-5).

Table 2-5. Autocorrelation of intercept, animal and units components in an MCMCglmm animal model (adapted from de Villemereuil, 2012).

Lag	Autocorrelation		
	model$Sol (fixed effect)	model$VCV (random effects)	
	Intercept	Animal	Units
0	1.0000000000	1.00000000	1.0000000000
10	0.0157137339	0.40759941	0.2729110582
50	-0.0035557380	0.02437235	0.0109679990
100	-0.0013154271	0.01591581	0.0043086559
500	0.0004660904	0.01007559	0.0001932177

The term ‘Lag 10’ stands for autocorrelation between every 10th point in parameter space explored by the MCMC algorithm. These outputs are derived from a model with a ‘thin’ argument set to 10. If the autocorrelation is too large the table of autocorelations (e.g. Table 2-3) shows how much the autocorrelation can be reduced by choosing a larger ‘thin’ value. These example outputs show that there is little autocorrelation for the Intercept. However, the autocorrelation for variance components of the random effects only falls to suitable levels at Lag 50. In practice, an autocorrelation less than 0.1 is acceptable (de Villemereuil, 2012) and therefore a ‘thin’ value is chosen based on the Table of autocorrelations and Lags to produce an approximately independent sample (Kass et al., 1997). Adjusting the ‘thin’ parameter may mean the algorithm needs to be run for longer to achieve a sufficiently large effective sample size (see below for discussion of the effective sample size).

2.10.6.5 Output from MCMC function

The summary of the model output is as follows:

```
Iterations = 10001:99991
Thinning interval  = 10
Sample size  = 9000

DIC: 3203.707

G-structure:  ~animal

                 post-mean         l-95% CI          u-95% CI          eff.samp
 Units           1.048             0.7683            1.334             3787


R-structure:  ~units

                 post-mean         l-95% CI          u-95% CI          eff.samp
 Units           0.9989            0.8256            1.178             4747

Location effects: FEC ~ MONTH + IGA

                   post-mean      l-95% CI      u-95% CI      eff.samp   pMCMC
 (Intercept)      10.13              9.91          10.35          8721   <1e-04 ***
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

(adapted from de Villemereuil, 2012)

The first part of the output shows the MCMC parameters: the number of iterations, the thinning interval and sample size. The Deviance Information Criterion (DIC) associated with the model is calculated by MCMCglmm() by default (Wilson et al., 2010).

DIC has similar function to Akaike Information Criterion (AIC) (Akaike, 1974) in that it provides an estimate of the relative quality of statistical models in terms of fit to the data for a given set of data. The AIC value for a model is given by

$$AIC = D_{min} + 2k$$

where D_{min} is the minimum deviance which corresponds to the point in parameter space that maximises the likelihood. A good model is selected based on the minimum AIC among all the other models (Mohammed, Naugler and Far, 2015).

The DIC is a Bayesian version of AIC given by

$$DIC = \widehat{D} + 2p_D$$

where $\widehat{D}$is the deviance when using the mean of the posterior distribution and $p_D$is the effective number of estimated parameters (McCarthy, 2007). The maximum log likelihood of the posterior mean is the expectation of the log posterior predictive density which will be drawn approximately from the posterior distribution with MCMC (Paun et al., 2018). DIC balances model fit and model complexity simultaneously. Although the properties of DIC are not completely understood in the case of complex models, model comparisons can be performed using DIC where the minimum values of DIC are preferred (Wilson et al., 2010).

The effective sample size is important for ensuring a sufficiently accurate estimate of the posterior distribution of the parameter of interest. The effective sample size depends on the number of iterations, the thinning interval and also the autocorrelation between samples. The effect of autocorrelation will be to reduce the effective sample size. The larger the effective sample size the better, but in practice, an effective sample size above 1000 is recommended (de Villemereuil, 2012).

The output of the model is in three sections namely the G-structure, R-structure and location effects. Information about the variance estimates of the random effect are given by G-structure. In this example, the random effect is referred to as ‘animal’. The R-structure gives the information on the residual variance estimation which referred to as as ‘units’. Finally, the location effects section gives the information about the fixed effects. In this output, the location effects section shows the ‘Intercept’ or population mean μ.

These sections give the values of posterior mean of the posterior distribution (or MCMC sample mean), the lower and upper limits of 95% credible interval as well as the effective sample size for the particular parameters. The column called pMCMC provides the Bayesian p-value: it is the posterior probability that the effect parameter is different from zero. In this example, the values of credible interval and pMCMC indicate that the population mean is significantly different from zero (de Villemereuil, 2012).

Chapter 3: Response to gastrointestinal nematode infection among naturally infected Boer goats

3.1 Background

Gastrointestinal nematode infection is one of the most important diseases affecting small ruminants such as sheep and goats all over the world. However, the majority of the evidence about host-nematode interactions and control approaches have been derived from studies on sheep. The immune response is the major mechanism of defence against gastrointestinal nematodes (McRae et al., 2015).

In goats, the browsing habit is thought to make them less susceptible to gastrointestinal nematode infection (Malan, 2000) as it is helps them to reduce the chance of ingesting infective larvae during grazing (Hoste et al., 2010). Goats that are kept with sheep and allowed to browse often have lower FEC than sheep (Hoste et al., 2008). In contrast, goats that co-graze with sheep often have higher FEC than sheep, which led to the hypothesis that the immune response to gastrointestinal nematode infection is less effective in goats than sheep (Le Jambre and Royal, 1976).

However, there is still little published evidence about the resistance status of goats. The ability to reduce larval establishment and survival is weaker in goats than in sheep (Huntley et al., 1995). Relatively resistant goats have more inhibited larvae than their susceptible counterparts because the larvae unable to develop to adult stage (Patterson et al., 1996a, b). There is also a possibility that nematodes can develop more slowly in the resistant goats as a consequence of going into temporary inhibition (Huntley et al., 1995).

The Boer goat is an important meat breed as studies have revealed that farmers prefer the Boer breed to the other goat breeds due to its rapid growth (Vatankhah and Talebi, 2008; Zhang et al.,2008). Their excellent meat quality and high fertility also make them a highly favourable production animal (Malan, 2000). Boer goats may be somewhat resistant to gastrointestinal nematodes based on some preliminary evidence in a study in the Philippines (Baker and Gray, 2004).

The nematode *T. circumcincta* is the dominant nematode in cool temperate areas of the world (Bartley et al. 2004; Taylor et al., 2007). As it has been extensively studied, especially in sheep, there is more known about the mechanisms of protective immunity to this nematode species than any other (Stear et al., 1995). Strong parasite-specific IgA responses against *T. circumcincta* are associated with decreased FEC in naturally infected

Scottish Blackface sheep (Strain et al. 2002). In contrast, high parasite-specific IgA responses against *T. circumcincta* were associated with high FEC in natural infection among Scottish Cashmere goats that were selectively bred for improved nematode resistance (McBean et al., 2016). The selection for nematode resistance line of this breed of goats was based on low FEC. In the same study, there was only a weak correlation between eosinophilia and FEC in the selected goats although their peripheral eosinophil counts were greater than the control animals. The study demonstrated that selection based on FEC was successful in producing a line of more resistant goats against nematode infection. However, there was no clear evidence pertaining to the mechanism of response to nematode infection as observed in sheep.

Selection programs require traits of interest to have heritable variation. Generally, the heritability of a trait is different in each set of environments and in each population; therefore, heritability cannot be extrapolated from one population and set of environments to another (Griffiths et al., 2000). Hence, heritability estimates for a trait can vary as influenced by different experimental environments. FEC heritability estimates are typically higher in sheep breeds (Watson et al., 1995; Morris et al., 1998; Bishop and Stear, 2001; Goldberg et al., 2012) than the goat breeds (Morris et al., 1997; Mandonnet et al., 2001; Mandal and Sharma, 2008; Mandal et al., 2012) but still unknown in Boer goats. A study at the Boer Goat Breeding Station in China shows that heritability estimates of bodyweight ranged from low to moderate levels between 0.10 ± 0.08 and 0.57 ± 0.19 among different models that fitted direct additive, maternal genetic and permanent maternal environmental effects (Zhang et al., 2009). An understanding of factors affecting the bodyweight including resistance to nematode infection is needed to implement optimal breeding and selection programs in the future.

Overall, this study aimed to address the lack of knowledge surrounding the immune responses to infection in goats particularly the Boer breed. Specifically, this study investigated phenotypic markers of immunity and response to gastrointestinal nematode infection (FEC, bodyweight, IgA activity, peripheral eosinophil counts and PCV) in Boer goats in a natural infection of predominately *T. circumcincta*. The aims were to estimate variance components and genetic parameters for characteristics related to the response against nematode infection and growth rate. Estimating variance components then allowed the estimation of repeatability and heritability for FEC and bodyweight. The repeatability not only provides a measure of quality control where it indicates the similarity between measurements taken at different times during the infection but also provides the upper limit of heritability for a given trait (Falconer and Mackay, 1996).

There are challenges in analysing the data from such experiments. Since FEC data are typically right-skewed and exhibit many zero-counts, analysing FEC as the response variable in classical linear models is mostly inappropriate even after transformation (O'Hara and Kotze, 2010). A common approach for count data is Poisson regression but the choice of Poisson distribution is limited to the data that is not overdispersed (Chipeta et al., 2014). Accounting for overdispersion can be done in different ways and differs between the standard GLM or GLMM approach and the Bayesian method used in this thesis.

In GLM or GLMM approaches an additional multiplicative overdispersion parameter is estimated. This allows the variance to be greater than the absolute value of and standard errors of estimated coefficients are adjusted accordingly. The coefficients themselves are not altered. Under the MCMCglmm approach, overdispersion is handled automatically through the residual term in the linear predictor (Hadfield, 2017).

Zero-inflation occurs when the observed number of zero counts under the parametric model being considered is larger than expected which can also cause overdispersion (Dean and Lundy, 2016). This can be handled within MCMCglmm via zero-inflated Poisson models in which FEC with zero-observations can be arising from both zero-inflated (binary; zero or non-zero) and Poisson (count; zero and non-zero) components (Chipeta et al., 2014).

Zeroes arised from binary component are known as true zeroes while those that arised from Poisson component are known as false zeroes (Yang et al., 2017). In this study, true zeroes were obtained from uninfected goats while false zeroes were obtained from infected goats but the nematode eggs could not be detected or counted under the microscope during McMaster method.

The objectives of this chapter are as follows:

1. To validate the reliability of laboratory assays used to measure FEC, IgA activity, peripheral eosinophil count and PCV conducted in this study.
2. To determine the relationships within and between phenotypic measurements across different months of sampling following natural infection in Boer goats.
3. To explore the distribution of FEC among Boer goats in mixed natural infection dominated by *T. circumcincta,* in particular assessing whether zero-inflated models are required.
4. To identify significant explanatory variables for FEC and bodyweight as the response variables, applying zero-inflated models where necessary.
5. To estimate the repeatability and heritability of FEC and bodyweight in Boer goats following natural infection.

3.2 Materials and methods

3.2.1 Study sites and animals

This study was conducted in a Boer goat farm in Lancaster, England. A total of 158 goats of both sexes aged 3 to 5 months were sampled from this farm once every four weeks from August to October 2014. Information collected on each goat included date of birth, type of birth (either singleton or twin), sex, as well as pedigree data (here, the identity of the father, mother and grandparents as shown in Appendix B). The goats were allowed to graze on the pasture throughout summer, provided water ad libitum and given supplements of commercial pelleted feed. All goats were drenched once with 2.5 % benzimidazole (7.5 mg/kg bodyweight) in August and 0.1 % moxidectin (0.2 mg/kg bodyweight) once in September and once in October according to the manufacturer's recommended dosages. Blood and faecal samples were collected within one week before drenching.

Each goat was weighed prior to sampling. Faecal samples were collected from the rectum and blood samples were collected from the jugular vein from each goat in the morning. The samples were transported to School of Veterinary Medicine, University of Glasgow on the same day of sampling for laboratory analysis. The faecal samples were used to determine the FEC (Appendix C). Pooled faecal samples collected in each month were cultured to harvest L3 for identification to genus level. The blood samples were used to measure IgA activity, peripheral eosinophil counts and PCV (Appendix C).

3.2.2 Modified McMaster technique

The Modified McMaster technique was performed as described in Section 2.1.

3.2.3 Faecal culture

Faecal culture was conducted as described in Section 2.2.

3.2.4 Identification and enumeration of infective stage larvae

L3 identification and enumeration was conducted as described in Section 2.3.

3.2.5 Peripheral eosinophil counts

Peripheral eosinophil counts was measured as described in Section 2.5.

3.2.6 Packed cell volume estimation

Packed cell volume was measured as described in Section 2.6.

3.2.7 ELISA IgA

Nematode antigen was prepared for ELISA IgA as described in Section 2.8. The primary L3 stock was a gift from Moredun Institute, Edinburgh that was used to maintain the passage of L3 in twin lambs reared in Cochno Farm, Glasgow. The pooled faeces of the lambs were subjected for larval culture three times a week. The L3 was harvested as the source of parasite antigen for ELISA assay in this study. ELISA IgA assay was performed as described in Section 2.9.

3.2.8 Statistical analysis

3.2.8.1 Reliability test

Reliability of the methods used to measure FEC (McMaster technique), IgA (ELISA), peripheral eosinophil counts and PCV assays in this study was determined by Cronbach's alpha coefficient using Spearman's Rank Correlation by the CORR procedure in SAS 9.4 software. Cronbach's alpha coefficient is an index of reliability that estimates the internal consistency of a method or the average correlation of items within the test (Cronbach, 1951). It describes the extent to which all replicates in each assay are measured consistently and thus connected to the inter-relatedness of all replicates within the assay.

In order to determine how each measurement reflects the reliability of the method, an alpha coefficient was calculated after deleting each variable. The alpha was expressed as a number between 0 and 1. If the replicates in an assay are correlated with each other, the value of alpha is high. However, if the alpha is low, the replicate that shows, a low correlation with the total score can be deleted to increase alpha value. Cronbach's alpha coefficients above 0.70 are considered acceptable (Nunnally and Bernstein, 1994). Nonetheless, the maximum alpha value of 0.90 has been recommended (Tavakol and Dennick, 2011). If the alpha value is higher than 0.90, it could be that the method is highly reliable, or the person that conduct the method is highly skilful, or the data have been amended so the replicates will have similar results.

3.2.8.2 Descriptive statistics

The means, standard deviations, variance, minimum values, maximum values and skewness of the response variables (FEC and bodyweight) were measured by the UNIVARIATE procedure in SAS 9.4 statistical software. The Shapiro-Wilk statistic was used via the shapiro.test() function in R to test the assumption of normality. All distributions were plotted using the ggplot2 package in R (Wickham, 2016).

3.2.8.3 Comparison of variables between months

The differences among phenotypic variables namely FEC, IgA, PCV, peripheral eosinophil counts and body weight between month of sampling were assessed. One-way analysis of variance (ANOVA) was used for PCV that had normal distribution. The non-parametric Kruskal-Wallis test were used for variables that were not normally distributed such as IgA, peripheral eosinophil counts and bodyweight. The t-test and Wilcoxon rank-sum test were then applied for pairwise comparison for PCV and non-Gaussian variables (IgA, peripheral eosinophil counts and bodyweight), respectively.

3.2.8.4 Correlation analyses

Correlation analyses between FEC, IgA, peripheral eosinophil counts, PCV and bodyweight in each month of sampling were determined by Spearman's rank correlation and presented in a correlogram using the corrplot() function in R. Correlation analyses can also determine the repeatability of a phenotypic trait measured at different time. To account for the large number of comparisons, the Benjamini-Hochberg procedure was used to determine the false discovery rate as the Bonferroni correction is considered too severe for this type of analysis and can lead to large numbers of false negatives (MacDonald, 2014). The Benjamini-Hochberg procedure works as follows.

First order the p-values from small to large, giving p_1 the smallest to p_m the largest, where m is the number of comparisons. If the desired false discovery rate is Q, find the first *k* p-values where

$$p_i \leq \frac{i}{m} Q \quad \text{for } i = 1,2,3..k$$

The method can also be used in reverse to calculate the false discovery rate associated with a particular choice of p-value cut-off.

3.2.8.5 Repeated measures analysis using the animal model

The data collected in this study comprise repeated measures on individual goats of a set of phenotypic variables (FEC, IgA, eosinophils, bodyweight, PCV), along with pedigree data and information on time of sampling, type of birth and sex. These data are suitable for analysis with the animal model using the MCMCglmm. The response variables modelled were FEC and bodyweight.

FEC models

Two sets of models were analysed for the FEC data. The original count data for FEC was used in the repeated measures models. The first set treated IgA and eosinophils as independent variables (Model a and b); the second set combined them into an immune index (Model c and d). The other fixed effects examined for FEC models were time of sampling (sampling month: TIME1, 2 and 3 for August, September and October respectively), sex (1 for female and 2 for male), type of birth (singleton and twin). The immune index was created from IgA activity and peripheral eosinophil counts by subtracting the mean from each observation, dividing by the standard deviation and then adding together these standardised variables, following Basripuzi et al. (2018). The immune index was examined as there was a possibility that both variables (IgA and eosinophils) were independently correlated with the protective immune response against gastrointestinal nematode at the site of infection although no strong correlation was observed between the variables (Table 3-5).

Bodyweight models

The bodyweight models were run in MCMCglmm using the standard Gaussian family of distributions. Four sets of models were used to examine the effect on bodyweight of month of sampling, FEC and their interaction (Model e and f); month of sampling, IgA, peripheral eosinophil counts and their interactions (Model g and h); month of sampling, immune index and their interaction (Model i and j); and month of sampling, parasite index and their interaction (Model k and l). The parasite index was created following Basripuzi et al. (2018) by adding together the standardised FEC and PCV as both variables were correlated with bodyweight on at least three occasions and may be correlated with each other (Table 3-5). The FEC and PCV were standardised by subtracting the mean from each observation and dividing by the standard deviation.

<u>Random effects</u>

As each animal was measured at three monthly time points, the ID of the goats was treated as a random effect. This also allows estimation of repeatability. In addition, as pedigree data were available, the additive genetic effect for each goat on FEC was

included as another random effect termed 'animal'. Each set of FEC and bodyweight models was run with ID; and with and without animal as a random effect. MCMCglmm recognizes the term animal and used it to relate each individual to their records in the pedigree data while the term ID will dissociate individual records from the pedigree thus allowing the permanent environment effects and additive genetic effects to be separated (Wilson et al., 2010).

Specifying a zero-inflated Poisson distribution in MCMCglmm

In the case of zero-inflated data, a Poisson model cannot adequately capture the number of zeroes in the observed FEC data and a zero-inflated Poisson model within MCMCglmm is chosen. In the final part of the methods, Section 3.2.8.9 (Poisson Model 4), it is demonstrated that a zero-inflated model is required for these FEC data.

A zero-inflated model allows the observed FEC to be modelled as a mixture distribution of zeroes arising through zero-inflation (binary component) and counts originating from the Poisson distribution (count component) (Hadfied, 2017). This is achieved by using the MCMCglmm helper function 'at.level()'. For example, including the interaction of 'at.level (trait,1)' with time (e.g. at.level (trait,1): TIME), allows the effect of TIME on the count component of the distribution to be investigated. To investigate the effect of time variable on the binary component of the FEC distribution, the interaction 'at.level (trait,2)' would be used (e.g. at.level (trait, 2): TIME) to consider the effect of TIME on the binary component of the FEC distribution. The intercepts for the count and binary components would be specified as 'trait_FEC' and 'traitzi_FEC', respectively.

Prior specification

For models using either the Gaussian or Poisson family, the priors were as described in Section 2.10.6.3. They consisted of default priors on the fixed effects and a weakly informative prior on the random effects and residual variance. The prior specification for a model with two random effects (denoted G1 and G2) and residual variance (R) would take the form:

```
prior <- list(R = list(V = 1, nu = 0.002),
              G = list (G1 = list(V = 1, nu = 0.002),
                        G2 = list(V = 1, nu = 0.002))).
```

In the case of a zero-inflated distribution being used, there are additional random effects and residual variance associated with the binary component of the zero-inflated model. MCMCglmm fit the same random effect models and residual error terms to both count and binary components of the model. Specification of prior aided to turn off the random effects for the binary component of the model. Following Bolker et al (2012), as the

residual error for the binary component is non-identifiable (*i.e.* cannot be estimated), it was fixed at 1 by setting fix=2, while the 'degree of belief' parameter was specified as nu=0.002 (a small, non-zero value) to be a very weak prior for the other (non-fixed) variance term in order to get reasonable mixing of the chain. The random effects were effectively turned off by fixing the variance to be 10^{-6}. The specification in this case would take the form:

```
prior <- list(R = list(V = diag(1,1), nu=0.002, fix = 2),
           G = list(G1 = list(V = diag(1,1e-6), nu = 0.002, fix =2),
                    G2 = list(V = diag(1,1e-6), nu = 0.002, fix = 2))).
```

Model diagnostics

The MCMC simulations provide the posterior distribution for parameters of interest as well as the trace, which is the sequence of values forming the posterior distribution. If there is autocorrelation in the chain, samples will not satisfy the requirement of being independent samples from the distribution. This can be revealed by three things: 1) a small effective sample size; 2) high autocorrelation values returned by the autocorr.diag() function; and 3) patterns in the trace when no trend should be observed (de Villemereuil, 2012) sometimes referred to as a 'fuzzy caterpillar' appearance. Autocorrelation can be reduced by thinning which means only using every Nth sample from the chain and is specified using the parameter 'thin' in the MCMCglmm function call.

In all of the simulations conducted, the number of iterations, the burn-in and the thinning interval were selected for each model set so that the effective sample size exceeded 1000; the autocorrelation was less than 0.1 and no trend could be seen in the trace plots (de Villemereuil, 2012).

Model selection

Choosing the model that minimises the DIC (the Bayesian version of AIC) is often used as a model selection criterion. AIC is a straightforward combination of the log-likehood and the number of estimated parameters. DIC is the Bayesian analogue but the effective number of parameters can be difficult to determine especially when there are random effects or informative priors (McCarthy, 2007). In particular, when random effects are present, they can dominate the DIC, sometimes leading to the effective numbers of parameters being smaller for more complex models than for the simpler models leading to a lower DIC for the more complex model than desired (McDonald, 2014). For this reason, it was decided that model selection would be conducted by removing the least significant fixed effects one by one based on the largest p-values until arriving at the simplest model.

3.2.8.6 Repeatability and heritability estimation

Repeatability and heritability are calculated from the variance components generated by the mixed model repeated measures analysis as described by Wilson et al. (2010).

Using the term 'ID' as a random effect within the MCMCglmm R package (described in Section 2.10.5.2) will dissociate individual records from the pedigree, thus allowing estimation of repeatability. In models without the 'animal' random effect, repeatability is given by

$$Repeatability = \frac{V_{ID}}{V_{ID} + V_R}.$$

In models with the 'animal' random effect it is given by

$$Repeatability = \frac{V_A + V_{ID}}{V_A + V_{ID} + V_R}.$$

and heritability by

$$h^2 = \frac{V_A}{V_A + V_R}$$

where V_A is the additive genetic variance, V_{ID} is the between-individual variance due to permanent environmental effects and V_R is the residual variance.

3.2.8.7 Creating predicted data from a fitted zero-inflated Poisson model

For the count component of the zero-inflated Poisson model with a log-link function and linear predictor l_1 , the probability of observing a count Y is

$$\text{Prob}(pois(exp(l_1)) = Y) \tag{3.1}$$

For the binary component of the model with a logit-link and linear predictor l_2 the probability of observing a zero is

$$\frac{\exp(l_2)}{1 + \exp(l_2)} \tag{3.2}$$

Combining the two elements for the zero-inflated Poisson model (Hadfield, 2017), the probability, p_0, of an observation $Y = 0$ is given by

$$p_0 = \Pr(y = 0) = \frac{\exp(l_2)}{1+\exp(l_2)} + \left(1 - \frac{\exp(l_2)}{1+\exp(l_2)}\right) \Pr(\text{pois}(\exp(l_1)) = 0) \tag{3.3}$$

This expression makes it clear that a zero-count can come from either the binary component of the model or the Poisson part. The probability of obtaining a count Y > 0 is given by

$$\Pr(y = Y) = \left(1 - \frac{\exp(l_2)}{1+\exp(l_2)}\right) \Pr(\text{pois}(\exp(l_1)) = Y) \quad (3.4)$$

This process was used in R to generate predicted data from the final model parameters generated in MCMCglmm. The first step was to calculate p_0 (the probability of a zero) for each observation from the final model parameters and create the binary component (0 or 1) via

$$\text{zeroes <- rbinom(N, 1, } p_0\text{)} \quad (3.5)$$

Then a set of predicted counts, Y, is generated using

$$\text{Y <- zeroes * rpois(N, exp(} l_2 \text{))} \quad (3.6)$$

Generating predicted data from the fitted model this way allows the true zeroes (those created by the binary component) to be distinguished from the false zeroes (coming from the Poisson part). The predicted data were used to compare the means of the observed and predicted data and also the observed and predicted numbers of zeroes as an additional check on model fit to the data. Predicted true zeroes were obtained from the binary component in the prediction function of the models at time point 1, 2, and 3 as well as from the overall counts. The number of predicted true zeroes was then divided by the number of predicted zeroes to obtain the probability that a predicted zero is a true zero.

3.2.8.8 Modelling of simulated data using MCMCglmm

This section of the methods uses simulated datasets to describe the interpretation of MCMCglmm outputs and the decision-making process in determining the model type (whether zero-inflated or not) to be run in MCMCglmm. The first step was to explore the MCMCglmm package by its use on the simulated data for which the coefficients are known.

Example data sets were created using the following equation as a starting point:

$$l_i \sim (1 + 2\text{IgA}_i) \quad \text{when } \textit{MONTH}_i = 1 \text{ or } 3 \quad (3.4a)$$
$$l_i \sim (1 + 2\text{IgA}_i + 3MONTH_I) \quad \text{when } \textit{MONTH}_i = 2 \quad (3.4b)$$
$$\lambda_i = \exp(l_i) \quad (3.5)$$

where l_i is a latent variable for each observation i where $i \epsilon [1, N]$ and N is the total number of observations. The exponential function is the canonical link function for Poisson regression. This means that a linear model is described (3.4a, b) and then the exponential function is used to convert these values to the mean of the Poisson distribution, λ_i (3.5). IgA_i gives the value of IGA and $MONTH_i$ denotes the MONTH associated with observation i. To follow the structure of the field data, $MONTH_i$ is allowed to take the values of 1, 2 or 3.

If data is generated using equations 3.4a, b the regression coefficient associated with IgA should be approximately 2. There is no effect of MONTH if MONTH is 1 or 3, but an effect of magnitude 3 when MONTH is 2. Therefore, a coefficient of 3 associated with MONTH 2 is expected.

Simulated counts were created by sampling from a Poisson distribution with the mean λ_i as follows:

$$y_i \sim Pois(\lambda_i) \tag{3.6}$$

The characteristics and types of simulated data are shown in Table 3-1:

Table 3-1. Characteristics and types of simulated data in MCMCglmm argument

Data	Variable in MCMCglmm call	Types	Used to create simulated data
FEC	Response variable	Continuous	Yes
Month	Explanatory variable, fixed effect	Categorical	Yes
IgA	Explanatory variable, fixed effect	Continuous	Yes
Animal	Random effect	Categorical	No
ID	Random effect	Categorical	No

Subsequent explorations will fit random effects associated with “ID” and “animal” to the data. Three explorations of the simulated data are presented below (Poisson model 1, Poisson model 2 and Poisson model 3) to demonstrate the fitting of a Poisson model to overdispersed data and to demonstrate that posterior checks on the number of observed and predicted zeroes can be used to assess whether the Poisson model is suitable.

Poisson model 4 then fits a Poisson model to the observed field data and uses the same approach to assess whether a Poisson model is appropriate for the observed data or whether a zero-inflated model is required.

Poisson Model 1: Poisson model of simulated data without residual variation.

A simple MCMCglmm model (Poisson model 1) without random effects was run on the simulated data with month and IgA set as the fixed effects for response variable FEC by using the following prior and R code:

```
prior1 <- list(R = list(V=diag(1), nu=0.002))
Poisson_Model1 <- MCMCglmm(FEC ~ MONTH + IGA , family = "poisson",
                    data = sim_data1, prior = prior1,
                    pr =  TRUE, saveX = TRUE, saveZ = TRUE,
                    nitt = 100000, burnin = 10000, thin = 100)
```

The prior for the residual variance (prior1) follows a standard choice for a weakly informative prior using the inverse-gamma prior distribution with a variance of 1 and degree of belief parameter nu=0.002 (here a small value indicates that the prior is weakly informative) (Villemereuil 2012; Hadfield 2017).

In the MCMCglmm function call, saveX=TRUE and saveZ=TRUE were specified to save the design matrices. These matrices can be combined into the design matrix W and multiplied by the parameter vector θ to obtain predictions (Hadfield, 2017). The summary of the model output for Poisson model 1 is shown in Table A.

The first three lines of the results summary give the information about the chain parameters of Poisson model 1 where 10000 'burn-in' samples have been taken and followed by 100,000 steps (number of iterations-nitt) that were sampled every 100 steps (thin). The Deviance Information Criterion (DIC) which is a measure of model fit is also given.

The R-structure section gives the information on the residual variance estimation, V_R which is referred to by MCMCglmm as 'units'. As no residual variation was present in the simulated data, the V_R was expected to be very small as shown in R-structure. The small posterior mean of V_R (0.001) and associated posterior distribution can be seen in the density plot of the residual variance (Figure 3-2).

The location effects section of the result summary gives information about the fixed effects. In this model, the first month of sampling (MONTH1) served as the base. The FEC in the second month of sampling (MONTH2) was significantly higher than MONTH1 ($p<0.001$) while FEC in the third month of sampling (MONTH3) was not significantly different from MONTH1. The result also shows that IgA was positively associated FEC ($p<0.001$). The estimates for the effect of MONTH2 (2.989) and IGA (1.977) correspond well to the values used for prediction (3 and 2 respectively as described for Equations 3.4a and b) indicating a good model fit.

When conducting MCMC analyses it is important to check the trace for autocorrelation. The calculation of autocorrelation (achieved using autocorr.diag()) is shown in the lower part of Table A. This indicates that the autocorrelation would be sufficiently low at Lag 1000 (i.e. when using a thin parameter of 1000) but is too high at current Lag of 100 (i.e. the choice of thin = 100). It is recommended to achieve an autocorrelation of less than 0.1 and an effective sample size of more than 1000 (de Villemereuil, 2012). In this example, the MCMC should be run for more iterations (nitt) to reduce autocorrelation and to increase the effective sample size which is low (<200) for some variables.

The trace and posterior density plots for the fixed effects and residual variance are shown in Figures 3-1 and 3-2. These also show evidence of autocorrelation with some trends seen in the trace of each variables. The posterior distributions show a peak around 2 for IGA and around 3 for MONTH2, in reasonable agreement with the values used to create the data.

Table A: Model outputs summary for Poisson model 1.

```
Iterations = 10001:99901
Thinning interval  = 100
Sample size  = 900

DIC: 1683.214

R-structure:  ~units
               post-mean        l-95% CI        u-95% CI       eff.samp
 Units          0.0008           0.0002          0.0015         476.9


Location effects: FEC ~ MONTH + IGA
                   post-mean     l-95% CI     u-95% CI     eff.samp        pMCMC
 (Intercept)           1.024        0.934        1.104        154.2       <0.001 **
 MONTH2                2.989        2.934        3.058        126.5       <0.001 **
 MONTH3               -0.024       -0.111        0.063        122.6        0.593
 IGA                   1.977        1.909        2.041        536.0       <0.001 **
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

autocorr.diag(Poisson_Model1$VCV)
                  units
 Lag 10           1.000
 Lag 100          0.449
 Lag 500          0.034
 Lag 1000         0.015
 Lag 5000         0.091

autocorr.diag(Poisson_Model1$Sol)
                  (Intercept)       MONTH2           MONTH3            IGA
 Lag 10             1.000          1.000           1.000          1.000
 Lag 100            0.413          0.529           0.605          0.141
 Lag 500            0.024          0.034           0.140          0.032
 Lag 1000          -0.070         -0.082          -0.033          0.006
 Lag 5000          -0.071         -0.060          -0.099         -0.044
```

post-mean = posterior mean; l-95% CI = lower 95% credible interval; u-95% CI = upper 95% credible interval; eff.samp = effective sample size; pMCMC = p-values for MCMC; autocorr.diag = code to estimate autocorrelation; VCV = variance components; Sol = fixed effects.

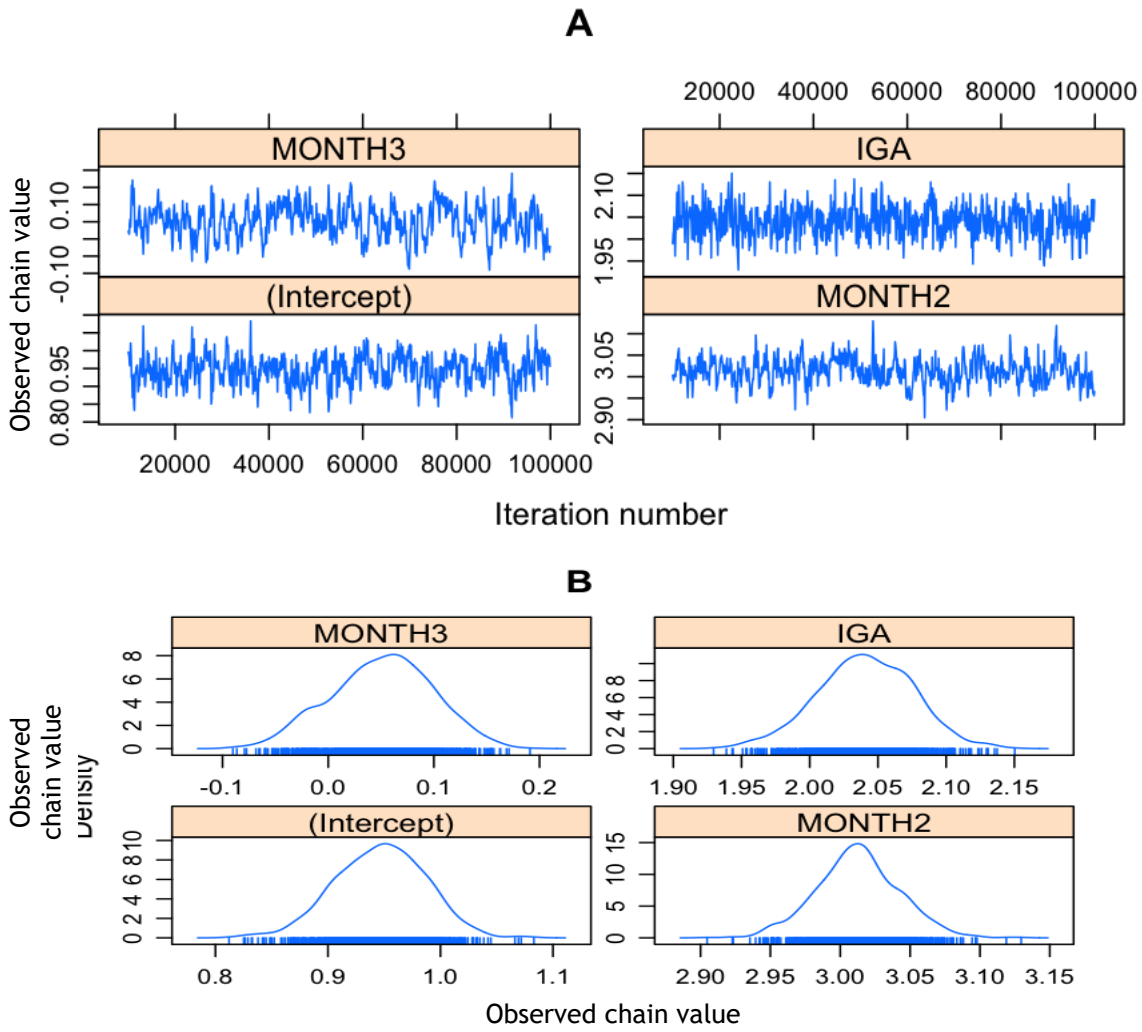


Figure 3-1. Trace plots of parameter value taken during runtime of the chain (A) and posterior density plots of parameter value distributions in the chain (B) for fixed effects in Poisson Model 1.

IGA = Immunoglobulin A; MONTH2= September; MONTH3 = October

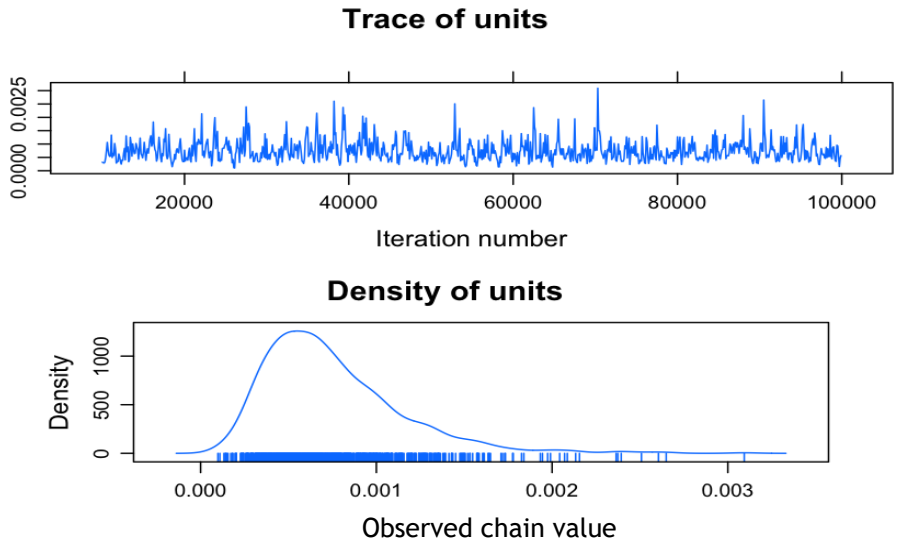


Figure 3-2. Trace plot of parameter value taken during runtime of the chain (top) and posterior density plot of parameter value distribution in the chain (bottom) for residual variance in Poisson Model 1.

The fitted model can be used to generate predicted datasets. A thousand datasets were generated and the number of zeroes in each recorded. This is the posterior predictive distribution of zeroes. The histogram of posterior predictive distribution of zeroes (nz) compared to the observed number of zeroes (vertical red line) from the simulated data is shown in Figure 3-3. In this example, the simulated data that the model was fitted to had 1 zero. Here, the observed number of zeroes is located within the posterior predictive distribution of zeroes (within the histogram). This means that observed and predicted zeroes are comparable with each other indicating that the Poisson model is appropriate for these data.

For illustration purposes, Poisson Model 1 was deliberately simple without residual variation or random effects. More complex model structures were explored by adding the residual variation in Poisson Model 2 and then random effects in Poisson Model 3. These models were also run with more iterations than Poisson Model 1 to reduce the autocorrelation to desired levels.

Figure 3-3. Posterior predictive distribution of zeroes (nz) from Poisson Model 1 with the vertical red line showing the observed number of zeroes (1 in this set of simulated data).

Poisson Model 2: Poisson model of overdispersed simulated data with residual variation.

For Poisson Model 2, the simulated FEC was designed to be overdispersed by including residual variation in the simulated data as follows:

$$l_i \sim (1 + 2\mathrm{IgA}_i + \epsilon) \quad \text{when } \mathit{MONTH}_i = 1 \text{ or } 3 \quad (3.7a)$$
$$l_i \sim (1 + 2\mathrm{IgA}_i + 3\mathit{MONTH}_I + \epsilon) \quad \text{when } \mathit{MONTH}_i = 2 \quad (3.7b)$$
$$\lambda_i = \exp(l_i) \quad (3.8)$$

where ϵ stands for residuals that were sampled from a normal distribution with mean $\mu = 0$ and standard deviation $\sigma = \sqrt{0.9}$. Note that because MCMCglmm captures overdispersion via a residual term in the linear predictor, the Poisson family can be used to capture this overdispersion (Hadfield 2017).

Poisson Model 2 was run without random effects using the following prior and R code, with model output shown below.

```
> prior1 <- list(R = list(V=diag(1), nu=0.002))
> Poisson_Model2 <- MCMCglmm(FEC ~ MONTH + IGA , family = "poisson",
                             data = sim_data2, prior = prior1,
                             pr =  TRUE, saveX = TRUE, saveZ = TRUE,
                             nitt = 500000, burnin = 10000, thin = 100)
```

A larger number of iterations was used (nitt = 500000) in order to reduce autocorrelation, resulting in a larger expected sample size (4900) as can be seen in the results summary below. The summary of the model output for Poisson Model 2 is shown in Table B.

Table B: Model outputs summary for Poisson Model 2.

```
Iterations = 10001:499901
Thinning interval  = 100
Sample size  = 4900

DIC: 1931.895

R-structure:  ~units
                post-mean        l-95% CI         u-95% CI         eff.samp
 Units          1.056            0.875            1.268            4671

Location effects: FEC ~ MONTH + IGA
                  post-mean      l-95% CI     u-95% CI      eff.samp         pMCMC
 (Intercept)        0.955          0.522        1.425          4900        <2e-04 ***
 MONTH2             2.968          2.663        3.286          4492        <2e-04 ***
 MONTH3            -0.001         -0.324        0.325          5104             1
 IGA                2.010          1.449        2.559          4900        <2e-04 ***
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

autocorr.diag(model2$VCV)
                units
 Lag 10          1.000
 Lag 100        -0.005
 Lag 500         0.029
 Lag 1000       -0.013
 Lag 5000       -0.024

autocorr.diag(model2$Sol)
                (Intercept)        MONTH2          MONTH3           IGA
 Lag 10           1.000          1.000           1.000          1.000
 Lag 100          0.001          0.015           0.001         -0.011
 Lag 500          0.003         -0.004           0.005          0.009
 Lag 1000         0.018          0.017          -0.017          0.017
 Lag 5000         0.002          0.006           0.008          0.015
```

post-mean = posterior mean; l-95% CI = lower 95% credible interval; u-95% CI = upper 95% credible interval; eff.samp = effective sample size; pMCMC = p-values for MCMC; autocorr.diag = code to estimate autocorrelation; VCV = variance components; Sol = fixed effects.

As for Poisson Model 1, the location effects of Poisson Model 2 correspond well with the values used to create the simulated data, confirming an appropriate model structure and fit, whilst the estimate of the residual variance is consistent with the value 0.9 used to create the data. As expected, the estimate for V_R markedly increased when the residual variation was included in the simulated data as shown in the R-structure. The posterior mean of V_R (1.056) and associated 95% credible interval of 0.875 to 1.27 shows that V_R is significantly different from zero.

Note that the effective sample size of V_R has increased greatly in Poisson Model 2 (4671) than Poisson Model 1 (477) exceeding the recommended value of 1000 (see Section 2.10.3.6). The trace (Figure 3-4, A and Figure 3-5 top) is improved without visible trends compared to the Poisson Model 1 output (appearing more as the desired 'fuzzy caterpillar') which is in agreement with the reduction in autocorrelation (Table B) to acceptable levels (below 0.1).

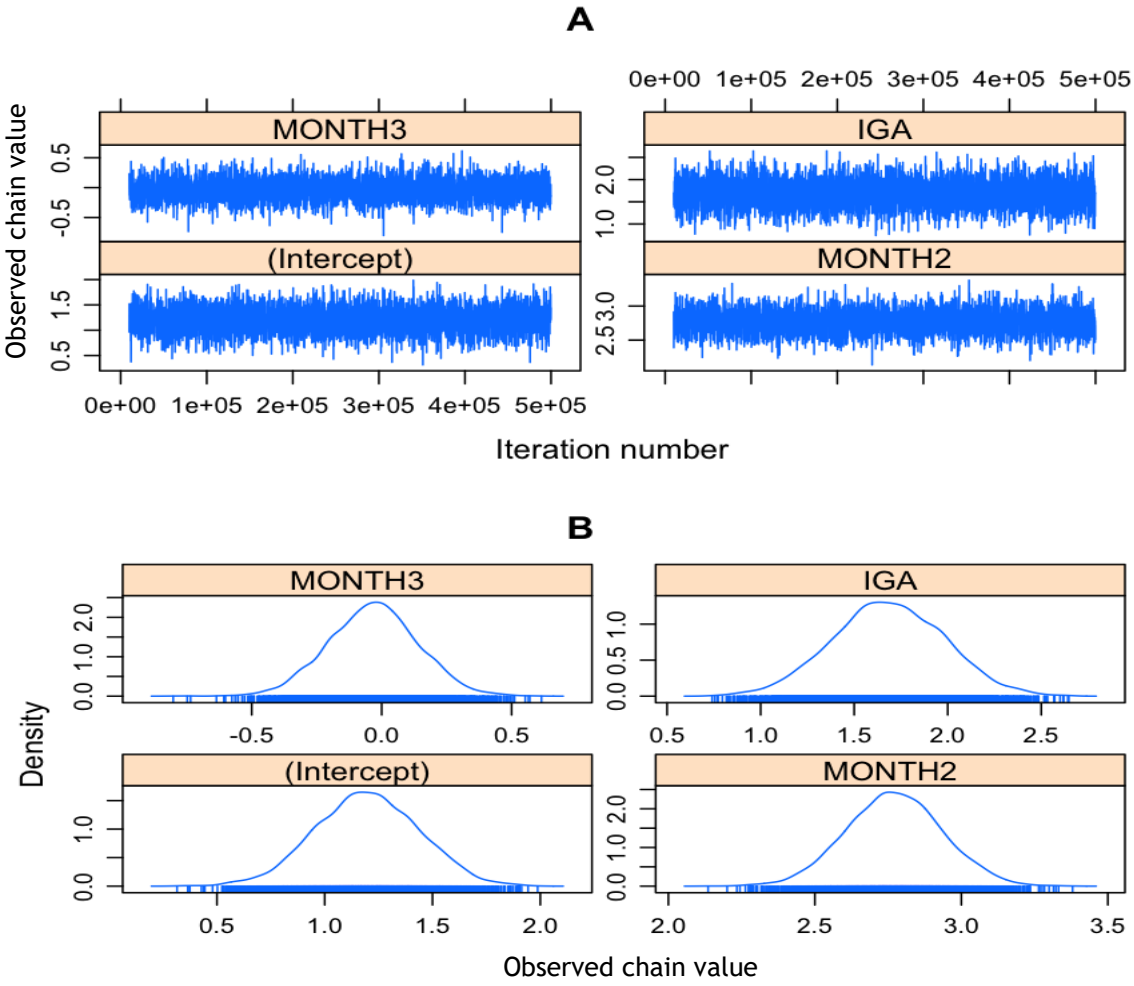


Figure 3-4. Trace plots of parameter value taken during runtime of the chain (A) and posterior density plots of parameter value distributions in the chain (B) for fixed effects in Poisson Model 2.
IGA = Immunoglobulin A; MONTH2= September; MONTH3 = October

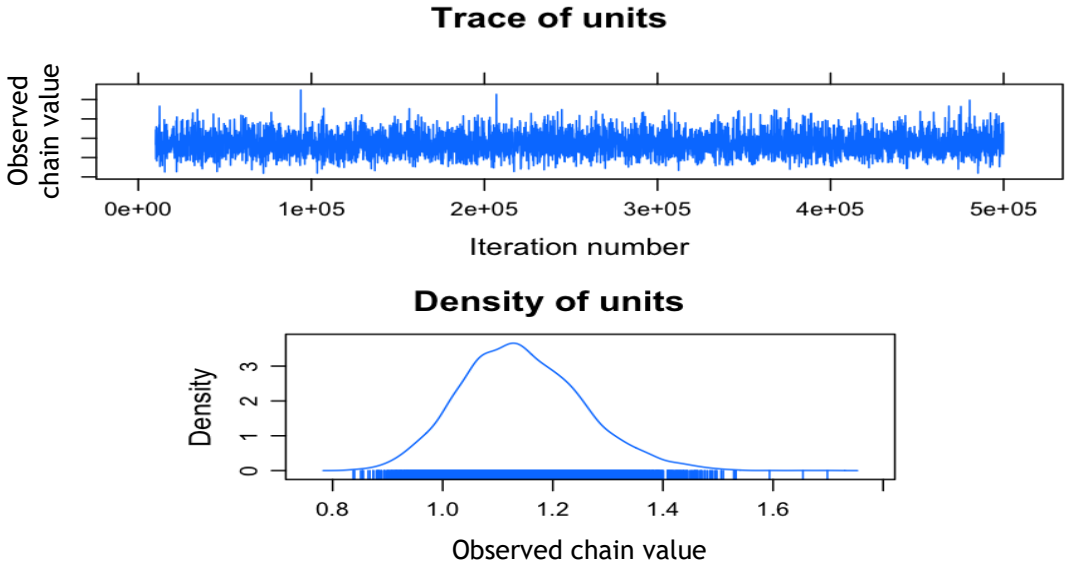


Figure 3-5. Trace plots of parameter value taken during runtime of the chain (top) and posterior density plots of parameter value distributions in the chain (bottom) for residual units in Poisson Model 2.

The observed number of zeroes from the simulated data (vertical red line) is again located within the posterior predictive distribution of zeroes (nz) (Figure 3-6), again indicating that a Poisson model is suitable for these data.

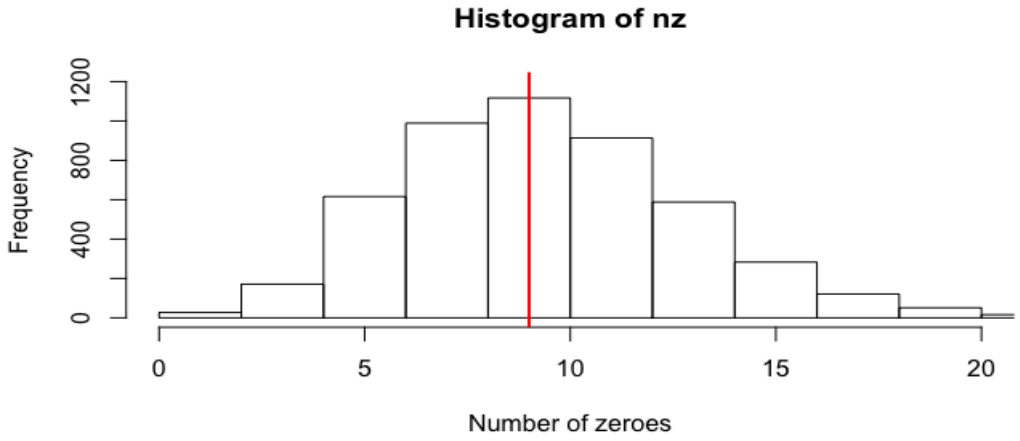


Figure 3-6. Posterior predictive distribution of zeroes (nz) from Poisson Model 2 with the vertical red line showing the observed number of zeroes (9 in this set of simulated data).

The next section shows the extension of the model and priors to encompass random effects in the model.

Poisson Model 3: Poisson model of overdispersed simulated data with random effects and residual variation.

Animal and ID were added as random effects in Model 3 using the same simulated data set as for Poisson Model 2. This model was run with larger number of iterations and thinning parameter than for Poisson Model 2. Poisson Model 3 was run with the priors and R code as shown below, with the result summary shown afterward (Table C). The prior specification has now been extended to include priors for the animal and ID variance components.

```
> prior2 <- list(R = list(V=diag(1), nu=0.002),
                         G = list(G1 = list(V=diag(1), nu=0.002),
                                  G2 = list(V=diag(1), nu=0.002)))

> Poisson_Model3 <- MCMCglmm(FEC ~ MONTH + IGA, random = ~animal + ID,
                      family = "poisson",pedigree = pedigree,
                       data = current_data, prior = prior2,
                       pr =  TRUE, saveX = TRUE, saveZ = TRUE,
                       nitt = 2500000, burnin = 10000, thin = 2000)
```

Table C: Model outputs summary for Poisson Model 3.

```
Iterations = 10001:2498001
Thinning interval  = 2000
Sample size  = 1245

DIC: 1927.250

G-structure:  ~animal

       post.mean  l-95% CI u-95% CI eff.samp
animal    0.014     0.001    0.0580     1245

               ~ID

   post.mean  l-95% CI u-95% CI eff.samp
ID    0.019      0.001   0.070     1245

 R-structure:  ~units

      post.mean l-95% CI u-95% CI eff.samp
units     0.712   0.570    0.860     1245

 Location effects: FEC ~ MONTH + IGA

            post.mean l-95% CI u-95% CI eff.samp  pMCMC
(Intercept)    1.018    0.634    1.431     1245   <8e-04 ***
MONTH2         2.926    2.652    3.153     1245   <8e-04 ***
MONTH3        -0.250   -0.526    0.018     1245    0.0691 .
IGA            2.062    1.571    2.525     1245   <8e-04 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> autocorr.diag(Poisson_model3$Sol)

          (Intercept)  MONTH2     MONTH3      IGA
Lag 0       1.000       1.000      1.000      1.000
Lag 2000   -0.021       0.014      0.004     -0.028
Lag 10000  -0.028       0.011     -0.027     -0.022
Lag 20000  -0.030       0.00      -0.058     -0.022
Lag 1e+05  -0.015       0.00      -0.024     -0.010

> autocorr.diag(Poisson_model3$VCV)

             animal     ID       units
Lag 0        1.000    1.000      1.000
Lag 2000    -0.032    0.022      0.028
Lag 10000   -0.021    0.002      0.010
Lag 20000   -0.023   -0.021     -0.009
Lag 1e+05   -0.062    0.009      0.005
```

post-mean = posterior mean; l-95% CI = lower 95% credible interval; u-95% CI = upper 95% credible interval; eff.samp = effective sample size; pMCMC = p-values for MCMC; autocorr.diag = code to estimate autocorrelation; VCV = variance components; Sol = fixed effects.

The summary of the model output for Poisson Model 3 is shown in Table C. A larger number of iterations (nitt = 2500000) and a larger thinning interval (thin = 2000) were chosen to avoid problems with autocorrelation. The results summary shows the autocorrelation to be less than 0.1 for a lag (thinning interval) of 2000. The effective sample sizes for all fixed and random effects exceeded the recommended value of 1000. In agreement with these numerical assessments of autocorrelation, no trends can be seen in the trace of the fixed effects (Figure 3-7, A and Figure 3-8, left).

The G-structure section in the model summary (Table C) shows the variance estimates for the random effects of animal and ID. The term "animal" is recognised by MCMCglmm and uses it to relate each goat to its own pedigree record. This random effect is the additive genetic variance, V_A. In this model, the posterior mean for the animal component was small (0.014) as would be expected when the data were not generated with an animal effect. Similarly, the posterior mean for variance associated with the random effect of ID was small (0.018).

The posterior mean of V_R (0.71 within 95% CI of 0.57 to 0.86) indicates substantial overdispersion. This is slightly lower than the variance used to create the overdispersion ($\sigma = \sqrt{0.9}$) in the simulated data but this may be because some of this extra variation is now being captured via the small but non-zero random effects of ID and animal. As for Poisson Model 1 and 2, the location effects for Poisson Model 3 correspond to the values used to create the simulated data, confirming an appropriate model structure and fit.

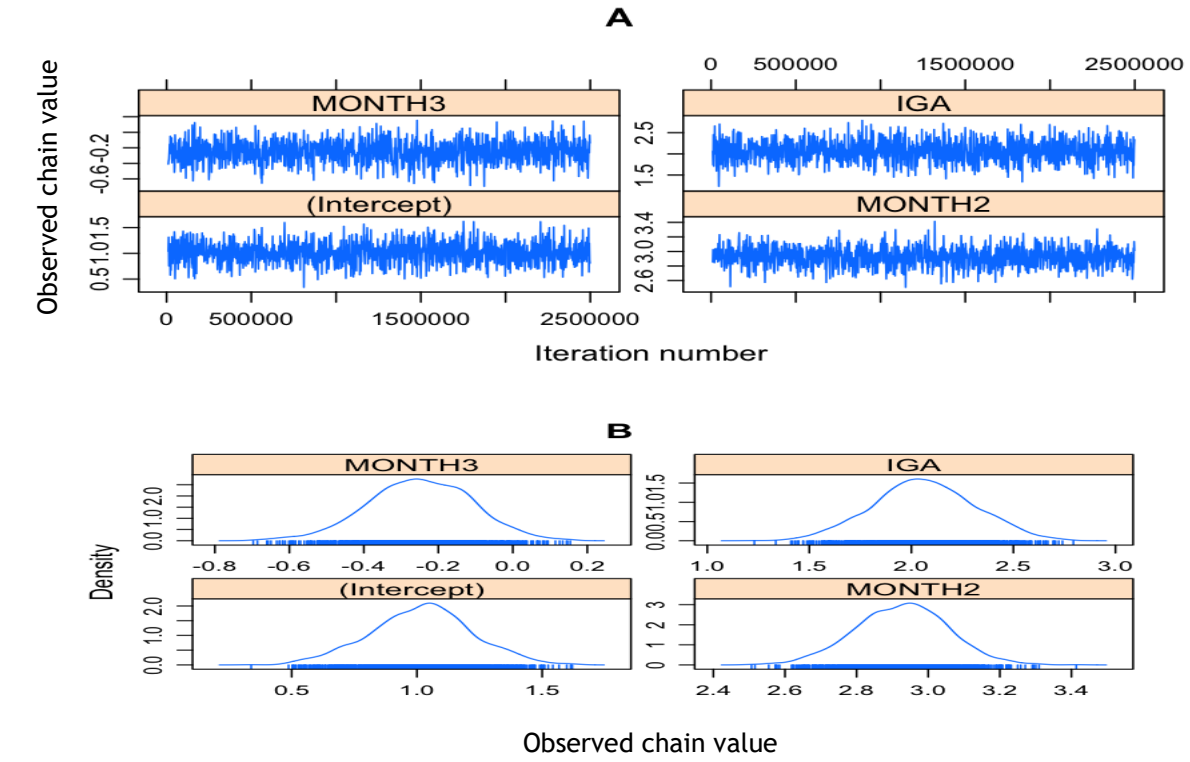


Figure 3-7. Trace plots of parameter value taken during runtime of the chain (A) and posterior density plots of parameter value distributions in the chain (B) for fixed effects in Poisson Model 3.

IGA = Immunoglobulin A; MONTH2= September; MONTH3 = October

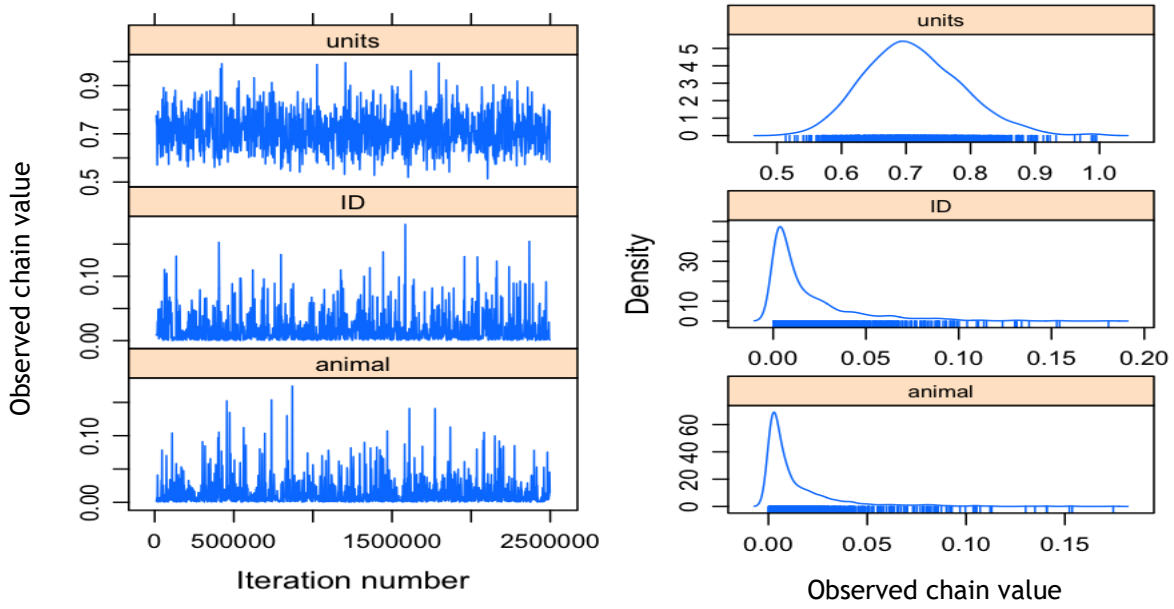


Figure 3-8. Trace plots of parameter value taken during runtime of the chain (left) and posterior density plots of parameter value distributions in the chain (right) for random effects and residual units in Poisson Model 3.

The histogram of the posterior predictive distribution of zeroes (nz) compared to the observed number of zeroes (vertical red line) from the simulated data are shown in Figure 3-9. The observed number of zeroes is located within the posterior predictive distribution of zeroes (nz), indicating that the Poisson Model 3 is appropriate for the simulated overdispersed data.

Figure 3-9. Posterior predictive distribution of zeroes (nz) with the observed number of zeroes (vertical red line) in Poisson Model 3.

3.2.8.9 Poisson Model 4: Assessing the need for a zero-inflated Poisson model for the observed data

The final step was to use the procedure above to examine whether the observed data could be appropriately modelled with a Poisson model or whether a zero-inflated model is required.

Therefore, a full starting model was run on the observed data with sex, type of birth, interactions between IgA and peripheral eosinophil counts, interaction between time (month) and IgA as well as interaction between month and peripheral eosinophil counts. Animal and ID were set as the random effects. The insignificant fixed effects with the largest p-values were then iteratively removed one by one to select the best model. The MCMC running time was chosen to achieve desired levels of autocorrelation (less than 0.1) and effective sample sizes (more than 1000). The final model was run with the following prior and R code:

```
prior2 <- list(R = list(V=diag(1), nu=0.002),
               G = list(G1 = list(V=diag(1), nu=0.002),
                        G2 = list(V=diag(1), nu=0.002)))

Poisson_Model4 <- MCMCglmm(FEC ~ 1 + TIME + IGA + EO + TIME*EO + EO*IGA,
                    random = ~animal + ID,
                    family = "poisson", prior = prior2,
                    pedigree = pedigree, data = data1,
                    pr =  TRUE, saveX = TRUE, saveZ = TRUE,
                    nitt = 2*10^6, burnin = 10000, thin = 1000)
```

The summary of the model output for Poisson Model 4 is shown in Table D. The effective sample sizes all exceeded 1000 and the autocorrelation was below 0.1 as required. Accordingly, no trends can be seen in the trace of the fixed effects (Figure 3-10, A) and random effects (Figure 3-11, left). The G-structure (Table D) shows that the variance estimates for random effects of animal and ID are small (each around 0.05). In contrast, the posterior mean of residual variance, V_R (2.50) was high from the R-structure with 95% CI between 1.997 and 3.148 indicating that the original data are highly overdispersed.

Table D: Model outputs summary for Poisson Model 4.

```
Iterations = 10001:1999001
Thinning interval  = 1000
Sample size  = 1990

DIC: 1731.775

G-structure:  ~ID

    post.mean  l-95% CI  u-95% CI  eff.samp
ID   0.042    0.001      0.185      1560

               ~animal

       post.mean   l-95% CI  u-95% CI  eff.samp
animal   0.056       0.001    0.236      1808

R-structure:  ~units

      post.mean l-95% CI u-95% CI eff.samp
units     2.496    1.997    3.148     2084

Location effects: FEC ~ 1 + TIME + IGA + EO + TIME * EO + EO * IGA

            post.mean  l-95% CI  u-95% CI  eff.samp   pMCMC
(Intercept)   5.021      3.631    6.470      1990    <5e-04 ***
TIME3        -4.544     -5.556   -3.508      1990    <5e-04 ***
TIME1        -3.787     -4.963   -2.668      1990    <5e-04 ***
IGA          -0.148     -0.558    0.305      1742     0.511
EO           -0.810     -1.495   -0.225      1990     0.015 *
TIME3:EO      0.575      0.107    1.051      1990     0.020 *
TIME1:EO      0.428     -0.100    0.916      1990     0.102
IGA:EO        0.223      0.036    0.435      1990     0.030 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

autocorr.diag(Model4$Sol)

            (Intercept)   TIME3     TIME1     IGA       EO
Lag 0         1.000       1.000     1.000    1.000     1.000
Lag 1000     -0.006      -0.012    -0.026    0.009    -0.011
Lag 5000     -0.024       0.023     0.022   -0.015    -0.007
Lag 10000     0.037       0.008    -0.008    0.023     0.013
Lag 50000     0.020      -0.020    -0.020    0.029     0.015

             TIME3:EO    TIME1:EO      IGA:EO
Lag 0         1.000       1.000        1.000
Lag 1000     -0.007      -0.022        0.003
Lag 5000      0.017       0.027        0.013
Lag 10000    -0.012      -0.001        0.001
Lag 50000    -0.022      -0.005        0.021


autocorr.diag(Model4$VCV)

             ID     animal   units
Lag 0      1.000   1.000    1.000
Lag 1000   0.048   0.089    0.019
Lag 5000  -0.013  -0.021   -0.012
Lag 10000 -0.023   0.001   -0.003
Lag 50000 -0.015  -0.016    0.057
```

post-mean = posterior mean; l-95% CI = lower 95% credible interval; u-95% CI = upper 95% credible interval; eff.samp = effective sample size; pMCMC = p-values for MCMC; autocorr.diag = code to estimate autocorrelation; VCV = variance components; Sol = fixed effects;TIME1 = August; TIME3 = October; IGA = Immunoglobulin A; EO = peripheral eosinophil counts.

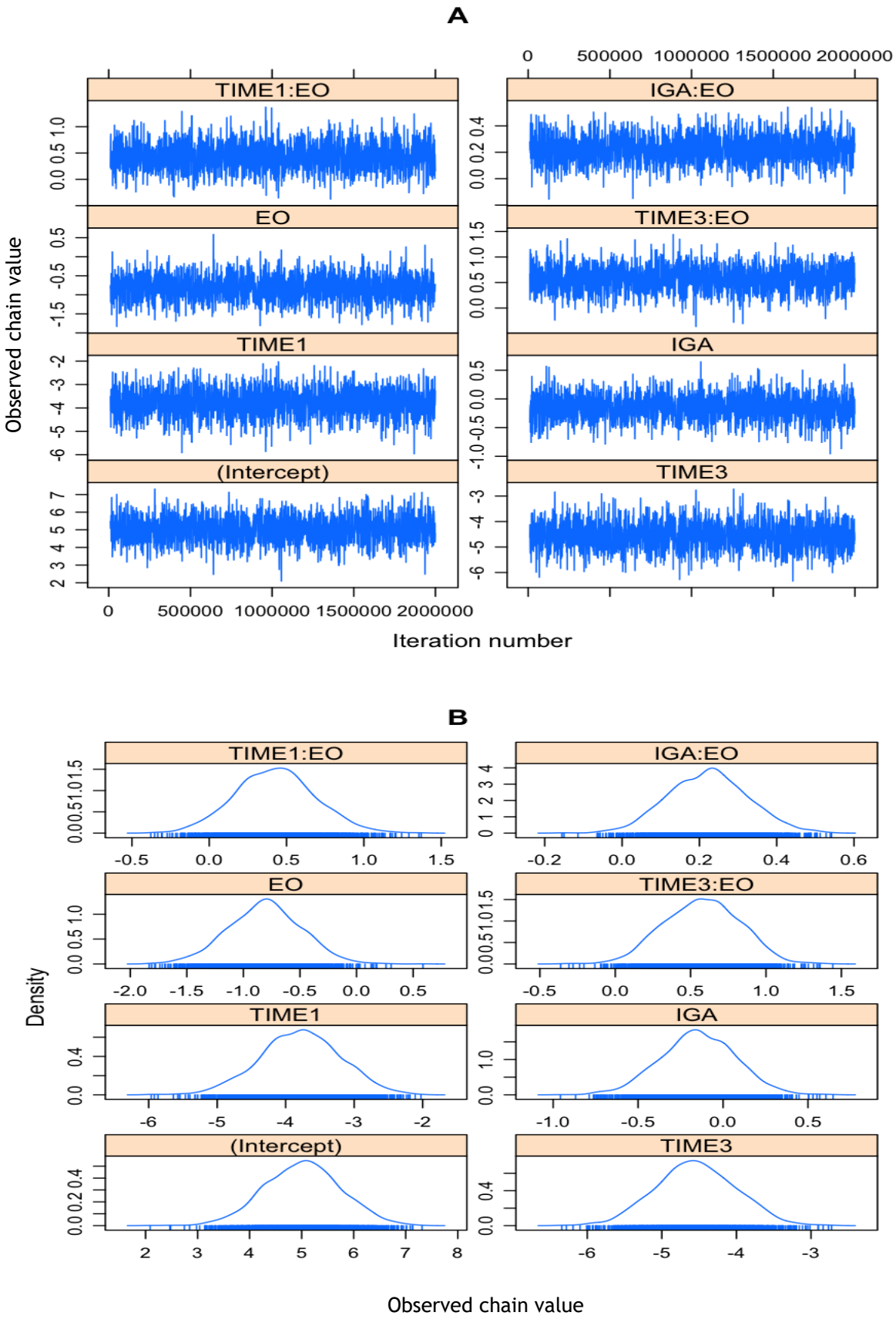


Figure 3-10. Trace plots of parameter value taken during runtime of the chain (A) and posterior density plots of parameter value distributions in the chain (B) for fixed effects in Poisson Model 4.

TIME1 = August; TIME3 = October; IGA = Immunoglobulin A; EO = peripheral eosinophil counts.

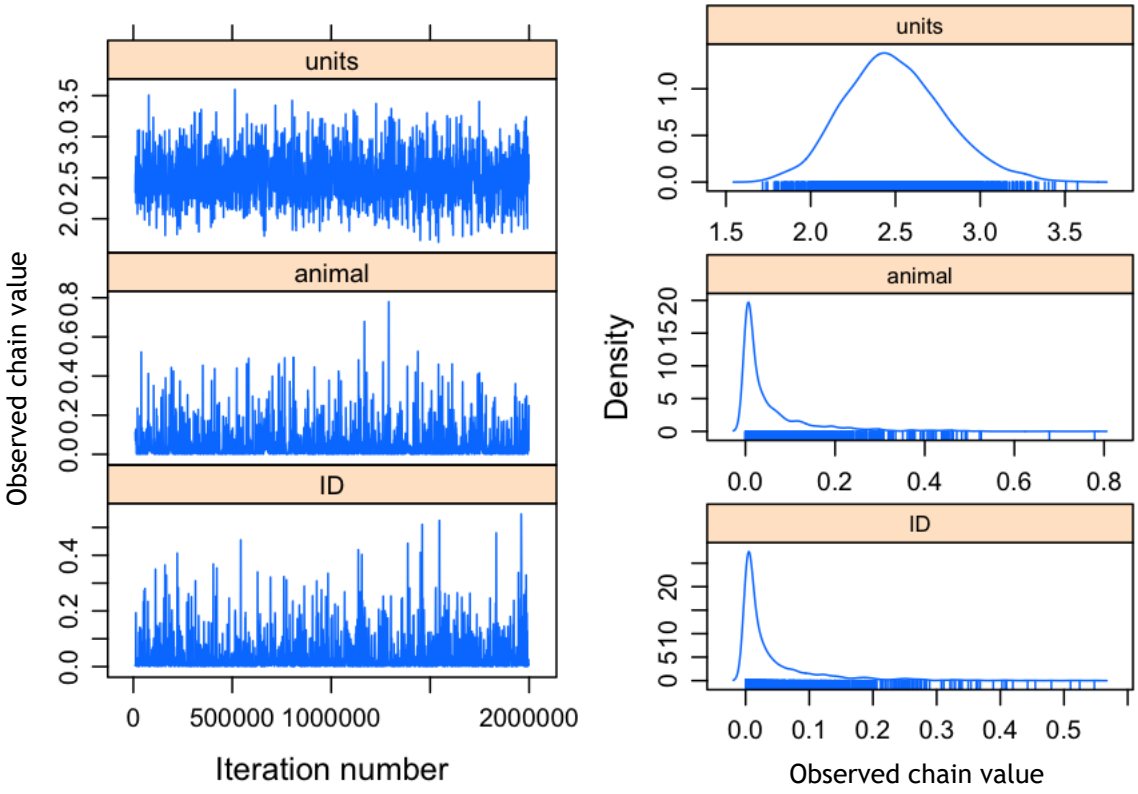


Figure 3-11. Trace plots of parameter value taken during runtime of the chain (left) and posterior density plots of parameter value distributions in the chain (right) for random effects and residual units in Poisson Model 4.

The histogram of the posterior predictive distribution of zeroes (nz) compared to the observed number of zeroes (73, indicated by vertical red line) from original data are shown in Figure 3-12. In contrast to Poisson Models 1, 2 and 3, the observed number of zeroes in Poisson Model 4 as illustrated by the vertical red line (Figure 3-12) was in the right-hand tail of the predicted distribution and not located within the 95% CI of posterior predictive distribution of zeroes which lies in the range 45 to 69. This suggests that the original data cannot be adequately modelled with a Poisson model and therefore that a zero-inflated model would be more appropriate. The repeated measures models shown in the results section therefore make use of the zero-inflated Poisson model to model the egg count data.

Figure 3-12. Posterior predictive distribution of zeroes (nz) with the observed number of zeroes (vertical red line) in Poisson model 4.

3.3 Results

The Cronbach's alpha coefficient for the methods used to measure FEC, IgA and PCV of naturally infected Boer goats were above 0.70 (Table 3-2), showing these procedures to be reliable. The Cronbach's alpha coefficient for peripheral eosinophil counts however was lower than the acceptable value at 0.51. This appeared to be due to one replicate in October, in which the peripheral eosinophil counts of the replicate had substantial difference than the counts of the other replicates from the same sample. The Cronbach's alpha coefficient increased to an acceptable value of approximately 0.80 when the replicate has been removed.

Table 3-2: Cronbach's alpha coefficient of variables collected in England.

Data	Method	Cronbach's apha coefficient
Faecal egg counts	McMaster technique	0.80
IgA	ELISA	0.91
Peripheral eosinophil counts	Peripheral eosinophil counts assay	0.81*
Packed cell volume	Packed cell volume assay	0.81

*improved to acceptable value (>0.7) after one out of two replicates was deleted. Initial alpha value = 0.51.

The proportions of different L3 genera recovered from faecal culture show that *Teladorsagia* dominated the number of L3 followed by *Trichostrongylus* sp. This finding suggests that the number or length of female *T. circumcincta* that produce eggs was greater than *Trichostrongylus* sp. (Table 3-2).

Table 3-3. Percentage of L3 recovery from faecal culture of Boer goats in a farm in England in each month of sampling.

Genera of gastrointestinal nematode	August	September	October
Teladorsagia sp.	93	90	92
Trichostrongylus sp.	7	10	8

Summary statistics and results from normality tests for FEC and bodyweight are shown in Table 3-4. FEC was not normally distributed and had a high positive skewness. Bodyweight in August was not significantly different from a normal distribution. Bodyweight in September and October did not quite follow a normal distribution but the skewness measures were low. These findings were reflected in Figure 3-13 where the FEC distribution was strongly right-skewed (A) while bodyweight had almost a bell-shaped distribution (B). The goats were drenched with broad spectrum anthelmintics namely monepantel and moxidectin in July and September, as well as narrow spectrum benzimidazoles in August.

Table 3-4. Summary statistics for FEC and bodyweight of naturally infected Boer goats in a farm in England for each month of sampling.

Variable/ Month	Mean ± SEM	SD	Minimum	Maximum	Prob (Norm)	Skewness
FEC						
August	213 ± 30.8	332	0	2025	<0.0001	3.3
September	3240 ± 295.2	3124	0	13850	<0.0001	1.3
October	177 ± 37.4	409	0	3300	<0.0001	4.9
Bodyweight						
August	16.0 ± 0.3	4.1	7.5	29.5	0.067	0.4
September	17.8 ± 0.4	4.7	8.5	35.0	0.003	0.6
October	20.0 ± 0.3	4.1	11.0	34.0	0.019	0.5

SEM = standard error of the mean; SD = standard deviation; Prob (Norm) = probability that the distribution was significantly different from normal distribution by Shapiro-Wilk test ($p<0.05$); FEC: faecal egg counts.

Figure 3-14 shows the distributions of IgA OD index, peripheral eosinophil counts, PCV and bodyweight by month of sampling. Based on the Kruskal-Wallis test, there were significant differences in IgA between months (Kruskal-Wallis = 36.616, df = 2, $p<0.001$) but only between August and October as well as between September and October (pairwise Wilcoxon rank sum test, $p<0.001$). The IgA distribution (Figure 3-14, A) is similar in August and September (pairwise Wilcoxon rank sum test, $p = 0.49$). Eosinophil counts differed between months (Kruskal-Wallis = 28.131, df = 2, $p< 0.001$) and appeared highest in September (Figure 3-14, B) while the bodyweight distribution (Figure 3-14, D) shows an increasing trend from August to October with significant differences between month (Kruskal-Wallis = 44.337, df = 2, $p<0.001$). The PCV (Figure 3-14, C) was normally distributed in each month of sampling with significant differences observed between months (ANOVA: $F = 9.891$, df = 2, $p<0.001$).

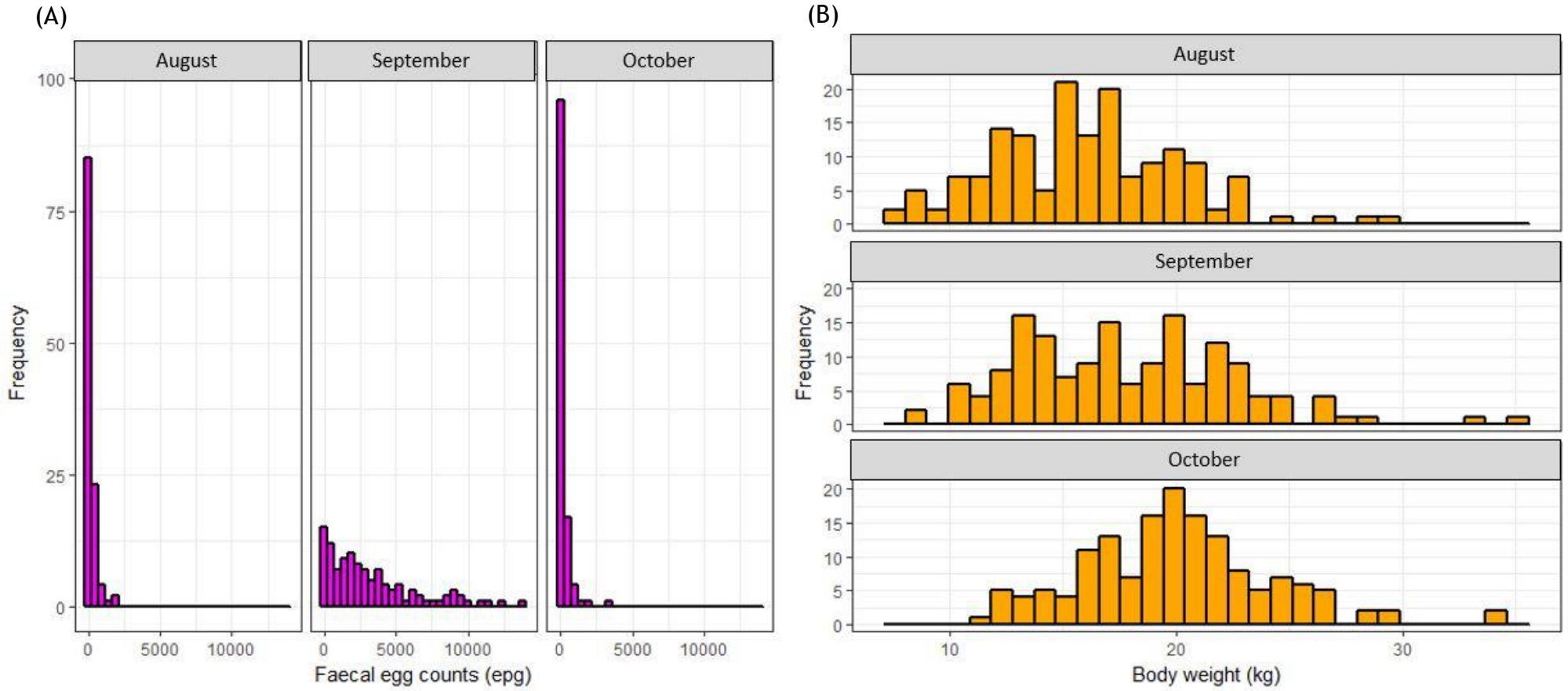


Figure 3-13. Distribution and frequency of faecal egg counts (A) and bodyweight (B) of naturally infected Boer goats in each month of sampling.

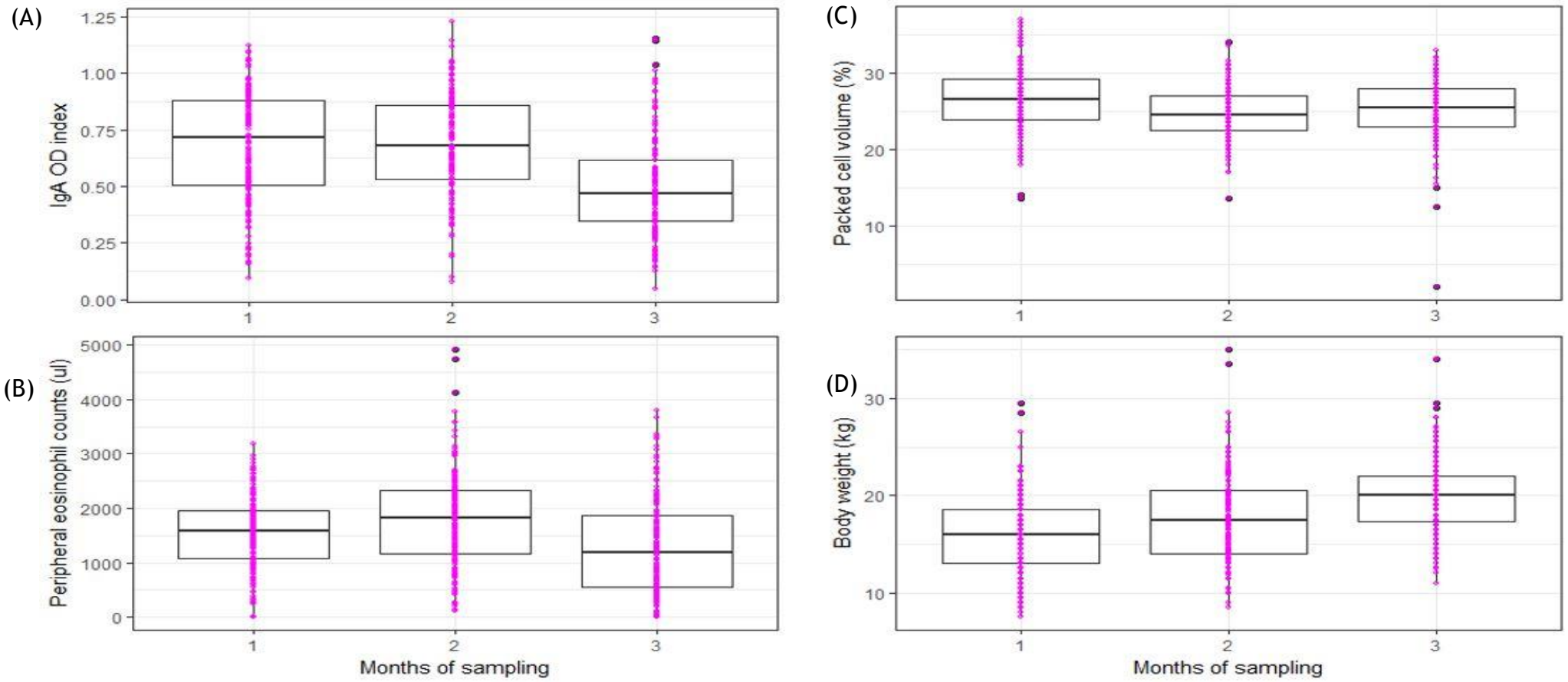


Figure 3-14. Distribution of IgA OD index (A), peripheral eosinophil counts (B), packed cell volume (C) and bodyweight (D) of naturally infected Boer goats by months of sampling (1 = August, 2 = September, 3 = October). The horizontal line gives the median value and the boxes indicate the 25th and 75th percentiles.

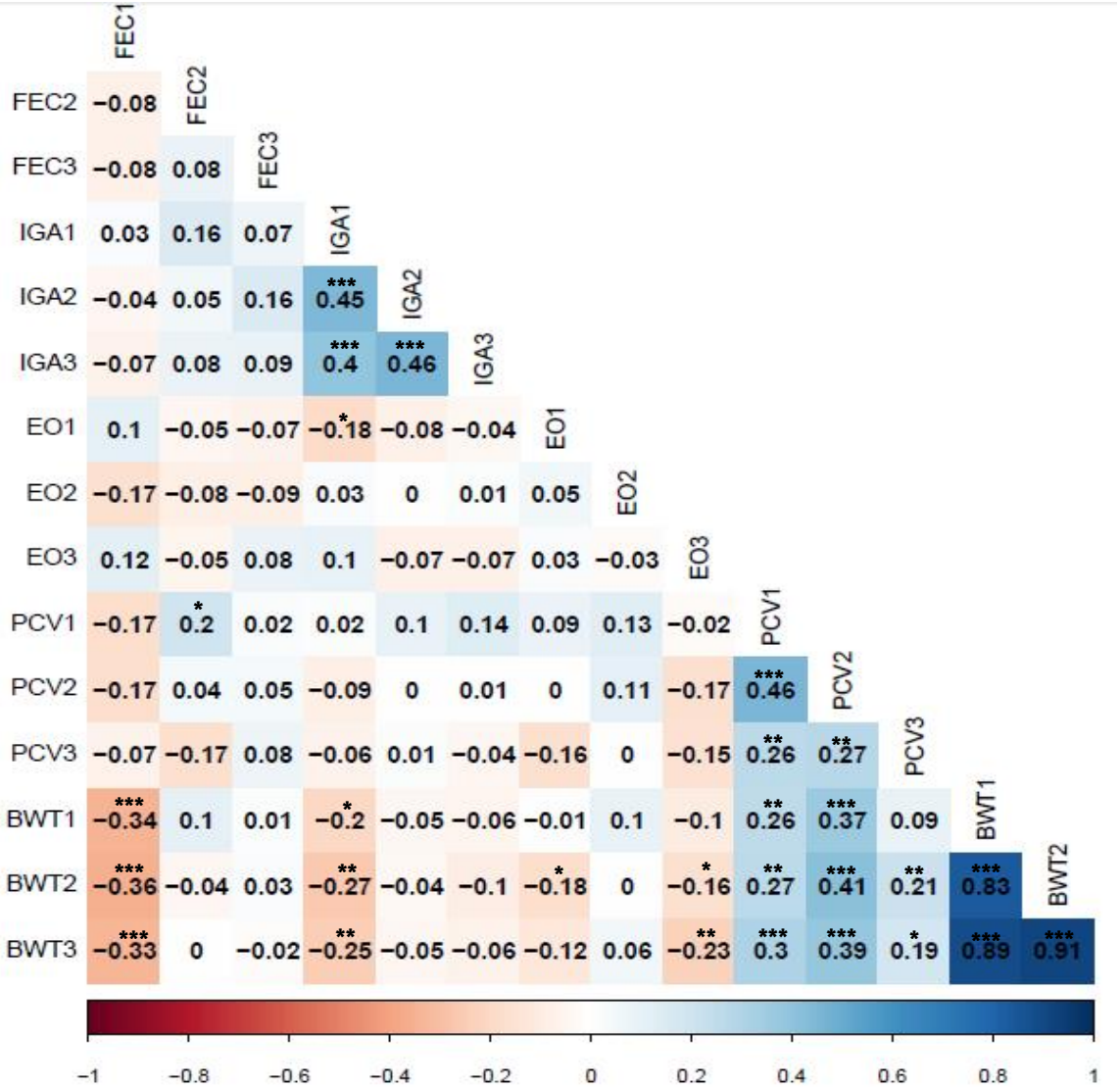
Correlations among FEC, IgA activity, peripheral eosinophil counts, PCV and bodyweight in each month of sampling are shown in Table 3-5 (p-values shown for the Spearman rank correlation test). Bodyweight had the highest correlations among adjacent months at 0.83, 0.89 and 0.91 ($p<0.05$) and followed by correlations of IgA activity at 0.40, 0.45 and 0.46 ($p<0.05$). The correlations of PCV between adjacent months were 0.26, 0.27 and 0.40 ($p<0.05$). The correlations among FEC and peripheral eosinophil counts were low and not significant ($p>0.05$).

There were some significant correlations among different variables (Table 3-5). A lower bodyweight in each month of sampling was associated with a higher FEC ($r=-0.34$, -0.36, -0.33; $p<0.001$) and higher IgA activity ($r=-0.20$, -0.27, -0.25; $p<0.05$) in August. Increased peripheral eosinophil counts in August were associated with lower bodyweight in September ($r=-0.18$; $p<0.05$) and increased peripheral eosinophil counts in October were associated with decreased bodyweight in September ($r=-0.16$; $p<0.05$) and October $r=-0.23$; ($p<0.1$). Increased bodyweight was associated with increased PCV in 8 of 9 comparisons (significant correlations ranged from -0.16 to 0.46; $p<0.01$).

Other than bodyweight, the only associations with FEC was a single association of high PCV in August with high FEC in September ($p<0.05$). There were no associations between the different immunological measures other than one of nine comparisons between IgA and peripheral eosinophil counts ($p<0.05$). This weak negative correlation between both variables was observed in August.

Together, there were 28 statistically significant ($p<0.05$) correlations in 105 comparisons. The Benjamini-Hochberg procedure was used to examine false discovery rate. Using $p<0.05$ as a cut-off in this correlation analysis corresponds to a false discovery rate of 0.19, suggesting that around 5 of the correlations are likely to be false positives.

Table 3-5. Spearman rank correlations among faecal egg counts, IgA activity, peripheral eosinophil counts, packed cell volume and bodyweight in each month of sampling.



FEC = faecal egg counts; IGA = IgA optical density; EO = peripheral eosinophil counts; PCV = packed cell volume; BWT = bodyweight; 1 = August; 2 = September; 3 = October; * = p< 0.05; ** = p<0.01, *** = p <0.001.

3.3.1 Mixed effects repeated measures models

The following sections report the results from the two sets of FEC models (with and without an animal random effect) and the four sets of bodyweight models (also with and without an animal random effect).

3.3.1.1 FEC models

a) Mixed effects models of FEC with time (TIME), IgA, peripheral eosinophils counts (EO), types of birth (TB), sex of goat (SEX) and their interactions and random effect of ID.

Model selection and diagnostics Table 3-6 shows the process of model selection from the full zero-inflated Poisson FEC model (1a) to the simplest model (5a) by removing variables from the fixed effects with the largest p-values until only significant predictors remain (see section 3.2.8.5). Autocorrelations and effective sample size for all models were checked and are shown here only for the final model, Model 5a. For a 'nitt' of 7500000, a 'burnin' of 10000 and thinning interval of 5000, the effective sample sizes for the fixed and random effects (Table 3-7) are greater than 1000 and the autocorrelations at 5000 are all below 0.1 as required (Table 3-8). No trend is seen in the trace for either the fixed effects (Figure 3-15, A) or random effects (Figure 3-16, left). In addition, there was no evidence of autocorrelation in the estimate of repeatability based on the trace (Figure 3-17, left). The corresponding posterior distributions are shown in Figure 3-15 (B), Figure 3-16 (right) and Figure 3-17 (right).

Fixed effects The posterior means and 95% credible intervals for the fixed effects for Model 5a are shown in Table 3-7. There is an effect of time on both the count and binary component of the model. The count component of the model has a significant effect of time which results in increased FEC at time point 2 compared to time point 1 and 3. There is no main effect of IgA on FEC ($p = 0.286$) but eosinophilia had a significant negative association with FEC ($p = 0.029$). An interaction between IgA and eosinophils shows significant positive relationship with FEC ($p = 0.024$). FEC data were predicted with and without the interaction term to assess its affect. Figure 3-19 (A) shows that the predicted mean FEC increases with IgA in each month of sampling when including the interaction between IgA and eosinophils. In comparison, the predicted mean FEC without interaction shows a slight downward trend with IgA (Figure 3-19 B). When plotted against eosinophils rather than IgA, the predicted mean FEC versus eosinophil counts decreases in each month of sampling when excluding the interaction between IgA and eosinophils (Figure 3-20 B). In comparison, no clear trend can be seen in the predicted mean FEC when the interaction is included in the prediction (Figure 3-20 A).

The binary component of the fixed effects was used to calculate the probability of observing a zero (see 3.2.8.7) and shown in Table 3-10. The proportion of zeroes is highest at time point 3 and lowest at time point 2. The overall predicted numbers of zeroes (71.9) match well with the observed numbers of zeroes (71) and the model predicts that the overall proportion of true zeroes is 74%.

Random effects The posterior means and 95% credible intervals for the random effects and residuals for Model 5a are shown in Table 3-7. The random effect of ID was small (0.039) compared to the residual variance (1.494). This large residual variance suggests overdispersion. The binary component of the random effects and residual variance were fixed by the priors and therefore not discussed further.

Repeatability The repeatability estimate of FEC using the posterior mode was 0.001 with a highest posterior density (HPD) interval of 0.0 and 0.107 (Table 3-9; Figure 3-17, right). The posterior mean for FEC repeatability was higher at 0.025.

Observed versus predicted data Figure 3-18 shows that the observed FEC data (top) were consistent with the predicted FEC data (bottom). This finding confirm that the FEC data were following zero-inflated Poisson distribution. Table 3-10 shows that the numbers of observed and predicted zero FEC are in generally reasonable agreement. Table 3-10 also shows the probability of a true zero count (those coming from the binary rather than count component of the model) and that the overall proportion of zeroes which are true zeroes is high at around 74%. Table 3-11 shows that the observed and predicted means of FEC are generally comparable.

Table 3-6. Model selection process for mixed effects models of FEC with time, type of birth, IgA, eosinophil counts and sex as fixed effects, ID as a random effect.

Variable/DIC	Model 1a	Model 2a	Model 3a	Model 4a	Model 5a
DIC	1802.5	1802.6	1806.8	1805.6	1806.7
TIME (B)	Y	Y	Y	Y	Y
TIME (C)	Y	Y	Y	Y	Y
TB (C)	Y				
IGA (C)	Y	Y	Y	Y	Y
EO (C)	Y	Y	Y	Y	Y
SEX (C)	Y	Y	Y	Y	
TIME:EO (C)	Y	Y			
TIME:IGA (C)	Y	Y	Y		
IGA:EO (C)	Y	Y	Y	Y	Y
ID (B,C)*	Y	Y	Y	Y	Y

DIC= Deviance Information Criterion; (B) = binary component; (C) = count component; Y = included; '*' = random effects; TB = types of birth; IGA = immunoglobulin A; EO = peripheral eosinophil counts.

Table 3-7. Model coefficients of association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction with ID as a random effect (Model 5a).

Structure	Variable	Posterior mean (Binary)	Posterior mean (Count)	Lower 95% CI	Upper 95% CI	Eff. sample	p-value	
Fixed effects	Intercept		2.158	0.987	3.451	1498	0.001	**
		-3.503		-5.805	-1.868	1258.457	0.001	***
	TIME2	-0.354		-2.564	2.127	1498	0.653	
	TIME3	2.960		1.224	5.298	1324.547	0.001	***
	TIME2		2.855	2.463	3.253	1498	0.001	***
	TIME3		0.285	-0.201	0.751	1498	0.254	
	IGA		-0.220	-0.627	0.149	1498	0.286	
	EO		-0.601	-1.123	-0.054	1498	0.029	*
	IGA:EO		0.211	0.028	0.388	1498	0.024	*
Random effects	ID		0.039	0	0.162	1498	-	-
		0		0	0	0	-	-
Residuals	Units		1.494	1.137	1.890	1619.301	-	-
		1		1	1	0	-	-

CI = credible intervals; Eff. sample = effective sample size; TIME2 = September; TIME3 = October; IGA = immunoglobulin A; EO = peripheral eosinophil counts; Significance codes: '***' < 0.001; '**' < 0.01; '*' <0.05; '.' < 0.1; ' ' <=1

Table 3-8. Autocorrelation of fixed and random effects in Model 5a.

	trait_FEC	traitzi_FEC	TIME2 (B)	TIME3 (B)	TIME2 (C)
Lag 0	1.000	1.000	1.000	1.000	1.000
Lag 5000	-0.023	0.087	0.012	0.072	-0.036
Lag 25000	0.034	0.037	0.027	0.047	0.020
Lag 50000	-0.026	0.007	0.016	0.015	-0.008
Lag 250000	0.022	0.012	0.017	0.001	-0.057
	TIME3 (C)	IGA(C)	EO(C)	IGA:EO (C)	
Lag 0	1.000	1.000	1.000	1.000	
Lag 5000	0.028	-0.014	-0.036	-0.024	
Lag 25000	-0.026	0.026	0.033	0.019	
Lag 50000	-0.057	-0.026	0.028	0.013	
Lag 250000	-0.019	0.005	0.006	-0.018	
	trait_FEC.ID	trait_FEC.units			
Lag 0	1.000	1.000			
Lag 5000	-0.009	-0.039			
Lag 25000	0.059	0.026			
Lag 50000	0.025	-0.040			
Lag 250000	0.008	0.009			

trait_FEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component; TIME2 = September; TIME3 = October; IGA = immunoglobulin A; EO = peripheral eosinophil counts; (B) = binary component; (C) = count component; trait_FEC.ID = ID variance component; trait_FEC.units = residual variance.

Table 3-9. FEC repeatability estimate for Model 5a.

Posterior mode for repeatability	Lower HPD interval	Upper HPD interval	Posterior mean for repeatability
0.001	0	0.107	0.025

HPD = highest posterior density.

Table 3-10. Observed and predicted zero FEC for Model 5a.

Time point	Number of counts	Observed zeroes	Predicted zeroes	Predicted true zeroes	Probability of predicted zeroes that are true zeroes
1	110	18	23.720	11.930	0.50
2	91	4	4.170	3.660	0.88
3	110	49	44.100	38.030	0.86
All	311	71	71.985	53.611	0.74

Table 3-11. Observed and predicted means of FEC for Model 5a.

Time point	Number of counts	Observed means	Predicted means
1	110	7.760	8.750
2	91	138.710	168.040
3	110	7.380	17.130
All	311	45.950	58.320

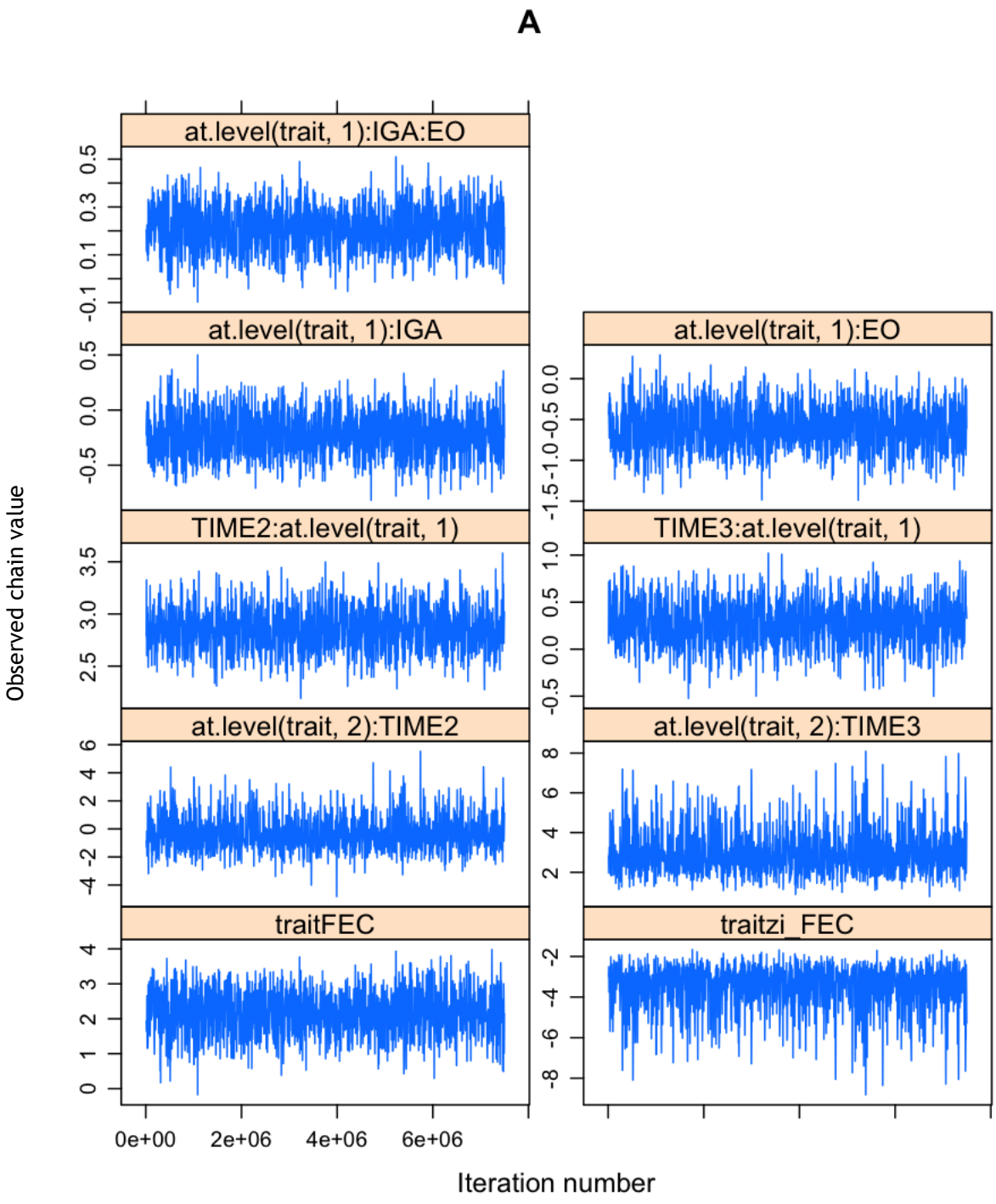


Figure 3-15 (A). Trace plots of parameter value explored by the MCMC chain of fixed effects for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction, with ID as a random effect (Model 5a).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = September; TIME3 = October; IGA = immunoglobulin A; EO = peripheral eosinophil counts; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

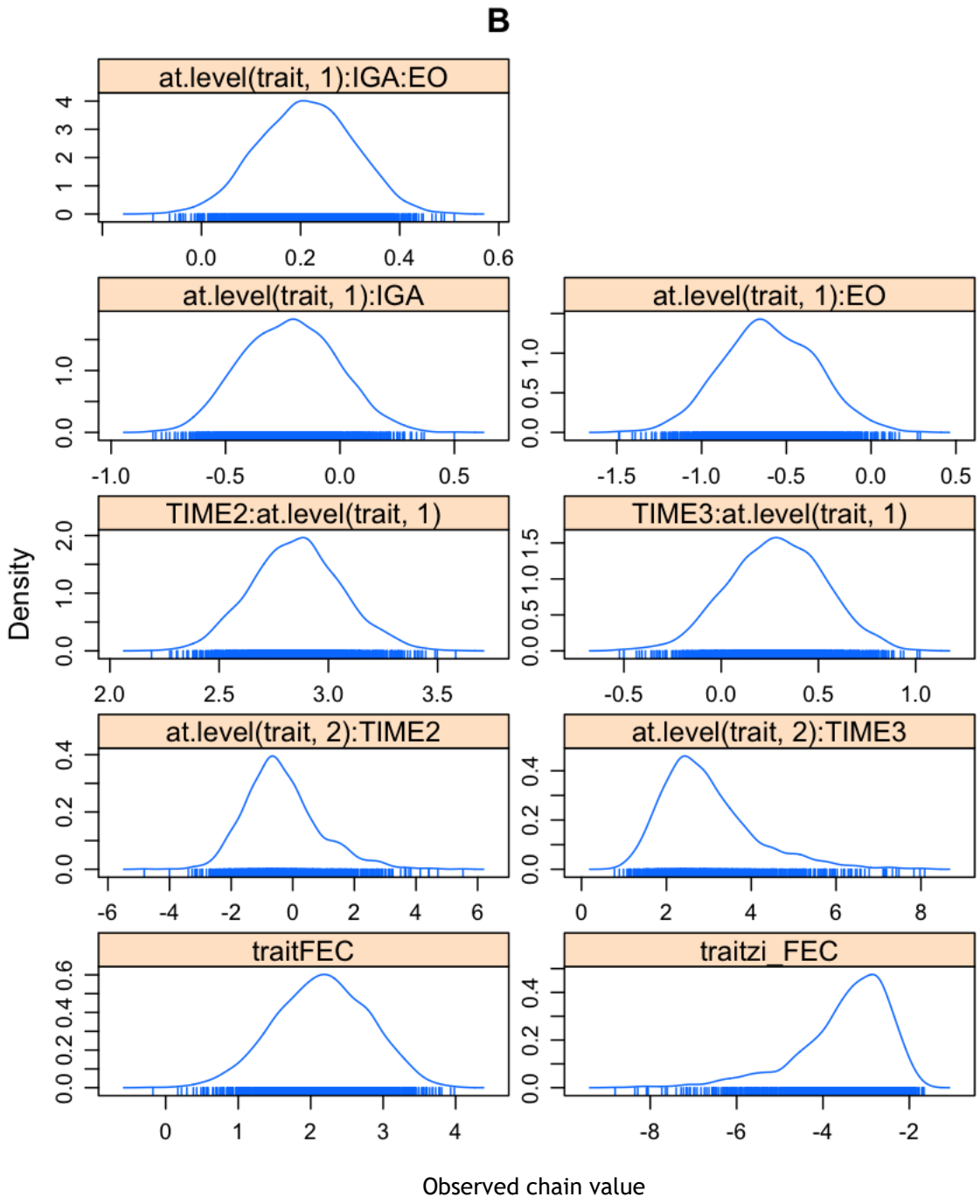


Figure 3-15 (B). Posterior density plots of parameter value distributions in the MCMC chain for fixed effects for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction, with ID as a random effect (Model 5a).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = September; TIME3 = October; IGA = immunoglobulin A; EO = peripheral eosinophil counts; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

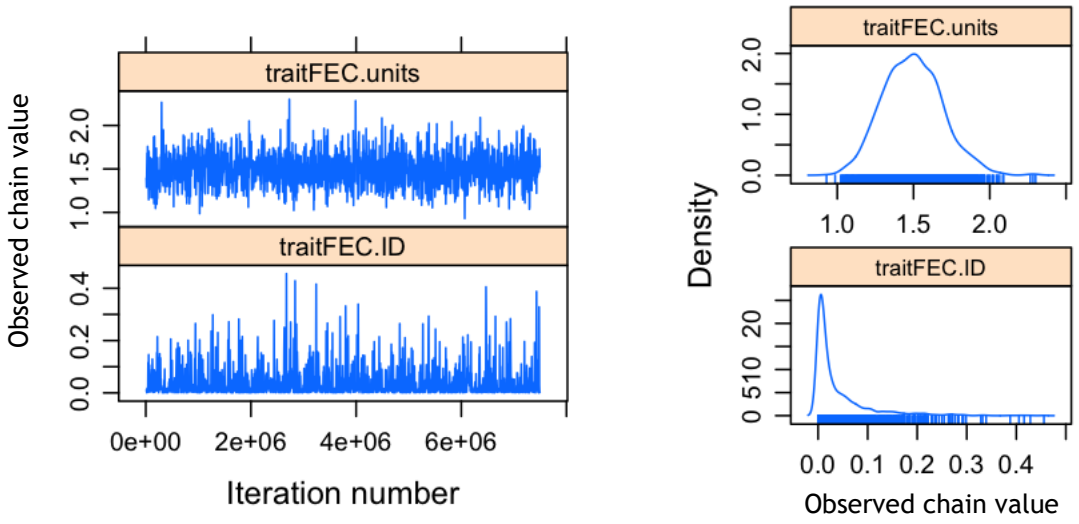


Figure 3-16. Trace plots of parameter value explored by MCMC chain (left) and corresponding posterior density plots (right) for the random effect of ID and residual variance (denoted) units for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID as a random effect (Model 5a).

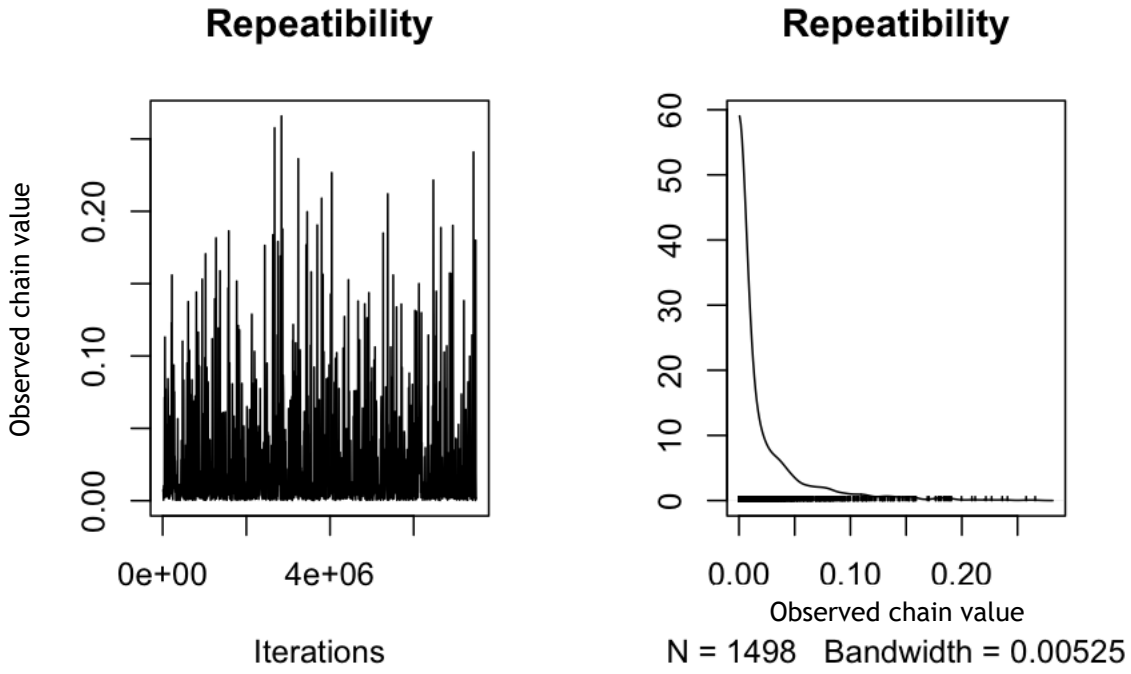


Figure 3-17. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID as a random effect (Model 5a).

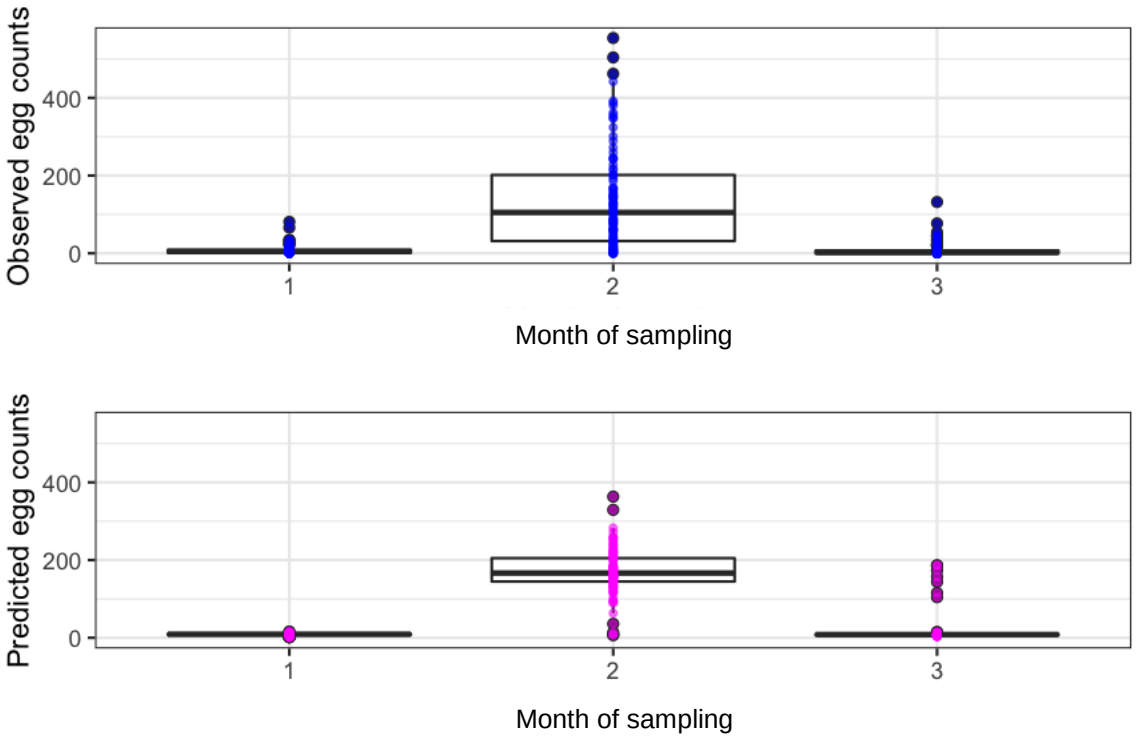


Figure 3-18. Observed (top) and predicted (bottom) FEC data using the model of association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID as a random effect (Model 5a).

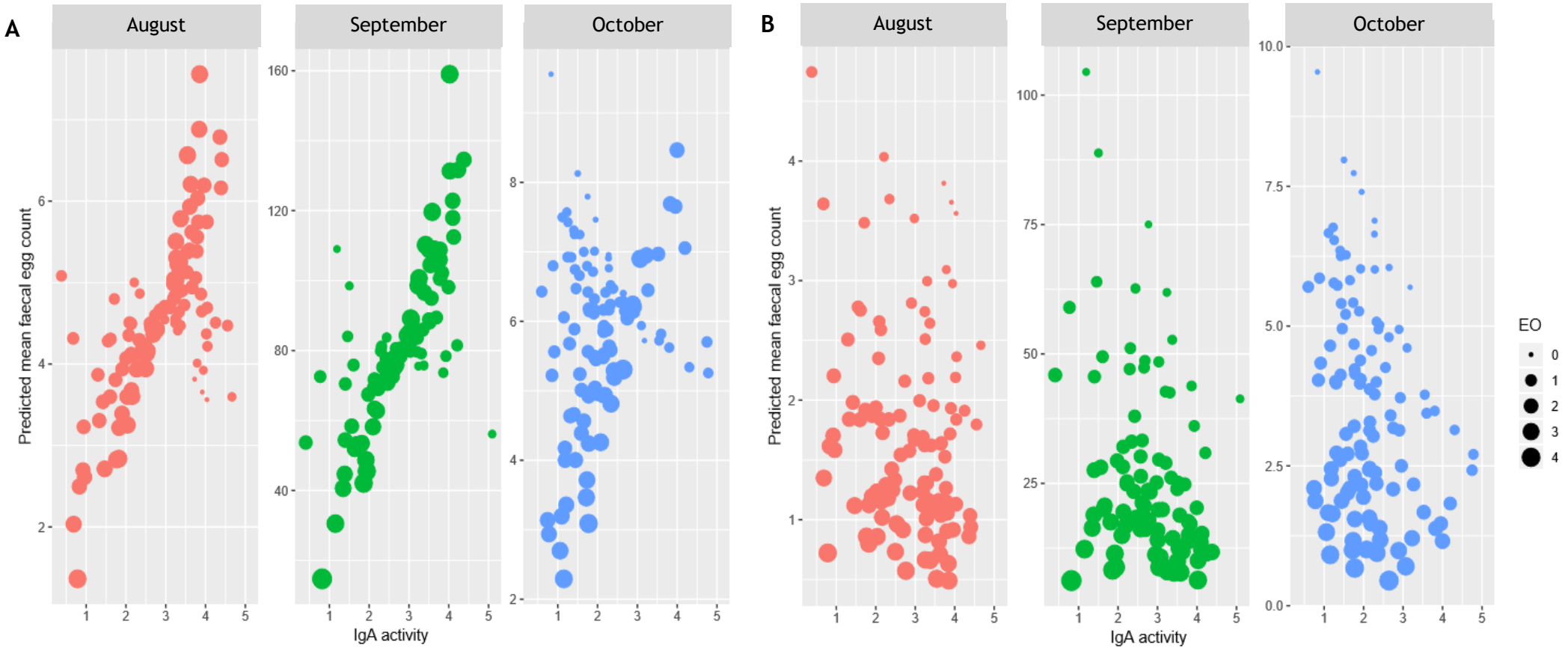


Figure 3-19. Predicted mean FEC against IgA activity (A) with and (B) without the interaction with peripheral eosinophil counts using the model of association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID as a random effect (Model 5a).

EO = Peripheral eosinophil counts.

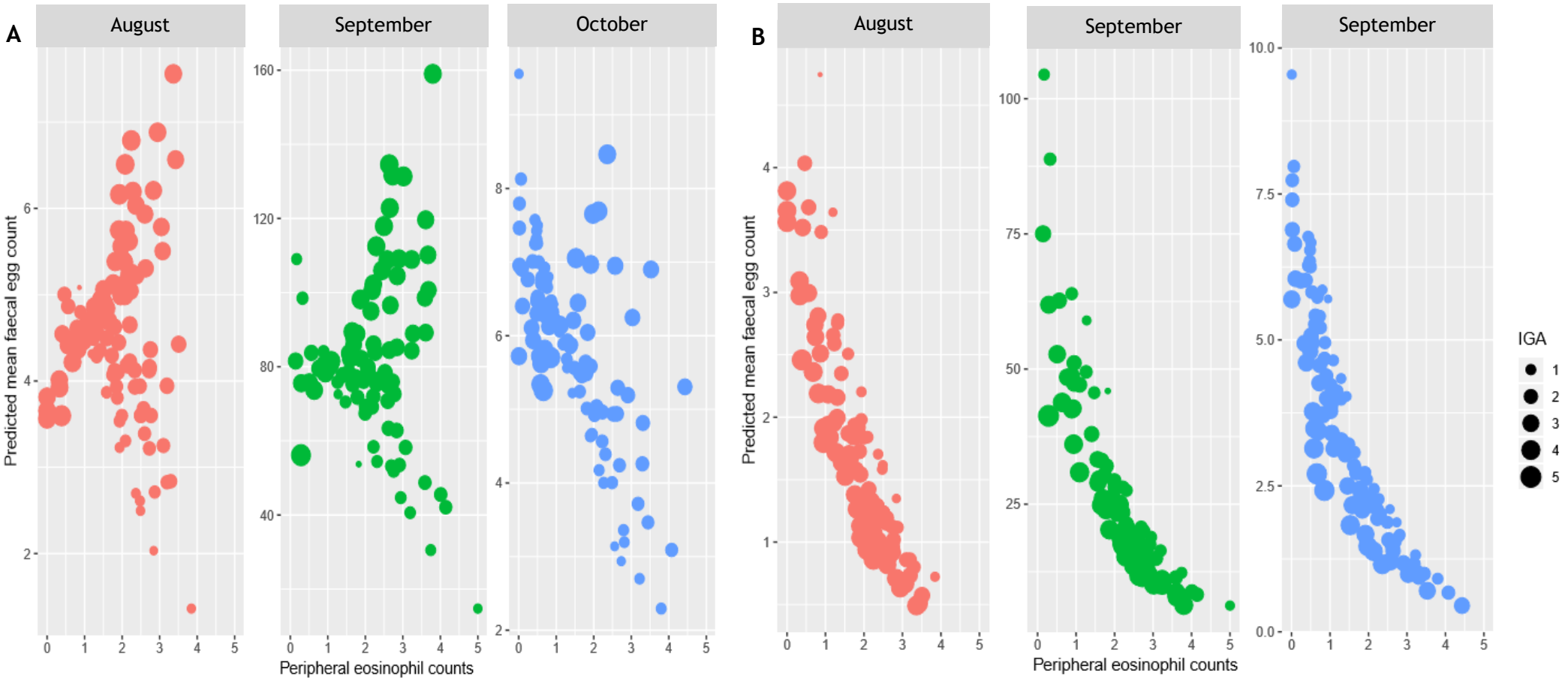


Figure 3-20. Predicted mean FEC against peripheral eosinophil counts (A) with and (B) without the interaction with IgA activity using the model of association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID as a random effect (Model 5a).

IGA = IgA activity.

(b) Mixed effects models of FEC with time (TIME), IgA, peripheral eosinophil counts (EO), types of birth (TB), sex of goats (SEX) and their interactions and random effects of ID and animal.

Model selection and diagnostics Animal as an additional random effect was included in the next set of models. The model selection process, leading to the final model known as Model 5b is shown in Table 3-12. Autocorrelations (not shown) and effective sample sizes were checked as for the previous models. No trend was seen in the trace of the fixed effects (Figure 3-21, A), random effects (Figure 3-22, left), FEC repeatability (Figure 3-23, left) and FEC heritability (Figure 3-24, left). The corresponding posterior densities for Model 5b are shown in Figure 3-21 (B), Figure 3-22 (right), Figure 3-23 (right) and Figure 3-24 (right).

Fixed effects The fixed effects for Model 5b (Table 3-13) have similar attributes (coefficients; positive and negative relationships with FEC) as the fixed effects for Model 5a (Table 3-7). In Model 5b, there is an effect of time on both the count and binary components of the model. The count component of the model has a significant effect of time which results in increased FEC at time point 2 compared to time point 1 and 3. Similar to Model 5a, there is no main effect of IgA on FEC (p=0.24) but eosinophilia had a significant negative association on FEC (p = 0.036). The interaction between IgA and eosinophils has positive effect on FEC (p=0.04). To assess the combined effects of the main effects plus the interaction (as for Model 5a) FEC was predicted with and without the interaction term. Similar patterns to those for Model 5a were seen. The predicted mean FEC increases with IgA in each month of sampling when the interaction of IgA and eosinophils is present (Figure 3-26 A). In comparison, the predicted mean FEC shows a slight downward trend with IgA without the interaction between IgA and eosinophils (Figure 3-26 B). When the predicted mean FEC is plotted again eosinophils, the predicted mean FEC shows a clear downward trend with eosinophil counts in each month of sampling in the absence interaction (Figure 3-27 B). In comparison, no clear trend can be seen in the predicted mean FEC when the interaction is included in the prediction (Figure 3-27 A).

The binary component of the fixed effects was used to calculate the probability of observing a zero (see 3.2.8.8) and shown in Table 3-16. The proportion of zeroes is highest at time point 3 and lowest at time point 2. The predicted numbers of overall zeroes (72.5) match well with the observed numbers of zeroes (71) and the model predicts that the overall proportion of true zeroes is 74%.

Random effects The posterior means and 95% credible intervals for the random effects and residuals for Model 5b are shown in Table 3-13. The estimate for the residual variance (1.442) is similar to Model 5a (1.494); again this large residual variance suggests overdispersion. The posterior mean of ID is small (0.035) and also similar to that for Model 5a (0.039). The posterior mean of the animal random effect is small at 0.061.

Repeatability and heritability The posterior mode for repeatability estimate of FEC is small at 0.009 with a larger, although still small, estimate for the posterior mean (0.062) as shown in Table 3-14. The heritability of FEC can be estimated because the animal random effect was included in the model. Table 3-15 shows that the posterior mode for FEC heritability is very small (0.001) but the posterior mean is substantially larger, although still small at 0.039. The posterior distributions for both the repeatability (Figure 3-23, right) and heritability (Figure 3-24, right) are very skewed with most values located around zero, suggesting that there is insufficient data to estimate them more precisely.

Observed versus predicted data Similar to Model 5a, the observed FEC data in Model 5b (Figure 3-25, top) were consistent with the predicted FEC data (Figure 3-25, bottom). Table 3-16 shows that the numbers of observed and predicted zeroes have only small differences between each other. Table 3-16 also shows the probability of a true zero count (those coming from the binary component rather than count component of the model) and that the overall proportion of zeroes which are true zeroes is high at 74%. Table 3-17 shows that the means of observed and predicted FEC are generally in reasonable agreement with each other.

Table 3-12. Model selection process for mixed effects models of FEC with time, type of birth, IgA, eosinophil counts and sex as fixed effects, with ID and animal as random effects.

Variable/DIC	Model 1b	Model 2b	Model 3b	Model 4b	Model 5b
DIC	1802.4	1802.4	1806.3	1805.5	1806.1
TIME (B)	Y	Y	Y	Y	Y
TIME (C)	Y	Y	Y	Y	Y
TB (C)	Y				
IGA (C)	Y	Y	Y	Y	Y
EO (C)	Y	Y	Y	Y	Y
SEX (C)	Y	Y	Y	Y	
TIME:EO (C)	Y	Y			
TIME:IGA (C)	Y	Y	Y		
IGA:EO (C)	Y	Y	Y	Y	Y
ID* (B,C)	Y	Y	Y	Y	Y
Animal* (B,C)	Y	Y	Y	Y	Y

DIC= Deviance Information Criterion; (B) = binary component; (C) = count component; Y = included; '*' = random effects; TB = types of birth; IGA = immunoglobulin A; EO = peripheral eosinophil counts.

Table 3-13. Model coefficients of association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction with ID and animal as random effects (Model 5b).

Structure	Variable	Posterior mean (Binary)	Posterior mean (Count)	Lower 95% CI	Upper 95% CI	Eff. sample	p-value	
Fixed effects	Intercept		2.162	0.903	3.348	1412.702	0.001	**
		-3.476		-5.789	-1.926	1205.828	0.001	***
	TIME2	-0.421		-2.715	2.105	1150.954	0.619	
	TIME3	2.923		1.305	5.261	1248.414	0.001	***
	TIME2		2.850	2.442	3.254	1498	0.001	***
	TIME3		0.264	-0.204	0.752	1306.896	0.275	
	IGA		-0.234	-0.651	0.144	1498	0.240	
	EO		-0.592	-1.120	-0.062	1498	0.036	*
	IGA:EO		0.210	0.017	0.394	1736.985	0.040	*
Random effects	ID		0.035	0	0.146	1498	-	-
		0		0	0	0	-	-
	Animal		0.061	0	0.229	1498	-	-
		0		0	0	0	-	-
Residuals	Units		1.442	1.075	1.795	1596.084	-	-
		1		1	1	0	-	-

CI = credible intervals; Eff. sample = effective sample size; TIME2 = September; TIME3 = October; IGA = immunoglobulin A; EO = peripheral eosinophil counts; Significance codes: '***' < 0.001; '**' < 0.01; '*' <0.05; '.' < 0.1; ' ' <=1

Table 3-14. FEC repeatability estimate for Model 5b.

Posterior mode for repeatability	Lower HPD interval	Upper HPD interval	Posterior mean for repeatability
0.009	0.001	0.179	0.062

HPD = highest posterior density

Table 3-15. FEC heritability estimate for Model 5b.

Posterior mode for heritability	Lower HPD interval	Upper HPD interval	Posterior mean for heritability
0.001	0	0.142	0.039

HPD = highest posterior density

Table 3-16. Observed and predicted zero FEC for Model 5b.

Time point	Number of counts	Observed zeroes	Predicted zeroes	Predicted true zeroes	Prop of predicted zeroes that are true zeroes
1	110	18	24	11.860	0.49
2	91	4	4	3.640	0.91
3	110	49	44.220	37.930	0.86
All	311	71	72.540	53.430	0.74

Table 3-17. Observed and predicted means of FEC for Model 5b.

Time point	Number counts	Observed means	Predicted means
1	110	7.760	8.600
2	91	138.710	164.840
3	110	7.380	17.040
All	311	45.950	57.300

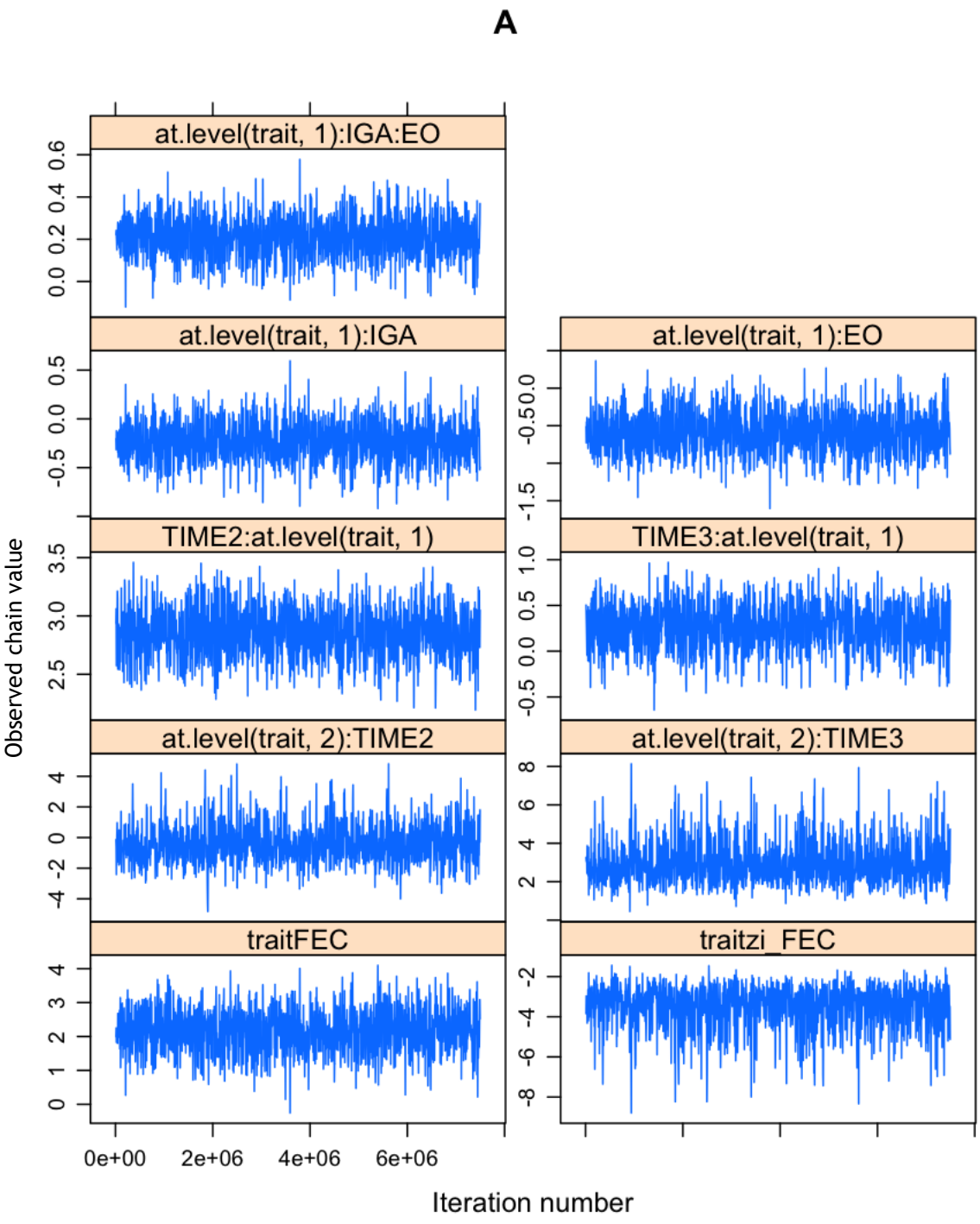


Figure 3-21 (A). Trace plots of parameter value explored by the MCMC chain of fixed effects for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction, with ID and animal as a random effects (Model 5b).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = September; TIME3 = October; IGA = immunoglobulin A; EO = peripheral eosinophil counts; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

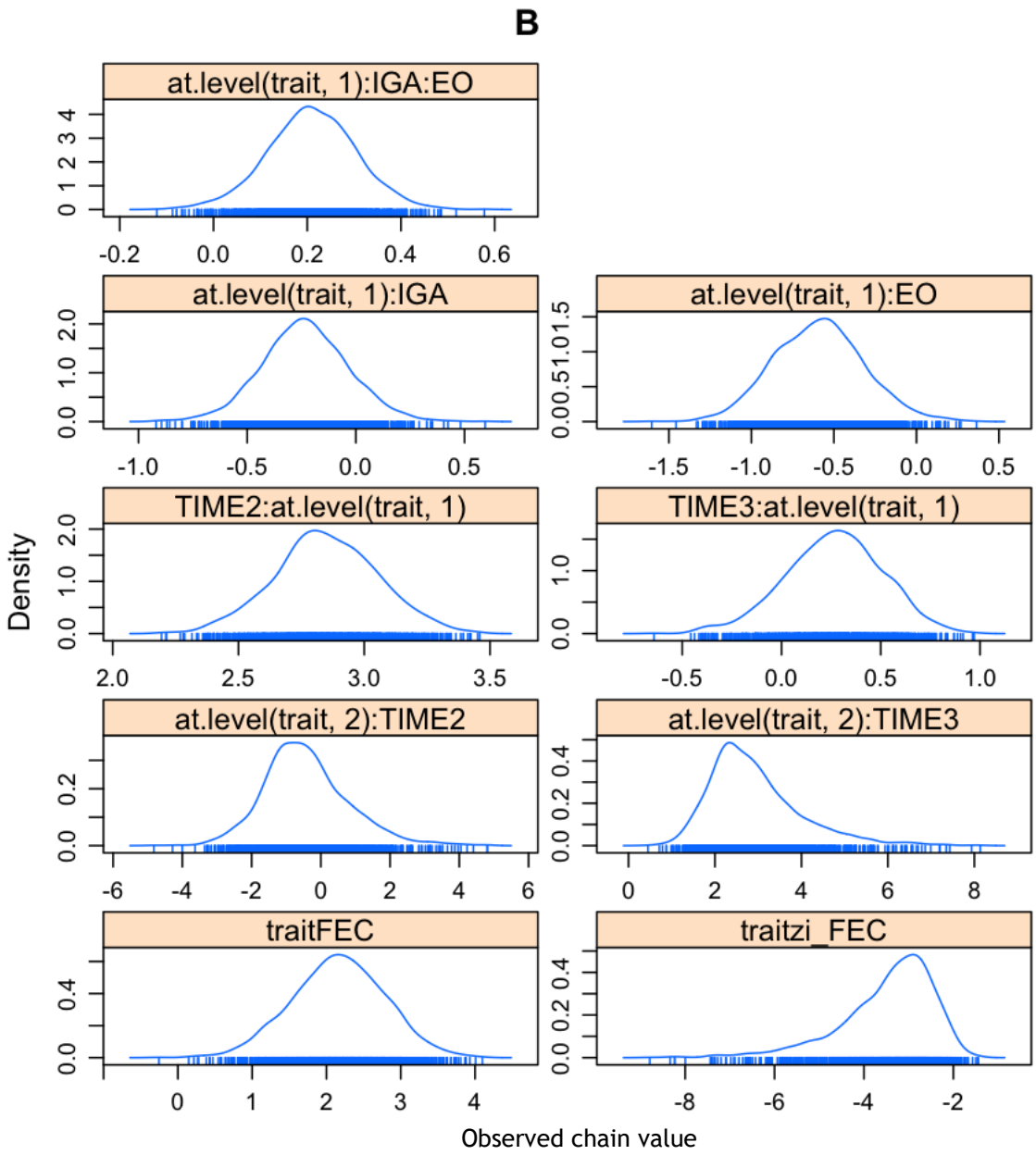


Figure 3-21 (B). Posterior density plots of parameter value distributions in the MCMC chain for fixed effects for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction, with ID and animal as a random effects (Model 5b).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = September; TIME3 = October; IGA = immunoglobulin A; EO = peripheral eosinophil counts; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

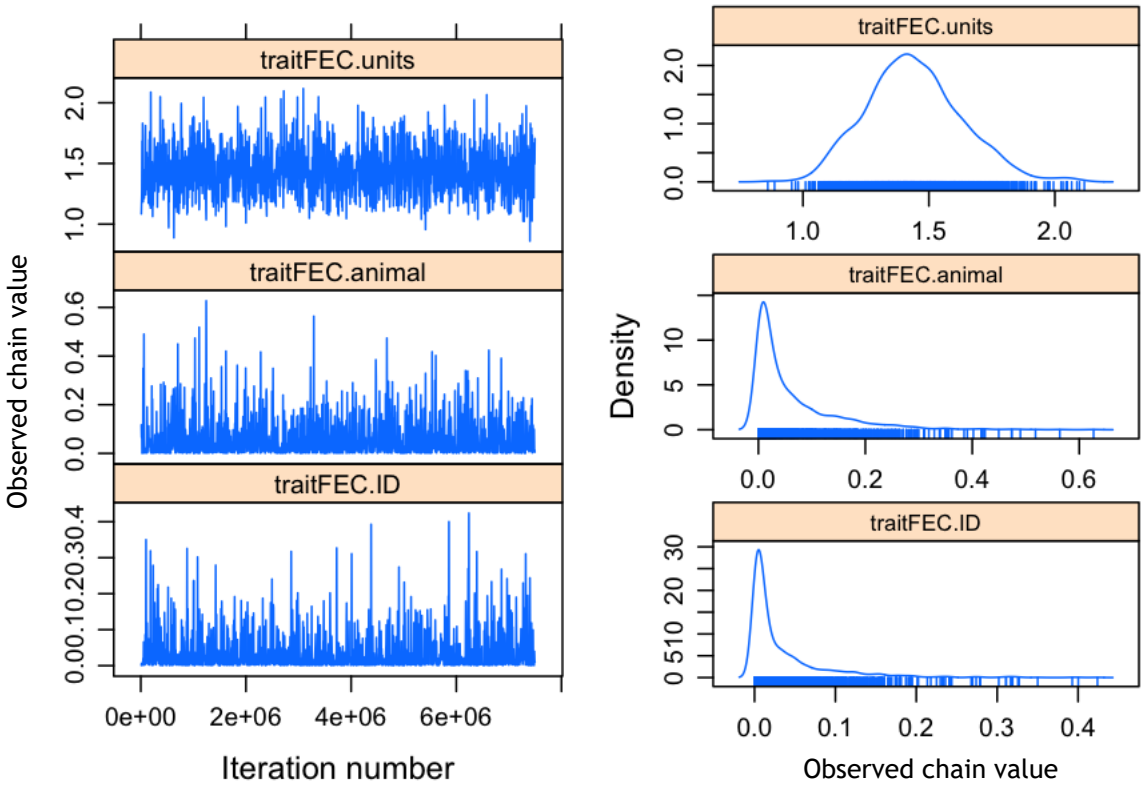


Figure 3-22. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for the random effects and residual variance (denoted units) for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID and animal as random effects (Model 5b).
traitFEC.units = residual variance; ; traitFEC.ID = ID variance component.

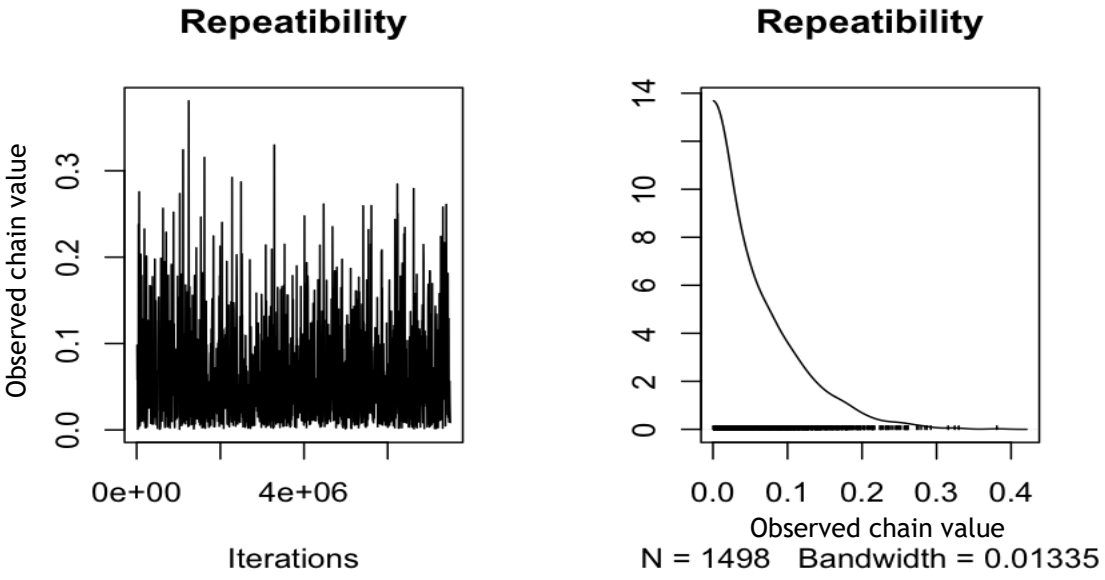


Figure 3-23. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID and animal as random effects (Model 5b).

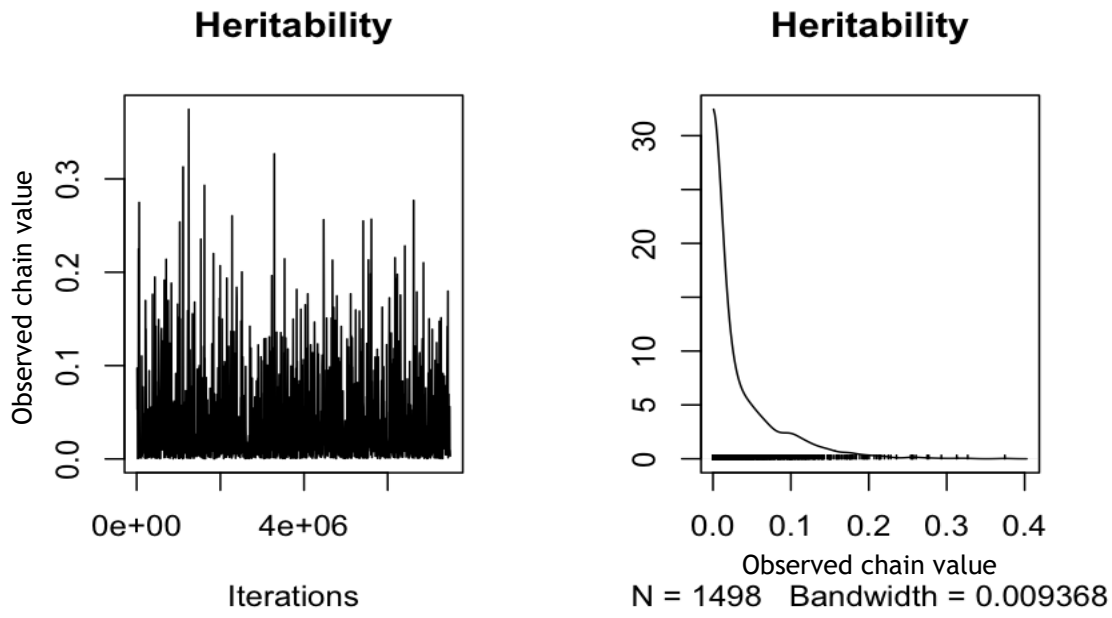


Figure 3-24. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC heritability for the association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID and animal as random effects (Model 5b).

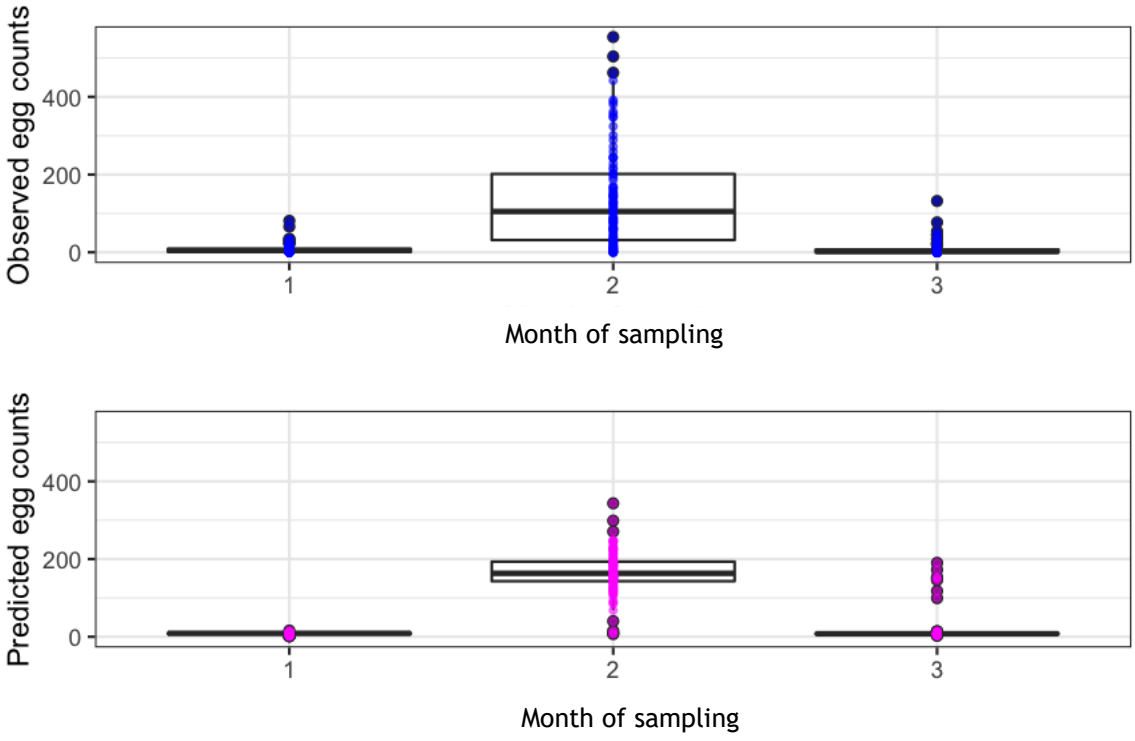


Figure 3-25. Observed (top) and predicted (bottom) FEC data using the model of association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID and animal as a random effects (Model 5b).

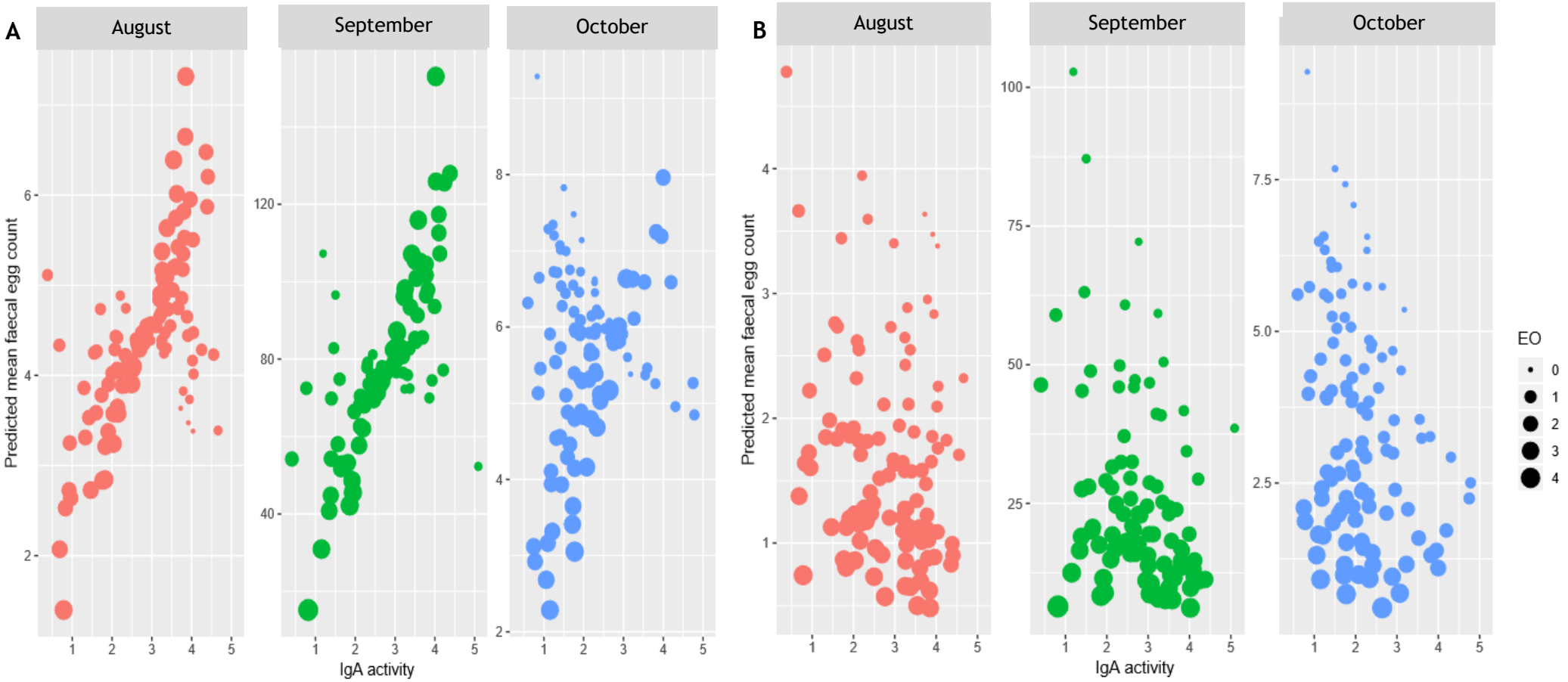


Figure 3-26. Predicted mean FEC against IgA activity (A) with and (B) without interaction with peripheral eosinophil counts using the model of association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID and animal as a random effects (Model 5b)..

EO = Peripheral eosinophil counts.

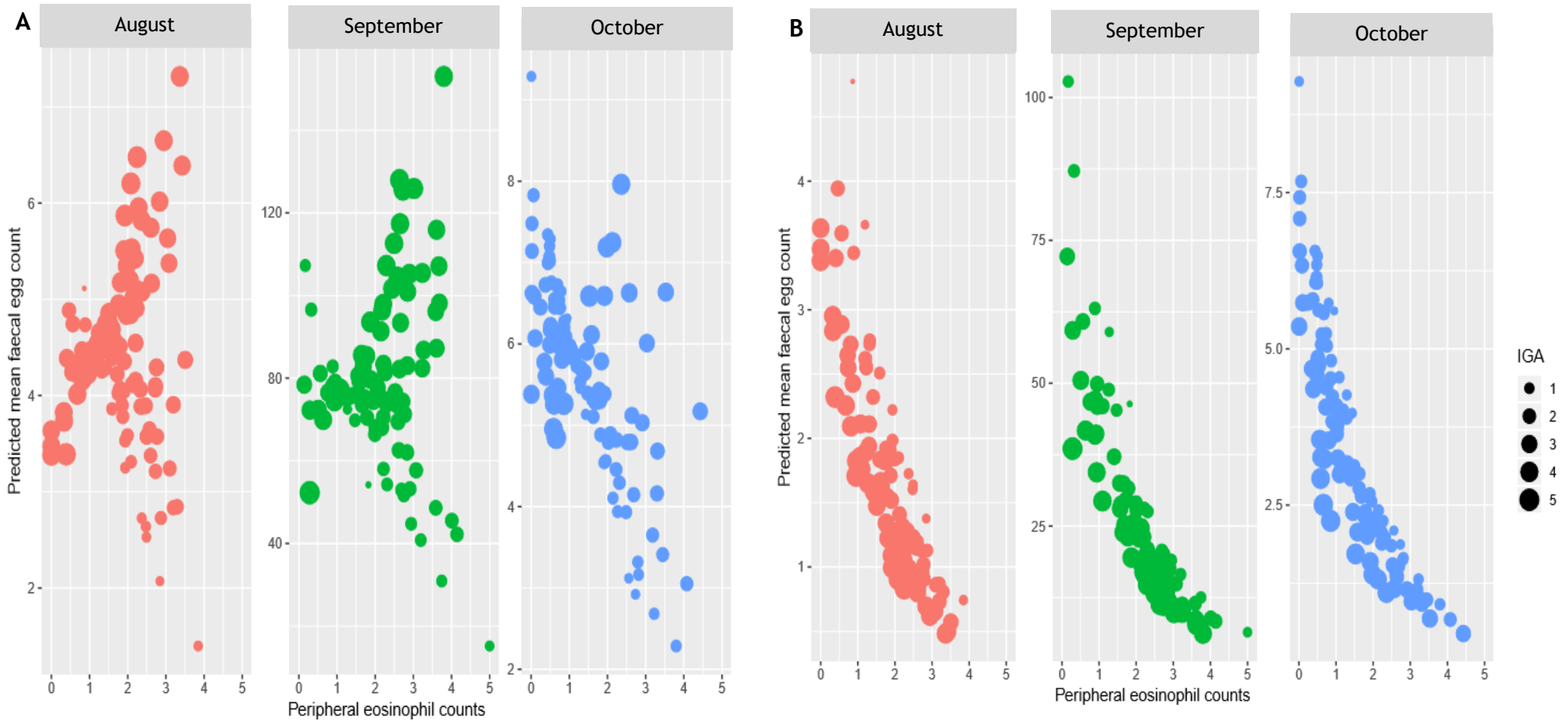


Figure 3-27. Predicted mean FEC against peripheral eosinophil counts (A) with and (B) without interaction with IgA activity using the model of association of FEC with time of sampling, IgA, peripheral eosinophils and their interaction; with ID and animal as a random effect (Model 5b).
IGA = IgA activity

c) Mixed effects models of FEC with time (TIME), immune index, types of birth (TB), sex (SEX) and their interactions and random effect of ID.

Model selection and diagnostics Table 3-18 shows the process of model selection. Autocorrelations (not shown) and effective sample size for all models were checked as for previous models. No trend is seen in the trace for either the fixed effects (Figure 3-28, A) or random effects (Figure 3-29, left). In addition, there was no evidence of autocorrelation in the estimate of repeatability based on the trace (Figure 3-30, left). The corresponding posterior distributions are shown in Figure 3-28 (B), Figure 3-29 (right) and Figure 3-30 (right).

Fixed effects The posterior means and 95% credible intervals for the fixed effects for Model 4c are shown in Table 3-19. In this model, time effects had similar attributes to Models 5a and 5b on both binary and count components. In addition, Model 4c had a significant effect of sex, but no effect of the immune index on FEC.

Random effects The posterior means and 95% credible intervals for the random effects and residuals for Model 4c are shown in Table 3-19. The random effect of ID was small (0.036) and the residual variance was large (1.526) indicating overdispersion, showing very similar patterns to Models 5a and 5b.

Repeatability The posterior mode and mean estimates for the repeatability were small (0.001 and 0.023 respectively; Table 3-20), similar to the results for Models 5a and 5b.

Observed versus predicted data Figure 3-31 shows that the observed FEC data (top) were consistent with the predicted FEC data (bottom). Table 3-21 shows that the numbers of observed and predicted zero FEC are in generally reasonable agreement. Similar to Models 5a and 5b, Table 3-21 also shows the overall proportion of zeroes which are true zeroes is high at around 73%. Table 3-22 shows that the observed and predicted means of FEC are generally comparable.

Table 3-18. Model selection process for mixed effects models of FEC with time, type of birth, IgA, immune index and sex as fixed effects, ID as a random effect.

Variable/DIC	Model 1c	Model 2c	Model 3c	Model 4c
DIC	1807.1	1806.6	1806.4	1807.8
TIME (B)	Y	Y	Y	Y
TIME (C)	Y	Y	Y	Y
TB (C)	Y			
ImmIndex (C)	Y	Y	Y	
SEX (C)	Y	Y	Y	Y
TIME:ImmIndex (C)	Y	Y		
ID* (B,C)	Y	Y	Y	Y

DIC= Deviance Information Criterion; (B) = binary component; (C) = count component; Y = included; '*' = random effects; TB = types of birth; ImmIndex = immune index.

Table 3-19. Model coefficients of association of FEC with time of sampling and sex of goats in Model 4c with ID as a random effect.

Structure	Variable	Posterior mean (Binary)	Posterior mean (Count)	Lower 95% CI	Upper 95% CI	Eff. sample	p-value	
Fixed effects	Intercept		1.479	1.169	1.761	1498	0.001	***
		-3.679		-6.080	-1.870	1044.990	0.001	***
	TIME2	-0.013		-2.203	2.572	1268.889	0.852	
	TIME3	3.050		1.323	5.622	1061.542	0.001	***
	TIME2		2.969	2.572	3.377	1498	0.001	***
	TIME3		0.226	-0.237	0.697	1610.898	0.339	
	SEX2		-1.076	-2.112	-0.062	1498	0.048	*
Random effects	ID		0.036	0	0.155	1545.878	-	-
		0		0	0	0	-	-
Residuals	Units		1.526	1.175	1.886	1498	-	-
		1		1	1	0	-	-

CI = credible intervals; Eff. sample = effective sample size; TIME2 = September; TIME3 = October; SEX2 = male; Significance codes: '***' < 0.001; '**' < 0.01; '*' <0.05; '.' < 0.1; ' ' <=1.

Table 3-20. FEC repeatability for Model 4c.

Posterior mode for repeatability	Lower HPD interval	Upper HPD interval	Posterior mean for repeatability
0.001	0	0.097	0.023

HPD = highest posterior density

Table 3-21. Observed and predicted zero FEC for Model 4c.

Time	Number of counts	Observed zeroes	Predicted zeroes	Predicted true zeroes	Probability of predicted zeroes that are true zeroes
1	110	18	23.060	11.260	0.49
2	91	4	4.440	3.990	0.90
3	110	49	43.200	36.610	0.85
All	311	71	70.703	51.862	0.73

Table 3-22. Observed and predicted means of FEC for Model 4c.

Time	Number counts	Observed means	Predicted means
1	110	7.760	8.560
2	91	138.710	169.120
3	110	7.380	18.540
All	311	45.950	59.070

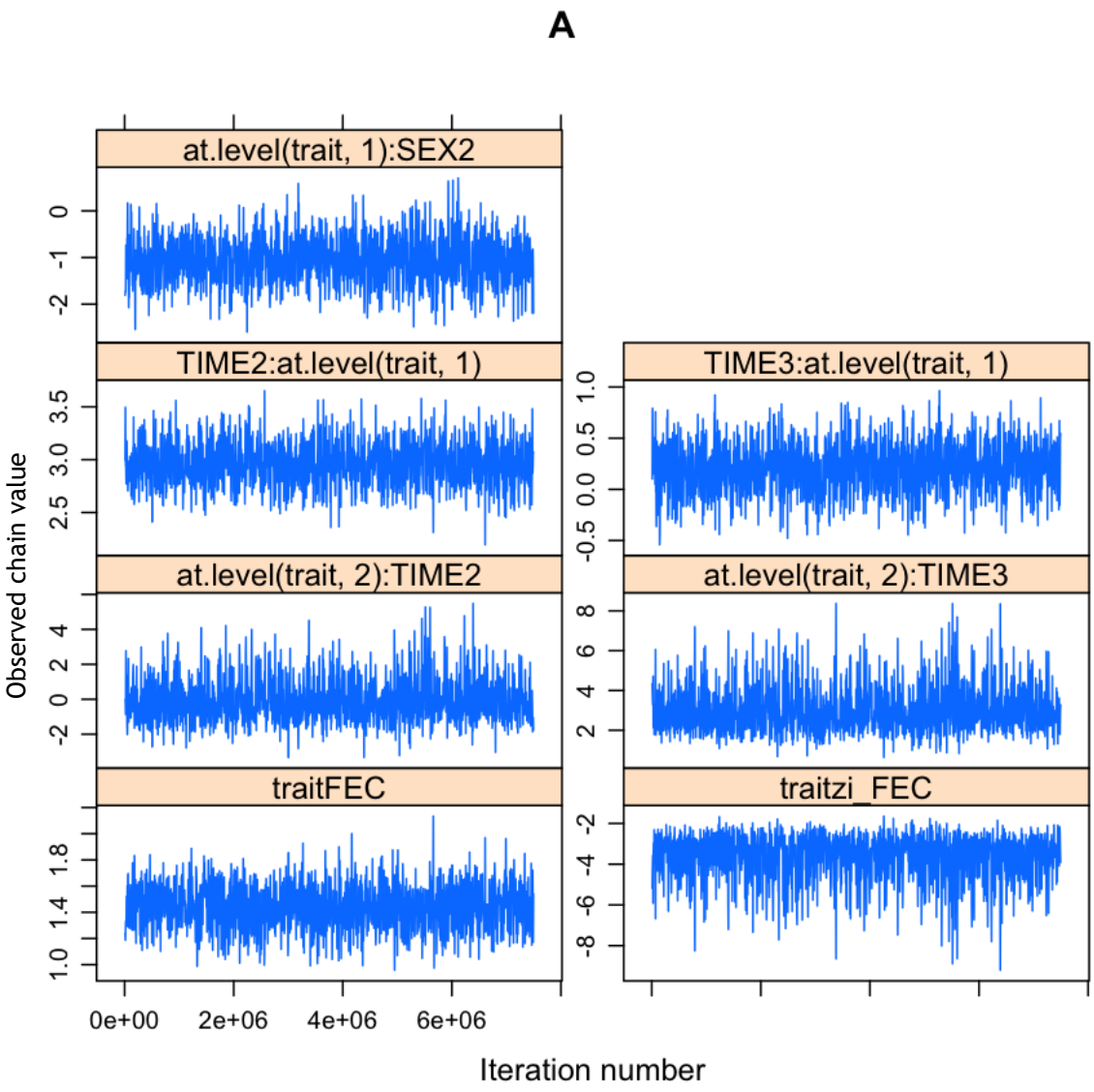


Figure 3-28 (A). Trace plots of parameter value explored by the MCMC chain of fixed effects for the association of FEC with time of sampling, IgA, and sex of the goats, with ID as a random effect (Model 4c).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = September; TIME3 = October; SEX2 = male; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

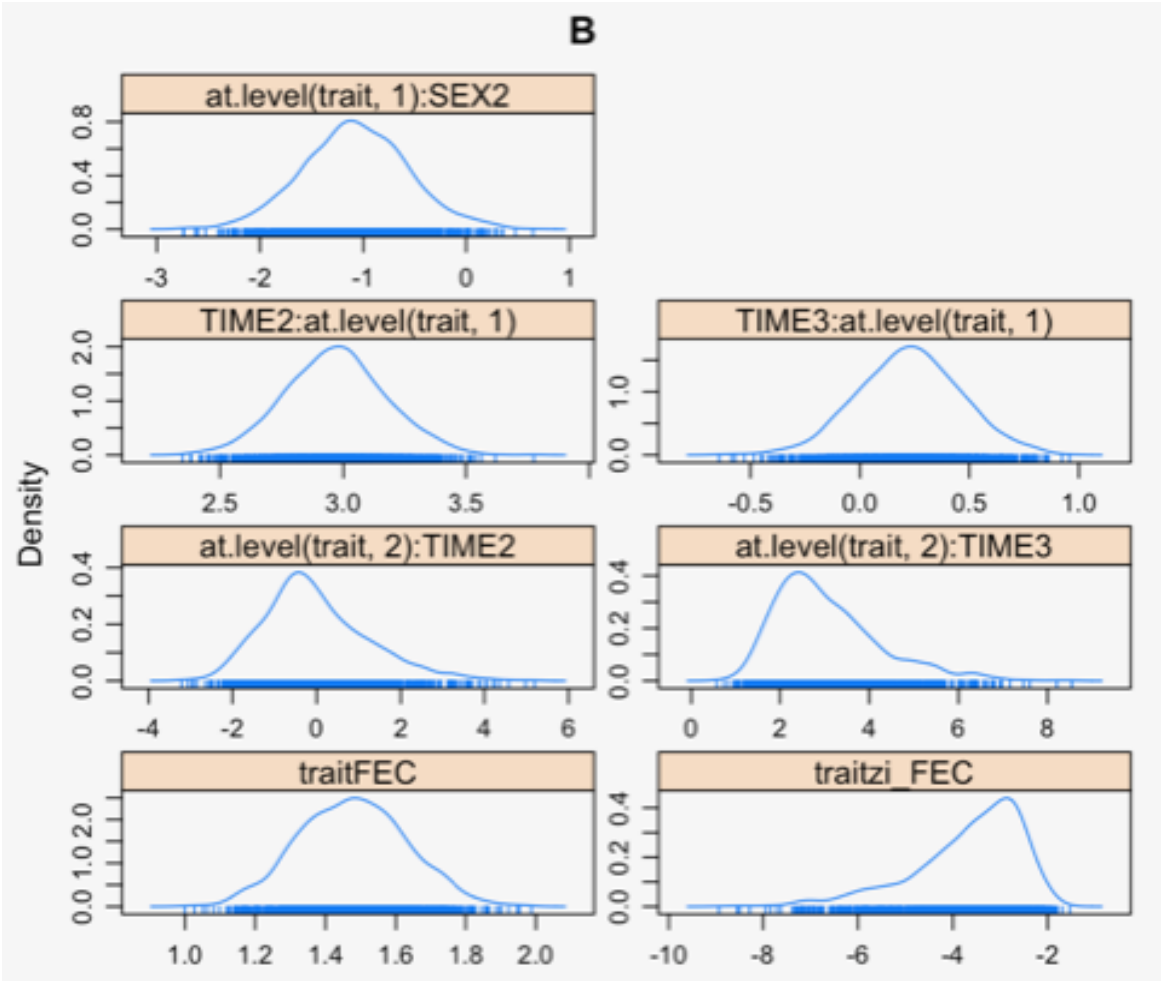


Figure 3-28 (B). Posterior density plots of parameter value distributions in the MCMC chain for fixed effects for the association of FEC with time of sampling and sex of the goats, with ID as a random effect (Model 4c).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = September; TIME3 = October; SEX2 = male; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

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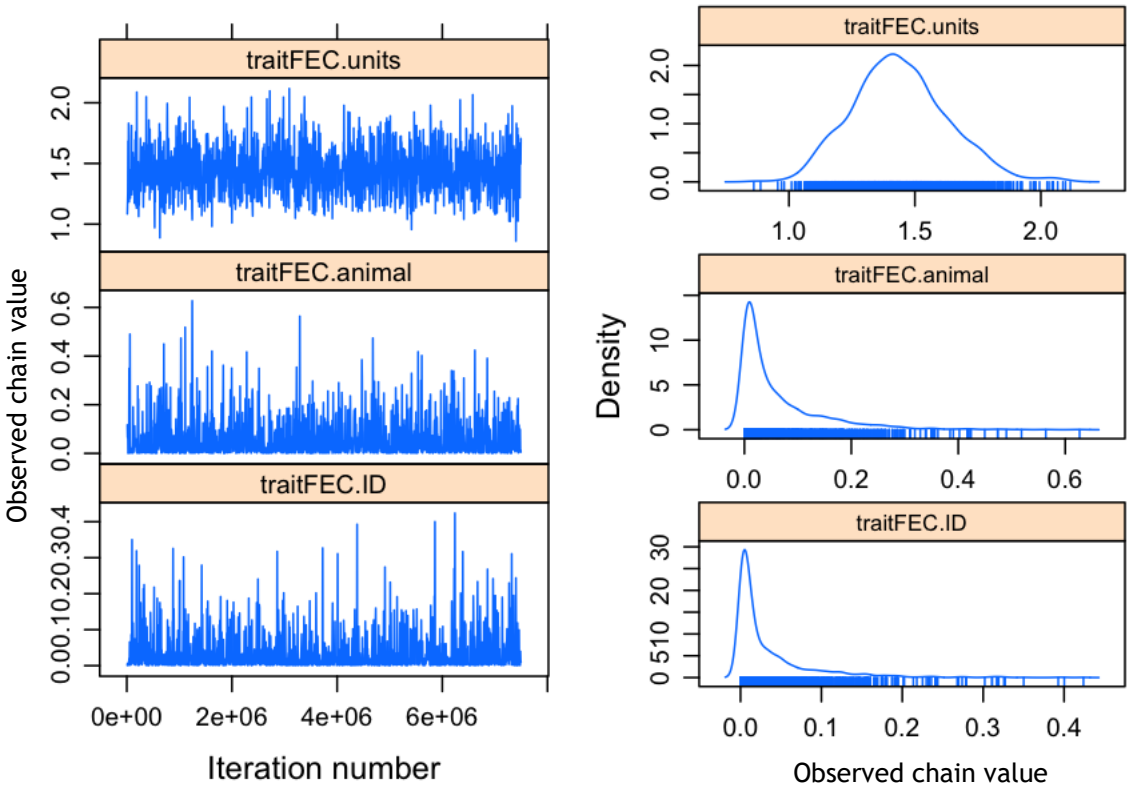


Figure 3-29. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for random effect and residual variance for the association of FEC with time of sampling and sex of goats; with ID as a random effect (Model 4c).

traitFEC.units = residual variance; ; traitFEC.ID = ID variance component.

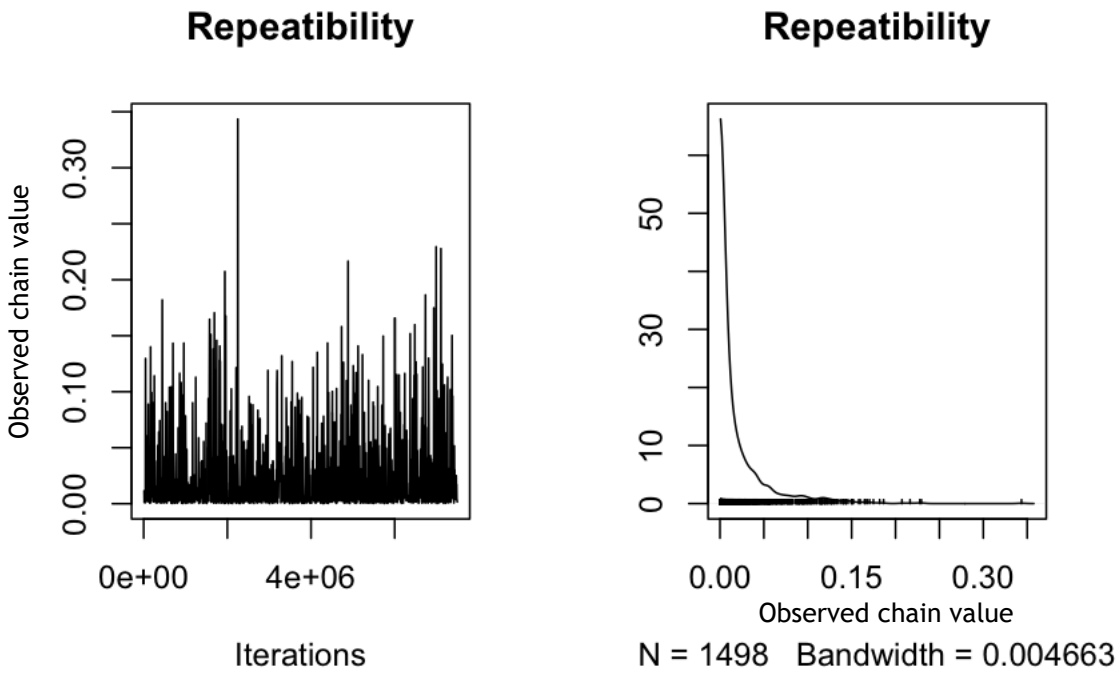


Figure 3-30. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots of parameter value distributions in the chain (right) for FEC repeatability for the association of FEC with time of sampling and sex of goats; with ID as a random effect (Model 4c).

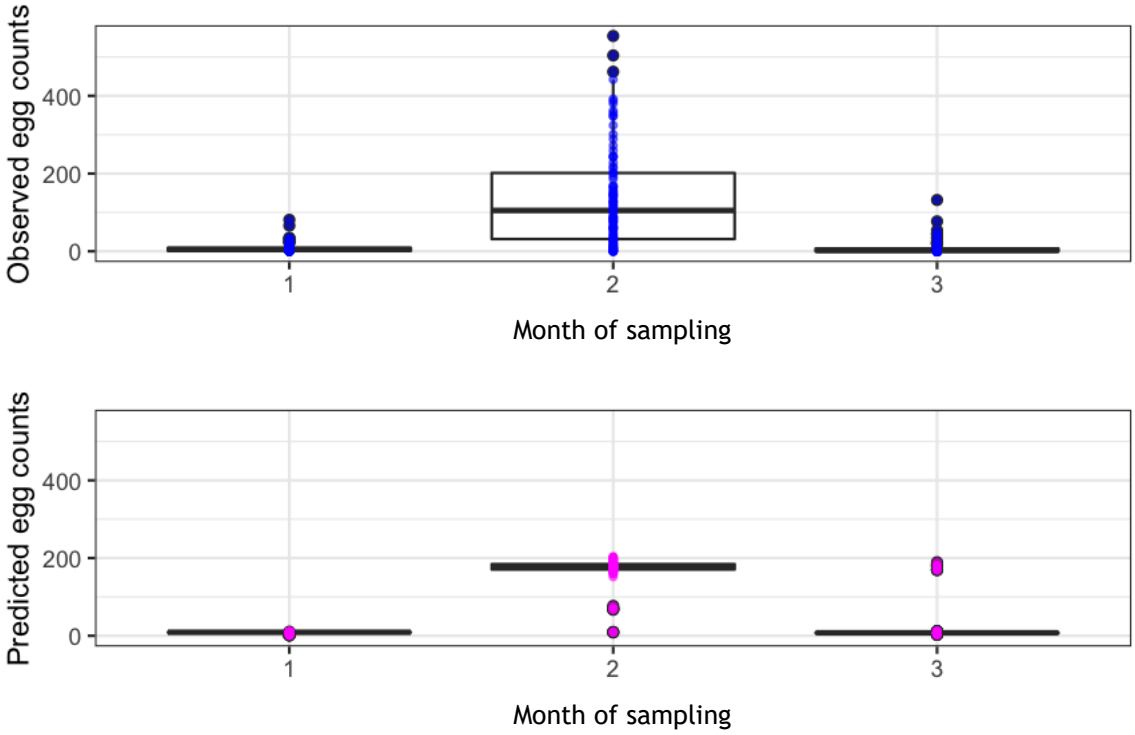


Figure 3-31. Observed (top) and predicted (bottom) FEC data using the model for the association of FEC with time of sampling and sex of goats; with ID as a random effect (Model 4c).

d) Mixed effects models of FEC with time (TIME), immune index, types of birth (TB), sex (SEX) and their interactions and random effect of ID and animal.

Model selection and diagnostics Table 3-23 shows the process of model selection. Autocorrelations (not shown) and effective sample size for all models were checked as for previous models with no trend seen in the trace for fixed effects (not shown), random effects (not shown), repeatability (Figure 3-32, left) and heritability (Figure 3-33, left).

Fixed effects The fixed effects in Model 4d were very similar to those in Model 4c (Table 3-24), with significant fixed effects of time and sex on FEC, but no effect of the immune index.

Random effects The posterior means and 95% credible intervals for the random effects and residuals for Model 4d are shown in Table 3-24. The random effects of ID and animal were small (0.036 and 0.071 respectively), whilst the residual variance was large (1.468), showing very similar patterns to Model 5b.

Repeatability and heritability The posterior mode and mean estimates for the repeatability and heritability were small (Tables 3-24 and Table 3-26), similar to the results for Models 5a and 5b. The posterior distributions for repeatability (Figure 3-28) and heritability (Figure 3-29) were skewed towards zero as seen in Models 5a and 5b.

Observed versus predicted data Figure 3-34 shows that the observed FEC data (top) were consistent with the predicted FEC data (bottom). The observed and predicted zeroes for Model 4d were similar to Models 5a, 5b and 4c (Table 3-27), showing that the overall proportion of zeroes which are true zeroes to be high at around 73%. Table 3-28 shows that the observed and predicted means of FEC are generally comparable.

Table 3-23. Model selection process for mixed effects models of FEC with time, type of birth, IgA, immune index and sex as fixed effects, ID and animal as random effects.

Variable/DIC	Model 1d	Model 2d	Model 3d	Model 4d
DIC	1806.6	1806.5	1806.3	1807.5
TIME (B)	Y	Y	Y	Y
TIME (C)	Y	Y	Y	Y
TB (C)	Y			
ImmIndex (C)	Y	Y	Y	
SEX (C)	Y	Y	Y	Y
TIME:ImmIndex (C)	Y	Y		
ID* (B,C)	Y	Y	Y	Y
Animal* (B,C)	Y	Y	Y	Y

DIC= Deviance Information Criterion; (B) = binary component; (C) = count component; Y = included; '*' = random effects; TB = types of birth; ImmIndex = immune index.

Table 3-24. Model coefficients of association of FEC with time of sampling and sex of goats in Model 4d with ID and animal as random effects.

Structure	Variable	Posterior mean (Binary)	Posterior mean (Count)	Lower 95% CI	Upper 95% CI	Eff. sample	p-value	
Fixed effects	Intercept		1.443	1.104	1.759	1498	0.001	***
		-3.636		-6.044	-1.976	1309.478	0.001	***
	TIME2	-0.079		-2.218	2.523	1328.927	0.828	
	TIME3	3.009		1.257	5.485	1295.405	0.001	***
	TIME2		2.961	2.544	3.338	1498	0.001	***
	TIME3		0.21	-0.260	0.704	1498	0.377	
	SEX2		-1.053	-2.031	-0.061	1285.543	0.039	*
Random effects	ID		0.036	0	0.124	1498	-	-
		0		0	0	0	-	-
	Animal		0.071	0	0.249	1498	-	-
		0		0	0	0	-	-
Residuals	Units		1.468	1.097	1.836	1498	-	-
		1		1	1	0	-	-

CI = credible intervals; Eff. sample = effective sample size; TIME2 = September; TIME3 = October; SEX2 = male; Significance codes: '***' < 0.001; '**' < 0.01; '*' <0.05; '.' < 0.1; ' ' <=1.

Table 3-25. FEC repeatability for Model 4d.

Posterior mode for repeatability	Lower HPD interval	Upper HPD interval	Posterior mean for repeatability
0.007	0.001	0.182	0.064

HPD = highest posterior density

Table 3-26. FEC heritability for Model 4d.

Posterior mode for heritability	Lower HPD interval	Upper HPD interval	Posterior mean for heritability
0.001	0	0.163	0.045

HPD = highest posterior density

Table 3-27. Observed and predicted zero FEC for Model 4d.

Time point	Number counts	Observed zeroes	Predicted zeroes	Predicted true zeroes	Probability of predicted zeroes that are true zeroes
1	110	18	23.370	11.330	0.48
2	91	4	4.470	3.930	0.88
3	110	49	43.440	36.680	0.84
All	311	71	71.276	51.934	0.73

Table 3-28. Observed and predicted means of FEC for Model 4d.

Time point	Number of counts	Observed means	Predicted means
1	110	7.760	8.550
2	91	138.710	163.520
3	110	7.380	17.890
All	311	45.950	57.200

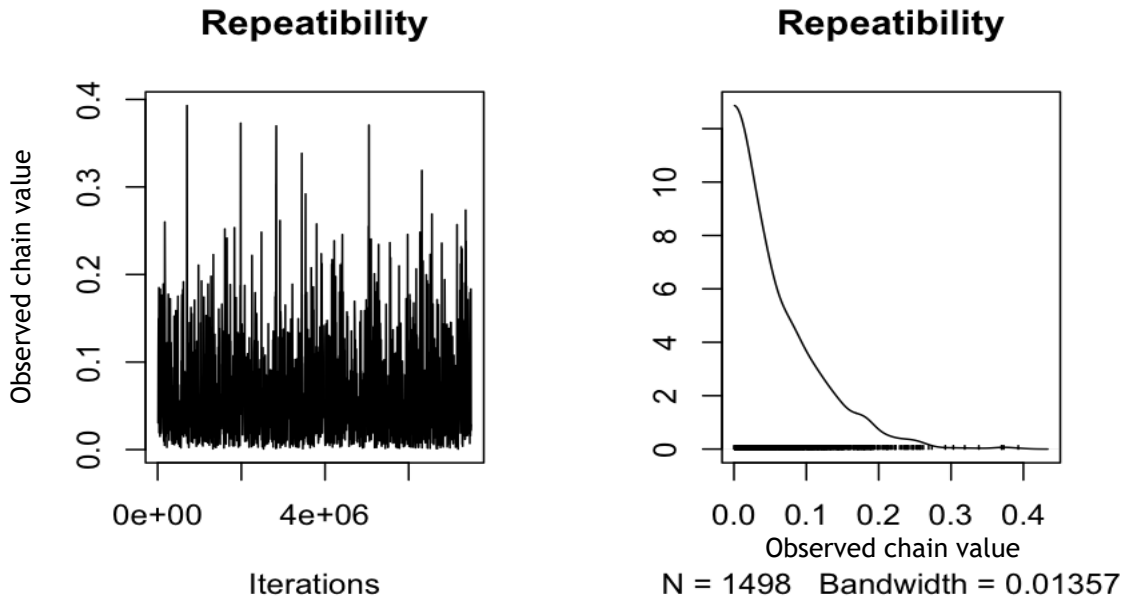


Figure 3-32. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability for the association of FEC with time of sampling and sex of goats; with ID and animal as random effects (Model 4d).

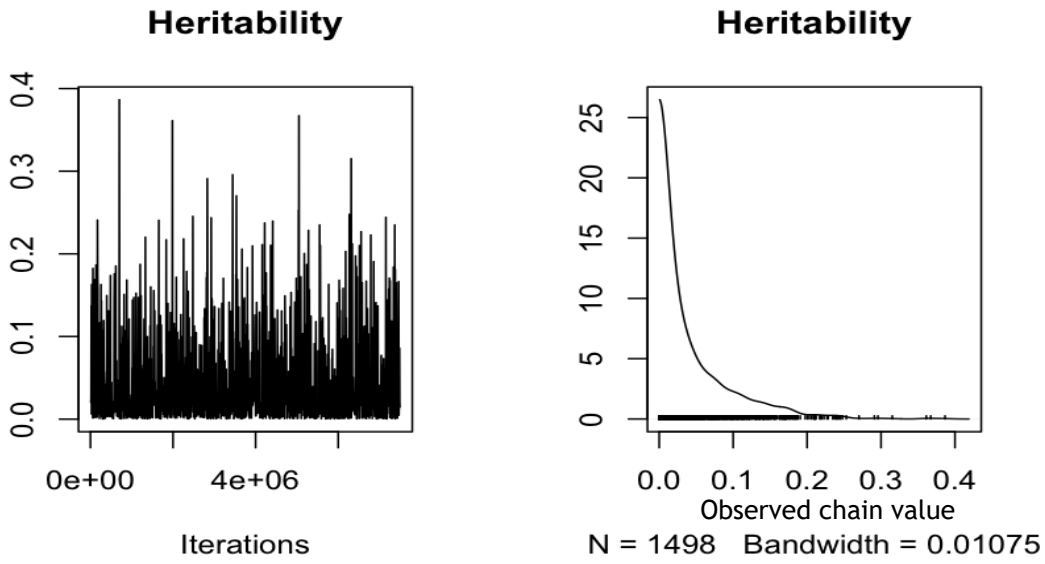


Figure 3-33. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC heritability for the association of FEC with time of sampling and sex of goats; with ID and animal as random effects (Model 4d).

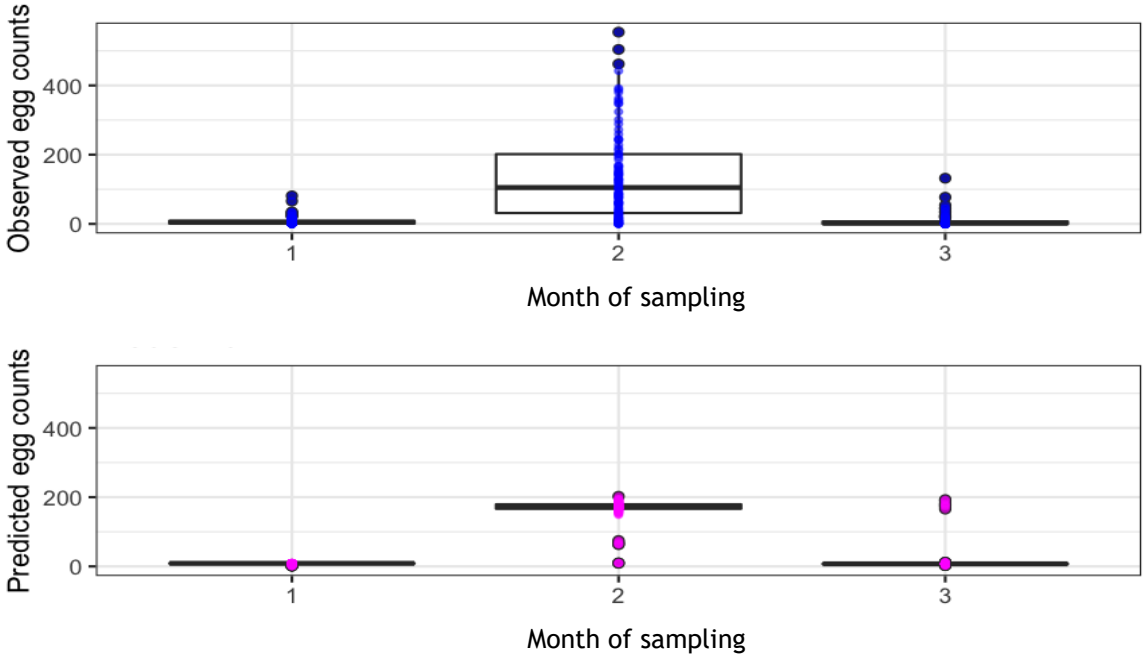


Figure 3-34. Observed (top) and predicted (bottom) FEC data using the model for the association of FEC with time of sampling and sex of goats; with ID and animal as random effects (Model 4d).

3.3.1.2 Summary of FEC models

The model sets a-d explored the role of immune response on FEC. The first two sets of models (sets a and b) included IgA and eosinophils as independent variables and showed a significant negative effect of eosinophils on FEC. IgA had no significant effect on FEC but its interaction with eosinophils would act to moderate the main effect of eosinophils in controlling nematode infection. In the other words, IgA reduces the protective effect of eosinophils in reducing FEC. In the last two sets of models (sets c and d), male goats show significantly lower FEC than female goats. In all final models, time that indicated month of sampling showed significant effect on FEC. The random effects of ID and animal were small in these models, but the residual variation for each model was comparatively large indicating overdispersion of FEC. Additionally, both repeatability and heritability for FEC were small.

3.3.1.3 Bodyweight models

e) Mixed effects models of bodyweight with FEC, month and their interaction as fixed effects and ID as a random effect.

f) Mixed effects models of bodyweight with FEC, month and their interaction as fixed effects; ID and animal as random effects.

Model sets e and f differ in whether animal is included as a random effect and are discussed together in this section.

Model selection and diagnostics All models were checked for autocorrelation and convergence as for previous models in this chapter. The selected parameters were run to ensure autocorrelation to be less than 0.1 and effective samples sizes exceeding 1000.

Fixed effects Tables 3-29 and 3-30 show the process of model selection for model sets e and f respectively. In both cases, the final models have fixed effects of month and FEC. The model summaries for final models, Model 2e and 2f are shown in Tables 3-31 and 3-32. In both models, the month of sampling was associated with differences in bodyweight ($p<0.001$). In addition, as the FEC decreased, the bodyweight increased.

Random effects The main difference between the models was in the estimates for the random effects, residuals (Figure 3-38) and therefore the estimates of repeatability and heritability. In Model 2e, there was a large random effect of ID (15.880) and a comparatively small residual variance (1.970). In Model 2f, the residual variance was similar in size with Model 2e (1.955), but there was a large effect of animal (16.030) and a much smaller effect of ID (2.225). These results indicate that much of the repeatability among animals in bodyweight results from additive genetic effects that were captured in Model 2f.

Repeatability and heritability The repeatability (Models 2e and 2f) and heritability (Model 2f) of bodyweight derived from the variance components are shown in Figures 3-35, 3-36 and 3-37. In Model 2e, the estimate for the repeatability is high at 0.91 within the HPD interval of 0.87 and 0.94 (Figure 3-35 (right)). In Model 2f, the estimate of bodyweight repeatability was similar at 0.89 within the HPD interval of 0.85 and 0.92 (Figure 3-36, right). The heritability estimates of bodyweight in Model 2f was high at 0.90 within a wider HPD interval of 0.33 to 0.94 (Figure 3-37 (right).

Table 3-29. Model selection process for mixed effects models of body weight with FEC and month as fixed effects, ID as a random effect.

DIC/Variable/ Random effect	Model	
	1e	2e
DIC	1125.4	1122.3
FEC	✓	✓
MONTH	✓	✓
MONTH : FEC	✓	
ID*	✓	✓

'*' = random effects; DIC= Deviance Information Criterion; FEC = faecal egg counts; ID= identification.

Table 3-30. Model selection process for mixed effects models of body weight with FEC and month as fixed effects, ID and animal as random effects.

DIC/Variable/ Random effect	Model	
	1f	2f
DIC	1123.5	1120.3
MONTH	✓	✓
FEC	✓	✓
MONTH : FEC	✓	
ID*	✓	✓
Animal*	✓	✓

'*' = random effects; DIC= Deviance Information Criterion; FEC = faecal egg counts; ID= identification.

Table 3-31. Model coefficients of association of body weight with month of sampling and faecal egg counts in Model 2e with ID as a random effect.

Structure	Variable	Posterior means	Lower 95% CI	Upper 95% CI	Eff. sample	p-value
Intercept		16.230	15.550	16.980	1980	<5e-04***
Fixed effects	Month 2	2.005	1.389	2.589	1980	<5e-04***
	Month 3	3.953	3.534	4.363	1980	<5e-04 ***
	FEC	-1.263e-04	-2.369e-04	-1.697e-05	1980	0.028*
Random effects	ID	15.880	11.920	19.830	2134	-
Residuals	Units	1.970	1.553	2.439	1980	-

CI = credible intervals; Eff. sample = effective sample size; Month 2 = September; Month 3 = October; FEC = faecal egg counts; ID = identification; p-value: '*' = p<0.05; '**' = p<0.01; '***' = p<0.001.

Table 3-32. Model coefficients of association of body weight with month of sampling and faecal egg counts in Model 2f with ID and animal as random effects.

Structure	Variable	Posterior means	Lower 95% CI	Upper 95% CI	Eff. sample	p-value
Intercept		17.310	15.320	19.350	1980	<5e-04***
Fixed effects	Month 2	1.967	1.401	2.536	2161	<5e-04***
	Month 3	3.962	3.580	4.389	1902	<5e-04 ***
	FEC	-1.188e-04	-2.220e-04	-8.441e-05	1980	0.027*
Random effects	ID	2.225	0.001	9.624	1203	-
	Animal	16.030	4.719	24.280	1209	
Residuals	Units	1.955	1.529	2.398	1980	-

CI = credible intervals; Eff. sample = effective sample size; Month 2 = September; Month 3 = October; FEC = faecal egg counts; ID = identification; p-value: '*' = p<0.05; '**' = p<0.01; '***' = p<0.001.

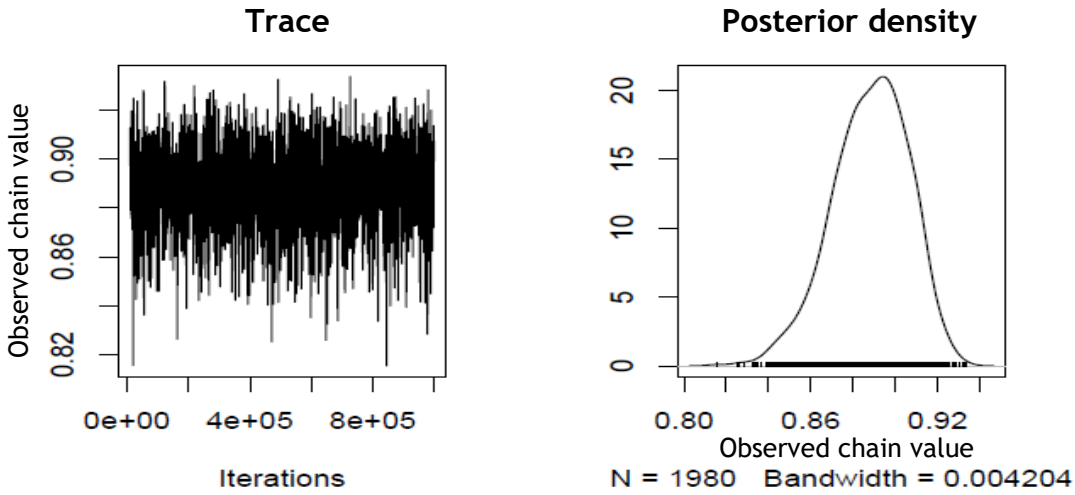


Figure 3-35. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for body weight repeatability for the association of body weight with month of sampling; with ID as a random effect (Model 2e).

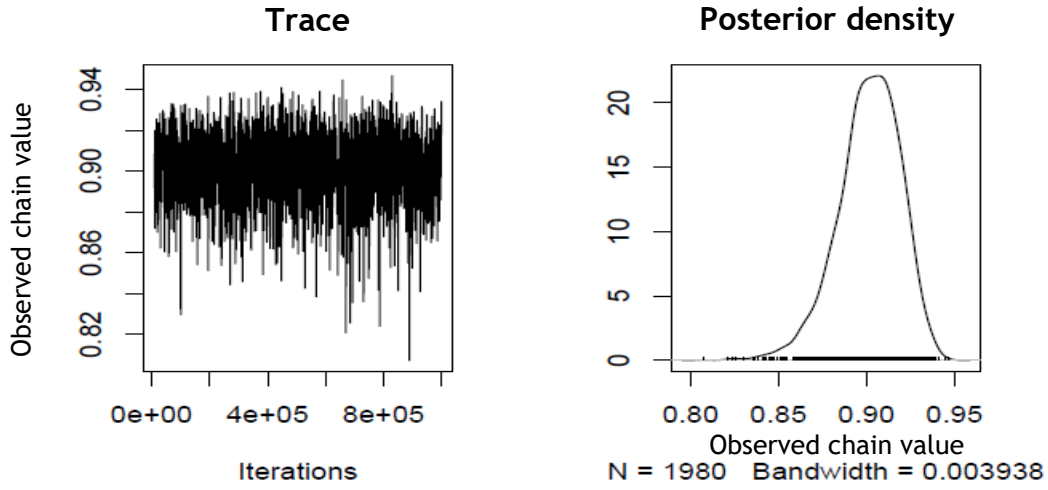


Figure 3-36. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for body weight repeatability for the association of body weight with month of sampling; with ID and animal as random effects (Model 2f).

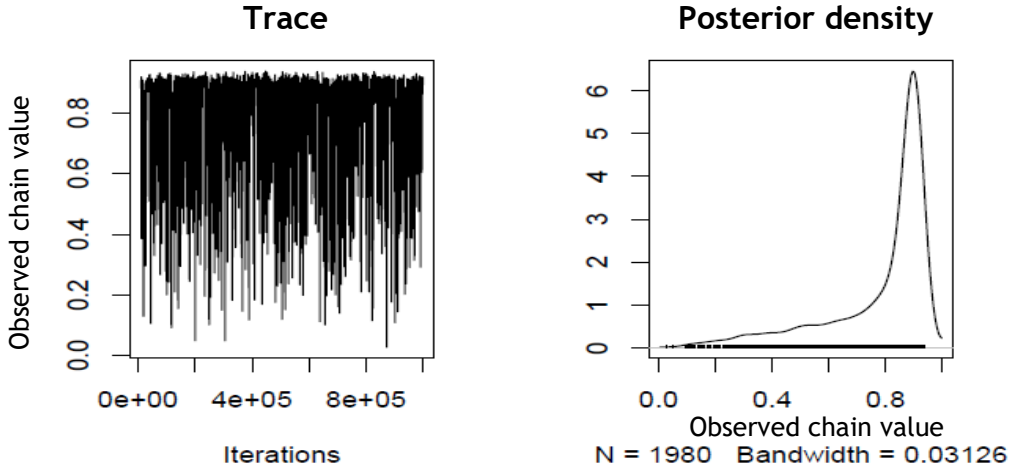


Figure 3-37. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for body weight heritability for the association of body weight with month of sampling; with ID and animal as random effects (Model 2f).

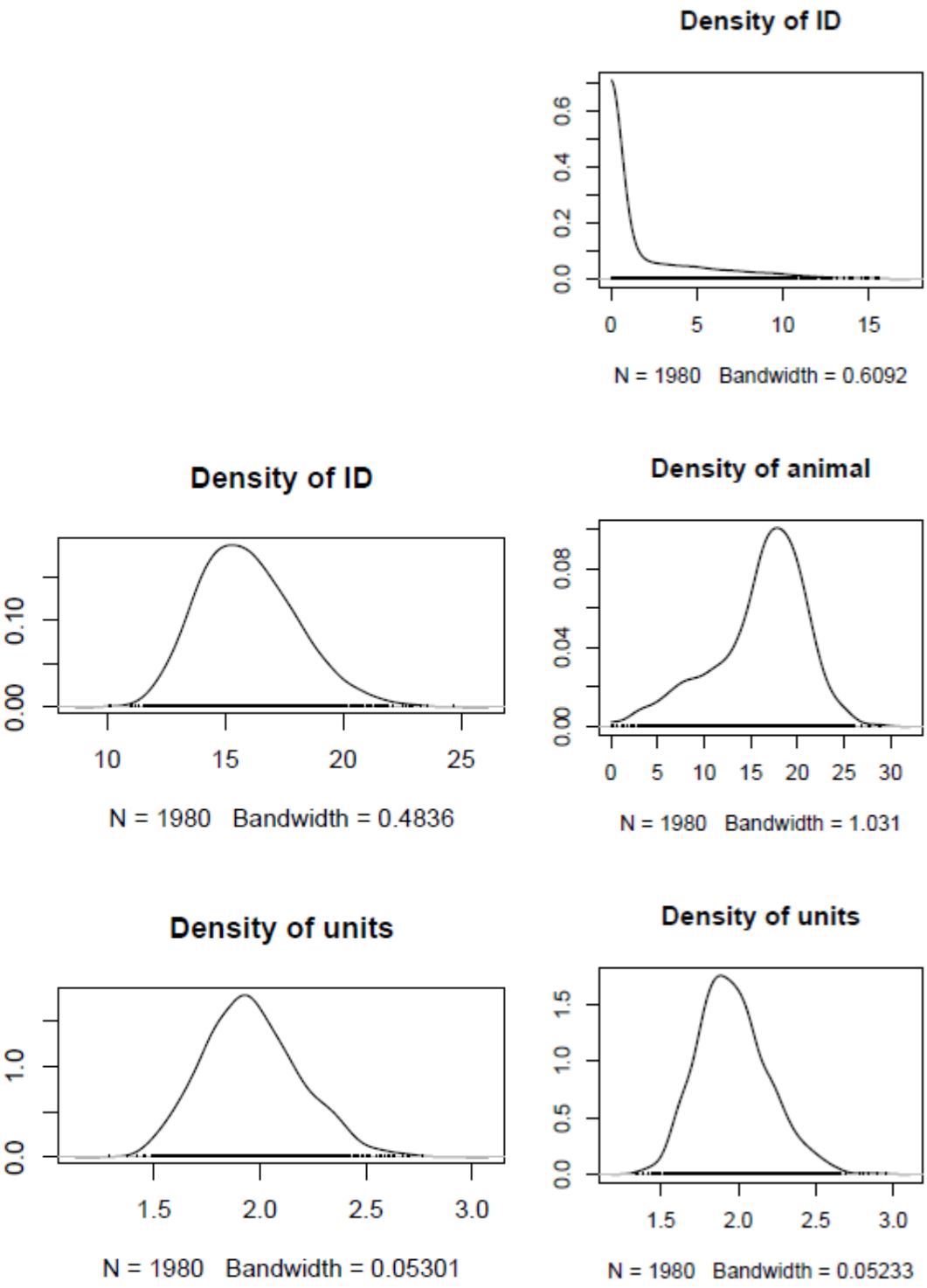


Figure 3-38. Posterior density plots of parameter value distributions in the MCMC chain for random effects and residual variance (denoted 'units') for the association of body weight with month of sampling; with ID as a random effect (Model 2e, left) and with both animal and ID as random effects (Model 2f, right).

g) Mixed effects models of bodyweight with month, IgA, peripheral eosinophil counts and their interactions as fixed effects; ID as a random effect.

h) Mixed effects models of bodyweight with month, IgA, peripheral eosinophil counts and their interactions as fixed effects; ID and animal as random effects.

Model sets g and h differ in whether animal was included as a random effect. As the results of the previous section (model set e and f) show a substantial additive genetic effect shown by the animal random effect, we will only discuss the results for model set h (with both random effects) here.

Model selection and diagnostics Table 3-33 shows the process of model selection. All models were checked for autocorrelation, trends in the trace (Figure 3-39, left) and effective sample size as in previous sections. The model summary for the final model (Model 5h) is shown in Table 3-34.

Fixed effects There were significant effects of month of sampling ($p<0.001$), peripheral eosinophil counts ($p<0.05$) as well as the interaction of Month 2 ($p<0.05$) and Month 3 ($p<0.01$) with peripheral eosinophil counts on bodyweight of the goats (Table 3-34).

Random effects Table 3-34 shows that the posterior mean variance for the random effect of animal was high in Model 5h (15.380) compared to the random effect of ID (2.670) and residual variance (1.942).

Repeatability and heritability This resulted in a high posterior mode for FEC heritability of 0.91 with a HPD interval of 0.33 to 0.94 (Figure 3-40). The posterior mode for the repeatability estimate of FEC in this model was also high at 0.91 with a HPD interval of 0.87 and 0.94 (Figure 3-41).

Table 3-33. Model selection process for mixed effects models of bodyweight with month, IgA and peripheral eosinophil counts as fixed effects; ID and animal as random effects.

DIC/Variable/ Random effect	Model				
	1h	2h	3h	4h	5h
DIC	**1118.9**	**1125.9**	**1122.5**	**1118**	**1118.9**
MONTH	✓	✓	✓	✓	✓
IgA	✓	✓	✓	✓	
EO	✓	✓	✓	✓	✓
Month : IgA	✓	✓	✓		
Month : EO	✓	✓	✓	✓	✓
IgA : EO	✓	✓			
Month : IgA : EO	✓				
ID*	✓	✓	✓	✓	✓
Animal*	✓	✓	✓	✓	✓

'*' = random effects; DIC= Deviance Information Criterion; EO = peripheral eosinophil counts; ID= identification.

Table 3-34. Model coefficients of association of body weight with month of sampling, peripheral eosinophil counts and their interactions in Model 5h with ID and animal as random effects.

Structure	Variable	Posterior means	Lower 95% CI	Upper 95% CI	Eff. sample	p-value
Intercept		16.280	14.060	18.390	1829	<5e-04***
Fixed effects	Month 2	2.882	1.656	4.173	1996	<5e-04***
	Month 3	5.404	4.388	6.407	1870	<5e-04***
	EO	6.968e-04	1.832e-04	1.213e-03	1996	0.017*
	Month 2 : EO	-8.318e-04	-1.525e-03	-8.718e-05	1996	0.035*
	Month 3 : EO	-1.039e-03	-1.781e-03	-4.072e-04	1996	0.003**
Random effect	ID	2.670	0.001	10.640	1574	-
	Animal	15.380	2.999	23.600	1787	-
Residuals	Units	1.942	1.509	2.381	2313	-

CI = credible intervals; Eff. sample = effective sample size; Month 2 = September; Month 3 = October; EO = peripheral eosinophil counts; ID = identification; p-value: '*' = p<0.05; '**' = p<0.01; '***' = p<0.001.

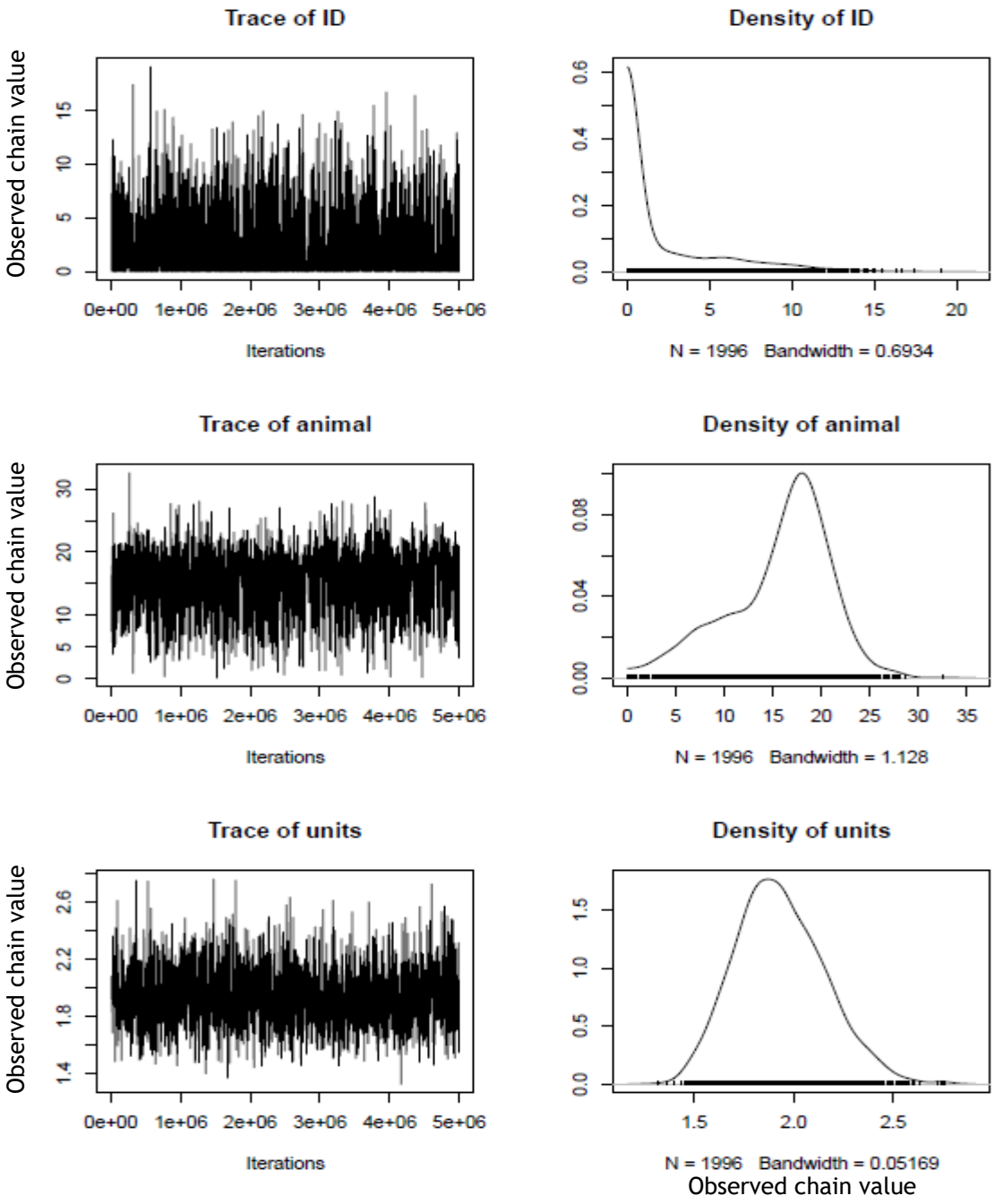


Figure 3-39. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for random effects and residual variance (denoted units) for the association of body weight with month of sampling, peripheral eosinophil counts and their interactions; with ID and animal as random effects (Model 5h).

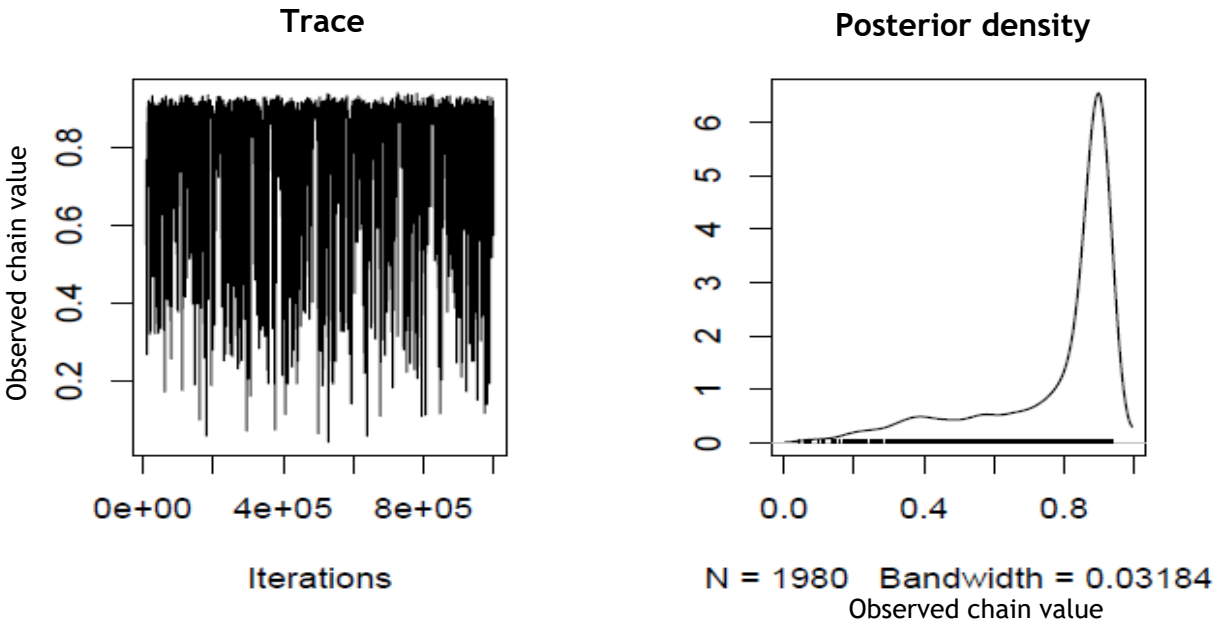


Figure 3-40. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for body weight heritability for the association of body weight with month of sampling, peripheral eosinophil counts and their interactions; with ID and animal as random effect (Model 5h).

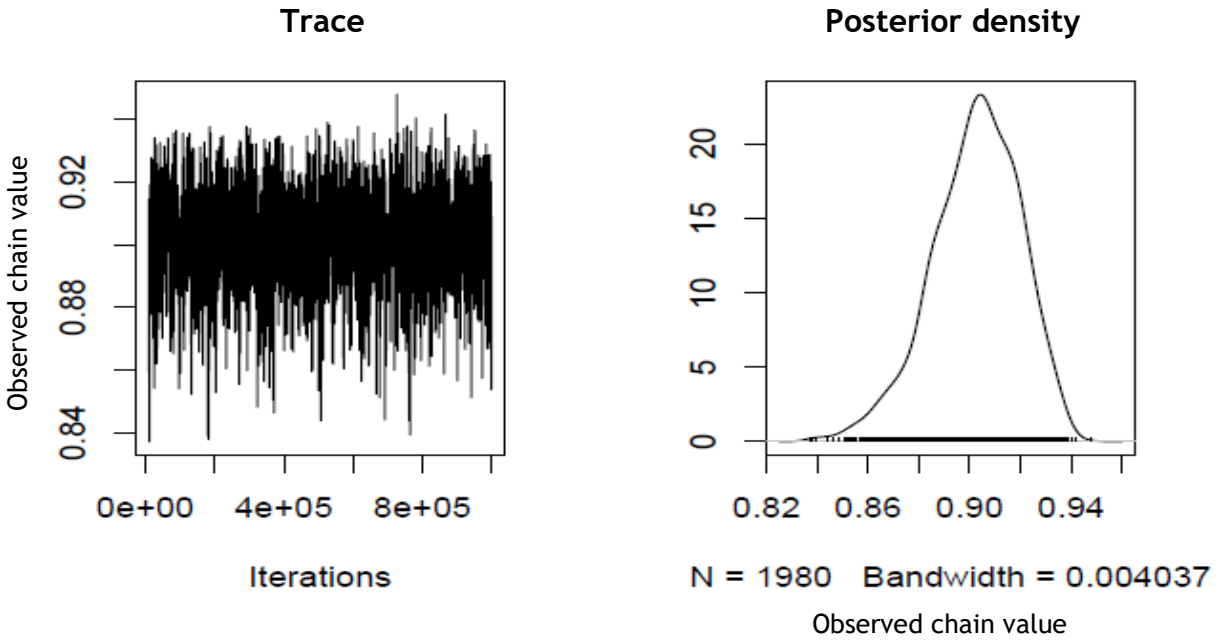


Figure 3-41. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for body weight repeatability for the association of body weight with month of sampling, peripheral eosinophil counts and their interactions; with ID and animal as random effect (Model 5h).

i) Mixed effects models of bodyweight with month, immune index and their interaction as fixed effects; ID as a random effect.

j) Mixed effects models of bodyweight with month, immune index and their interaction as fixed effects; ID and animal as a random effects.

Model sets i and j differ in whether animal was included as a random effect. As the results from model set e and f show a substantial additive genetic effect, we will only discuss the results for model set j (with both random effects) here.

Model selection and diagnostics Table 3-35 shows that all effects were retained in the final model (Model 1j) which was checked for autocorrelation, trends in the trace and effective sample size as in previous sections.

Fixed effects The model summary for Model 1j is shown in Table 3-36. There were significant effects of month of sampling ($p<0.001$), immune index ($p<0.05$) and the interaction of Month 3 with immune index ($p<0.05$).

Random effects The posterior mean variance for the random effect of animal was high in Model 1j (16.09) compared to the residual variance (1.945) and the random effect of ID (2.40) (Table 3-44).

Repeatability and heritability The high posterior mean variance for animal as a random effect resulted in a high posterior mode for bodyweight heritability of 0.91 with a HPD interval of 0.34 to 0.94 (Figure 3-42, right). The posterior mode for the repeatability of bodyweight in this model was also high at 0.91 with a HPD interval of 0.87 and 0.93 (Figure 3-43, right).

Table 3-35. Model selection process for mixed effects models of bodyweight with month and immune index as fixed effects; ID and animal as random effects.

DIC/Variable/ Random effect	Model 1j
	1
DIC	1120.89
MONTH	✓
IMMIDX	✓
MONTH : IMMIDX	✓
ID*	✓
Animal*	✓

'*' = random effects; DIC= Deviance Information Criterion; IMMIDX = immune index; ID= identification.

Table 3-36. Model coefficients of association of body weight with month of sampling, immune index and their interactions in Model 1j, ID and animal as random effects.

Structure	Variable	Posterior means	Lower 95% CI	Upper 95% CI	Eff. sample	p-value
Intercept		17.281	15.379	19.315	1996	<5e-04***
Fixed effects	Month 2	1.571	1.130	2.044	1996	<5e-04***
	Month 3	3.975	3.538	4.400	1996	<5e-04***
	IMMIDX	0.346	0.023	0.659	1562	0.038*
	Month 2 : IMMIDX	-0.364	-0.758	0.025	1996	0.072
	Month 3 : IMMIDX	-0.438	-0.794	-0.011	1996	0.027*
Random effect	ID	2.397	0.0002	9.889	1823	-
	Animal	16.090	4.406	23.920	1625	-
Residuals	Units	1.945	1.518	2.3920	1831	-

CI = credible intervals; Eff. sample = effective sample size; Month 2 = September; Month 3 = October; IMMIDX = immune index; ID = identification; p-value: '*' = p<0.05; '**' = p<0.01; '***' = p<0.001.

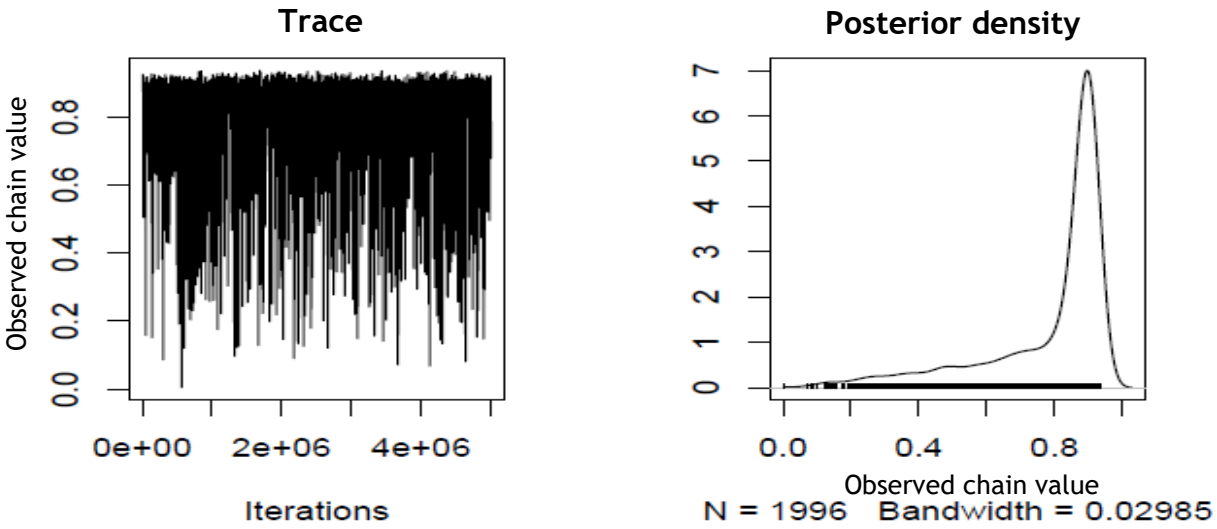


Figure 3-42. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for body weight heritability for the association of body weight with month of sampling, immune index and their interactions; with ID and animal as random effects (Model 1j).

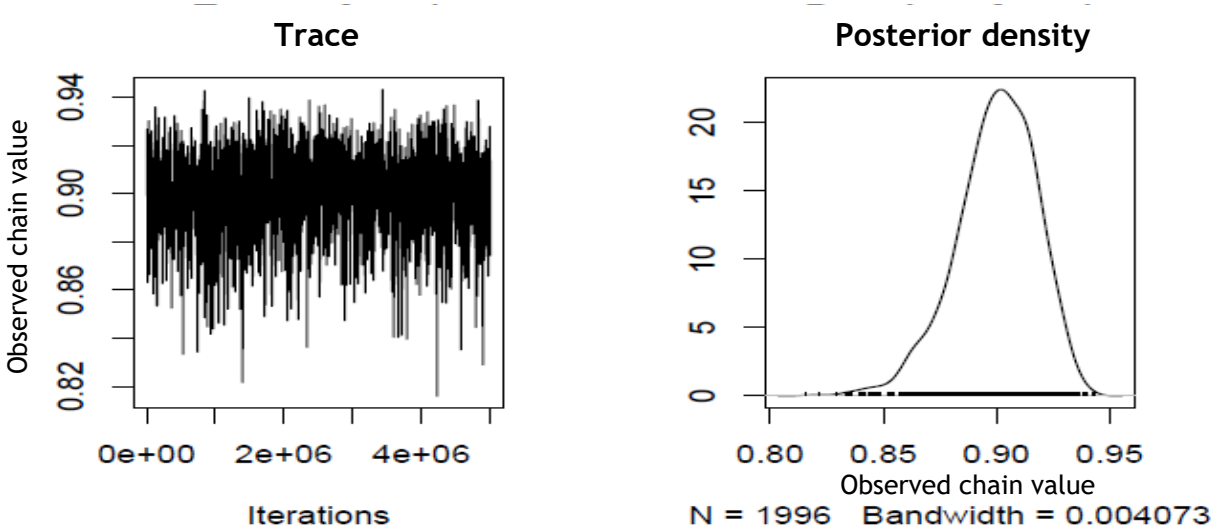


Figure 3-43. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for body weight repeatability for the association of body weight with month of sampling, immune index and their interactions; with ID and animal as random effects (Model 1j).

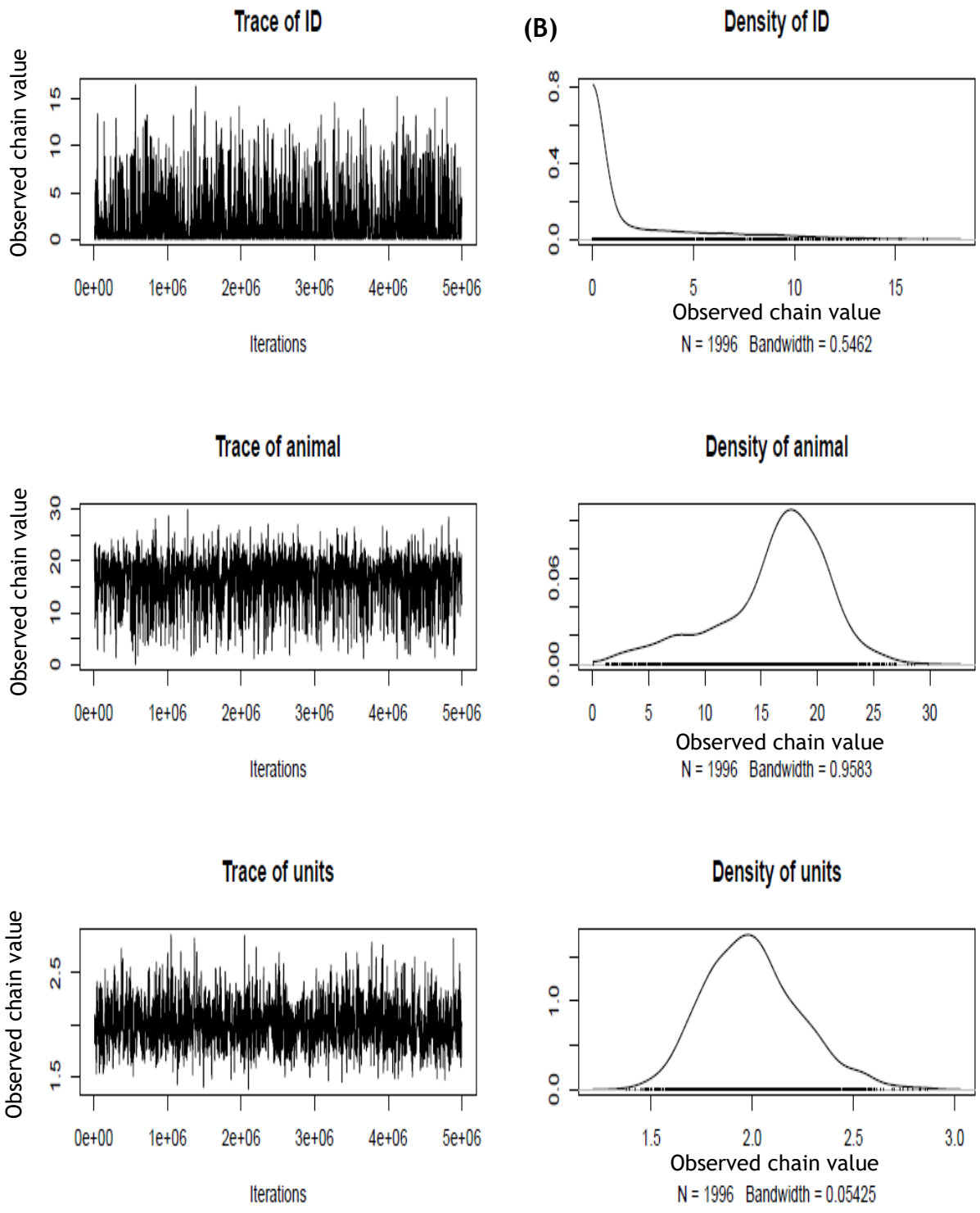


Figure 3-44. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for random effects and residual variance (denoted units) for the association of body weight with month of sampling, immune index and their interactions; with ID and animal as random effects (Model 1j).

k) Mixed effects models of bodyweight with month, parasite index and their interaction as fixed effects; ID as a random effect.

l) Mixed effects models of bodyweight with month, parasite index and their interaction as fixed effects; ID and animal as random effects.

Model sets k and l differ in whether animal was included as a random effect. As the results from model set e and f show a substantial additive genetic effect, we will only discuss the results for model set l (with both random effects) here.

Table 3-37 shows the process of model selection. All models were checked for autocorrelation, trends in trace and effective sample size as in previous sections. Table 3-38 shows the model summary for the final model, Model 3l. Only the month of sampling was associated with differences in bodyweight ($p<0.001$). As bodyweight models in previous sections had greater explanatory power (i.e much lower DIC) we do not discuss Model 3l further.

Table 3-37. Model selection process for mixed effects models of bodyweight with month and parasite index as fixed effects; ID and animal as random effects.

DIC/Variable/ Random effect	Model		
	1l	2l	3l
DIC	1127.9	1128.3	1125.1
MONTH	✓	✓	✓
PARAIND	✓	✓	
MONTH : PARAIND	✓		
ID*	✓	✓	✓
Animal	✓	✓	✓

DIC= Deviance Information Criterion; PARAIND = parasite index; ID = identification.

Table 3-38. Model coefficients of association of body weight with month of sampling in Model 3l with ID and animal as random effects.

Structure	Variable	Posterior means	Lower 95% CI	Upper 95% CI	Eff. sample	p-value
Intercept		17.271	15.377	19.453	1327	<8e-04***
Fixed effects	Month 2	1.561	1.092	1.993	1497	<8e-04 ***
	Month 3	3.957	3.535	4.395	1327	<8e-04 ***
Random effect	ID	2.120	0.001	9.304	1059	-
	Animal	16.230	4.451	24.370	1108	-
Residuals	Units	2.004	1.593	2.506	1327	-

CI = credible intervals; Eff. sample = effective sample size; Month 2 = September; Month 3 = October; ID = identification; p-value: '*' = $p<0.05$; '**' = $p<0.01$; '***' = $p<0.001$.

3.3.1.4 Summary of bodyweight models

The model sets e-l explored the role of several sets of potential predictors on bodyweight: FEC (set e and f); IgA and eosinophil responses (sets g and h); an immune index (sets i and j); and a parasite index (k and l). Comparison between the final models for set e (Model 2e) and f (Model 2f) show that animal random effect has a larger estimate than the ID random effect indicating that variation in bodyweight of the goats is influenced by additive genetic effect. Therefore, the results section focused on the final models with random effects of ID and animal. All final models with random effects of both ID and animal showed a large estimate for the random effect of animal which generated high estimates for bodyweight heritability (around 0.9).

Month of sampling was a highly significant predictor ($p<0.001$) in all models. In addition, the results showed that low FEC was associated with high bodyweight (set e and f); that eosinophils were associated with higher bodyweight but that there was negative interactions with Months 2 and 3 with Month 1 as the baseline (set g and h); with similar results seen for the immune index which was positively associated with bodyweight but with a negative interaction with Month 3. These findings suggest that the positive associations of eosinophils and immune index on bodyweight of the goats decreased with time.

3.4 Discussion

This study examined the responses of Boer goats to natural infection with a mixture of gastrointestinal nematodes, predominantly *T. circumcincta* with small numbers of *T. colubriformis* based on the percentage of L3 recovery from faecal culture in each month of sampling. This study was conducted in England, thus consistent with Bartley et al. (2004) and Taylor et al. (2007), in which *T. circumcincta* was found to be the dominant gastrointestinal nematode that survived anthelmintic treatments in the sheep farms throughout England, Scotland and Wales.

Cronbach's alpha coefficient was used to assess the reliability of laboratory methods used in this study. The values of the Cronbach alpha coefficient for the methods used to determine FEC, IgA activity and PCV in this study were within the acceptable range from 0.70 to 0.95 (Tavakol and Dennick, 2011). The alpha coefficient for the method used to measure peripheral eosinophil counts increased to 0.80 when one of the replicates was deleted. Therefore, all assays conducted in this study were considered as reliable. This procedure does not appear to have been applied to goat parasitological assays before, and provides some assurance that the observed differences between sheep and goats in response to gastrointestinal nematode infection are not simply a consequence of poor quality assays in goats.

Phenotypic markers of resistance to gastrointestinal nematodes

Over the three months of sampling, it was seen that FEC peaked in September, IgA decreased, bodyweight increased, peripheral eosinophil counts were highest in September and PCV appeared relatively constant. As change in these variables is likely to be dependent on each other, correlations among the variables were explored using Spearman rank correlation which is a powerful non-parametric procedure.

Correlations among the same variables measured at different times provide a measure of repeatability and can provide a guide for the strength of genetic control because it presents an upper limit for heritability (Falconer and MacKay, 1996). The correlations between months were high for bodyweight, moderate for PCV and IgA activity but very low at around zero for both FEC and peripheral eosinophil counts.

In comparison, the correlations between months for FEC in lambs at 4 to 6 months of age are usually higher and above 0.2 (Bishop et al., 1996). The correlations among IgA in the present study ranged from 0.4 to 0.46 which is not dissimilar to the correlations among IgA activity in lambs at 4 to 6 month of age: 0.55 between August and September and 0.50 between September and October (Strain et al., 2002). In the same lambs, the

correlation among eosinophil counts in August and September was 0.4 (Stear et al., 2002), which is much larger than the correlations seen in this study that were around zero (-0.07 to 0.05).

High IgA responses were associated with increased FEC in natural infections of *H. contortus* and *T. circumcincta* in Creole goats and Scottish Cashmere goats, respectively (de la Chevrotière et al., 2012; McBean et al., 2016). These associations suggest that high intensities of infection may be driving high IgA responses. Conversely, low FEC was associated with high peripheral eosinophil counts in Creole goats (de la Chevrotiere et al., 2012) which can be bred for improved resistance to gastrointestinal nematodes (Mandonnet et al., 2001). In the present study, there were only weak correlations of FEC with IgA and peripheral eosinophil counts that were observed among Boer goats in a natural infection predominated by *T. circumcincta*.

Gastrointestinal nematode infection is usually assumed to be detrimental to the growth of animals. Previous research has indicated that exposure to nematode infection reduces growth rate in lambs (Coop et al., 1985). Therefore, it was expected that growth would decrease as the FEC increases and vice versa. The FEC in August represents the early infection loads with strong negative correlations with bodyweight of the goats throughout three months of sampling ($p<0.001$). The other weak and inconsistent associations between FEC and bodyweight in this study may be a consequence of the regular and effective anthelmintic treatments.

Levels of immunoglobulins (IgG, IgA, IgE) and peripheral eosinophil counts increased significantly in both resistant and susceptible groups of Creole kids after deliberate infection with *H. contortus* but no significant difference was observed between both groups (Bambou et al., 2008). These findings suggest that cellular and humoral immune responses occurred but are not related to resistance against infection. In the present study, negative correlations observed between bodyweight and IgA response as well as between bodyweight and eosinophil response at certain times suggest that the responses to infection could affect the animal growth. However, the correlations do not account for all the sources of variation in the system, and an effect of IgA is not observed in the repeated measures analysis when multiple fixed and random effects are accounted for.

Different classes of anthelmintic used before each month of sampling may lead to the variation of FEC between months (Figure 3-13). The broad spectrum anthelmintic such as monepantel and moxidectin that were used to drench the goats in July and September seemed to be effective against infection that were reflected from low FEC in August and

October, while high FEC in September maybe due to the used of narrow spectrum benzimidazoles that was ineffective against nematode infection in August.

Repeated measures models of FEC – using the animal model

The next stage of the analyses was to go beyond correlations and use mixed models repeated measures analyses to examine predictors for FEC and bodyweight. Based on the summary statistics and histograms, the bodyweight data was assumed to follow a normal distribution. However, this was not the case for FEC data that had a typical skewed distribution in each month of sampling with a large number of low counts but a long tail to the distribution. For these data, it was shown that a Poisson model cannot capture the observed number of zeroes and therefore the analysis was conducted with a zero-inflated Poisson model.

One of the key components in this chapter was four sets of models that were run to explore the impact on FEC of immune mechanism (IgA activity, peripheral eosinophil counts and in combination as an immune index) as well as the other potential explanatory variables such as time (month of sampling), type of birth, sex and the interactions.

One set of models examined the immune mechanism via the immune index: the sum of the standardised IgA and eosinophil measurements. The idea was to capture the immune response at the site of infection (i.e abomasum) by using the immune responses (IgA and eosinophils) that were measured in peripheral blood. This was because both peripheral IgA and eosinophils are assumed to be correlated with their protective immune counterparts in the abomasum. Hence, it was assumed that the combination of both could give a better reflection of response to infection. However, there was no significant effect of immune index on FEC. The fixed effects in the final models for FEC only contained month of sampling and sex of goat.

The other set of FEC models included IgA and eosinophils as independent predictors. In the count component of the final models, there was no significant effect of IgA, a negative association with eosinophil counts, and a positive interaction between IgA and eosinophils on FEC. These findings indicate that IgA was not effective to control nematode infection. Although eosinophils was protective on its own, there is a positive interaction of IgA with eosinophils. This positive association of FEC with the interaction between IgA and eosinophils can be interpreted as reduced effectiveness of eosinophils in reducing FEC when IgA is high. In other words, the protective effect of eosinophils against nematode infection would decrease when IgA increased. The lack of eosinophil receptors for IgA in goats (Basripuzi et al., 2018) could be the main reason for the ineffective synergistic effects of IgA and eosinophils in controlling FEC. These findings were in contrast to

Scottish Blackface lambs at 4 to 6 month-old where strong IgA and eosinophil responses were associated with decreased FEC (Stear et al., 2002; Strain et al., 2002).

The animal model was run when the 'animal' term that was accompanied by pedigree data was included as a random effect. The other random effect was ID. The small posterior means within the estimated 95% CI for each random effect in the count component of the final models suggest that the FEC variance among naturally infected Boer goats in this study was not greatly affected by individual variance (ID) and genetic variance (animal). Additionally, the comparatively large residual variances which were around 1.3 in all final models indicate that FEC was overdispersed (Bolker et al., 2012).

The binary component of the model captured the effect of month of sampling on the probability of observing a zero count. Overall, there was reasonable agreement between the observed and predicted numbers of zeroes, with the highest observed zero FEC in October (Month 3) and the lowest in September (Month 2). These model fits indicate that around 73% of the zeroes were true zeroes.

Based on the description by Yang et al. (2017), the true zeroes observed in this study were presumably coming from faecal samples that were free from nematode eggs whereas the false zeroes arise from the faecal samples that contained nematode eggs but were not counted microscopically during the McMaster technique. In practice, an egg will not be counted if it is located outside of the McMaster grids (Ministry of Agriculture, Fisheries and Food of Great Britain, 1986).

FEC Repeatability

Repeated measurements were conducted on each individual goat, and therefore it was of interest to see how repeatable a trait was – particularly for FEC among the goats. The FEC repeatability was estimated as the proportion of FEC variance explained by the goats' individual identity (Wilson et al., 2010). The FEC repeatability from each final model was very low, ranging from 0.001 to 0.009. These findings are consistent with findings from other breeds of goats such as Saanen and Creole goats which also had relatively low FEC repeatability that ranged from 0.09 ± 0.06 to 0.17 ± 0.02 (Morris et al., 1997; Mandonnet et al., 2006). In contrast, the FEC repeatability in sheep breeds such as Scottish Blackface and Romney sheep ranged from 0.25 ± 0.04 to 0.46 ± 0.04 (Morris et al., 1998; Bishop and Stear, 2001).

FEC heritability

As expected, whether looking at posterior mean or mode, the FEC heritability estimates were consistently lower than the FEC repeatability estimates in the final animal models. The low heritability of FEC suggests that FEC was not heritable among naturally infected Boer goats in this study, or in the other words, that FEC in the goats was not influenced by additive genetic variation.

Low FEC heritability (although higher than in our study) was also found in Creole (0.10 ± 0.02), Saanen (0.06 ± 0.04 to 0.09 ± 0.05), Barbari (0.05 ± 0.05 and 0.13 ± 0.04) and Jamunapari (0.11 ± 0.06 to 0.16 ± 0.06) breeds of goats (Morris et al., 1997; Mandonnet et al., 2006; Mandal and Sharma, 2008; Mandal et al., 2012). In sheep breeds, FEC heritability is typically higher, ranging in sheep breeds between 0.23 ± 0.05 and 0.37 ± 0.06 for Scottish Blackface, Parendale and Romney sheep (Watson et al., 1995; Morris et al., 1998; Bishop and Stear, 2001; Goldberg et al., 2012).

Repeated measures models of bodyweight – using the animal model

The other key component of this chapter was the bodyweight models. Four sets of models (each set with and without the animal effect) were run to explore the impact of time (month of sampling), immune response (IgA, peripheral eosinophil counts, immune index), parasitism (FEC, PCV, parasite index) and their interactions on bodyweight of the goats. The parasite index (a combination of FEC and PCV) was analysed because both variables were significantly correlated with bodyweight on at least three occasions. FEC and PCV are the consequences of gastrointestinal nematode infection. Thus, combinations of both variables could be a stronger indicator for bodyweight of infected goats than any single variable.

The first set of models examined the effect of FEC on bodyweight. Of these, the model without the animal random effect had a large effect of ID, which generated a high repeatability. The repeatability of a trait can be estimated as the proportion of a phenotypic variance explained by the individual identity as represented by ID, which dissociates individual records from the pedigree (Section 2.10.5.2). The pedigree record can be incorporated into the model with the animal term as a random effect. The second set of models had a much smaller posterior mean variance for the random effect of ID but a large posterior mean variance for the random effect of animal. This generated a high estimate for heritability of bodyweight (around 0.9) indicating that bodyweight is under substantial additive genetic control. Therefore, the discussion of these and subsequent models will focus on the animal models i.e. those with the random effect of ID and animal.

In each of the final bodyweight models, month of sampling was associated with the increase in bodyweight of the Boer goats ($p<0.001$). This indicates that bodyweight of the goats increased as they aged. As expected, increased FEC was also associated with a decrease in bodyweight ($p<0.05$). This has also been shown by significantly higher bodyweight ($p<0.01$) and lower FEC ($p<0.05$) in resistant Creole kids compared to their susceptible counterparts experimentally infected with *H. contortus* (Bambou et al., 2009). The negative association between FEC and bodyweight is also consistent with findings in sheep, where exposure to nematode infection has been shown to reduce growth rate in lambs (Coop et al., 1985).

The pathology of nematode infection in sheep may be mainly mediated by the immune system rather than directly by the parasite (Greer, 2008; Stear et al., 2003). In this study, high peripheral eosinophils counts ($p<0.05$; Model 5h) and immune index ($p<0.05$; Model 1j) were associated with the increased bodyweight of the goats (. However, the interactions of peripheral eosinophil counts and immune index with month (Month2 and Month3 against Month1) show that the positive effects of both variables on bodyweight of the goats decreased with time. The parasite index examined in the final set of models has no association with bodyweight.

Repeatability and heritability of bodyweight

The estimate of repeatability captures both the permanent environmental effects and the additive genetic effect and is derived from the random effects of ID, animal the residual variance. As expected, the random effect of ID was much smaller in the second set of models (with both ID and animal as random effects). The repeatability and heritability estimates were generally high – around 0.9 in all models. These results confirm that bodyweight was highly heritable among the Boer goats. This finding was expected for Boer breed as a highly meat productive goat breed due to its high growth rates, good body conformation (Casey and Van Niekerk, 1988), better quality meat and heavier carcass (Malan, 2000).

Conclusion

As a conclusion, the aims for this chapter have been achieved. FEC distribution among naturally infected Boer goats was shown to follow and modelled as a zero-inflated Poisson distribution. The FEC variation was affected by time (month of sampling), eosinophilia and interaction of eosinophils with IgA. There was an interaction of IgA and eosinophil counts that reduced the protective effects of eosinophils to regulate FEC. Low heritability estimates for FEC indicates that FEC among the goats was not influenced by additive genetic effect. In addition, as FEC decreased the bodyweight of the goats increased. The bodyweight of the goats also increased as the response of eosinophils and

immune index increased; but their effects on bodyweight decreased with time. Furthermore, this study also confirmed that bodyweight is a highly heritable trait in Boer goats despite being infected by gastrointestinal nematodes, predominantly *T. circumcincta*.

Chapter 4: Response to gastrointestinal nematode infection among deliberately infected Boer goats

4.1 Background

Gastrointestinal nematode infection is one of the most severe diseases faced by sheep and goats (Hoste et al., 2011). The two species have developed different ways to reduce the severity of infection, in which the immune response of sheep is protective against gastrointestinal nematodes (Stear et al., 1999) but goats adopted different feeding habit by browsing instead of grazing (Malan, 2000; Hoste et al., 2010). In both sheep and goats (Mugambi et al., 1996; Bahirathan et al., 2000; Baker et al., 1998; Fakae et al., 1999, Chiejina et al., 2002), some breeds mount much more effective immune responses than the others. However, most of the studies to explore the use of genetically resistant animals to control nematode infection have been focused on sheep (Paterson et al., 1996b; Stear et al., 2007; McBean et al., 2016).

For Boer goats, there is some evidence that this breed may be somewhat resistant to *H. contortus* infection, based on FEC and PCV traits (Baker and Gray, 2004). The Boer is an indigenous productive meat goat breed of South Africa (Casey and Van Niekerk, 1988) and the most popular meat goat breed in the United States (APHIS-USDA, 2005). This breed has also been reared widely in Malaysia for meat purpose but the genetic variability of the fullblood Boer breed in resistance to gastrointestinal nematode infection has not been studied.

H. contortus is the predominant gastrointestinal nematodes in small ruminants in the tropical and subtropical regions (Waller and Chandrawathani, 2005). In Malaysia, *H. contortus* has been reported as the most prevalent species in mixed infection of gastrointestinal nematodes on both government and private small ruminant farms (Khadijah et al., 2006a,b). The other species regarded as common gastrointestinal nematodes in small ruminants in Malaysia are *Trichostrongylus* spp. and *Oesophagostomum* spp. but with lower prevalence than *H. contortus* (Israf et al., 1996; Khadijah et al., 2006a,b; Basripuzi et al., 2012). Gastrointestinal nematode infections are usually mixed, with additive pathogenic effects when different nematodes inhabit different niches in the gastrointestinal tract (Emery et al., 2016).

High FEC with high IgA responses were observed in both Scottish Cashmere and Creole goats in natural infections of *T. circumcincta* and *H. contortus*. Weak correlations between FEC and eosinophilia were also observed in these breeds (de la Chevrotière et al., 2012; McBean et al., 2016). These findings were in contrast to the resistant breeds of

sheep that have strong protective IgA and eosinophil responses to nematode infection (Strain et al., 2002; Amarante et al., 2005; Henderson and Stear, 2006; Terefe et al., 2009). The synergistic response of IgA and eosinophil against gastrointestinal nematode infection may be not protective in goats, as they were in sheep, due to the lack of IgA receptors expressed by eosinophils, in comparison to sheep and human which have high affinity IgA receptors (Basripuzi et al., 2018). Based on bioinformatic analysis, the study suggests that Boer goats appear to lack a functional IgA and eosinophil responseagainst gastrointestinal nematode infection.

As an important nematode, assessment of resistance against *H. contortus* based on FEC has been long and widely documented particularly in sheep where St. Croix, Santa Ines, Merino and Red Masai are among identified resistant breeds (Sreter et al., 1994, Mugambi et al., 1997; Gauly et al., 2002; Amarante et al., 2009, Goncalves et al., 2018). Although the corresponding information is comparatively limited in goat breeds, the available reports suggest that the heritability of FEC in goats is typically lower than in sheep.

In sheep breeds, the FEC heritability varied between 0.2 and 0.4 (Watson et al., 1995; Morris et al., 1998; Bishop and Stear, 2001; Goldberg et al., 2012). In goat breeds such as Saanen, Jamunapari, Barbari and Creole; the FEC heritability estimates ranged from 0.05 ± 0.05 to 0.33 ± 0.06 in which Creole breed has the highest heritability estimate for FEC (Morris et al., 1997; Mandonnet et al., 2001; Mandal and Sharma, 2008; Mandal et al., 2012). Despite the economic importance of Boer goat, the FEC repeatability and heritability are still unknown.

The goal of this chapter is to examine the immune responses in Boer goats to deliberate infection with gastrointestinal nematodes, to quantify the repeatability which provides the upper limit for heritability (Falconer and MacKay, 1996; Wilson et al., 2010), and to estimate the heritability of FEC. Specifically, in this study, phenotypic markers of immunity against GIN infection such as FEC, IgA activity, peripheral eosinophil counts and PCV were investigated among deliberately infected Boer goats, a follow-on study to that in naturally infected Boer goats in the previous chapter.

The objectives of this chapter are as follows:

1. To identify the relationship between FEC and explanatory variables, especially the immune responses, measured via IgA and peripheral eosinophil counts.
2. To estimate the repeatability of FEC, IgA activity, peripheral eosinophil count and PCV in Boer goats following deliberate infection.
3. To estimate the heritability of FEC.

4.2 Materials and methods

4.2.1 Study sites and animals

This study was conducted on a Boer breeder farm in Selangor, Malaysia from April to October 2015. Initially, a total of 100 goats of both sexes aged from 3 to 7 months old were sampled from this farm from April to June 2015 for gastrointestinal nematode screening and collection of faecal samples to prepare parasite antigen for ELISA assay. Following the herd health program in the farm, all goats were drenched with 2.5 % benzimidazole in February 2015 according to the manufacturer's recommended dosage (0.5 ml/10 kg bodyweight; single drenching). The goats were kept in wooden slatted-floor houses and provided with water ad libitum 24-hours a day. The goats were given fresh cut grasses, silage and a supplement of commercial pelleted feed. Faecal samples were collected per rectum and blood samples were collected via jugular vein from each goat in the morning once every four weeks from April to June 2015. All goats were confirmed to have zero FEC from April to June 2015. The information collected on each goat included date of birth, type of birth (either singleton or twin), sex, and identity of sire, dam and grandparents (Appendix D).

During the sampling in June 2015, 30 out of 100 goats were selected randomly for deliberate infection from the ID record. The farm workers shepherded the selected goats from their current houses to the experimental house through the ground covered by grasses. The goats were then kept isolated in five pens with six goats each. To eliminate any possible GIN infections during the transfer, the goats were drenched with 2.7% levamisole with the dosage as recommended by the manufacturer (2.5 ml/10 kg bodyweight). All goats were confirmed to have zero FEC after four weeks of a single levamisole drenching. Levamisole was the anthelmintic of choice before the start of deliberate infection because it was found to be more effective than the anthelmintics from other classes namely albendazole, ivermectin and closantel based on faecal egg count reduction tests conducted in smallholder goat farms in Malaysia to detect anthelmintic resistance (Basripuzi et al., 2012).

The initial plan was to infect the goats with a high dose of monospecies of *H. contortus* L3. However, the plan had to be changed due to a lack of L3 stock. Instead, a monospecific culture of *H. contortus* from the Veterinary Research Institute, Malaysia was combined with a mixed culture from the Parasitology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The L3 used to infect the goats were kept at 4^{o}C for three to six months before the start of the experiment. Five goats in each pen were infected with 2400 L3 of *H. contortus, Trichostrongylus* spp. *and Oesophagostomum* spp. at a 6:1:1

ratio while the other goats (one per pen) served as intended negative controls. The purpose of setting control animals in the experiment was to investigate the differences in phenotypic measurements such as FEC, IgA, PCV and peripheral eosinophil counts between infected and non-infected goats that were kept in the same environment.

Faecal and blood samples were collected twice a week for eight weeks at three-four-three day intervals. The faecal samples were subjected to the modified McMaster technique which is sensitive to 25 epg of faeces to determine FEC. The blood samples were used to measure IgA, PCV and peripheral eosinophil counts (Appendix E). The goats were slaughtered on the farm after the 16th sampling (day 52 post-infection) to collect the gastrointestinal tracts. The goats were humanely slaughtered according to halal slaughtering procedure as outlined in the MS1500:2009 (Department of Standards Malaysia, 2009) and the research was conducted with similar procedures that were approved by Animal Ethics Committee of the University of Glasgow and the UK Home Office for the works published in Basripuzi et al. (2018). All samples were transported to the Faculty of Veterinary Medicine, Universiti Putra Malaysia on the same day of sampling for laboratory analysis.

4.2.2 Modified McMaster technique

The Modified McMaster technique was conducted as described in Section 2.1.

4.2.3 Faecal culture

Faecal culture was conducted as described in Section 2.2.

4.2.4 Identification and enumeration of infective stage larvae

Infective stage larval identification and enumeration was performed as described in Section 2.3.

4.2.5 Peripheral eosinophil counts

Eosinophil counts were obtained as described in Section 2.5.

4.2.6 Packed cell volume estimation

Packed cell volume was measured as described in Section 2.6.

4.2.7 ELISA IgA

Parasite antigen was prepared for ELISA IgA as described in Section 2.8. The pooled faeces of deliberately infected goats with 6:1:1 ratio of *Haemonchus* spp. : *Trichostrongylus* spp. : *Oesophagostomum* spp. were subjected for larval culture to obtain L3 stock as the source of parasite antigen for ELISA assay in this study. The final ratio of L3 stock of *Haemonchus* spp. : *Trichostrongylus* spp. : *Oesophagostomum* spp. was 14:2:1. The ELISA IgA assay was conducted as described in Section 2.9.

4.2.8 Total worm counts, identification, sexing and measurement of nematode length

Total worm counts, identification, sexing and measurement of nematode length were conducted as described in Section 2.4.

4.2.9 Statistical analysis

4.2.9.1 Reliability test

The reliability of the laboratory methods used in this study was evaluated using Cronbach's alpha coefficient for FEC (McMaster technique), IgA (ELISA), peripheral eosinophil counts and PCV assays using Spearman's Rank Correlation by the CORR procedure in SAS 9.4 software. The principle of Cronbach's alpha coefficient that estimates the internal consistency of a method (Cronbach, 1951) has been described in Section 3.2.8.1. The maximum Cronbach's alpha coefficient of 0.90 has been recommended (Tavakol and Dennick, 2011) and alpha values above 0.70 are considered acceptable (Nunnally and Bernstein, 1994).

4.2.9.2 Comparison of distributions of variables between time points

The distributions of variables (FEC, IgA, peripheral eosinophil counts and PCV) were compared across time points using the Kruskal-Wallis test, which is a non-parametric version of ANOVA for data that are not normally distributed. The Wilcoxon rank-sum test was then applied to make pairwise comparisons between time points.

4.2.9.3 Repeatability estimation of phenotypic variables

Spearman correlation analyses applied to each variable (FEC, IgA, peripheral eosinophil counts and PCV) between each pair of sampling times (3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49 and 52 days) were conducted to estimate their repeatability. Correlations were not measured for FEC until day 21 post-infection when FEC became

non-zero following the pre-patent period. Consequently, there were 45 possible pairwise correlations for FEC between the sampling events from day 21 until day 52 post-infection. Each of the other variables had 120 possible pairwise correlations between each sampling event from day 0 to day 52. The repeatabilities of these variables were estimated by cor.test() function and their trend over time presented using the ggplotRegression() function in R. The repeatability estimation that was analysed by regression analysis used FEC from both binary and count components of the data.

The repeatability of FEC was also estimated via the Mixed Model Repeated Measures analysis described below. Using the term 'ID' within the MCMCglmm R package dissociates individual records from the pedigree, thus allowing estimation of repeatability (described in Section 2.10.5.2). The ID component represents between-individual variance while the within-individual variance is given by the residual component (Wilson et al., 2010). The repeatability estimation that was analysed by Mixed Model Repeated Measures analysis only used FEC from count component of the data.

4.2.9.4 Repeated measures analysis using the animal model

The MCMCglmm R package (Hadfield, 2010) was used to fit the mixed model with a zero-inflated Poisson distribution to FEC data as described in Section 2.10.6. The MCMCglmm package was specifically chosen to allow me to make use of the pedigree data and an animal model. The data collected in this study comprise repeated measures on individual goats of a set of phenotypic variables (FEC, IgA, eosinophils and PCV), along with pedigree data and information on time of sampling, type of birth and sex. The FEC was modelled as the response variable.

In Chapter 3, a Poisson model (Poisson model 4) was applied to the observed data to examine the FEC distribution (Section 3.2.8.9). It was shown that the Poisson model for FEC in naturally infected Boer goats was unable to capture the observed numbers of zeroes and therefore that the data follow a zero-inflated Poisson distribution. In other words, the distribution can be thought of as having a count component and a binary component. Because of these findings, in this chapter, the FEC among deliberately infected Boer goats was assumed to follow a zero-inflated Poisson distribution.

FEC models

These analyses were restricted to the 25 deliberately infected animals. The intended control goats in each pen were excluded as they were subsequently found to be at least temporarily infected during the experiment. The sampling days were grouped into 4 sets of 4 consecutive sampling points namely time point 1 (Days 0, 3, 7, 10); time point 2 (Days

14, 17, 21, 24); time point 3 (Days 28, 31, 35, 38) and time point 4 (Days 42, 45, 49, 52). The pre-patent period corresponded to time point 1 when the FEC was consistently zero, and therefore time point 1 was excluded from the analyses.

The original count data for FEC was used in the repeated measures models to analyse two sets of FEC models. The first set treated IgA and eosinophils as independent variables (Model a and b); and the second set combined them into an immune index (Model c and d) as explained in Section 3.2.8.5. The other fixed effects examined for FEC models were time point of sampling (TIME2, 3 and 4) and type of birth (singleton and twin). The immune index was created because there is a possibility that IgA and eosinophils were independently correlated with the protective immune response at the site of infection in the gastrointestinal tract.

Random effects

As the goats were grouped into 5 pens and repeated measures were conducted on each of them, the pen number and ID of the goats were treated as random effects. In addition, because pedigree information was available, an 'animal' random effect was included which allows the additive genetic effect for FEC for each goat to be calculated.

The first set of models was divided into two categories based on random effects. In the first category, ID and pen were included as random effects to estimate FEC repeatability without the additive genetic effect. In the second category; ID, animal and pen were included as random effects to obtain both repeatability and heritability estimates.

Specifying a zero-inflated Poisson distribution in MCMCglmm

The zero-inflated Poisson family allows variables to differentially effect the binary component (zero or non-zero) and the count component (zero and non-zero) of the FEC outcome variable. The effect of time on the count component of FEC was specified by the MCMCglmm helper function 'at.level' as the interaction of 'at.level (trait,1)' with time point (eg. at.level (trait,1): TIME_POINT). The effect of time point on the binary component of FEC was specified as the interaction of 'at.level (trait,2)' with time point (eg. at.level (trait, 2): TIME_POINT). The intercepts for the binary and count components of FEC were specified as 'traitzi_FEC' and 'trait_FEC', respectively.

Prior specification

The priors were chosen as described in Section 3.2.8.5 following Bolker et al. (2012). As the FEC data was assumed to follow a zero-inflated Poisson distribution, there were random effects and residual error associated with the count and binary component of a zero-inflated model. For the count component of each model, a very weak prior was

specified on the random effects and residual error (by setting nu=0.002; as described in Section 2.10.6.3). For the binary component, as the residual error is non-identifiable (i.e. cannot be estimated), it was fixed to be 1, whilst the random effects were effectively negated by fixing the variance to be 10^{-6}.

Model diagnostics

The models were run with a total number of iterations (nitt) of 2500000, number of iterations during the burn-in phase (burnin) of 10000, and a thinning interval (thin) which determines the values that are retained for analysis; for example, a thinning interval of 100 means that 1 in every 100 values is kept for analysis. This is to avoid autocorrelation in samples from the posterior distribution. These values for nitt, burnin and thin were determined after a series of explorations to reduce autocorrelation in the zero-inflated Poisson FEC models. Autocorrelation was monitored by trace plots and autocorrelation estimation as described in Section 3.2.8.5.

Model selection

Minimising DIC (the Bayesian version of AIC) is often used as a model selection criterion. However, when random effects are present, they can dominate the DIC, sometimes leading to DIC values being smaller for more complex models than for the simpler models (Bolker, 2009). For this reason, it was decided that model selection would be conducted by removing the least significant fixed effects one by one based on the largest p-values until arriving at the simplest model.

4.2.9.5 Repeatability and heritability estimation of FEC from mixed model repeated measures analysis

Repeatability and heritability are calculated from the variance components generated by the mixed model repeated measures analysis as described by Wilson et al. (2010).

Using the term 'ID' as a random effect within the MCMCglmm R package (described in Section 2.10.5.2) will dissociate individual records from the pedigree, thus allowing estimation of repeatability. For models with just the random effect of ID, repeatability is given by

$$Repeatability = \frac{V_{ID}}{V_{ID} + V_R}.$$

For models including both random effects of ID and animal it is given by

$$Repeatability = \frac{V_A + V_{ID}}{V_A + V_{ID} + V_R}$$

where V_A is the additive genetic variance, V_{ID} is the between-individual variance due to permanent environmental effects and V_R is the residual variance. The first approach simply provides an estimate of repeatability that is an upper limit for heritability (Wilson et al., 2010). The second approach allows the repeatability to be decomposed into the permanent environmental effects and the additive genetic variance. Heritability is given by

$$h^2 = \frac{V_A}{V_A + V_{ID} + V_R}.$$

4.2.9.6 Creating predicted data from a fitted zero-inflated Poisson model

The process of predicting data from simulated zero-inflated Poisson distribution has been described in Section 3.2.8.7. Predicted true zeroes were obtained from the binary component in the prediction function of the models at time point 2, 3 and 4 as well as from the overall counts. The number of predicted true zeroes was then divided by the number of predicted zeroes to obtain the probability that a predicted zero is a true zero. The predicted data were used to compare the means of the observed and predicted data and also the observed and predicted numbers of zeroes as an additional check on model fit to the data.

4.3 Results

The Cronbach's alpha coefficient for the methods used to measure FEC, IgA, peripheral eosinophil counts and PCV were above 0.70 as shown in Table 4-1, indicating good levels of consistency of the laboratory protocols.

Table 4-1. Cronbach's alpha coefficient of variables collected in Malaysia.

Data	Method	Cronbach's apha coefficient
Faecal egg counts	McMaster technique	0.93
IgA	ELISA	0.78
Peripheral eosinophil counts	Peripheral eosinophil counts assay	0.79
Packed cell volume	Packed cell volume assay	0.97

The composition of L3 recovered from faecal culture of the deliberately infected goats throughout the 52 days of study is shown in Table 4-2. *Haemonchus* spp. (82%) was the most common, followed by *Trichostrongylus* spp. (12%) and *Oesophagostomum* spp. (6%). Identification was at genus level based on the manual provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986).

Table 4-2. L3 recovery from faecal culture of deliberately infected Boer goats.

Genera of gastrointestinal nematode	Percentage of L3 (%)
Haemonchus spp.	82
Trichostrongylus spp.	12
Oesophagostomum spp.	6

Table 4-3 shows the FEC and total worm counts in the 25 deliberately infected and 5 intended control goats (shown in red). Although not all goats harboured nematodes or were shedding nematode eggs at the final sampling point, all 30 were shedding nematode eggs at some point before day 52 of sampling. The FEC on day 52 ranged from the lowest of zero epg to the highest of 1325 epg. Ten goats were not detected to be shedding nematode eggs (zero epg) on day 52. These included three control animals (RB45, RB53 and DE30). Six of the 10 manifested with *H. contortus* spp. and/or *Trichostrongylus* spp. on day 52, whilst one goat (BR57) was negative for both nematodes and eggs on day 52. Two controls (BR03 and FA38) were detected with only low FEC (125 and 25 epg, respectively) on day 52. One of the controls (BR03) was positive for nematodes during the total worm count. No nematodes were detected from the gastrointestinal tract of four other controls. In addition, none of the goats were harbouring *Oesophagostomum* sp. during total worm count.

Table 4-3. Faecal egg counts and total worm counts in deliberately infected and control goats.

Goat ID	Gastrointestinal nematode eggs			Gastrointestinal nematodes						
	Presence/ Absence before Day 52	Presence/ Absence on Day 52	FEC (epg) Day 52	Presence/ Absence	Genus					
					Haemonchus contortus (Number)		*Trichostrongylus* colubriformis (Number)		*Oesophagostomum spp.* (Number.)	
					F	M	F	M	F	M
M	✓	✓	1325	✓	170	210	20	10	0	0
RB41	✓	--	0	✓	0	10	0	10	0	0
RB40	✓	✓	425	✓	390	240	30	0	0	0
RB47	✓	✓	250	✓	90	100	0	0	0	0
RB44	✓	--	0	✓	0	0	10	0	0	0
RB45	✓	--	0	--	0	0	0	0	0	0
RB67	✓	✓	200	✓	190	120	20	0	0	0
RB58	✓	✓	450	✓	10	0	20	10	0	0
BR57	✓	--	0	--	0	0	0	0	0	0
RB36	✓	✓	125	✓	80	40	0	0	0	0
RB53	✓	--	0	--	0	0	0	0	0	0
BR03	✓	✓	125	✓	150	160	60	20	0	0
CY22	✓	--	0	✓	10	10	20	0	0	0
CY32	✓	--	0	✓	30	30	0	0	0	0
FA31	✓	--	0	✓	150	110	0	0	0	0
FA36	✓	✓	100	✓	60	160	10	0	0	0
FA32	✓	✓	25	✓	80	70	10	0	0	0
FA37	✓	✓	200	✓	220	180	0	10	0	0
FA38	✓	✓	25	--	0	0	0	0	0	0
BR08	✓	✓	875	✓	50	20	30	40	0	0
BR25	✓	✓	50	✓	0	0	90	60	0	0
BR17	✓	--	0	✓	290	310	20	10	0	0
BR01	✓	✓	150	✓	70	120	30	10	0	0
RB64	✓	✓	825	✓	160	120	50	40	0	0
DE30	✓	--	0	--	0	0	0	0	0	0
CY40	✓	✓	50	--	0	0	0	0	0	0
CY26	✓	✓	600	--	0	0	0	0	0	0
CY27	✓	✓	150	✓	270	260	10	10	0	0
CY30	✓	✓	525	✓	110	90	30	20	0	0
BR26	✓	✓	50	✓	80	40	30	0	0	0

✓ = presence of either eggs or nematodes; -- = absence of either eggs or nematodes; F = female; M = male; red font = the control goats.

The development of infection based on FEC, IgA OD index, peripheral eosinophil counts and PCV distribution throughout 52 days of sampling is shown in Figure 4-1 for the deliberately infected goats (Group 1, red dots) and intended controls (Group 2, blue dots). A zero FEC was observed during the pre-patent period until day 20 post-infection (Fig. 4-1, A). IgA appears relatively constant although some goats had increased IgA activity towards the end of the experiment (Fig. 4-1, B). During the pre-patend period, the IgA OD indices of the goats were below 1.5. Peripheral eosinophil counts seems to have a downward trend in eosinophil counts from the pre-patent period through to the end of the experiment (Fig. 4-1, C). PCV was also relatively constant with suggestion of a drop at day 28 (Fig. 4-1, D).

Figure 4-2 shows the distributions of FEC, IgA OD index, peripheral eosinophil counts and PCV by time points of sampling starting from time point 2, which marked the commencement of egg shedding after the pre-patent period. Based on the Kruskal-Wallis test, there were significant differences in FEC between time points (Kruskal-Wallis = 62.165, df = 2, $p<0.001$) but only between time points 2 and 3 and between time point 2

and 4. The FEC distribution (Figure 4-2, A) is similar between time point 3 and 4 (pairwise Wilcoxon rank sum test, p = 0.60). There were also significant differences in IgA between time points (Kruskal-Wallis = 13.595, df = 2, p=0.001) which was the lowest in time point 4 (Figure 4-2, B). Eosinophil counts differed between months (Kruskal-Wallis = 62.693, df = 2, p<0.001) and appeared highest in time point 2 (Figure 4-2, C). The PCV was consistent throughout the study (Figure 4-2, D) without significant difference between time points (Kruskal-Wallis = 0.660, df = 2, p = 0.719).

The correlations between observations provides a measure of repeatability (Figure 4-3). The repeatability of each variable decreased as the interval between sampling increased but the decline was not significant for FEC (p=0.705) and PCV (p=0.070). The mean repeatability of FEC was around 0.2 (Figure 4-3, A). Similarly, the PCV repeatabilities were quite consistent at around 0.6 throughout the experiment (Figure 4-3, D). The repeatability of IgA (Figure 4-3, B) declined significantly (p<0.001) as the number of days between sampling increased. The repeatability of IgA activity declined from around 0.75 to be around 0.4 at the end of the experiment. The repeatability of peripheral eosinophil counts (Figure 4-3, C) initially dropped and then plateau at larger time intervals.

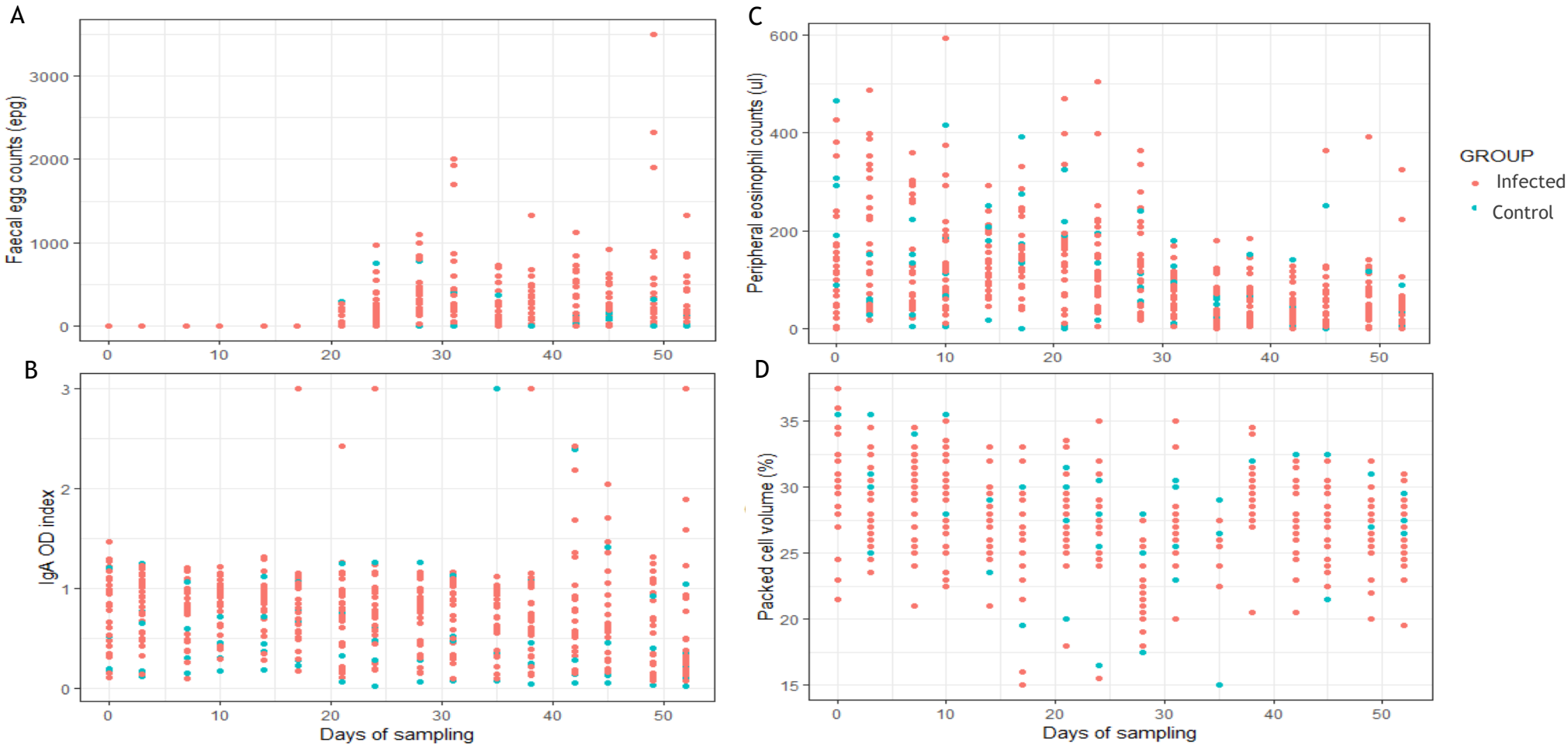


Figure 4-1. Distribution of faecal egg counts (A), IgA OD index (B), peripheral eosinophil counts (C) and packed cell volume (D) of deliberately infected and control groups of Boer goats by days of sampling.

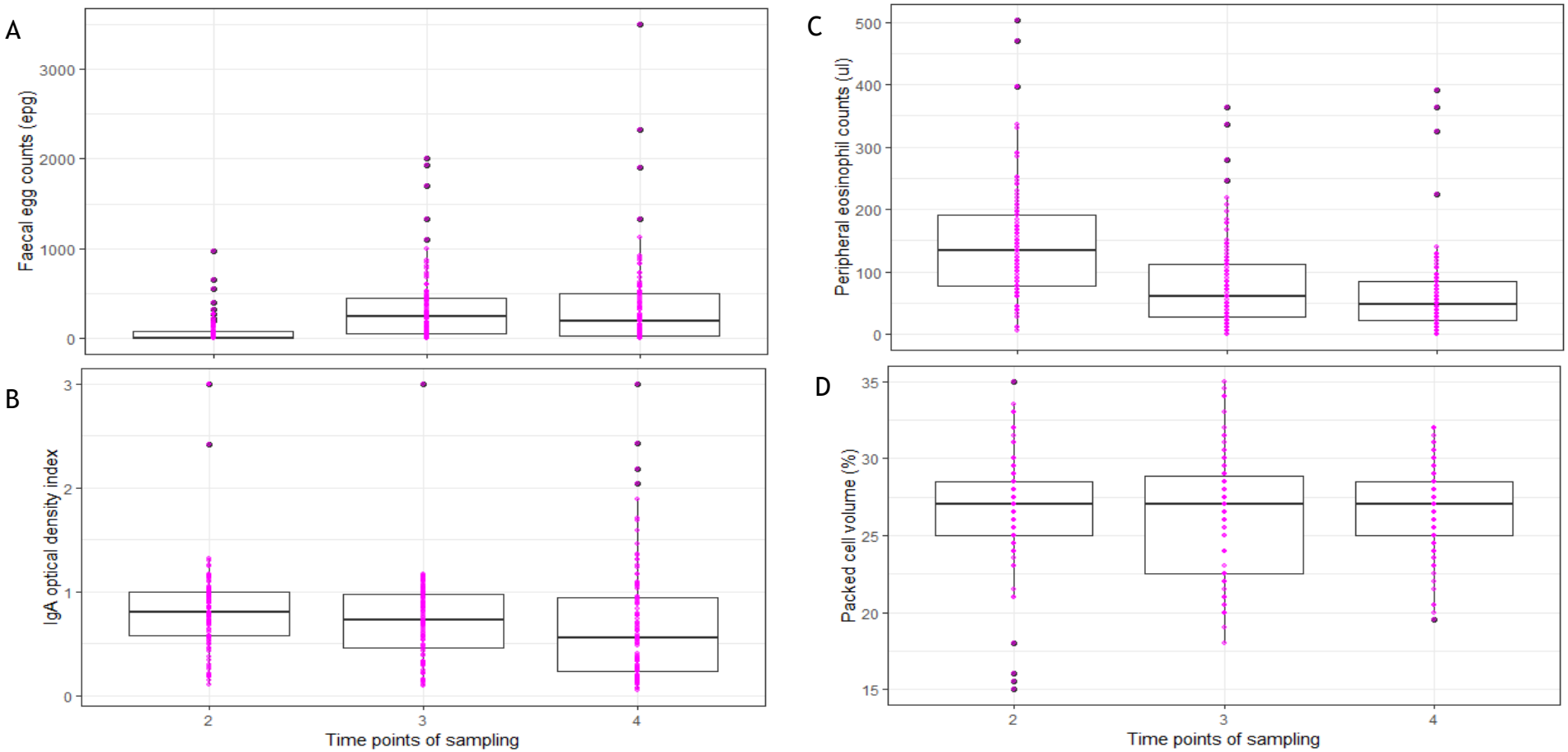


Figure 4-2. Distribution of faecal egg counts (A), IgA OD index (B), peripheral eosinophil counts (C) and packed cell volume (D) of deliberately infected Boer goats by time points of sampling. The horizontal line gives the median value and the box encompasses points between the 25th and 75th percentiles.

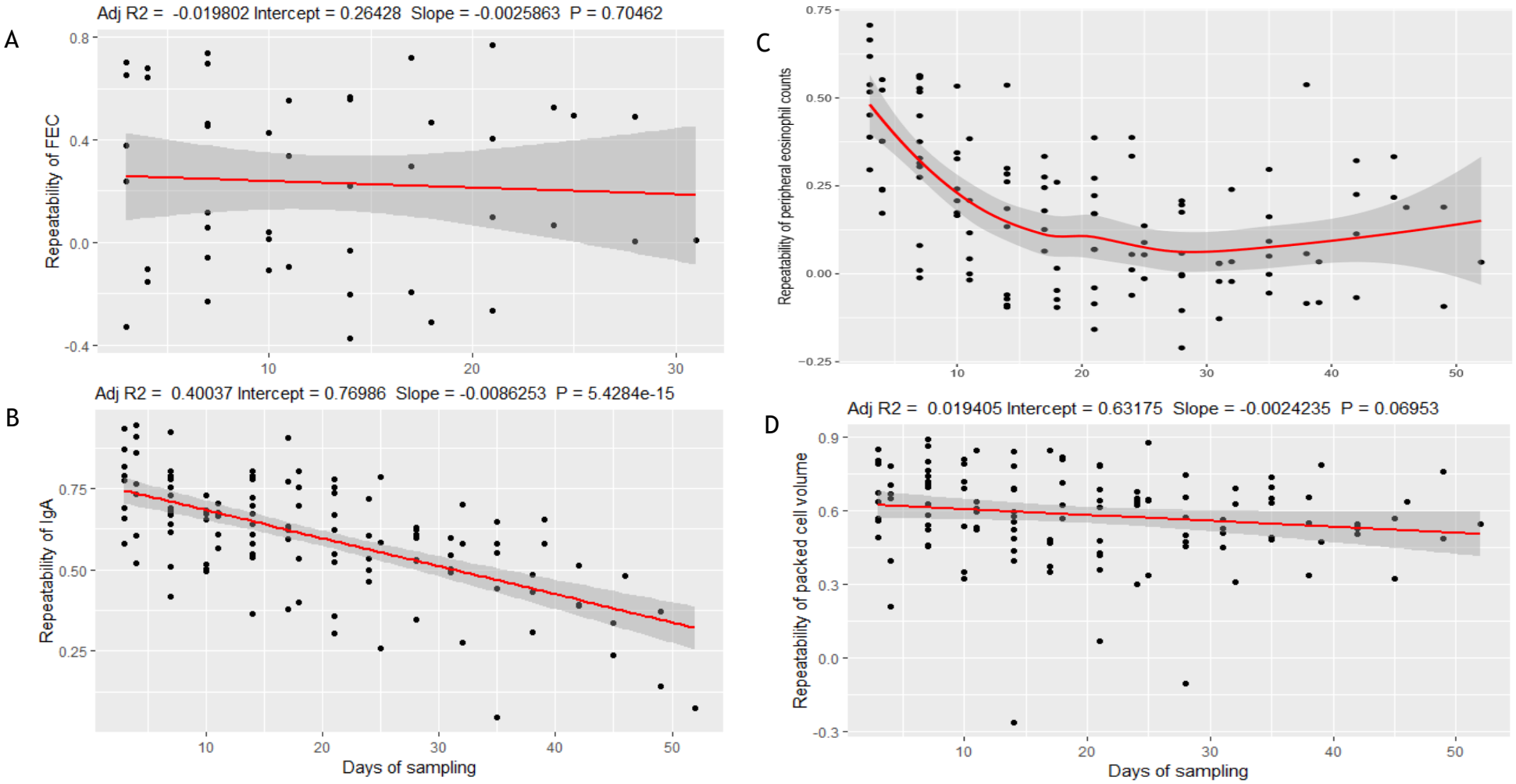


Figure 4-3. Linear regression to examine the change over time in correlations between measurements at different sampling intervals for faecal egg counts (A), IgA OD index (B), peripheral eosinophil counts (C) and packed cell volume (D) in deliberately infected Boer goats.

Mixed model repeated measures analysis

Four sets of models will be presented in this section. They are:

a) Mixed effects models of FEC with time, IgA, peripheral eosinophils counts, type of birth and their interactions; and random effects of ID and pen.

b) Mixed effects models of FEC with time, IgA, peripheral eosinophils counts, types of birth and their interactions; and random effects of ID, animal and pen.

c) Mixed effects models of FEC with immune index, time, type of birth and their interactions; and random effects of ID and pen.

d) Mixed effects models of FEC with immune index, time, type of birth and their interactions; and random effects of ID, animal and pen.

a) Mixed effects models of FEC with time (TIME), IgA, peripheral eosinophil counts (EO), types of birth (TB) and their interactions and random effects of ID and pen (PEN).

Model selection and diagnostics Table 4-4 shows the process of model selection from the full zero-inflated Poisson FEC model (1a) to the simplest model (5a) by removing variables from the fixed effects with the largest p-values until only significant predictors remain. Autocorrelations and effective sample size for all models were checked and are shown here only for the final model, Model 5a. For a 'nitt' of 5000000, a 'burnin' of 10000 and thinning interval of 5000, the effective sample sizes for the fixed and random effects (Table 4-5) are greater than 1000 and the autocorrelations at 5000 are all below 0.1 as required (Table 4-6). No trend is seen in the trace for either the fixed effects (Figure 4-4, A) or random effects (Figure 4-5) except for a small spike in the pen effect. In addition, there was no evidence of autocorrelation in the estimate of repeatability based on the trace (Figure 4-6, left). The corresponding posterior distributions are shown in Figure 4-4 (B), Figure 4-5 (right) and Figure 4-6 (right).

Fixed effects The posterior means and 95% credible intervals for the fixed effects for Model 5a are shown in Table 4-5. There is an effect of time on both the count and binary component of the model. The count component of the model has a significant effect of time which results in reduced FEC at time points 2 and 3 compared to time point 4. There is a small but significant interaction between time and eosinophils. Eosinophils alone do not have a significant effect ($p = 0.563$) but at time point 4, eosinophilia reduces FEC ($p < 0.05$). The binary component of the fixed effects was used to calculate the probability of observing a zero (see 4.2.9.4) and shown in Table 4-8. There is a high probability of a zero at time point 2 (>0.6) and a reduction at time points 3 and 4 compared to time point 2. The overall probability of zero is 0.87.

Random effects The posterior means and 95% credible intervals for the random effects and residuals for Model 5a are shown in Table 4-5. The random effect of pen was small (with a mean variance of 0.064) compared to variance among individual goats of 0.529 and the residual variance of 0.766 (Table 4-5). This large residual variance suggests overdispersion. The binary component of the random effects and residual variance were fixed by the priors and therefore not discussed further.

Repeatability The repeatability estimate of FEC was 0.381 within the Highest Posterior Density (HPD) interval of 0.05 and 0.40 (Table 4-7; Figure 4-6, right).

Observed versus predicted data Figure 4-7 shows that the observed FEC data (top) were consistent with the predicted FEC data (bottom). Table 4-8 shows that the numbers of observed and predicted zero FEC have only small differences between each other, and Table 4-9 shows that the observed and predicted means of FEC are generally comparable. Table 4-8 also shows the probability of a true zero count (those coming from the binary rather than count component of the model) and shows that the overall proportion of zeroes which are true zeroes is high at just under 0.9.

Table 4-4. Model selection process for mixed effects models of FEC with time, type of birth, IgA and peripheral eosinophil counts as fixed effects, ID and pen as random effects.

Variable/DIC	Model 1a	Model 2a	Model 3a	Model 4a	Model 5a
DIC	1274.4	1275	1274.5	1275.4	1276.9
TIME (B)	Y	Y	Y	Y	Y
TIME (C)	Y	Y	Y	Y	Y
TB (C)	Y	Y	Y	Y	
IGA (C)	Y	Y	Y		
EO (C)	Y	Y	Y	Y	Y
TIME:EO (C)	Y	Y	Y	Y	Y
TIME:IGA (C)	Y				
IGA:EO (C)	Y	Y			
ID (B,C)*	Y	Y	Y	Y	Y
PEN (B,C)*	Y	Y	Y	Y	Y

DIC= Deviance Information Criterion; (B) = binary component; (C) = count component; Y = included; '*' = random effects; TB = types of birth; IGA = immunoglobulin A; EO = peripheral eosinophil counts

Table 4-5. Model coefficents of association of FEC with time of sampling, peripheral eosinophil counts and their interactions in Model 5a with ID and pen as random effects.

Structure	Variable	Posterior mean (Binary)	Posterior mean (Count)	Lower 95% CI	Upper 95% CI	Eff. sample	p-value	
Fixed effects	Intercept		2.740	2.270	3.310	1460	<0.001	***
		-1.840		-2.560	-1.100	1200	<0.001	***
	TIME2	2.240		1.310	3.120	1200	<0.001	***
	TIME3	-0.937		-2.230	0.580	1070	0.152	
	TIME2		-1.210	-1.920	-0.491	1200	<0.001	**
	TIME3		-0.478	-0.971	-0.014	1200	0.045	*
	EO		-0.001	-0.004	0.002	948	0.563	
	TIME3:EO		0.0004	-0.003	0.005	1200	0.855	
	TIME4:EO		-0.007	-0.012	0.001	1300	0.048	*
Random effects	ID		0.529	0.190	1.060	1060	-	-
		1e-06		1e-06	1e-06	0	-	-
	Pen		0.064	0.0001	0.255	1200	-	-
		1e-06		1e-06	1e-06	0	-	-
Residuals	Units		0.766	0.515	1.050	1200	-	-
		1		1	1	0	-	-

CI = credible intervals; Significance codes: '***' < 0.001; '**' < 0.01; '*' <0.05; '.' < 0.1; ' '

Table 4-6. Autocorrelation of fixed and random effects in Model 5a.

Fixed effects	traitFEC	traitzi_FEC	TIME2 (B)	TIME3 (B)	TIME2 (C)
Lag 0	1.000	1.000	1.000	1.000	1.000
Lag 5000	-0.027	0.023	-0.002	0.057	-0.027
Lag 25000	-0.001	0.010	0.016	0.009	0.005
Lag 50000	-0.044	0.002	0.017	0.013	0.046
Lag 250000	0.022	-0.039	-0.047	-0.020	0.027

Fixed effects	TIME3 (C)	EO (C)	TIME3: EO (C)	TIME4:EO (C)
Lag 0	1.000	1.000	1.000	1.000
Lag 5000	-0.020	0.012	-0.007	-0.041
Lag 25000	0.043	0.010	-0.025	0.031
Lag 50000	0.019	0.012	0.011	0.002
Lag 250000	0.025	-0.007	-0.010	-0.012

Random effects	traitFEC.ID	traitFEC.PEN	traitFEC.units
Lag 0	1.000	1.000	1.000
Lag 5000	0.060	0.005	0.026
Lag 25000	-0.012	0.010	0.007
Lag 50000	0.018	-0.014	-0.021
Lag 250000	0.006	-0.004	0.029

Table 4-7. FEC repeatability estimate for Model 5a.

Posterior mode for repeatability	Lower HPD interval	Upper HPD interval	Posterior mean for repeatability
0.335	0.187	0.598	0.381

HPD = highest posterior density.

Table 4-8. Observed and predicted zero FEC for Model 5a.

Time point	Observed zeroes	Predicted zeroes	Predicted true zeroes	Probability of predicted zeroes that are true zeroes	Number of counts
2	63	62.73	57.29	0.91	99
3	12	10.54	7.56	0.72	81
4	21	19.63	16.24	0.83	90
Overall	96	92.90	81.09	0.87	270

Table 4-9. Observed and predicted mean of FEC for Model 5a.

Time point	Observed means	Predicted means	Number of counts
2	2.82	3.55	99
3	13.73	17.39	81
4	14.24	19.13	90
All	9.90	12.89	270

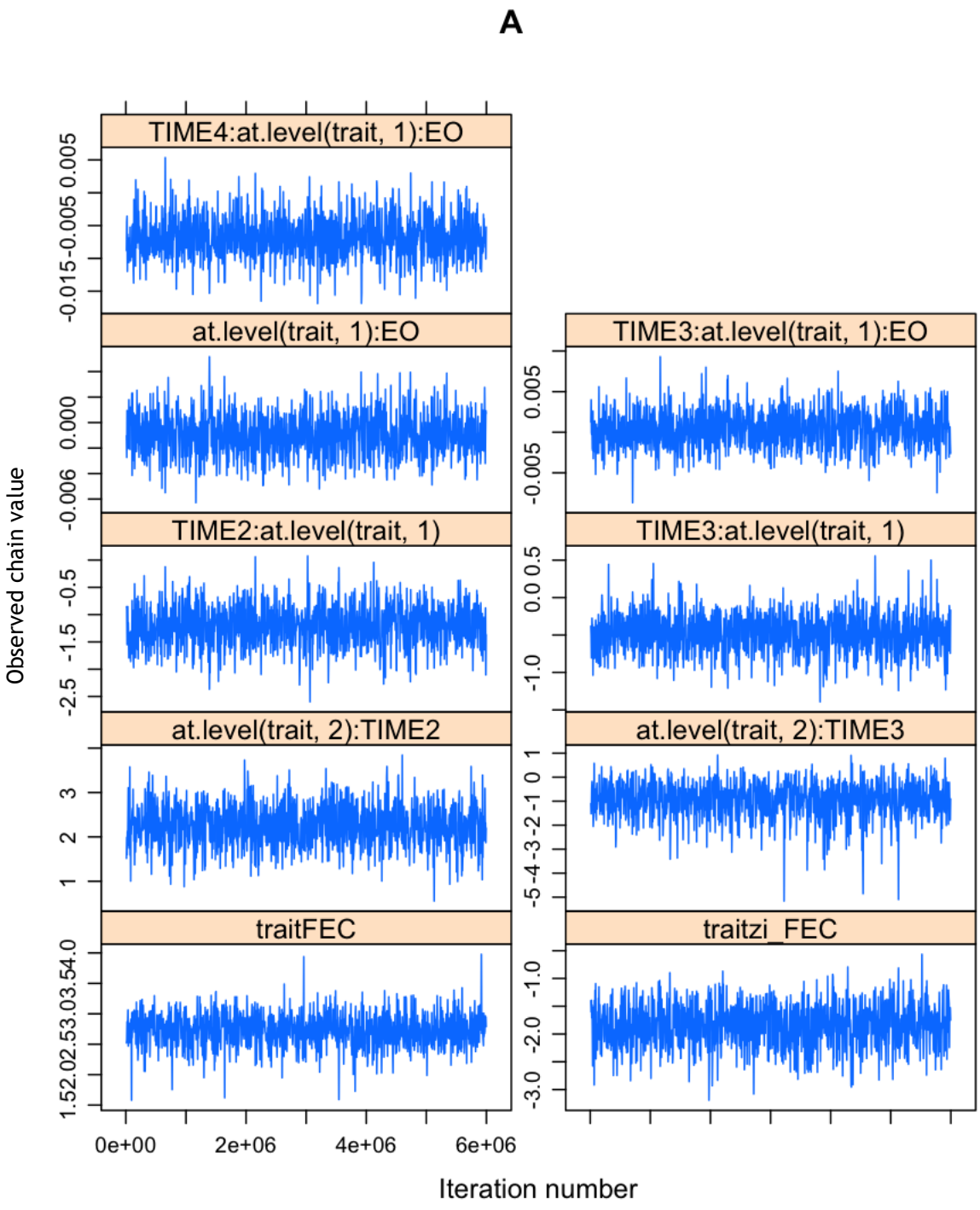


Figure 4-4 (A). Trace plots of parameter value explored by the MCMC chain of fixed effects for the association of FEC with time of sampling, peripheral eosinophil counts and their interaction; ID and pen as random effects (Model 5a).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = time point 2; TIME3 = time point 3; TIME4 = time point 4; EO = peripheral eosinophil counts; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

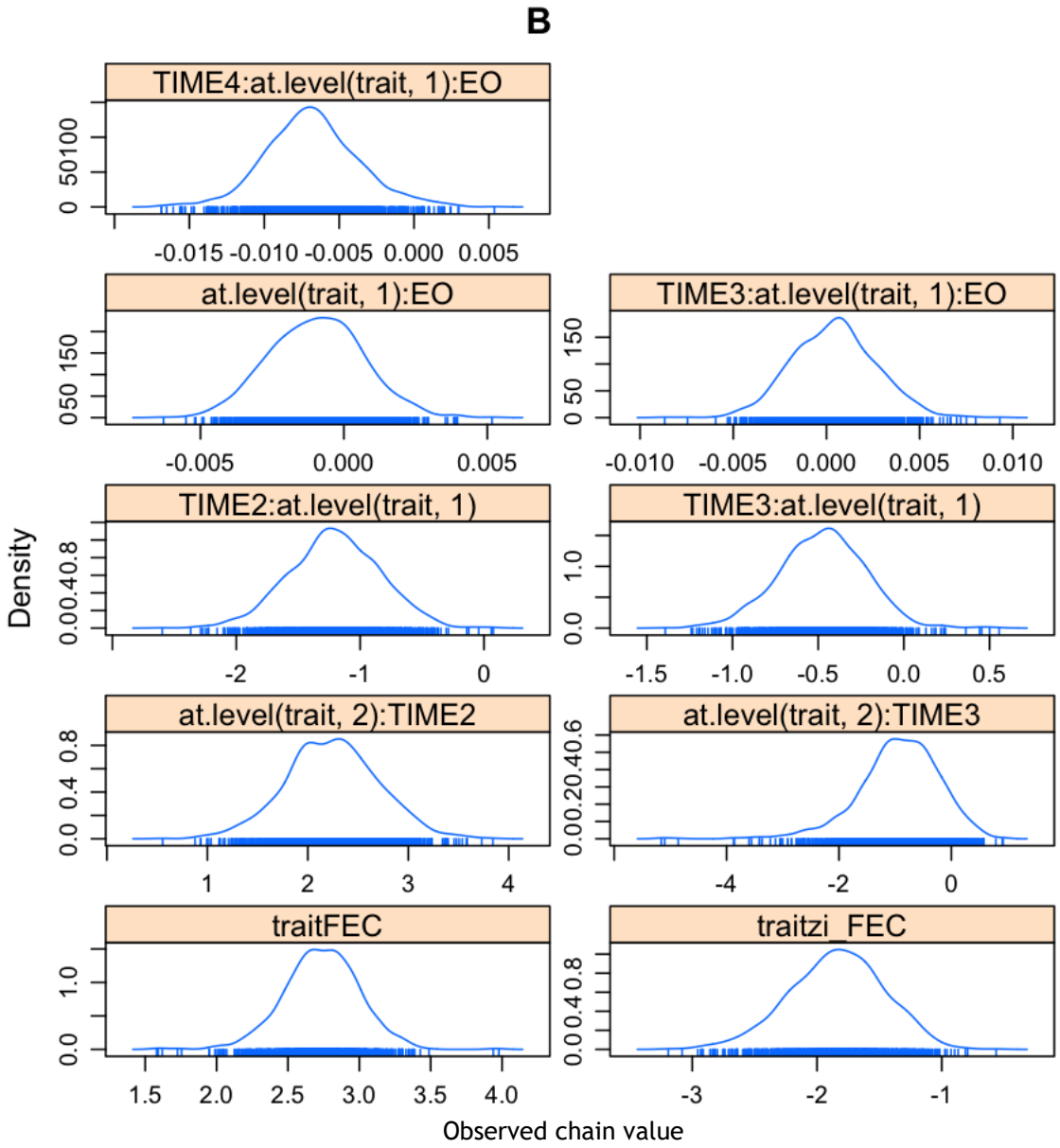


Figure 4-4 (B). Posterior density plots of parameter value distributions in the MCMC chain for fixed effects for the association of FEC with time of sampling, peripheral eosinophils and their interaction; ID and pen as random effects (Model 5a).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = time point 2; TIME3 = time point 3; TIME4 = time point 4; EO = peripheral eosinophil counts; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

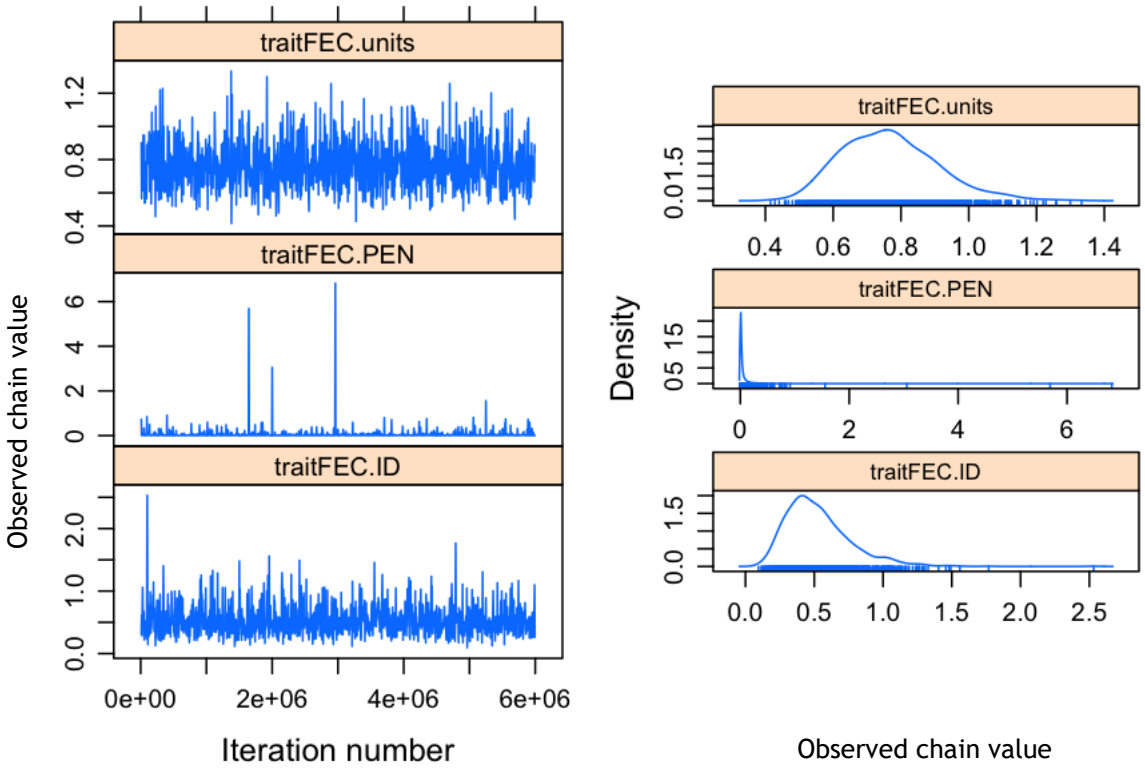


Figure 4-5. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for random effects and residual variance (denoted units) for the association of FEC with time of sampling, peripheral eosinophil counts and their interactions with ID and pen as random effects (Model 5a).
traitFEC.units = residual variance; ; traitFEC.ID = ID variance component, traitFEC.PEN = Pen variance component.

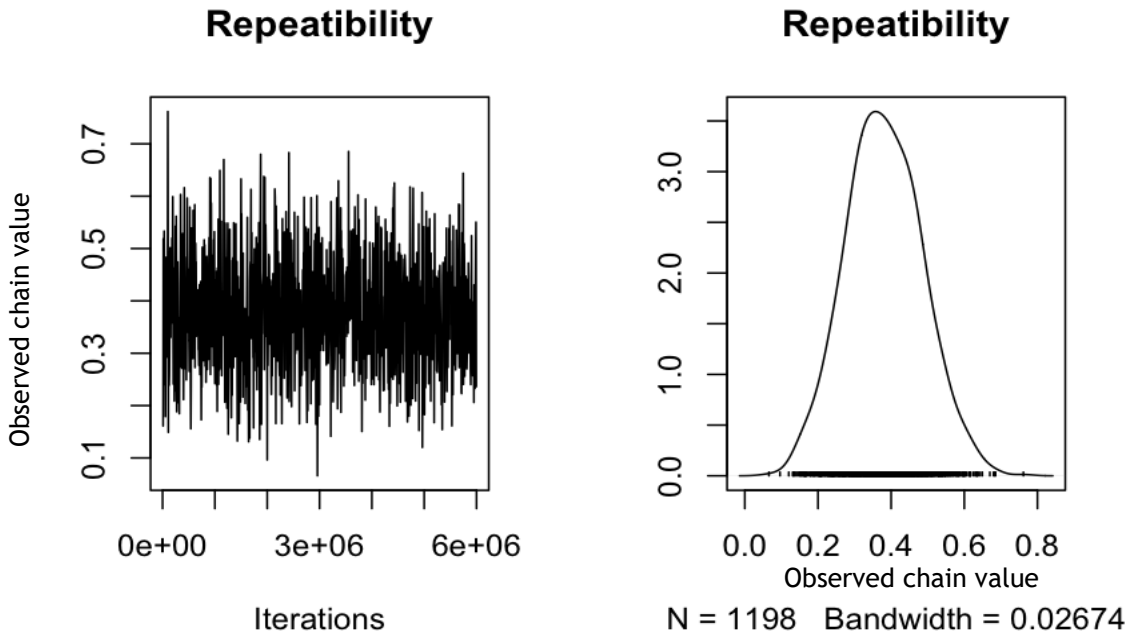


Figure 4-6. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability for the association of FEC with time of sampling, peripheral eosinophil counts and their interactions with ID and pen as random effects (Model 5a).

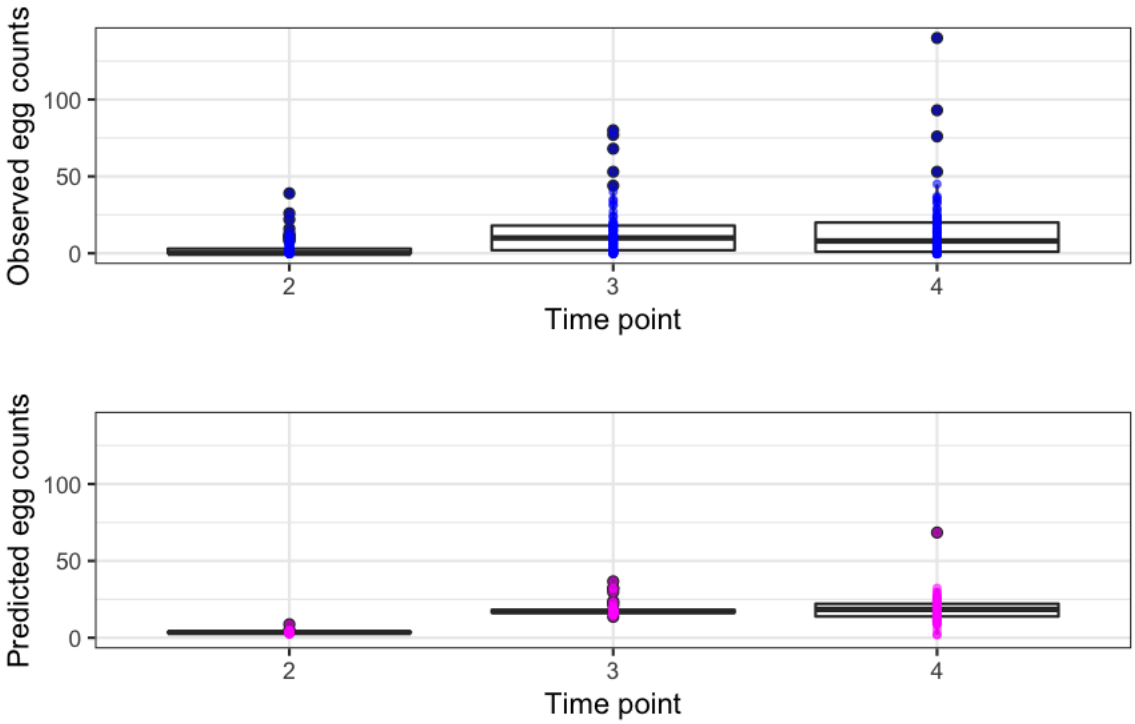


Figure 4-7. Observed (top) and predicted (bottom) FEC data using the model for the association of FEC with time of sampling, peripheral eosinophil counts and their interactions with ID and pen as random effects (Model 5a).

b) Mixed effects models of FEC with time, IgA, peripheral eosinophils counts, types of birth and their interactions as fixed effects and random effects of ID, animal and pen.

Model selection and diagnostics Animal as an additional random effect was included in the next set of models. The model selection process, leading to the final model known as Model 5b is shown in Table 4-10. Autocorrelations (not shown) and effective sample sizes were checked as for the previous models. No trend was seen in the trace of the fixed effects (Figure 4-8, A), random effects (Figure 4-9, left), FEC repeatability (Figure 4-10, left) and FEC heritability (Figure 4-11, left). The corresponding posterior densities for Model 5b are shown in Figure 4-8 (B), Figure 4-9 (right), Figure 4-10 (right) and Figure 4-11 (right).

Fixed effects The fixed effects are very similar in direction and size as the fixed effects for Model 5a (Table 4-11). There is an effect of time on both the count and binary components of the model. There is also a small but significant interaction between time and eosinophils ($p<0.05$) in which FEC reduces as eosinophil increases at time point 4. As for Model 5a, the binary component of the fixed effects in Model 5b was used to calculate the probability of observing a zero (Table 4-14). Very similar to the results for Model 5a are seen (Table 4-15) with the probability of a zero being high at time point 2 (> 0.6) and declining for time point 3 and 4.

Random effects The posterior means and 95% credible intervals for the random effects and residuals for Model 5b are shown in Table 4-11. Estimates for the random effect of pen and the residual variance are similar to Model 5a, showing again that the effect of pen is small and large residual variance suggesting overdispersion. The posterior mean of ID is smaller with the inclusion of animal as another random effect in this model (reduced from 0.53 in Model 5a to 0.35 in Model 5b). The posterior mean of animal random effect corresponds to an additive genetic variance of 0.23.

Repeatability and heritability The posterior mode for repeatability (Figure 4-10) estimate of FEC is 0.4 with a very similar estimate for the mean (Table 4-12). The heritability of FEC can be estimated as the animal random effect was included in the model. Table 4-13 shows that the posterior mode for FEC heritability is very small (0.0037) but the mean is substantially larger (0.157). This is because the posterior distribution is very skewed with most values are located around zero but some are much larger ones, suggesting that there is insufficient data to estimate the heritability more precisely.

Observed versus predicted data Similar to Model 5a, the observed FEC data in Model 5b (Figure 4-12, top) were consistent with the predicted FEC data (Figure 4-12, bottom). The numbers of observed and predicted zeroes have only small differences between each other (Table 4-14), and that the means of observed and predicted FEC are generally comparable with each other (Table 4-15). Table 4-14 also shows the probability of a true zero count (those coming from the binary rather than count component of the model) and shows that the overall proportion of zeroes which are true zeroes is high at just under 0.9.

Table 4-10. Model selection process for mixed effects models of FEC with time, type of birth, IgA and eosinophils as fixed effects; ID, pen and animal as random effects.

Variable/DIC	Model 1b	Model 2b	Model 3b	Model 4b	Model 5b
DIC	1275.1	1275.2	1275	1275.4	1277.6
TIME (B)	Y	Y	Y	Y	Y
TIME (C)	Y	Y	Y	Y	Y
TB (C)	Y	Y	Y	Y	
IGA (C)	Y	Y	Y		
EO (C)	Y	Y	Y	Y	Y
TIME:EO (C)	Y	Y	Y	Y	Y
TIME:IGA (C)	Y				
IGA:EO (C)	Y	Y			
ID (B,C)*	Y	Y	Y	Y	Y
PEN (B,C)*	Y	Y	Y	Y	Y
Animal (B,C)*	Y	Y	Y	Y	Y

DIC= Deviance Information Criterion; (B) = binary component; (C) = count component; Y = included; '*' = random effects; TB = types of birth; IGA = immunoglobulin A; EO = peripheral eosinophil counts.

Table 4-11. Model coefficients of association of FEC with time of sampling, eosinophil counts and their interactions in Model 5b with ID, pen and animal as random effects.

Structure	Variable	Posterior mean (Binary)	Posterior mean (Count)	Lower 95% CI	Upper 95% CI	Eff. sample	p-value	
Fixed effects	Intercept		2.710	2.090	3.250	1200	<0.001	***
		-1.820		-2.520	-1.050	1200	<0.001	***
	TIME2	2.230		1.410	3.150	1070	<0.001	***
	TIME3	-0.881		-2.160	0.321	1150	0.177	
	TIME2		-1.210	-1.870	-0.488	1200	<0.001	***
	TIME3		-0.471	-0.958	0.040	1210	0.070	.
	EO		-0.001	-0.004	0.002	1200	0.566	
	TIME3:EO		0.0004	-0.004	0.005	1200	0.878	
	TIME4:EO		-0.007	-0.013	-0.001	1330	0.030	*
Random effects	ID		0.347	0.001	0.899	1170	-	-
		1e-06		1e-06	1e-06	0	-	-
	Pen		0.051	0.0002	0.217	1200	-	-
		1e-06		1e-06	1e-06	0	-	-
	Animal		0.233	0.0003	0.842	1200	-	-
		1e-06		1e-06	1e-06	0	-	-
Residuals	Units		0.761	0.523	1.030	1200	-	-
		1		1	1	0	-	-

CI = credible intervals; Significance codes: '***' < 0.001; '**' < 0.01; '*' <0.05; '.' < 0.1; ' ' <=1; EO = peripheral eosinophil counts.

Table 4-12. FEC repeatability estimate for Model 5b.

Posterior mode for repeatability	Lower HPD interval	Upper HPD interval	Posterior mean for repeatability
0.408	0.191	0.614	0.401

HPD = highest posterior density

Table 4-13. FEC heritability estimate for Model 5b.

Posterior mode for heritability	Lower HPD interval	Upper HPD interval	Posterior mean for heritability
0.0037	0.000222	0.505	0.157

HPD = highest posterior density

Table 4-14. Observed and predicted zero FEC for Model 5b.

Time point	Observed zeroes	Predicted zeroes	Predicted true zeroes	Probability of predicted zeroes that are true zeroes	Number of counts
2	63	62.97	57.87	0.92	99
3	12	11.47	8.06	0.70	81
4	21	20.38	16.39	0.80	90
Overall	96	94.82	82.32	0.87	270

Table 4-15. Observed and predicted mean of FEC for Model 5b.

Time point	Observed means	Predicted means	Number of counts
2	2.82	3.54	99
3	13.73	17.11	81
4	14.24	17.53	90
All	9.90	12.28	270

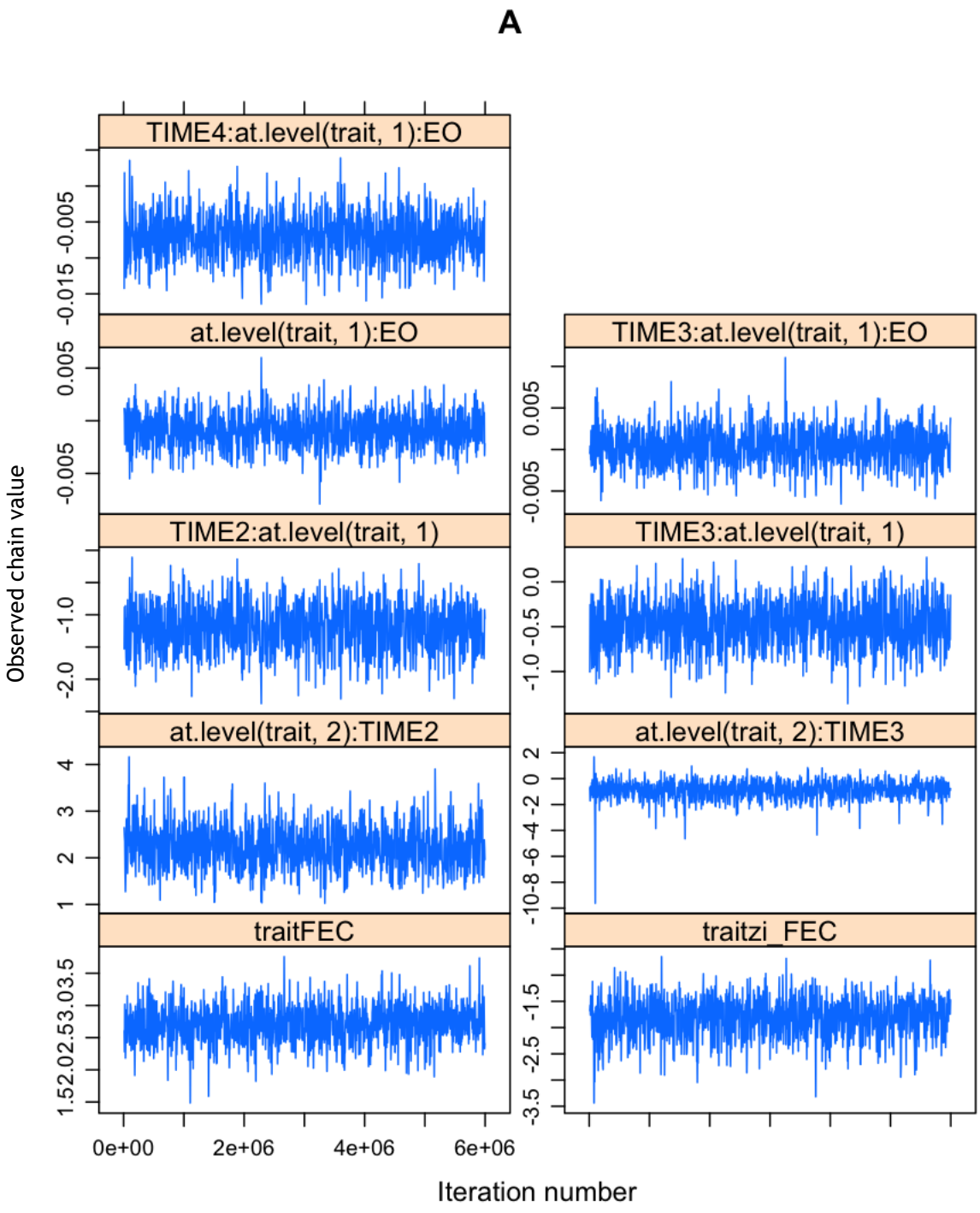


Figure 4-8 (A). Trace plots of parameter value explored by the MCMC chain of fixed effects for the association of FEC with time of sampling, peripheral eosinophil counts and their interaction; ID, pen and animal as random effects (Model 5b).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = time point 2; TIME3 = time point 3; TIME4 = time point 4; EO = peripheral eosinophil counts; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

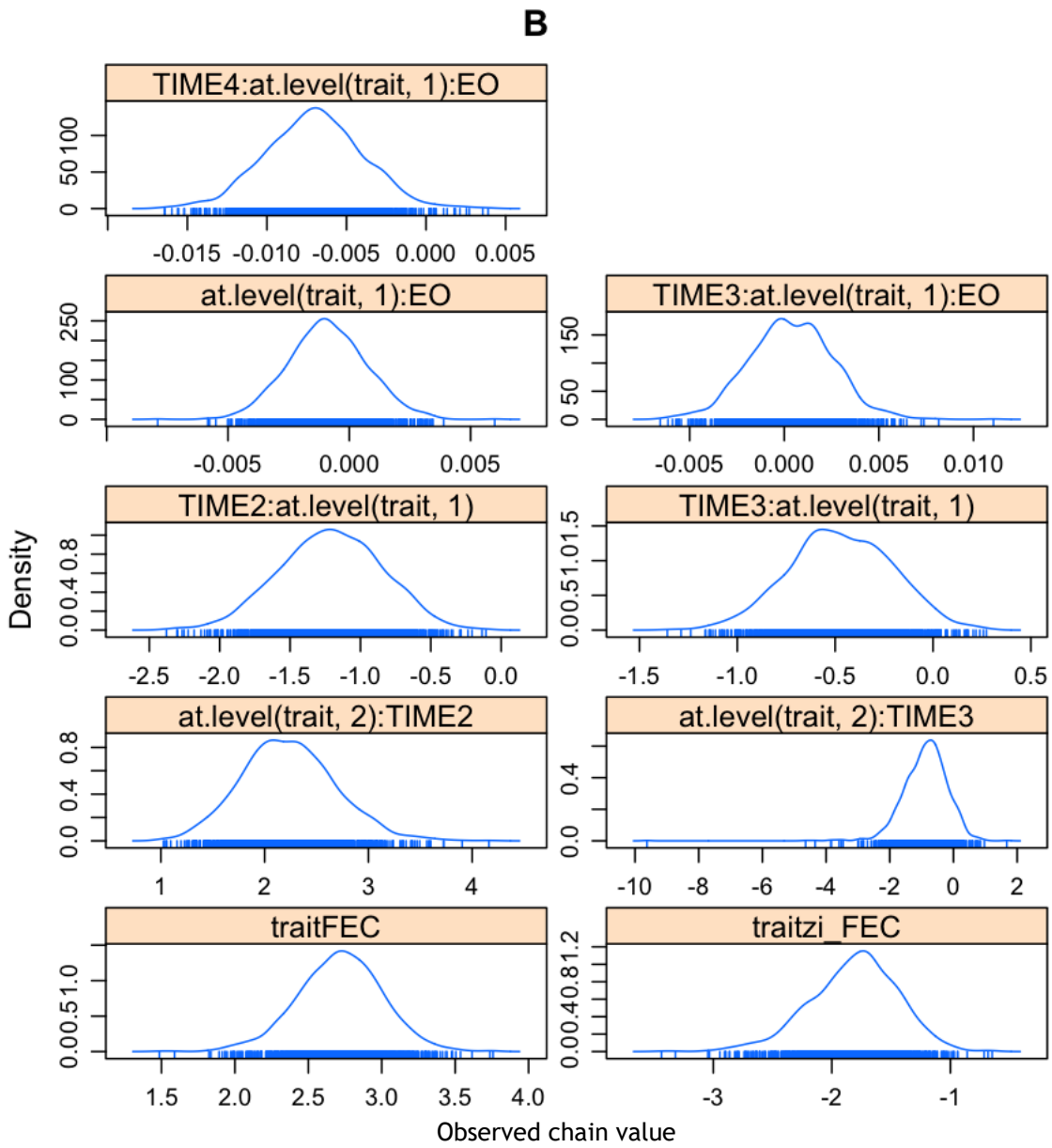


Figure 4-8 (B). Posterior density plots of parameter value distributions in the MCMC chain for fixed effects for the association of FEC with time of sampling, peripheral eosinophils and their interaction; ID, pen and animal as random effects (Model 5b).

at.level(trait,1) = count component; at.level(trait,2) = binary component; TIME2 = time point 2; TIME3 = time point 3; TIME4 = time point 4; EO = peripheral eosinophil counts; traitFEC = intercept for FEC in count component; traitzi_FEC = intercept for FEC in binary component.

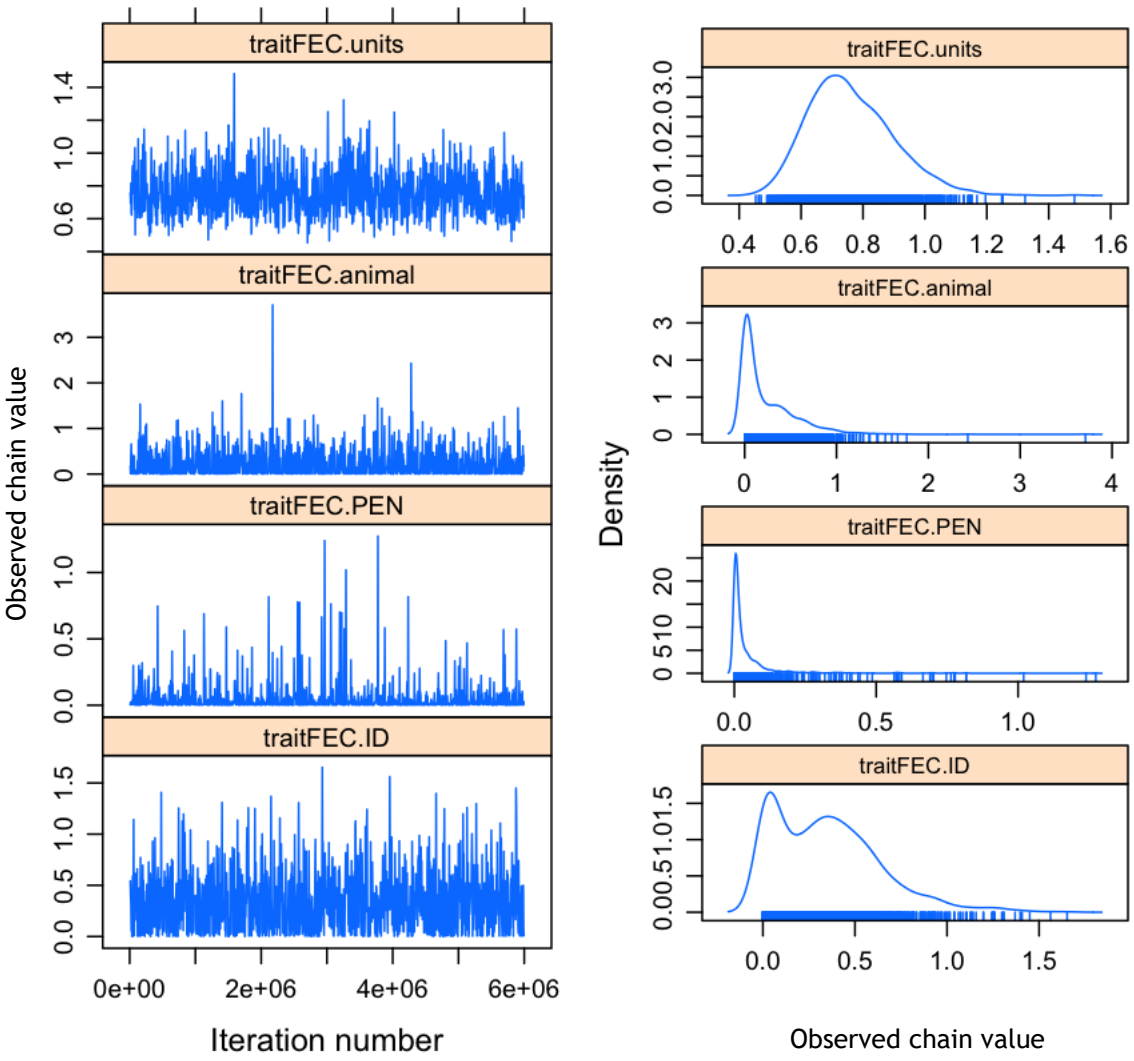


Figure 4-9. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for random effects and residual variance (denoted units) for the association of FEC with time of sampling, eosinophil counts and their interactions; with ID, pen and animal as random effects (Model 5b).

traitFEC.units = residual variance; ; traitFEC.ID = ID variance component, traitFEC.PEN = Pen variance component. TraitFEC.animal = Animal variance component.

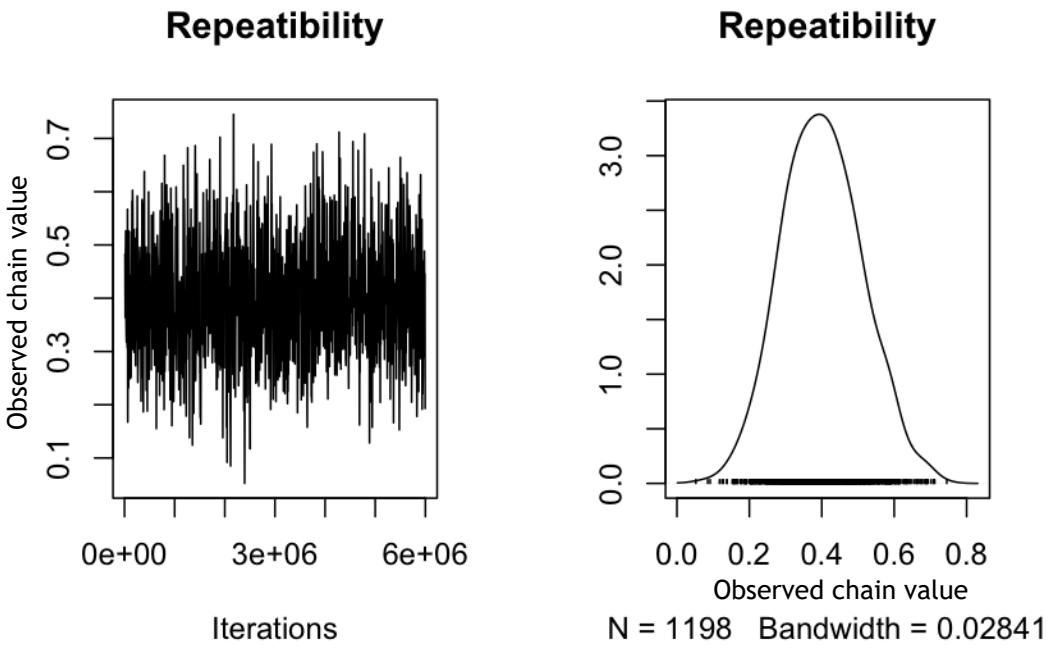


Figure 4-10. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability for the association of FEC with time of sampling, eosinophil counts and their interactions; with ID, pen and animal as random effects (Model 5b).

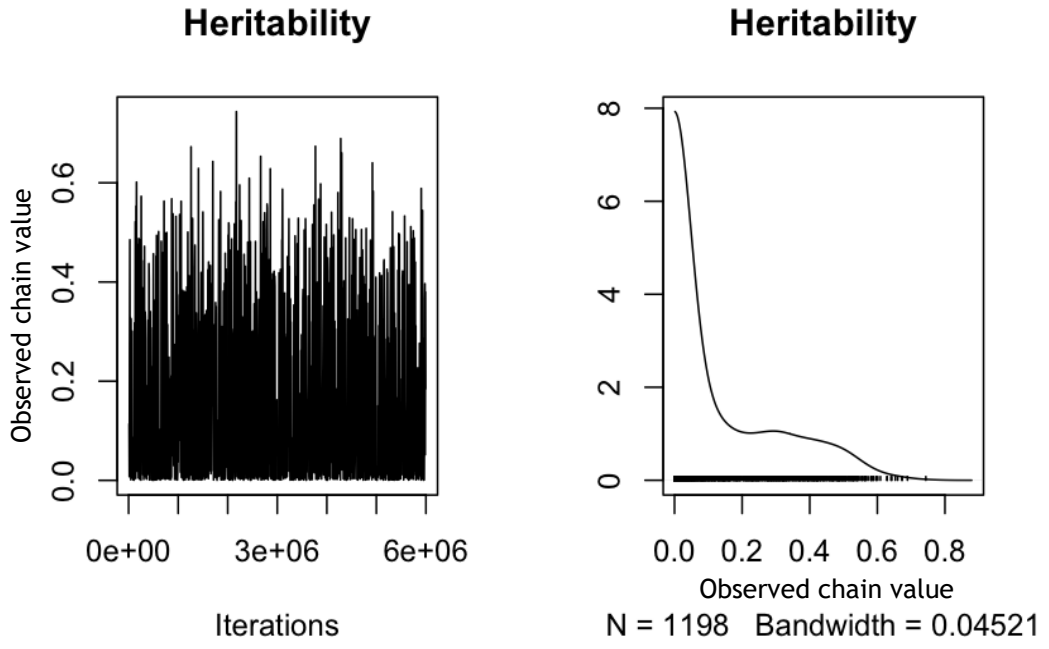


Figure 4-11. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability for the association of FEC with time of sampling, eosinophil counts and their interactions; with ID, pen and animal as random effects (Model 5b).

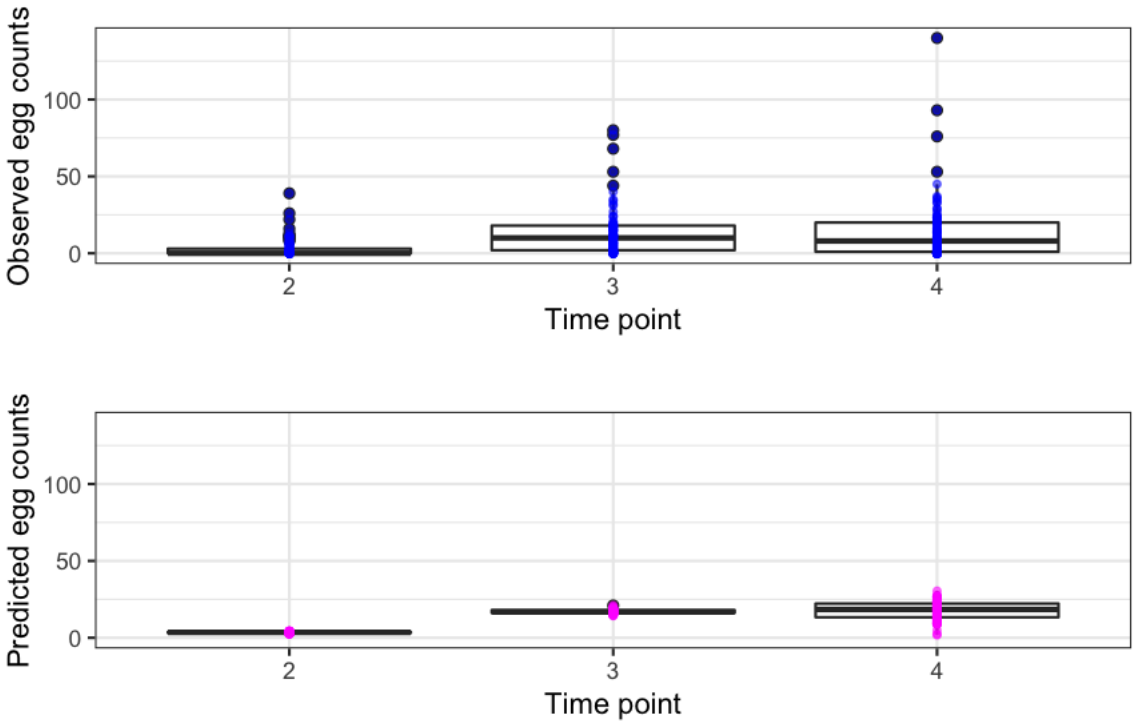


Figure 4-12. Observed (top) and predicted (bottom) FEC data for the association of FEC with time of sampling, eosinophil counts and their interactions; with ID, pen and animal as random effects (Model 5b).

c) Mixed effects models of FEC with immune index, time and their interactions as fixed effects and random effects of ID and pen.

Model selection and diagnostics The model selection process that leads to the final model known as Model 4c is shown in Table 4-16. Autocorrelations (not shown) and effective sample sizes were checked as for the previous models. For an 'nitt' of $2.5x10^6$, a 'burnin' of 10000 and thinning interval of 2000, the effective sample sizes for the fixed and random effects (Table 4-17) are greater than 1000 and the autocorrelations at 2000 are all below 0.1 as required. No trend was seen in the trace of the fixed effects (Figure 4-13, A), random effects (Figure 4-14, left) or the repeatability (Figure 4-15, left). The corresponding posterior densities for Model 4c are shown in Figure 4-13 (B), Figure 4-14 (right) and Figure 4-15 (right).

Fixed effects The only significant fixed effect in Model 4c is the effect of time on both the count and binary component (Table 4-17). The FEC increases with time on the count component of the model relative to the baseline of time point 2. As for Models 5a and 5b, the effect of the fixed effects on the binary component can be seen by predicting the number of zero counts. Model 4c has similar results to Models 5a and 5b (Table 4-19) with the probability of a zero being high at time point 2 (> 0.6) and declining for time point 3 and 4.

Random effects The posterior means and 95% credible intervals for the random effects and residuals for Model 4c are shown in Table 4-20. Estimates for the random effect of pen and the residual variance are similar to Model 5a and Model 5b, showing again that the effect of pen is small and large residual variance suggesting overdispersion. The random effect of ID (0.45) lies between the estimates for Models 5a and 5b.

Repeatability The posterior mode for repeatability (Figure 4-15, right) was very similar with the mean estimates for repeatability, both at 0.35 (Table 4-18). The repeatability estimates of FEC in sheep breeds are around 0.2 to 0.4 while in goat breeds around 0.1 to 0.2 (Table 1-3).

Observed versus predicted data Similar to Models 5a and 5b, the observed FEC data (Figure 4-16, top) were consistent with the predicted FEC data (Figure 4-16, bottom). Table 4-19 shows that the numbers of observed and predicted zeroes have only small differences between each other, and Table 4-20 shows generally comparable means between the observed and predicted counts. Table 4-19 also shows that the probability of a true zero count (those coming from the binary rather than count component of the model) and shows that the proportion of zeroes which are true zeroes is high at 0.9.

Table 4-16. Model selection process for mixed effects models of FEC with time, type of birth and immune index as fixed effects; ID and pen as random effects.

Variable/DIC	Model 1c	Model 2c	Model 3c	Model 4c
DIC	1282.1	1284.2	1284.7	1286.1
TIME (B)	Y	Y	Y	Y
TIME (C)	Y	Y	Y	Y
TB (C)	Y	Y	Y	
ImmIndex (C)	Y	Y		
TIME:ImmIndex (C)	Y			
ID (B,C)	Y	Y	Y	Y
PEN (B,C)	Y	Y	Y	Y

DIC= Deviance Information Criterion; (B) = binary component; (C) = count component; Y = included; '*' = random effects; TB = types of birth; ImmIndex = immune index.

Table 4-17. Model coefficients of association of FEC with time of sampling as a fixed effect in Model 4c with ID and pen as random effects.

Structure	Variable	Posterior mean (Binary)	Posterior mean (Count)	Lower 95% CI	Upper 95% CI	Eff. sample	p-value	
Fixed effects	Intercept		1.4000	0.916	1.890	1110	<0.001	***
		0.416		-0.146	0.981	1250	0.159	
	TIME3	-3.040		-4.350	-1.830	1240	<0.001	***
	TIME4	-2.040		-2.890	-1.140	1240	<0.001	***
	TIME3		0.824	0.426	1.270	1080	0.002	**
	TIME4		0.936	0.503	1.360	1240	<0.001	***
Random effects	ID		0.45	0.110	0.915	1240	-	-
		1e-06		1e-06	1e-06	0	-	-
	Pen		0.052	0.0002	0.229	1160	-	-
		1e-06		1e-06	1e-06	0	-	-
Residuals	Units		0.751	0.523	0.997	1250	-	-
		1		1	1	0	-	-

CI = credible intervals; Significance codes: '***' < 0.001; '**' < 0.01; '*' <0.05; '.' < 0.1; ' ' <=1

Table 4-18. FEC repeatability estimate for Model 4c.

Posterior mode for repeatability	Lower HPD interval	Upper HPD interval	Posterior mean for repeatability
0.350	0.141	0.559	0.348

HPD = highest posterior density

Table 4-19. Observed and predicted zero FEC for Model 4c.

Time point	Observed zeroes	Predicted zeroes	Predicted true zeroes	Probability of predicted zeroes that are true zeroes	Number of counts
2	63	62.59	57.68	0.92	99
3	12	11.12	8.46	0.76	81
4	21	20.93	18.67	0.89	90
Overall	96	94.64	84.81	0.90	270

Table 4-20. Observed and predicted mean of FEC for Model 4c.

Time point	Observed means	Predicted means	Number of counts
2	2.82	3.27	99
3	13.73	16.36	81
4	14.24	15.71	90
All	9.90	11.34	270

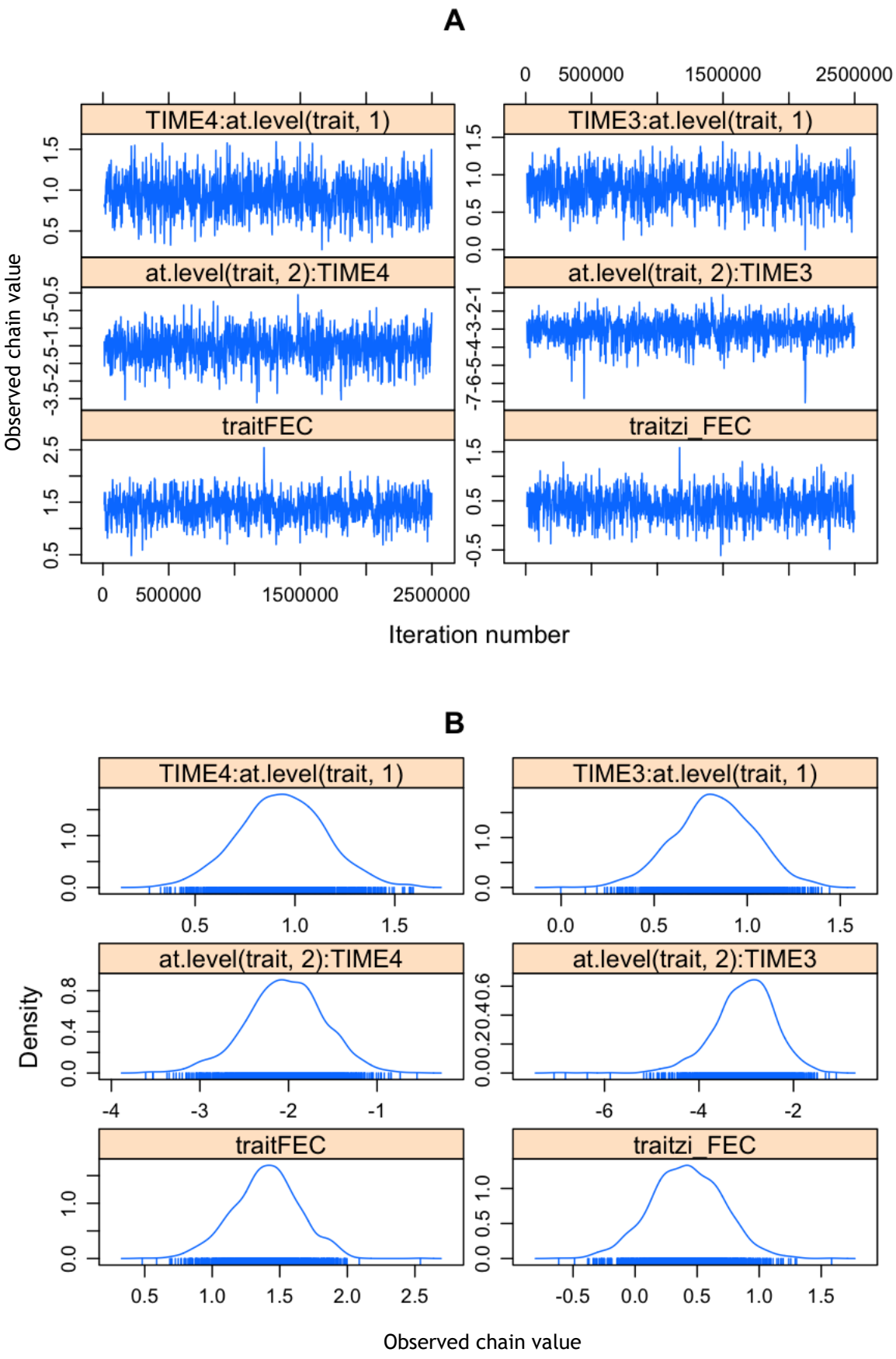


Figure 4-13. Trace plots (A) and posterior density plots (B) of parameter value explored by the MCMC chain of fixed effects for the association of FEC with time of sampling as a fixed effect, ID and pen as random effects (Model 4c).

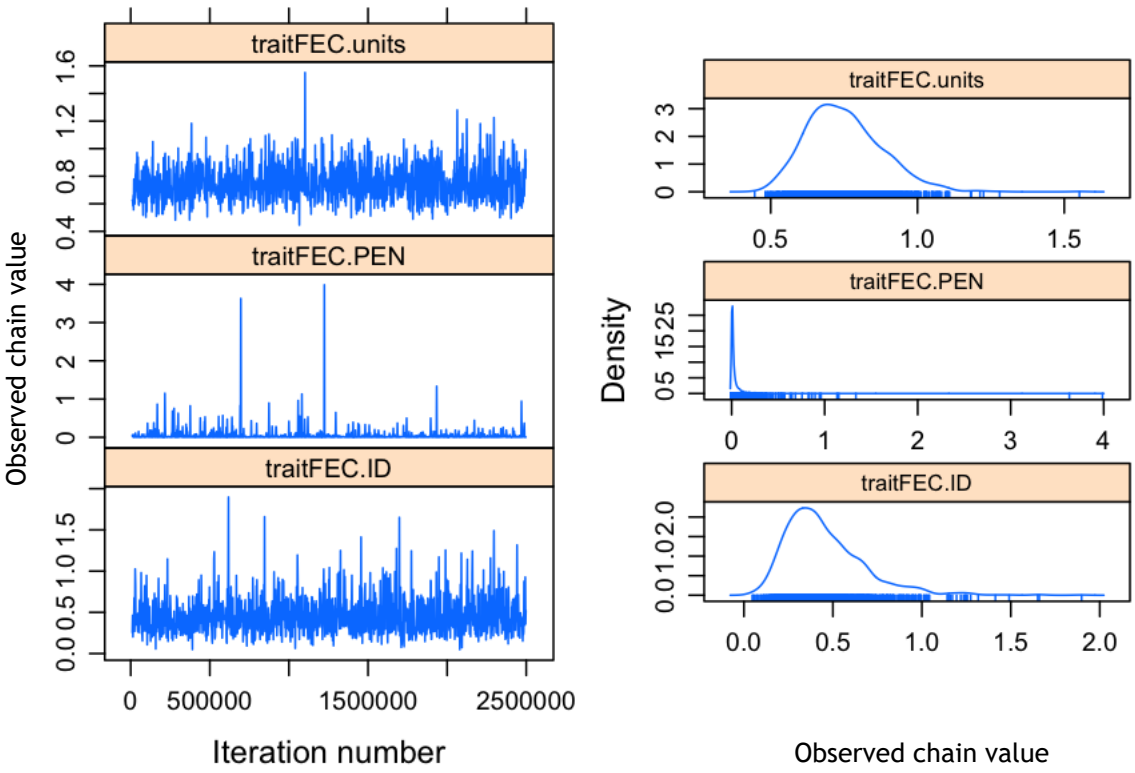


Figure 4-14. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for random effects and residual variance (denoted units) for the association of FEC with time of sampling as a fixed effect with ID and pen as random effects (Model 4c).

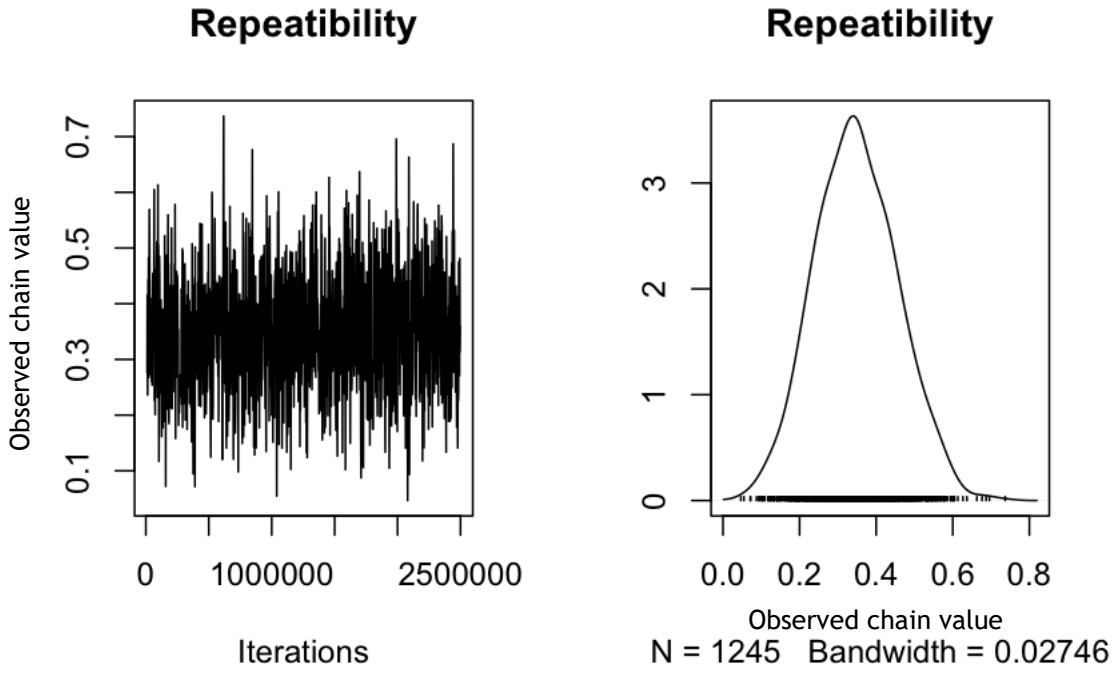


Figure 4-15. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability for the association of FEC with time of sampling as a fixed effect with ID and pen as random effects (Model 4c).

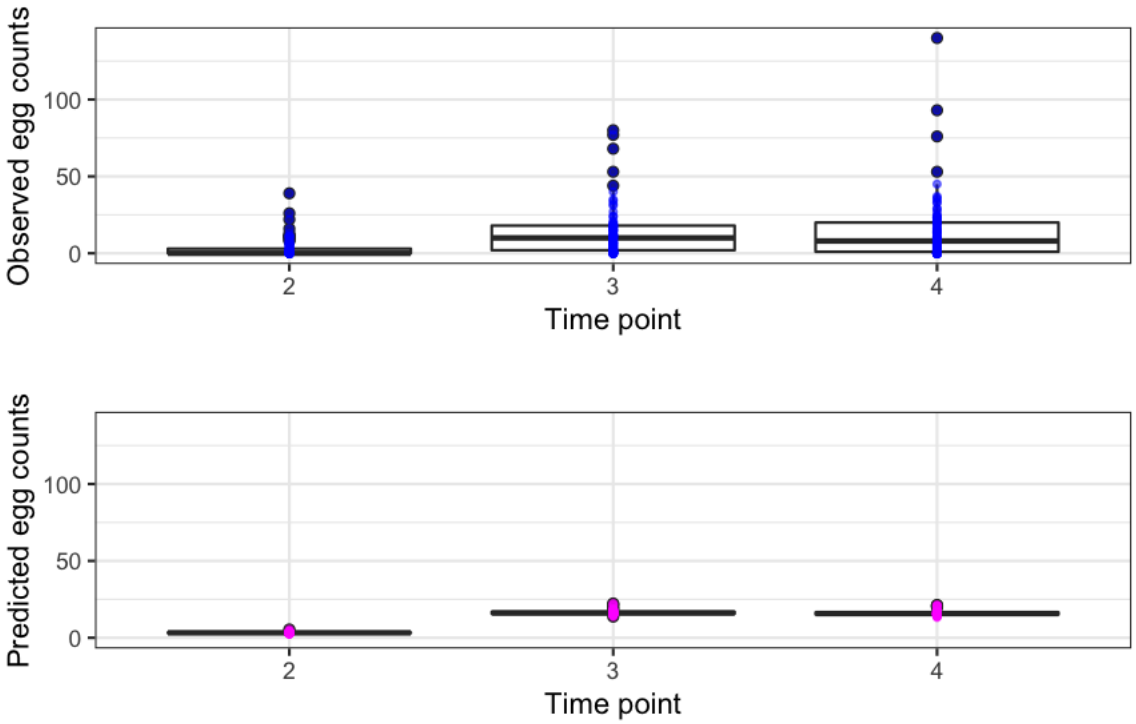


Figure 4-16. Observed (top) and predicted (bottom) FEC data for the association of FEC with time of sampling as a fixed effect with ID and pen as random effects (Model 4c).

d) Mixed effects models of FEC with immune index, time and their interactions as fixed effects and random effects of ID, animal and pen.

Model selection and diagnostics This was conducted as for Models 5a, 5b and 4c. Model behaviours and parameter estimates were also very similar to the previous models so the trace and posterior plots are not presented in this section. Table 4-21 shows the process of model selection and Table 4-22 shows the parameter estimates for the final model known as Model 4d.

Fixed effects The only significant fixed effects in Model 4d are the effect of time on the count and binary components and take very similar to the values for Model 4c (Table 4-22); the FEC increases with time relative to the baseline of time point 2. As for the previous models (Models 5a, 5b, 4c), the effect of the fixed effects on the binary components can be seen by predicting the number of zero counts. Very similar to the results for the previous models, the probability of a zero for Model 4d is high at time point 2 (> 0.6) and declining for time point 3 and 4 (Table 4-25).

Random effects The random effect of pen and the residual variance (Table 4-22) are similar to Model 5a, 5b and 4c and indicate the small effect of pen and overdispersion. The inclusion of animal as another random effect in this model results in a decrease in the posterior mean of ID (from 0.45 in Model 4c to 0.287 in Model 4d) and estimates a posterior mean of the animal random effect that corresponds to an additive genetic variance of 0.21.

Repeatability and heritability The addition of the animal random effect allows the estimation of FEC repeatability and heritability for Model 4d. The FEC repeatability has a posterior mode of approximately 0.40 and mean of 0.37 (Figure 4-17, right; Table 4-23). The FEC heritability has a posterior mode of 0.004 but a much larger mean of 0.15 due to the skewed posterior distribution (Figure 4-18, right; Table 4-24).

Observed versus predicted data As for the three previous models, there is a good match between the observed data (Figure 4-19, top) and the predicted data (Figure 4-19, bottom) of Model 4d that is also shown in Table 4-26. The observed and predicted numbers of zeroes are also similar (Table 4-25). Again, the proportion of zeroes that are true zeroes is just under 0.9 which is similar to the previous models.

Table 4-21. Model selection process for mixed effects models of FEC with time, type of birth and immune index as fixed effects; ID and pen as random effects.

Variable/DIC	Model 1d	Model 2d	Model 3d	Model 4d
DIC	1282.2	1284	1284.5	1286.1
TIME (B)	Y	Y	Y	Y
TIME (C)	Y	Y	Y	Y
TB (C)	Y	Y	Y	
ImmIndex (C)	Y	Y		
TIME:ImmIndex (C)	Y			
ID (B,C)	Y	Y	Y	Y
Animal (B,C)	Y	Y	Y	Y
PEN (B,C)	Y	Y	Y	Y

DIC= Deviance Information Criterion; (B) = binary component; (C) = count component; Y = included; '*' = random effects; TB = types of birth; IGA = immunoglobulin A; EO = peripheral eosinophil counts

Table 4-22. Model coefficients of association of faecal egg counts with time of sampling in Model 4d with ID, pen and animal as random effects.

Structure	Variable	Posterior mean (Binary)	Posterior mean (Count)	Lower 95% CI	Upper 95% CI	Eff. sample	p-value	
Fixed effects	Intercept		1.390	0.826	1.950	1240	0.002	**
		0.415		-0.138	0.940	1250	0.149	
	TIME3	-3.030		-4.260	-1.900	1250	<0.001	***
	TIME4	-2.04		-2.930	-1.220	1240	<0.001	***
	TIME3		0.819	0.368	1.290	1240	<0.001	***
	TIME4		0.935	0.512	1.370	1240	<0.001	***
Random effects	ID		0.287	0.0003	0.740	1240	-	-
		1e-06		1e-06	1e-06	0	-	-
	Animal		0.210	0.0002	0.792	1090	-	-
		1e-06		1e-06	1e-06	0	-	-
	Pen		0.042	0.0002	0.169	1240	-	-
		1e-06		1e-06	1e-06	0	-	-
Residuals	Units		0.758	0.524	1.040	1240	-	-
		1		1	1	0	-	-

CI = credible intervals; Significance codes: '***' < 0.001; '**' < 0.01; '*' <0.05; '.' < 0.1; ' ' <=1

Table 4-23. FEC repeatability estimate for Model 4d.

Posterior mode for repeatability	Lower HPD interval	Upper HPD interval	Posterior mean for repeatability
0.397	0.15	0.575	0.367

HPD = highest posterior density

Table 4-24. FEC heritability estimate for Model 4d.

Posterior mode for heritability	Lower HPD interval	Upper HPD interval	Posterior mean for heritability
0.00423	0.000151	0.474	0.15

HPD = highest posterior density

Table 4-25. Observed and predicted zero FEC for Model 4d.

Time point	Observed zeroes	Predicted zeroes	Predicted true zeroes	Probability of predicted zeroes that are true zeroes	Number of counts
2	63	62.50	57.78	0.92	99
3	12	11.69	8.42	0.72	81
4	21	21.35	18.67	0.87	90
All	96	95.54	84.87	0.89	270

Table 4-26. Observed and predicted mean of FEC for Model 4d.

Time point	Observed means	Predicted means	Number of counts
2	2.82	3.42	99
3	13.73	16.17	81
4	14.24	16.19	90
All	9.90	11.50	270

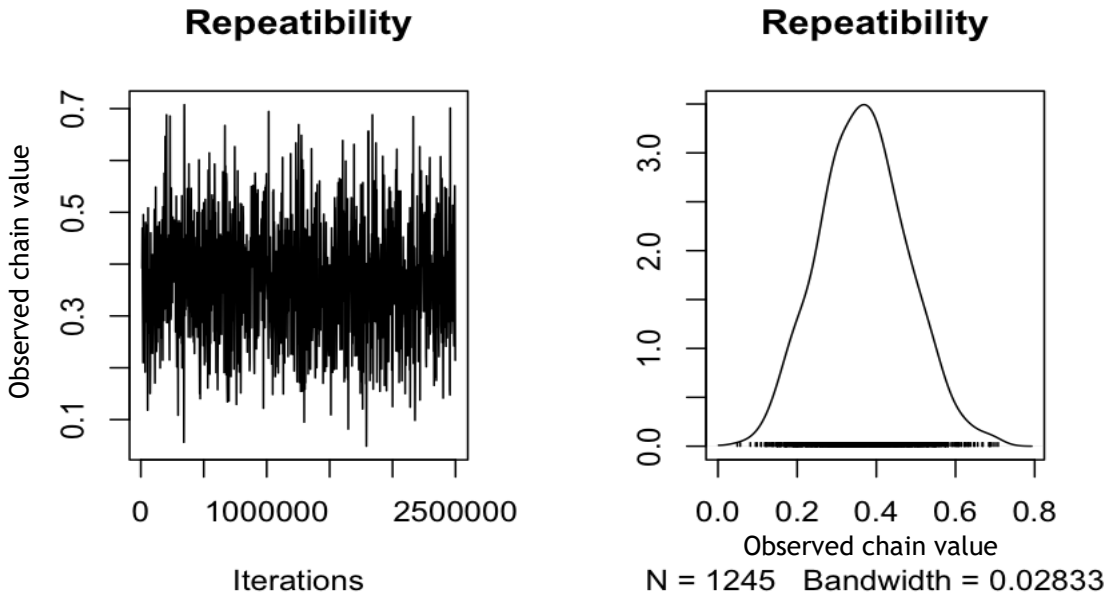


Figure 4-17. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability Model 4d for the association of faecal egg counts with time of sampling with ID, pen and animal as random effects (Model 4d).

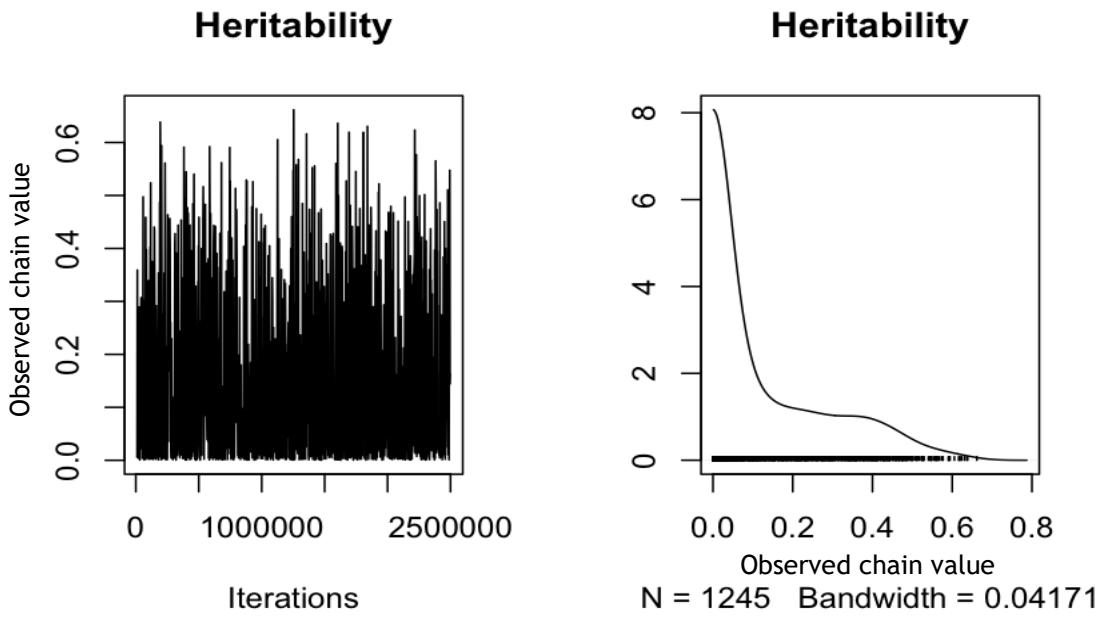


Figure 4-18. Trace plots of parameter value explored by MCMC chain (left) and posterior density plots (right) for FEC repeatability for the association of faecal egg counts with time of sampling with ID, pen and animal as random effects (Model 4d).

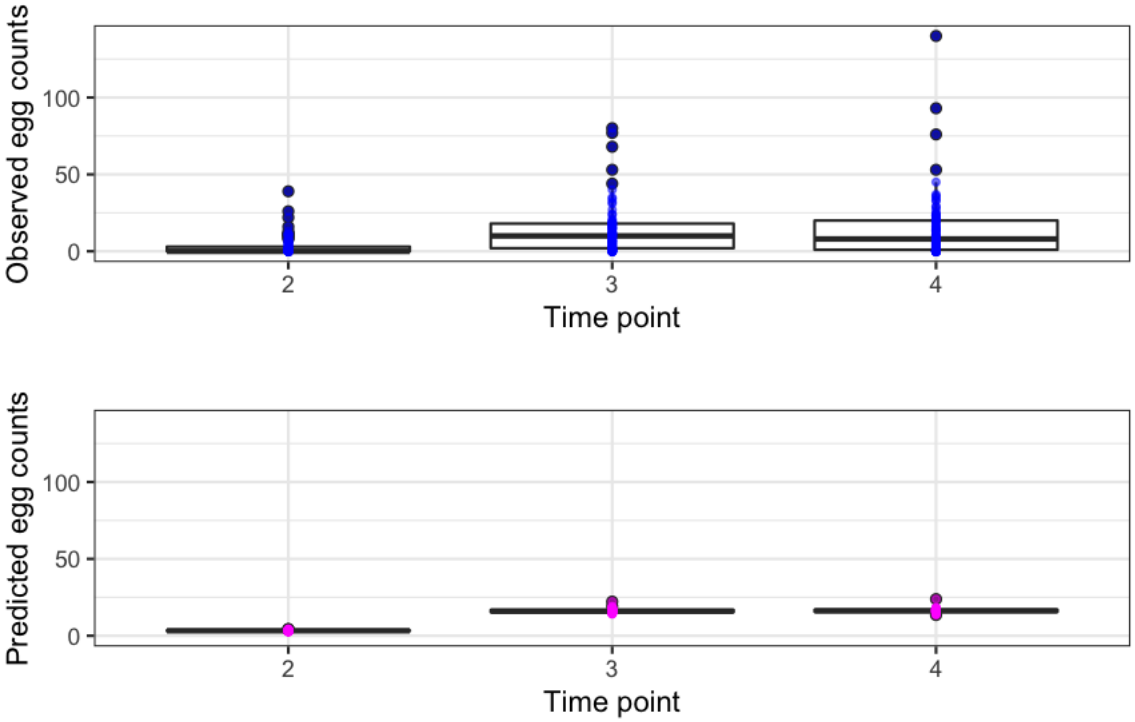


Figure 4-19. Observed (top) and predicted (bottom) FEC data for the association of faecal egg counts with time of sampling with ID, pen and animal as random effects (Model 4d).

4.4 Discussion

This study examined the responses of Boer goats to deliberate infection with a mixture of gastrointestinal nematodes predominantly *H. contortus* with smaller numbers of *T. colubriformis* and *Oesophagostomum spp*. Besides different infection types, this study was also conducted in a different location and climate to the England based study of the previous chapter. One of the key components of this chapter was the repeated measures model analyses to explore the impact of immune mechanisms, specifically IgA activity and peripheral eosinophil counts on FEC, as well as the other explanatory variables such as time and types of birth on FEC. The models were also used to estimate repeatability and heritability of FEC.

Cronbach's alpha was used to assess the reliability of laboratory methods used in this study and does not appear to have been applied to goat parasitological assays before. Acceptable values range from 0.70 to 0.95 (Tavakol and Dennick, 2011). Therefore, finding alpha coefficents above 0.7 indicates that the methods used to determine FEC, IgA activity, peripheral eosinophil counts and PCV were reliable. This offers assurance that observed differences between sheep and goats in response to gastrointestinal nematode infection are not simply a consequence of poor quality assays in goats.

Larval establishment to adult nematodes

The initial plan to infect the goats with a high monospecies dose of *H. contortus* L3 had to be changed due to a lack of L3 stock. A mixture of L3 was then used to infect the goats in a 6:1:1 ratio of *Haemonchus* spp. : *Trichostrongylus* spp. : *Oesophagostomum* spp. Unlike the adult nematode, identification of L3 could only be done at genus level. Based on genera, the percentage of L3 recovered from the faecal culture of deliberately infected Boer goats for *Haemonchus* spp., *Trichostrongylus* spp. and *Oesophagostomum* spp. was 82%, 12% and 6% respectively, or approximately a 14:2:1 ratio (Table 4-2). This ratio was different from the 6:1:1 ratio used to establish the infection. This is likely because the female *Haemonchus* spp. is a much more prolific egg layer with production of up to 5000 eggs per day per nematode (Le Jambre, 1995) than *Trichostrongylus* spp. and *Oesophagostomum* spp.

Mixed infections are not often used for deliberate infections of small ruminants but they do have the advantage of providing a more realistic model of natural infection. In particular, the use of several species of gastrointestinal nematodes makes it less likely that any unusual features of the goat response are specific to a single species of nematode.

All of the 30 goats including the control animals became infected based on the presence of gastrointestinal nematode eggs before day 52 post-infection. Nonetheless, some of the goats were found to have zero epg on day 52 post-infection. The control animals were likely to have been naturally infected by faecal-oral transmission because they were kept in the same pen as deliberately infected goats. Therefore, the control animals could not be used as the intended control group and were excluded from further analyses in this chapter. Only the 25 goats that were deliberately infected were included in the statistical analyses. The control and infected animals should be kept in separate pens to prevent subsequent infection to the control animals in future studies.

Although all of the goats were found to be infected at some point based on FEC prior to slaughter, adult nematodes were not found in some of them at post-mortem examination (Table 4-3). Twenty-three goats were found with *H. contortus* in the abomasum and/or *T. colubriformis* in small intestines of either male, female or both sexes. Seven goats, including four control animals, were not harbouring any nematodes at slaughter. These results suggest that some of the goats had the ability to suppress the development of adult nematodes.

The numbers of adult *H. contortus* were always higher than the numbers of adult *T. colubriformis* in goats infected with both species. These findings were expected because a previous study in Malaysia found *H. contortus* as the most prevalent species (overall prevalence of 78%) followed by *Trichostrongylus* spp. (22%) from the culture of faecal samples collected from small ruminants in five government farms located in Peninsular Malaysia (Khadijah et al., 2006a).

In this study, only 2400 L3 were administered to each goats and yielded the maximum total number of 630 for adult male and female *H. contortus,* giving a maximum establishment of 35% for *H. contortus* from 6:1:1 infection ratio with the L3 of *Trichostrongylus* spp. and *Oesophagostomum* spp. (Table 4-3). This finding is higher than a study of 36 goats of Saanen, Anglo Nubian, Toggenburg and British Alpine breeds that were administered with 40000 *H. contortus* L3 each (Rahman and Collins, 1991), where establishment of *H. contortus* ranged between 3.1% and 20.2% (1259 to 8065 adult *H. contortus*). In the present study, the establishment of L3 to adult nematodes may not be suppressed by the density-dependent regulation of nematode population due to a low infection rate (Roberts, 1995) whereas the establishment of *H. contortus* in Rahman and Collins (1991) may be affected by density-dependent mechanism due to high challenge of L3. Surprisingly, *T. colubriformis* had higher total number of male and female nematodes than *H. contortus* (740) which indicates 247% of establishment from L3 infection ratio of

1:8 (6:1:1 = *Haemonchus* : *Trichostrongylus* : *Oesophagostomum*). This finding suggests that *T. colubriformis* may not be affected by co-infection and host immune responses.

In the present study, no goats were found with *Oesophagostomum* spp. during total worm counts. This finding was expected because previous studies in Malaysia have also found *Oesophagostomum* spp. at low prevalence among naturally infected small ruminants. In 16 private small ruminant farms the prevalence of *Oesophagostomum* spp. was only 4% in comparison to *H. contortus* (56%), *Trichostrongylus* spp. (30%), *Bunostomum* spp. (7%) and *Cooperia* spp. (3%) based on faecal culture (Khadijah et al. 2006b). In eight smallholder goat farms, the prevalence of *Oesophagostomum* spp. was only 1% whereas *H. contortus* and *Trichostrongylus* spp. had prevalences of 73% and 26%, respectively (Basripuzi et al., 2012).

The absence of adult *Oesophagostomum* spp. with the presence of its L3 from faecal culture of deliberately infected goats suggests that this nematode could survive during co-infection. However, *Oesophagostomum* spp. was unable to survive in large numbers as it could not be detected during total worm counts at post-mortem although they were originated from the same family of *Trichostrongylidae* and reside in different niches of the gastrointestinal tract. The prolific nature of *H. contortus* in the abomasum and the presence of *T. colubriformis* in the small intestines probably ceased the development of *Oesophagostomum* spp. in the large intestines.

Phenotypic markers of resistance to gastrointestinal nematodes

The FEC was at zero epg until day 20 post-infection (Figure 4-1, A), consistent with the completion of *H. contortus* prepatent period which is approximately 17 to 21 days (Machen et al., 1998). Several of the IgA observations seemed to be substantially higher than the others after day 40 post-infection as illustrated in Figure 4-1 (B). According to Stear et al. (1999b), resistant sheep produce more IgA as well as parasite-specific IgA molecules than their susceptible counterparts. A significant decline in peripheral eosinophil counts (Figure 4-2, C) suggests recruitment of eosinophils to the sites of infection such as at the abomasal mucosa and small intestine induced by the immune response as a characteristic of gastrointestinal nematode infection (Hendawy, 2018).

A drop in PCV level on day 28 post-infection (Figure 4-1, D) may indicate that most of the L3 had developed into adult nematodes, predominantly the hematophagous *H. contortus* that continuously fed on blood. However, PCV rapidly returned to the previous levels in subsequent days and no overall decline was observed. Low PCV is commonly related to the blood sucking activity of adult nematodes in the abomasa of infected animals (Baker et al., 2003) but progressive anaemia only occurs for heavy nematode burdens (Bowman,

2003). All goats were infected, either deliberately or via faecal-oral transmission from deliberately infected goats to the intended control goats (Table 4-3). There were no patterns observed in Figure 4-1 for FEC, IgA activity, peripheral eosinophil counts and PCV levels among control goats that could distinguish their response to infection from the deliberately infected goats.

Similar to FEC, PCV was initially considered as another response variable because it is a common marker for selective breeding for nematode resistance particularly to infection caused by *H. contortus* (Gauly and Erhardt, 2001). However apart from the temporary drop in PCV at day 28 post-infection, there was no indication that *H. contortus* was able to cause anaemia in the infected goats based on the consistent median PCV values of 27% at each time point (Figure 4-2, D). This value falls within the reference range of PCV in goats of 22% to 38% (Kahn et al., 2005). For these reasons, PCV was not modelled as a response variable in this study.

Using correlations between sampling time points, the repeatability of the response to infection was determined for FEC, IgA activity, peripheral eosinophil counts and PCV (Figure 4-3). In the present study, the repeatability decreased as the interval between sampling increased which is similar to Scottish Blackface sheep infected with *T. circumcincta* (Stear et al., 1995b). The repeatability significantly declined over time for IgA activity ($p<0.001$) but not significantly declined for FEC ($p=0.705$) and PCV ($p=0.070$). The repeatability for IgA was initially high at 0.75 and only decreased to 0.25 over 52 days post-infection (Figure 4-3, B), which could be considered as a long interval between sampling. This finding suggests that some goats were mounting consistently higher IgA responses than the others throughout the study. The repeatability of peripheral eosinophil counts initially declined and then plateau at larger intervals between sampling time (Figure 4-3, C). The repeatability of FEC was not significant because at each time of sampling the FEC varied at low level. This could be due to the low challenge of L3 and possible variation of L3 doses given to each goats.

Goetsch et al. (2018) finds that PCV in deliberately infected Boer goats with 10000 *H. contortus* L3 was 24.9% ± 0.42 while Coutinho et al. (2015) finds that PCV in resistant group of crossbreed goats originating from ½ Saanen and ½ Anglo-Nubian breeds naturally infected with *H. contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. presented a higher average of PCV (26.48% ± 1.25) than their susceptible counterparts (24.04% ± 1.56). These findings suggest that Boer goats in this study with mean PCV of 26.67% ± 0.21 similar to that in the resistant breeds above may be relatively resistant to gastrointestinal nematode infection.

Repeated measures models – using the animal model

Repeated measures models were run to examine the independent effects of IgA and eosinophils (Model set (a) and (b)) and their effect when combined into an immune index (Model set (c) and (d)). In the first two models, there was a significant effect of time on zero-inflation and FEC. In addition, although there was no main effect of eosinophils, there was a significant interaction between eosinophils and time which can be interpreted as a reduction in FEC associated with eosinophilia at time point 4. This finding may be because the peripheral eosinophil response occurred at a later stage of infection as the eosinophils were recruited to the local site of infection (eg. abomasum and small intestines) during the early stage of infection. In the immune index models (Model set (c) and (d)), time point was the only fixed effect that significantly affected FEC.

In all four models, there was a significant zero-inflation with the probability of a zero count dependent on the time point. The probability of a zero count was around 0.3 overall in all 4 models and around 0.6, 0.1 and 0.2 for time points 2, 3 and 4 respectively. Following description by Yang et al. (2017), the true zeroes observed in this study were presumably originating from faecal samples that were free from nematode eggs while the remaining zeroes are false zeroes from the faecal samples that contained nematode eggs, which were not counted microscopically during the McMaster technique. In practice, a nematode egg will not be counted if it is located outside of the McMaster grids (Ministry of Agriculture, Fisheries and Food of Great Britain, 1986).

Model set (b) and (d) were the same as Model sets (a) and (c) except for the addition of 'animal' as a random effect which allows the inclusion of pedigree data. The other random effects were ID and pen. The posterior means for the random effect of pen were consistently small. The posterior means for random effect of ID (capturing individual to individual variability) ranged from 0.29 to 0.53 across models, taking smaller values in models that also included the animal random effect. The posterior means for the animal random effect was around 0.2 in Models 5b and 4d. However, the posterior distribution for this random effect was heavily skewed towards 0, resulting in different values for the heritability depending on whether the mean or mode are estimated (see below: Repeatability of FEC). The posterior mode of FEC repeatability was located around zero but the posterior mean of FEC repeatability was larger and located towards the right tail of the posterior distribution. In all models, there were large residual variances (between 0.75 and 0.77) which indicate that FEC was overdispersed. The skewed distribution of heritability means that the heritability was likely to be small. However, there was insufficient data to exclude the possibility that the heritability was large. Therefore, follow-up studies with a larger sample size would be needed. In all models, there were

large residual variances (between 0.75 and 0.77) which indicate that FEC was overdispersed.

Repeatability of FEC

The values of FEC repeatability in Boer goats in this study did not resemble the values of FEC repeatability in Scottish Blackface sheep. The regression analysis on the correlations between time points showed that FEC repeatability in Boer goats was approximately 0.2 at any interval between sampling (Figure 4-3, A) but the mean repeatability of FEC in Scottish Blackface sheep was 0.48 at seven day intervals (Stear et al., 1995b).

The repeated measures model would be expected to produce a similar estimate. The estimates of repeatability in the repeated measures models are derived using the variance of the count component only but the repeatability estimates in the regression analysis are derived from both count and binary components (irrespective of whether zeroes might be true zeroes or not). The estimates are higher when derived by the repeated measures analysis, presumably because this accounts for some of the variance due to the fixed effects, giving a better estimate of repeatability. Both approaches provide a mean repeatability estimate that is lower than that seen in Scottish Blackface sheep (r=0.48). The similarity between different measurements, or also known as repeatability will provide the upper limit for heritability or genetic variation (Falconer and MacKay, 1996; Wilson et al., 2010). Therefore as FEC repeatability was in the range 0.33 to 0.41, the FEC heritability was expected to fall below this value.

Heritability of FEC

The posterior mode estimates for the heritability were low (< 0.005) across all models. However, the posterior distribution for the heritability was heavily skewed towards zero (as was the animal random effect), such that the upper HPD (higher posterior density) limit was approximately 0.5 and the mean estimate was around 0.15. This suggests that heritability is likely to be very small but there is insufficient evidence to exclude the possibility that the heritability is larger.

Previous studies have shown that the FEC heritability of other breeds of goats is lower than in sheep. In natural *H. contortus* infection, FEC heritability of Barbari goats ranged between 0.05 ± 0.05 and 0.13 ± 0.04 (Mandal and Sharma, 2008) while in Jamunapari goats it ranged between 0.11 and 0.16 ± 0.06 (Mandal et al., 2012). The FEC heritability of Creole goats in natural mixed infection of *H. contortus* and *T. colubriformis* ranged between 0.14 ± 0.05 and 0.33 ± 0.06 (Mandonnet et al., 2001). In Saanen does, the FEC heritability ranged from 0.06 ± 0.04 and 0.15 ± 0.12 from early to late lactation (Morris et al., 1997). The FEC heritability in sheep ranged approximately between 0.2 and 0.4

(Watson et al., 1995; Morris et al., 1998; Bishop and Stear, 2001; Goldberg et al., 2012). The findings in the present study do not provide evidence for genetic variation in the FEC in Boer goats – or in the other words, the FEC among deliberately infected Boer goats was not heritable. However, low challenge of infecting larvae potentially contributed to low FEC heritability estimate in this study.

Conclusion

As a conclusion, the aims for this chapter have been achieved. FEC variation among deliberately infected Boer goats was affected by time and its interaction with eosinophils. The repeatability of FEC, IgA activity, peripheral eosinophil counts and PCV decreased as the interval between sampling increased but the decrease was only significant for IgA and eosinophilia responses. The FEC was not heritable among Boer goats in this study but the repeatability estimates of FEC in response to infection suggests that FEC may be influenced by non-genetic effects (eg. nutrition, supplementary diet and age). There could be a possibility that the variation in FEC was also due to the variation in the dosage of L3 that was deliberately infected to the goats. This could be countered by using a higher challenge dose. In addition, the unaffected PCV levels suggest that this breed may be more resilient to the blood feeder *H. contortus* infection.

Chapter 5: *Haemonchus contortus* and *Trichostrongylus colubriformis* interactions during co-infection in Boer goats.

5.1 Background

H. contortus and *T. colubriformis* are reported as the main gastrointestinal nematodes of sheep and goats in Southeast Asia (Sani and Gray, 2004) including in Malaysia (Khadijah et al., 2006a; 2006b). It is undeniable that *H. contortus* is the most pathogenic nematode of small ruminants particularly in tropical and subtropical countries due to its high biotic potential and blood sucking ability (Waller and Chandrawathani, 2005), and thus it has been a focus of scientific research. Anaemia, lack of appetite, lethargy, loss of weight, dehydration, oedema and subsequent death in heavy infections are the common signs of haemonchosis (Getachew et al, 2007, Besier et al., 2016a; Emery et al., 2016). In contrast, little attention has been paid to *T. colubriformis* (Cardia et al., 2011), which shows milder signs such as inappetence, weight loss, poor body condition, emaciation, diarrhoea and hypoproteinaemia that impair productivity in ruminants (Holmes et al., 1985).

Infection by mixed gastrointestinal nematodes is common in natural infections among small ruminants and is known to affect productivity in goats. Milk production in Saanen goats that have been treated for natural infection with *H. contortus* and *T. colubriformis* is significantly higher (399.5 L ± 34.0 L; $p<0.05$) than the untreated groups (281.6 L ± 37.5 L) (Suarez et al., 2017). In repeated deliberate infection with *H. contortus* and *T. colubriformis* on naïve dairy goats, the high milk producers suffer from severe pathophysiological disturbances that lead to low milk yield (Chartier and Hoste, 1997). However, most of the research on natural infection has been conducted in sheep (Doligiska et al., 1999; Bishop and Stear, 2000, Mugambi et al., 2005) rather than goats, and no studies have investigated the potential interactions in goats.

The interaction between *H. contortus* and *T. colubriformis* in deliberately co-infected sheep has been explored and it has been shown that co-infection affects both nematodes species as well as cellular immune response of the host (Lello et al., 2018). In this study, the immune response induced by *T. colubriformis* had negative impacts on the number of *H. contortus*. In contrast, immune suppression caused by *H. contortus* had a positive impact on the number of *T. colubriformis*. To my knowledge, there is no similar study in goats and this chapter will investigate potential interactions between *H. contortus* and *T. colubriformis* in Boer goats.

Assessing potential interactions between the nematode species requires information on a number of characteristics in addition to nematode number, in particular nematode index and nematode length. Nematode length is a marker of its fecundity but both characteristics (length and fecundity) are influenced by the number of nematodes within the host as well as by the strength and specificity of IgA response (Stear et al. 1999b). Reduction in the length and fecundity of adult nematodes is one of the signs of immunity in sheep which is strongly associated with the local IgA and eosinophil response (Stear et al., 1995; Henderson and Stear, 2006). Therefore, the effects of IgA and eosinophils responses were included in the analyses exploring the effect on and of *H. contortus* and *T. colubriformis* index, number and length.

The effects of gastrointestinal nematode infection are often reflected in their FEC and PCV. Therefore, both FEC and PCV were fitted as response variables to explore the effects of *H. contortus* and *T. colubriformis* index, number and length of variables as well as the effects of IgA and eosinophil responses. The objectives of this chapter as follows:

1. To explore the association of *H. contortus* and *T. colubriformis* index, length and numbers; and the effect of IgA and peripheral eosinophil counts on these variables during co-infection.

2. To identify significant explanatory variables for FEC and PCV during co-infection with *H. contortus* and *T. colubriformis*.

5.2 Materials and methods

5.2.1 Animals

Thirty goats were selected randomly for deliberate infection with mixed L3 nematodes namely *H. contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. in a 6:1:1 ratio as described in Section 4.2.1. Faecal and blood samples were collected twice a week for eight weeks at three-four-three day intervals. The faecal samples were subjected to the modified McMaster technique which was sensitive to 25 epg of faeces to determine FEC. The blood samples were used to measure IgA (as described in Section 4.2.7), PCV and peripheral eosinophil counts (as described in Section 2.5 and 2.6). The goats were slaughtered on the farm after the 16th sampling (day 52 post-infection) to collect the gastrointestinal tracts for total worm counts, identification, sexing and nematode measurement. All samples were transported to the Faculty of Veterinary Medicine, Universiti Putra Malaysia on the same day of sampling for laboratory analysis.

5.2.2 Modified McMaster technique

The Modified McMaster technique was conducted as described in Section 2.1.

5.2.3 ELISA IgA

The ELISA IgA assay was conducted as described in Section 4.2.7.

5.2.4 Peripheral eosinophil counts

Peripheral eosinophil counts were obtained as described in Section 2.5.

5.2.5 Packed cell volume

Packed cell volume was estimated as described in Section 2.6.

5.2.6 Total worm counts, identification, sexing and measurement of nematode length

Total worm counts, identification, sexing and measurement of nematodes were conducted as described in Section 2.4. Briefly, these procedures were based on protocols in the Manual of Veterinary Parasitological Laboratory Techniques provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986). The abomasum from a slaughtered goats was ligated and opened along the greater curvature. The abomasal contents and

washing were collected into a graduated bucket and made up to 1 L with tap water. After mixing, 100 ml were transferred into a glass jar with a sieve-like lid. The jar was inverted and vigorously shaken until most of the fluid contents were removed. This process was repeated until the contents appeared clear with a final volume of approximately 100 ml. The final contents of the jar were then transferred into a 120 ml specimen bottle. The nematodes were preserved by adding 5 ml of 75 % ethanol into the specimen bottle. The nematodes were identified, counted, sexed and measured under a stereo microscope at x15 magnification. Based on the dilution factor of abomasal contents, each nematode counted represented 10 nematodes (Lyndall-Murphy, 1993). The same process was used to count and identify nematodes in the small and large intestines. The only difference was that the ligation was made to separate the small intestine from duodenum and large intestine.

There were only two nematode species that were recovered during total worm count procedure namely *H. contortus* and *T. colubriformis;* thus *Oesophagostomum* spp. was not modelled in this chapter. The absence or inability of *Oesophagostomum* spp. to develop into adult nematodes during co-infection with two other species was discussed in Section 4.4.

5.2.6.1 Nematode index

The nematode index were calculated separately for each nematode species, by first calculating the female and male nematode index and then combining to produce the total nematode index as below:

$$Female\ nematode\ index = (number\ x\ length)\ of\ female\ nematodes$$

$$Male\ nematode\ index = (number\ x\ length)\ of\ male\ nematodes$$

$$Total\ nematode\ index = Female\ nematode\ index + Male\ nematode\ index$$

5.2.7 Statistical analyses

All analyses in this chapter were conducted in R version 3.5.2.

5.2.7.1 Correlation analyses between variables

Correlations between the means of the observations at necropsy (number, length and index of nematodes) and the means of the phenotypic variables measured over the course of the infection (FEC, IgA, peripheral eosinophil counts and PCV) were generated using Pearson correlation following transformation on non-normally distributed variables and presented in a correlogram using the corrplot() function.

5.2.7.2 Multiple linear regression models

Multiple linear regression models conducted using the function lm() used to examine the relationship between the observations at necropsy which were the nematode number, mean nematode length and mean nematode index as well as the means of FEC, IgA activity, peripheral eosinophil count and PCV averaged over the 16 times of sampling.

Three sets of models were fitted for *H. contortus* variables in which *H. contortus* index (set A), *H. contortus* length (set B) and *H. contortus* number (set C) were fitted as the response variables. The explanatory variables for these models were *T. colubriformis* number, length and their interaction; *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction. *H. contortus* index, *H. contortus* number and *T. colubriformis* number were transformed by taking logarithm to the base 10 of the data to normalize the residuals of the models whereas *T. colubriformis* index was transformed by taking logarithm to the base 10 + 1.

Three sets of models were also fitted for *T. colubriformis* variables in which *T. colubriformis* index (set D), *T. colubriformis* length (set E) and *T. colubriformis* number (set F) were fitted as the response variables. The explanatory variables for these models were *H. contortus* number, length and their interaction; *H. contortus* index; IgA, peripheral eosinophil counts and their interaction. *T. colubriformis* index was transformed by taking logarithm to the base 10 + 1 while *T. colubriformis* number, *H. contortus* index and *H. contortus* number were transformed by taking logarithm to the base 10 of the data to normalize the residuals of the models.

Each of FEC and PCV data was fitted as a response variable in two separate set of models (set G and set H respectively). The explanatory variables for FEC were the number and length of female *H. contortus* numbers; the number and length of female *T.*

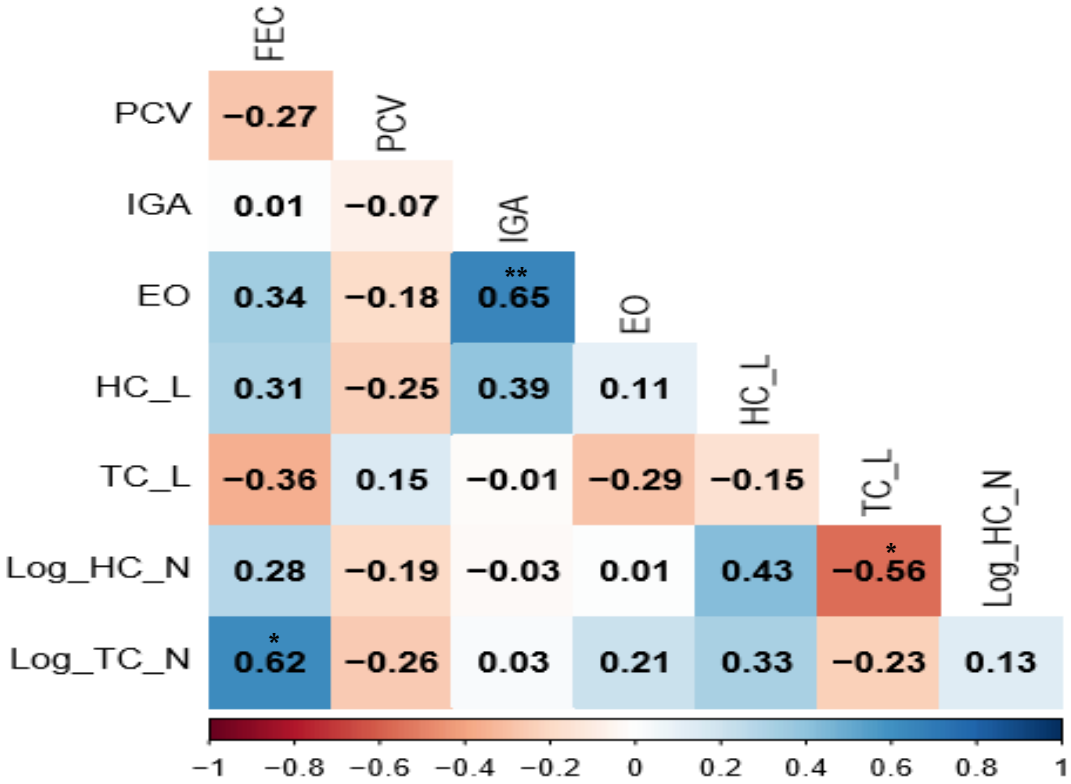
colubriformis; IgA, peripheral eosinophil counts and their interaction. The explanatory variables for PCV include *H. contortus* number, length and their interaction; *T. colubriformis* number, length and their interaction; *H. contortus* index, *T. colubriformis* index and their interaction; IgA, peripheral eosinophil counts and their interaction. No transformations were applied for either the FEC or PCV models.

The variables in the final models were standardized by using the scale() function. Residuals were checked for normality based on visual inspection of the Q-Q plots and by testing the residuals using the Shapiro-Wilk test. The best model was selected on the basis of AICc, a corrected version of AIC that is suitable for small sample sizes. Model selection was conducted using the dredge() function which explores all models that are sub-models of the full model to identify the one with the lowest AICc. The model that best fit the data were selected based on AICc value in this chapter because it was not affected by random effects as in the previous chapters.

5.3 Results

Table 5-1 shows the correlations among the observations at necropsy (*H. contortus* and *T. colubriformis* number, length and index) and the means of phenotypic variables measured over the course of infection (FEC, IgA, peripheral eosinophil counts and PCV). FEC was positively associated with Log *T. colubriformis* number (r=0.62, p<0.05) while IgA had a positive correlation with peripheral eosinophil counts (r=0.65, p<0.05). The *T. colubriformis* length also had a negative correlation with Log *H. contortus* number (r=-0.56, p<0.05). In addition, Log *T. colubriformis* index had positive correlations with *H. contortus* length (r=0.69, p<0.01) and Log *H. contortus* index (r=0.52, p<0.05).

Table 5-1. Pearson correlations among the means of phenotypic variables and nematode variables in deliberately infected Boer goats.



Abbreviations and symbols in Table 5-1	Definition
FEC	Faecal egg counts
PCV	Packed cell volume
IGA	Immunoglobulin A
EO	Peripheral eosinophil counts
HC_L	*H. contortus* length
TC_L	*T. colubriformis* length
Log_HC_N	Log of *H. contortus* numbers
Log_TC_N	Log of *T. colubriformis* numbers
*	p < 0.05
**	p < 0.01
***	p < 0.001

Eight multiple regression linear models will be presented in the following section.

- Model sets A, B and C examine the effect on *H. contortus* index (set A), length (set B) and number (set C) of *T. colubriformis* number, length and their interaction; *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction.

- Model sets D, E and F examine the effect on *T. colubriformus* index (set A), length (set B) and number (set C) of *H. contortus* number, length and their interaction; *H. contortus* index; IgA, peripheral eosinophil counts and their interaction.

- Model set G examines the effect on average FEC of female *H. contortus* number and length; female *T. colubriformis* number and length; IgA, peripheral eosinophil counts and their interaction.

- Model set H examines the effect on average PCV of *H. contortus* number and length; *T. colubriformis* number and length; *H. contortus* index and *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction.

A) Final model of *H. contortus* index with *T. colubriformis* number, length and their interaction; *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.

Figure 5-1 shows the comparison between the full model (AICc = 81.34) and final model (AICc = 44.74) to predict *H. contortus* index from *T. colubriformis* variables and their interactions; as well as from IgA, peripheral eosinophil counts and their interaction. The length of *T. colubriformis* has significant negative relationship with Log *H. contortus* index which is the combination of length and number of male and female *H. contortus* ($F_{1, 13} = 5.106$, $p = 0.042$) as shown in Table 5-2. The final model had an adjusted R^2 of 0.23 indicating that the model explains 23% of the variation in Log *H. contortus* index.

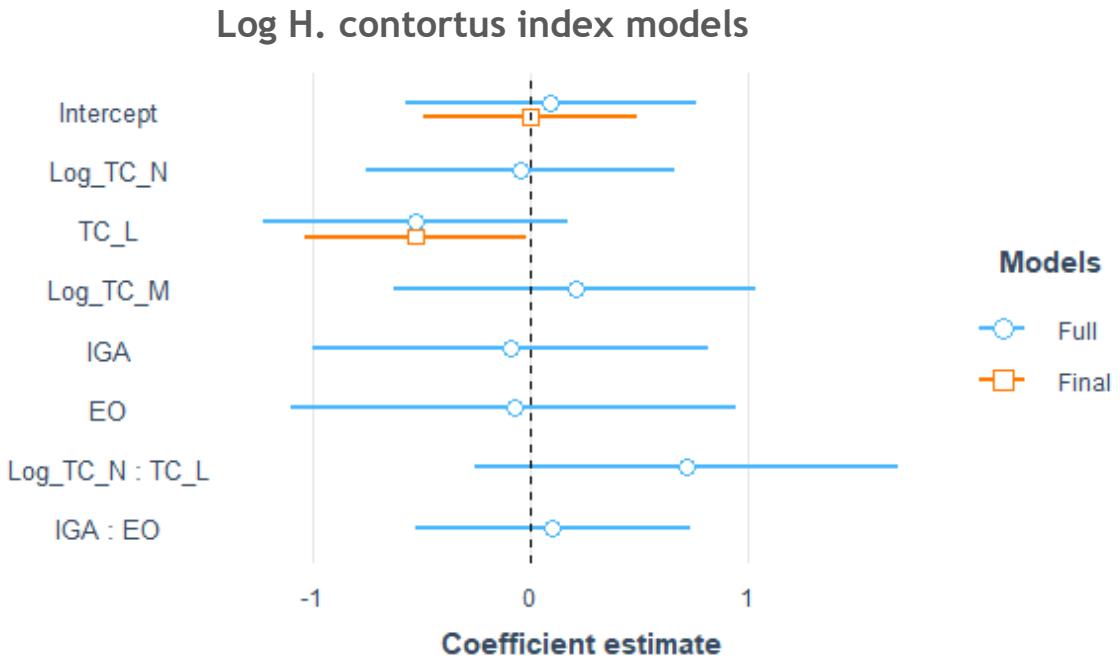


Figure 5-1. Full and final models of *H. contortus* index with *T. colubriformis* numbers, length and their interaction; *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.
Log_TC_N = Log of *T. colubriformis* number; TC_L = *T. colubriformis* length; Log_TC_M = log of *T. colubriformis* index +1; IgA = IgA optical density; EO = peripheral eosinophil counts.

Table 5-2. Final model to predict Log *H. contortus* index from *T. colubriformis* length.

Explanatory variables	Estimate	95% CI	p-value
Intercept	-3.135e-16	-0.490, 0.490	1.000
T. colubriformis length	-0.531	-1.039, -0.023	0.042 *

CI = confidence interval; * = $p < 0.05$.

B) Final model of *H. contortus* length with *T. colubriformis* number, length and their interaction; *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.

Comparison between the full model (AICc = 71.43) and final model with the lowest AICc (33.23) to predict *H. contortus* length from *T. colubriformis* variables and their interactions; as well as from IgA, peripheral eosinophil counts and their interaction is shown in Figure 5-2. Table 5-3 shows that *H. contortus* length has positive relationship withLog *T. colubriformis* index and IgA ($F_{2, 12}$ = 17.22, p <0.001). The final model had an adjusted R^2 of 0.70 indicating that the model explained 70% of the variation in *H. contortus* length. This model indicates that IgA does not reducing the length of *H. contortus* as expected.

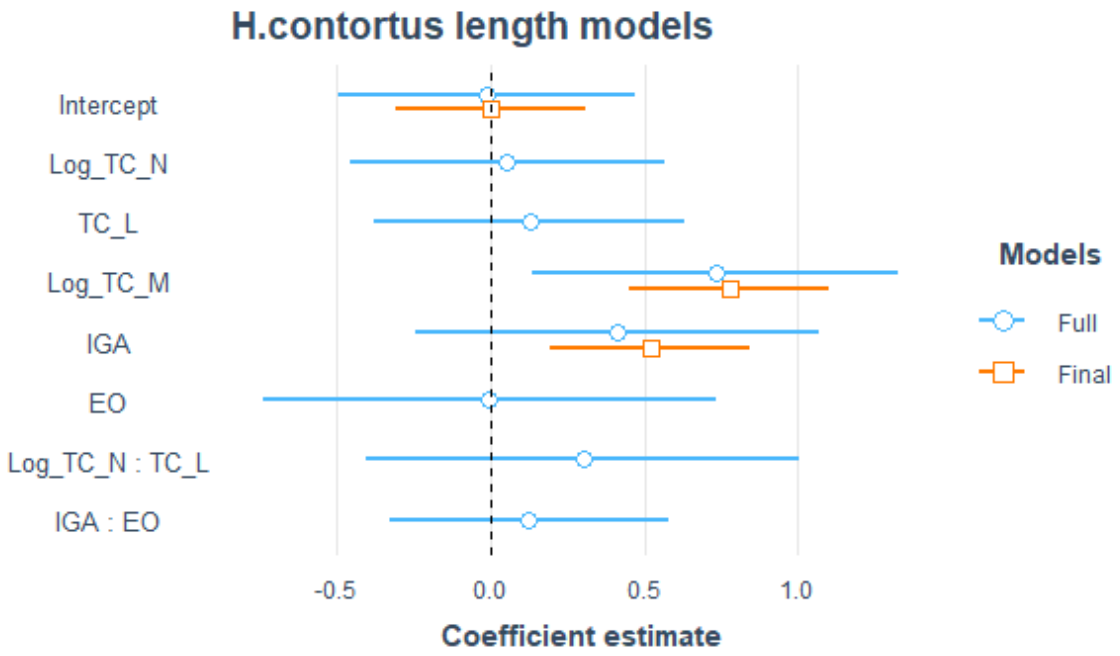


Figure 5-2. Full and final models of *H. contortus* length with *T. colubriformis* numbers, length and their interaction; *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.
Log_TC_N = Log *T. colubriformis* number; TC_L = *T. colubriformis* length; Log_TC_M = Log of *T. colubriformis* index +1; IgA = IgA optical density; EO = peripheral eosinophil counts.

Table 5-3. Final model to predict *H. contortus* length from Log *T. colubriformis* index and IgA.

Explanatory variables	Estimate	95% CI	p-value
Intercept	-9.938e-17	-0.309, 0.309	1.000
Log of *T. colubriformis* index	0.777	0.453, 1.102	< 0.001 ***
IgA	0.521	0.196, 0.845	< 0.01 **

CI = confidence interval; ** = p < 0.01; *** = p < 0.001.

C) Final model of *H. contortus* numbers with *T. colubriformis* number, length and their interaction; *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.

The full (AICc = 83.31) and final (AICc = 44.15) models to predict *H. contortus* number from *T. colubriformis* variables and their interactions; as well as from IgA, peripheral eosinophil counts and their interaction are compared in Figure 5-3. In the final model (Table 5-4), only the length of *T. colubriformis* has significant relationship with Log *H. contortus* number ($F_{1, 13}$ =5.833; p = 0.031). The adjusted R^2 for the final model was 0.26 indicating that 26% of the variation in Log *H. contortus* number was explained by the model.

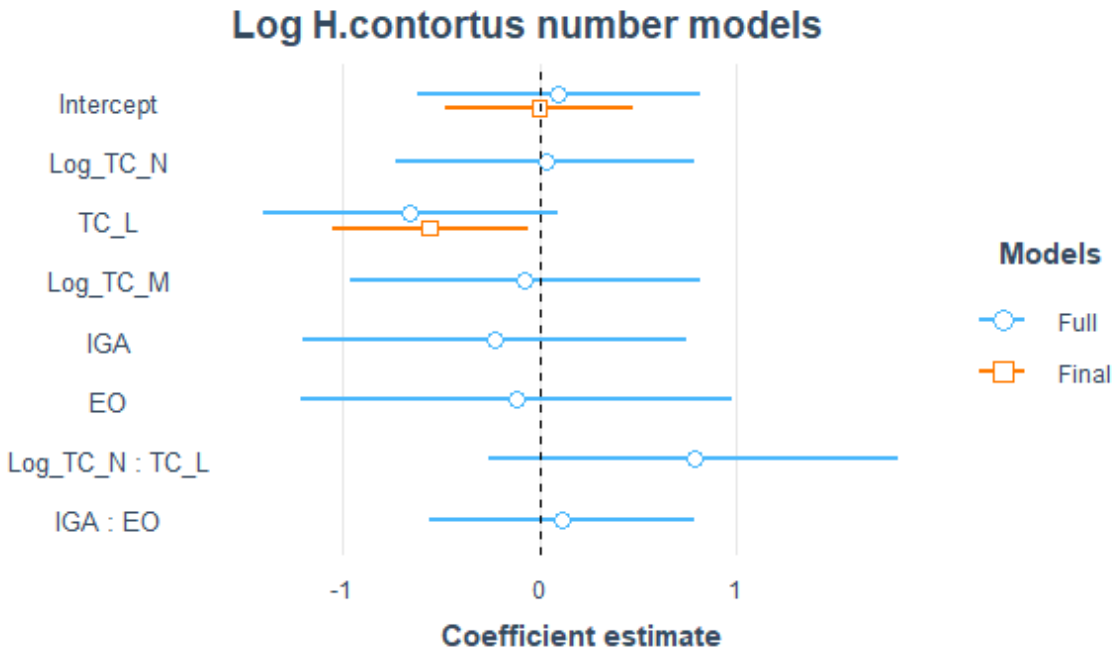


Figure 5-3. Full and final models of *H. contortus* number with *T. colubriformis* numbers, length and their interaction; *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.
Log_TC_N = Log of *T. colubriformis* number; TC_L = *T. colubriformis* length; Log_TC_M = Log of *T. colubriformis* index +1; IgA = IgA optical density; EO = peripheral eosinophil counts.

Table 5-4. Final model to predict Log *H. contortus* number from *T. colubriformis* length.

Explanatory variables	Estimate	95% CI	p-value
Intercept	3.472e-16	-0.481, 0.481	1.000
T. colubriformis length	-0.557	-1.054, -0.059	0.031 *

CI = confidence interval; * = p<0.05.

D) Final model of *T. colubriformis* index with *H. contortus* number, length and their interaction; *H. contortus* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.

Figure 5-4 shows the comparison between the full and final models of Log (*T. colubriformis* index + 1) with AICs of 71.91 and 33.32 respectively. The models were analysed to predict Log (*T. colubriformis* index + 1) from Log *H. contortus* number, length and their interaction; Log *H. contortus* index; IgA, peripheral eosinophil counts and their interaction. The (*T. colubriformis* index + 1) was affected positively by *H. contortus* length and negatively by IgA activity ($F_{2,12}$ = 14.20, p = 0.001) as shown in Table 5-5. The final model had an adjusted R^2 of 0.65 indicating that 65% of the variable Log (*T. colubriformis* index + 1) was explained by the model.

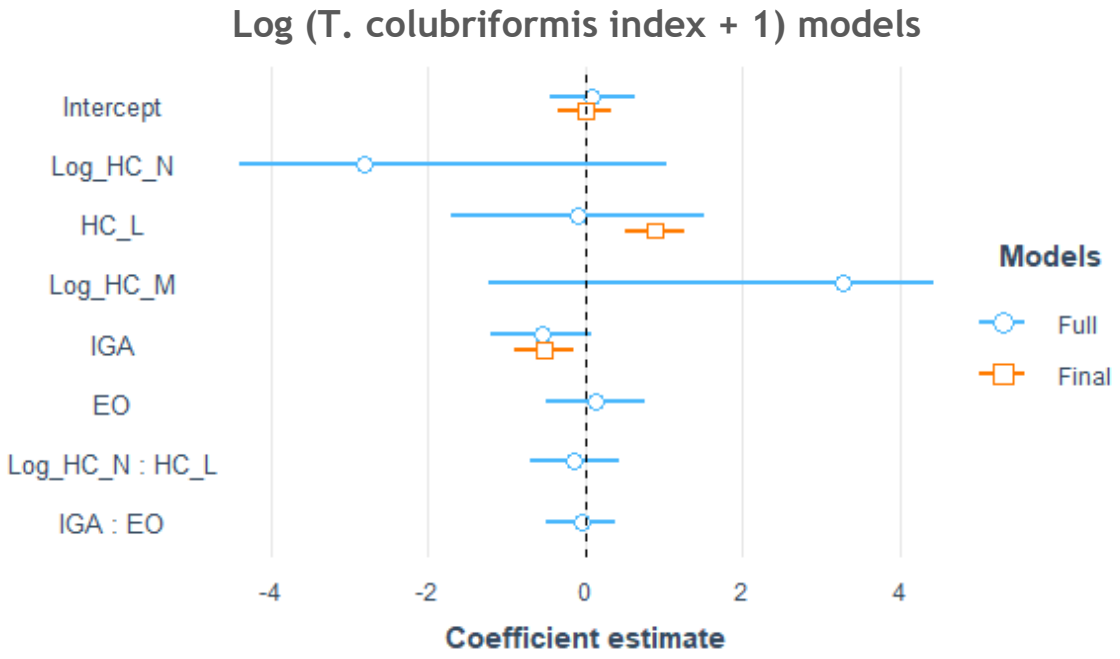


Figure 5-4. Full and final models of *T. colubriformis* index with *H. contortus* number, length and their interaction; *H. contortus* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.
Log_HC_N = Log of *H. contortus* number; HC_L = *H. contortus* length; Log_HC_M = Log of *H. contortus* index; IgA = IgA optical density; EO = peripheral eosinophil counts.

Table 5-5. Final model to predict Log (*T. colubriformis* index + 1) from *H. contortus* length and IgA.

Explanatory variables	Estimate	95% CI	p-value
Intercept	8.037e-17	-0.331, 0.331	1.000
H. contortus length	0.894	0.521, 1.266	< 0.001 ***
IgA	-0.516	-0.888, -0.143	0.011 *

CI = confidence interval; * = p < 0.05; *** = p < 0.001.

E) Final model of *T. colubriformis* length with *H. contortus* number, length and their interaction; *H. contortus* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.

Comparison between the full model (AICc = 80.52) and final model with the lowest AICc (44.15) to predict *T. colubriformis* length from *H. contortus* variables and their interactions; as well as from IgA, peripheral eosinophil counts and their interaction is shown in Figure 5-5. Table 5-6 shows that Log *H. contortus* number significantly affect the length of *T. colubriformis* ($F_{1, 13} = 5.833$, $p = 0.031$). The adjusted R^2 for the final model was 0.26 indicating that 26% of the variation in *T. colubriformis* length was explained by the model.

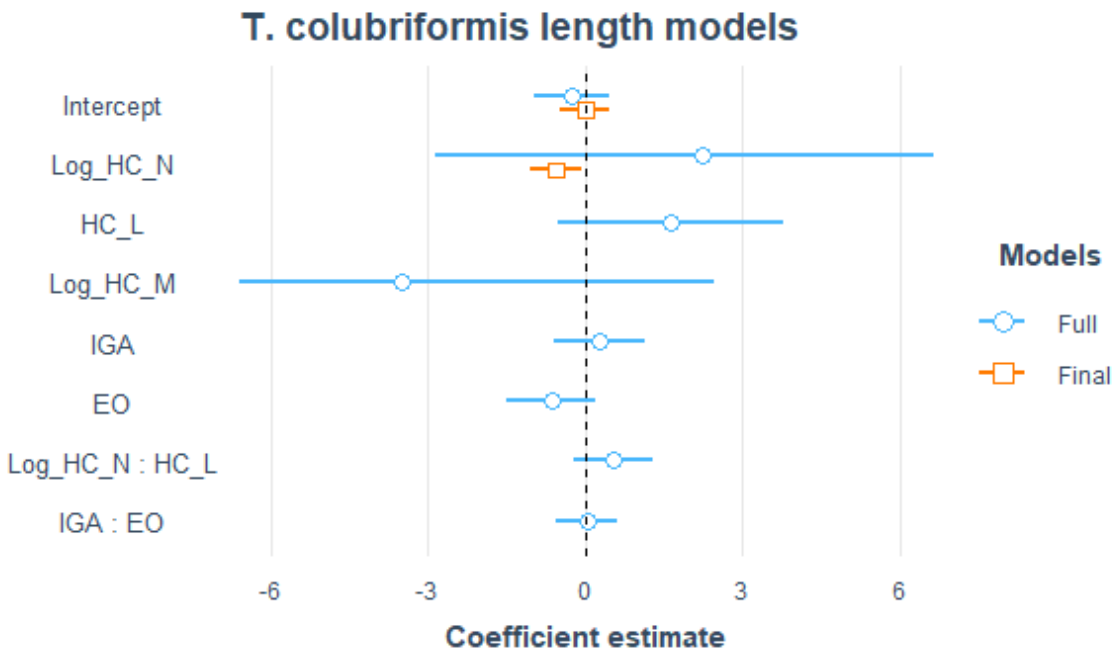


Figure 5-5. Full and final models of *T. colubriformis* length with *H. contortus* number, length and their interaction; *H. contortus* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.
Log_HC_N = Log of *H. contortus* number; HC_L = *H. contortus* length; Log_HC_M = Log of *H. contortus* index; IgA = IgA optical density; EO = peripheral eosinophil counts.

Table 5-6. Final model to predict *T. colubriformis* length from Log *H. contortus* number.

Explanatory variables	Estimate	95% CI	p-value
Intercept	2.328e-16	-0.481, 0.481	1.000
Log of *H. contortus* number	-0.557	-1.054, -0.059	0.031 *

CI = confidence interval; * = $p < 0.05$.

F) Final model of *T. colubriformis* number with *H. contortus* number, length and their interaction; *H. contortus* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.

The full (AICc = 84.46) and final (AICc =46.53) models to predict Log *T. colubriformis* number from *H. contortus* variables and their interactions; as well as from IgA, peripheral eosinophil counts and their interaction are compared in Figure 5-6. In the final model, none of the variables significantly affect the number of *T. colubriformis* as shown in Table 5-7.

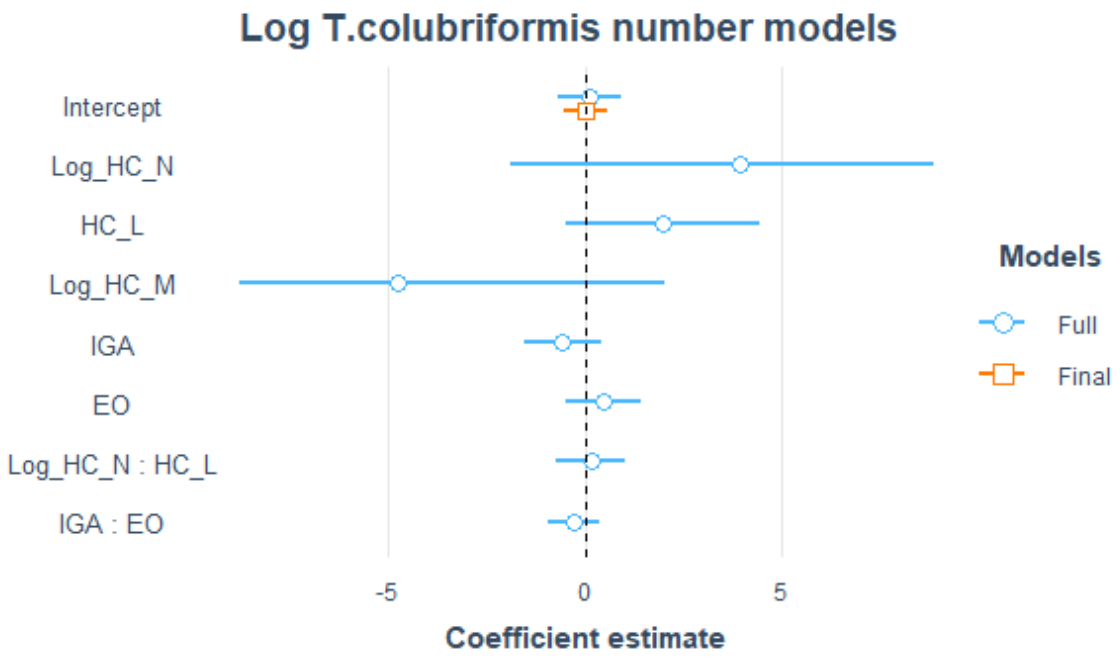


Figure 5-6. Full and final models of *T. colubriformis* number with *H. contortus* number, length and their interaction; *H. contortus* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.
Log_HC_N = Log of *H. contortus* number; HC_L = *H. contortus* length; Log_HC_M = Log of *H. contortus* index; IgA = IgA optical density; EO = peripheral eosinophil counts.

Table 5-7. Final model to predict Log *T. colubriformis* number.

Explanatory variables	Estimate	95% CI	p-value
Intercept	7.166e-17	-0.554, 0.554	1.000

CI = confidence interval.

G) Final model of mean FEC with number and length of female *H. contortus*; number and length of *T. colubriformis*, IgA and peripheral eosinophil counts as explanatory variables.

The comparison between full (AICc = 80.44) and final (AICc = 34.43) models of mean FEC is shown in Figure 5-7. In the final model (Table 5-8), only the number of female *T. colubriformis* significantly affects FEC but the other variables are not significant ($F_{1, 10}$ = 8.806, p = 0.014). The final model had an adjusted R^2 of 0.42 indicating that 42% of the variation in FEC was explained by the model.

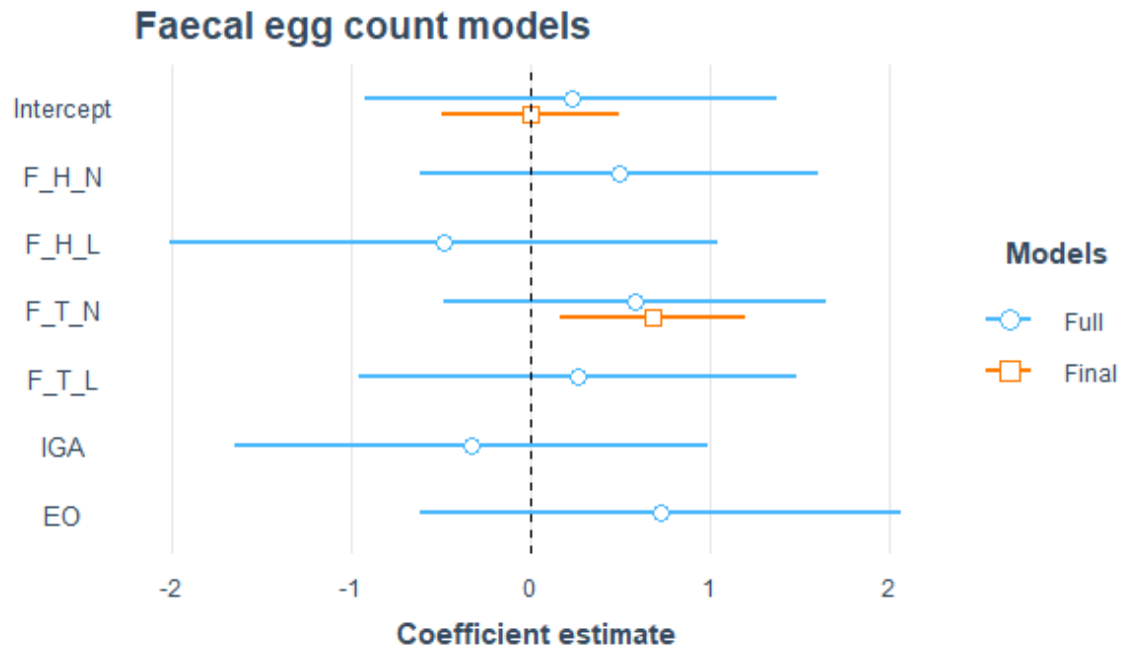


Figure 5-7. Full and final models of FEC with female *H. contortus* number and length; female *T. colubriformis* number and length; IgA, peripheral eosinophil counts and their interaction as explanatory variables.
F_H_N = female *H. contortus* number; F_H_L = female *H. contortus* length; F_T_N = female *T. colubriformis* number; F_T_L = female *T. colubriformis* length; IgA = IgA optical density; EO = peripheral eosinophil counts.

Table 5-8. Final model to predict mean FEC from female *H. contortus* number.

Explanatory variables	Estimate	95% CI	p-value
Intercept	7.506e-17	-0.492, 0.492	1.000
Female *T. colubriformis* number	0.684	0.170, 1.198	0.014 *

CI = confidence interval; * = p < 0.05.

H) Final model of mean PCV with *H. contortus* number and length; *T. colubriformis* number and length; *H. contortus* index and *T. colubriformis* index; IgA, peripheral eosinophil counts and their interaction as explanatory variables.

Figure 5-8 shows the comparison between full and final models for mean PCV with AICs of 416.10 and 46.53 respectively. None of the explanatory variables have significant effect on PCV as shown in Table 5-9.

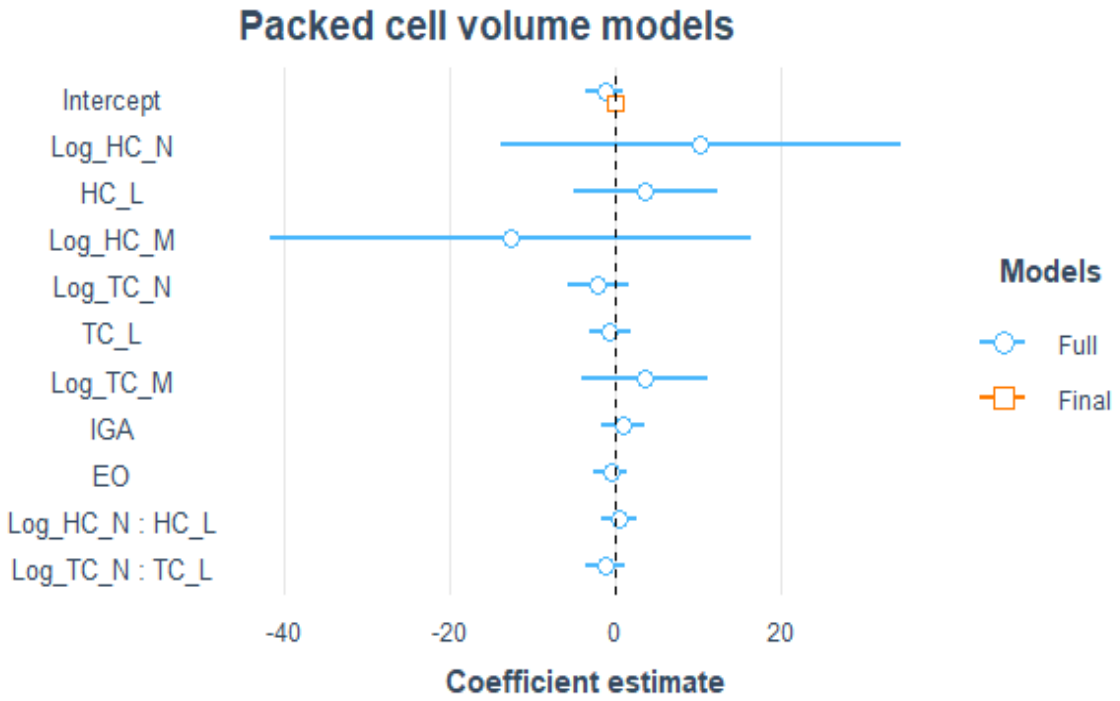


Figure 5-8. Comparison of full and final models of PCV with *H. contortus* number, length and their interaction; *T. colubriformis* number, length and their interaction; *H. contortus* index, *T. colubriformis* index and their interaction; IgA, peripheral eosinophil counts and their interaction as explanatory variables.

Log_HC_N = Log of *H. contortus* number; HC_L = *H. contortus* length; Log_HC_M = Log of *H. contortus* index; Log_TC_N = Log of *T. colubriformis* number; TC_L = *T. colubriformis* length; Log_TC_M = Log of *T. colubriformis* index; Log_TC_M = Log *T. colubriformis* index; IgA = IgA optical density; EO = peripheral eosinophil counts.

Table 5-9. Final model to predict mean PCV.

Explanatory variables	Estimate	95% CI	p-value
Intercept	2.365e-16	-0.554, 0.554	1.000

CI = confidence interval.

5.4 Discussion

This study explored the effects of a single, low-dose deliberate infection of mixed gastrointestinal L3 in naïve Boer goats. This infection was predominately *H. contortus* followed by *T. colubriformis* with the L3 of *Oesophagostomum* spp. failing to develop to adult stage as discussed in Section 4.4. Therefore, this chapter focused on analysing the patterns in gastrointestinal nematodes that were recovered during total worm count procedure, namely *H. contortus* and *T. colubriformis*.

The first molecular identification (using polymerase chain reaction (PCR)) of strongylids among Malaysian livestock revealed a high co-infection rate of *H. contortus* and *Trichostrongylus* spp. in goats in comparison to the other livestock such as deer, cattle and swine (Tan et al., 2014). In the same study, sequencing of DNA from faecal samples collected from the goats showed high infection rates with *H. contortus* followed by *T. colubriformis* and *T. axei*. Therefore, the deliberate infection among Boer goats conducted in this study resembled natural co-infection that commonly occurs among goats in Malaysia.

Correlations between phenotypic variables and nematode variables.

Female *H. contortus* are known to be a prolific eggs producer (Coyne and Smith, 1992; Le Jambre, 1995). Previous studies have shown a significant correlation between FEC and the number of *H. contortus* in natural infections among Katjang kids in Malaysia ($r=0.9$; $p<0.05$) (Israf et al., 1996) and dairy goats in Italy ($r=0.9$; $p<0.05$) (Rinaldi et al., 2009). Thus, initial expectation was that *H. contortus* number would be positively correlated with FEC among Boer goats in this study. However, FEC was more strongly correlated with *T. colubriformis* number($r=0.62$; $p<0.05$) than *H. contortus* number ($r=0.28$; $p>0.05$) in the present study. Together these findings with the other significant positive and negative correlations between *H. contortus* and *T. colubriformis* variables (Table 5-1) suggest that there might be interactions between the blood feeder and mucosal browser nematodes respectively, during co-infection which were then explored in a series of linear regression models. In addition, a significant positive correlation between IgA activity and peripheral eosinophils counts ($r=0.65$, $p<0.01$) suggests that both variables have similar kinetics in response to gastrointestinal nematode infection.

Multiple linear regression models:

a) Interaction of *H. contortus* and *T. colubriformis* during co-infection.

Multiple linear regression models were run to examine the interactions between *H. contortus* (Model A, B and C) and *T. colubriformis* (Model D, E and F) variables at necropsy such as the index, length and number. In addition, the effects of index, length and number of *H. contortus* and *T. colubriformus* species as well as IgA activity and peripheral eosinophil counts on the mean FEC (Model G) and mean PCV (Model H), were also examined.

The results show that the blood feeder *H. contortus* and mucosal browser *T. colubriformis* affected each other during co-infection. Specifically, the results show negative relationships for *T. colubriformis* length with the index (Model A) and number (Models C and E) of *H. contortus*. These relationships have the appearance of density-dependent effects, but the mechanisms by which this could operate are unclear since *H. contortus* and *T. colubriformis* occupy different niches in the gastrointestinal tract. Normally, the negative density-dependent mechanism retards the growth rates at high population densities thus help stabilizing the natural nematode communities (Churcher et al., 2006). However in the present study, reduction in the growth rate or in this case, the length of mucosal browser *T. colubriformis* was associated with increases in the population density (as represented by number and index) of the blood feeding nematode *H. contortus* in the abomasum (Model A and C). Similarly, reduction in the number of *H. contortus* was associated with the growth (length) of *T. colubriformis* in the small intestine (Model E). The negative effect may act to control the nematode population in the gastrointestinal tract. Thus, this would prevent detrimental impact by both nematode species on the host that could occur concurrently during co-infection.

As the number of *T. colubriformis* was unaffected by *H. contortus* variables (number, length and index) or the IgA and eosinophils responses (Model F), the female nematodes of *T. colubriformis* species had the ability to significantly affect FEC of the goats in comparison to the more fecund females of *H. contortus* species (Model G). The stable and unaffected PCV suggest that *H. contortus* was unable to cause anaemia among the goats (Model H). This may be due to either the single and low dose infection rate, or due to the presence of mucosal browser *T. colubriformis* that could impair the pathogenicity of *H. contortus* as a blood feeder species of nematodes in goats, or both.

In this study, although *H. contortus* is generally considered a much more fecund parasite, only *T. colubriformis* was associated with a significant effect on FEC (Model G). However, this does not mean that the female nematodes of *H. contortus* species do not produce eggs but that the variation in FEC is driven more by variation in *T. colubriformis* number or density than in *H. contortus*.

Following the veterinary parasitological laboratory techniques manual provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986), the eggs of many nematode species including *H. contortus* and *T. colubriformis* have the same appearance hence the eggs are not readily distinguishable between nematode species. Therefore, FEC is often refers as the contribution from all species of nematodes involve in an infection which may differ widely in their pathogenicity and fecundity.

To determine the proportion of different nematode species of those eggs, the eggs could be allowed to develop under faecal culture technique (Section 2.2) to be identified as the third stage larvae, L3 (Section 2.3). In this study, percentage of *H. contortus, T. colubriformis* and *Oesophagostomum* spp. L3 recovered from the faecal cultures of deliberately infected Boer goats were 82%, 12% and 6% respectively (Table 4-2). These results indicate that *Oesophagostomum* spp. also producing eggs even though there was no recovery of adult nematode of this species from the gastrointestinal tracts of the goats during total worm counts.

In comparison to the findings in this chapter, the presence of intestinal mucosal browsers *T. colubriformis* of sheep and *Trichostrongylus retortaeformis* of wild rabbits were associated with a reduced intensity of *H. contortus* and *Graphidium strigosum* respectively during co-infection (Lello et al., 2004 and 2018). In these studies, the host immune response was induced by mucosal browsers and negatively affected the number of blood feeders. At the same time, the mucosal browsers increased in number as they benefitted from the suppression of host immune response by the blood feeder.

b) IgA and eosinophil responses against *H. contortus* and *T. circumcincta* co-infection.
Parasite-specific IgA has been associated with resistance in several host-gastrointestinal nematode relationship such as between Scottish Blackface sheep and *T. circumcincta* (Strain et al, 2002), Holstein Friesian cattle and *Cooperia oncophora* (Kanobana et al., 2002) as well as Wistar rat and *Strongyloides ratti* (Wilkes et al., 2007). During infection in sheep, the IgA response has a negative effect on nematode length which in turn is also affected by density-dependent mechanisms of nematode burden (a higher number of nematodes leads to shorter nematodes) (Bishop and Stear, 2000). Nevertheless, the

pathogenicity of an infection is more closely related to the length of infecting gastrointestinal nematodes rather than their number, as suggested by Stear et al. (1999a).

The negative relationship between IgA and nematode length has been observed in Santa Ines, Suffolk, and Ile de France lambs infected with *H. contortus* (Amarante et al., 2005), Scottish Blackface sheep infected with *H. contortus* (Strain and Stear, 2001) and *T. circumcincta* (Strain et al., 2002) as well as Churra lambs infected with *T. circumcincta* (Martínez-Valladares et al., 2005). Increased IgA activity was associated with decreased FEC ($p<0.001$) and shorter length of adult female *T. circumcincta* ($p<0.01$) in Scottish Blackface sheep (Strain et al., 2001) but elevated FEC ($p<0.01$) in Scottish Cashmere goats (McBean et al., 2016). In addition, a strong correlation between mucosal IgA and plasma IgA levels has been observed in Scottish Blackface sheep infected with *T. circumcincta* ($r=0.66$; $p=0.0007$) (Henderson and Stear, 2006).

The findings from the present study contrast with the findings in sheep. In this study, the length of *H. contortus* and the index of *T. colubriformis* were positively associated but *H. contortus* and *T. colubriformis* appeared to be affected differently by IgA activity (Models B and D). Here, the index of *T. colubriformis* was negatively associated with IgA (Model D). This finding suggests that a protective IgA response had been induced that controls the density of this mucosal browser species in the small intestine. On the other hand, a protective effect of IgA response against *H. contortus* was not apparent as there was a positive association of IgA with the length of this blood feeder species (Model B). In other words, IgA appeared not to be effective in controlling the growth of *H. contortus* in the abomasum. Additionally during a co-infection, there is also a possibility that a host with favourable predilection site for a nematode species also possesses a favourable environment for another species. This condition contributes to the existence of both nematode species infecting the host, in which the host could be considered as susceptible for co-infection.

Thus, these results indicate that the IgA response during co-infection has different effects on different nematode species that differ in their feeding habits and occupy different niches of gastrointestinal tract of the goats. Specifically, these findings suggest that co-infection of both nematode species induces IgA activity in the goats but the IgA response was effective against intestinal mucosal browser *T. colubriformis* but not the abomasal blood feeder *H. contortus*. The results also suggest that mucosal IgA in the intestine that affected *T. colubriformis* index might have been more effective than the circulating IgA. The absence of association between IgA response and the number of *H. contortus*, as well as the number of *T. circumcincta* (Model C and F), suggests that IgA activity does not determine nematode numbers which was consistent with Stear et al. (1995a).

Additionally, IgA has no significant effect on FEC of the goats in which the infection was dominated by *H. contortus* (Model G) suggesting that it was ineffective in controlling the consequences of infection. The intensity of infection is reflected in FEC which also reflects the ability of adult nematodes to reproduce hence sustain the infection by mating and laying eggs.

IgA has been shown to have a synergistic effect with eosinophilia where the combination of both variables accounted for 53% of the variation in the length of *T. circumcincta* in the resistant Scottish Blackface sheep (Henderson and Stear, 2006). This synergistic effect occurs when eosinophils express receptors for IgA that can be activated by binding of nematode antigen to IgA cell surface receptors, thus helping target eosinophils to nematodes (Lee et al., 2011). A bioinformatic analysis that compared the high affinity IgA receptor between goat, sheep and human indicated that goats lack eosinophil receptors for IgA (Basripuzi et al., 2018). In this study, the receptor in humans (with high affinity for IgA) and sheep have similar form, but the goat receptor has a different structure. This may explain the relative ineffectiveness of eosinophils in the present study in which no significant effect was observed for peripheral eosinophils counts on *H. contortus* and *T. colubriformis* variables.

Resistance to gastrointestinal nematode infection in sheep is generally associated with Th2 cells response, which stimulate cellular activities such as infiltration of eosinophils as well as mast cell proliferation and maturation. This will also activate the humoral response by signalling B cells to synthesize parasite-specific antibodies such as IgA and IgE. Then, it will cause larval exclusion and adult nematode expulsion which subsequently lead to low FEC, low number of adult nematode and low female nematode fecundity (Venturina et al., 2013, Sweeney et al., 2016). In contrast, susceptibility to nematode infection is generally associated with Th1 and Th17 cell responses. These cells will release cytokines that inhibit Th2 response thus reducing the number of globular leucocytes, mast cells and eosinophils as well as IgA activity. This will lead to high FEC, high number of adult nematode and increase in female nematode fecundity (Venturina et al., 2013).

Hence in the present study, the negative association between IgA activity and *T. colubriformis* index suggests involvement of Th2 cells from the protective immune response; an indication of resistance to this mucosal browser species. Although the positive association between IgA activity and *H. contortus* length suggests that IgA was not protective against the blood feeder, it is difficult to determine whether the Boer goats are resistant (based on high IgA) or susceptible (based on increase in *H. contortus* length) to this nematode species. Therefore, further investigation is needed to confirm this.

Furthermore, this study could not confirm that Boer goats are susceptible to *H. contortus* infection because the multiple linear regression analysis showed that none of the variables from this species was significantly associated with PCV which is the marker for hematophagous parasite infection. However, it is also worth noting here that the normal range and consistent PCV estimates could be due to the low infection intensity. It is possible that the impact of the blood feeder species on Boer goats or the ability of Boer goats to resist its infection can only be seen in an extended period of deliberate infection study with a higher dosage of infective larvae or through a continuous exposure to *H. contortus* infection in the natural environment.

Conclusion

In conclusion, the findings show that the abomasal blood feeder *H. contortus* and the intestinal mucosal browser *T. colubriformis* interacted with each other. The mucosal browser *T. colubriformis* appeared to contribute significantly to the variation in FEC than *H. contortus* while the blood feeder species was unable to affect PCV levels in the goats.

Additionally, immune responses were induced by co-infection, as seen via significant associations of IgA with both nematode species but with the effect of IgA differing for *H. contortus* and *T. colubriformis*. In this study, IgA appeared to have a protective effect against the mucosal browser but not the blood feeder nematodes. It is worth noting here that *H. contortus* infection was not detrimental to the goats, with no observed impact on PCV. This could be because the growth of *T. colubriformis* as represented by its length was associated with reduced number and index of the blood feeder during co-infection, or possibly due to low infection dosage. Therefore, this study could not confirm whether the Boer goats are resistant, resilient or susceptible to gastrointestinal nematode infection particularly with the blood feeder *H. contortus*.

Consequently, the resistance, resilient or susceptibility status of Boer goats to both nematode species need to be confirmed in future studies. This could be achieved by using a higher dose of L3 to conduct deliberate infection study. It is also important to compare the response of goats infected with monospecies of *H. contortus* and monospecies of *T. colubriformis* with those that are co-infected with both nematode species. In addition, it would be informative to study the consequence of *H. contortus* and *T. colubriformis* species in natural co-infection studies, for a better understanding of the goat-parasites interaction and its potential impacts on the health and productivity of animals.

Chapter 6: Conclusion

The problem of gastrointestinal nematode infection among small ruminants with the consequences of production loss and mortality have been emphasized in many studies around the world. *T. circumcincta* has been identified as the most important nematode of small ruminants in cool temperate regions while *H. contortus* as its most significant counterpart in the hot and humid subtropic and tropical countries. These major nematodes are mainly controlled by chemical anthelmintics but effective control is restricted by the development of anthelmintic resistance. Thus, non-chemotherapeutic control such as selective breeding for resistance against gastrointestinal nematode infection has become a more desirable approach for this problem.

Many studies have focussed on sheep breeds to solve the problem of teladorsagiosis and haemonchosis although goats with increasing populations are claimed to be more susceptible to gastrointestinal nematode infection (Onzima et al, 2017). The present study utilized some of the methods used in sheep studies to explore the interaction of goats with *T. circumcincta* and *H. contortus* to address the gaps surrounding resistance or susceptibility of goats to gastrointestinal nematode infection.

Boer goats were specifically chosen in this study because they are commonly reared for meat and their importance can be seen from the establishment of Boer Goat Societies around the world (e.g. British Boer Goat Society, American Boer Goat Association, Boer Goat Breeders' Association of Australia, South Africa Boer Goat Breeders' Society). However, prior to the studies described in this thesis, there was no scientific evidence on the variation in resistance to gastrointestinal nematode infection among Boer goats.

A number of potential phenotypic markers for resistance against gastrointestinal nematodes have been investigated to assist in selective breeding programmes including FEC, anti-nematode antibodies (eg. IgA, IgE), eosinophilia, pepsinogenaemia, PCV in the case of haematophagous nematodes as well as the length, number and fecundity of adult nematodes collected from dead animals.

The methods used in this study to measure FEC, IgA, PCV and peripheral eosinophil counts were shown to be reliable based on Cronbach's alpha values (Nunnally and Bernstein, 1994). Therefore, the methods can be applied confidently in future research. The same methods have been utilised by Stear et al. (1995c) and Strain et al. (2002) to measure phenotypic variables in sheep before MHC studies were established.

In the present study, the responses of Boer goats by means of FEC, IgA, PCV and peripheral eosinophil counts to natural gastrointestinal nematode infection predominated by *T. circumcincta* (Chapter 3) and to deliberate infection predominated by *H. contortus* (Chapter 4) were explored. The infections were studied in different locations and climates, namely England and Malaysia, where *T. circumcincta* and *H. contortus* were respectively found in previous studies to be the dominant nematodes (Bartley et al., 2004; Taylor et al., 2007; Khadijah et al., 2006a; 2006b). In both studies, *T. colubriformis* was found to be the main co-infecting nematode. Consequently, interactions between *T. colubriformis* and the main nematode species were expected to occur during co-infection. This interaction was explored by multiple linear regression models between *H. contortus* and *T. colubriformis* following deliberate co-infection in Boer goats (Chapter 5).

This chapter will summarise and draw together the key findings from the previous chapters and also suggests possible improvement for future research. There are five key findings from this study, as listed below:

1. FEC were found to follow a zero-inflated Poisson distribution in both natural and deliberate gastrointestinal nematode infection in Boer goats.

2. FEC variation was influenced by time, eosinophilia, interaction of peripheral eosinophil counts with IgA and interaction of peripheral eosinophil counts with time.

3. The bodyweight of Boer goats was highly heritable and PCV remained within normal ranges despite being infected with gastrointestinal nematodes.

4. Interaction between *H. contortus and T. colubriformis* during co-infection.

5. Co-infection induced an IgA response which was only effective against *T. colubriformis* but not protective against *H. contortus* infection.

1. FEC were found to follow a zero-inflated Poisson distribution in both natural and deliberate gastrointestinal nematode infection in Boer goats.

In this study, FEC data from natural infection was confirmed to follow a zero-inflated Poisson distribution after a series of model exploration by MCMCglmm R package for modelling in Chapter 3. Simulated data were first used to illustrate the MCMCglmm modelling approach and to show how to determine when a zero-inflated model is required. This approach was then applied to the observed data, showing that the observed zeroes fell outside of the 95% credible interval for the predicted distribution of zeroes, indicating the need for a zero-inflated Poisson model. The FEC of Boer goats was also assumed to follow a zero-inflated Poisson distribution following deliberate infection in Chapter 4. This means that zero observations from FEC data were assumed to arise from a mixture of both Poisson (count component) and zero-inflated (binary component) distributions regardless of the modes of infection (natural or deliberate infection) and dominant nematode species infecting the goats (*T. circumcincta* versus *H. contortus*). The results then showed significant Poisson and zero-inflated parts of the models, confirming that the zero-inflated Poisson model was appropriate for these analyses.

In a zero-inflated Poisson model, two types of zeroes, known as true zeroes and false zeroes are thought to exist in the data. The true zeroes (also known as structural zeroes) are assumed to come from uninfected individuals while the false zeroes (also known as random zeroes) come from an infected group (Yang et al., 2017). In the present study, the true zeroes were expected to come from faecal samples of uninfected goats that were entirely free from nematode eggs; while the false zeroes were expected to arise from the faecal samples of infected goats that contained nematode eggs but were not counted microscopically during the McMaster technique. According to the manual for veterinary parasitology laboratory techniques provided by Ministry of Agriculture, Fisheries and Food of Great Britain (1986), a nematode egg will not be counted if it is located outside of the McMaster grids which presumably contributed to the false zeroes observation in this study.

The MCMCglmm package was specifically chosen for modelling analyses in Chapter 3 and Chapter 4 because it enabled exploration of animal model by utilizing the available pedigree records from the goat farms where the study was conducted. This package also supports many types of distribution including zero-inflated Poisson distribution (Hadfield, 2010). Since the goats were measured repeatedly, zero-inflated Poisson generalized linear mixed effects models were fitted to the data. The random effects were the 'animal' term that made use of the pedigree record to generate the additive genetic variance; the animal 'ID' term that represented individual to individual variation allowing

the repeatability to be calculated; and the pen in which the goats were kept. Time, IgA, peripheral eosinophil counts and their interactions as well as sex and types of birth were fitted as fixed effects.

2. FEC variation was influenced by time, eosinophilia, interaction of peripheral eosinophil counts with IgA and interaction of peripheral eosinophil counts with time.

All of the final models for natural and deliberate infections had extremely consistent results where FEC variation was affected by time in both counts and binary components of zero-inflated Poisson models. In the natural infection (Chapter 3), the observed FEC showed a peak in Month 2 (September) that was significantly higher than Month 1 as the baseline (August) in the Poisson part of the model which contributed to the variation in FEC by time variable. The estimates of zero-inflation component of the models also show that zero-inflation is greatest at the final sampling times (October), reflecting the high number of zeroes as the goats recover from infection.

In the deliberate infection (Chapter 4), although FEC was not extremely high due to a low level of infection, the FEC was large enough to show significant differences between time levels. In the Poisson part of the models, the FEC during the earlier times of sampling (time points 2 and 3; or from day 14 to 24 post-infection and from day 28 to 38 post-infection) was significantly lower than the FEC at later times of sampling (time point 4 as the baseline; or from day 42 to 52 post-infection) that contributed to the variation in FEC by time variable. The estimates of zero-inflation component of the models show that zero-inflation is the highest in time point 2 (day 14 to 24 post-infection) which reflects the highest number of zeroes. This finding is relevant as time point 2 marked the early establishment of infection after the pre-patent period with continuous absence of nematode eggs in time point 1 (day 0 to day 13 post-infection).

During the natural infection study, different anthelmintics were used to drench the goats in each month, affecting the FEC during the subsequent months. The observation suggests that the nematodes were already resistant to narrow-spectrum benzimidazole that was used to drench the goats in August, leading to a high FEC in September. The broad-spectrum anthelmintics such as monepantel (an amino-acetonitrite derivative) and moxidectin (a macrocylic lactone) that were used to drench the goats in July and September seemed to be effective, based on the much lower FEC in August and October. In this study, I could not conduct a faecal egg count reduction test (FECRT) (Coles et al., 1992) to evaluate anthelmintic resistance status because the test requires untreated animals as control group which would not have been compatible with the farm practices.

In future studies, I would propose evaluation of anthelmintic resistance status by FECRT on the farm where research is going to be conducted.

As expected, eosinophilia caused a significant reduction in FEC of Boer goats in natural co-infection that was predominated by *T. circumcincta* (Chapter 3). This is consistent with findings in Scottish Blackface sheep that were infected with the same nematode species (Stear et al., 2002). However, the negative effect of eosinophilia on FEC was moderated by its interaction with IgA that has a positive effect on FEC.

In Creole and Scottish Cashmere goats that were infected naturally with *H. contortus* and *T. circumcincta* respectively, high FEC was associated with high IgA responses (de la Chevrotière et al., 2012; McBean et al., 2016). These findings from other breeds of goats suggest that IgA has been induced in response to infection but it is not protective in controlling gastrointestinal nematode infection in goats in the way that it does in sheep (Strain et al., 2002). Instead, control of infection relied on the protective effect of eosinophils to regulate FEC. In this study, the positive association of FEC with the interaction between eosinophils and IgA could be due to the reduction in the effectiveness of eosinophils in moderating FEC when IgA was high. Presumably, the apparent lack of eosinophil receptors for IgA in goats (Basripuzi et al., 2018) leads to the inability for synergistic action of IgA and eosinophils to control nematode length, and therefore its fecundity, which leads to increased FEC.

There was no main effect of peripheral eosinophils observed in the deliberate infection study (Chapter 4) except its negative interaction with time point 4 (day 42 to 52 post-infection) in comparison to its interaction with time point 2 (day 14 to 24 post-infection). This could be due to the migration of eosinophils to the peripheral blood that only occurred at a later stage of infection. The eosinophils were recruited to the local site of infection such as abomasum and small intestines during the early stage of infection.

A key finding from both the natural and deliberate infection studies is that the FEC heritability of these goats was very low ($h^2 < 0.01$). However, there could be a possibility that the heritability is larger based on the skewed posterior distributions. Therefore, a study with a larger sample size would be needed to confirm the result. The hypothesis that derives from the fundamental theorem of natural selection (Fisher, 1930) states that fitness traits should have very low heritability. Genes that positively influence these traits (eg. disease resistance) will be subjected to natural selection and increase in frequency until they become fixed. Subsequently, this will lead to loss of heritable genetic variation hence resulting in low heritability estimates for fitness traits (Stear et al., 2011) which may have occurred for FEC heritability among Boer goats.

3. The bodyweight of Boer goats was highly heritable and PCV remained within normal ranges despite being infected with gastrointestinal nematodes.

Resistance to gastrointestinal nematode infection can be shown by low FEC or low nematode challenge while resilience is commonly assessed by normal PCV and maintenance or increase in bodyweight (Morris and Bisset, 1996; Onzima et al., 2017). Boer goats have been shown to be somewhat resistant to gastrointestinal nematode infection from a preliminary study in Philippines (Baker and Gray 2004). In the present study, the bodyweight of Boer goats was positively associated with eosinophilia ($p=0.017$) and the immune index ($p=0.038$); and had a negative association with FEC ($p=0.027$). Interestingly, the bodyweight of the infected goats increased significantly with time ($p<0.001$) but the effect of eosinophilia ($p<0.01$) and immune index ($p=0.027$) on bodyweight decreased with time as the goats aged or because the infection diminished over time.

In this study, the models that fitted the 'animal' term as one of the random effects showed substantial additive genetic effects on bodyweight of the goats. In the models, the estimates for the animal random effect was much larger than the estimates for individual variance as represented by 'ID' term as another random effect. Therefore, the findings indicate that bodyweight of the goats was more affected by genetic variance than the individual variance, suggesting that bodyweight is a highly heritable trait of Boer goats. In order to confirm the bodyweight heritability, the repeatability of bodyweight which serves as the upper limit for heritability (Falconer and MacKay, 1996) was estimated first. The repeatability estimates for bodyweight of the goats were consistently high in all final models ($r=0.91$; HPD interval = 0.87-0.94). The investigation proceeded to heritability estimation which also revealed high heritability ($h^2=0.90$-0.91; HPD interval = 0.28-0.94). Unlike FEC that has low heritability, the body weight was highly heritable among the goats because they were selected for high body weight from a few generations and thus this trait has been affected by additive genetic effect.

These results confirm that the bodyweight was a highly heritable trait of Boer goats. This finding was expected as Boer breed is an important meat breed of goats with desirable characteristics of high growth rates, good body conformation (Casey and Van Niekerk, 1988), better quality meat and heavier carcass (Malan, 2000). Furthermore, their bodyweight significantly increased with time, particularly just within three months of sampling the fact that they were continuously exposed to gastrointestinal nematodes infection predominated by *T. circumcincta* during grazing. For Scottish Blackface sheep, the most important genes for their growth rate are those that have the ability to control gastrointestinal nematode infection which is based on the strong negative genetic

correlation between FEC and bodyweight (Bishop et al., 1996) that may also occur among Boer goats. It would offer useful insights into the relationship between bodyweight and gastrointestinal nematode infection if a similar study to the natural infection study in Chapter 3 could be conducted in Boer goats with natural infection predominated by *H. contortus* in the future.

The packed cell volume (PCV) was not affected in either the natural or deliberate infection studies. PCV measures the volume of circulating erythrocytes in the blood compared to the whole blood, thereby serving as a marker for anaemia which is commonly used in the case of blood feeder *H. contortus* infection. The normal range for PCV in goats is estimated to be between 22% and 38% (Byers and Kramer, 2010) while mild anaemia is suspected when the ruminants have a lower PCV estimation of 20% to 26% (Tvedten, 2010).

The resistant group of crossbreed goats originating from ½ Saanen and ½ Anglo-Nubian breeds naturally infected with *H. contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. have higher mean PCV (26.48% ± 1.25) than their susceptible counterparts (24.04% ± 1.56) (Coutinho et al., 2015) while the Boer goats in the present study that were deliberately infected with similar species of nematodes had a mean PCV of 26.67% ± 0.21 (Chapter 4). These findings indicate that the PCV of Boer goats were similar to that of resistant goats in previous studies or could be due to low challenge of nematodes

Therefore, both findings on bodyweight and PCV suggest that Boer goats may be relatively resistant and resilient to gastrointestinal nematode infection. However, it is worth noting here that the infection dosage for deliberate infection study was relatively low and this could explain the unaffected PCV among the goats.

4. Interaction between *H. contortus* and *T. colubriformis* during co-infection.

Co-infection by gastrointestinal nematodes is common during natural infection among small ruminants. Thus, it is important to explore the potential interactions between nematode species and the significant consequences that may occur during a co-infection with a nematode community. The scope of this study was narrowed to deliberate co-infection of Boer goats with *H. contortus* and *T. colubriformis* in a single, low dose infection. Nevertheless, this study still resembled natural co-infection in that goats in Malaysia have been confirmed by molecular identification to be highly co-infected with *H. contortus* and *T. colubriformis* (Tan et al., 2014).

As expected, *H. contortus* and *T. colubriformis* affected each other during co-infection. The growth (increase in length) of *T. colubriformis* in the small intestine was negatively associated with the density (number and index) of *H. contortus* in the abomasum and vice versa. Even though these negative interactions appear similar to a density-dependent effect, the mechanisms underlying the action are very unclear because *H. contortus* and *T. colubriformis* occupy different niches of the gastrointestinal tract of the host.

Initially, *H. contortus* was expected to have association with high FEC due to the typical prolific characteristic of the female nematodes. However, in this study, only the female nematodes of *T. colubriformis* species had a significant positive effect on FEC. This suggests that the fecundity of female *H. contortus* may have been affected by co-infection with *T. colubriformis*.

Additionally, in the future, it would be informative to conduct a similar study of natural co-infection among sheep and goats in order to reveal the impacts of interactions between nematodes on their communities' dynamics. The findings from the present study suggest that co-infection may have some role in the control of dominant nematode species in natural infection.

5. Co-infection induced an IgA response which was only effective against *T. colubriformis* but not protective against *H. contortus* infection.

IgA activity in the plasma depends mainly on mucosal IgA activity and the nematode index. de Cisneros et al. (2014) finds that increased mucosal IgA activity was associated with increased plasma IgA activity while decreased in plasma IgA activity was associated with the increased in the nematode index among naturally infected sheep. Low plasma IgA activity was either associated with a low infection intensity that generates low immune response; or because most of the mucosal IgA is bound to nematode antigens during heavy infections hence a relatively small proportion of IgA is transferred into the plasma.

In this study, IgA activity could still be detected during deliberate infection even though the infection rate was low. However, IgA only had negative associations with *T. colubriformis* index but positive associations with *H. contortus* length. Given that *T. colubriformis* is a mucosal browser in the intestine, the fourth stage larvae (L4) of this species would be more likely to be affected by mucosal IgA activity than *H. contortus,* which feeds on blood in the abomasum. Although mucosal IgA was not measured in this study, plasma IgA activity provided an indication of IgA activity in the gastrointestinal tract of the goats in response to co-infection with *H. contortus* and *T. colubriformis.*

Limitations to the present studies

In the present study, only 2400 L3 consisting of *H. contortus, Trichostrongylus* spp. *and Oesophagostomum* spp. at a 6:1:1 ratio were used to infect the goats due to the insufficient L3 stock. In future research, deliberate infection studies could be improved by increasing the infection dosage as suggested by Emery et al. (2016). A deliberate infection study conducted by Stear et al. (1995) shows that FEC, red blood cell counts, bodyweight and sex of naïve Scottish Blackface lambs infected with three dosages of 10000 L3 of *H. contortus* at eight week intervals accounted for the variation in the nematode burden among the lambs which could also be followed in future studies. In addition, the infected animals should be separated from the uninfected control animals to avoid infection occurring in the controls. This would allow comparison of the immune responses between exposed and naïve animals providing a better understanding of immune mechanisms.

IgA activity against fourth-stage larvae (L4) and IgE activity against third-stage larvae (L3) are the most important antibody responses in protection against gastrointestinal nematodes in sheep (Stear et al., 2011). In the present study, the lack of a protective IgA response in the analyses of the FEC dynamics may be due to the use of L3 instead of L4 as the antigen for IgA ELISA assay. The studies on Scottish Cashmere goats (McBean et al., 2016) and Creole goats (Chevrotiere et al., 2012) that found positive association between IgA and FEC also used L3 as the nematode antigen for IgA ELISA assay. Therefore, the use of specific antibody against appropriate nematode antigens (eg. L4 antigen for the IgA ELISA; L3 antigen for the IgE ELISA) in future research may demonstrate significant protective antibody effects on FEC. In this study, it was not possible to carry out the digestion technique for recovery of L4 from the abomasum and intestines (Lyndall-Murphy, 1993) of the goats as these organs were required by another research team for histology studies following nematode infection. In addition, the lack of eosinophil receptors for IgA in goats (Basripuzi et al., 2018) may impede the effectiveness of immune response against gastrointestinal nematode infection as discovered in this study.

The ELISA optimization protocol for IgE was conducted and working concentrations for plasma pepsinogen levels against gastrointestinal nematodes had been assessed before the start of the experiment. However, these preliminary assays could only detect very low levels of IgE and pepsinogen, and thus neither assay was considered feasible in this study. The reason for low IgE and pepsinogen levels from the samples remains unclear. It would be beneficial if IgE activity and pepsinogen concentration responses against gastrointestinal nematode infection could be investigated in future research.

The fecundity of female nematodes was not assessed in the present study. Although length is correlated with fecundity, when multiple nematode species are present it would be helpful to measure fecundity in female *H. contortus* which are known as prolific eggs producers, as well as the *T. colubriformis*, in order to investigate more precisely the effect of co-infection on fecundity and FEC. I would suggest following the method described by Rowe et al. (2008) to determine the individual female *H. contortus* fecundity in future research. In addition, it would be more informative if a comparative study could be conducted between goats infected with monospecies of *H. contortus* and *T. colubriformis* with those that are infected with both nematode species to gain more understanding of goats-nematodes interactions.

It is worth noting that the most appropriate models to examine the immune response and the host–parasite interaction with gastrointestinal nematodes are derived from either natural infections or continuous deliberate infection with graded doses of infecting larvae; for example 500 L3 of *H. contortus* three times per week or a single large dose of more than 8000 L3 of *H. contortus* will be adequate to trigger the immune response (Emery et al. 2016). However, it was not feasible to follow these suggestions in the present study due to insufficient L3 stock for deliberate infection but could be followed in future studies.

Conclusion

In conclusion, the studies carried out in this thesis have contributed to expanding knowledge of the host-parasite relationship and of interspecific nematode interactions in goats. The findings of consistent and normal range of PCV (Chapter 4) as well as no significant association observed between PCV and nematode variation (number, length and index) (Chapter 5) suggest that the goats were not strongly affected by gastrointestinal nematode infection, particularly by the blood feeder *H. contortus* during deliberate infection. These findings suggest that Boer goats could be considered resilient to nematode infection; however, it is possible that the infection dosage was too low to cause significant signs of infection.

Although there was a negative association between FEC and bodyweight of the goats (Chapter 3), the significant increase of bodyweight throughout natural infection study also suggests that the goats are relatively resilient to gastrointestinal nematode infection. Peripheral eosinophil counts have been shown to be associated with reduction in FEC but this effect was moderated by IgA which was not protective against infection on its own. Additionally, both repeatability and heritability for FEC were small among naturally and deliberately infected Boer goats whereas the heritability of their bodyweight was

considerably high suggesting that the Boer breed are naturally resistant to nematode infection.

The abomasal blood feeder *H. contortus* and the intestinal mucosal browser *T. colubriformis* were found to interact with each other but *Oesphagostomum* spp. may have failed to establish their population in large intestine during co-infection. IgA responses against *H. contortus* and *T. colubriformis* were induced during co-infection but it wereonly effective against the mucosal browser species. Even so, the infection caused by the blood feeder species was not detrimental to the goats, as shown by PCV. This could also be due to the ability of *T. colubriformis* to moderate the infection intensity of *H. contortus*.

This thesis could only suggest but not yet confirm that Boer goats in this study are relatively resilient and resistant to gastrointestinal nematode infection. Thus, further studies are needed to confirm this suggestion, but the limitations of the present study must be addressed as suggested above.

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Appendices

Appendix A : Preparation of buffers and solutions.

a) PBS pH 7.4.
   i. 80 g of NaCl, 2 g of KCl, 14.4 g of Na_2HPO_4 and 2.4 g of K_2HPO_4 were dissolved in distilled water to make 1 L of 10x PBS.
   ii. 100 ml of 10x PBS was diluted with 900 ml of distilled H_20.
   iii. The solution was adjusted for pH 7.4 by adding acid or alkali.
   iv. Stored at 4°C

b) PBS/Antibiotic.
   i. 1 ml of 5mg/ml Streptomycin, 5000 i.u. Penicillin, 50 µl of 10 mg/ml Gentamycin and 0.5 ml of 250 µg/ml Amphotericin B were mixed to make a solution.
   ii. The solution was made up to 50 ml with sterile PBS pH 7.4.

c) Tris Poisons in 10 mM Tris pH 8.3.

   i. Preparation of the following stock solutions:
      - 0.5 M of EDTA (2.92 g made up to 20 ml by distilled H_20)
      - 0.5 M of EGTA (3.80 g made up to 20 ml by distilled H_20)
      - 1.0 M of NEM (1.25 g made up to 10 ml by distilled H_20)
      - 1.0 mM of Pepstatin (6.85 mg made up to 10 ml by ethanol)
      - 0.33 M of PMSF (581 mg made up to 10 ml by ethanol)
      - 0.1 M of TPCK (353 mg made up to 10 ml by ethanol)
   ii. 1 ml of each water soluble inhibitor (EDTA, EGTA, NEM) were mixed together and made up to 5 ml with distilled H_20. The solution was designated as PI-A (aqueous) and stored at -20°C.
   iii. 3 ml of 0.33 M PMSF was mixed with 1 ml of 0.1 M TPCK then added with 1 ml of 1 mM Pepstatin. The solution was then dissolved by water bath at 65°C. This solution was designated was PI-B (organic).
   iv. 24.2 g of Tris was added in 200 ml H_20 and adjusted for pH 8.3 to give a 1M solution of Tris-HCl.
   v. 2 ml of Tris-HCl, 1 ml of PI-A and 1 ml of PI-B were mixed together and made up to 200 ml by H_20. This solution was known as Tris Poisons solution.

EDTA = Ethylene Glycol-bis (ß-Aminoethylether)-N,N,N',N'-Tetraacetic Acid
EGTA = Ethylene Diamine Tetraacetic Acid
NEM = N-Ethylmaleimide
Pepstin A = (Isovaleryl-Val-Val-Sta-Ala-Sta) Sta=statine=(3S,4S)-4-Amino-3-hydroxyl-6-methylheptonic Acid
PMSF = Phenyl Methyl Sulfonyl Flouride
TPCK = N-Tosyl-$_L$-Phenylalanine Chloromethyl Ketone

d) Tris-Poisons + DOC.
   i. The same steps to prepare Tris-Poisons solution were used but 2 g of sodium deoxycholate (DOC) was added in the final step.

e) 0.06 M carbonate buffer pH 9.6.
   i. Preparation of the following stock solutions:
      - 1 M of $NaHCO_3$ (84 g in 1 L H_20)
      - 1 M of Na_2CO_3 (106 g in 1 L H_20)

ii. 45.3 ml of 1 M $NaHCO_3$ was mixed with 18.2 ml 1 M Na_2CO_3.
iii. The solution was made up to 1 L with H_20 and adjusted for pH 9.6.

f) PBS-Tween solution
   i. 1 ml of Tween 20 was added to 1 L of PBS.

g) Blocking buffer
   i. 4.0 g of dried skimmed milk was dissolved in 100 ml PBS-Tween to make a 4% solution.

h) Stop solution (2 M H_2SO_4)
   i. 56 ml of 95% H_2SO_4 was added slowly to 250 ml of distilled H_2O.
   ii. The solution was made up to 500 ml with distilled H_2O.

Appendix B: Pedigree data frame of Boer goats in a farm in England.

Animal	Dam	Sire
Bispham Oswald	NA	NA
Cockerham Ob805	NA	NA
Cockerham Ob806	NA	NA
Cockerham Ob807	NA	NA
Cockerham Ob808	NA	NA
Cockerham Ob809	NA	NA
Broadland Sam	NA	NA
Harperley Charles	NA	NA
Topknot groucho	NA	NA
Bispham Sarah	NA	NA
Chaz	NA	NA
Sam Cream Eyebrows	NA	NA
Sam Brown White	NA	NA
Colin Long Hair White	NA	NA
Colin Blue Cashmere	NA	NA
Sam Eyebrows	NA	NA
Sam 513	NA	NA
Alf	NA	NA
Groucho	NA	NA
Sam	NA	NA
Alf Rosie	NA	NA
Oswald	NA	NA
Bispham Joe	NA	NA
Colin 5281	NA	NA
Colin	NA	NA
White tail	NA	NA
Kevin	NA	NA
Colin Blue	NA	NA
Grouch	NA	NA
Colin 8251	NA	NA
Colin 501	NA	NA
Chas	NA	NA
Sam Can I Have a Go	NA	NA
Groucho Snotty	NA	NA

Sam 532	NA	NA
Colin WTLF	NA	NA
Colin Can I Have a Go	NA	NA
Chaz 23	NA	NA
Alf Claire No Blaze	NA	NA
Colin Brown Black	NA	NA
Oke	NA	NA
Cockerham Blue367	NA	NA
Cockerham Blue368	NA	NA
Cockerham Blue369	NA	NA
Cockerham Blue370	NA	NA
Cockerham Blue371	NA	NA
Cockerham Blue372	NA	NA
Cockerham Blue373	NA	NA
Cockerham Blue374	NA	NA
Cockerham Blue375	NA	NA
Cockerham Blue376	NA	NA
Cockerham Blue377	NA	NA
Cockerham Green 524	NA	NA
Bispham Eevee	NA	NA
Jannus Hattie	NA	NA
Twenty 5 of Cockerham	NA	NA
Rosedale Oswald	NA	NA
206	NA	NA
5337Blue	NA	NA
Blue	NA	NA
Girl	NA	NA
Cashmere	NA	NA
5281	NA	NA
8	NA	NA
6359	NA	NA
GT	NA	NA
420	NA	NA
Cream Eyebrows	NA	NA
Girl 1	NA	NA
383	NA	NA
Brown Black	NA	NA
8251	NA	NA
531	NA	NA
Smashie	NA	NA
559	NA	NA
5165	NA	NA
139	NA	NA
Frankendale	NA	NA
5210 Can I Have a Go	NA	NA
130	NA	NA
443	NA	NA

Palendale	NA	NA
SC Wonkey	NA	NA
Snotty	NA	NA
Twelve	NA	NA
155	NA	NA
112	NA	NA
Bluff	NA	NA
Smashiedale	NA	NA
165	NA	NA
Suzie	NA	NA
White	NA	NA
Lankydale	NA	NA
5357 Cash	NA	NA
Gucci	NA	NA
Wilma	NA	NA
Spotty	NA	NA
Creamandale	NA	NA
Lucky	NA	NA
5246 Brown Black	NA	NA
Clares Blaze	NA	NA
181	NA	NA
455	NA	NA
Biscuit	NA	NA
169	NA	NA
Orchid	NA	NA
Ibo	NA	NA
brown/white	NA	NA
nine	NA	NA
1384	NA	NA
1385	NA	NA
1386	NA	NA
1387	NA	NA
1388	NA	NA
1389	NA	NA
1433	NA	NA
1550	NA	NA
1551	NA	NA
1552	NA	NA
1553	NA	NA
1554	NA	NA
1614	NA	NA
1638	NA	NA
1555	NA	NA
1556	NA	NA
1390	NA	NA
1391	NA	NA
Cockerham 1196	NA	Cockerham Ob805

396	Cashmere	Alf Rosie
Rosie	Girl	Oswald
149	Suzie	Sam
Gaia	8	White tail
Darkendale	6359	Sam
375	GT	Grouch
374	Girl 1	Grouch
463	Cashmere	Groucho Snotty
367	Twelve	Grouch
556	Brown Black	Colin
777	Darkendale	Alf
501	149	Bispham Oswald
362	White	Grouch
399	Lankydale	Alf
536	5246 Brown Black	Colin Brown Black
829	Clares Blaze	Colin
553	Cream Eyebrows	Colin
GHT	Bluff	Oke
115	Girl 1	Oswald
1177	556	Chaz
881	216	Sam Cream Eyebrows
883	206	Sam Brown White
551	5337Blue	Colin Long Hair White
541	5337Blue	Colin Blue Cashmere
880	216	Sam Cream Eyebrows
795	553	Chaz
885	216	Sam Eyebrows
876	216	Sam 513
820	Blue	Alf
476	396	Groucho
1163	Gaia	Sam
424	Cashmere	Bispham Joe
522	5281	Colin 5281
838	501	Colin
1099	367	Oswald
796	420	Chaz
1185	536	Chaz
1062	375	Kevin
1098	383	Oswald
879	Brown Black	Sam
1186	531	Chaz
792	Smashie	Chas
814	559	Alf
807	5165	Alf
1114	531	Alf
469	139	Groucho
472	Frankendale	Grouch

791	GHT	Chaz
822	Blue	Colin WTLF
533	5210 Can I Have a Go	Colin Can I Have a Go
790	5337Blue	Chaz 23
1089	130	Oswald
477	443	Groucho
1069	Palendale	Kevin
769	5337Blue	Alf Claire No Blaze
201	SC Wonkey	Sam
1119	362	Alf
458	Snotty	Groucho
216	Gaia	Sam
499	362	Kevin
1061	375	Kevin
386	155	Grouch
1279	777	Groucho
1167	829	Sam
805	112	Alf
274	Bluff	Sam
1218	Darkendale	Kevin
830	165	Colin
776	Rosie	Alf
1063	463	Kevin
874	5357 Cash	Sam
409	Cashmere	Alf
179	Gucci	Sam
166	Wilma	Sam
1159	Darkendale	Sam
806	375	Alf
1120	Spotty	Alf
1280	Creamandale	Groucho
158	GT	Sam
1060	Palendale	Kevin
182	Lucky	Sam
1274	Cream Eyebrows	Colin
1153	115	Sam
1217	Frankendale	Kevin
449	Bluff	Alf
770	Gaia	Alf
864	399	Oswald
1081	181	Oswald
495	455	Kevin
176	Spotty	Sam
439	Wilma	Alf
198	Biscuit	Sam
833	169	Colin
1216	Smashiedale	Kevin

159	Orchid	Sam
841	Ibo	Colin
1071	399	Oswald
866	216	Oswald
1195	brown/white	Chaz
1278	399	Groucho
Yorkvale Chaz	Jannus Hattie	Harperley Charles
Cockerham Oswald	Cockerham Blue367	Bispham Oswald
Cockerham Alf	Bispham Eevee	Broadland Sam
Cockerham Kevin	Cockerham Green 524	Broadland Sam
Cockerham Colin	Twenty 5 of Cockerham	Topknot groucho
1211	386	Yorkvale Chaz
1212	556	Yorkvale Chaz
1213	1279	Yorkvale Chaz
1214	1167	Yorkvale Chaz
1225	375	Cockerham Kevin
1248	769	Cockerham Oswald
1249	769	Cockerham Oswald
1250	201	Cockerham Oswald
1251	1119	Cockerham Oswald
1253	409	Cockerham Oswald
1296	476	Cockerham 1196
1297	1163	Cockerham 1196
1299	396	Cockerham 1196
1300	396	Cockerham 1196
1315	796	Cockerham Oswald
1316	1185	Cockerham Oswald
1317	1185	Cockerham Oswald
1318	881	Cockerham Oswald
1319	1062	Cockerham 1196
1321	541	Cockerham Oswald
1322	374	Cockerham Alf
1323	1098	Cockerham Alf
1324	879	Cockerham Oswald
1325	879	Cockerham Oswald
1327	522	Cockerham Alf
1329	885	Cockerham Oswald
1331	792	Cockerham Alf
1332	876	Cockerham Oswald
1336	814	Cockerham Oswald
1337	791	Cockerham Alf
1339	822	Cockerham Kevin
1340	533	Cockerham Alf
1341	790	Cockerham Alf
1342	1089	Cockerham Kevin
1344	477	Cockerham Oswald
1346	1069	Yorkvale Chaz

1348	1218	Yorkvale Chaz
1349	1218	Yorkvale Chaz
1350	830	Cockerham Oswald
1351	386	Yorkvale Chaz
1352	556	Yorkvale Chaz
1354	201	Cockerham Oswald
1355	1119	Cockerham Oswald
1360	Rosie	Cockerham Alf
1361	374	Cockerham Alf
1363	522	Cockerham Alf
1364	838	Cockerham Alf
1365	Gaia	Cockerham Alf
1366	Smashiedale	Cockerham Alf
1368	1099	Cockerham Alf
1369	1099	Cockerham Alf
1370	Darkendale	Cockerham Alf
1375	274	Cockerham Colin
1376	501	Cockerham Colin
1377	1153	Cockerham Colin
1378	1217	Cockerham Colin
1379	1217	Cockerham Colin
1380	449	Cockerham Colin
1381	449	Cockerham Colin
1382	866	Cockerham Colin
1383	866	Cockerham Colin
1431	463	Cockerham Kevin
1432	375	Cockerham Kevin
1456	805	Yorkvale Chaz
1457	182	Yorkvale Chaz
1458	536	Yorkvale Chaz
1459	536	Yorkvale Chaz
1460	829	Yorkvale Chaz
1461	brown/white	Yorkvale Chaz
1462	1274	Yorkvale Chaz
1463	1071	Yorkvale Chaz
1464	1071	Yorkvale Chaz
1507	458	Cockerham Oswald
1508	776	Yorkvale Chaz
1509	1279	Cockerham Colin
1511	149	Cockerham Oswald
1512	499	Cockerham Oswald
1513	1061	Cockerham Oswald
1514	362	Yorkvale Chaz
1515	399	Cockerham Oswald
1516	399	Cockerham Oswald
1517	1063	Cockerham Oswald
1518	149	Cockerham Oswald

1519	274	Cockerham Colin
1521	501	Cockerham Colin
1522	874	Cockerham Oswald
1523	874	Cockerham Oswald
1525	770	Cockerham Oswald
1526	553	Yorkvale Chaz
1527	182	Yorkvale Chaz
1529	864	Cockerham Colin
1530	179	Cockerham Oswald
1531	1081	Yorkvale Chaz
1532	166	Cockerham Oswald
1533	GHT	Cockerham Colin
1534	1159	Cockerham Oswald
1535	495	Yorkvale Chaz
1536	176	Yorkvale Chaz
1537	439	Yorkvale Chaz
1539	brown/white	Yorkvale Chaz
1540	158	Cockerham Oswald
1541	198	Yorkvale Chaz
1543	833	Cockerham Oswald
1544	1216	Yorkvale Chaz
1545	159	Bispham Oswald
1546	159	Bispham Oswald
1547	1186	Cockerham Oswald
1548	1278	Cockerham Colin
1549	1278	Cockerham Colin
1611	Frankendale	Cockerham Alf
1612	469	Cockerham Alf
1613	472	Cockerham Alf
1621	216	Cockerham Oswald
1622	499	Cockerham Oswald
1623	1061	Cockerham Oswald
1624	367	Cockerham Oswald
1625	367	Cockerham Oswald
1626	179	Cockerham Oswald
1627	166	Cockerham Oswald
1628	1159	Cockerham Oswald
1629	806	Cockerham Oswald
1630	806	Cockerham Oswald
1631	1120	Cockerham Oswald
1632	1120	Cockerham Oswald
1633	1280	Cockerham Oswald
1634	158	Cockerham Oswald
1636	841	Bispham Oswald
1642	1177	Cockerham Oswald
1643	881	Cockerham Oswald
1644	883	Cockerham Oswald

1645	551	Cockerham Oswald
1646	541	Cockerham Oswald
1647	880	Cockerham Oswald
1648	795	Cockerham Oswald
1649	885	Cockerham Oswald
1650	876	Cockerham Oswald
1651	820	Cockerham Oswald
1652	807	Cockerham Oswald
1653	1114	Cockerham Oswald
1654	1060	Cockerham Oswald

Appendix C: Phenotypic data frame of Boer goats in a farm in England.

Animal	Dam	Sire	Sex	TB	Month	FEC	IGA	EO	PCV	BWT
1211	386	Yorkvale Chaz	1	1	1	125	0.81548	1797.6	23	15.5
1212	556	Yorkvale Chaz	1	1	1	0	1.064375	1719.2	29.5	16.5
1213	1279	Yorkvale Chaz	1	1	1	NA	0.899619	1904	25.5	18
1214	1167	Yorkvale Chaz	1	1	1	25	0.795613	1601.6	34	18
1225	375	Cockerham Kevin	1	2	1	125	0.914314	1629.6	25.5	18
1248	769	Cockerham Oswald	2	2	1	0	0.091329	711.2	21.5	21
1249	769	Cockerham Oswald	1	2	1	575	0.785796	2542.4	23	10
1250	201	Cockerham Oswald	1	1	1	NA	1.094314	1360.8	22.5	15.5
1251	1119	Cockerham Oswald	1	1	1	200	0.52483	1556.8	26	13
1253	409	Cockerham Oswald	1	1	1	0	0.580919	1752.8	34	15.5
1296	476	Cockerham 1196	1	1	1	475	0.598883	1820	26	28.5
1297	1163	Cockerham 1196	1	2	1	200	0.563427	1422.4	25	15
1299	396	Cockerham 1196	1	2	1	0	0.459367	2144.8	23.5	23
1300	396	Cockerham 1196	1	2	1	25	0.513761	1484	30	17.5
1315	796	Cockerham Oswald	1	1	1	125	0.226248	1596	19.5	14.5
1316	1185	Cockerham Oswald	1	1	1	775	0.927234	2430.4	27	15.5
1317	1185	Cockerham Oswald	1	1	1	325	0.63006	1316	30.5	12
1318	881	Cockerham Oswald	1	1	1	0	0.792643	1960	30	19.5
1319	1062	Cockerham 1196	1	1	1	475	0.804718	884.8	25.5	17
1321	541	Cockerham Oswald	1	1	1	0	0.918258	1954.4	31.5	17
1322	374	Cockerham Alf	1	1	1	250	0.851921	1456	25.5	20
1323	1098	Cockerham Alf	1	1	1	1650	0.389346	1086.4	26.5	21
1324	879	Cockerham Oswald	1	2	1	NA	0.788432	1898.4	27	19.5
1325	879	Cockerham Oswald	1	2	1	50	NA	2318.4	28	16
1327	522	Cockerham Alf	1	1	1	350	0.615817	2251.2	28.5	19
1329	885	Cockerham Oswald	1	1	1	NA	0.516981	1551.2	25	18.5
1331	792	Cockerham Alf	1	1	1	50	0.165389	2346.4	28	21
1332	876	Cockerham Oswald	1	1	1	850	0.648969	2273.6	30	13
1336	814	Cockerham Oswald	1	1	1	50	0.881535	1187.2	26	23
1337	791	Cockerham Alf	1	1	1	250	0.231361	2044	24	17
1339	822	Cockerham Kevin	1	1	1	25	0.780831	1618.4	23.5	20
1340	533	Cockerham Alf	1	1	1	0	0.974263	0	23.5	16.5
1341	790	Cockerham Alf	1	1	1	175	0.667539	2895.2	26	17
1342	1089	Cockerham Kevin	1	1	1	25	0.771234	1276.8	24	21.5

1344	477	Cockerham Oswald	1	2	1	NA	0.552128	1394.4	28.5	19.5
1346	1069	Yorkvale Chaz	1	1	1	50	0.660435	1080.8	13.5	16
1348	1218	Yorkvale Chaz	1	2	1	125	0.719138	336	23.5	11.5
1349	1218	Yorkvale Chaz	1	2	1	200	1.053032	1853.6	28	13
1350	830	Cockerham Oswald	1	1	1	NA	0.898952	0	24.5	18.5
1351	386	Yorkvale Chaz	1	2	1	75	0.716794	1332.8	28.5	16
1352	556	Yorkvale Chaz	1	1	1	0	0.374525	1092	32	17
1354	201	Cockerham Oswald	1	1	1	50	0.719885	1360.8	27.5	19.5
1355	1119	Cockerham Oswald	1	1	1	200	0.973553	1579.2	18	14.5
1360	Rosie	Cockerham Alf	1	1	1	575	0.419268	1545.6	31	15
1361	374	Cockerham Alf	1	1	1	50	0.899372	0	29.5	17
1363	522	Cockerham Alf	1	1	1	25	0.977504	560	27.5	18.5
1364	838	Cockerham Alf	1	1	1	NA	0.580919	1456	23	17.5
1365	Gaia	Cockerham Alf	1	1	1	NA	0.614071	1349.6	14	21
1366	Smashiedale	Cockerham Alf	1	2	1	225	0.521763	2284.8	33.5	17.5
1368	1099	Cockerham Alf	2	2	1	NA	0.894811	952	25.5	15.5
1369	1099	Cockerham Alf	1	2	1	175	0.440002	2721.6	28	13.5
1370	Darkendale	Cockerham Alf	1	1	1	225	0.544808	1932	29.5	22.5
1375	274	Cockerham Colin	1	1	1	0	0.785683	716.8	25.5	15.5
1376	501	Cockerham Colin	1	1	1	1175	0.971557	2699.2	NA	12
1377	1153	Cockerham Colin	1	1	1	NA	0.619714	1859.2	26	15.5
1378	1217	Cockerham Colin	1	1	1	NA	0.831931	845.6	25.5	13.5
1379	1217	Cockerham Colin	1	1	1	NA	1.056272	2973.6	25.5	17
1380	449	Cockerham Colin	1	2	1	150	1.059762	1590.4	31	12
1381	449	Cockerham Colin	1	2	1	75	0.787188	2167.2	30	12.5
1382	866	Cockerham Colin	1	2	1	75	0.836453	996.8	33.5	15
1383	866	Cockerham Colin	1	2	1	NA	1.037659	1668.8	26.5	15
1384	NA	NA	1	1	1	NA	0.716597	901.6	20.5	10
1385	NA	NA	1	1	1	1675	NA	2710.4	26.5	9.5
1386	NA	NA	1	1	1	125	0.603286	2637.6	29.5	15
1387	NA	NA	1	1	1	25	0.426089	2637.6	35.5	17
1388	NA	NA	1	1	1	NA	0.278183	1187.2	22	8
1389	NA	NA	1	1	1	NA	0.428616	1405.6	22.5	7.5
1431	463	Cockerham Kevin	1	1	1	NA	0.716562	974.4	37	26.5
1432	375	Cockerham Kevin	1	2	1	NA	0.779086	1573.6	24.5	18
1433	NA	NA	1	1	1	NA	0.815978	1853.6	29.5	17.5
1456	805	Yorkvale Chaz	1	1	1	NA	0.342416	1528.8	25.5	16.5
1457	182	Yorkvale Chaz	1	1	1	NA	0.621232	2284.8	26.5	14.5
1458	536	Yorkvale Chaz	2	2	1	NA	0.411809	1892.8	25	25
1459	536	Yorkvale Chaz	1	2	1	NA	NA	NA	NA	17
1460	829	Yorkvale Chaz	2	1	1	NA	0.447998	2055.2	29.5	18.5
1461	brown/white	Yorkvale Chaz	1	1	1	NA	0.375664	2497.6	28	13
1462	1274	Yorkvale Chaz	1	1	1	0	0.200137	2055.2	25	20
1463	1071	Yorkvale Chaz	1	2	1	225	0.855894	1708	21	16.5
1464	1071	Yorkvale Chaz	1	2	1	0	0.490724	2060.8	23.5	15
1507	458	Cockerham Oswald	1	1	1	250	0.441609	2251.2	20	13
1508	776	Yorkvale Chaz	1	1	1	25	0.491964	2559.2	25	18.5

1509	1279	Cockerham Colin	1	2	1	50	0.971204	672	24	17
1511	149	Cockerham Oswald	1	1	1	75	0.943143	879.2	27.5	14
1512	499	Cockerham Oswald	1	1	1	475	0.781817	1321.6	23.5	9.5
1513	1061	Cockerham Oswald	1	1	1	NA	0.727841	1741.6	23.5	13
1514	362	Yorkvale Chaz	1	1	1	NA	0.560254	1064	22	20
1515	399	Cockerham Oswald	1	2	1	NA	0.244487	240.8	23.5	18
1516	399	Cockerham Oswald	1	2	1	50	0.32087	1724.8	27	22.5
1517	1063	Cockerham Oswald	1	1	1	100	0.77055	1713.6	24	12
1518	149	Cockerham Oswald	1	1	1	100	0.912427	1500.8	26.5	15
1519	274	Cockerham Colin	1	1	1	0	1.099282	784	24.5	16
1521	501	Cockerham Colin	1	1	1	325	0.688192	1478.4	26	16
1522	874	Cockerham Oswald	1	1	1	25	0.867691	1674.4	34.5	17.5
1523	874	Cockerham Oswald	1	2	1	100	0.882195	1808.8	28.5	16.5
1525	770	Cockerham Oswald	1	2	1	850	0.875873	2340.8	26.5	13
1526	553	Yorkvale Chaz	1	1	1	625	0.946097	0	22	12.5
1527	182	Yorkvale Chaz	1	1	1	175	0.586295	1926.4	22.5	15
1529	864	Cockerham Colin	1	1	1	175	1.026638	789.6	24	15.5
1530	179	Cockerham Oswald	1	1	1	275	0.566738	464.8	26.5	10.5
1531	1081	Yorkvale Chaz	1	1	1	25	0.490519	2060.8	24.5	22.5
1532	166	Cockerham Oswald	1	1	1	400	0.856143	2828	26.5	12
1533	GHT	Cockerham Colin	1	1	1	125	0.504242	991.2	35	20
1534	1159	Cockerham Oswald	1	1	1	NA	NA	NA	NA	13.5
1535	495	Yorkvale Chaz	1	1	1	NA	0.54025	NA	29	17
1536	176	Yorkvale Chaz	1	1	1	350	0.483533	1450.4	27.5	20.5
1537	439	Yorkvale Chaz	1	2	1	50	0.80389	1752.8	26.5	16.5
1539	brown/white	Yorkvale Chaz	1	1	1	0	0.460227	1528.8	30.5	12
1540	158	Cockerham Oswald	1	1	1	300	0.77211	1836.8	24	13.5
1541	198	Yorkvale Chaz	1	1	1	200	0.412643	733.6	32	20
1543	833	Cockerham Oswald	1	1	1	25	0.516335	2122.4	27.5	17.5
1544	1216	Yorkvale Chaz	1	1	1	0	0.975767	907.2	31.5	20.5
1545	159	Bispham Oswald	1	2	1	125	0.533396	380.8	22	11.5
1546	159	Bispham Oswald	1	2	1	375	0.952944	274.4	23	11
1547	1186	Cockerham Oswald	1	1	1	NA	0.15686	1926.4	27	21.5
1548	1278	Cockerham Colin	1	2	1	NA	0.870914	1663.2	23.5	9
1549	1278	Cockerham Colin	1	2	1	2025	0.693485	1820	23	8.5
1550	NA	NA	1	1	1	100	0.222058	1954.4	25.5	13.5
1551	NA	NA	1	1	1	200	0.814237	610.4	27	11
1552	NA	NA	1	1	1	50	0.312699	1310.4	31.5	19
1553	NA	NA	1	1	1	125	0.915068	268.8	30	21
1554	NA	NA	1	1	1	0	0.783433	599.2	29	15
1611	Frankendale	Cockerham Alf	1	1	1	NA	0.921687	1909.6	32	19
1612	469	Cockerham Alf	1	1	1	425	0.751078	1075.2	30.5	17
1613	472	Cockerham Alf	1	1	1	NA	0.197489	1265.6	28.5	29.5
1614	NA	NA	1	3	1	0	0.343105	1596	23.5	19
1621	216	Cockerham Oswald	1	1	1	0	0.979526	1993.6	NA	9
1622	499	Cockerham Oswald	1	1	1	100	0.884253	1176	29.5	15
1623	1061	Cockerham Oswald	2	1	1	NA	NA	NA	NA	11.5

1624	367	Cockerham Oswald	2	2	1	450	0.55931	2038.4	18.5	12
1625	367	Cockerham Oswald	1	2	1	300	0.797682	459.2	26.5	12
1626	179	Cockerham Oswald	1	1	1	50	0.921916	1736	27	10
1627	166	Cockerham Oswald	1	1	1	50	0.938114	1047.2	27.5	10.5
1628	1159	Cockerham Oswald	1	1	1	225	0.906223	1248.8	28	10
1629	806	Cockerham Oswald	1	2	1	150	0.189031	3175.2	20	12.5
1630	806	Cockerham Oswald	1	2	1	NA	0.863213	1758.4	23.5	15
1631	1120	Cockerham Oswald	1	2	1	NA	0.910738	873.6	19	12
1632	1120	Cockerham Oswald	1	2	1	425	0.701605	666.4	21.5	11.5
1633	1280	Cockerham Oswald	1	1	1	250	0.807134	1864.8	28	15
1634	158	Cockerham Oswald	1	1	1	NA	1.052561	1400	22.5	12
1636	841	Bispham Oswald	1	1	1	125	1.126133	319.2	30.5	12
1638	NA	NA	1	1	1	NA	NA	0	23	14
1642	1177	Cockerham Oswald	1	2	1	25	0.87051	2150.4	25	11.5
1643	881	Cockerham Oswald	1	1	1	NA	0.365593	1271.2	36.5	23
1644	883	Cockerham Oswald	1	1	1	0	0.821825	1271.2	36	18
1645	551	Cockerham Oswald	1	1	1	50	0.500171	1164.8	32	19.5
1646	541	Cockerham Oswald	1	1	1	NA	0.84715	946.4	30	20.5
1647	880	Cockerham Oswald	1	1	1	175	0.815791	2514.4	25.5	16
1648	795	Cockerham Oswald	1	1	1	200	0.385991	1646.4	27.5	17
1649	885	Cockerham Oswald	1	1	1	25	0.514493	1013.6	19	15.5
1650	876	Cockerham Oswald	1	1	1	100	0.635704	1573.6	26.5	13
1651	820	Cockerham Oswald	1	1	1	50	0.353767	2368.8	27	16.5
1652	807	Cockerham Oswald	1	1	1	50	0.610115	2245.6	27.5	17.5
1653	1114	Cockerham Oswald	1	1	1	NA	0.668343	1293.6	24	18
1654	1060	Cockerham Oswald	1	1	1	175	0.956895	1887.2	34.5	20.5
1555	NA	NA	1	1	1	NA	0.538947	2727.2	30.5	8.5
1556	NA	NA	1	1	1	25	0.93012	2777.6	25.5	23
1390	NA	NA	1	1	1	0	0.162523	985.6	28	8.5
1391	NA	NA	1	1	1	275	0.440491	1976.8	NA	10
1211	386	Yorkvale Chaz	1	1	2	425	0.75443	1831.2	20.5	14.5
1212	556	Yorkvale Chaz	1	1	2	3250	0.860675	1775.2	23.5	19.5
1213	1279	Yorkvale Chaz	1	1	2	3650	0.990064	2060.8	22.5	19.5
1214	1167	Yorkvale Chaz	1	1	2	5700	0.536866	1797.6	25.5	19.75
1225	375	Cockerham Kevin	1	2	2	150	NA	868	25	22.25
1248	769	Cockerham Oswald	2	2	2	3600	0.098088	1506.4	25	27
1249	769	Cockerham Oswald	1	2	2	950	0.772673	761.6	22	11.5
1250	201	Cockerham Oswald	1	1	2	0	0.584348	1159.2	24.5	19.5
1251	1119	Cockerham Oswald	1	1	2	225	0.849734	1394.4	21.5	17
1253	409	Cockerham Oswald	1	1	2	NA	NA	823.2	22	19
1296	476	Cockerham 1196	1	1	2	725	0.972583	2492	28.5	33.5
1297	1163	Cockerham 1196	1	2	2	850	1.016809	901.6	23.5	16
1299	396	Cockerham 1196	1	2	2	9050	0.622068	1612.8	28.5	26.5
1300	396	Cockerham 1196	1	2	2	1500	0.506308	2536.8	24	21.5
1315	796	Cockerham Oswald	1	1	2	6050	0.512195	1657.6	24	13.5
1316	1185	Cockerham Oswald	1	1	2	11550	0.932733	526.4	23	17
1317	1185	Cockerham Oswald	1	1	2	4650	0.643583	1926.4	24.5	13.75

1318	881	Cockerham Oswald	1	1	2	4150	0.774615	1293.6	26	22.5
1319	1062	Cockerham 1196	1	1	2	2175	0.62069	1428	23	19
1321	541	Cockerham Oswald	1	1	2	200	0.853207	NA	28	14
1322	374	Cockerham Alf	1	1	2	2625	0.740145	2699.2	24.5	23
1323	1098	Cockerham Alf	1	1	2	250	0.377121	1831.2	27	24.5
1324	879	Cockerham Oswald	1	2	2	8675	0.971405	3136	24.5	17.75
1325	879	Cockerham Oswald	1	2	2	7225	0.336978	1909.6	23.5	15.25
1327	522	Cockerham Alf	1	1	2	1575	1.229605	235.2	25	22.5
1329	885	Cockerham Oswald	1	1	2	4000	0.734487	2979.2	28.5	20.5
1331	792	Cockerham Alf	1	1	2	700	0.681262	1708	27.5	23.25
1332	876	Cockerham Oswald	1	1	2	NA	NA	NA	NA	NA
1336	814	Cockerham Oswald	1	1	2	NA	0.887226	2497.6	25	18
1337	791	Cockerham Alf	1	1	2	NA	0.362697	2598.4	26.5	18.5
1339	822	Cockerham Kevin	1	1	2	NA	0.850789	1696.8	22.5	20.5
1340	533	Cockerham Alf	1	1	2	NA	0.805165	2531.2	17	17.8
1341	790	Cockerham Alf	1	1	2	NA	0.422956	201.6	26.5	14.5
1342	1089	Cockerham Kevin	1	1	2	1925	0.598246	3572.8	NA	22.25
1344	477	Cockerham Oswald	1	2	2	4250	0.620947	2284.8	28.5	21.5
1346	1069	Yorkvale Chaz	1	1	2	300	0.530576	2340.8	27.5	20.5
1348	1218	Yorkvale Chaz	1	2	2	NA	0.566547	1041.6	27.5	27.5
1349	1218	Yorkvale Chaz	1	2	2	925	0.948924	772.8	25	14.5
1350	830	Cockerham Oswald	1	1	2	1100	0.800025	733.6	25	22.75
1351	386	Yorkvale Chaz	1	2	2	3100	0.649354	2245.6	21	17
1352	556	Yorkvale Chaz	1	1	2	NA	0.626349	610.4	27.5	21.5
1354	201	Cockerham Oswald	1	1	2	2475	NA	268.8	NA	22
1355	1119	Cockerham Oswald	1	1	2	500	0.579914	1825.6	24	17
1360	Rosie	Cockerham Alf	1	1	2	3225	0.28777	140	25.5	17.5
1361	374	Cockerham Alf	1	1	2	4825	0.854519	2352	29	17.5
1363	522	Cockerham Alf	1	1	2	2525	0.98967	2189.6	26.5	21.5
1364	838	Cockerham Alf	1	1	2	3525	0.732239	638.4	25.5	19.5
1365	Gaia	Cockerham Alf	1	1	2	12600	0.514838	1478.4	26	18.5
1366	Smashiedale	Cockerham Alf	1	2	2	100	0.665136	1708	33.5	14.25
1368	1099	Cockerham Alf	2	2	2	NA	0.473458	1008	24	16.5
1369	1099	Cockerham Alf	1	2	2	2025	0.395683	2273.6	24	13.25
1370	Darkendale	Cockerham Alf	1	1	2	9500	0.713056	2671.2	30.5	20.25
1375	274	Cockerham Colin	1	1	2	1825	NA	868	NA	17.75
1376	501	Cockerham Colin	1	1	2	9000	NA	1209.6	22.5	10.5
1377	1153	Cockerham Colin	1	1	2	4150	0.449354	3421.6	27	14
1378	1217	Cockerham Colin	1	1	2	NA	NA	NA	NA	NA
1379	1217	Cockerham Colin	1	1	2	2650	1.057611	2178.4	24	16.75
1380	449	Cockerham Colin	1	2	2	6100	0.725395	2346.4	20	14.25
1381	449	Cockerham Colin	1	2	2	2075	NA	481.6	26	16.5
1382	866	Cockerham Colin	1	2	2	3200	0.825456	3029.6	31	19.25
1383	866	Cockerham Colin	1	2	2	1700	1.029268	1142.4	NA	17.5
1384	NA	NA	1	1	2	NA	0.50771	1904	19.5	10.5
1385	NA	NA	1	1	2	1625	0.785534	3040.8	22.5	13
1386	NA	NA	1	1	2	NA	0.723297	235.2	26	16

1387	NA	NA	1	1	2	300	0.277924	3091.2	29.5	20
1388	NA	NA	1	1	2	NA	NA	NA	NA	NA
1389	NA	NA	1	1	2	NA	NA	NA	NA	NA
1431	463	Cockerham Kevin	1	1	2	NA	0.636669	2492	30.5	26.5
1432	375	Cockerham Kevin	1	2	2	2950	NA	1288	26	21.5
1433	NA	NA	1	1	2	NA	0.678441	2324	28.5	17.5
1456	805	Yorkvale Chaz	1	1	2	0	NA	2307.2	26	20.25
1457	182	Yorkvale Chaz	1	1	2	0	0.197365	4127.2	22	12
1458	536	Yorkvale Chaz	2	2	2	75	0.18587	1052.8	31	28.5
1459	536	Yorkvale Chaz	1	2	2	NA	0.857277	2032.8	21	17
1460	829	Yorkvale Chaz	2	1	2	175	0.334455	2430.4	27.5	21.5
1461	brown/white	Yorkvale Chaz	1	1	2	2575	NA	1097.6	26.5	15.5
1462	1274	Yorkvale Chaz	1	1	2	1300	NA	2660	22	20.25
1463	1071	Yorkvale Chaz	1	2	2	25	0.4631	2962.4	17	14
1464	1071	Yorkvale Chaz	1	2	2	2025	0.647168	739.2	20	13.5
1507	458	Cockerham Oswald	1	1	2	0	0.387902	1041.6	21	10
1508	776	Yorkvale Chaz	1	1	2	3475	0.846676	2671.2	23	17
1509	1279	Cockerham Colin	1	2	2	NA	0.811539	1545.6	21	19.5
1511	149	Cockerham Oswald	1	1	2	NA	0.59646	4732	27.5	15.75
1512	499	Cockerham Oswald	1	1	2	NA	0.610435	1691.2	21.5	12
1513	1061	Cockerham Oswald	1	1	2	2150	0.515539	2161.6	24.5	14.75
1514	362	Yorkvale Chaz	1	1	2	5025	0.477295	1646.4	20	17.25
1515	399	Cockerham Oswald	1	2	2	1325	0.325082	2637.6	21	24.5
1516	399	Cockerham Oswald	1	2	2	5450	0.363013	268.8	27	26.5
1517	1063	Cockerham Oswald	1	1	2	5325	0.729164	1864.8	20.5	11.5
1518	149	Cockerham Oswald	1	1	2	6800	0.817662	2200.8	25.5	16.15
1519	274	Cockerham Colin	1	1	2	11050	0.845345	1467.2	23	15
1521	501	Cockerham Colin	1	1	2	2925	0.554003	901.6	22.5	13.5
1522	874	Cockerham Oswald	1	1	2	5050	0.566381	1372	25.5	20.5
1523	874	Cockerham Oswald	1	2	2	3850	0.890464	1360.8	22.5	16.5
1525	770	Cockerham Oswald	1	2	2	625	0.636976	2144.8	21	17
1526	553	Yorkvale Chaz	1	1	2	1525	0.781859	240.8	24	13.5
1527	182	Yorkvale Chaz	1	1	2	8900	0.718374	1556.8	20	14.5
1529	864	Cockerham Colin	1	1	2	NA	1.046187	974.4	20	17.3
1530	179	Cockerham Oswald	1	1	2	600	0.882744	NA	22	13.5
1531	1081	Yorkvale Chaz	1	1	2	NA	0.567989	1999.2	24	24
1532	166	Cockerham Oswald	1	1	2	9675	0.338258	1215.2	19	12
1533	GHT	Cockerham Colin	1	1	2	NA	0.504093	2520	23.5	22.5
1534	1159	Cockerham Oswald	1	1	2	NA	0.324416	1153.6	13.5	12.5
1535	495	Yorkvale Chaz	1	1	2	NA	0.40161	2060.8	23.5	15.75
1536	176	Yorkvale Chaz	1	1	2	8725	0.589446	464.8	24.5	21
1537	439	Yorkvale Chaz	1	2	2	NA	0.902708	1937.6	30.5	19.5
1539	brown/white	Yorkvale Chaz	1	1	2	NA	1.143209	2038.4	23.5	12.5
1540	158	Cockerham Oswald	1	1	2	NA	0.852714	599.2	21	15.5
1541	198	Yorkvale Chaz	1	1	2	2850	0.913118	2133.6	24	25
1543	833	Cockerham Oswald	1	1	2	NA	1.022187	3589.6	29	21.5
1544	1216	Yorkvale Chaz	1	1	2	8100	0.558106	784	27	22.5

1545	159	Bispham Oswald	1	2	2	NA	0.41887	1192.8	27	15
1546	159	Bispham Oswald	1	2	2	NA	0.637016	414.4	23.5	13.5
1547	1186	Cockerham Oswald	1	1	2	4225	NA	2016	21	25
1548	1278	Cockerham Colin	1	2	2	NA	0.764132	2592.8	20	9
1549	1278	Cockerham Colin	1	2	2	875	0.93429	1528.8	NA	10
1550	NA	NA	1	1	2	425	0.913373	1803.2	23	16
1551	NA	NA	1	1	2	1800	NA	NA	NA	13.5
1552	NA	NA	1	1	2	450	NA	NA	NA	21.5
1553	NA	NA	1	1	2	NA	0.911675	1971.2	25	24
1554	NA	NA	1	1	2	NA	0.991392	NA	24.5	19.25
1611	Frankendale	Cockerham Alf	1	1	2	6475	1.023437	2251.2	31	20
1612	469	Cockerham Alf	1	1	2	1850	0.814347	420	27.5	19.5
1613	472	Cockerham Alf	1	1	2	0	0.438464	2402.4	28	35
1614	NA	NA	1	3	2	1925	0.641894	784	22.5	22.5
1621	216	Cockerham Oswald	1	1	2	1600	NA	3785.6	25.5	12.5
1622	499	Cockerham Oswald	1	1	2	NA	1.12109	1971.2	22	18.3
1623	1061	Cockerham Oswald	2	1	2	7525	0.780066	2962.4	18	12.5
1624	367	Cockerham Oswald	2	2	2	450	0.53802	1775.2	23	14.25
1625	367	Cockerham Oswald	1	2	2	1875	0.723696	2178.4	21.5	14.25
1626	179	Cockerham Oswald	1	1	2	175	0.876758	2391.2	24	11.75
1627	166	Cockerham Oswald	1	1	2	1475	0.865466	2979.2	27	13.5
1628	1159	Cockerham Oswald	1	1	2	13850	0.630584	1282.4	28.5	10
1629	806	Cockerham Oswald	1	2	2	NA	0.728597	1052.8	21	11.5
1630	806	Cockerham Oswald	1	2	2	NA	1.05192	1439.2	25.5	17.5
1631	1120	Cockerham Oswald	1	2	2	3075	0.893831	1920.8	NA	12.5
1632	1120	Cockerham Oswald	1	2	2	1425	0.995891	1892.8	25	13.5
1633	1280	Cockerham Oswald	1	1	2	3000	0.630273	1898.4	22.5	18.5
1634	158	Cockerham Oswald	1	1	2	NA	0.577161	2335.2	24.5	13.5
1636	841	Bispham Oswald	1	1	2	4750	NA	NA	NA	13
1638	NA	NA	1	1	2	NA	NA	NA	18.5	14
1642	1177	Cockerham Oswald	1	2	2	175	0.923726	1825.6	27.5	13.5
1643	881	Cockerham Oswald	1	1	2	NA	0.706794	1624	30.5	23.5
1644	883	Cockerham Oswald	1	1	2	3675	0.914313	2016	29	18.75
1645	551	Cockerham Oswald	1	1	2	NA	0.868183	2156	30	22.5
1646	541	Cockerham Oswald	1	1	2	NA	0.860974	1327.2	31.5	22.3
1647	880	Cockerham Oswald	1	1	2	2175	0.964067	1545.6	23	14
1648	795	Cockerham Oswald	1	1	2	NA	0.509683	1243.2	26	17.8
1649	885	Cockerham Oswald	1	1	2	675	0.732183	1316	19	19
1650	876	Cockerham Oswald	1	1	2	9800	0.594642	2144.8	29	10.5
1651	820	Cockerham Oswald	1	1	2	2325	0.401263	2223.2	24	20
1652	807	Cockerham Oswald	1	1	2	6125	0.669281	117.6	26	20
1653	1114	Cockerham Oswald	1	1	2	3800	0.669396	2083.2	23.5	15
1654	1060	Cockerham Oswald	1	1	2	3825	NA	2682.4	34	21.5
1555	NA	NA	1	1	2	NA	0.582783	4916.8	29	8.5
1556	NA	NA	1	1	2	25	0.46858	3309.6	23.5	20.5
1390	NA	NA	1	1	2	1150	0.352397	733.6	23.5	19.5
1391	NA	NA	1	1	2	NA	0.076535	NA	NA	13.5

1211	386	Yorkvale Chaz	1	1	3	0	0.808163	1394.4	19	18.5
1212	556	Yorkvale Chaz	1	1	3	375	0.463177	744.8	23	20
1213	1279	Yorkvale Chaz	1	1	3	400	NA	873.6	25	20
1214	1167	Yorkvale Chaz	1	1	3	0	0.501442	2721.6	30.5	20.5
1225	375	Cockerham Kevin	1	2	3	NA	0.505562	2251.2	28.5	25
1248	769	Cockerham Oswald	2	2	3	0	0.144221	784	24	28
1249	769	Cockerham Oswald	1	2	3	0	0.292813	2307.2	23	12.5
1250	201	Cockerham Oswald	1	1	3	0	0.342604	980	24.5	20
1251	1119	Cockerham Oswald	1	1	3	NA	0.385166	879.2	28.5	18
1253	409	Cockerham Oswald	1	1	3	100	0.713804	1204	23.75	20.5
1296	476	Cockerham 1196	1	1	3	NA	0.474535	1456	24	34
1297	1163	Cockerham 1196	1	2	3	1350	0.519521	1136.8	21.5	19.5
1299	396	Cockerham 1196	1	2	3	NA	NA	NA	NA	NA
1300	396	Cockerham 1196	1	2	3	525	0.637365	403.2	30	22.5
1315	796	Cockerham Oswald	1	1	3	25	NA	207.2	23.5	17.5
1316	1185	Cockerham Oswald	1	1	3	0	0.954987	1635.2	17.5	18
1317	1185	Cockerham Oswald	1	1	3	0	0.339254	392	26	17
1318	881	Cockerham Oswald	1	1	3	3300	0.550213	16.8	22.5	26.5
1319	1062	Cockerham 1196	1	1	3	200	0.384206	1909.6	24	21
1321	541	Cockerham Oswald	1	1	3	NA	0.659769	2749.6	22	17.5
1322	374	Cockerham Alf	1	1	3	0	0.541916	1153.6	22	24.5
1323	1098	Cockerham Alf	1	1	3	NA	0.5252	1747.2	25.5	27
1324	879	Cockerham Oswald	1	2	3	50	NA	3315.2	28	21
1325	879	Cockerham Oswald	1	2	3	0	0.352263	716.8	26.5	18
1327	522	Cockerham Alf	1	1	3	NA	NA	50.4	29.5	25.5
1329	885	Cockerham Oswald	1	1	3	350	0.39929	1836.8	28.5	21.5
1331	792	Cockerham Alf	1	1	3	0	0.422431	16.8	30	26
1332	876	Cockerham Oswald	1	1	3	NA	NA	NA	NA	NA
1336	814	Cockerham Oswald	1	1	3	175	0.346312	604.8	21	21.5
1337	791	Cockerham Alf	1	1	3	25	0.220437	1064	23.5	19.5
1339	822	Cockerham Kevin	1	1	3	0	0.531628	604.8	16.25	22
1340	533	Cockerham Alf	1	1	3	100	0.460432	884.8	28	21
1341	790	Cockerham Alf	1	1	3	0	0.46428	308	25	22
1342	1089	Cockerham Kevin	1	1	3	0	0.300799	571.2	25	21
1344	477	Cockerham Oswald	1	2	3	NA	0.170911	957.6	24.5	23
1346	1069	Yorkvale Chaz	1	1	3	NA	0.448252	1848	30.5	22
1348	1218	Yorkvale Chaz	1	2	3	25	0.339514	1612.8	22	18.5
1349	1218	Yorkvale Chaz	1	2	3	0	0.399119	436.8	26	19.5
1350	830	Cockerham Oswald	1	1	3	275	0.560254	750.4	24.5	24.5
1351	386	Yorkvale Chaz	1	2	3	0	0.255413	2660	24	20
1352	556	Yorkvale Chaz	1	1	3	50	0.285744	1870.4	31.5	24.5
1354	201	Cockerham Oswald	1	1	3	0	0.265181	2324	27	24
1355	1119	Cockerham Oswald	1	1	3	0	0.519711	845.6	20.5	21
1360	Rosie	Cockerham Alf	1	1	3	NA	0.194987	2234.4	26	19.5
1361	374	Cockerham Alf	1	1	3	325	0.314586	1064	25	18.5
1363	522	Cockerham Alf	1	1	3	0	0.923469	1758.4	26	23.5
1364	838	Cockerham Alf	1	1	3	1125	0.748876	319.2	30	21.5

1365	Gaia	Cockerham Alf	1	1	3	825	0.85679	459.2	25.5	22.5
1366	Smashiedale	Cockerham Alf	1	2	3	100	0.467037	1713.6	25.5	19.5
1368	1099	Cockerham Alf	2	2	3	NA	0.165225	257.6	31	19
1369	1099	Cockerham Alf	1	2	3	NA	0.228261	476	27	16
1370	Darkendale	Cockerham Alf	1	1	3	NA	0.471364	235.2	22	23
1375	274	Cockerham Colin	1	1	3	NA	0.638193	1276.8	22	20
1376	501	Cockerham Colin	1	1	3	NA	0.709955	1601.6	25	14.5
1377	1153	Cockerham Colin	1	1	3	NA	0.451518	1310.4	12.5	16.5
1378	1217	Cockerham Colin	1	1	3	NA	NA	NA	NA	NA
1379	1217	Cockerham Colin	1	1	3	0	0.483585	NA	23.5	20.5
1380	449	Cockerham Colin	1	2	3	325	0.638022	3656.8	27	17.5
1381	449	Cockerham Colin	1	2	3	300	0.455413	504	28	18
1382	866	Cockerham Colin	1	2	3	1125	0.788723	1304.8	25.5	21
1383	866	Cockerham Colin	1	2	3	575	1.012815	1260	29.5	18.5
1384	NA	NA	1	1	3	0	0.187886	2256.8	27	14.5
1385	NA	NA	1	1	3	0	0.66428	935.2	27.5	16.5
1386	NA	NA	1	1	3	50	0.414663	2844.8	25	18.5
1387	NA	NA	1	1	3	0	0.3751	364	22	21.5
1388	NA	NA	1	1	3	NA	NA	NA	NA	NA
1389	NA	NA	1	1	3	NA	NA	NA	NA	NA
1431	463	Cockerham Kevin	1	1	3	NA	0.442446	3796.8	27.5	27
1432	375	Cockerham Kevin	1	2	3	75	0.424357	705.6	24.5	21.5
1433	NA	NA	1	1	3	50	0.564365	2732.8	23	19.5
1456	805	Yorkvale Chaz	1	1	3	NA	0.58014	NA	28	20
1457	182	Yorkvale Chaz	1	1	3	25	0.58426	2402.4	20.5	17.5
1458	536	Yorkvale Chaz	2	2	3	0	0.206428	1181.6	30	29
1459	536	Yorkvale Chaz	1	2	3	NA	0.689869	2962.4	25.5	19
1460	829	Yorkvale Chaz	2	1	3	0	0.481671	1842.4	31	24.5
1461	brown/white	Yorkvale Chaz	1	1	3	500	0.179726	2111.2	26.5	18.5
1462	1274	Yorkvale Chaz	1	1	3	150	0.380213	NA	25	22.5
1463	1071	Yorkvale Chaz	1	2	3	75	0.316646	1579.2	26	17
1464	1071	Yorkvale Chaz	1	2	3	0	1.154676	543.2	23.5	18.5
1507	458	Cockerham Oswald	1	1	3	75	0.463842	1338.4	15.5	13
1508	776	Yorkvale Chaz	1	1	3	125	0.278327	3136	27	19.5
1509	1279	Cockerham Colin	1	2	3	150	0.521295	1069.6	21.5	21
1511	149	Cockerham Oswald	1	1	3	0	0.36266	50.4	27	20.5
1512	499	Cockerham Oswald	1	1	3	0	0.573741	442.4	25.5	15
1513	1061	Cockerham Oswald	1	1	3	375	0.428303	3365.6	25	17.5
1514	362	Yorkvale Chaz	1	1	3	50	0.310878	1058.4	27.5	23
1515	399	Cockerham Oswald	1	2	3	75	0.4711	16.8	25.5	25.5
1516	399	Cockerham Oswald	1	2	3	NA	0.423469	862.4	2	29.5
1517	1063	Cockerham Oswald	1	1	3	0	0.580392	2178.4	19	12.5
1518	149	Cockerham Oswald	1	1	3	275	0.473159	1388.8	21.5	18.5
1519	274	Cockerham Colin	1	1	3	125	0.696983	2503.2	21	20
1521	501	Cockerham Colin	1	1	3	NA	0.485991	1954.4	18	15.5
1522	874	Cockerham Oswald	1	1	3	0	0.561978	420	29.5	21.5
1523	874	Cockerham Oswald	1	2	3	900	0.74094	2912	21	18

1525	770	Cockerham Oswald	1	2	3	100	0.779566	2122.4	28	19
1526	553	Yorkvale Chaz	1	1	3	300	0.385661	1500.8	21	17
1527	182	Yorkvale Chaz	1	1	3	NA	0.289042	1590.4	20	20
1529	864	Cockerham Colin	1	1	3	0	NA	2525.6	22	18.5
1530	179	Cockerham Oswald	1	1	3	50	0.200682	5.6	33	17
1531	1081	Yorkvale Chaz	1	1	3	625	0.372953	621.6	25	25
1532	166	Cockerham Oswald	1	1	3	450	0.418418	2626.4	25	16
1533	GHT	Cockerham Colin	1	1	3	0	0.296505	358.4	23.5	26
1534	1159	Cockerham Oswald	1	1	3	25	0.515948	1489.6	29.5	18
1535	495	Yorkvale Chaz	1	1	3	0	NA	2122.4	21	20
1536	176	Yorkvale Chaz	1	1	3	NA	0.141342	1730.4	25.5	22
1537	439	Yorkvale Chaz	1	2	3	0	0.549032	67.2	30.5	20
1539	brown/white	Yorkvale Chaz	1	1	3	125	0.561466	1579.2	26	16
1540	158	Cockerham Oswald	1	1	3	0	0.513219	NA	22.5	20.5
1541	198	Yorkvale Chaz	1	1	3	0	0.638855	84	26.5	28
1543	833	Cockerham Oswald	1	1	3	225	0.303017	397.6	25.5	23.5
1544	1216	Yorkvale Chaz	1	1	3	200	0.616457	593.6	32	24.5
1545	159	Bispham Oswald	1	2	3	100	0.292748	NA	29.5	17
1546	159	Bispham Oswald	1	2	3	75	0.348492	2055.2	29	13.5
1547	1186	Cockerham Oswald	1	1	3	0	0.212955	660.8	28.5	27
1548	1278	Cockerham Colin	1	2	3	NA	0.459849	1265.6	22.5	12.5
1549	1278	Cockerham Colin	1	2	3	0	0.428128	1668.8	25	12
1550	NA	NA	1	1	3	325	0.343076	408.8	28.5	18
1551	NA	NA	1	1	3	50	0.580036	414.4	25	17
1552	NA	NA	1	1	3	NA	0.274486	2251.2	25.5	25.5
1553	NA	NA	1	1	3	1925	0.851076	1584.8	27	26.5
1554	NA	NA	1	1	3	0	0.918039	492.8	26	21
1611	Frankendale	Cockerham Alf	1	1	3	NA	0.880645	28	NA	24
1612	469	Cockerham Alf	1	1	3	250	0.425575	1220.8	26.5	20.5
1613	472	Cockerham Alf	1	1	3	0	0.278602	896	30.5	34
1614	NA	NA	1	3	3	0	0.37545	1344	21.5	23
1621	216	Cockerham Oswald	1	1	3	0	0.519833	1467.2	25.5	14
1622	499	Cockerham Oswald	1	1	3	0	0.528616	2161.6	28	19.5
1623	1061	Cockerham Oswald	2	1	3	0	0.419396	509.6	22	17
1624	367	Cockerham Oswald	2	2	3	50	0.28492	1769.6	27	16
1625	367	Cockerham Oswald	1	2	3	125	0.663265	1517.6	24	17
1626	179	Cockerham Oswald	1	1	3	150	0.868526	571.2	30	11
1627	166	Cockerham Oswald	1	1	3	150	0.701685	907.2	23	16.5
1628	1159	Cockerham Oswald	1	1	3	50	0.427895	840	29.5	13.5
1629	806	Cockerham Oswald	1	2	3	200	0.425834	NA	20	16
1630	806	Cockerham Oswald	1	2	3	0	1.039793	481.6	23	19
1631	1120	Cockerham Oswald	1	2	3	0	0.430779	862.4	25.5	15.5
1632	1120	Cockerham Oswald	1	2	3	50	0.648125	862.4	28.5	14.5
1633	1280	Cockerham Oswald	1	1	3	0	0.767463	5.6	25	19
1634	158	Cockerham Oswald	1	1	3	75	0.518129	2083.2	25	15
1636	841	Bispham Oswald	1	1	3	50	1.146583	705.6	28	18.5
1638	NA	NA	1	1	3	0	0.965395	1948.8	23	16

1642	1177	Cockerham Oswald	1	2	3	NA	0.842455	117.6	28.5	14
1643	881	Cockerham Oswald	1	1	3	NA	0.742131	1624	32	25.5
1644	883	Cockerham Oswald	1	1	3	0	0.550799	840	27.5	21
1645	551	Cockerham Oswald	1	1	3	NA	0.977935	1545.6	28.5	22
1646	541	Cockerham Oswald	1	1	3	50	0.707509	666.4	30.5	23
1647	880	Cockerham Oswald	1	1	3	NA	0.615175	2744	20	16
1648	795	Cockerham Oswald	1	1	3	0	0.553507	1472.8	26.5	18.5
1649	885	Cockerham Oswald	1	1	3	175	0.26819	414.4	26.5	20
1650	876	Cockerham Oswald	1	1	3	0	0.429106	2217.6	22.5	12.5
1651	820	Cockerham Oswald	1	1	3	0	0.46584	851.2	20	22
1652	807	Cockerham Oswald	1	1	3	175	0.699744	285.6	27	20.5
1653	1114	Cockerham Oswald	1	1	3	0	0.551272	201.6	24	20
1654	1060	Cockerham Oswald	1	1	3	0	0.322827	554.4	26.5	24
1555	NA	NA	1	1	3	NA	NA	NA	NA	NA
1556	NA	NA	1	1	3	NA	0.282012	3074.4	NA	21.5
1390	NA	NA	1	1	3	NA	0.127025	3264.8	NA	16
1391	NA	NA	1	1	3	NA	0.044501	NA	15	13.5

Sex : 1 = Female, 2 = Male; TB = Types of birth, 1= Single, 2 = Twin, 3 =Triplet; Month : 1 = August, 2 = September, 3 = October; FEC = Feacal egg counts (original count has been converted to 25 where an egg represent 25 eggs per gram of faeces) ; IGA = Immunoglobulin A optical density; EO = Peripheral eosinophil counts; PCV = Packed cell volume; BWT = body weight.

Appendix D: Pedigree data frame of Boer goats in a farm in Malaysia.

Animal	Dam	Sire
AUS Doe	NA	NA
X 78/09	NA	NA
ar 189	NA	NA
RN 07/09	NA	NA
CY 09/09	NA	NA
AA 85	NA	NA
ar 440	NA	NA
ar 83	NA	NA
ar 773	NA	NA
AA 121	NA	NA
X 116/09	NA	NA
ar 595	NA	NA
ar 86	NA	NA
ar 87	NA	NA
CH 199	NA	NA
AUS Buck	NA	NA
Bravo	NA	NA
FA 02/09	NA	NA
BY 7.10/09	NA	NA
BA	NA	NA
RN 23/09	NA	NA
Robin	NA	NA
Amar	NA	NA
ar 167	NA	NA
ar 122	NA	NA

RN 23/10	NA	NA
ar 17	NA	NA
Cyclone	NA	NA
Falcon	NA	NA
Delta	NA	NA
AA 177	AUS Doe	AUS Buck
X 17/13	AUS Doe	AUS Buck
X 19/13	AUS Doe	AUS Buck
BR 02/13	AUS Doe	Bravo
FA 02 11/13	X 78/09	FA 02/09
BY 7.10 04/13	ar 189	BY 7.10/09
BR 13/13	RN 07/09	Bravo
BR 09/13	CY 09/09	Bravo
BA (NT)	AUS Doe	BA
BR 12/13	AA 85	Bravo
RN 05/13	ar 440	Robin
/12	AUS Doe	AUS Buck
ar 253	AUS Doe	AUS Buck
ar 373	AUS Doe	AUS Buck
RN 23 07/13	ar 83	RN 23/09
RN 23 02/12	ar 773	RN 23/09
RN 18/12	AA 121	Robin
RB 07/13	X 116/09	Robin
ar 107	AUS Doe	AUS Buck
Cross	AUS Doe	AUS Buck
AA 131	AUS Doe	AUS Buck
ar 846	ar 595	AUS Buck
AA 142	AUS Doe	AUS Buck
ar 701	AUS Doe	AUS Buck
ar 922	AUS Doe	AUS Buck
AM 82/12	ar 86	Amar
AA 175	AUS Doe	AUS Buck
AM 49/12	CH 199	Amar
RB 17/12	ar 167	Robin
RB 37/12	ar 122	Robin
BR 01/12	ar 17	Bravo
CY 12 05/12	RN 23/10	Cyclone
FA 02/09	AUS Doe	Falcon
RB 17/12	ar 167	Robin
DE 164/10	AUS Doe	Delta
RB68/14	AA 177	RB 17/12
RB41/14	X 17/13	RB 37/12
RB40/14	X 19/13	RB 37/12
RB47/14	BR 02/13	RB 37/12
RB44/14	FA 02 11/13	RB 37/12
RB45/14	BY 7.10 04/13	RB 37/12
RB67/14	AA 177	RB 17/12

RB58/14	BR 13/13	RB 17/12
BR57/14	BR 09/13	RB 17/12
RB36/14	BA (NT)	RB 37/12
RB53/14	BR 12/13	RB 17/12
BR03/14	RN 05/13	BR 01/12
CY 22/14	/12	CY 12 05/12
CY 32/14	ar 253	CY 12 05/12
FA 31/14	ar 373	CY 12 05/12
FA36/14	RN 23 07/13	FA 02/09
FA32/14	RN 23 02/12	FA 02/09
FA37/14	RN 18/12	FA 02/09
FA38/14	RB 07/13	FA 02/09
BR08/14	ar 107	BR 01/12
BR25/14	Cross	BR 01/12
BR17/14	AA 131	BR 01/12
BR01/15	ar 846	BR 01/12
RB64/14	AA 142	RB 17/12
DE30/14	ar 701	DE 164/10
CY40/14	ar 922	CY 12 05/12
CY26/14	AM 82/12	CY 12 05/12
CY27/14	BY 7.10 12/13	CY 12 05/12
CY30/14	AA 175	CY 12 05/12
BR 26/14	AM 49/12	BR 01/12

Appendix E: Phenotypic data frame of Boer goats in a farm in Malaysia.

Animal	Pen	TB	Day post-infection	Time point	FEC	IGA	EO	PCV
RB68/14	5	2	14	2	0	1.0319	89.6	23.5
RB41/14	1	1	14	2	0	0.6936	61.6	25
RB40/14	1	1	14	2	0	0.8928	123.2	28.5
RB47/14	5	1	14	2	0	0.9674	168	30
RB44/14	4	1	14	2	0	0.2769	78.4	28
RB67/14	2	2	14	2	0	1.3179	140	32
RB58/14	4	1	14	2	0	1.1718	196	28
BR57/14	3	1	14	2	0	0.7771	67.2	25.5
RB36/14	1	2	14	2	0	0.8451	106.4	25
CY22/14	2	1	14	2	0	NA	95.2	30
CY32/14	5	1	14	2	0	1.024	112	33
FA31/14	2	1	14	2	0	0.9132	67.2	27
FA36/14	1	1	14	2	0	0.5698	61.6	28
FA32/14	1	1	14	2	0	0.3423	44.8	29.5
FA37/14	5	1	14	2	0	0.9408	201.6	21
BR08/14	5	1	14	2	0	0.7953	156.8	26
BR25/14	2	1	14	2	0	1.0024	134.4	24.5
BR17/14	4	1	14	2	0	0.9526	207.2	27
BR01/15	3	2	14	2	0	0.8555	134.4	25.5
RB64/14	3	2	14	2	0	0.9173	212.8	21
CY40/14	3	3	14	2	0	0.9807	78.4	27.5

CY26/14	3	1	14	2	0	1.0327	291.2	28
CY27/14	4	1	14	2	0	1.2932	291.2	27.5
CY30/14	4	2	14	2	0	0.7698	67.2	26
BR26/14	1	1	14	2	0	0.5234	240.8	27.5
RB68/14	5	2	17	2	0	3	240.8	23
RB41/14	1	1	17	2	0	0.5045	106.4	27
RB40/14	1	1	17	2	0	0.7802	39.2	28
RB47/14	5	1	17	2	0	1.1294	134.4	26
RB44/14	4	1	17	2	0	0.1764	123.2	28
RB67/14	2	2	17	2	0	3	190.4	30
RB58/14	4	1	17	2	0	1.0996	330.4	26
BR57/14	3	1	17	2	0	0.5697	39.2	26.5
RB36/14	1	2	17	2	0	0.5569	229.6	29
CY22/14	2	1	17	2	0	0.6408	89.6	29
CY32/14	5	1	17	2	0	0.806	168	33
FA31/14	2	1	17	2	0	0.8889	44.8	21.5
FA36/14	1	1	17	2	0	0.5572	117.6	32
FA32/14	1	1	17	2	0	0.2937	84	29.5
FA37/14	5	1	17	2	0	0.9998	145.6	16
BR08/14	5	1	17	2	0	0.4907	168	27
BR25/14	2	1	17	2	0	1.0417	61.6	24
BR17/14	4	1	17	2	0	1.0138	117.6	25
BR01/15	3	2	17	2	0	0.8872	246.4	24
RB64/14	3	2	17	2	0	0.7575	145.6	15
CY40/14	3	3	17	2	0	0.8459	61.6	24
CY26/14	3	1	17	2	0	1.1478	162.4	26.5
CY27/14	4	1	17	2	0	1.0478	285.6	25
CY30/14	4	2	17	2	0	0.6899	151.2	23
BR26/14	1	1	17	2	0	0.3692	140	29
RB68/14	5	2	21	2	175	1.2569	470.4	28.5
RB41/14	1	1	21	2	0	0.4569	162.4	26
RB40/14	1	1	21	2	50	0.7429	100.8	29.5
RB47/14	5	1	21	2	50	1.1401	179.2	27.5
RB44/14	4	1	21	2	0	0.1065	336	27
RB67/14	2	2	21	2	25	1.0902	184.8	33
RB58/14	4	1	21	2	225	1.1572	196	28
BR57/14	3	1	21	2	0	0.1459	11.2	25
RB36/14	1	2	21	2	0	0.7116	173.6	28
CY22/14	2	1	21	2	175	0.708	11.2	31.5
CY32/14	5	1	21	2	275	0.8575	173.6	31
FA31/14	2	1	21	2	275	0.9803	128.8	25
FA36/14	1	1	21	2	0	0.4248	196	33.5
FA32/14	1	1	21	2	50	0.1889	72.8	29
FA37/14	5	1	21	2	25	0.9897	173.6	18
BR08/14	5	1	21	2	50	0.6691	67.2	26
BR25/14	2	1	21	2	0	0.7832	162.4	28
BR17/14	4	1	21	2	125	0.9437	117.6	29.5

BR01/15	3	2	21	2	0	0.6102	151.2	26
RB64/14	3	2	21	2	0	0.8025	397.6	24
CY40/14	3	3	21	2	125	0.9175	28	25
CY26/14	3	1	21	2	50	2.4192	39.2	26.5
CY27/14	4	1	21	2	0	0.8428	134.4	26.5
CY30/14	4	2	21	2	0	0.7211	168	25.5
BR26/14	1	1	21	2	125	0.214	173.6	26.5
RB68/14	5	2	24	2	550	1.2388	504	25.5
RB41/14	1	1	24	2	150	0.7301	100.8	26.5
RB40/14	1	1	24	2	275	0.5332	84	28
RB47/14	5	1	24	2	100	3	224	27.5
RB44/14	4	1	24	2	75	0.1992	207.2	27
RB67/14	2	2	24	2	75	1.0085	218.4	35
RB58/14	4	1	24	2	650	1.142	151.2	27
BR57/14	3	1	24	2	0	0.2534	44.8	27
RB36/14	1	2	24	2	150	0.5803	252	27
CY22/14	2	1	24	2	275	0.7188	72.8	32
CY32/14	5	1	24	2	400	0.7615	78.4	26.5
FA31/14	2	1	24	2	225	0.9727	33.6	24
FA36/14	1	1	24	2	0	0.4458	100.8	31
FA32/14	1	1	24	2	0	0.1805	39.2	28.5
FA37/14	5	1	24	2	175	0.9545	117.6	15.5
BR08/14	5	1	24	2	200	0.5763	112	25
BR25/14	2	1	24	2	125	0.627	252	24.5
BR17/14	4	1	24	2	50	0.9076	173.6	27.5
BR01/15	3	2	24	2	75	0.7633	145.6	26.5
RB64/14	3	2	24	2	975	0.7005	190.4	25
CY40/14	3	3	24	2	275	0.9723	39.2	27
CY26/14	3	1	24	2	0	1.1586	106.4	27
CY27/14	4	1	24	2	25	0.7836	397.6	26.5
CY30/14	4	2	24	2	25	0.8319	67.2	24.5
BR26/14	1	1	24	2	325	0.1915	5.6	29
RB68/14	5	2	28	3	1100	1.1281	364	22.5
RB41/14	1	1	28	3	25	0.7639	95.2	21
RB40/14	1	1	28	3	850	0.4323	22.4	22.5
RB47/14	5	1	28	3	175	0.8954	140	20
RB44/14	4	1	28	3	325	0.1613	280	21.5
RB67/14	2	2	28	3	525	0.9896	117.6	27.5
RB58/14	4	1	28	3	400	1.164	207.2	25
BR57/14	3	1	28	3	0	0.3327	33.6	22
RB36/14	1	2	28	3	225	0.2969	95.2	22.5
CY22/14	2	1	28	3	NA	0.6528	218.4	22
CY32/14	5	1	28	3	425	0.4797	151.2	26
FA31/14	2	1	28	3	1000	0.9986	134.4	20
FA36/14	1	1	28	3	125	0.3051	16.8	24
FA32/14	1	1	28	3	150	0.1493	28	22.5
FA37/14	5	1	28	3	275	0.8676	16.8	22

BR08/14	5	1	28	3	450	0.4671	44.8	25.5
BR25/14	2	1	28	3	225	0.7074	246.4	20
BR17/14	4	1	28	3	0	0.7848	50.4	21
BR01/15	3	2	28	3	25	0.8125	336	20.5
RB64/14	3	2	28	3	800	0.906	196	18
CY40/14	3	3	28	3	475	0.9621	100.8	24
CY26/14	3	1	28	3	25	1.1515	100.8	21
CY27/14	4	1	28	3	375	1.0992	117.6	20
CY30/14	4	2	28	3	275	0.8467	128.8	19
BR26/14	1	1	28	3	300	0.208	78.4	23
RB68/14	5	2	31	3	2000	1.1154	100.8	28
RB41/14	1	1	31	3	50	0.8887	67.2	26.5
RB40/14	1	1	31	3	1700	0.4522	22.4	27
RB47/14	5	1	31	3	425	1.1343	168	28.5
RB44/14	4	1	31	3	275	0.2916	106.4	26.5
RB67/14	2	2	31	3	450	1.0684	117.6	35
RB58/14	4	1	31	3	600	1.1598	112	26.5
BR57/14	3	1	31	3	0	0.2436	44.8	26
RB36/14	1	2	31	3	175	0.5812	61.6	27.5
CY22/14	2	1	31	3	450	0.8341	39.2	30
CY32/14	5	1	31	3	225	0.6806	112	33
FA31/14	2	1	31	3	0	1.0167	22.4	25
FA36/14	1	1	31	3	50	0.3337	44.8	28.5
FA32/14	1	1	31	3	175	0.3019	50.4	30
FA37/14	5	1	31	3	375	0.9506	28	20
BR08/14	5	1	31	3	875	0.4955	5.6	27.5
BR25/14	2	1	31	3	0	0.9441	179.2	27
BR17/14	4	1	31	3	25	0.8766	84	27.5
BR01/15	3	2	31	3	450	0.7254	61.6	27.5
RB64/14	3	2	31	3	1925	0.7272	5.6	20
CY40/14	3	3	31	3	125	1.0518	78.4	27
CY26/14	3	1	31	3	50	1.0882	28	28.5
CY27/14	4	1	31	3	775	1.1008	145.6	27
CY30/14	4	2	31	3	250	1.0348	89.6	24
BR26/14	1	1	31	3	250	0.1004	50.4	28
RB68/14	5	2	35	3	725	0.8946	123.2	NA
RB41/14	1	1	35	3	0	0.7115	0	NA
RB40/14	1	1	35	3	425	0.5666	11.2	NA
RB47/14	5	1	35	3	125	0.9857	112	NA
RB44/14	4	1	35	3	100	0.1401	22.4	26
RB67/14	2	2	35	3	275	0.9388	16.8	NA
RB58/14	4	1	35	3	75	0.9947	11.2	NA
BR57/14	3	1	35	3	0	0.3795	179.2	26.5
RB36/14	1	2	35	3	175	0.5474	39.2	25.5
CY22/14	2	1	35	3	125	0.8106	117.6	26
CY32/14	5	1	35	3	0	0.5977	84	NA
FA31/14	2	1	35	3	50	0.9382	28	NA

FA36/14	1	1	35	3	175	0.3087	0	27.5
FA32/14	1	1	35	3	250	0.2116	5.6	NA
FA37/14	5	1	35	3	700	0.8084	33.6	NA
BR08/14	5	1	35	3	525	0.3272	16.8	NA
BR25/14	2	1	35	3	50	0.6312	78.4	NA
BR17/14	4	1	35	3	25	0.9346	72.8	24
BR01/15	3	2	35	3	300	0.7001	112	NA
RB64/14	3	2	35	3	600	NA	16.8	NA
CY40/14	3	3	35	3	0	1.0306	33.6	NA
CY26/14	3	1	35	3	25	1.1135	28	NA
CY27/14	4	1	35	3	0	0.8504	28	22.5
CY30/14	4	2	35	3	525	0.9492	33.6	NA
BR26/14	1	1	35	3	0	0.0988	5.6	NA
RB68/14	5	2	38	3	425	1.0421	145.6	31.5
RB41/14	1	1	38	3	100	0.5331	28	32
RB40/14	1	1	38	3	600	0.6069	11.2	29.5
RB47/14	5	1	38	3	275	0.6782	72.8	30.5
RB44/14	4	1	38	3	75	0.1248	33.6	29
RB67/14	2	2	38	3	500	0.712	61.6	34
RB58/14	4	1	38	3	300	1.1469	44.8	28
BR57/14	3	1	38	3	25	0.3114	123.2	30
RB36/14	1	2	38	3	0	0.3933	112	27.5
CY22/14	2	1	38	3	375	0.8552	22.4	34
CY32/14	5	1	38	3	75	0.5759	184.8	34.5
FA31/14	2	1	38	3	0	0.8472	72.8	29.5
FA36/14	1	1	38	3	0	0.3253	5.6	31.5
FA32/14	1	1	38	3	475	0.2181	5.6	30.5
FA37/14	5	1	38	3	275	1.0102	56	28.5
BR08/14	5	1	38	3	375	0.5357	84	29
BR25/14	2	1	38	3	0	0.7535	145.6	27.5
BR17/14	4	1	38	3	0	0.5679	5.6	27
BR01/15	3	2	38	3	300	0.6982	72.8	27
RB64/14	3	2	38	3	1325	0.694	16.8	20.5
CY40/14	3	3	38	3	75	1.0664	5.6	29
CY26/14	3	1	38	3	150	3	61.6	34
CY27/14	4	1	38	3	350	0.6707	33.6	29
CY30/14	4	2	38	3	675	1.1052	61.6	27.5
BR26/14	1	1	38	3	225	0.1511	78.4	31
RB68/14	5	2	42	4	50	0.9167	95.2	28
RB41/14	1	1	42	4	0	0.5415	128.8	28
RB40/14	1	1	42	4	75	0.3293	56	30.5
RB47/14	5	1	42	4	250	1.0273	16.8	29.5
RB44/14	4	1	42	4	250	0.1467	95.2	25
RB67/14	2	2	42	4	475	0.5719	28	31.5
RB58/14	4	1	42	4	500	2.1784	50.4	27
BR57/14	3	1	42	4	0	0.7686	117.6	27
RB36/14	1	2	42	4	325	0.5145	128.8	28

CY22/14	2	1	42	4	150	0.7785	39.2	30
CY32/14	5	1	42	4	25	0.6689	106.4	32
FA31/14	2	1	42	4	0	0.7914	5.6	23
FA36/14	1	1	42	4	350	0.5355	16.8	30
FA32/14	1	1	42	4	375	0.1796	5.6	29.5
FA37/14	5	1	42	4	725	0.4065	11.2	20.5
BR08/14	5	1	42	4	600	0.3732	28	26.5
BR25/14	2	1	42	4	NA	1.3583	39.2	27.5
BR17/14	4	1	42	4	675	1.3098	72.8	24.5
BR01/15	3	2	42	4	1125	0.5371	33.6	24.5
RB64/14	3	2	42	4	0	0.7666	61.6	20.5
CY40/14	3	3	42	4	75	0.8916	0	26
CY26/14	3	1	42	4	325	2.4209	39.2	26
CY27/14	4	1	42	4	725	1.6865	22.4	26.5
CY30/14	4	2	42	4	75	NA	11.2	25
BR26/14	1	1	42	4	0	0.1519	11.2	30
RB68/14	5	2	45	4	250	1.352	106.4	30.5
RB41/14	1	1	45	4	400	0.1404	72.8	28
RB40/14	1	1	45	4	125	0.1934	16.8	27
RB47/14	5	1	45	4	225	0.6551	89.6	28.5
RB44/14	4	1	45	4	275	0.0487	16.8	23.5
RB67/14	2	2	45	4	575	0.3534	16.8	32
RB58/14	4	1	45	4	25	1.7019	28	23.5
BR57/14	3	1	45	4	350	0.6154	56	26
RB36/14	1	2	45	4	525	0.274	61.6	25.5
CY22/14	2	1	45	4	575	0.8378	128.8	30
CY32/14	5	1	45	4	0	0.7408	364	32
FA31/14	2	1	45	4	925	0.9377	11.2	22.5
FA36/14	1	1	45	4	200	0.1644	11.2	27.5
FA32/14	1	1	45	4	200	0.1714	11.2	29.5
FA37/14	5	1	45	4	0	0.5866	123.2	21.5
BR08/14	5	1	45	4	0	0.183	78.4	26
BR25/14	2	1	45	4	NA	1.4598	56	24
BR17/14	4	1	45	4	0	0.506	28	27
BR01/15	3	2	45	4	200	0.3577	33.6	25.5
RB64/14	3	2	45	4	625	0.179	61.6	28
CY40/14	3	3	45	4	NA	1.0502	56	26
CY26/14	3	1	45	4	500	2.0442	44.8	28
CY27/14	4	1	45	4	0	0.5678	16.8	24.5
CY30/14	4	2	45	4	25	1.1736	123.2	25.5
BR26/14	1	1	45	4	0	0.2871	5.6	28.5
RB68/14	5	2	49	4	350	0.878	112	30
RB41/14	1	1	49	4	225	0.1557	28	29
RB40/14	1	1	49	4	1900	0.1092	0	27
RB47/14	5	1	49	4	275	1.0979	140	31
RB44/14	4	1	49	4	0	0.1248	95.2	25.5
RB67/14	2	2	49	4	575	0.3399	0	32

RB58/14	4	1	49	4	500	1.2503	22.4	25
BR57/14	3	1	49	4	0	0.35	67.2	27
RB36/14	1	2	49	4	50	0.633	NA	26.5
CY22/14	2	1	49	4	0	1.1662	392	29
CY32/14	5	1	49	4	200	0.5581	39.2	32
FA31/14	2	1	49	4	NA	1.057	44.8	22
FA36/14	1	1	49	4	150	0.2569	16.8	26
FA32/14	1	1	49	4	100	0.1178	33.6	28.5
FA37/14	5	1	49	4	500	0.693	128.8	20
BR08/14	5	1	49	4	825	0.2346	50.4	27.5
BR25/14	2	1	49	4	25	0.9548	95.2	28
BR17/14	4	1	49	4	0	1.0682	78.4	26
BR01/15	3	2	49	4	400	0.6261	123.2	25
RB64/14	3	2	49	4	2325	0.2352	72.8	23
CY40/14	3	3	49	4	100	1.3078	28	26
CY26/14	3	1	49	4	150	1.0847	84	26
CY27/14	4	1	49	4	900	0.6804	84	28
CY30/14	4	2	49	4	NA	0.7088	78.4	25.5
BR26/14	1	1	49	4	3500	0.0738	5.6	30
RB68/14	5	2	52	4	1325	0.2613	44.8	27.5
RB41/14	1	1	52	4	0	0.149	106.4	29
RB40/14	1	1	52	4	425	0.2004	39.2	27
RB47/14	5	1	52	4	250	NA	56	28.5
RB44/14	4	1	52	4	0	0.1458	39.2	26.5
RB67/14	2	2	52	4	200	0.2673	33.6	31
RB58/14	4	1	52	4	450	1.2304	67.2	24.5
BR57/14	3	1	52	4	0	0.1641	50.4	27
RB36/14	1	2	52	4	125	0.2416	56	24
CY22/14	2	1	52	4	0	NA	324.8	25.5
CY32/14	5	1	52	4	0	0.486	89.6	30.5
FA31/14	2	1	52	4	0	1.5833	16.8	23
FA36/14	1	1	52	4	100	0.1926	0	27
FA32/14	1	1	52	4	25	0.1893	11.2	28
FA37/14	5	1	52	4	200	0.5022	28	24
BR08/14	5	1	52	4	875	0.3253	11.2	28
BR25/14	2	1	52	4	50	0.8973	89.6	25.5
BR17/14	4	1	52	4	0	0.9311	44.8	25.5
BR01/15	3	2	52	4	150	0.3803	NA	24
RB64/14	3	2	52	4	825	0.2935	224	19.5
CY40/14	3	3	52	4	50	3	50.4	25
CY26/14	3	1	52	4	600	1.8839	39.2	26
CY27/14	4	1	52	4	150	0.1294	61.6	26
CY30/14	4	2	52	4	525	0.7745	39.2	24
BR26/14	1	1	52	4	50	0.0758	56	27

TB = Types of birth, 1= Single, 2 = Twin, 3 =Triplet; FEC = Feacal egg counts (original count has been converted to 25 where an egg represent 25 eggs per gram of faeces); IGA = Immunoglobulin A optical density; EO = Peripheral eosinophil counts; PCV = Packed cell volume.