A genome-wide association study on mechanisms underlying genetic resistance to gastrointestinal parasites in goats, Zimbabwe

by

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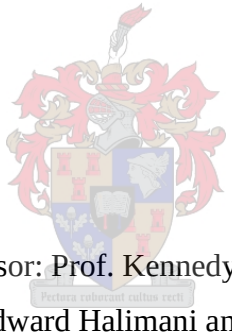

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Abstract

Genome wide association studies (GWAS) have evolved into powerful tools for investigating the genetic association of complex traits, such as gastrointestinal parasite (GIN) resistance. Knowledge on genes associated with GIN resistance can provide information for use in breeding programs. The objective of the study was to identify markers associated with resistance in goats, through the following specific objectives: i) assessing the level of knowledge on GIN, management and control of GIN, ii) determining the prevalence and risk factors of GIN, iii) determining genetic diversity and population structure of goats in Zimbabwe and iv) investigating genomic loci associated with GIN resistance traits using a genome-wide association analyses (GWAS). Surveys were conducted in 135 households, using a pre-tested questionnaires in Chipinge (natural region (NR) I and II), Shurugwi (NR III), Binga and Tsholotsho (NR IV) and Matobo (NR V). GIN were ranked highest as the most common disease, with 57% of farmers not controlling or treating animals and 63% of farmers not having knowledge on the spread of GIN. A total of 580 blood and faecal samples were collected from goats from the same households, with additional sampling being conducted in the Research station flock. Highest prevalence was determined for *Eimeria* oocysts (43%) and *Strongyles* (31%). Area, season, sex and age significantly influenced patterns of GIN infections ($P < 0.05$). Prevalence was highest in goats from Chipinge and Binga, greater in wet than dry season and in males than females. High prevalences were observed for goats aged 1 and 6 years and the least for goats aged 3. Associated risk factors were also evaluated per area. A subset of the sampled animals (253) was genotyped using the Illumina Goat 50 K SNP beadchip. Population structure analyses were performed using ADMITXURE and PLINK. Five clusters were identified, with distinct populations of Binga and high levels of shared ancestry in goats from

Tsholotsho and Matobo districts. Genetic parameters indicated high levels of genetic diversity based on observed (H_E) and expected (H_O), low linkage disequilibrium (r^2 = 0.03 - 0.18) and low F_{ST} (0.01 – 0.04). For genome-wide analyses, two approaches were used: i) single-SNP association using logarithm transformed faecal egg counts, ii) within-population association using case/control data. After quality control, 49 984 SNPs and 44 918 SNPs were available for genome-wide association analyses in GenAbel and PLINK respectively. The study confirmed that GIN resistance traits were heritable (0.27 - 0.56 i.e low - moderate). The analyses revealed significant multiple SNPs that were associated with *Eimeria* and *Strongyles* at the genome-wide level. Regions on chromosomes (chr) 4 (P = 2.66 $x10^{-6}$ and P = 1.45 $x10^{-5}$) for *Eimeira* and chr 29 (P = 9.93 $x10^{-6}$) were found to be associated with GIN resistance, for the *Eimeria* and *Strongyles* traits. Genes annotated to the SNP positions were *ORC5*, *DGKB* and *HRASLS5*, respectively. The role of the genes have not been reported in previous studies or implicated in the involvement of biological pathways that have roles in eliciting responses towards GIN infections. Overally, the study demonstrates the utility of the Illumina Goat 50 K SNP, despite that the animals used in the study were not represented in the SNP discovery breeds. Knowledge of these genes and understanding the underlying mechanisms to GIN resistance can be used in the development of breeding programs and hence improve productivity.

Opsomming

Genoom wye assosiasie studies (GWAS) het ontwikkel in ‘n kragtige instrument vir die ondersoek van genetiese verwantskappe van komplekse eienskappe, soos gastro-parasiet weerstand. Kennis oor gene wat verband hou met gastro-parasiet weerstand kan inligting verskaf wat gebruik kan word in teeltprogramme. Die doel van hierdie studie was om merkers geassosieer met weerstand in bokke te identifiseer, deur die volgende spesifieke doelwitte: (i) die bepaling van die vlak van kennis oor gastro-parasiete onder kleinboere, hul bestuur en beheer van gastro-parasiete (ii) die bepaling van die voorkoms en risikofaktore van gastro-parasiete (iii) bepaling van genetiese diversiteit en populasisestruktuur van bokke in Zimbabwe (iv) die ondersoek van genomiese lokusse wat verwant is aan gastro-parasiet weerstand eienskappe met behulp van ‘n genoom wye assosiasie studie (GWAS). Opnames is in 135 huishoudings, met behulp van ‘n pre-toetse vraelyste in Chipinge (natuurlike gebied (NG) I en II), Shurugwi (NG III), Binga enTsholotsho (NG IV), en Matobo (NG V) distrikte, wat vyf landbou-ekologiese streke in Zimbabwe verteenwoordig. Gastro-parasiete was die hoogste geklas as die mees algemeenste siekte, met meerderheid van die boere (57%) wat nie beheer toepas of siek diere behandel nie en 63% van die boere wat geen kennis het oor die verspreiding van gastro-parasiet siektes nie. ‘n Totaal van 580 bloed en fekale monsters was versamel van bokke vanuit dieselfde huishoudings, met bykomede monsterversameling gedoen in die Navorsingstasie kudde. Hoogste voorkoms was *Eimeria* oösiste (43%) en *Strongyles* (31%). Gebied, seisoen, geslag en ouderdom het die patroon van gastro-parasiete infeksies beduidend beïnvloed ($P < 0.05$). Voorkoms was die hoogste in bokke vanaf Chipinge en Binga, asook hoër in die nat teenoor droë seisoen en hoër in bokramme teenoor bokooie. Hoë voorkoms is ook waargeneem vir bokke 1 en 6 jaar oud en die minste vir bokke 3 jaar oud. Geassosieerde risikofaktore is ook geëvalueer per area. ‘n Subset van die gemonsterde diere (253) was

genotipeerd met behulp van die Illumina Bok 50 K SNP beadchip. Populasiestruktuur analise is uitgevoer met behulp van ADMITXURE en PLINK. Vyf klusters is geïdentifiseerd, elk met sy eie bevolkings van Binga en hoë vlakke van gedeelde afkoms in die bokke vanaf Tsholotsho en Matobo. Genetiese parameters is aanduided van hoë vlakke van genetiese diversiteit gebaseerd op die waargeneemde (H_E) en verwagte (H_O), lae koppeling onewewigtigheid (r^2 = 0.03 - 0.18) en lae F_{ST} (0.01 – 0.04). Vir genoomwye ontledings is twee benaderings gebruik: i) enkel-SNP assosiasie met behulp van logaritme veranderde fekale eiertellings ii) binne-populasie assosiasie met behulp van gevalle/kontrole data. Na gehalte beheer, 49 984 SNPs en 44918 SNPs was beskikbaar vir die genoomwye assosiasie analise in GenAbel en PLINK onderskeidelik. Die studie het bevestig dat gastro-parasiete weerstand eienskappe is oorerflik (0.27 - 0.56 d.w.s lae tot gemiddeld). Die analise het beduidende verskeie SNP's openbaar wat verband hou met *Eimeria* en *Strongyles* by die genoomwye vlak. Streke op chromosome (chr) 4 ($P = 2.66 \times 10^{-6}$ and $P = 1.45 \times 10^{-5}$) vir *Eimeira* en chr 29 ($P = 9.93 \times 10^{-6}$) is gevind wat verband hou met die gastro-parasiete weerstand, vir die *Eimeria* en *Strongyles* eienskappe. Gene geannoteerd naby hierdie SNP posisies was *ORC5, DGKB* en *HRASLS5* onderskeidelik. Die rol van die gene is nog nie aangemeld in vorige studies of hul betrokkenheid by biologiese weë wat reaksie lok teenoor gastro-parasiete infeksie nie. In geheel, toon die studie die nut van Illumina Bok 50 K SNP, ten spyte daarvan dat die diere gebruik in die studie nie die diere verteenwoordig wat gebruik was in die SNP ontdekking rasse nie. Kennis van hierdie gene en die begrip van die onderliggende meganismes van gastro-parasiete weerstand kan gebruik word in die ontwikkeling van teelprogramme en sodoende produktiwiteit verbeter.

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List of Abbreviations

AAD	Aminoacetonitriles
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variances
AVM	Avermectins
BZ	Benzimidazoles
CNV	Copy number variants
CV	Cross validation
DEGs	Differentially expressed genes
EBV	Estimated breeding value
EHH	Extended haplotype homozygosity
FAO	Food and Agriculture Organisation of the United Nations
FEC	Faecal egg counts
F_{ST}	Fixation index (inbreeding coefficient of sub-population)
F_{IS}	Inbreeding coefficients of an individual relative to the sub-populations they belongs to
GIN	Gastrointestinal parasites
GEBV	Genomic estimated breeding value
GWAS	Genome-wide association study
Hc	*Haemonchus contortus*
HWE	Hardy Weinberg equilibrium
IFN-γ	Interferon gamma- γ
H_O	Observed heterozygosity
H_E	Expected heterozygosity
iHS	Integrated haplotype score
IMID	Imidothiazoles
LD	Linkage disequilibrium

LRH	Long-range haplotype
MAF	Minor allelic frequency
MDS	Multi-dimension scaling
MHC	Major histo-compatibility complex
MLB	Milbemycin
ML	Macrocyclic lactone
N_e	Effective population size
NG	Natural/agro-ecological regions
NGS	Next-generation sequencing
OAR	Ovine chromosomes
PCA	Principal component analysis
PCV	Packed cell volumes
QC	Quality control
QTL	Quantitative trait loci
QQ	Quantile-quantile
RI	Ranking index
SAS	Statistical Analysis Systems
SCL	Salicylanilides
SNPs	Single-nucleotide polymorphisms
Tc	*Trichostrongylus colubriformis*
TETR	Tetrahydropyrimidines
XP-EHH	Cross population extended haplotype homozygosity

Chapter 1

1 Background

1.1 General introduction

Gastrointestinal parasites (GIN) impose severe economic constraints on goat production (Saddiqi et al., 2011; Várady et al., 2011). Control strategies are based almost entirely on the frequent use of dewormers (anthelmintic drugs), which are increasingly regarded as unsustainable, given the emergence of multiple drug-resistant parasites (Bishop and Morris, 2007; McManus et al., 2014). In addition, consumer demands for organically produced commodities (Moreno et al., 2012) and reduction in drug residues in the environment (Alba-Hurtado; Muñoz-Guzmán, 2012), has led to increased restrictions on the use of chemicals. This has led to the need for new control measures, such as selection for increased GIN resistance with available field data. Current knowledge about GI parasite infections in Zimbabwe are derived primarily from epidemiological data (Mukaratirwa et al., 2001; Pfukenyi et al., 2007; Marufu et al., 2008). Globally, several studies have demonstrated that at least part of the natural variation in resistance to nematode infection is under genetic control (Vagenas et al., 2002; Crawford et al., 2006; Gutiérrez-Gil et al., 2009). Exploring the host's genetic resistance to parasites can be used as an alternative strategy for controlling GIN. In addition to that, the physiological and underlying genetic mechanisms conferring resistance to GIN which are complex, are not fully understood.

Goat breeds reared in Zimbabwe include Boer, Mashona, Matabele and several kinds of crossbreeds, with a large proportion of the population being indigenous. Overall, indigenous goat genetic breeds in Southern Africa are known for their hardiness, prolificacy, early attainment of puberty and low requirement for inputs (Gwaze et al., 2009a). Despite these

advantages, indigenous goats are largely neglected for commercal production. Exploitation of these genetic resources can be vital for improvement of resistance to GIN, as well as goat productivity.

1.2 Problem statement

Goats are markedly susceptible to infection with gastrointestinal parasites, as such that the frequency of anthelmintic resistance is higher compared to sheep, with which they share the same nematode parasites (Mandonnet et al., 2001). Integrated control of strongylosis in goats necessitates incorporation of genetic resistance into control systems. Limited studies exist globally on resistance to GIN in goats compared to sheep (Bolormaa et al., 2010a); (Vagenas et al., 2002). In Zimbabwe, no studies have been conducted to estimate the genetic parameters associated with parasite resistance in goats. However, there are reports of quantitative trait loci (QTL) for nematode resistance in goats (Bolormaa et al., 2010a; de la Chevrotière et al., 2012) and sheep (Dominik et al., 2010; Rout et al., 2012).

The genetic control of complex traits in livestock has been studied without identifying the genes or gene variants underlying observed variation, with selection being conducted on the basis of estimated breeding values (EBVs) calculated from phenotypic and pedigree information (Goddard and Hayes, 2009). This may pose a serious challenge in smallholder farming systems, where there is no record keeping. Selection for parasite resistance has mainly been based on indicator traits, such as faecal egg count (FEC) (Davies et al., 2005; Dominik, 2005), packed cell volumes (Janssen et al., 2002) i.e. degree of anaemia or immunological activity e.g. circulating eosinophils and antibody level (Castillo et al., 2011). Results from these studies were highly inconsistent, thus posing threats to their uses.

Collection and quantifying indicator traits can be costly and time-consuming; therefore it would be advantageous if the selection can be conducted without rigorous phenotyping. The use of genetic markers in selection programs could be more effective. This can be achieved by collecting blood or tissue samples from young animals, then selection is performed based on their genotypes, although a low level of phenotyping would be required. The use of genome-wide data can be utilized as a means of overcoming some of these mentioned problems. In addition to identifying markers associated with GIN resistance, data can also be used to understand the mechanisms underlying the pathways that increase resistance.

1.3 Justification

Genome wide association studies (GWAS) have recently evolved into powerful tools for investigating the genetic association to diseases in livestock. This has been made possible by the introduction of high-density single nucleotide polymorphisms (SNPs) genotyping platforms. These studies take a systematic 'unbiased' approach by interrogating the entire genome for associations between common gene variants (SNPs) and a phenotype (Visscher, 2008). All the potential genetic variation for a trait could be picked up due to the extent of linkage disequilibrium (LD) between the SNPs on the panel and causative QTL. This explains whether polymorphisms associated with resistance are closely linked to the resistance-conferring mutation or are a large physical distance away in the genome. Evidence where GWAS have already identified significant regions associated are documented for GIN resistance (Kemper et al., 2011; Riggio et al., 2013; Pickering et al., 2015), and production traits (Kijas et al., 2013; Martin et al., 2016; Matika et al., 2016).

The advantage of using GWAS in low-input/output systems is that it can be used without pedigree information. Unlike the candidate approach which tests each marker independently

for an association with the trait, the genome-wide association studies have a potential of shrinking the estimated effect of each marker and predict genetic merit using a linear combination of their effects (Kemper et al., 2011). Information at molecular level generated in this study can be used in selection and breeding programs of goats and will also help determine the mechanism of parasite resistance. Selection of goats that are genetically resistant to parasites may lead to vast epidemiological benefits. There can be reduced pasture larval contamination, which will lead to reduced challenge and lower FEC as well as improved production.

1.4 Objectives

The overall objective of the study was to identify markers associated with resistance to gastrointestinal parasites (GIN) infection in goat populations in Zimbabwe

The specific objectives of the study were:

i) To assess the level of knowledge on GIN, management and control of the disease among smallholder goat farmers in Zimbabwe;
ii) To determine the prevalence and risk factors of gastrointestinal parasites in different agro-ecological regions in Zimbabwe;
iii) To determine genetic diversity and population structure of goats reared in low-input/output farming systems of Zimbabwe; and,
iv) To investigate markers associated with resistance to gastrointestinal parasites using genome-wide association analysis (GWAS).

1.5 Thesis overview and layout

The study was conducted with the aim of identifying genetic markers associated to GIN resistance in indigenous goats reared in low-input/ output farming systems in Zimbabwe. This analyses was made possible by the use of the Illumina Goat 50K SNP beadchip. The use of genome-wide tools has been demonstrated in most sheep studies, with little known in goats. The thesis is structured into seven chapters, consisting of the general background of the study, literature review, four research chapters and a general discussion and conclusion. Each chapter is structured as a manuscript with its abstract and list of references.

In chapter 1 the background of the study and the motivation of the study were highlighted.

Chapter 2 reviewed the current control methods of GIN, the motivations of GWAS being elaborated and its potential benefits are also discussed. The work in this chapter was published in *Veterinary Parasitology*.

Chapter 3 explored the management and control practises of GIN in low-input/output farming systems. Results indicated that the majority of the farmers were not controlling parasites and most of them lacked knowledge in GIN. This work was published in *Tropical Animal Health and Production*.

In chapter 4, prevalence of gastrointestinal parasitic infections was determined in different age groups and sex using faecal egg counts data. The effects of area, season, sex and age were evaluated *vs* the occurrence of infection. Association of these risk factors were then evaluated for each area. The work from this chapter was published in *Small Ruminants Research*.

In chapter 5 the Goat 50 k SNP beadchip was used to assess the genomic population structure of 253 indigenous breeds/ecotypes goats. After quality control, ADMIXTURE and Principal

Components were used to perform the population structure analyses. Level of linkage disequilibrium (LD), LD decay, effective population sizes and F_{ST} were determined. The work from this chapter is being prepared for submission in an international peer reviewed journal.

In chapter 6 genomewide analyses were conducted using GenAbel and PLINK. Analyses was performed using results from Chapter 4 to explain phenotypes and Chapter 5 to infer population structure. Regions associated with the phenotypes were then annotated onto the goat genome in the National Centre for Biotetechnology Information (NCBI) website. Assumed mechanisms or pathways proposed to be linked to genetic resistance were drawn. This work is being compiled in preparation for submission in an international peer reviewed journal.

Chapter 7 presents the general discussion, linking all the work conducted in the study.

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Chapter 2

2 Literature Review

2.1 Introduction

Small ruminants make important contributions to human livelihoods, particularly in developing economies. In 2012, 37 and 22% of the 1.2 billion world sheep population were located Asia and Africa respectively, as well as 56 and 30% of the approximately 1 billion world goat population (FAO, 2015). In most low-input/output smallholder farming systems goats serve as household assets with multiple livelihood functions, providing food, income and important non-market services (Ruto et al., 2008). However, gastrointestinal parasitic infestations impose severe constraints on small ruminant production in marginal systems (Periasamy et al., 2014). Control strategies worldwide are based on the use of anthelmintic drugs, which have often been associated with cases of multiple drug resistant parasites and drug residues in the food and environment. However, most small ruminant farmers in the tropics and sub-tropics are resource-constrained, and do not have access to either anthelmintics or land management practices to mitigate the influence of gastrointestinal parasites (GIN). Therefore, there is a need for alternative methods of parasite control in these farming systems, with genetic improvement offering a more sustainable option. Although resistance to GIN is well studied in both experimental (Davies et al., 2006; Riggio et al., 2013) and commercial flocks (Matika et al., 2011), a few studies have focused on low-input/output smallholder systems in developing countries. This review offers an overview of current practices and potential control methods for GIN resistance.

2.2 Value of indigenous farm animal genetic resources

Farm animal genetic resources refer to all animal species and breeds that are of economic, scientific and cultural interest to humankind in terms of food and agricultural production for the present or the future (Rege and Okeyo, 2006; Rege et al., 2010). Livestock make a particularly important contribution to human livelihoods by serving as household assets with multiple livelihood functions, providing food, income and important non-market services such as draught power and manure (Kohler-Rollefson, 2004; Ruto et al., 2008; Rege et al., 2011). Livestock provides capital stock with insurance functions and contribute to social and traditional structures, forming the root of cultural identity for many societies (Zander, 2006). Indigenous breeds have superior adaptive attributes compared to exotic breeds (Rege et al., 2011). They have good maternal qualities, are fertile with long productive life spans, experience low mortality and good feed conversion rates (Kohler-Rollefson, 2004). All these qualities form the basis for low-input, sustainable agriculture (Philipson et al., 2011).

2.3 Control methods for GIN

2.3.1 Non-genetic methods of internal parasite control

Gastrointestinal nematode control methods previously proposed include chemical and management or biological approaches (Jackson and Miller, 2006). Chemical control is the most widely used method. Alternative approaches, such as use of copper oxide wire particles, have been reported in the control of *Haemonchus contortus* in small ruminants (Torres-Acosta and Hoste, 2008). Copper toxicity is however a problem particularly in sheep (Hoste and Torres-Acosta, 2011), but the potential risk is lower in goats.

Use of ethno-veterinary products, dietary and nutritional supplementation have also been reported to reduce parasite infections (Hoste et al., 2006; Terrill et al., 2009). Paolini et al.

(2003) reported a reduction by 50-60% in faecal egg counts (FEC) in small ruminants following condensed tannin-rich diets supplementation. However, some condensed tannin extracts have been found to reduce small intestine burdens (*Trichostrongylus colubriformis, Cooperia, Nematodirus, Bunostomum* spp.) but not those from the abomasum (*H. contortus, Teladorsagia circumcinta*) (Athanasiadou et al., 2001). Anti-parasitic action has been also demonstrated in chicory (*Cichorium intybus*), sulla (*Hedysarum coronarium*), sainfoin (*Onobrychus viciifolia*) and sericea lespedeza (*Lespedeza cuneata*) (Houdijk et al., 2012). Biological control methods using nematophagous microfungus *Duddingtonia flagrans* have the ability to break the lifecycle of parasites by trapping and killing infective GIN larvae in faeces before they migrate to pasture (Terrill et al., 2012).

Rotational resting and grazing as a means of parasite control limits the host-parasite contact thus reducing pasture contamination and increasing productivity in common grazing rangelands. The strategy of rotational resting and grazing is considered as being either preventative, evasive or diluting (Jackson and Miller, 2006). According to Cabaret et al. (2002) and Younie et al. (2004), the preventative strategy involves turning out parasite-free animals onto clean pastures. The evasive strategy involves moving animals from contaminated to clean pastures within the same season and alternating grazing of different species. The diluting strategy allows worm challenge to be relieved by diluting pasture infectivity by reducing stocking rates, allowing mixed species grazing of animals of different age groups. However, these above mentioned methods are difficult to apply at all times, especially in extensive production systems and in systems with common grazing. Improved nutrition through supplementation of by-pass protein in small ruminants improves resistance and resilience to GIN (Torres-Acosta et al., 2012). Studies by Steel (2004), Colvin et al. (2012) and Marume et

al. (2012) also provided evidence of the benefits of protein supplementation as a means of parasite control.

Internal parasites can also be controlled by making use of vaccines. Some of these vaccines are based on antigens of the parasite stage that adheres to the gut wall and these antigens induce immune responses that interfere with successful attachment in the gut. One of the vaccination methods for example, focuses on identifying protective hidden antigens derived from the worm's intestinal gut cells (Terrill et al., 2012). When the parasites feed on the host they ingest antibodies that bind to functional proteins on the brush border of their intestinal cells, so that the digestive processes are compromised, leading to starvation, loss of fecundity, weakness and death. Eventually, the parasites detach and are lost from the predilection site (Jackson and Miller, 2006). Until recently, the use of hidden antigens was only thought to be effective on cestodes (Waller and Thamsborg, 2004) and not on nematodes. In 2014, a new vaccine against *H. contortus*, (Barbervax®) was commercially available. This is an alternative to the drench–based control method and it has the ability to manage drench resistance (Maxwell, 2015). The problem associated with the use of this vaccine could be related to cost, i.e. for initial use in an animal, three priming doses are required to achieve an effective level of antibody protection and this protection lasts only approximately 6 weeks; thus an animal requires 4-5 vaccinations annually. This poses problems in low-input/output farming systems not only in terms of cost but also for vaccine storage (limited refrigeration capacity) and handling.

The main constraint for the use of anthelmintics is the development of drug resistance, which may be a consequence of host-pathogen co-evolution, in which the parasites survive exposure to standard recommended doses of anthelmintics and are able to thrive and reproduce under existing dosing regimes. The frequency and dosage of treatment are usually the main factors

driving development of resistance to anthelmintics. However, under-dosing, which is a common practice in resource limited smallholder farms, particularly in goats, may be the one of the leading forces to parasite resistance. The continuous development of new classes of anthelmintics has for several decades compensated for parallel development of resistance (von Samson-Himmelstjerna and Blackhall, 2005), in several genera such as *Haemonchus*, *Trichostrongylus* and *Ostertagia* spp. (Kaplan, 2004; McKellar and Jackson, 2004) in sheep and goats. Examples drawn worldwide of anthelmintic resistance across chemical compound classes in small ruminants are summarised in Table 2.1.

2.3.2 Genetic control of GIN

The genetic control methods involve selection of individuals resistant to GIN (Vagenas et al., 2002) and this relies on the existence of host genetic variation and the predominating environmental conditions. Most goat breeds that are highly resistant to parasite infections are found in the tropics reared under extensive farming (Hohenhaus and Outteridge, 1995), but these breeds remain greatly under-utilized (Baker, 1998). Few studies have been conducted on breeding for resistance to GIN in the tropics and subtropics. These include work conducted in Kenya by Baker et al. (1998) in goats (Small East African and Galla breeds) and sheep (Red Masaai and Dorper breeds) and also work conducted in Zimbabwe by Matika et al. (2003) in sheep (Sabi and Dorper breeds).

To date, little work has been undertaken in utilizing these genetic resources as a means of parasite control via selection and breeding for the resistant lines. Although breeding for GIN resistance is an appealing technique, such approaches are difficult to implement in low-input/output smallholder farming systems, mainly due to lack of record keeping and pedigree data in the open mating systems. This aspect is discussed in detail later in this review.

2.4 Resistance to GIN in small ruminants

Resistance is the animal host's ability to counter the adverse effects of pathogens by developing immune-mediated resistance to the pathogen (Kelly et al., 2013). It is often the result of changes in genes other than the immediate drug target, including transporters and drug metabolism. The ability to reduce worm infection differs between sheep and goats depending on their immunological, physiological and behavioural characteristics. Goats have a weaker immune response to GIN compared to sheep (Ahmed et al., 2011) leading to higher infestation under grazing conditions. However, in conditions where browse is available, their feeding behaviour minimises exposure, as they avoid contact with the infective stages of GIN (Torres-Acosta and Hoste, 2008). Anthelmintic resistance problems are greater in goats than in sheep due to the higher requirement for treatment in adults and also goats' ability to metabolise and inactivate anthelmintics faster (Walken-Brown et al., 2008).

2.4.1 Phenotypic indicators of resistance

Common indicators of resistance include faecal egg counts (FEC) which is a function of both parasite burden and fecundity. Other traits include the immune response factors such as eosinophilia and antibody response (IgA, IgG and IgM).

Table 2.1:Cases of anthelmintic resistance in sheep and goats

Species	Country	Anthelmintic[1] (Class)	Nematode genera	Reference(s)
Goats	Ethiopia	Albendazole, Tetramisole, Ivermectin (BZ, IMID, AVM)	*H. contortus, Trichostrongylus, Teladorsagia spp*	Sissay et al., 2006; Kumsa and Abebe, 2009
	Uganda	Albendazole, Levamisole, Ivermectin (BZ, IMID, AVM)	*H. contortus, Cooperia spp. Oesophagostomum spp*	Byaruhanga and Okwee-Acai, 2013
	Nigeria		*H. contortus*	Chiejina et al., 2010
	Pakistan	Oxfendazole, Levamisole (BZ, IMID)	*H. contortus, T. colubriformis*	Saeed et al., 2010
Sheep	Zimbabwe	Fenbendazole, Albendazole, Oxfendazole, Levamisole (BZ, IMID)	*H. contortus, Cooperia spp.*	Mukaratirwa et al., 1997; Matika et al., 2003
	Zimbabwe	Fenbendazole, Levamisole, Rafoxanide (BZ, IMID, SCL)	*H. contortus*	Boersema and Pandey, 1997
	Zambia	Ivermectin , Albendazole (AVM, BZ)	*H. contortus*	Gabriel et al., 2001
	Germany	Levamisole, Ivermectin (IMID, AVM)	*Trichostrongylus spp*	Voigt et al., 2012
	Brazil	Ivermectin (AVM)	*H. contortus,*	Fortes et al., 2013
	Northern Ireland	Benzimidazole, Moxidectin, Avermectin Levamisole (BZ, MLB, AVM, IMID)	*Trichostrongylus Teladorsagia, Cooperia spp.*	McMahon et al., 2013
Sheep/goats	South Africa	Albendazole, Closantel, Ivermectin, Levamisole (BZ, SCL, AVM, IMID)	*H. contortus, Trichostrongylus, Oesophagostomum spp*	Bakunzi et al.,2013 Tsotetsi et al., 2013
	Kenya	Ivermectin ,Fenbendazole (AVM, BZ)	*H.contortus, Trichostrongylus, Oesophagostomum spp.*	Mwamachi et al., 1995
	Switzerland	Avermectin (AVM)	*Haemonchus contortus, Trichostrongylus spp*	Artho et al., 2007
	Norway	Albendazole (BZ)	*Teladorsagia, Trichostrongylus spp*	Domke et al., 2012
	India	Fenbendazole, Benzimidazole (BZ)	*H. contortus, Trichostrongylus spp*	Rialch et al., 2013
	India	Thiabendazole, Tetramisole (BZ, IMID)	*H. contortus*	Swarnkar and Singh, 2011
	Philippines	Benzimidazoles (BZ)	*H. contortus*	Ancheta et al., 2004

[1]Benzimidazoles -BZ; Macrocyclic lactones- ML (Avermectins-AVM or Milbemycin –MLB; Nicotinic agonists (Imidothiazoles-IMID or Tetrahydropyrimidines-TETR); Aminoacetonitriles derivatives-AAD; Salicylanilides-SCL

It also includes the impact of infection such as anaemia, pepsinogen or fructosamine concentrations and resilience in form of growth rate and required treatment frequency (Bishop, 2011).

2.4.2 Genetic resistance to parasites, from a classical selection approach

Gastrointestinal parasite resistance is under genetic control and the existence of genetic variation among individuals with regards to resistance to GIN has been studied extensively (Table 2.2). Conventional breeding strategies are based on the use of indicator traits such as FEC and packed cell volumes (PCV), which are costly and time consuming to collect. Whilst FEC have been the main indicator for resistance to GIN, significant levels of infection are required for genetic variation in FEC to be expressed and in drier parts of the world, this increase in FEC may not occur for several years, or may be masked by parasite control measures aimed at limiting the infection.

Nematode resistance assessed by using FEC has a low to high heritability in small ruminants, ranging from 0.01 to 0.65 (Table 2.3). The heritability of a trait indicates the potential of obtaining genetic gain through selection (Lôbo et al., 2009). For example, selecting animals with the lowest FEC would increase host resistance to parasites. However, resilient animals are not targeted by this approach. Hence, selection and breeding for resistance to GIN is feasible; and a case example of 69% reduction in FEC following genetic selection was reported by Eady et al. (2003).

Although selection for resistance is possible and effective for sheep and goats; this has not been fully adopted in most developing countries, but restricted to research flocks, due to complexity

in collecting phenotypes and pedigree information and limitations associated with costs involved in running the breeding schemes. Moreover, there are other factors to be taken into account. Technical and infrastructural related issues, for example, are the greatest bottlenecks in genetic improvement programmes for most of the sheep and goat farming systems: small flock sizes, lack of clear breeding goals, lack of or poor infrastructures. These are all factors that contribute to the low participation of farmers in breeding schemes, which in turn makes achieving within-breed genetic improvement highly challenging. It has to be kept in mind, however, that the implementation of a breeding program requires an accurate pedigree. It has been indeed shown that even in dairy cattle, which have well established breeding program, over 20% of registered animals have paternity errors (Ron et al., 1996) and this percentage is probably even higher in small ruminants.

In smallholder properties in tropical and subtropical environments usually there is no pedigree recording and no data recording at any time. Mating systems are often not planned with all year round kidding/lambing with community animals mixing in communal shared grazing lands. This renders the conventional breeding practices as we know them currently impossible to implement. However, there are other possibilities with the modern technologies that may remedy some of these shortfalls.

2.4.3 Identification of QTL associated with GIN resistance

Quantitative trait loci (QTL) mapping can help in understanding the complexity of parasite resistance by identifying candidate genomic regions. Studies using microsatellite markers (Beh et al., 2002; Davies et al., 2006; Gutiérrez-Gil et al., 2009; Marshall et al., 2009) have been conducted to understand the mechanisms underlying parasite resistance. Candidate gene

studies, together with microarray and gene association studies have also been conducted in several small ruminant breeds in an effort to identify genes that are involved in the control of resistance and susceptibility (Crawford et al., 2006; Brown et al., 2013). The candidate gene approach focuses on identifying DNA markers within candidate genes, which may not necessarily be causative mutations for resistance themselves, but may be in linkage disequilibrium (LD) with the causative mutation (Sayers and Sweeney, 2005). Candidate genes implicated included those that regulate the immune response, e.g. major histo-compatibility complex (*MHC)* and interferon gamma-y (*IFN-γ)* genes. Several studies confirmed markers associated with GIN resistance close to *MHC* (Miller and Horohov, 2006; Bolormaa et al., 2010a; Alba-Hurtado and Muñoz-Guzmán, 2013) and *IFN-γ* genes (Coltman et al., 2001; Crawford et al., 2006; Miller and Horohov, 2006; Bolormaa *et al.*, 2010b; Alba-Hurtado and Muñoz-Guzmán, 2013).

Although, no causative mutations have been identified in published QTL studies, *IFN-γ* and *MHC* are possible plausible functional and positional candidate genes (Stear et al., 2009). In contrast to the classical selection, the marker-assisted selection can utilize identified QTL to accelerate selection even in cases where the desirable alleles for the trait are found in low frequencies. Several QTL on different regions and chromosomes (OARs) have been reported in the literature for sheep, indicating a polygenic nature for the trait (OAR1, 3, 6, 14 and 20) (Beh et al., 2002; Dominik, 2005; Crawford et al., 2006; Davies et al., 2006; Matika et al., 2011; Salle et al., 2012). In a few studies, some potential candidate genes were identified on OAR8 (Crawford et al., 2006), OAR13 (Beraldi et al., 2007), and OAR22 (Silva et al., 2012). The lack of consensus across studies may be due to parasite resistance being a genetically complex trait (Kemper et al., 2011; Riggio et al., 2013) as well as other reasons discussed in the following section.

Table 2.2: Small ruminant breeds with reported resistance traits against gastrointestinal parasites

Species	Resistant Breed	Susceptible breed	Infection[1]	Parasite(s)[2]	References
Goats	Sabi	Dorper	N	Hc	Matika et al., 2003
	Small East African (SEA)	Galla	N	Hc	Baker et al., 1994; 1998
	Jamunapari	Barbari	N	Hc, *Strongyloides Oesophagostomum spp*	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., 2012b
	West African	-	N	Mixed	Behnke et al., 2011
Sheep	Gulf Coast Native	-	N	Hc	Peña et al., 2004
	F_1 and F_2 Suffolk X Gulf Coast Native	-	N	Hc	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 Masaai	Blackheaded Somali, Dorper, Romney Marsh	A/N	Hc	Mugambi et al., 1997
	Barbados black belly	INRA401	A	Trichostrongyles	Gruner et al., 2003
	Santa Ines	Ile de France, Suffolk	N	Hc, *Oesophagostomum columbianum*	Amarante et al., 2004
	Texel	Suffolk	N	T*richostrongyle; Teladorsagia, Nematodirus*	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., 2010
	Caribbean Hair, Katahdin	Crossbred-Dorper	A	Hc	Vanimisetti et al., 2004

(-) indicates trials which only involved one breed, within-breed differences; [1]N – natural infection; A – artificial challenge [2]Hc-*Haemonchus contortus*; Tc-*Trichostrongylus colubriformis*

Table 2.3: Faecal egg counts (FEC) and packed cell volume (PCV) heritability estimates in small ruminants

Species	Breed(s)	h^2	Age (mo)	Country	References
Goats	Galla and SEA	0.13	4.5-8	Kenya	Baker et al., 1994
	Cross-bred Cashmere	0.2-0.3	12-18	Scotland	Vagenas et al., 2002
	Creole	0.14-0.33	4-10	French west indies	Mandonnet et al.,2001
	Creole	0.10	>11	French west indies	Mandonnet et al.,2006
Sheep	Dorper vs Red Masaai	0.18 vs. 0.35	8	Kenya	Baker, 1998
	Menz and Horro	0.01-0.15	1-12	Ethiopia	Rege et al., 2002
	Rhon and German Merino	0-0.35	3-5	Germany	Gauly et al., 2002
	Merino	0.2-0.65	4-13	Australia	Pollot et al.,2004
	Dorset-Rambouillet-Finn (Lambs–ewes)	0.15-0.39	4 (1-10yrs)	Australia	Vanimisetti et al., 2004
	Soay	>0.10-0.26		Scotland	Beraldi et al., 2007
	Santa Ines lambs	0.01-0.52	-	Brazil	Lobo et al., 2009
	Scottish Blackface	0.14	6-7	Scotland	Stear et al., 2009

2.4 Inconsistencies across studies

The lack of consistency across the results of nematode studies may be in part due to the weaknesses associated with the use of different methods of evaluation. The candidate gene approach relies on prior knowledge, however, a large majority of genes have their functions yet to be defined (Singh et al., 2014). In addition, previously identified QTL seem to disappear with new ones emerging between populations. A possible explanation for this is the differences in the analytical or experimental approaches used in different studies. Examples of these include the use of within-family microsatellite-based linkage (Beraldi et al., 2007; Gutiérrez-Gil et al., 2009; Marshall et al., 2013) *vs.* LD approaches using SNPs in genome-wide association studies (GWAS) (Riggio et al., 2013). Most of the published QTL studies were conducted using half-sib family experimental designs which uses within family linkage as opposed to a population LD. Other factors that may also contribute to these inconsistencies could be the animal population studied (i.e., different breeds, age, sex, immune and physiological status), sample size, nature of infection (i.e. natural infection *vs.* artificial challenge), climatic conditions (i.e. wet *vs.* dry, tropical *vs.* temperate), the production system (i.e. extensive *vs.* intensive), nematode species and indicator traits measured. Despite the added advantages of utilizing QTL as a means of increasing genetic progress, there are still practical problems associated with the use of genetic markers as no major QTL have been identified associated with GIN resistance (i.e. GIN resistance seem to be polygenic trait, with many loci with small effect spread across the genome).

2.4.4 Using GWAS to identify loci underlying variation in GIN resistance

Advances in genomics, technology, statistics and bioinformatics have led to the implementation of GWAS which aim at understanding the genetic basis of complex traits, such as resistance to diseases and production traits (e.g. growth, feed intake and milk yield). Previous FEC studies utilizing within family linkage have been criticised for the inability to replicate results. GWAS aim at overcoming some of these limitations by searching the whole genome for genetic variants associated with quantitative traits, without prior assumptions, thus limiting bias (Hirschhorn and Daly, 2005). In cases where there is no evidence for a positional candidate, LD is exploited to further refine the location of the QTL to target functional mutations in causal genes (Raadsma and Fullard, 2006). The SNP arrays such as the Goat SNP 50k chip with a capacity to genotype 52,295 SNPs (Tosser-Klopp et al., 2014) and Ovine SNP 600k chip with a capacity to genotype 603,350 SNPs (Anderson, 2014) are becoming important tools for GWAS. Setting up GWAS for parasite resistance requires genotyping and phenotyping large numbers of animals to obtain sufficient sample sizes (McCarthy et al., 2008).

Other methods can be used to search for QTL, such as the Wright's fixation index (F_{ST}), which utilizes allele frequencies between resistant *vs.* susceptible individuals and measures the degree of population differentiation. Comparisons of F_{ST} from different parts of the genome can also provide insights into the demographic history of populations and selective sweeps (Kijas et al., 2012). Few studies have been published on host resistance to parasites in small ruminants, mostly in sheep, using SNP chips (Table 2.4).

2.4.4.1 Limitations of the GWAS methodology

In most cases, SNP chips failed to replicate results previously obtained using microsatellites. Discrepancies may be due to different factors, such as the method used (linkage analysis where markers are phased within families *vs.* LD), SNP density, lack of LD between markers and causative mutations, breeds being analysed (which may not be well represented in the reference populations used to create the SNP chips), polygenic nature of the traits of interest, and sample size. Large confidence intervals in the linkage analyses makes it difficult to compare the results across studies (Höglund et al., 2012). Manolio et al. (2009) reported the problem of missing heritability in GWAS for complex traits. Missing heritability refers to heritability estimates of complex traits that cannot be accounted for by use of markers in GWAS, but may be attributable to non-additive genetic variances such presence of copy number variants (CNV) and epigenetics (for a detailed review on missing heritability see Vinkhuyzen et al., 2013).

A meta-analysis conducted by Riggio et al. (2014a) highlighted how some of the challenges could be addressed by aggregation of data from several independent studies, thereby increasing power of detection of genetic variants with small effects. Work done by Kemper et al. (2012) also highlighted how some of the differences between GWAS and family-based linkage studies can be overcome, i.e. through adjusting differences in LD, and fitting all markers simultaneously instead of individually.

Table 2.4: Published QTL studies on host resistance to nematodes in small ruminants

Species	Markers[1]	Breed	Chromosome	References
Goats	M	Australian Angora and Cashmere	23	Borlomaa et al., 2010
	M	Creole	22, 26	de la Chevrotiere et al., 2012b
Sheep	M	Romney- Coopworth	8, 23	Crawford et al., 2006
	M	Scottish Blackface	2, 3, 14 and 20	Davies et al., 2006
	M	Soay	1*, 6*, 12*	Beraldi et al., 2007
	M	Scottish Blackface	3, 20	Stear et al., 2009
	M	Spanish Churra	1, 6, 10, 14	Gutiérrez-Gil et al., 2009
	SNP	Merino		Marshall et al., 2009
	M	Romney-Merino Backcross	3*, 21, 22*	Dominik et al., 2010
	M	Suffolk and Texel	3, 14	Matika et al., 2011
	M, SNP	Romane-Martinik Blackbelly Backcross	5, 12, 13, 21	Salle et al., 2012
	M	Red Masaai, Dorper	2, 26	Marshall et al., 2013
	SNP	Soay	1, 9*	Brown et al., 2013
	SNP	Scottish Blackface	6, 14	Riggio et al., 2013
	SNP	Scottish Blackface, Sarda-Lacaune Backross, Martinik Blackbelly-Romane Backcross	4*, 6, 14, 19*, 20*	Riggio et al., 2014a
	SNP	Red Maasai-Dorper Backcross	6, 7	Benavides et al., 2015

*Suggestive associations; [1]M – Microsatellites; SNP – OvineSNP50 chip

2.4.4.2 Challenges of setting up GWAS in low-input/output smallholder systems

The first hurdle in conducting GWAS in low-input/output smallholder systems, where records are scarce, is obtaining accurate indicator traits. Other challenges include cases of co-infection, mixed or poorly defined breeds, and requirements for large sample sizes (Hayward, 2013). Selective genotyping and selective DNA pooling can be done to reduce number of individuals to be genotyped; however, this may lead to loss of individual information (Singh et al., 2014). In low-input/output smallholder systems it may not be feasible to meet some of these requirements. In general, it is not possible to extrapolate results across distantly related populations. The genetically fragmented nature of sheep and goat populations/ecotypes makes it challenging to use the results on anything other than the population in which they are derived.

One of the key shortcomings of using the SNP technology in low-input/output systems is the cost associated with it. To mitigate this, one could exploit the advantages of imputations, in which key individuals are genotyped using higher SNP chips or sequenced to form the basis from which animals genotyped with low density SNP are imputed to the same density as the former. The power for detection of genetic associations can also be improved by performing 2-stage joint analyses which involve genotyping a proportion of the available samples in the first stage and the remaining in the second stage, with the second stage acting as replication (Skol et al., 2006). Furthermore, data sets from different studies can be combined and data imputation (after rigorous data checking) can be used as a tool to avoid bias and false-negative results (Ioannidis et al., 2009).

2.4.5 Application of genome-wide SNP data in parasite resistance

2.4.5.1 Selective sweeps/signatures

The term selective sweeps/signatures refers to advantageous alleles being fixed in a population on a particular haplotype background due to selection, leading to changes in gene frequencies of variants associated with traits (Gurgul et al., 2014). Statistical methods used for detecting selective sweeps are the F_{ST} (Weir et al., 2005), LD approach, extended haplotype homozygosity (EHH) test, integrated haplotype score (iHS), long-range haplotype (LRH) (Qanbari et al., 2011) and cross population EHH (XP-EHH) test. The XP-EHH detects selective sweeps in which the selected allele has different frequency to the other population. A study by McRae (2012) using selective sweeps on loci associated with resistance or susceptibility to GIN infection identified nine regions showing the highest signals in both Romney and Perendale lines. In another GWAS study on divergent lines selectively bred for high and low FEC, McRae et al. (2014) identified sixteen regions harbouring candidate genes associated with immunological responses to parasite infection i.e. Chitinase activity and cytokine response.

2.4.5.2 Copy number variation (CNV)

Copy number variants are defined as DNA segments which are 1 kb or larger and have variable numbers of copies to those in the reference genome (Iafrate et al., 2004). These variants exhibit similar demographic patterns to SNPs. CNV analysis on genome-wide SNP data can lead to identification of chromosomal regions containing structural variations affecting complex traits (Zarlenga and Gasbarre, 2009).

A GWAS between CNVs and resistance to GIN in Angus cattle resulted in haplotype blocks containing immune-related genes being detected (Xu et al., 2014). According to these authors, when the CNV co-segregates with linked SNPs and associated genes, it contributed to the detected variations in gene expression and thus difference in host parasite resistance. Studies in sheep performed to investigate differentially expressed genes (DEGs) have identified various DEGs related to parasite resistance (Diez-Tascon et al., 2005; Keane et al., 2006; Ingham et al., 2008). A study conducted by Liu et al. (2011) in cattle identified 20 CNVs, 85% of which were associated with parasite resistance. Another large scale analysis of CNVs using SNP genotyping data by Hou et al. (2012) detected 297 CNV regions which were validated by qPCR and overlapped with 437 Ensembl genes associated to GIN infection. Current high-throughput genome scan technologies such as next-generation sequencing (NGS) or SNP genotyping microarrays enables CNV identification at a genome-wide scale (Gheyas and Burt, 2013). The NGS has a potential of reducing ascertainment bias. Despite some of the highlighted potentials, these technologies have not been applied widely to small ruminants.

2.4.6 Genomic selection

Classical genetic improvement programs have relied on the use of phenotypes and pedigree information to generate estimated breeding values (EBV). The increasing use of SNP markers in studying complex traits also avails the potential to calculate genomic estimated breeding values (GEBVs) for traits such as parasite resistance when adequate genotypes and phenotypes are available. Understanding genetic architecture underlying resistance will enable the prediction of genetic risk or selective breeding (Spencer et al., 2009; Hayes et al., 2010). The genomic selection approach was first proposed by Meuwissen et al. (2001) in an attempt to use all SNPs in predictions

and has since become a powerful tool especially for genomic predictions in polygenic traits. Furthermore, accuracy of estimated SNP effects is influenced by the size of the reference population and genetic variance is explained by markers influenced by the effective population size (N_e) and density at which the SNP chip covers the genome.

The accuracy of GEBV has been evaluated in experiments involving other livestock species, such as sheep (Daetwyler et al., 2012, Riggio et al., 2014b), with minimal work being conducted in goats. For reliable genomic prediction, the population under evaluation should have a close relationship with the reference population (Habier et al., 2010). To date, limited studies have been reported on the use of high density genomic information to select for nematode resistance in small ruminants. This may be due to low animal value, and high cost of genotyping. According to Kemper et al. (2011), genomic prediction of nematode resistance suggests only moderate accuracy with currently available SNP arrays; however, the potential of genomic selection warrants that the concept be further investigated. Riggio et al. (2014b) reported moderate accuracies in a within breed approach; however, they also noted that across breed accuracies were low or close to zero. Within breed genomic selection provides the benefits such as improved genetic progress and reduced generation interval.

Genomic selection is now well established in the dairy cattle sector (for milk production) with examples in New Zealand and Ireland where GEBVs are now being routinely used by farmers (Spelman et al., 2013). The genomic selection programmes for sheep are starting to be rolled out to farmers also in Australia. Due to limited goats studies, lessons from sheep studies can be adopted for goats. This may require cheap genotyping (low density SNP chip) of large numbers of animals

combined with imputation from high density information in targeted animals in order to facilitate predictions across breeds (van der Werf et al., 2009). This could be a potential tool for low-input/output farming systems, in which well phenotyped and genotyped animals from the same "breed" could be used as the training set to predict village animals genotyped using a lower coverage of 5k SNPs or less. Imputation from the 5k to 50k or higher SNP coverage can then be done to allow better prediction. The other option would be to create low density "custom" SNP chip which then incorporates the main GWAS hits from genome-wide association meta-analyses studies.

Such an approach was successful in human data albeit the "hits" were generated from high powered studies (Spiliopoulou et al., 2015). These approaches will have the potential to reduce costs; however no low density arrays are commercially available. For now, the challenges of setting up such breeding schemes are great and genomic selection at least with current technologies, is likely to be expensive and logistically difficult to implement in tropical sheep and goats. Despite all these limitations, in systems where records are scarce, genomic selection is the only tool that still offers real potential in improving breeding. In these scenarios, a few farmers can be incentivised to collect data which then can be used to predict genetic merit from non-recorded communal flocks.

2.5 Integrated control, eradication to manipulation of host-parasite equilibrium

Anthelmintic resistance in nematode populations may have resulted partially from the recurrent use and over reliance on drugs. As a result of this, concerns have been raised as to whether host

genetic resistance would similarly breakdown over a period of time, with nematodes evolving to adapt to the resistant hosts (McManus et al., 2014). According to Bishop (2012), the polygenic nature of host-parasite resistance suggests that worm evolution should be slower than that of anthelmintic resistance, as worms would have to evolve against many more targets. In addition to that, there is no published evidence for apparently resistant breeds losing their relative advantage compared with those that are more susceptible.

The strategy of nematode control has evolved to a more logical manipulation of host-parasite equilibrium in grazing systems by implementation of various actions, which include genetic resistance of small ruminants. According to Mandonnet et al. (2014), different strategies can be implemented for nematode control especially in the tropics; these include short-term strategies like reducing host contact with infective larvae through grazing management. It also involves other strategies such as extending the efficiency of current anthelmintics molecules through targeted selective treatments (TSTs) which rely on the assumption that some animals are more infected than others. In addition to that, it also relies on a longer-term strategy which involves enhancing the ability of the host to tolerate the negative effects of worm through genetic selection.

Use of markers in genomic selection dispenses with the need to record pedigrees since these can be reconstructed from the markers. However, accurate phenotypes for the reference populations will still need to be collected. These could be through creating some “phenotype farms” where farmers are incentivised to collect the phenotypes. Some possibilities would be to use existing research institute facilities or form breeding schemes (in low-input/output smallholder farming systems) through centralised nucleus flocks and village or community-based flocks. By using these

strategies, the problems associated with cost of using genomic tools may be mitigated. Village flocks can then be improved for parasite resistance using the genetic merits of the animals in the nucleus flock. Selection for resistant hosts can thus be considered as a sustainable control strategy as it leads to reduced pasture contamination and increased overall flock productivity. However, whatever method will be implemented, success will be most likely achieved if they are being used to complement other control strategies.

2.6 Summary

Different control strategies for GIN can be put in place and these include improved nutrition, reducing host contact with infective stages, use of vaccination, extending efficiency of anthelmintic through target selective treatment and in the long term enhancing ability of the host to tolerate negative effects of the worm. Given the reviewed candidate gene, QTL mapping and GWAS studies, the genetic architecture of GIN is a trait influenced by many loci with small effects. The overall lack of consensus in different studies can be explained by the diversity in studies involving different breeds, parasites species and experimental procedures.

The use of sustainable genetic tools is not the ultimate solution but its use in combination with other integrated control methods could yield positive results. Conventional breeding systems involves phenotyping traits of importance and based on the availability of pedigree information, EBVs are computed and used as a basis for selection. The use of genomic tools has the potential to be explored in low-input/output farming systems, where no records are kept. The identification of SNPs associated with GIN resistance can be used to develop customised chips for the low-

input/output farming systems. In the long-run it is possible to consider the use of genomic tools as an alternative means of parasite control.

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Chapter 3

3 A survey on management and control of gastrointestinal nematodes in communal goat farms in Zimbabwe

3.1 Abstract

Goats are an important source of livelihood especially in smallholder subtropical communities. Infections with gastrointestinal nematodes (GIN) remain the most prevalent parasitic diseases affecting small ruminants. The study was conducted to assess management, the level of knowledge and control of gastrointestinal nematodes. Surveys were conducted in Chipinge (Natural region (NR) I and II), Shurugwi (NR III), Binga and Tsholotsho (NR IV) and Matobo (NR V). Data was collected in 135 households using a pretested semi-structured questionnaire. High flock sizes were found in NR IV and V which are low rainfall areas. Partitioning of gender roles was such that the adult males were involved in decision-making while adult females and children were involved in day-to-day management of animals. Farmers showed low levels of input use, with natural pasture (98.4%) being the main feed source and indigenous breeds (73.2%) being kept. Mashona breeds were reared in NR I, NR II (100% each) and 40% in NR III; Tonga and Matabele reared in NR IV (50% each), and Matabele (100%) in NR V. Farmers ranked food and financial benefits as the main reasons for keeping goats. Gastrointestinal nematodes ranked highest as the most common disease, with 57% of farmers not controlling or treating animals and 63% of farmers not having knowledge on the spread of GIN. Access to veterinary services, anthelmintic class and breeds used by the farmers had the highest effects on parasitic infections in households. There is need for incorporating training workshops for farmers, so as to improve their level of knowledge on GIN control This has a potential of improving goat productivity due to improved animal management.

Keywords: gastrointestinal parasites, anthelmintic, goats, smallholder farmers

3.2 Introduction

Goats are among the main livestock ruminants reared in rural communities in Africa (Nabukenya et al., 2014). In Zimbabwe, goats are a means of livelihood with 97% of the 3.3 million goats being reared by smallholder farmers (Homann et al., 2007). Goats are a major source of income, and food protein for rural farmers in the tropics and sub-tropics. However, the full exploitation of these resources is hindered by drought, traditional systems of husbandry and the presence of numerous prevalent diseases (Sheferaw et al., 2015). Goats have an ability to survive and maintain condition in harsh environment compared to other ruminants (Emiru et al., 2013). Despite their hardiness, studies have shown that infections caused by gastrointestinal parasites (GIN) are a major factor hindering small ruminant productivity globally (Calvete et al., 2014; Zanzani et al., 2014). Economic losses are due to stunted growth, poor weight gain and poor feed utilization (Pedreira et al., 2006; Shija et al., 2014). The impact of helminths is manifested through high morbidity, mortality (Gwaze et al., 2009b), cost of treatment and control measures against helminths (Nwosu et al., 2007; Miller et al., 2011).

Presently there is little or no attention given to the problem of parasitism of goats in the extensive farming systems. Although several studies have been conducted in Zimbabwe, a few have contributed to the control programmes used by the smallholder farmers and little is known about the level of awareness of the disease. Knowledge on these control practices may assist in revising

control strategies. The objective of the study was to assess the knowledge on GIN, management and control of the disease in different agro-ecological regions in Zimbabwe.

3.3 Material and methods

3.3.1 Study sites

Surveys were conducted in Chipinge, Shurugwi, Binga, Tsholotsho and Matopo districts, representing the five agro-ecological regions/natural regions (NR) in Zimbabwe. Selection was based on the densities of goat numbers in the communal area (using information from the Livestock Production Department) targeting areas with minimal urban influence. Table 3.1 gives a summary of characteristics of the natural regions.

Table 3.1: Agro-ecological zones/ natural regions (NR) of Zimbabwe and farming systems

NR	District	Rainfall (mm yr^{-1})	Mean annual Temperature (^oC)	Farming system
I	Chipinge	> 1000 (very high)	18.2	Specialised and diversified
II	Chipinge	750-1000 (high)	18.2	Intensive
III	Shurugwi	650-800 (moderate)	17.6	Semi-intensive
IV	Binga	450-650 (low)	25.3	Semi-extensive
IV	Tsholotsho	450-650 (low)	20.9	Semi-extensive
V	Matobo	< 450 (very low)	19.9	Extensive

A modification from (Vincent et al., 1960)
*Sampling in NR IV was conducted in two districts; this is where most of the goat populations are found in Zimbabwe

3.3.2 Household sampling and data collection methods

One hundred and thirty-five households were randomly selected, with the majority located in NR III, IV and V (Table 3.2). Sampling of households was based on goat ownership and the willingness to participate of farmers. Information was collected through individual interviews with household heads and in their absence the most senior member. The questionnaire collected information on farm characteristics, production, management practices, farmer knowledge on internal parasites and methods of control. Open-ended questionnaires were pre-tested to adapt the content to the local socio-economic environment and for clarity of the interview session. It was also done to remove any ambiguous question, to improve the flow of questioning and refine the questionnaire.

Table 3.2: Summary of households sampled across geographical locations

Province	NR	District	No. of villages	No. of households
Manicaland	I	Chipinge	4	15
Manicaland	II	Chipinge	4	13
Midlands	III	Shurugwi	8	27
Matabeleland North	IV	Binga	6	28
	IV	Tsholotsho	8	26
Matabeleland South	V	Matopo	4	26
Total		**5**	**33**	**135**

3.4 Statistical analyses

Data was analysed using Statistical Analysis Systems (SAS) 9.3 (SAS, 2011), using the SURVEYMEANS and SURVEYFREQ procedures. Descriptive statistics and χ^2 values were computed to investigate the relationship between natural regions and use of anthelmintic, and gender of household head and goat ownerships. The Generalised Linear Models procedure was used to analyse the effects of farmers' socio-economic profiles and natural regions on livestock flock sizes. Pair-wise comparisons of the least square means were performed using the PDIFF option. Indices were calculated to ranked data such as livestock species importance, traits perceived important by farmers, purpose of keeping goats, culling reasons and buck choice as important according to the formula:

$$Index = \frac{\sum(n\ for\ rank\ 1 + (n-1) for\ rank\ 2 + (n-2) for\ rank\ 3 \ldots rank\ i)\ for\ variable\ X}{\sum(n\ for\ rank\ 1 + (n-1) for\ rank\ 2 + (n-2) for\ rank\ 3 \ldots rank\ i)\ \ for\ all\ variables}$$

Where *n* highest value given to a variable based on the number of ranks (e.g. ranks are 1-3, then *n* = 3) and *i* = least rank (if least rank is 3, then *i* = 3) (Mbuku et al., 2006). A binary ordinal logistic regression model (PROCSURVEY LOGISTIC) was used to predict the probability of a household to experience gastrointestinal parasite infections. The logit model used for analysis was:

$$log\frac{p}{1-p} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \dots\dots\dots \beta_n X_n + \varepsilon_{ijkl}$$

where ***p*** is the probability of a household experiencing gastrointestinal parasite infections; $\frac{p}{1-p}$ is the odds ratio, which refers to the odds of a household experiencing GIN infections; β_0 is the intercept; $\beta_1 \dots \beta_n$ are the regression coefficients of predictors; $X_1 \dots X_n$ are the predictor variables (age of household head, gender of household head, goat flock sizes, goat breeds owned, availability of supplementary feed, vaccines, a housing structure and accessibility to veterinary services, anthelmintic treatment, anthelmintic class); ε is the random residual error distributed as N (0, 1 σ^2_E).

3.5 Results

3.5.1 Livestock production

Livestock rearing was a major activity (77 %) in most households. Mean livestock populations and goat flock composition across regions are shown in Table 3.3. Goats were the most reared livestock (92.1 %) followed by chickens (84.3 %) and the least were pigs (7.9 %). Goats were ranked the most important livestock Ranking index (RI) = 0.35), followed by chickens (RI = 0.22), cattle (RI = 0.18) and the least were pigs (RI = 0.02). Total goat numbers ranged from 1-132 in households with average flock sizes of 13.9 ± 1.61. Goat flock sizes and composition differed by NRs, with highest total flock sizes being observed in NR IV and V (21 ± 2.55 and 16.2 ± 3.40) and the least

in NR 1 and 2 (6 ± 4.47 and 5.7 ± 4.81), respectively. Across the NRs majority of the farmers (59.3 %) had flock sizes ranging 1- 10, 23.4 % (11-20) and 17.2 % having flock sizes of at least 20 goats.

3.5.2 Goat flock composition, ownership and participation in rearing activities

Flock size significantly varied by gender ($P < 0.05$) with male-headed households keeping larger flocks (15.3 ± 1.83) compared to female-headed households (6.4 ± 1.38). Goats were reared extensively (92.1%) and majority of the households rearing goats had at least 2 does whereas bucks were found in limited households. Majority of the goats were owned by adult males (53 %) and the least by children (19 %) in most natural regions and in NR IV women and children had high goat ownership (Figure 3.1). Rating of goat importance in relation to other livestock species was not affected by the total flock size ($\chi^2 = 0.88$). However goats were ranked the most important livestock by farmers who had the least number of goats (i.e. flock size range 1 – 10). Adult males were greatly involved in the rearing activities (50.8 %), female (37.9 %) and children to a lower extent (11.4 %). Partitioning of roles was in such a way that the adult males were mainly involved in decision-making processes such as purchasing, selling and decision on when to slaughter, whereas the day-to-day activities such as health management and herding were done by adult females and children (Figure 3.2).

3.5.3 Perceptions of farmers on reasons for keeping goats

Across the regions, goats were mainly reared for meat, cash, manure and to a lesser extent for milk, breeding and cultural reasons (Figure 3.3). Farmers ranked food and financial benefits as the main reasons for keeping goats.

Table 3.3: Livestock numbers and goat flock composition (± SE)

Species	**Average per household**					**% total livestock**
Natural region	**I**	**II**	**III**	**IV**	**V**	**%**
Cattle	1.4 ± 2.03^{c}	4.3 ± 2.18^{b}	8.2 ± 1.52^{a,b}	10.0 ± 1.16^{a}	5.4 ± 1.52^{b}	20.5
Goats	5.1 ± 3.61^{b}	4.15 ± 3.88^{b}	6.3 ± 2.69^{b}	14.7 ± 2.06^{a}	14.3 ± 2.69^{a}	28.0
Sheep	0	0	1.1 ± 1.32	3.5 ± 1.01	1.4 ± 1.32	4.5
Chickens	24.3 ± 13.45	17.1 ± 5.42	18.8 ± 4.59	14.8 ± 3.51	10.4 ± 4.59	40.3
Pigs	2.2 ± 1.04	0	0	0.2 ± 0.24^{b}	0	0.9
Donkeys	0	0.5 ± 0.62^{b}	0	1.8 ± 0.33^{a}	2.3 ± 0.43^{a}	3.2
Other poultry	3.5 ± 1.28^{a}	2.2 ± 1.16^{a,b}	0.6 ± 0.54^{c}	0.6 ± 0.48^{c}	0.5 ± 0.54^{c}	2.6
Goat flock composition						**% total flock**
Kids	1.2 ± 1.03^{b}	1.8 ± 1.04^{b}	1.7 ± 0.76^{b}	5.4 ± 0.58^{a}	1.5 ± 0.76^{b}	21.7
Mature bucks	1.1 ± 1.43^{b}	0.5 ± 1.54^{b}	1.1 ± 1.07^{b}	4.5 ± 0.82^{a}	3.6 ± 1.07^{a,b}	20
Mature does	3.7 ± 2.69^{b}	3.4 ± 2.89^{b}	5.1 ± 2.0^{b}	11.1 ± 1.54^{a}	10.7 ± 2.0^{a}	58.3

^{a,b,c} Means in the same row with different superscripts are significantly different ($P < 0.05$)

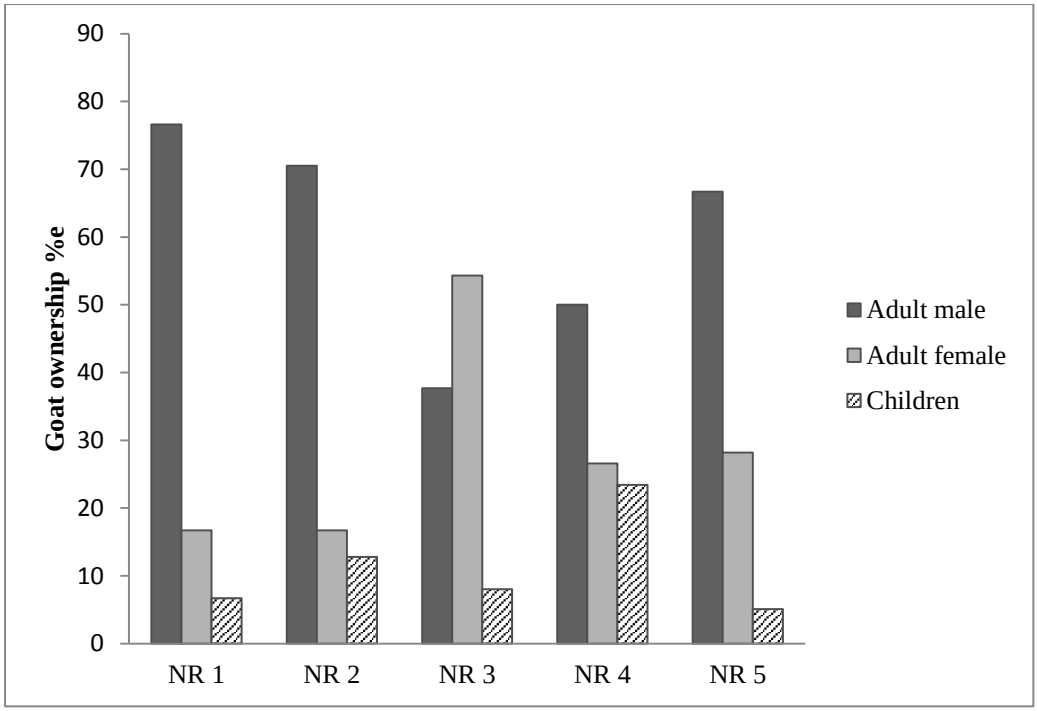

Figure 3.1: Goat ownership by household members in communal in different agro-ecological regions

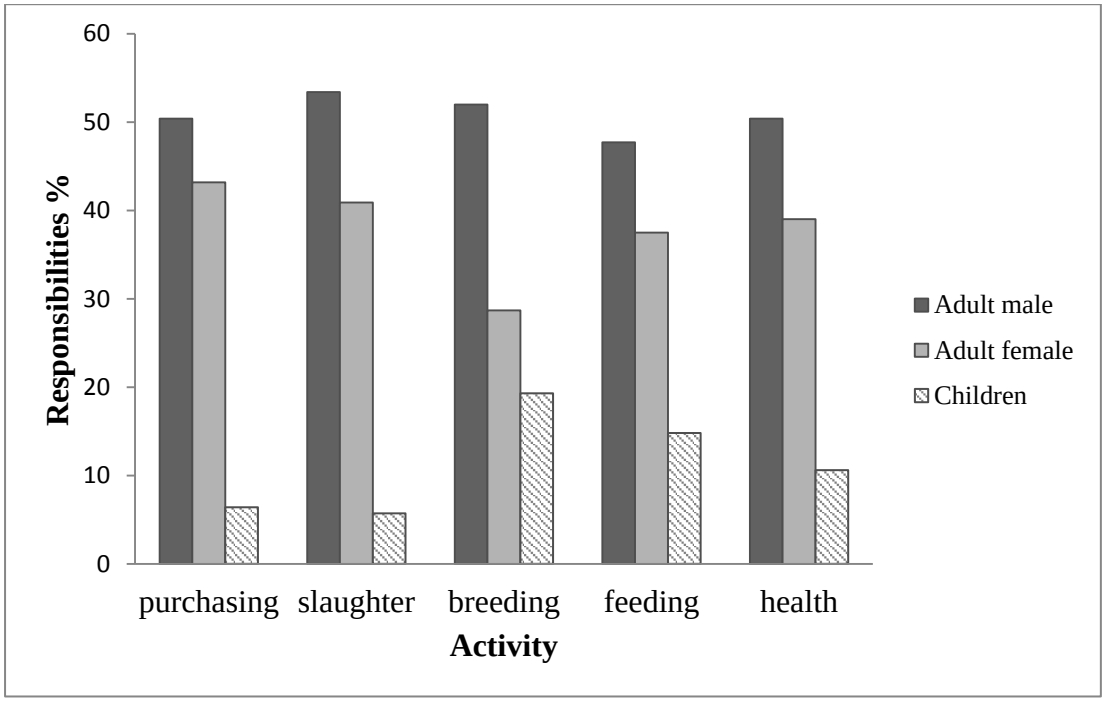

Figure 3.2: Management activities by household members in communal households

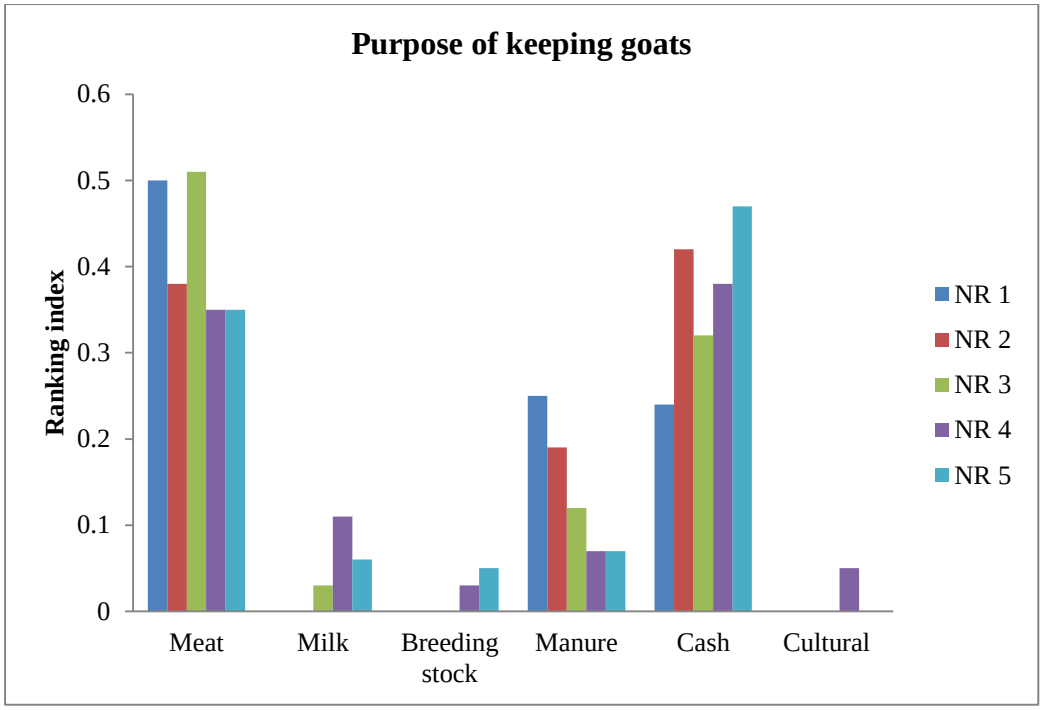

Figure 3.3: Reasons for keeping goats in communal households in different agro-ecological regions

3.5.4 Goat management

3.5.4.1 Nutrition, housing and breeding practices

Natural pasture was the dominant source of feed (98.4%), followed by stover (24.2%), with a few buying commercial feeds (6.6%) and about 5.5% supplementing with forage legumes. The majority of farmers housed their goats in pens (95%). The breeds among the farmers were Matabele (34.7%), Mashona (31.5%), crossbreeds (20.2%) and Tonga (13.7%). In different natural regions proportions of indigenous breeds reared were Mashona in NR I, NR II (100% each) and 40% in NR III, Tonga and Matabele in NR IV (50% each), and Matabele (100%) in NR V. Goat breeds were obtained from local communities (92%), with a few farmers obtaining their goats from commercial farms (0.80%) and neighbouring countries (0.80%) in districts lying closer to the borders of the country. Most of the farmers practiced uncontrolled breeding (96%) and the remaining, hand mating. Buck sources were from unknown origins (60%), with

about 17% of the households using their own bucks. Culling was not a common practice (11%) across the regions and in areas where it was practiced, old age (19.7%) was the main cause.

3.5.4.2 Goat health and management of gastrointestinal parasites

Most of the farmers had access to healthcare (69.5%), with the government veterinary services being the primary healthcare provider (24.2%), extension (15.6%), and the least were drug suppliers and private veterinary specialists. Internal parasites were a prevalent health problem (89.5% of respondents). Pulpy kidney (62.9% of respondents), skin problems (46.9%), tick borne diseases (41%), orf (27.6%) and eye problems (23.6%) were also reported. Other diseases included feet, respiratory and reproductive problems. The main methods of disease control, involved use of traditional medicines (50.4%), antibiotics (41.7 %), tick control (41.1 %), and anthelmintics (27.3%). About 19.8% did not know the names of specific drugs they use and 12.8% did not treat their animals.

3.5.1 Knowledge on GIN

Most of the farmers did not know how internal parasites are spread from one animal to the next (62.8%), while 19.6% suspected it could be from contaminated feed, 16.7% from contaminated water and the remainder from contaminated kraals. On perception of internal parasites infections symptoms; 33% had no idea, while 27.3% used loss in body condition as an indicator, 21.9% identifying worms after slaughter, 14.8% worms in faeces and the remainder by seeing worms in feaces, diarrhea, ruffled coat and bottle jaw.

3.5.4.4 Control of GIN

Majority of the households did not provide any medical intervention for internal parasite control (57.9%), while 34.6% were dosing, and the remainder vaccinated or used traditional methods. Frequency of parasitic control was on a routine basis (52.7%) or on per-need basis (45.7%) and the rest was based on availability of funds. For those using anthelmintics, 31.7% treated adult animals, whereas 8.5% treated kids and 29.4% treated the whole flock. Overall, farmers used Benzimidazoles (56.8%), Salicylanilides (27.3%) and to a lesser extent Imidothiazoles and macrocyclic lactones to control gastrointestinal parasites (Figure 3.4). The drugs used by the majority of the farmers were Valbazen® (56.8%), Systamex® (20.5%) and Albex® (15.9%). Proportions of farms using Benzimidazoles and Salicylanilides significantly differed by natural regions ($P < 0.05$). The use of Benzimidazoles and Salicylanilides was highest in NR I, III, IV and NR I, II, V respectively. Of the 44 farms relying on use of anthelmintics, 34% changed drugs within one year. Among these farmers, 13.6% changed the drug class between years.

Factors considered affecting occurrences of parasitic infections in the different households were investigated and the availability of veterinary services, goat breeds and the anthelmintic class were used by farmers to predict the occurrence of GIN in the selected areas (Table 3.4). Male-headed households (OR = 1.58) and households with heads less than 40 years (OR = 1.05) had the highest probability of experiencing GIN infections ($P > 0.05$). In addition to that, households owning at least 20 goats (OR = 1.73), and those owning crossbreeds (OR = 3.89) had higher chances of experiencing GIN infections than those with less animals and rearing indigenous breeds.

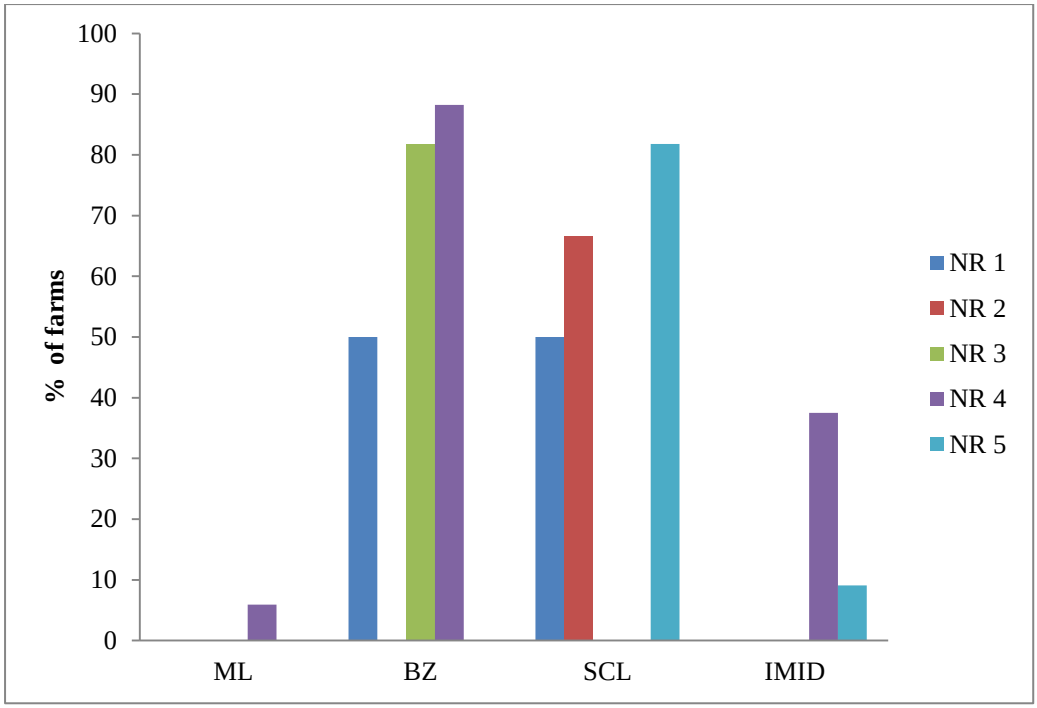

Figure 3.4: Proportion of households using different classes of anthelmintics in different agro-ecological regions; ML = Macrocyclic lactones, BZ = Benzimidazoles, SCL = Salicylanilides, IMID = Imidothiazoles

Goats that were not provided with housing had higher odds of infection (OR = 1.14) than those with. Farmers who had access to veterinary services had higher chances of experiencing parasitic infections than those who did not. In terms of GIN control methods, households not treating their animals had the highest odds of infection, followed by those who were dosing and vaccinating and the least were the ones relying on traditional methods. The probability of parasitic infections was also higher in goats that were not given nutritional supplements. Households using Benzimidazoles experienced high parasitic infections as compared to those that used Salicylanilides, Imidothiazoles, and Macrocyclic lactones.

Table 3.4: Odds ratio estimates of a household gastrointestinal parasite challenges in the selected areas in Zimbabwe

Effect	Odds Ratio	95% Wald Confidence limit	
		Lower limit	Upper limit
Age of household head (> 40 vs < 40 years)	0.767	0.180	3.275
Gender of household head (male vs female)	0.820	0.184	3.652
Goat flock size (1 -10 vs 11-20 vs > 20)	0.792	0.442	1.419
Goat breeds (indigenous vs crossbreeds)	1.658	0.625	4.396
Availability of goat housing (no vs yes)	0.888	0.088	8.968
Availability of supplementary feed (no vs yes)	0.836	0.198	3.524
Availability of veterinary services(no vs yes)	1.062	0.350	3.221
Gastrointestinal parasitic control (no vs yes)	0.795	0.445	1.423
Anthelmintic treatment (no vs yes)	0.186	0.035	1.000
*Class of anthelmintic treatment (BZ vs either SCL/IMID/ML)	2.300	0.890	5.949

*ML - Macrocyclic lactones; BZ - Benzimidazoles; SCL - Salicylanilides; IMID – Imidothiazoles

3.6 Discussion

The study showed gender disparities in goat ownership with male-headed households having larger stock numbers. Despite the larger numbers of goats owned by men, the partitioning of roles was in such a way that men were mainly involved in decision making process while women and children were the main source of labour. Findings were similar to research by Oluka et al. (2005), who attributed this to men having disproportionate control over livestock resources and benefits. Livestock production is culturally a male-dominated sector, even though women provide the labour. According to Sinn et al. (1999), women are face difficulties in access to land, credit, inputs and services, such that to access agricultural extension services

is about one in twenty to that of men. This has a potential to negativily affect productivity in rural households.

The level of animal management (nutrition, breeds reared, mating systems, disease control) were similar to findings by Kosgey et al. (2008). A characteristic of most smallholder farming systems: uncontrolled mating systems, indigenous breeds, minimal input use i.e. feeding and health management and animals reared for family needs *vs*. commercial purposes (Kosgey et al., 2008). High goat numbers in these natural regions can be associated with the availability of natural pasture, and according to Devendra (1999), goat population size and distribution favour the arid and semiarid conditions. According to Nwosu et al. (2007), localized contamination of watering and feeding areas may predispose the animals to parasitic infections. Improved feeding facilitates resistance to parasites. In addition, access to tanniferous feeds such as tree legumes also reduces levels of infection in goats (Gray et al., 2012). High goat flock sizes were noted in NRs IV and V, which are areas characterized by high temperatures and low rainfall, patterns also described by Omoike et al. (2014).

There was limited level of knowledge and perception on causes of parasitic infections, modes of transmission, symptoms of infection and parasite control. This could be associated with the limited awareness of GIN infection. Current results were in contrast with findings by Moore et al. (2016), where farmers had higher awareness of parasite control and the need for proper diagnosis of animals before treatment. Despite farmers in this study highlighting that they had access to animal healthcare from government veterinary services, the extent of farm visits were very minimal. Most of the farmers were not controlling GIN, while a few relied on anthelmintics, vaccinations and traditional means. Frequency of anthelmintic treatments was relatively moderate, with farmers treating their goats only when animals exhibited clinical

signs. Results from this study were in agreement with some studies conducted elsewhere (Aga et al., 2013; Zanzani et al., 2014). Poor healthcare management can be mainly attributed to limited knowledge, or shortages of funds for procuring drugs. Shija et al. (2014) observed that many GIN infections rarely get veterinary attention because of their chronic and insidious nature and also clinical signs may be evident only during the terminal stages.

For those using phamaceuticals as a means of parasitic control, the majority had limited knowledge of the drugs they were using and they mostly relied on the extension workers for health management. In this study, identification of the different anthelmintics was mainly based on the availability of residual containers in the households making it difficult amongst particpants who had disposed such containers. Benzimidazoles class were the most commonly used drugs/ antehilmintics across natural regions, and this was in line with the findings from Tsotetsi et al. (2013). This could have been attributed to the low cost of Benzimidazoles in relation to other anthelmintics (Tsotetsi et al., (2013; Leignel et al., 2010). A small proportion of households unknowingly alternated between drugs of the same or different anthelmintic class within the same year or between years; supporting findings by Zanzani et al. (2014) and Rojo-Vázquez and Hosking (2013). This, coupled with improper drug use could potentially introduce anthelmintic resistance despite the lower frequencies of use. According to Pedreira et al. (2006)**,** efficient parasite-control programmes involve more than three treatments per year, which was not the case among the interviewd farmers. Some farmers used non-anthelmintic drugs for treatment such as antibiotics, highlighting the need for farmer education on parasitic control and/or prevention methods, drug use and dosage. Considering that use of drugs has a potential of reducing parasitic infections. According to Aga et al. (2013), phamaceuticals remains the basis of helminths control programs, therefore proper use is very important.

Goats from male headed-households had higher occurrences of infection, which could have been disproportionate allocation of resources biased towards other livestock such as cattle. The probability of infection was higher for households using Benzimidazoles as compared to other classes, this maybe due to development of resistance by parasites. This supports studies conducted by Rialch et al. (2013) and Kumar et al. (2015) who reported reduced efficacy of Fenbendazole, a variant of Benzimidazoles class against GIN. Households that were providing nutrient supplements had lower infection rates. Studies conducted by Torres-Acosta et al. (2012), Raju et al. (2015) and Garate-Gallardo et al. (2015) have shown that nutrient supplementation improves resilience against GIN. Poorly nourished animals are more susceptible to GIN infection leading to reduced productivity (Pathak et al., 2013). In this study, households with access to veterinary services showed high chances of having infections than those without; in this study it was because extension workers interacted less with farmers. On the other hand, indigenous breeds showed lower infection. According to Aumont et al. (2003), local tropical breeds are adapted to constraints of poor-quality grass, high temperatures and local diseases, and thus thrive under such conditions.

The disconnection between level of awareness of gastrointestinal parasitic diseases, their cause and control, warrant an urgent need to bridge this gap.

3.7 Conclusion

Goat farming is important for smallholder farmers in terms of improving livelihoods by providing food and serving as a source of insurance in cases of emergencies. Flock sizes were greater in the semi-arid agro ecological regions and numbers were also high in male-headed

households, though women and children were found to be playing an active role in goat management. The disconnection between level of awareness of gastrointestinal parasitic diseases, their cause and control, warrant further studies. The survey demonstrated that the level of awareness in GIN disease, transmission methods and control was very low. Most of the farmers were not treating their goats and some were using non-anthelmintic drugs to treat infections. Capacitation of farmers via trainings was necessary for the effective control of GIN. The next chapter provides insights on prevalence of GIN in the same study sites described in this chapter. The initial sample collection was conducted at the same time when the survey was conducted and then conducted different at various times, taking seasons into consideration.

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Chapter 4

4 Prevalence and risk factors of gastrointestinal nematodes in low-input, low output farming systems in Zimbabwe

4.1 Abstract

A longitudinal study was conducted in low-input, low-output farming systems to determine the prevalence of gastrointestinal parasitic infections in different age groups, sex and associated risk factors in goats. A total of 580 indigenous goats were randomly selected in areas representing the five agro-ecological regions of Zimbabwe in the dry and wet seasons. Blood and faecal samples were collected from each animal and egg/oocyst per gram of faeces (epg/opg), larval culture, and packed cell volumes (PCV) were determined. Factors affecting parasitic infections were evaluated. Highest prevalence was determined for *Eimeria* oocysts (43%), strongyles (31%) and lower levels in trematodes and cestodes. Parasites identified were *Haemonchus* spp, *Strongyloides* spp and *Oesophagostomum* spp. Area, season, sex and age significantly influenced patterns of gastrointestinal infections ($P < 0.05$). Prevalence was highest in goat populations from Chipinge and Binga. Cannonical correlations indicated that parasite species composition varied by area and impacts of risk factors also differed. Risk of infection was very high for goats sampled in natural regions (NR) I, II, III (OR = 6.6 - 8.2; $P < 0.05$) as compared to those in NR IV and V. Highest helminths and *Eimeria* infections were observed in the wet season ($P < 0.05$). Young animals were more susceptible to parasitic infections ($P < 0.05$). High prevalences were observed for goats aged 1 and 6 years with the least, in goats aged 3. Prevalence was higher in males than females, with odds of infection for males being almost three times to that for females ($P < 0.0001$). Knowledge concerning gastrointestinal helminth biology and epidemiological infection patterns caused by these

parasites is essential in the development of appropriate control strategies and this has a potential to reduce production losses.

Keywords: faecal floatation, gastrointestinal tract, helminth, coccidian, risk assessment

4.2 Introduction

Goats make important contributions to human livelihoods in developing economies, since they are extremely hardy animals that can survive and reproduce under extremely high temperatures and low humidity with minimum available feed (Baker and Gray, 2004). Of the approximately 1 billion world goat population, 56 and 30% are located in Asia and Africa, respectively (FAO, 2015). The majority of the goats in Zimbabwe are owned by smallholder farmers in mixed crop-livestock systems (Rooyen and Tui, 2009). In this farming system, goats are increasingly used to augment cash income and enhance food security, thus serve as an important component in the household's livelihood strategies. Socio-economic importance is attached to goat ownership such that, in some instances, they may be the only realisable wealth of a rural household (Nwosu et al., 2007). In addition, goats have other functions such as provision of manure, cultural roles, thus playing a significant role in livelihoods.

Gastrointestinal nematode (GIN) infections are the main prevalent parasitic diseases affecting small ruminant productivity worldwide, especially in tropics and sub-tropics (Torres-Acosta and Hoste, 2008; Calvete et al., 2014). Globally the most common nematode species known to affect small ruminants are *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Teladorsagia circumcincta* and some species such as *Nematodirus* spp., which are not found in sub-Saharan Africa (Bishop and Morris, 2007). Large numbers of internal parasites and their prevalence have been documented in different studies of goats including Zimbabwe (Pandey

et al., 1994), Namibia (Kumba et al., 2003), Nigeria (Nwosu et al., 2007), Kenya (Odoi et al., 2007), Ethiopia (Sissay et al., 2007), South Africa (Gwaze et al., 2009b), Cameroon (Ntonifor et al., 2013); Tanzania (Sharma and Mandal, 2013). The most common nematode genera detected in mixed infections in these studies were *Haemonchus*, *Trichostrongyles*, *Strongyloides*, *Trichuris*, *Bunostomum*, *Oesophagostomum*, *Cooperia*, *Nematodirus* spp. (Badaso and Addis, 2015). Trichostrongyle nematodes are considered among the most pathogenic and economically important parasites of small ruminants (Jurasek et al., 2010) and further studies on these are needed in order to devise programmes for preventing managing these parasitic diseases.

Gastrointestinal parasitism is associated with economic losses, low productivity, reduced animal performance (Badran et al., 2012), high mortality and morbidity (Negasi et al., 2012). Goats infected with internal parasites show a rough dull-coat, weakness, diarrhea, apathy, tail rubbing, signs of hypo-proteinaemia, submandibular oedema (bottle jaw), loss of appetite and weight loss (Risso et al., 2015). In addition, some trichostrongyle nematodes cause anaemia due to their haematophagous effect and protein loss, which can lead to ill-thrift in animals. In addition to gastrointestinal nematodes, coccidiosis (especially *Eimeria* species) have also been known to infect livestock in Zimbabwe, having moderate to high pathogenic effects (Radfar et al., 2011). However, co-infection with other trichostrongyle nematodes makes diagnosis of clinical coccidiosis difficult (Chhabra and Pandey, 1991; Zainalabidin et al., 2015).

Host and environment risk factors (agro-ecological conditions, animal husbandry practices such as housing system, and pasture management) play an important role on the onset of GIN infections (Ratanapob et al., 2012). These largely determine the type, incidence and severity of various parasitic diseases (Badran et al., 2012). Other risk factors such as the host species, sex

of the animal, age, body condition and breed/genotype (Badaso and Addis, 2015), parasite species and intensity of the worm population, also have an effect on the development of gastrointestinal parasitic infections (Tariq et al., 2010). Lack of area-specific studies conducted in Zimbabwe (Pandey et al., 1994; Matika et al., 2003) have generated limited information on gastrointestinal parasite prevalence in the different agro-ecological regions and associated risk factors to parasite infection. Knowledge on the prevalence, specific composition of the gastrointestinal fauna can provide baseline information which can be used to control parasite infections. The objectives of this study were to: i) determine GIN present in diverse farming systems; ii) determine level of prevalence of the parasites considered, and iii) evaluate risk factors on parasite infections in goats reared in low-input, low-output systems in Zimbabwe.

4.3 Material and methods

4.3.1 Study sites and animals

The study was conducted between November 2014 and June 2015 in low-input low-output farming systems in five districts of Zimbabwe: Chipinge, Shurugwi, Binga, Tsholotsho and Matobo, representing the five agro-ecological regions. Table 4.1 shows a description of the study districts.

Table 4.1: Agro-ecological zones/natural regions (NR) of Zimbabwe and vegetation

NR	District	Rainfall (mm yr^{-1})	Temp (^oC)	Altitude (m)	Vegetation
I	Chipinge	> 1000	18.2	> 1600	**Mountain grassveld:** *Themeda, Loudetia, Andropogon, Monocymbium, Eragrostis* spp. Shrubs*: Senecio* spp.
II	Chipinge	750-1000	18.2	1200 – 1675	**Hyparrhenia tall grassveld:** *Hyparrhenia, Hyperthelia, Heteropogon, Brachiaria, Digitaria, Eragrostis, Andropogon* spp. Shrubs *Terminalia, Burkea, Combretum, Vachellia* spp.
III	Shurugwi	650-800	17.6	> 1200	**Hyparrhenia and Eragrostis veld**: *Eragrostis, Heteropogon, Themeda, Cymbopogon, Hyparrhenia* spp. Shrubs *Vachellia, Brachystegia, Julbernardia* spp.
IV	Binga	450-650	25.3	450 – 1050	**Eragrostis veld***: Eragrostis Schizachyrium, Heteropogon, Schmidtia, Pogonarthria, Brachiaria, Urochloa, Digitaria, Enneapogon, Aristida* spp. Shrubs: *Terminalia, Combretum, Vachellia, Commiphora, Colophospermum, Grewia, Brachystegia, Enneapogon* spp.
IV	Tsholotsho	450-650	20.9	450 - 1050	**Eragrostis veld***: Eragrostis Schizachyrium, Heteropogon, Schmidtia, Pogonarthria, Brachiaria, Urochloa, Digitaria, Enneapogon, Aristida* spp. Shrubs: *Terminalia, Combretum, Vachellia, Commiphora, Colophospermum, Grewia, Brachystegia, Enneapogon* spp.
V	Matobo	< 450	19.9	900 – 1200	**Aristida and Eragrostis veld:** *Aristida, Digitaria, Triraphis, Heteropogon, Eragrostis, Panicum, Baikiaea* spp. Shrubs: *Colophopsermum, Pterocarpus, Julbernardia, Brachystegia, Burkea, Terminalia, Guibourtia, Combretum* spp.

Modified from (Vincent et al., 1960) and (Gambiza and Nyama, 2000);

4.3.2 Animal management

Animals from Chipinge, Shurugwi, Binga, Tsholotsho and Matobo were owned by smallholder farmers who had small flock sizes, ranging from 1 to 10. The animals from these areas were maintained under extensive management systems, foraging on farm land or in communal

pastures during the day with minimum supplementation and kraaled during the night. In these areas, veterinary care was low to non-existent, with goats not treated/dewormed. Animals mated indiscriminately in communal grazing areas. Goats in these areas had contact with other animal species such as cattle and sheep in the communal grazing areas.

Animals at Matopos Research Station (in the district of Matobo) were managed semi-intensively. Goats foraged on the Research Station open rangeland throughout the year with some rotation in the paddocks during the day, minimum supplementation (1 kg of prepared meal of forage legumes + maize per animal) and penned at night. All animals were treated with an acaricide weekly during the wet season and fortnightly during the dry season to control ticks and tick-borne diseases. Ivermectin and closantel were used routinely to control for gastrointestinal parasites. Mating was done yearly from June to August, with each buck mated with 25 - 30 does.

4.3.3 Animal ethical clearance

Ethical clearance (certificate number 001/15/Animal) for animal use was approved by the Animal Ethics sub-committee of the Department of Livestock and Veterinary Services, Zimbabwe. This was based on international standards of animals use in research.

4.3.4 Study animals

Animals from households described in chapter 3 were used. Size for biological samples was determined using the equation $\boldsymbol{n} = 1.96^2\boldsymbol{pq/L}^2$ (Thrusfield, 1997), where $\boldsymbol{n}$ = sample size, $\boldsymbol{p}$ = expected prevalence, $\boldsymbol{q}$ = 1- $\boldsymbol{p}$ and $\boldsymbol{L}$ = limits of error on the prevalence (absolute precision at 95% confidence interval). The expected prevalence was estimated at 80% in the communal areas. In addition, a 10% allowance for non-response in the communal herds was made, giving

a total sample size of 270 goats. This led to goats of different ages being sampled depending on availability per farm. On the other hand, all the animals at Matopos Research Station were sampled, resulting in 310 additional animals of different age classes as summarised in Table 4.2.

Table 4.2: Summary of animals sampled across geographical locations

NR	District/area	Sample size	[a]Predominant breed
I	Chipinge	30	Mashona
II	Chipinge	26	Mashona
III	Shurugwi	54	Mashona
IV	Binga	56	Tonga
IV	Tsholotsho	52	Matabele
V	Matopos Research Station	52	Matabele
V	Matobo	310	Matabele
Total	**5**	**580**	

[a]In each of the communal areas farmers kept predominant breeds together with crossbreds

The Mashona and Tonga goats are small, compact and hardy indigenous breeds. According to Mason and Maule (1960), these are prototypes of the Small East African goats, with mature body mass of 25 – 30 kg. The Matabele type goats are larger than the Mashona, with mature body mass ranging from 40 - 65 kg for males and 30 - 45 kg for females.

4.3.5 Sample collection, examination and culture

Faecal and blood samples were collected directly from the rectum and jugular veins by venipuncture into airtight containers and EDTA vacutainer tubes, respectively. Sample

collection was conducted over two different seasons targeting the dry (late April - early October) and wet (late October - early April) seasons from 2014 to 2015. Sampling was conducted in January, June and July (Tsholotsho); February, June and July (Shurugwi); April, June and October (Chipinge), February, May and November (Binga); January, May and September (Matobo); January, May, July and September (Matopos Research Station). Collection was done on 1,872 records from 580 animals. Rainfall and temperature for the different areas was obtained from the Meteorological department.

Samples were kept between 2 - 4 °C during field sampling in cooler boxes prior and later refrigerated prior to analyses. Briefly, faecal egg counts (FEC) were determined by the modified McMaster technique, using floatation methods for nematodes, cestodes, and sedimentation methods for trematodes (MAFF, 1986). Faecal cultures were prepared by incubating 2 - 3 g of faeces between 26 - 28°C for 7 days at 80% humidity after which infective larvae were collected using a modified Baerman technique as described by Roberts and O'sullivan (1950). Distinguishable nematode eggs (*Nematodirus and Trichuris*), trematode and cestode eggs were identified directly. Identification of 3rd stage larvae of nematodes was only at the genus level according to Van Wyk et al. (2004). Packed cell volumes (PCV) were assessed using the capillary micro-hematocrit centrifuge method (Bull, 2000). Low PCV is usually associated with cases of helminthiasis (Zainalabidin et al., 2015), especially *H. contortus*, which causes anaemia. To complement the information on the samples collected, a questionnaire was administered and information on management practices, farmer's knowledge on internal parasites and methods of control, was also recorded.

4.4 Statistical analyses

Analyses were carried out with the Statistical Analysis System v 9.3 (SAS, 2011). Descriptive analysis was conducted on survey data. The traits analysed were FEC for nematodes and coccidiosis and PCV. Faecal egg counts for all nematodes and coccidia were transformed through a base 10 logarithm ($\log_{10}$FEC+25) to approximate a normal distribution. Data and the results were back-transformed by taking anti-logarithms and presented as geometric means (GFEC). All statistical tests for FEC were applied to the transformed data. Fixed effects were explored using PROC GLM procedure (SAS, 2011) using the following model:

$$Y_{ijkl} = \mu + S_i + T_j + U_k + A_l + A*T_{lj} + A*U_{lk} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the response variable of LFEC (Lstrongyles, L*Fasciola*, Lamphistomes, L*Trichuris, LStrongyloides*, L*Moniezia*) and L*Eimeria*, μ is the population mean; S_i is the effect of the i^{th} study area (Chipinge, Shurugwi, Binga, Tsholotsho and Matobo districts); T_j is the effect of the j^{th} sex (male or female); U_k is the effect of the k^{th} season (wet or dry); A_l is the effect of the l^{th} age (1-7years); $A*T_{lj}$ and $A*U_{lk}$ are the interactions (age*season and age*sex); ε_{ijkl} is the random residual effect. Pairwise comparisons were carried out using the PDIFF option in SAS (2011). An ordinal logistic regression was used to determine the odds of infection status of the different parasites using the PROC LOGISTIC procedure (SAS, 2011):

$$log\frac{p}{1-p} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \ldots\ldots\ldots \beta_n X_n + \varepsilon_{ijkl}$$

where $\boldsymbol{p}$ is the probability of experiencing GIN infections; [$\boldsymbol{p}$/1 − $\boldsymbol{p}$] is the Odds ratio, which refers to the odds of experiencing GIN infections; β_0 is the intercept; $\beta_1 \ldots \beta_n$ are the regression coefficients of predictors; $X_1 \ldots X_n$ are the predictor variables (sex, age, area, month, breeds, availability of housing, supplementary feeding, veterinary services, farmer knowledge on GIN,

parasite control method, use of anthelmintics, anthelmintic class used); ε is the random residual error distributed as N (0, 1 σ^2_E). The best model was then chosen using stepwise selection. Overall fit of the logistic regression models was assessed using the Hosmer-Lemeshow goodness-of-fit statistics. The CANCORR procedure was then used to assess the relationships between parasites and the effect of risk factors on parasites in different sites.

Prevalence was calculated as a percentage of ***d/n*** where ***d*** is the number of animals infected and ***n*** is the total number of animals examined through FEC.

4.5 Results

4.5.1 Animal management

During the year, goats relied on natural foraging for feed (98.4%), while the remainder also received nutritional supplements in addition to the natural pasture. Ninety-five percent of the farmers provided their goats with housing (kraals). Majority of the farmers in the communal areas (69.5%) has access to healthcare services i.e. government or private veterinary practitioners. Regardless of, 57.9% of the farmers did not control for gastrointestinal parasitic infections. Among the farmers interviewed, 62.8% lacked general knowledge of parasitic infections.

4.5.2 Prevalence of gastrointestinal helminths and *Eimeria*

The L_3 nematodes identified from faecal cultures of all animals, across all age groups were *Haemonchus* and *Oesophagostomum* in Chipinge, Matobo (communal and Research Station) and Tsholotsho. In Shurugwi parasites identified were *Haemonchus* and *Strongyloides*, while in Binga all faecal cultures were negative for any genera of nematodes. Mixed infections,

comprising of 14% of the faecal cultures, were composed of *Haemonchus*, *Oesophagostomum* and *Strongyloides*, with cases of mixed infections highest in Chipinge (NR 1). Faecal egg counts were highly variable for the different areas as summarised in Table 4.3. Over the study period, majority of the animals had FECs of zeros for all the parasites, with 43 to 80% (dry - wet season) for *Strongyles* being documented and 55 to 60% for coccidia (*Eimeria* species). Level of infection was low for all groups of parasites ranging from *Strongyles* (143.8 ± 14.87 epg), *Eimeria* spp (216.2 ± 21.44 opg) and even lower for the other species, ranging from 0.04 ± 0.00 to 6.0 ± 4.32 epg. The highest epg recorded for *Strongyles* was 370.2 ± 44.56 epg in the wet season and for *Eimeria* species in goats aged 1 (457.7 ± 82.31 epg).

The highest prevalence (43%) was observed for *Eimeria* spp., followed by nematodes (31%), trematodes (5%) and cestodes (0.4%). Prevalences for all parasites were generally low in the Research Station flock as compared to the communal areas ($P < 0.05$). Information from FEC was used in Table 4.4 to summarise the prevalence of different gastrointestinal parasite across study areas. Prevalence levels were higher for younger animals i.e. yearlings (76%) *vs.* older goats i.e. 7 years (38%). *Eimeria* infections were the most prevalent parasitic infection, followed by *Strongyles*, and the remainder from the remaining species in all age groups. The level of *Strongyles* and *Eimeria* infections were generally low in goats of all age groups, using the intensity scales (levels of infection) by Hansen and Perry (1994), also by Asha and Chebo (2015). The highest levels of *Eimeria* infection were among yearlings (457.7 ± 82.03 opg) and those aged 6 years (320.3 ± 146.7 opg). *Strongyles* infections were low, at 292.2 ± 134.7 epg, for six-year old goats and (129.5 ± 24.3 to 207.2 ± 46.1 epg) for 1 to 3 year-old goats. The overall prevalence of the total internal parasites was higher in males (77%) than in females

(55%) (Table 4.5; $P < 0.05$). Conversely, the prevalence of *Strongyloides* and *Moniezia* spp. infections was higher in females than in males.

Prevalence of infection was high in wet (64%) vs. dry season (36%). In addition, the means (epg/opg) were calculated to assess the distribution and level of infection by month (Figure 4.1). *Strongyles* and coccidian distribution followed the rainfall patterns in Zimbabwe with high counts obtained in hot-wet as compared to the cold-dry months. Least squares means by season and sex for PCV, LFEC, L*Eimeria* and GFEC at different ages are presented in Table 4.6. Packed cell volume, LFEC, GFEC were high for wet vs. dry season, males vs. females for all age groups and these were significantly different ($P < 0.05$) among each other and between their interactions (age*season and age*sex). Three percent of the animals had low PCV of less than 20 %. Phenotypic correlation between *Strongyles* and PCV was relatively very weak and non-significant ($r = 0.003$; $P > 0.05$). In this study the levels of PCV were low and not significant. Fixed effects and their interactions were also tested for their effect on different parasites using the model stated.

Table 4.3: Summary statistics (mean ± SE, range) of gastrointestinal parasitic infections in goats in different areas in Zimbabwe

Area		Strongyles	*Fasciola* spp.	Amphistomes	*Trichuris* spp.	*Strongyloides* spp.	*Moniezia* spp.	*Eimeria* spp.
Binga	[a]Mean FEC	191.7 ± 45.91	0.2 ± 0.18	3.5 ± 2.01	0	0	0	290.6 ± 54.66
	(Range)	(0 - 1950)	(0 – 9)	(0 - 90)				(0 – 1500)
Chipinge	Mean FEC	314.4 ± 60.78	0.04 ± 0.034	0.02± 0.01	0.31 ± 0.29	41.0 ± 34.64	6.6 ± 6.10	188.3 ± 28.44
	(Range)	(0 – 7700)	(0 – 6)	(0 – 2)	(0 – 50)	(0 – 6000)	(0 – 1050)	(0 – 2500)
Matopo	Mean FEC	309.3 ± 43.21	0	0.2 ± 0.10	0.6 ± 0.59	5.1 ± 4.20	0	103.0 ± 39.66
	(Range)	(0 – 3600)		(0 – 11)	(0 – 75)	(0 – 500)		(0 – 4500)
Shurugwi	Mean FEC	277.8 ± 70.71	0.2 ± 0.11	9.7 ± 5.14	0.4 ± 0.35	7.3 ± 5.15	2.0 ± 1.75	263.7 ± 43.88
	(Range)	(0 – 8650)	(0 – 11)	(0 – 703)	(0 – 50)	(0 – 700)	(0 – 250)	(0 – 2950)
Tsholotsho	Mean FEC	114.0 ± 23.22	0	0	0.4 ± 0.35	2.6 ± 1.50	28.1 ± 26.43	247.8 ± 68.68
	(Range)	(0 – 2350)			(0 – 50)	(0 – 150)	(0 – 3800)	(0 – 9000)
Research station	Mean FEC	56.1 ± 8.25	0.01 ± 0.005	0.2 ± 0.05	1.8 ± 1.00	0	1.6 ± 1.00	230.5 ± 25.62
	(Range)	(0 – 3800)	(0 – 3)	(0 – 34)	(0 – 1000)		(0 – 1000)	(0 – 17450)

[a]Mean FEC: means were calculated on non-transformed faecal egg counts so as to observe the levels/intensities of infection

Table 4.4: Prevalence (%) of gastrointestinal parasitic infections in goats in different areas in Zimbabwe

Area	Binga	Chipinge	Matopo	Shurugwi	Tsholotsho	Research station
Strongyles	61.5	62	77.6	50.8	43	15.7
Fasciola spp.	5.1	1.3	0	4.9	0	0.9
Amphistomes	12.8	0.6	3.45	24	0	3.1
Trichuris spp.	0	0.6	0.9	0.8	0.8	0.6
Strongyloides spp.	0	2.5	2.6	1.6	2.3	0
Moniezia spp.	0	0.7	0	0	0.7	0.4
Eimeria spp.	56.4	51.2	26.7	53.3	39.1	40

All prevalences were calculated using faecal egg counts

Table 4.5: Prevalence (%) for helminths and coccidian parasites by sex of goats in different areas in Zimbabwe

Area	Sex	Strongyles	*Fasciola* spp.	Amphistomes	*Trichuris* spp.	*Strongyloides* spp.	*Moniezia* spp.	*Eimeria* spp.
Binga	Male	66.7	11.1	16.7	0	0	0	55.6
	Female	57.1	0	9.5	0	0	0	57.1
Chipinge	Male	63.4	0	0	0	2.4	0	48.8
	Female	61.5	1.7	0.85	0.9	2.6	0.9	52.1
Matopo	Male	80.7	0	5.3	0	0	0	24.6
	Female	74.6	0	1.7	1.7	5.1	0	28.8
Shurugwi	Male	54	2	18	2	2	0	56
	Female	48.6	6.9	28.1	0	1.4	0	51.4
Tsholotsho	Male	38.9	0	0	1.7	3.4	1.7	42.4
	Female	46.4	0	0	0	1.5	0	36.2
Research station	Male	27.3	1.8	6.7	1.4	0	0	53.9
	Female	8	0.2	0.7	0	0	0.7	30.7

Area of sampling had significant effects ($P < 0.05$) on all parasites except L*Trichuris* and L*Moniezia*. Specific effects of area on GIN infections are further explained in the section below. Sex, age and the interaction of season*age had significant effects on L*Strongyles* and L*Eimeria* ($P < 0.05$). Season had significant effects on L*Strongyles*, L*Amphistomes* and *LStrongyloides* ($P < 0.05$). The interaction of sex*age had no effect on parasitic infections in all areas. Prevalence was highest in goat populations from Chipinge and Binga, greater in wet season and in males. High prevalences were observed for goats aged 1 and 6 years while the least prevalences for the 3 year olds.

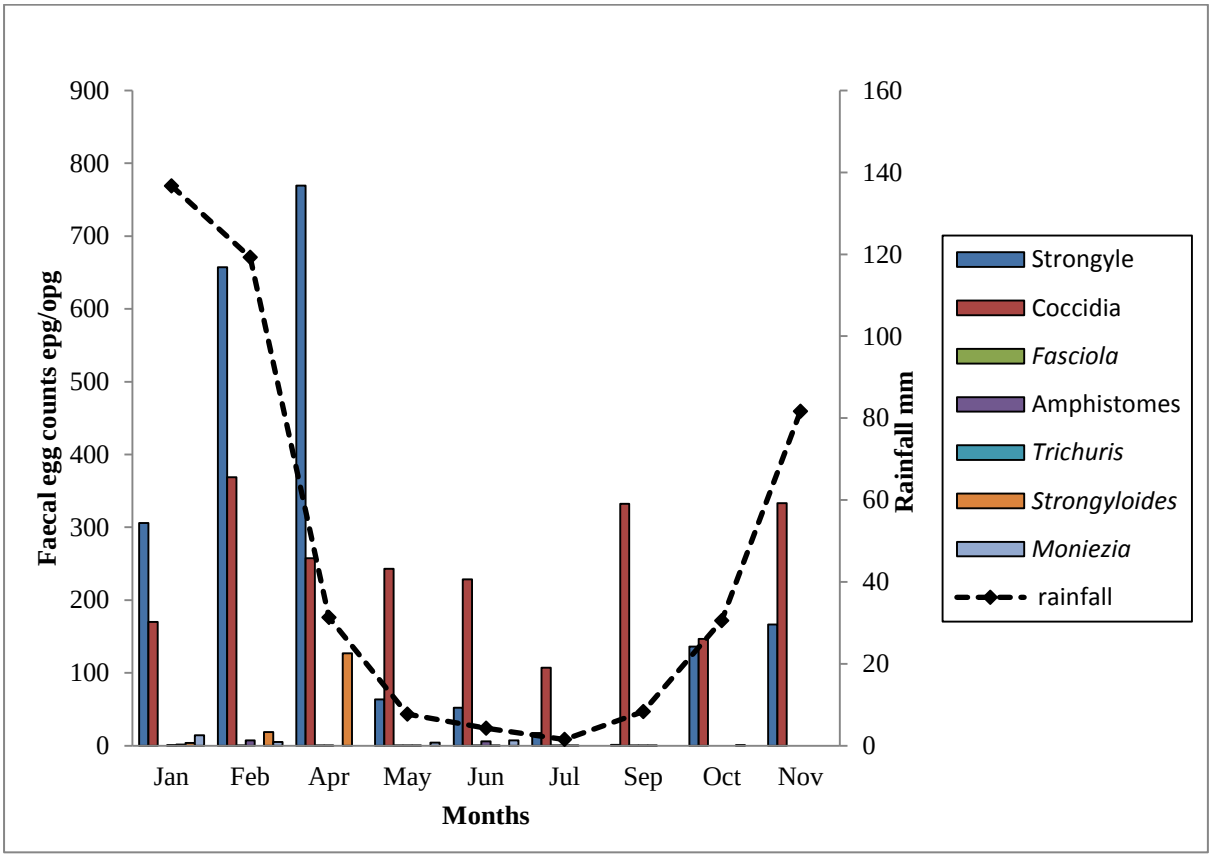

Figure 4.1: Rainfall patterns and mean monthly faecal egg counts for goats in all agro-ecological regions in Zimbabwe (There was no sampling in March, August and December), FECs for *Fasciola* spp., Amphistomes, *Trichuris* spp., *Moniezia* spp. were very low, hence the shape of the graph.

Table 4.6: Least squares means ± S.E. by season and sex for different ages for packed red cell volume (PCV (%)) logarithm transformed faecal egg counts (LFEC) for helminths/ coccidian oocysts and geometric mean of faecal egg counts (GFEC (EPG))

Age/ yrs	Trait	Season		Sex	
		Dry	Wet	Male	Female
1	PCV	25.7 ± 0.59	26.7 ± 0.77	24.9 ± 0.60	27.5 ± 0.71
	[b]LFEC	1.7 ± 0.03	2.1 ± 0.05	2.0 ± 0.03	1.9 ± 0.04
	L*Eimeria*	2.2 ± 0.04	1.8 ± 0.05	2.1 ± 0.04	1.9 ± 0.05
	GFEC	94	203	223	153
2	PCV	27.2 ± 0.57	30.1 ± 0.86	27.4 ± 0.68	30.0 ± 0.69
	LFEC	1.8 ± 0.03	2.2 ± 0.05	2.0 ± 0.03	1.9 ± 0.04
	L*Eimeria*	1.9 ± 0.04	1.8 ± 0.06	1.9 ± 0.05	1.8 ± 0.05
	GFEC	89	252	307	153
3	PCV	27.9 ± 0.58	28.0 ± 0.80	28.7 ± 0.82	27.3 ± 0.55
	LFEC	1.8 ± 0.03	2.1 ± 0.04	2.0 ± 0.04	1.9 ± 0.03
	L*Eimeria*	1.8 ± 0.04	1.9 ±0.06	1.9 ± 0.05	1.8 ± 0.04
	GFEC	135	257	171	201
4	PCV	27.3 ± 0.60	28.5 ± 0.83	28.0 ± 0.82	27.9 ± 0.60
	LFEC	1.8 ± 0.03	1.9 ±0.06	2.1 ± 0.04	1.9 ± 0.03
	L*Eimeria*	1.9 ± 0.04	1.9 ± 0.06	2.0 ± 0.06	1.8 ± 0.04
	GFEC	76	281	167	133
5	PCV	26.9 ± 0.90	29.5 ± 1.63	28.1 ± 1.65	28.2 ± 0.88
	LFEC	1.8 ± 0.04	1.9 ± 0.09	1.8 ± 0.09	1.8 ± 0.05
	L*Eimeria*	1.8 ± 0.06	2.1 ± 0.12	2.1 ± 0.12	1.9 ± 0.06
	GFEC	69	277	57	127
6	PCV	25.7 ± 1.72	27.1 ± 2.32	26.7 ± 1.80	26.2 ± 2.23
	LFEC	1.8 ± 0.09	2.6 ± 0.12	2.4 ± 0.09	2.0 ± 0.12
	L*Eimeria*	2.1 ± 0.12	1.7 ± 0.16	2.2 ± 0.13	1.6 ± 0.16
	[c]GFEC	32	289	157	59

[b]Infection with all the investigated parasites except coccidian oocysts. LFEC includes all helminthes infection; [c]GFEC- geometric faecal egg counts

4.5.3 Risk factors associated with gastrointestinal parasite infection

Factors affecting gastrointestinal parasitic infections are summarised in Table 4.7. Odds ratios indicated that area of sampling and age of animal had the highest effect on parasite infection. In addition to area of sampling and age; month of sampling and sex of goats also had significant effects on the distribution of parasites ($P < 0.05$). Odds ratios for the effect of month were generally low, but the highest/peak infection were in February (OR = 0.68) and the lowest from April to October (OR = 0.14 - 0.22), which indicates the start of the dry season. Goats sampled from Chipinge and Shurugwi (NR I, II and III) districts had the highest risk of parasitic infection (OR = 6.6 - 8.2; $P < 0.05$) as compared to those from dry and hot Tsholotsho, Binga and Matobo districts (NR IV and V). The risk of infection was highest at the extreme ages 1, 6 years; moderate at 2, 3, 5 years and lowest at 4 years ($P < 0.05$). The odds for males being infected with intestinal parasites were 2.8 higher than for females ($P < 0.0001$).

4.5.4 Association of risk factors with parasitic infections in different areas

Canonical analyses were used to further explore the parasite patterns and the impact of factors in different areas. Eigenvectors indicated that *Eimeria* and *Strongyles* were the most common parasites across the areas. In Binga, parasitic infections of *Strongyles*, *Eimeria* and amphistomes were the most common. Moderate to high correlations between breed (r = 0.50), month (r = 0.50) and availability of supplementary feed (r = 0.82) were associated with *Eimeria*, *Strongyles* and amphistomes infections. Comparing risk factors indicated that increasing supplementary feeding reduced the need for administering anthelmintic control (r = - 0.75). Infections in Chipinge included those from *Eimeria* and *Trichuris*. Low infections in *Eimeria* were associated with a decrease in age (r = - 0.36) and lack of parasitic control (r

= 0.31). Results indicated that the absence of anthelmintic treatment had low correlations (r = - 0.24) with *Trichuris* infections. The use of Salicylanilides and Macrocyclic lactones class of anthelmintics was highly negatively associated with *Trichuris* infections (r = - 0.91). *Eimeria* and *Strongyloides* infections were most common in Matobo. The absence of veterinary services was associated with *Eimeria* infections (r = -0.46) and the effect of month had a high negative relationship with *Strongyloides* infection. Common parasitic infections in Shurugwi included *Strongyles*, amphistomes and *Moniezia*. In this area, month had strong negative relationship with *Strongyles* and amphistomes infection. Low *Moniezia* infections (r = 0.46) were associated with use of indigenous breeds, while use of the same breeds showed an increase in amphistomes infections. Several risk factors were responsible for *Strongyles* and *Eimeria* infections in Tsholotsho. Low infections in *Strongyles* and *Moniezia* were positively associated with month, age, availability of supplementary feeds, with correlations ranging from 0.36 - 0.7, the converse of that was reported for *Eimeria*. The absence of anthelmintic treatment (r = - 0.46) favoured a decrease in *Strongyles* and *Moniezia* infection while *Eimeria* increased. Common parasitic infections in the Research Station flock included *Strongyles*, *Strongyloides Fasciola* and *Trichuris*. The availability of housing, supplementary feeding, veterinary services, had strong negative correlations (r = - 1) with the increase in all parasitic infections in the flock. Sex had a moderate effect (r = 0.48) on *Strongyles* and amphistomes infections, which were high in males. *Fasciola* infections were high in females and lack of anthelmintic use was associated with low *Fasciola* infections. Factors affecting parasitic infections differed according to area.

Table 4.7: Odds ratio estimates and confidence limits for fixed factors affecting gastrointestinal parasite infection

Effect	Odds Ratio	95% Wald Confidence limit		Significance
		Lower limit	Upper limit	
Area	23.562	10.904	52.746	***
Sex	0.365	0.286	0.467	***
Age	9.001	4.195	19.709	*
Month	2.106	0.187	23.989	*

* $P < 0.05$; *** $P < 0.001$

4.6 Discussion

The study identified goat internal parasite species in different geographical areas which were differentiated by annual rainfalls and vegetation patterns. The prevalence of parasites was quantified using FEC obtained in goats of different ages, in two seasons across study areas. The parasite prevalence in this study was similar to the one reported by Pandey et al. (1994), Nalumba et al. (1996), Odoi et al. (2007) and Shija et al. (2014). In these studies *Strongyles* and *Eimeira* species were the most prevalent parasites and prevalences were high during the rainy season. In this study, high infections with *Strongyles* and *Eimeira* species could be explained by the environment in which the goats were being reared, and also by poor animal management. Goats were reared in mixed crop-livestock systems, where a few numbers of goats were herded together in the same area during the dry and wet seasons. This results in higher rates of parasitic infection due to possibilities of re-infection in contaminated pastures. However, lower infections in the Research Station flock could be explained by improved management in terms of housing, feeding and healthcare. Another possibility is their access to browse forage such as *Vachellia* bush (new name for African *Acacia*, see Kyalangalilwa et al., 2013 for detail) which is dominant in the Research Station farm. *Vachellia* spp. contain

condensed tannins and evidence from a study by Costa-Júnior et al. (2014) showed a reduction in FECs upon supplementation with *Vachellia* forages.

The most prevalent nematodes were *Strongyles*, with *Haemonchus* being the most common. Previous studies on the epidemiology of gastrointestinal helminths of goats have also reported *Haemonchus* as the most important nematode (Vassilev, 1995; Tsotetsi and Mbati, 2003; Bakunzi et al., 2013; Ntonifor et al., 2013). Its higher prevalence could be due to the fact that adult females are capable of producing thousands of eggs per day, which can lead to rapid larval pasture contamination and associated outbreaks of haemonchosis (Roeber et al., 2013). There is also a role for climatic conditions since the parasite has high biotic potential and its pathogenicity which escalates the problem in humid tropics and subtropics (Waller and Chandrawathani, 2005). Another downside of *Haemonchus contortus* is its great ability to develop resistance to anthelmintic drugs (Kotze and Prichard, 2016), which pose a problem in terms of control.

The low prevalence of trematodes (amphistomes) was observed across the areas. However, high prevalence of amphistomes was noted in Shurugwi, this could be attributed to type of animal management and weather patterns of this region, characterized by average ambient temperatures of 20.3°C and 675 mm annual rainfall recorded during the sampling. These results recorded of high trematodes are in accordance with reports by Godara et al. (2014). Previous studies conducted in Shurugwi by Dube et al. (2002) identified *Paramphistomum microbothium* and *P. clavula* as the dominant trematodes. Results for *Fasciola* were as low as those reported by Khanjari et al. (2014). According to these authors, for the development of the intermediate host, temperature (> 9.5°C), rainfall and soil moisture are also important factors influencing the development of parasite from egg to miracidium. However, infections

may have been low in goats due to their browsing/foraging behavior, which minimizes chances of ingesting the metacercaria which are found on plants closer to the ground.

The only cestode observed in the study area was *Moniezia* spp., whose occurrence in the tropics is associated with the ingestion of oribatid mites infected with its larvocysts (Diop et al., 2015). In addition to *Haemonchus, Trichostrongylus, Oesophagostomum, Trichuris* and *Strongyloides* have been recorded in other studies (Tsotetsi and Mbati, 2003; Ayaz et al., 2013; Tsotetsi et al., 2013). It has been proposed that the prevalence of different species reported in literature can be explained by different geographical distribution, host factors and climatic conditions required for the development of free-living stages of different nematodes. In this study, only *Strongyle* eggs and *Eimeria* oocysts (Figure 4.1) showed a definite seasonal prevalence that corresponded to the rainfall patterns, similar to those previously reported by Nwosu et al. (2007) and Singh et al. (2013). An increase in FEC infection was observed from October with a peak in April, which gradually declines into the wet season. These observations were also reported by Chhabra and Pandey (1991) in Zimbabwe. Infections continued into the dry season, though the level of infection was low. This could be explained by the continued presence of worms in the host even during the dry season, when environmental conditions preclude the development and survival of their pre-parasitic stages. This observation indicates that rainfall and temperatures play a significant role in the epidemiology of gastrointestinal parasites as reported by Regassa et al. (2006). According to Magona and Musisi (2002) under satisfactory environmental conditions in the wet season, L_3 larva of *Haemonchus contortus* and other *Strongyles* that infect goats reach infective stages within 4 - 6 days, supporting the increased FEC in the wet period.

The findings that *Eimeira* spp. infestation was higher in young goats compared to adult goats, in terms of both prevalence and level/intensity of infection, and these findings were consistent with reports by Gwaze et al. (2009). High infections in young animals could be due to poor hygienic conditions and no prophylactic treatments against eimeriosis in smallholder farms (Ruiz et al., 2006). Most of the goats that had helminths infection also harboured coccidian oocysts, which confirms results obtained by Waruiru et al. (2000). According to Sharma and Mandal (2013), this may be associated to landholdings in the households, which directly determine the level of livestock management like sanitation, better living space and nutrition. In the current study, management in the smallholder system was characterized by overcrowding in small kraals/housing, poor nutrition, frequent exposure to communal grazing that have been contaminated and non-existent health control measures in place (Lone et al., 2012).

The observed high number of animals with zero FECs is in line with previous work (Odoi et al., 2007). In addition, there were low levels of infection because local breeds have acquired strong immunity to infection of GIT parasites due to recurrent infections. According to Baker et al. (1998), most goat breeds that are highly resistant to parasite infection are found in the tropics, but they lack desirable productivity traits.

Factors affecting the FEC i.e. study area, season, age were similar to those reported by Sissay et al. (2007), except for sex and time of sampling. In addition, age-wise prevalence revealed significant differences between age groups, with young animals being more susceptible and having higher FEC than adult animals. These findings are in agreement with previous work (Tariq et al., 2010; Lone et al., 2012 ; Ayaz et al., 2013) were naïve animals tended to be more susceptible to infections. The protective effect in older animals is therefore, attributed to acquired immunity through frequent exposure (Odoi et al., 2007).

Males of all ages were more susceptible than females as indicated in Table 6. These findings were similar to other studies (Tariq et al., 2008; Ayaz et al., 2013; Nabi et al., 2014; Badaso and Addis, 2015). The researchers attributed this to the genetic predisposition and differential susceptibility owing to hormonal control. In these studies young and adults were considered. However, these results were contrary to the findings of Emiru et al. (2013) and Vieira et al. (2014) in Ethiopia and Brazil. The preceeding authors reported that females were more susceptible to parasite infection than males. This was attributed to reduced resistance of female animals due to their reproductive events and insufficient/unbalanced diet against higher needs. There were different parasitic infections in different areas. Impact of various risk factors was assessed and these varied across area on different parasites. Each parasitic species was present in at least two different areas. Of all the factors assessed, the effects of month, age, supplementary feeding and anthelmintic use were the most dominant factors affecting parasitic infections. These differences can be explained by varying environmental and animal factors and also management systems in these areas.

Levels of infection for the indigenous goats were low, using the intensity scales by Hansen and Perry (1994) also by Asha and Chebo (2015). These findings were consistent with reports from Odoi et al. (2007). Low intensities could also be associated with the vegetation type that the goats were exposed to, in different areas as summarised in Table 4.1. Access to trees or shrubs with high levels of tannins e.g. *Vachellia* has the ability to reduce infection levels. Evidence of the anthelmintic properties of plants and plant-extracts is derived primarily from ethno-veterinary sources, most of which have been widely documented (Githiori et al., 2006). In addition, low infection levels can be attributed to individual host's ability to deter infection, or tolerate certain levels of infection without showing susceptibility. The impact of nematode

infection was not assessed, but the prevalence determined in this study may be regarded as a problem affecting productivity of animals especially in mixed livestock-crop farming systems where farmers do not provide nutritional supplements, or invest in acquiring drugs for controlling parasites (Kumba et al., 2003).

4.7 Conclusion

The results from the study indicate that prevalence was high for *Strongyles* and *Eimeria* oocysts, with *Haemonchus* being the most commonly identified parasite. Despite this, a lower percentage (3%) of these animals was anaemic. The study identified area, sex, age and month as the most relevant risk factors for the development of gastrointestinal parasites across agro-ecological regions. Furthermore, the effect of site was explored for impact of different risk factors on parasitic infections and common parasite species and risk factors differed with area. Knowledge on these gastrointestinal helminths species and of epidemiological parameters is important in the development of appropriate control strategies for different areas. This has a potential to reduce production losses and improve rural livelihoods. A subset of blood samples that were used for analysis in this chapter were selected for DNA analysis and further used for different analyses, presented in chapters 5 and 6.

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Chapter 5

5 Genetic diversity and population structure of goats reared in low-input/low-output farming systems of Zimbabwe

5.1 Abstract

Goats have evolved into a variety of locally adapted populations in response to different human and environmental pressures. Indigenous tropical goat breeds constitute a valuable genetic resource. In Zimbabwe majority of goat population is found in low-input/low output farming systems, where they are exposed to GIN to which there are considered to have developed some level of resistance. Genetic characterization of native breeds and investigation of indiscriminate breeding as well as resistance and adaptation to local selection pressures is important. This will contribute to improving management and conservation of available animal genetic resources in Zimbabwe and other countries. The objectives of the study were to assess genetic diversity, population genetic structure, linkage disequilibrium and trends in effective population size of goats raised in Zimbabwe. A total of 253 goat DNA samples from Chipinge (n = 33), Shurugwi (n = 22), Binga (n = 17), Matobo (n = 33), Tsholotsho (n = 25) and Matopos Research Station (n = 124) were genotyped using the Illumina goat SNP50k Bead chip. Approximately 90% of the single nucleotide polymorphism (SNPs) was available for downstream analyses. Genetic parameters indicated high levels of genetic diversity based on H_E and H_O estimates as well as low levels of inbreeding across populations. Populations partitioned into five clusters based on principal component analysis with distinct populations of Binga. ADMIXTURE indicated high levels of shared ancestry between and Tsholotsho populations. Linkage disequilibrium (LD) was on average very low in this study with r^2 ranging from 0.03 – 0.18. Chromosomes that had the highest mean r^2 were 3, 9, 15 16, 22, 27 and the least r^2 was observed on chromosome 21 across all populations. LD across all chromosomes ranged from 0.05 ± 0.09 to 0.11 ± 0.15,

with the highest in Binga and the least in Research Station goats. LD decay decreased with increase in marker distance in all populations. The effective population sizes were > 100 for all populations, 12 generations ago, with the exception of Binga, which had 82. This is the first analysis on population structure, diversity, linkage disequilibrium and effective population sizes of goat reared in low-input/low-output farming systems in Zimbabwe. The study demonstrates the utility of the Illumina Goat SNP50k Bead chip in population genetic studies of such and similar. The populations reared in the farming systems were genetically diverse, and there was evidence of shared ancestry among populations.

Keywords: genetic diversity, linkage disequilibrium, effective population, indigenous goats

5.2 Introduction

In Zimbabwe 90% of the 3.5 million-goat population is found in low-input/low-output farming systems. According to Homann et al. (2007), 40% of these households do not own cattle, hence complement their livestock resources with goats among other livestock species. Indigenous goats constitute a valuable genetic resource because of their ability to adapt to different environmental conditions, nutritional fluctuations, disease resistance and ability to survive under low-input/low-output systems (Webb and Mamabolo, 2004).

Selection has played an important role in the development of goat breeds which are widely dispersed and adapted to diverse biophysical and production environments. Continuous artificial selection for production, reproduction and biophysical traits in temperate regions has been conducted as a means of standardizing breeds (Kim et al. 2016). However, selection for such traits has not been conducted on indigenous tropical and subtropical breeds. Indigenous African goat breeds are most often exposed to natural selection for traits such as adaptation

under varied environments (Serrano et al., 2009). This has most likely selected multiple alleles, thus preserving genetic diversity in populations.

The extent of linkage disequilibrium (LD) defined as non-random association of alleles at two or more loci, is a useful tool in genetics and evolutionary biology (Qanbari et al., 2010). The extent of LD in a population is critical for the prediction of genetic merit from markers and for quantitative trait loci (QTL) detection (Goddard and Hayes, 2009; Roldán et al., 2014). It is also useful in providing insight into the evolutionary history of a population and inference on ancestral effective population size (N_e) (Sved, 1971). This is an important population parameter that may help to explain how populations evolved and can improve the understanding and modeling of genetic architecture underlying complex traits (Tenesa et al., 2007), such as gastrointestinal parasite resistance.

Extensive research in genetic diversity of goats has been reported globally using microsatellite markers (Liu et al., 2013; Kotze et al., 2014; Radhika et al., 2015; Hassen et al., 2016; Kim et al., 2016), SNP-based markers (Benjelloun et al., 2015; Kotze and Prichard, 2016; Lashmar et al., 2016; Manunza et al., 2016; Mdladla et al., 2016; Visser et al., 2016). In addition, high density panels have also been shown to be useful in genetic diversity analyses of other livestock species (Ai et al., 2013; Kijas et al., 2013; Makina et al., 2014; Khanyile et al., 2015). The availability of dense SNP-based markers has provided positive prospects for genetic analyses of goats, previously not possible and has an advantage of improving on limitations faced by traditional quantitative studies to accelerate genetic progress.

Mashona and Matabele are the predominant goat breeds in low-input/output farming systems of Zimbabwe. Others breeds include the Tonga and crossbreeds. Mashona and Tonga goats are

small, compact and hardy indigenous breeds. According to Mason and Maule (1960), these are prototypes of the small East African goats, with mature body mass of 25 – 30 kg. Mashona goats are mainly found in the North/South East parts of the country. The Tonga goats were mainly found in the North-west part of the country. The Matabele type goats are bigger than the Mashona or Tonga breeds with mature body mass ranging between 40 - 65 kg for males and 30 - 45 kg for females. The Matebele are mainly concentrated in the South-western part of the country. Knowledge on genetic diversity and possibly selected signatures in native goat populations can be used to preserve these animal genetic resources. The genetic relationships and differentiation between the native breeds and other products of indiscriminate breeding in Zimbabwe have not yet been characterized. Detection of genomic differences can thus provide basis of adaption to diverse environments and provide insights into functionally important genetic variants (Andersson and Georges, 2004). The objectives of this study were to i) investigate population genomic structure and genetic diversity ii) determine extent of linkage disequilibrium and trends in effective population sizes in goats reared in low-input/output farming systems in Zimbabwe.

5.3 Material and methods

5.3.1 Animal resources

A total of 253 blood samples were collected from goats in smallholder farms and a research station in Zimbabwe (a subset of samples collected in chapter 4). Sampling for possible Matabele goats was conducted in Matobo (n = 33, Tsholotsho (n = 25) and Matopos Research Station (n = 124). Mashona goats were sampled from Chipinge (n = 33) and Tonga goats from Binga (n = 17). Goats from Shurugwi (n = 22) were also sampled and were expected to be either Mashona or Matabele. It is however difficult to assign goats in different areas in

Zimbabwe to specific breeds, due to uncontrolled breeding systems especially in these farming systems and adaptation to different geographical areas. In this study supposedly breed names were ignored and animals were grouped based on their sampling areas.

Blood samples were collected by jugular venipuncture using 10-ml EDTA VACUETTE® tubes. Samples were kept between 2 - 4 °C on ice during field sampling period and later stored at -21 °C in the refrigerator to prevent formation of ice crystals, prior to laboratory analysis. Genomic DNA was extracted using the DNeasy® Blood and Tissue kit (Qiagen), as per manufacturer's protocol. DNA was quantified using Qubit® 3.0 Fluorometer (Life Technologies) and the Nanodrop Spectrophotometer (Nanodrop ND-1000). Gel electrophoresis was used to determine DNA integrity; samples were visually assessed on 1% agarose containing ethidium bromide and TAE buffer. DNA was then visualized by UV illumination.

5.3.2 SNP genotyping and quality control

SNP genotyping was conducted at the Agricultural Research Council-Biotechnology Platform in South Africa using the Illumina Goat 50K SNP Bead chip which features 53 347 SNP probes, distributed across the whole genome, with inter-SNP spacing of ~40kb (Tosser-Klopp, 2012). SNP calling was done using Illumina Genome Studio v 2.0 and the genotype input file was converted into PLINK v 1.07 input files (Purcell et al., 2007). The SNP positions were based on the goat genome assembly (CHI_1.0 goat) available from the International Goat Genome Consortium (http://www.goatgenome.org/).

The SNP data of all six populations was filtered to remove SNPs that were on sex chromosomes or had their positions unmapped to the latest reference assembly of the goat genome, resulting in 49 993 SNPs. Single nucleotide polymorphisms (SNP) quality control (QC) was conducted in different stages depending on the downstream analyses. Quality control thresholds were set to remove markers with missing data > 5%, that had MAF ≤ 5%, individuals with missing genotypes > 5% using PLINK v 1.07 (Purcell et al., 2007). This resulted in 246 goats and 44 918 SNPs across the six populations (89.9%). In addition to conducting quality control across populations, QC was also conducted per subpopulation (Table 5.2). The highest number of goats was removed from the Research Station flock (n = 53), Tsholotsho (n = 6), Shurugwi (n = 5), Chipinge (n = 5) and the least from Matobo (n = 4). For population structure analysis, 73 closely individuals, as inferred by a kinship estimate ≥ 0.45 were removed from downstream analyses. Single nucleotide polymorphisms were further excluded using PLINK v 1.07 (Purcell et al., 2007) for being in high linkage disequilibrium ($r^2 > 0.2$), removing 11 700 SNPs.

5.4 Data analysis

5.4.1 Minor allelic frequency

Minor allelic frequency (MAF) distribution per population was estimated using the 49 993 autosomal SNPs with chromosomal locations using PLINK v 1.07 (Purcell et al., 2007) under default settings. Means and standard deviations were calculated using PROC MEANS procedure of the Statistical Analysis System (SAS, 2011). MAF were categorized into fixed (MAF = 0), rare (0 - 0.01), intermediate (0.01 - 0.05) or polymorphic (MAF > 0.05) for which respective bins were set.

5.4.2 Within-population genetic diversity

Subsets of SNPs that had passed QC after quality control within populations were used to estimate observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficients (F_{IS}). The inbreeding coefficients, observed and expected heterozygosity indices were determined using PLINK v 1.07 software (Purcell et al., 2007) under default settings. Analysis of molecular variation was performed as a means of partitioning genetic diversity within and between populations, using the Arlequin v 3.1 program (Excoffier et al., 2007).

5.4.3 F_{ST} pairwise comparison

To assess genetic diversity between populations, population-specific fixation index (F_{ST}), were computed between 15 pairs of populations according to Weir and Cockerham (1984). Unbiased estimates of F_{ST} were calculated using SVS v 8.1 (Golden Helix Inc., 2014). Interpretation of the indices was based on guidelines proposed by Wright (1978). To determine variation in allele frequency between loci, per-marker F_{ST} values were calculated for all autosomal SNPs across populations.

5.4.4 Population structure analysis

Pairs of markers with high linkage disequilibrium (LD) provide redundant information and impose higher computational demands for population structure analyses. To correct for redundancy, the dataset was pruned based on LD between markers using PLINK v 1.07 (Purcell et al., 2007) command -indep-pairwise 50 5 0.2, which calculates LD for each pair of marker in a window of 50 SNP. The settings would prune out one of the SNPs pair that had $r^2 > 0.2$ on a sliding window of 5 SNP. The pruned genotypes defined a set including in 33 218 SNPs that were used to assess population structure using (i) principal components as implemented in

Golden Helix SNP Variation Suite (SVS) v 8.1 (Golden Helix Inc., 2014) and (ii) unsupervised clustering of individuals based on maximum likelihood using ADMIXTURE v 1.23 (Alexander et al., 2009). ADMIXTURE was used to infer the most probable number of ancestral populations based on SNP genotype data. Prior population information was ignored for identifying distinct genetic population and assigning individuals to populations. ADMIXTURE was run from K = 2 to K = 10 and the optimal number of clusters was determined as that which had the lowest cross validation error.

5.4.5 Linkage disequilibrium

Pairwise r^2 estimation was used to measure LD between pairs of SNPs within a chromosome and population using PLINK v 1.07 (Purcell et al., 2007) for SNPs on autosomes (chr1 – 29). The '--r2 --ld-window-kb 2000 --ld-window-r2 0'option in PLINK was used to estimate LD for SNP marker pairs up to a distance of 2000kb. Means and standard deviation were calculated using PROC MEANS procedure in SAS v 9.3 (SAS, 2011). An analysis of variance (ANOVA) was also conducted using the PROC GLM procedure of SAS (SAS, 2011) to determine the effects of chromosome, population, distance between SNP markers, the interaction between population and chromosome on r^2 using the model:

$$Y_{ijk} = \mu + C_i + D_j + (C*D)_{ij} + bE_k + \varepsilon_{ijk}$$

where Y_{ijk} is r^2, μ is the overall population mean, C_i is the effect of the i^{th} chromosome (chr 1-29), D_j the effect of the j^{th} goat population (Chipinge, Shurugwi, Binga, Tsholotsho and Matobo, Research), $(C*D)_{ij}$ is the effect of interaction between chromosome and population, E_k the effect of the k^{th} distance between SNP markers which was fitted as a covariate with a regression coefficient b and ε_{ijk} is the random residual effect. Linkage disequilibrium decay

was estimated for all subpopulations using sliding window bins set at 0-1, 1-10,10-20, 20-40, 40-60, 60-100, 100-200; 200-500, 500-1000 and 1000-2000 kb.

5.4.6 Effective population size

Effective population size (N_e) was estimated using SNeP v 1.1 (Barbato et al., 2015). SNeP estimates N_e from genome-wide linkage disequilibrium, using the following formula as suggested by Corbin et al. (2012):

$$N_{T(t)} = \frac{1}{(4f(c_t))}\left(\frac{1}{E\left[r^2_{adj}|c_t\right]}\right) - \propto$$

where $N_{T(t)}$ is the effective population size estimated t generations ago, c_t is the recombination rate *t* generations ago, r^2_{adj} is the linkage disequilibrium estimation adjusted for sampling bias and α is a constant. The recombination rate was calculated using the equation by (Sved, 1971).

Default minimum and maximum inter-SNP distances in SNeP v 1.1 (Barbato et al., 2015) were used for SNP data sets for each sub-population, as well as the merged dataset and grouped 30 distance bins of 50kb each. N_e estimates were subsequently calculated from the r^2 values obtained for the average distance of each distance bin.

5.5 Results

5.5.1 SNP marker characteristics

After quality control, 2679 markers with MAF ≤ 0.05; 803 SNP markers with missing genotypes ≥ 0.05; 2301 SNPs that deviated from HWE ($P < 0.001$) and eight animals with missing genotypes ≥ 0.05 were excluded from the dataset consisting of all the populations. The final working dataset included 246 animals and 44918 SNPs (89.9%) available for downstream

analysis. The highest number of SNPs eliminated from the analysis was based on low MAFs, with a range of 5 - 11 % across populations. Table 5.1 summarizes the quality control results for the different populations. MAF distribution was also assessed for each population. Binga populations had very low MAF (i.e. more animals in the MAF = 0 – 0.1 bin) and also highest levels (6.4%) of monomorphic SNPs (MAF = 0) (Figure 5.1).

5.5.2 Minor allelic frequency

The mean MAF was 0.31 ± 0.12 (Table 5.2) and 95.3 % markers were polymorphic across populations. The percentage of SNPs that were polymorphic (MAF > 0.05) ranged from 89 – 95 %, with the highest level of polymorphic SNPs in the Tsholotsho population (94.5 %) and the least in Binga population (89.2 %).

5.5.3 Within-population genetic diversity

Mean observed and expected heterozygosities were 0.61 ± 0.03 and 0.61 ± 0.00 respectively. Highest H_O and H_E were observed for Binga (0.64 ± 0.02; 0.63 ± 0.00) and Chipinge (0.64 ± 0.03; 0.63 ± 0.00). Heterozygosity estimates for most of the populations with the exception of Shurugwi indicated positive gene diversity ($H_E < H_O$), while those for Tsholotsho (0.60 ± 0.01; 0.60 ± 0.00) and the research station populations (0.60 ± 0.02; 0.60 ± 0.00) were similar ($H_E = H_O$). Inbreeding coefficients (F_{IS}) were generally low within populations, with high values in Chipinge, Binga and Matopo populations and random mating observed in Tsholotsho ($F_{IS} = 0$). Analysis of molecular variance (AMOVA) indicated that high variation within individuals as compared to among individuals within populations and also among different populations (Table 5.3).

5.5.4 Population structure analysis

Principal component analysis (PCA) was used to visualize individual relationships within and between populations. Goats from the different populations grouped into five clusters. Chipinge population had a distinct cluster, goats from Binga and Shurugwi grouped together, while the Tsholotsho and Matobo also grouped together with a mixture of animals from the research station flock. The first PC (Figure 5.2) showed that village goats from Binga, Shurugwi and Chipinge clustered in one axis whilst the Tsholotsho and Matobo village goats clustered in between the research station populations and Mashona goats. Generally the animals from the research station flock were dispersed into 3 separate clusters, while those from the villages in different districts had more compact clusters (Figure 5.2).

Table 5.1: SNP distribution of polymorphic markers, and within population diversity indicators for the different subpopulations

Population	Binga	Chipinge	Matopo	Research	Shurugwi	Tsholotsho	All
Total SNPs	49943	49943	49943	49943	49943	49943	49943
MAF≤ 0.05	5698	5081	2729	2912	4374	2924	2679
Geno ≥ 0.05	925	793	872	778	665	916	803
HWE ≥ 0.001	68	162	181	1503	77	675	2301
MIND > 0.05 (n)	16	32	29	121	22	26	246
SNPs available for analysis	43711	44381	46637	45372	45258	45929	44918
% SNPs available for analysis	87.52	88.86	93.38	90.85	90.62	91.96	89.94

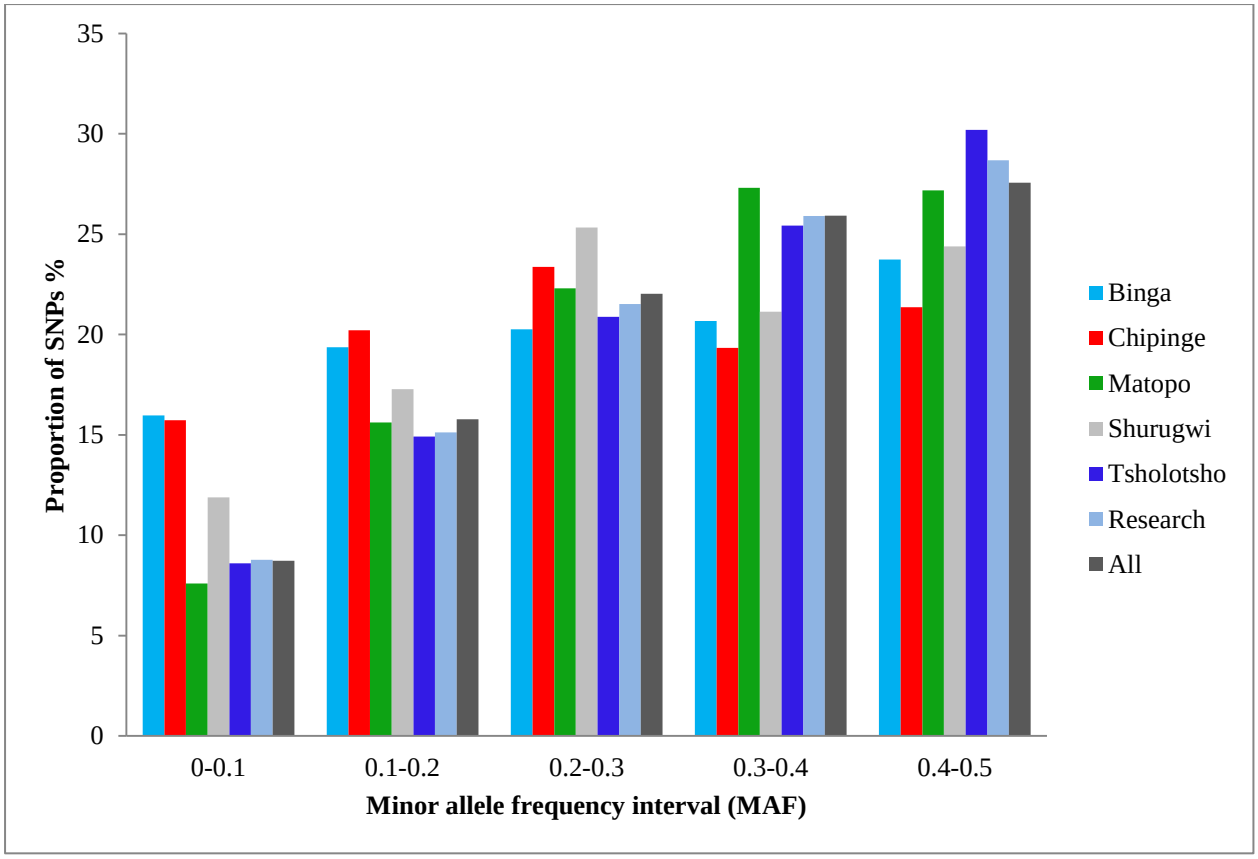


Figure 5.1: MAF distribution for each goat population

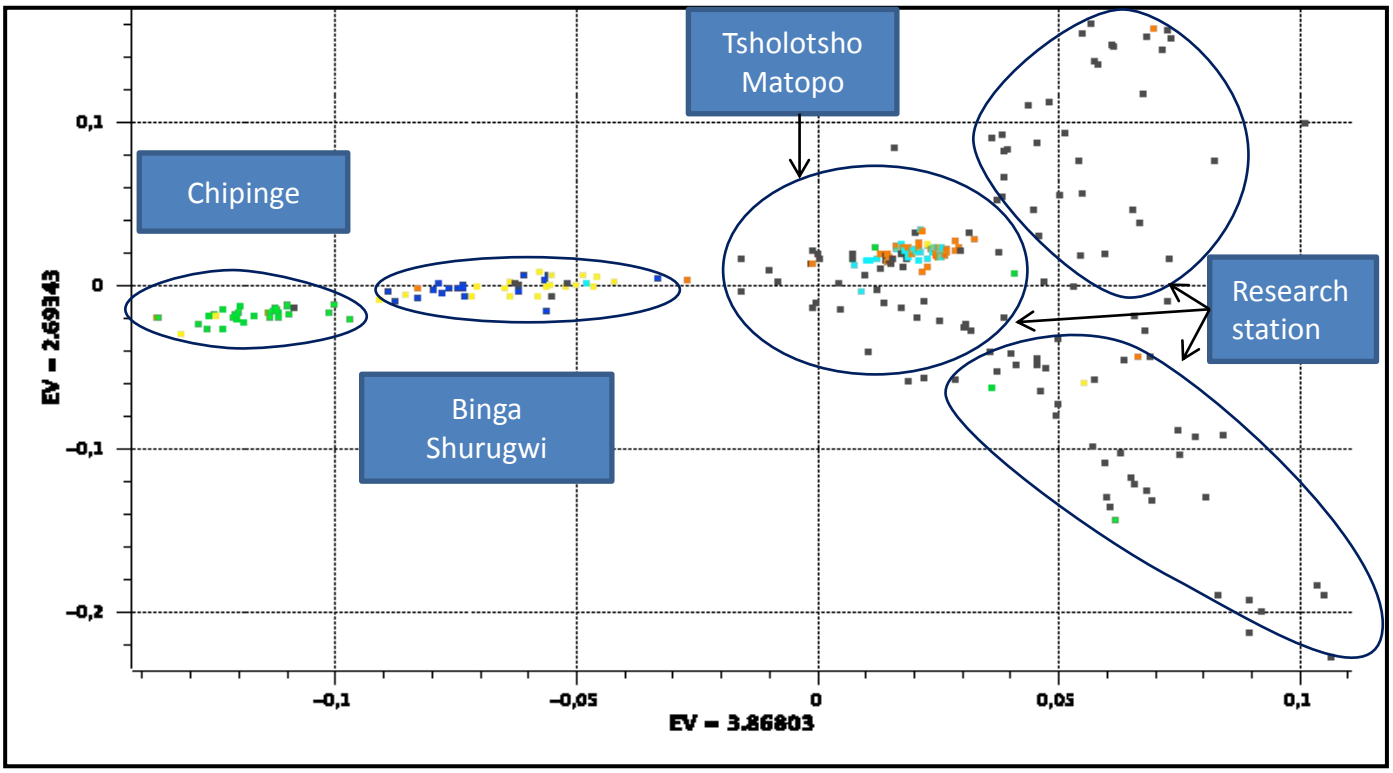
Table 5.2: Summary of polymorphic markers, and within-population diversity indicators for the different subpopulations

Population	n	P_N	MAF ± SD	H_O ± SD	H_E ± SD	F_{IS} ± SD
Binga	16	89.18	0.29 ± 0.12	0.64 ± 0.02	0.63 ± 0.00	0.02 ± 0.06
Chipinge	32	90.4	0.31 ± 0.12	0.64 ± 0.03	0.63 ± 0.00	0.03 ± 0.08
Matopo	29	93.74	0.28 ± 0.13	0.61 ± 0.02	0.60 ± 0.00	0.02 ± 0.05
Research	121	94.42	0.31 ± 0.12	0.60 ± 0.02	0.60 ± 0.00	-0.01 ± 0.04
Shurugwi	22	91.84	0.31 ± 0.12	0.61 ± 0.03	0.62 ± 0.00	0.01 ± 0.08
Tsholotsho	26	94.47	0.31 ± 0.12	0.60 ± 0.01	0.60 ± 0.00	0.00 ± 0.04
All	246	94.72	0.31 ± 0.12	0.61 ± 0.03	0.60 ± 0.00	0.03 ± 0.08

***P_N** polymorphic SNPs, MAF- minor allelic frequency, H_O and H_E –observed ad expected heterozygosity, F_{IS}- inbreeding coefficient

Table 5.3: Analysis of molecular variance using different goat population data

Population	Among populations	Among individuals within populations	Within individuals
All six populations	2.41	1.10	96.49
Village populations	2.57	2.65	94.78
Village and research populations	2.51	1.10	96.53



At K = 2 of ADMIXTURE analysis (Figure 5.3), the Chipinge goats are separated from the other goats. At K = 3, the animals were grouped into 3 clusters, a single cluster for Chipinge, Binga and Shurugwi; another with Matobo and Tsholotsho and the last cluster was for Research station which was genetically diverse. The four clusters at K = 4 corresponded to separation of Chipinge from its previous cluster, which remained the same in K = 5. In the study cross validation (CV) errors were plotted for comparison of K values, where K = 5 exhibited the lowest CV error the value thus was taken as the most probable number of inferred populations

(Figure 5.4). Clusters at K = 5 relate to Chipinge (blue), Binga (blue and green); Matopo and Tsholotsho, Shurugwi (green) and research station populations (mixed colours). K values beyond K = 5, were plotted so as to visualize any other kind of separations in the populations. At K = 6, the Binga population was more distinct. There is evidence of admixture with gene components being shared at different levels between populations, with an example of Matobo and sharing genetic components since K =2. The K = 6 to K = 10 were plotted so as the distinction of the clusters revealed Chipinge and Binga distinct groups, while the Research flock showed high levels of genetic diversity. Indications of outlier populations i.e. those with a different colour in a cluster were observed in Chipinge, Matobo and Shurugwi.

5.5.5 F_{ST} pairwise comparison

Pairwise population F_{ST} comparisons indicated low levels of genetic differentiation (F_{ST} = 0.01 – 0.04) across all populations (Figure 5.5). The genomic distribution of F_{ST} values for all autosomes is shown in Figure 5.6. The mean F_{ST} value for all 29 autosomes across populations was 0.038 ± 0.068 (range 0 – 0.54) indicating low genetic differentiation.

5.5.6 Linkage disequilibrium and extent of linkage disequilibrium decay

Information on the chromosomes, length, number of SNPs and average r^2 per chromosome are summarised in Table 5.4. Distribution of SNPs varied among chromosomes ranging from 875 – 2887 SNPs per chromosome. SNP interval per chromosome ranged from 248 – 288 kb, with the lowest and highest interval observed on chromosomes 18 and 19 respectively. Overall LD across all chromosomes ranges from 0.05 ± 0.09 to 0.11 ± 0.15. Highest LD was observed in Binga and the least in the research station flocks. The highest mean r^2 was observed on chromosomes 3, 9, 15 16, 22, 27 and the least in chromosome 21 across all populations.

Analysis of variance results (Table 5.5) indicated that population, chromosomes, population*chromosome interaction and SNP distance interval had significant effects on LD. An analysis of rate of LD decay over SNP distance indicated r^2 of 0.1 at SNP intervals less than 10kb that decayed to an average of $r^2 = 0.04$ at SNP intervals between 10 and 20kb across all populations. LD decay reduced with an increase in SNP distance, from 0.14- 0.17 at 20 – 40 kb to a range of 0.03 - 0.09 at 500 - 1000 kb (Figure 5.7). Highest LD values were observed at 20 - 40 kb (0.09 - 0.17) and the least at 10 - 20 kb (0.02 - 0.06) for the different populations. Binga had the highest LD with mean of 0.12 compared to the rest of the population. Least LD was observed in the research station (mean $r^2 = 0.06$).

5.5.7 Effective population size

Effective population size (N_e) at *t* generations in the past are summarised in Figure 5.8. N_e was estimated to be higher than 2000 animals 983 generations ago and decreased to below 450 animals within the last 12 generations ago. Across all populations a decrease in N_e was observed. The LD-based estimates of N_e indicated low effective population sizes ranging from 82 - 171 for all communal populations and slightly higher (437) for the research station flock. A decrease in N_e from at least 3800 and 4500 was observed for the communal and research populations respectively.

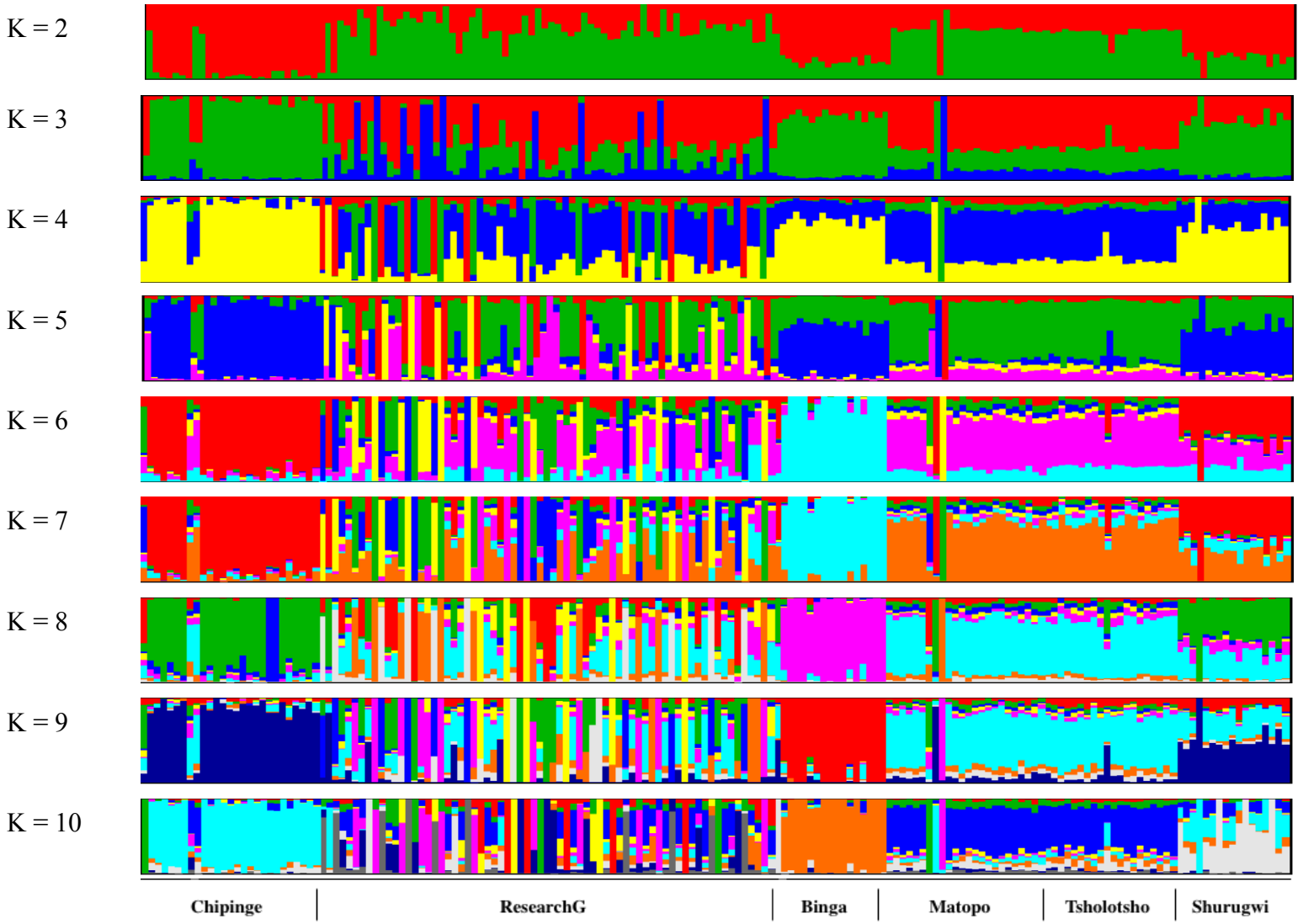


Figure 5.3: Admixture based clustering of goat populations in Zimbabwe

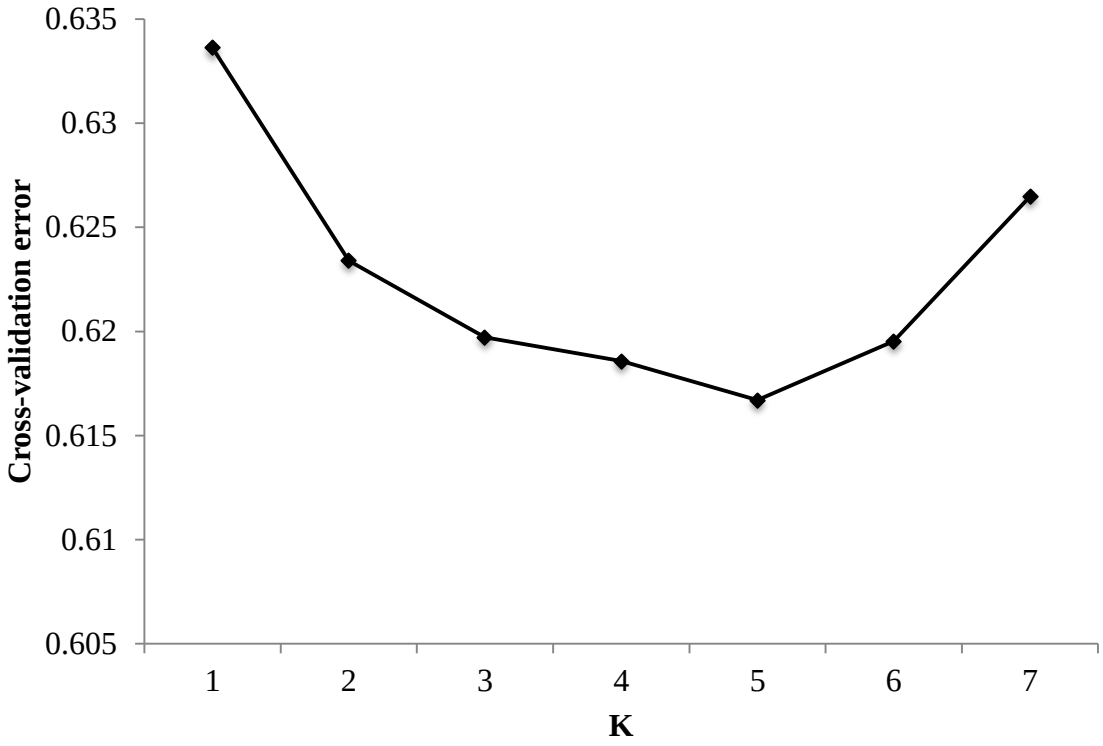


Figure 5.4: Cross validation plot for six goat populations in Zimbabwe

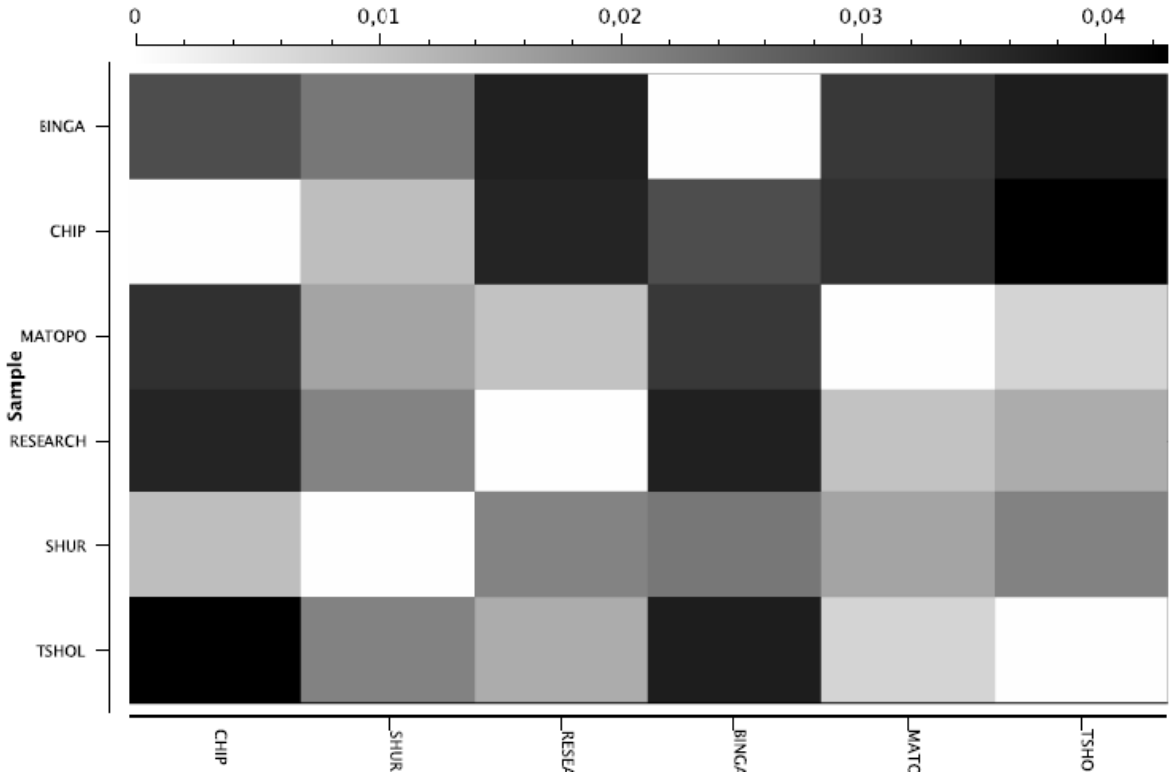


Figure 5.5: Genomic pairwise FST for goat populations in Zimbabwe

Table 5.4: Linkage disequilibrium (average r2) per chromosome in different goat populations in Zimbabwe

Chr.	Chr. length (Mb)	No. of SNPs	Binga	Chipinge	Matopo	Shurugwi	Tsholotsho	Research	All
1	155.01	2887	0.11±0.15	0.07±0.11	0.07±0.10	0.08±0.12	0.07±0.11	0.05±0.09	0.04±0.08
2	135.42	2547	0.11±0.15	0.07±0.11	0.06±0.10	0.08±0.11	0.07±0.10	0.04±0.09	0.03±0.08
3	116.8	2062	0.12±0.17	0.08±0.14	0.07±0.12	0.09±0.15	0.08±0.12	0.05±0.11	0.04±0.08
4	115.96	2150	0.11±0.15	0.07±0.11	0.07±0.10	0.08±0.12	0.07±0.10	0.05±0.09	0.04±0.08
5	111.06	1975	0.10±0.15	0.07±0.11	0.07±0.11	0.08±0.12	0.07±0.11	0.05±0.09	0.04±0.09
6	114.33	2189	0.11±0.15	0.07±0.11	0.06±0.10	0.08±0.12	0.07±0.11	0.05±0.09	0.03±0.08
7	106.55	2041	0.11±0.16	0.08±0.13	0.07±0.10	0.08±0.12	0.08±0.11	0.05±0.10	0.04±0.09
8	11.02	2184	0.11±0.16	0.08±0.13	0.07±0.11	0.08±0.13	0.08±0.12	0.05±0.10	0.04±0.09
9	90.29	1679	0.12±0.16	0.08±0.13	0.07±0.12	0.09±0.13	0.08±0.11	0.05±0.09	0.04±0.08
10	99.2	1810	0.12±0.16	0.08±0.13	0.07±0.12	0.09±0.13	0.08±0.12	0.05±0.10	0.04±0.09
11	105.31	1988	0.12±0.16	0.07±0.12	0.07±0.10	0.09±0.13	0.08±0.11	0.05±0.09	0.04±0.08
12	82.54	1539	0.11±0.15	0.07±0.12	0.07±0.11	0.09±0.13	0.08±0.11	0.05±0.08	0.04±0.08
13	80.63	1514	0.11±0.15	0.08±0.12	0.07±0.10	0.08±0.12	0.08±0.11	0.05±0.09	0.04±0.08
14	92.31	1793	0.11±0.14	0.07±0.11	0.07±0.10	0.08±0.11	0.07±0.10	0.05±0.09	0.04±0.08
15	78.99	1486	0.12±0.17	0.08±0.13	0.07±0.12	0.09±0.14	0.08±0.12	0.05±0.11	0.04±0.10
16	77.68	1305	0.12±0.17	0.08±0.13	0.08±0.12	0.09±0.13	0.08±0.12	0.05±0.10	0.04±0.09
17	71.88	1372	0.11±0.15	0.07±0.11	0.06±0.10	0.08±0.11	0.08±0.11	0.05±0.09	0.04±0.07
18	61.07	1226	0.11±0.15	0.07±0.12	0.07±0.10	0.08±0.13	0.08±0.11	0.05±0.09	0.04±0.09
19	62.13	1071	0.12±0.16	0.07±0.11	0.07±0.10	0.08±0.12	0.07±0.11	0.05±0.09	0.04±0.08
20	71.28	1374	0.11±0.15	0.07±0.11	0.07±0.10	0.08±0.12	0.07±0.11	0.05±0.08	0.04±0.08
21	66.77	1336	0.11±0.14	0.06±0.10	0.06±0.09	0.08±0.11	0.07±0.10	0.04±0.08	0.03±0.07
22	57.96	1072	0.11±0.16	0.08±0.12	0.07±0.11	0.09±0.12	0.08±0.11	0.05±0.10	0.04±0.09
23	49.4	966	0.11±0.15	0.07±0.12	0.06±0.10	0.08±0.11	0.07±0.11	0.05±0.09	0.04±0.08
24	61.76	1129	0.11±0.15	0.07±0.11	0.07±0.12	0.08±0.12	0.08±0.12	0.05±0.10	0.04±0.08
25	41.5	694	0.11±0.15	0.08±0.13	0.07±0.11	0.08±0.12	0.07±0.11	0.05±0.10	0.04±0.09
26	50.17	967	0.11±0.16	0.08±0.12	0.07±0.11	0.09±0.13	0.08±0.12	0.05±0.10	0.04±0.08
27	44.12	805	0.12±0.18	0.08±0.15	0.08±0.14	0.09±0.15	0.09±0.15	0.06±0.13	0.05±0.12
28	43.23	850	0.11±0.15	0.07±0.11	0.07±0.10	0.08±0.12	0.07±0.11	0.05±0.10	0.04±0.09
29	48.38	875	0.11±0.15	0.07±0.13	0.07±0.11	0.08±0.13	0.08±0.12	0.05±0.10	0.04±0.10
			0.11±0.15	0.07±0.12	0.07±0.11	0.08±0.12	0.08±0.11	0.05±0.09	0.04±0.08

Chr.-chromosome

Table 5.5: Effects of population, chromosome, SNP interval and the interaction between population and chromosome on linkage disequilibrium

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Population	5	2928.458	585.6916	35456.7	<.0001
Chromosome	28	171.0498	6.108921	369.82	<.0001
SNP interval	1	4143.923	4143.923	250866	<.0001
Population*chromosome	140	26.89004	0.192072	11.63	<.0001

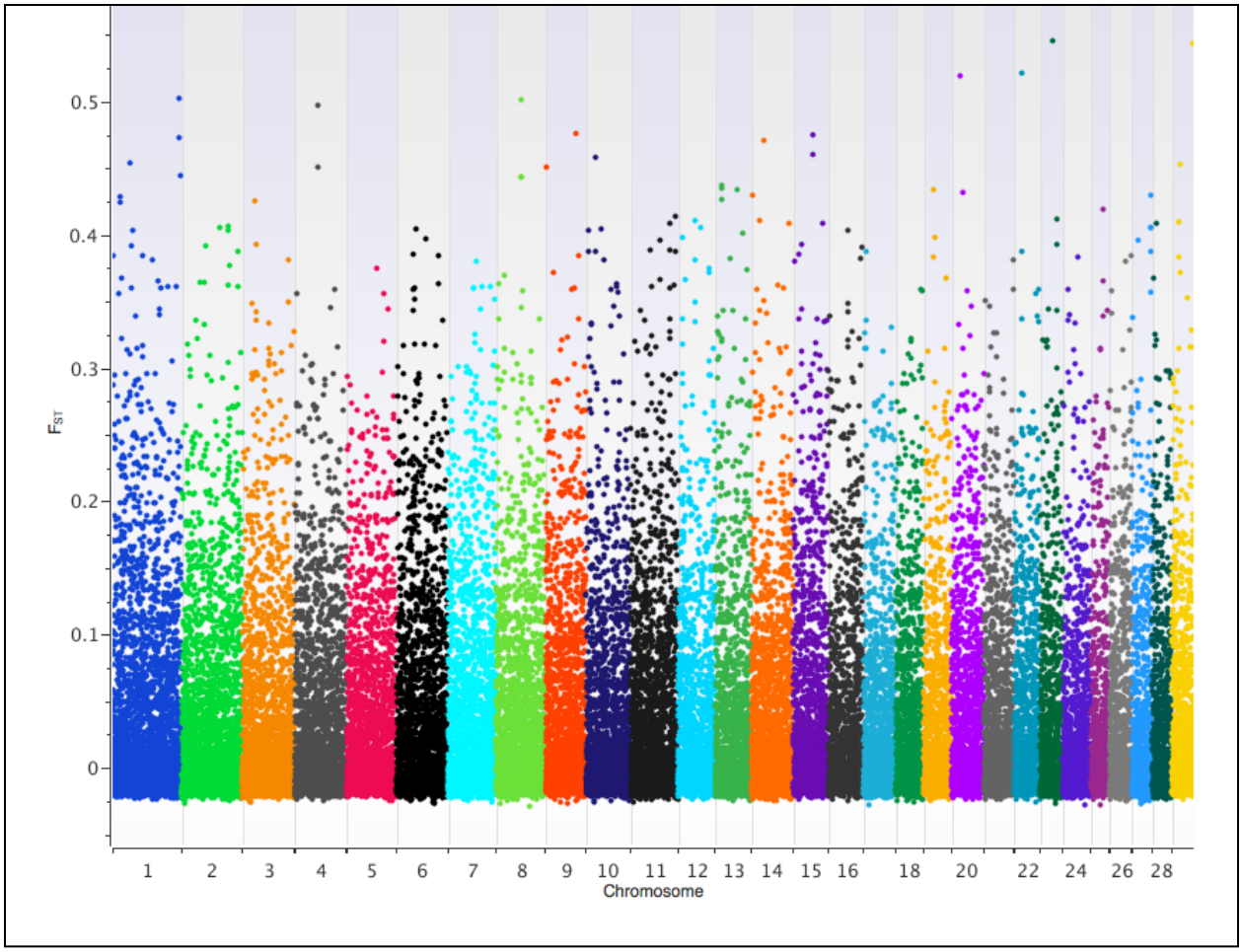


Figure 5.6: Genome distribution of FST values for autosomes across goat populations in Zimbabwe

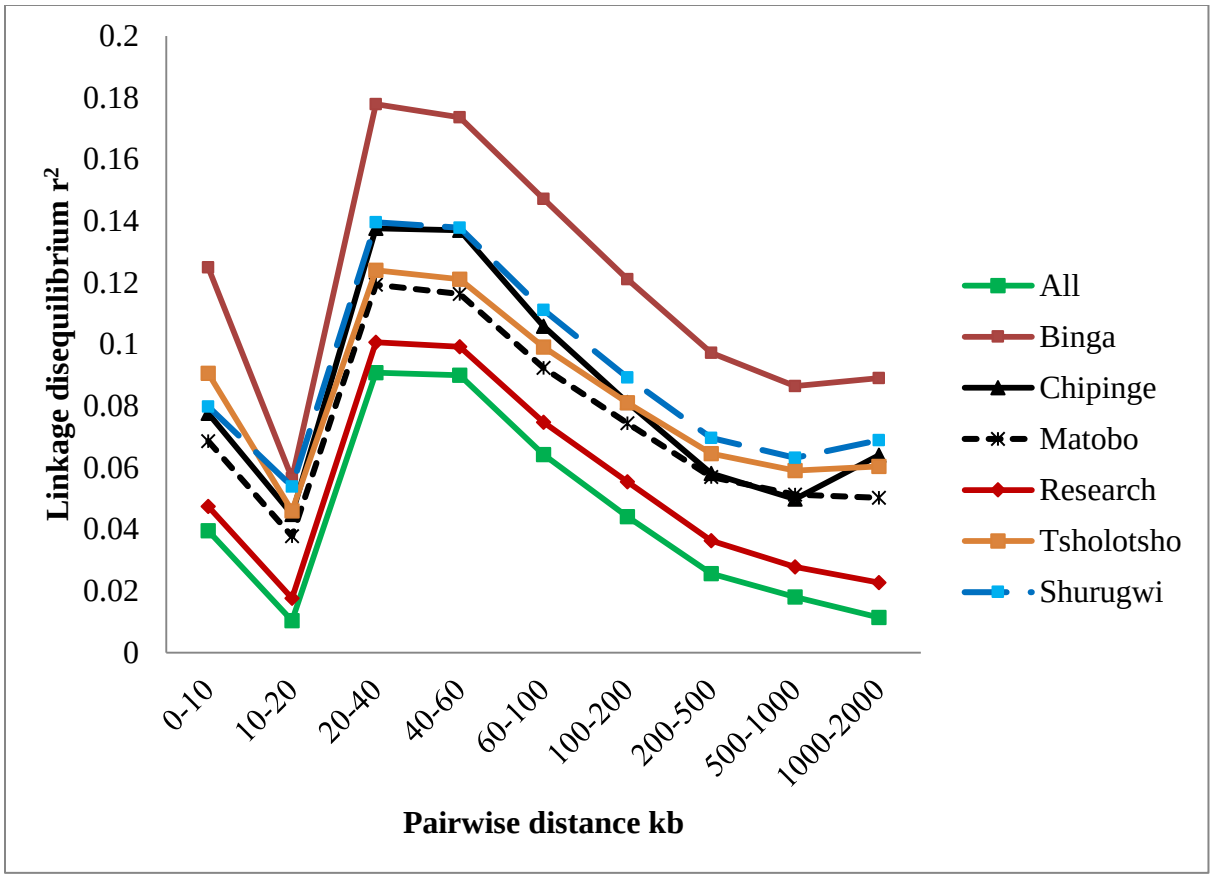


Figure 5.7: LD decay with increase physical distance between SNPs for autosomes in goat populations in Zimbabwe

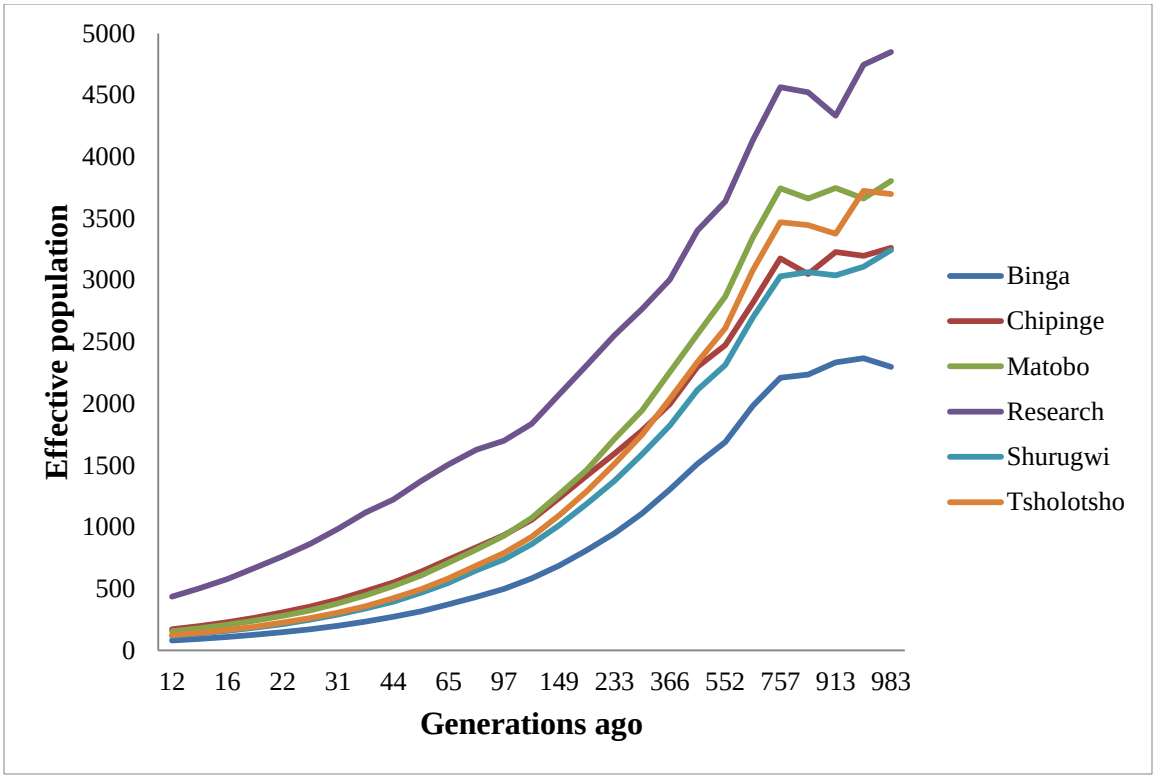


Figure 5.8: Trends in historic effective population size (Ne) over 983 generations ago

5.6 Discussion

Indigenous goats have been raised for a long time for various purposes and they have gradually accumulated several traits to be well adapted to local environments. Geographical isolation and human intervention has resulted in the formation of different subpopulations with different morphological traits. In Zimbabwe the majority of the goat population is reared by smallholder farmers. The dominant breeds reared in these systems are the Matabele and SEA prototypes which have been described above. Smallholder farmers are constrained in terms of land, capital/income for input procurement (De Sherbinin et al., 2008) and production losses such as mortalities (Rooyen and Tui, 2009). According to Gwaze et al. (2009a), these mortalities are caused by diseases and gastrointestinal parasites. Our preliminary work on gastrointestinal parasites in Zimbabwe (Zvinorova et al., 2016a), indicated that prevalence was 43% for Eimeria oocysts, 31% for strongyles 31%, with lower levels recorded for trematodes and cestodes. Parasites identified were *Haemonchus*, *Strongyloides* and *Oesophagostomum*, however, parasite species composition varied by area and impacts of risk factors also differed. This study was conducted as a means of understanding the genetic diversity and population structure of goats reared in these farming systems, linking that to the susceptibility or resistance of goats differentially challenged with gastrointestinal parasites, using the Illumina Goat 50K SNP Bead chip.

Genetic diversity is an essential component for population survival, evolution, genetic improvement and adaptation to changing environments (Kumar et al., 2015; da Rocha et al., 2016). Local goat populations in Zimbabwe have not been studied for population structure and diversity, thus genetic characterization of these populations is of paramount importance. Extensive studies

on genetic diversity in goats have been conducted based on a few markers (microsatellites). However, these do not provide whole genome coverage, which is essential for accurate predictions of population genetic parameters. The development of goat SNP chips has provided opportunities for population structure, diversity and association studies. The 50K SNP chip contains 53,347 SNPs and was developed using the Saanen, Alpine, Creole, Boer, Kacang, and Savanna breeds (Tosser-Klopp, 2012). The SNP chip utility has been demonstrated for different purposes including population genetic diversity studies (as previously highlighted in the introduction), including in genomic evaluation (Carillier et al., 2013; Brito et al., 2015; Mucha et al., 2015), QTL detection (An et al., 2012; Roldán et al., 2014). In the present study, the 50 K SNP chip was used to assess the level of LD, understanding the evolutionary history of populations and calculating genetic parameters. The use of high density markers makes this possible in the absence of pedigree data, especially in goats reared in smallholder farming systems, where record keeping is a not a common practice. The level of LD can then be used to detect QTLs associated to GIN resistance.

This is the first comprehensive study on population structure, diversity, linkage disequilibrium and effective population sizes of goats reared in low input/output farming systems in Zimbabwe, with a focus on indepth understanding of GIN resistance using genome-wide SNP markers. After quality control, 89.9% of the total SNPs were deemed informative on the Zimbabwean goat population. This is comparable to 87 % by Mdladla et al. (2016) for South African goats and by Visser et al. (2016) in South Africa, French and Argentinian breeds. The Research station goats had the largest number of SNPs (1503) that deviated from HWE suggestive of strong directional selection and non-random mating in this population. The level of polymorphism detected was high (i.e. > 89%) among the different populations studied, irrespective of the fact that the 50 K SNP

chip was developed from sequence data on exotic goat breeds in Zimbabwe. However, the proportion of SNPs with low MAF values was still high across populations indicating a higher proportion of fixed alleles within the Zimbabwean goats relative to the discovery populations.

Heterozygosity estimates obtained in this study were very high as compared to other studies (Kijas et al., 2013; Nicoloso et al., 2015; Mdladla et al., 2016), therefore, indicating even higher levels of genetic diversity. Low levels of inbreeding were observed across all populations. Genetic diversity was highest in village goats as compared to the Research station flock, as expected for village populations, since these goats rely on communal grazing and mating is indiscriminate, hence their low levels of inbreeding. In these farming systems goats are exposed to natural selection, where animals become genetically adapted for survival in their natural environments. According to Kim et al. (2016), natural selection and random mating of indigenous livestock has the ability to shape the genome while maintaining high within- and between-population genetic variability. This has a potential of fixing certain genes within populations, especially for complex traits such as gastrointestinal parasite resistance.

Heterozygosity estimates obtained in this study were higher as compared to other studies (Kijas et al., 2013; Nicoloso et al., 2015), therefore, indicating even higher levels of genetic diversity in the present study. Similarities in H_E and H_O in some of the populations may be due to the use of populations/breeds which were previously not used during the development of the 50K SNP panel. Molecular variance among all six populations (2.41%) was lower than the 6.39% reported for South African breeds (Mdladla et al., 2016) and 11.9 % reported for Angora goats (Visser et al., 2016). This indicates that there are low levels of genetic differences between the goat populations,

high individuals differences. This supported the hypothesis that genetic differences between individuals are greater than that between populations. This could be explained by high gene flow and heterogeneous characteristics of smallholder production systems. The genetic diversity among the populations increases the likelihood that at least some individuals will be able to survive parasitic infection by making the population more resistant.

The PCA and ADMIXTURE results clustered populations some village populations together and to some extent separating from the Research station flock. The analysis of population structure using PCA indicated Illumina Goat SNP50k Bead chip was able to discriminate some of the populations and a tendency of clustering together for village populations. According to Huson et al. (2014), Illumina Goat SNP50k Bead chip can effectively distinguish goat populations, specifically indigenous African goat populations. ADMIXTURE analyses confirmed the genetic relatedness of Binga and Shurugwi and also that of Matobo and Tsholotsho. According to Brito et al. (2015), this trend is consistent with the breeds' history.

High gastrointestinal parasite prevalence was observed in village populations (Binga, Chipinge, Matopo and Shurugwi) *vs.* the Research flock. This contradicts with high genetic diversity results observed in these populations. This could be explained by the management in place in smallholder farming systems; where animal were continuously re-infected in communal grazing lands. Levels of infection for the indigenous goats were low, using the intensity scales by Hansen and Perry (1994), also by Asha and Chebo (2015). This might be due to the animals acquiring strong immunity to infection of GIT parasites due to recurrent infections and development of resistance to parasitic infections by the animals. Chipinge population was distinct from the rest of the

populations at K=2 and beyond K=5 (lowest cross validation error), distinct populations of Binga were observed. However, Binga and Shurugwi showed some level of admixture, indicating that the populations may share common ancestry. These populations had the highest number of monomorphic SNPs and rare alleles (8 - 11%) and may suggest that a strong drift effect has taken place (Nicoloso et al., 2015). The genetic structure of Tsholotsho and Research was similar as shown by both PCA and ADMIXTURE results. Low parasite prevalence rates were also observed in both these populations, in addition to high levels of genetic diversity. This supports the work by King and Lively (2012), where the authors highlighted that high genetic diversity in host populations have a potential of reducing the risk of infection. The diversity in the Research station population could also be explained by them acquiring their animals from the nearby localities. This results in introduction of more genetic compositions, which may not necessarily be of indigenous breed origin. Subpopulations observed in the Research flock have a potential of reducing heterozygosity than there would be if the population was undivided. According to Muema et al. (2009) this could be as a result of founder effects, leading to subpopulations with allele frequencies that are different from the larger population.

Populations that share genomic proportions with each other indicate higher admixture and have a diverse genetic composition. Matobo and Tsholotsho had a distinct pattern in admixture which also signifies shared ancestry or it may suggest that certain alleles have become fixed in these populations due to adaptation in their environment. Overlapping of clusters confirm the ancestry the breeds of investigated animals, with those from Binga and Chipinge, mostly the Tonga and Mashona, respectively. However, those from Tsholotsho and Matobo were the Matabele, while Shurugwi had mixed genetic components. Research station animals have diverse genetic make-up

which may contain more than the mentioned indigenous breeds. In all the clusters only the Binga population was distinct, the other though indicative of their initial breed composition show different level of admixture.

Pairwise F_{ST} obtained across population were very low, indicating low population differentiation. According to Beaumont and Balding (2004), this may indicate regions of the genome that have been subject to stabilizing selection. The findings support the low levels of genetic variation explained by AMOVA where only 2.41% explained variation among populations. This makes it difficult to pick genetic difference between populations in terms of resistance to parasites. However, regions under selection can be detected by searching for outlier markers or haplotypes in either the distribution of allele frequencies within or between populations, or patterns of linkage disequilibrium along the genome (Holsinger and Weir 2005). Genomic F_{ST} values were computed. Using the guidelines by Wright (1978), the most differentiated regions representing with the highest F_{ST} (≥ 0.25) can be considered to be under selection.

Information about genome-wide linkage disequilibrium (LD) extent and decay is essential for GWAS mapping of loci affecting economically important traits and the implantation of genomic selection in farm animals (Ai et al., 2013). The average levels of LD in our study were very low. The highest observed levels of LD were in Binga and Shurugwi and with the Research station showing the. An observation, which might be associated with the level of sampling. Brito et al. (2014) suggests that it may be due to smaller effective population sizes in those breeds/populations, which is also confirmed by the effective population curves in the present study. A study by Meuwissen et al. (2001) using the Goat 50k SNP panel concluded that an r^2 value greater than 0.2

would be sufficient for genomic selection. The different goat populations in our study might benefit from a denser snp chip for conducting genome-wide association analyses, because the detection potential QTLs with such a panel is minimal. The marker density required for successful GWAS and subsequently genomic selection, depends on the extent of LD across the genome (Khatkar et al., 2008). Linkage disequilibrium also varied between chromosomes, suggesting a variation in autosomal recombination rates due to the effects of genetic drift and selection within populations (Qanbari et al., 2010). The extent of LD decay rapidly declined as genetic distances increased as calculated using equation by Sved (1971). The method takes into consideration recent and past recombinations, as well as past effective population size. Linkage disequilibrium at short distances is a function of effective population size many generations ago, while LD at long distances reflects more recent population history.

The high N_e observed in the past generations for the Research station reflects the great level of admixture observed for this population, as indicated by the ADMIXTURE analysis. However, Binga and Shurugwi had the lowest effective population sizes. According to Goddard and Hayes (2009), small N_e means that alleles in the current population coalesce in a common ancestor in a small number of generations, indicating that there are a few generations of recombination. The chromosome segments that are identical by descent are large, and so LD extends for a long distance. The N_e estimates for at least 12 generations ago in all populations except the research station were similar to those reported by Mdladla et al. (2016) for commercial and ecotype goats in South Africa. The current N_e for all populations except Binga is still of acceptable size. According to Meuwissen (2009), a threshold of $N_e = 100$ would be necessary to ensure that an animal population is long-term viable in terms of genetic diversity. The lower effective population

size in Binga goats implies that improved management should be taken into consideration to minimizes loss of diversity.

5.7 Conclusion

The study demonstrated the utility of the goat 50 K SNP panel in communal goats with highest levels of admixture, regardless of the fact that the panel was developed using a few breeds. Results from this study provided an insight on the population genetic structure, genetic diversity, extent of linkage disequilibrium and effective population sizes of goats in Zimbabwe. This indicates that there is potential for improving survival/tolerance of goats to GIN. There were high levels of genetic diversity based on H_E and H_O estimates, low levels of inbreeding across populations and low levels of population differentiation. The genetic population structure analysis indicated clusters of Binga, some degree of shared ancestry among Chipinge and Shurugwi, as well as Tsholotsho and Matobo populations. In all these clusters, the level of gastrointestinal parasite infection reported from a preliminary study was low, indicating a possibility of parasite resistance. Low levels of LD in this study makes it difficult to detect genetic difference between populations in terms of resistance to parasites as well as identify loci which may be associated to parasite resistance. The use of a denser SNP panel is required for the communal breeds for purposes of conducting GWAS genomic selection. Current effective population sizes were low for populations from Binga, indicating the need for improved management of animals to minimize loss of genetic diversity. DNA samples that were used in this section, were also used for further analyses in chapter 6.

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Chapter 6

6 Genome-wide association analyses for gastrointestinal parasite resistance in indigenous goats in Zimbabwe

6.1 Abstract

Gastrointestinal (GIN) parasitic infections pose a severe threat to small ruminant production, through weight losses and/or death. Exploiting host resistance to GIN as a control measure has been documented. Understanding the genetic architecture of parasite resistance is important for improved production. The aim of this study was to identify polymorphisms strongly associated with host resistance in goats reared in low-input/output farming systems in Zimbabwe. The study involved conducting genome wide association (GWAS) in goats from Chipinge (n = 33), Shurugwi (n = 22), Binga (n = 17), Matobo (n = 33), Tsholotsho (n = 25) and Matopo Research Station (n = 124) and also performing association analysis within-communal goat populations. The traits analysed were logarithm transformed faecal egg counts for *Eimeria* and *Strongyles*, level of PCV, loads of parasitic infection for both parasites. After quality control, 49 984 SNPs and 44918 SNPs were available for genome-wide association analysis in GenAbel and PLINK respectively. The study confirmed that GIN resistance traits had heritabilities ranging from 0.27 to 0.56. The GWAS analyses revealed multiple SNPs that were associated with *Eimeria* and *Strongyles* and were significant at the genome-wide level. In particular, the study identified regions on chromosomes (chr) 4 ($P = 2.66 \times 10^{-6}$) for *Eimeira* and chr29 ($P = 9.93 \times 10^{-6}$) for both *Eimeria* and *Strongyles* traits.

Keywords: Gastrointestinal parasites, GWAS, PCV, goats, genome-wide

6.2 Introduction

Gastrointestinal nematodes (GIN) infections in small ruminants are responsible for significant economic losses in tropical and temperate regions worldwide (Crawford et al., 2006). Control is largely based on use of anthelmintics (Zanzani et al., 2014). However, resistance to anthelmintics has been documented for all major classes of anthelmintics (Riggio et al., 2013; Pickering et al., 2015). Anthelmintic resistance is higher in goats than in sheep, with which they share the same nematode parasites (Mandonnet et al., 2001; Hoste et al., 2010). According to Chiejina and Behnke (2011), the ability of goats to control challenge infections following a primary infection is less efficient than that of sheep. The immunological memory following anthelmintic abbreviation of a primary infection and challenge does not last as long as in sheep. Heavy reliance on drug use to control parasites has also raised public concerns due to the presence of drug residues in animal food products (Benavides et al., 2015).

In tropical and sub-tropical countries, goats are owned by smallholder farmers and are managed extensively, with little or no routine worm control being practiced. In this farming system, goat productivity is low, which could be due to low level of chronic infections, with high prevalence levels of infections occurring all the year round (Chiejina and Behnke, 2011). According to Benavides et al. (2016), the longer animals are exposed to infective larvae in the pasture, the more likely host resistance will develop. It is important to develop alternative methods for controlling internal parasite infections, such as selection of resistant goats in Zimbabwe. Using host resistance as an alternative method of control has the potential to increase frequency of more resistant goats and to improve production.

Traditionally, the genetic control of complex traits in livestock has been studied without identifying the genes or gene variants underlying observed variation, with selection on the basis of estimated breeding values (EBVs) calculated from phenotypic and pedigree information (Mandonnet et al., 2006; Goddard and Hayes, 2009). Other genetic studies on GIN focused on identification of candidate genes (Miller and Horohov, 2006; Alba-Hurtado and Muñoz-Guzmán, 2012) and QTLs associated with parasite resistance (Matika et al., 2003; Bolormaa et al., 2010a; de la Chevrotière et al., 2012a) and using pedigreed populations. QTL studies typically localize the causative variant to a fairly large region. However, little overall consensus has emerged from these studies, an outcome compounded by the genetic complexity of the trait and the fact that these studies are very diverse, involving a variety of breeds, nematode species and experimental approaches.

Genome-wide association studies (GWAS) are a recent technology which employs thousands of single nucleotide polymorphism (SNP) markers to unveil genomic regions associated with the trait of interest. This technology has been possible after the release of genomic tools which in goats was the Illumina Goat 50 k SNP BeadChip, which features 53 347 SNP probes, distributed across the whole genome, with inter-SNP spacing of ~40 KB (Tosser-Klopp, 2012). GWAS can fit pedigreed populations but it can also be used in case-control studies with no pedigree information, which will be quite useful in low-input/output farming systems, where pedigree records are usually incomplete or nonexistent. Despite evidence for genetic resistance being studied extensively, fewer studies have been conducted in goats, mainly using microsatellite markers. Chronic infections are a major source of re-infection and a contributory factor to poor productivity goats in many countries. Knowledge on genes associated with parasite resistance can be incorporated in breeding

programs. This will benefit the smallholder farmer by reducing cost of drugs, reduce pasture contamination and improve animal performance. The objective of this study was to investigate SNP-markers associated with resistance to gastrointestinal parasites and infer roles of the genes found close to the markers, as a means of understanding mechanisms associated with parasite resistance in goats reared in low-input/output farming systems in Zimbabwe.

6.3 Material and methods

6.3.1 Population description

The study population consisted of 253 goats from smallholder farmers and a research station in Zimbabwe. Local ecotypes of Mashona/ Tonga and Matabele were sampled from Chipinge, Shurugwi, Binga, Tsholotsho, Matobo communal farms and from Matopos Research Station. Communal animals did not have pedigree records, while some of the Research Station animals had. Local ecotypes were maintained under extensive systems, where they foraged on farm land or on communal pastures during the day with minimum supplementation and kraaled at night. Animals mated indiscriminately and were continuously exposed to natural infections while foraging in the communal grazing areas. Animals at the research station (in the district of Matobo) were managed semi-intensively. Goats foraged on the research station open rangeland throughout the year with some rotation in the paddocks during the day, minimum supplementation, animal were naturally infected while foraging.

6.3.2 Phenotypic measurements

Faecal and blood samples were collected directly from the rectum and jugular veins into airtight containers and 10-ml EDTA VACUETTE® tubes, respectively. Samples were kept between 2 - 4 °C during field sampling period on ice in cooler boxes and later stored at -21 °C in the refrigerator to prevent formation of ice crystals, prior to laboratory analysis. Sample collection was conducted over two different seasons targeting the dry (late April - early October) and wet (late October - early April) seasons from 2014 to 2015. Faecal egg counts (FEC) were determined by the modified McMaster technique, using floatation methods for nematodes and protozoa (MAFF, 1986). Identification of 3rd stage larvae of nematodes was only at the genus level according to Van Wyk et al. (2004). Gastrointestinal parasites were classified according to *Eimeria* spp (oocysts) or nematode genera collectively termed *Strongyles*, which potentially included *Haemonchus* spp, *Oesophagostomum* spp, and *Strongyloides* spp. Packed cell volumes (PCV) were also assessed using the capillary micro-hematocrit centrifuge method (Bull, 2000). The FEC data was either used directly for analysis or used to classify animals into cases/controls and categorisation of animals by levels of infection according to scales used by Hansen and Perry (1994), also by Asha and Chebo (2015), i.e. FEC of 0 – 800 eggs per gram (epg) = low, FEC of 800 – 1200 epg = moderate and FEC > 1200 epg = high and PCV was either categorised as low or normal.

6.3.3 SNP genotypes and quality control

Animals were genotyped at the Agricultural Research Council-Biotechnology Platform (Oondersteport) in South Africa using the Illumina goat SNP50k Bead chip. The SNP positions were based on the current goat genome assembly (CHI_1.0 goat) available from the International Goat Genome Consortium (http://www.goatgenome.org). Quality control thresholds were set to

remove markers with missing data > 5%, that had MAF ≤ 5%, individuals with missing genotypes > 5%, those deviating from Hardy–Weinberg equilibrium (HWE; $P < 0.001$), across populations using PLINK v 1.07 (Purcell et al., 2007). For analyses involving genome-wide associations within populations (within-population genome-wide association), SNP data was filtered to remove SNPs that were on sex chromosomes or had their positions unmapped to the latest reference assembly of the goat genome, while on the single-SNP genome-wide association, these were not filtered off. After quality control, 49 984 SNPs were available for genome-wide association analysis in GeneAbel and 44918 for within-population GWA analysis in PLINK.

6.4 Statistical analyses

Initial model specifications and data exploration were conducted using SAS v 9.3 (SAS, 2011). Genetic parameters for packed cell volume (PCV), *Strongyles* and *Eimeria* resistance were determined using ASReml (Gilmour et al., 2009), with the latter two being transformed through a base 10 logarithm, $\log_{10}$ (FEC+25) to approximate a normal distribution. Genetic parameters were estimated by the fixed effects of sex (male and female), site (Binga, Chipinge, Matopo, Shurugwi, Tsholotsho and Research), also using the co-variates of age (1 – 7 years). The animal was fitted as a random effect by using either available pedigree for the research station animals or the genomic relationship matrix for all animals.

Since local ecotypes were from different localities, with no previous records and Research station animals with inadequate pedigree to account for population structure, it was important to identify and correct for population stratification. Accounting for population structure has a potential of

reducing false-positive associations due to population stratification (Lander and Schork, 1994). The population structure was inferred from marker data by using classical multi-dimension scaling (MDS) (see Figure 5.2), to explore population substructure and to verify the genetic homogeneity of the sample prior to analysis. To account for relatedness, the variance/covariance matrix was estimated from the genomic kinship matrix that was constructed by using pair-wise identities by state, and calculated for all samples based on all autosomal SNPs, as implemented in the GenABEL library (Aulchenko et al., 2007).

Genome-wide association (GWA) analyses was conducted by performing single SNP association analyses, using the GenABEL package in R environment (http://www.r-project.org) (Aulchenko et al., 2007) and by performing within-population GWA analyses using PLINK v 1.07 (Purcell et al., 2007). The single SNP association analysis utilized quantitative data and it involved fitting both fixed and polygenic effects for the traits, with the latter accounting for genetic relationships between animals. The fixed effects considered were explained above; also the first three principal components (PC1 - PC3) were included, to account for population stratification. Association analysis was also tested using the mmscore function (Chen and Abecasis, 2007) on the residuals which had been tested for familial relatedness. After Bonferroni correction, significant and suggestive thresholds were 1.24×10^{-6} and 2.47×10^{-5} for the genome-wide analysis ($P < 0.05$), respectively. For the within-population genome-wide association analyses, the FEC traits for *Strongyles* and *Eimeira* were classified as cases or controls, as well as high/low levels of infection based on faecal egg counts. Animals from the Research station were excluded from this association analyses due to their diverse population structure and high levels of admixture (see Fig 5.3). Animals were assigned as cases or controls for the various traits before allelic association using

the - - assoc function in PLINK. Association testing and chi square testing were performed in subpopulations within Binga, Chipinge, Matobo, Shurugwi and Tsholotsho goats and also in combined dataset.

6.5 Results

Descriptive statistics for FEC prevalence per population are summarised in Chapter 4, Table 4.4 and levels of infection are summarised in Table 6.1. Level/intensity of infection was generally low *Strongyles* (85.3%), *Eimeira* (95%) and mixed infection (79%), across populations. The remainder included either moderate and/or high levels of infection.

6.5.1 Estimation of genetic parameters

Heritability estimates for L*Strongyles*, LEimeria and PCV were generally higher when estimated by using the pedigree matrix rather than the kinship matrix. These estimates ranged from moderate to high (0.27 - 0.56) for both FEC and PCV when using the kinship matrix and high (0.63 - 0.75) when using the pedigree matrix (Table 6.2).

Table 6.1: Level of gastrointestinal infection in different areas

	Intensity	Binga	Chipinge	Matobo	Research	Shurugwi	Tsholotsho
Strongyles	Low	93.8	93.4	84.8	82.2	81.8	85.7
	Moderate	6.3	0.0	6.1	5.9	13.6	4.8
	High	0.0	3.6	9.1	11.9	4.6	9.5
Eimeria	Low	96.4	93.4	100.0	98.3	77.3	90.5
	Moderate	0.0	3.6	0.0	0.0	4.6	4.8
	High	3.6	0.0	0.0	1.7	18.2	4.8
Mixed	Low	96.4	92.9	78.1	80.3	68.2	76.2
	Moderate	3.6	3.6	12.5	6.0	9.1	14.3
	High	0.0	3.6	9.4	13.7	22.7	9.5

The level of infection was based on the faecal egg counts, where, FEC of 0 – 800 eggs per gram (epg) = low, FEC of 800 – 1200 epg = moderate and FEC > 1200 epg = high (Hansen; Perry, 1994); (Asha; Chebo, 2015)

Table 6.2: Heritability estimates for GIN using both the kinship and the pedigree-based relationship matrices

	Birth weight	L*Strongyles*	L*Eimeria*	PCV
Kinship				
σ^2_a	0.83 x 10^{-01}	0.19	0.19	4.57
σ^2_p	0.24	0.41	0.35	17.2
Se	0.41 x 10^{-01}	0.39 x 10^{-01}	0.34 x 10^{-01}	1.61
h^2	0.34	0.47	0.56	0.27
Se	0.29	0.20	0.18	0.18
Pedigree				
σ^2_a	0.36 x 10^{-01}	0.30	0.25	10.77
σ^2_p	0.23	0.40	0.35	17.0
Se	0.38 x 10^{-01}	0.39 x 10^{-01}	0.34 x 10^{-01}	1.61
h^2	0.15	0.75	0.72	0.63
Se	0.32	0.26	0.28	0.27

Pedigree data was only available for the Research station animals, kinship data was used for all the animals
L*Strongyles* = Log_{10}(*Strongyles*+25), L*Eimeria* = Log_{10}(*Eimeria*+25)

6.5.2 Genome-wide association analyses

Genome-wide association (GWA) analysis identified a strong genome-wide significant association for L*Eimeria*, having average animal effect on chromosome (chr) 4 (corrected P-value = 2.66 x 10^{-6}, $-\log_{10}$ *(P)* = 22.05). However, several SNPs reached the suggestive level for all traits according to definitions we used. A region on chr 4 was noted to have significant associations for L*Strongyles*, at the suggestive level. A summary of identified SNPs, the trait they were associated with, their map locations, their P-values and associated genes are reported in Table 6.3. The corresponding Manhattan plots and quantile-quantile (QQ) plots for all traits are presented in Figures 6.1 – 6.4. The QQ plots were constructed for each association to check the general

distribution of the test-statistics and to assess the degree of fit of the model for this analysis. Under the hypothesis that most SNP are not associated with the trait, the corresponding QQ plots should follow the 45° line y = x to confirm the good fit of the observed-to-expected (theoretical) distributions.

Using PLINK, the data was re-analysed with five phenotypes, first using the animals and then different subpopulations. The top 10 significant SNPs of different traits, from within-population comparisons, are summarised in Tables 6.4 – 6.8. Genome-wide association was observed for several SNPs, with two of the top five SNPs for *Strongyles* infection being located on chr 19 (snp10639-scaffold1377-1859736, P = 1.29 x 10^{-5} and snp13217-scaffold1507-522594, P = 7.72 x 10^{-6}) in Binga goats, chr 3 (snp44005-scaffold595-4718582, P = 6.35 x 10^{-5}) in Chipinge and chromosome 14 (snp19587-scaffold1975-373605, P = 1.71 x 10^{-4}; snp1952-scaffold1054-687207, P = 1.71 x 10^{-4}) in Tsholotsho. For *Eimeira* infection, chr 4 (snp55957-scaffold87-1026023, P = 2.2 x 10^{-5}) and chr 20 (snp49830-scaffold711-2279114, P = 9.68 x 10^{-5}) were found to be significant in Binga, on chr 23 (snp4884-scaffold1164-70551, P = 7.29 x 10^{-5}) for Chipinge goats and on chr 2 (snp9359-scaffold1341-265416, P = 4.98 x 10^{-5}) for Tsholotsho population. SNPs associated with loads/intensity of *Strongyle* infection were located on chromosomes 4, 17 and 29 being observed in Binga, Chipinge and Shurugwi. For *Eimera* intensity of infection association were located on chromosomes 4, 17 and 29 (P < 0.05). Common significant SNPs for PCV were located on chromosomes 3, 4 and 29 (P < 0.05) from individual sub-populations.

Table 6.3: List of SNPs associated with BWT FEC, PCV traits identified by genome-wide association analysis

Trait	SNP Marker	Chromosome	Position (bp)	-log$_{10}$ *(P)*	Pc1df (P)	Associated genes
L*Eimeria*	snp49152-scaffold701-194847	4	42887471	22.04742	2.66×10^{-6}	*ORC5*
L*Strongyle*	snp4990-scaffold117-145789	4	45522178	17.17311	3.41×10^{-5}	*RELN*
L*Eimeria*	snp11688-scaffold143-2734639	25	34779999	15.27857	9.28×10^{-5}	*NALCN*
L*Strongyle*	snp4026-scaffold1126-2473390	17	48644172	16.20155	5.69×10^{-5}	-
L*Strongyle*	snp42815-scaffold568-5657120	12	61651542	15.92779	6.58×10^{-5}	-
BWT	snp28134-scaffold300-5890340	8	40675422	15.64633	7.64×10^{-5}	-
L*Eimeria*	snp13629-scaffold1526-1243833	12	73014686	15.53893	8.08×10^{-5}	-
BWT	snp59084-scaffold969-2458316	9	30471671	15.45961	8.43×10^{-5}	-
PCV	snp53590-scaffold816-423811	22	36556377	15.11593	1.01×10^{-4}	*ns*
L*Strongyle*	snp1415-scaffold1038-2762722	19	3602327	15.09009	1.02×10^{-4}	*ns*
PCV	snp20198-scaffold20-1688380	11	57190566	14.71118	1.25×10^{-4}	*ns*
L*Eimeria*	snp49201-scaffold701-2256920	4	44949544	14.6866	1.27×10^{-4}	*ns*
L*Strongyle*	snp29977-scaffold327-1248091	17	54467125	14.53707	1.37×10^{-4}	*ns*
L*Eimeria*	snp47834-scaffold673-1856037	23	12980702	14.52212	1.39×10^{-4}	*ns*
PCV	snp16814-scaffold1760-528032	6	33884847	14.45462	1.44×10^{-4}	*ns*

The genome-wide significance threshold corresponded to a p value less than 1.24×10^{-6} and the suggestive significance threshold corresponded to a p-value less than 2.48×10^{-5}; (-) on associated genes refers to uncharacterized genes, *ns*- not significant

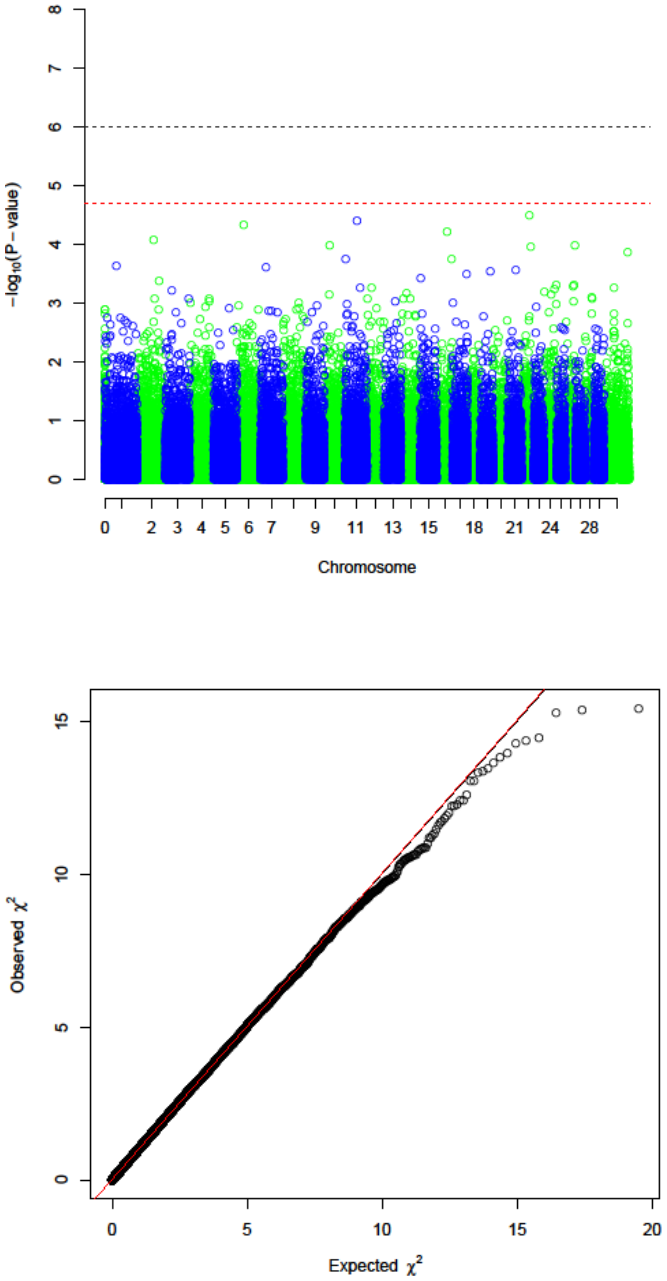

Figure 6.1: Manhattan plot displaying the GWA results (-log10 (P) of the corresponding Pc1df, P-values corrected for the genomic inflation factor λ) and Q–Q plot (below) of observed P-values against the expected P-values for $\log_{10}$ (*Strongyle*+25). Genome-wide P<0.05 (black dashed line) and suggestive (red dashed line) thresholds are shown**.**

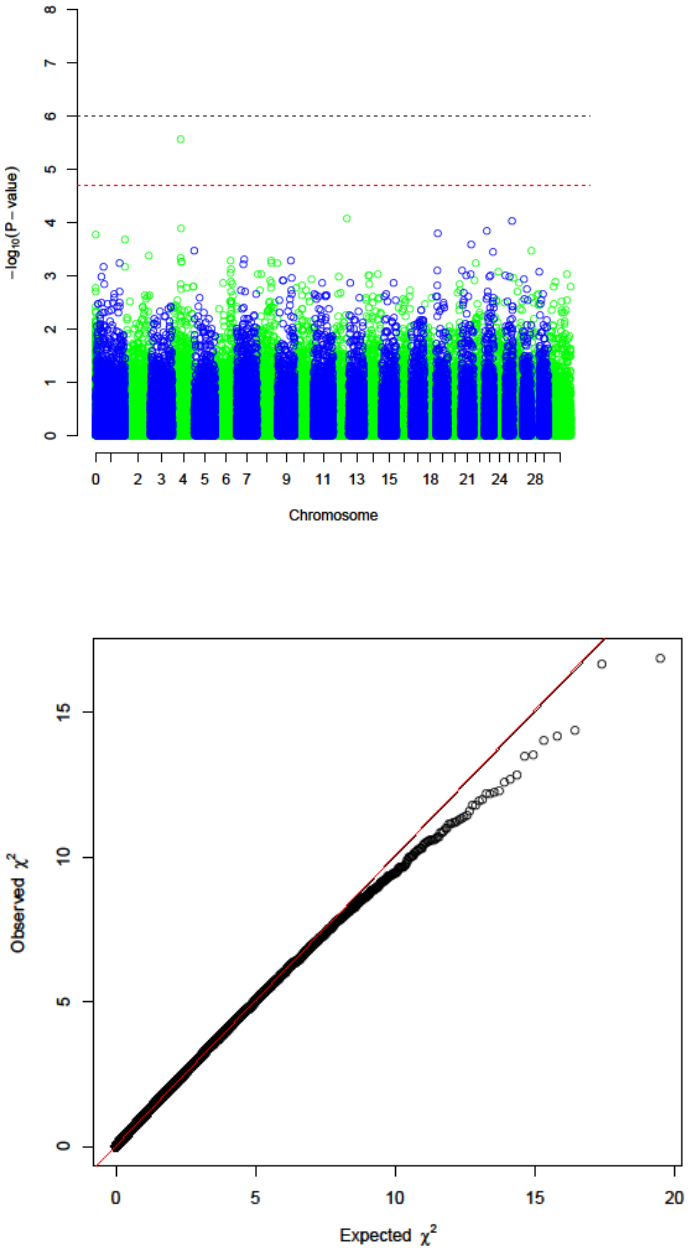

Figure 6.2: Manhattan plot displaying the GWA results (-log10 (P) of the corresponding Pc1df, P-values corrected for the genomic inflation factor λ) Q–Q plot (below) of observed P-values against the expected P-values for $\log_{10}$ (*Eimeria*+25). Genome-wide Genome-wide P<0.05 (black dashed line) and suggestive (red dashed line) thresholds are shown.

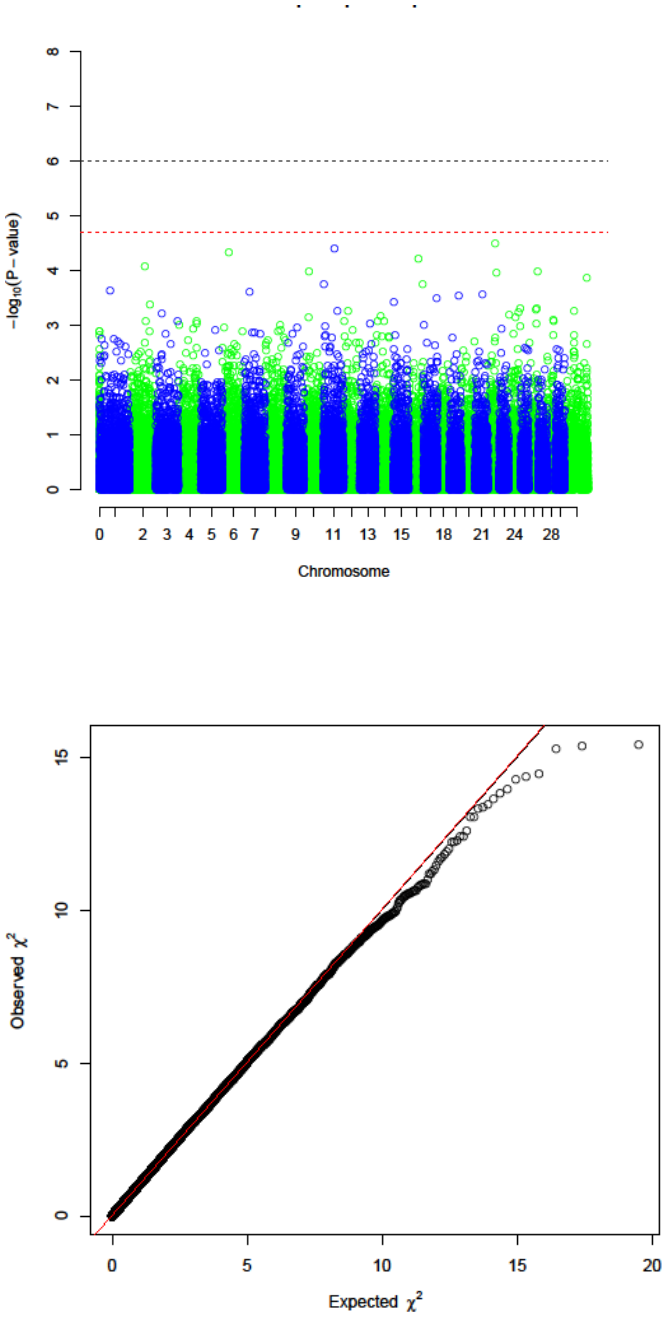

Figure 6.3: Manhattan plot displaying the GWA results (-log10 (P) of the corresponding Pc1df, P-values corrected for the genomic inflation factor λ) and Q–Q plot (below) of observed P-values against the expected P-values for packed cell volume. Genome-wide P<0.05 (black dashed line) and suggestive (red dashed line) thresholds are shown**.**

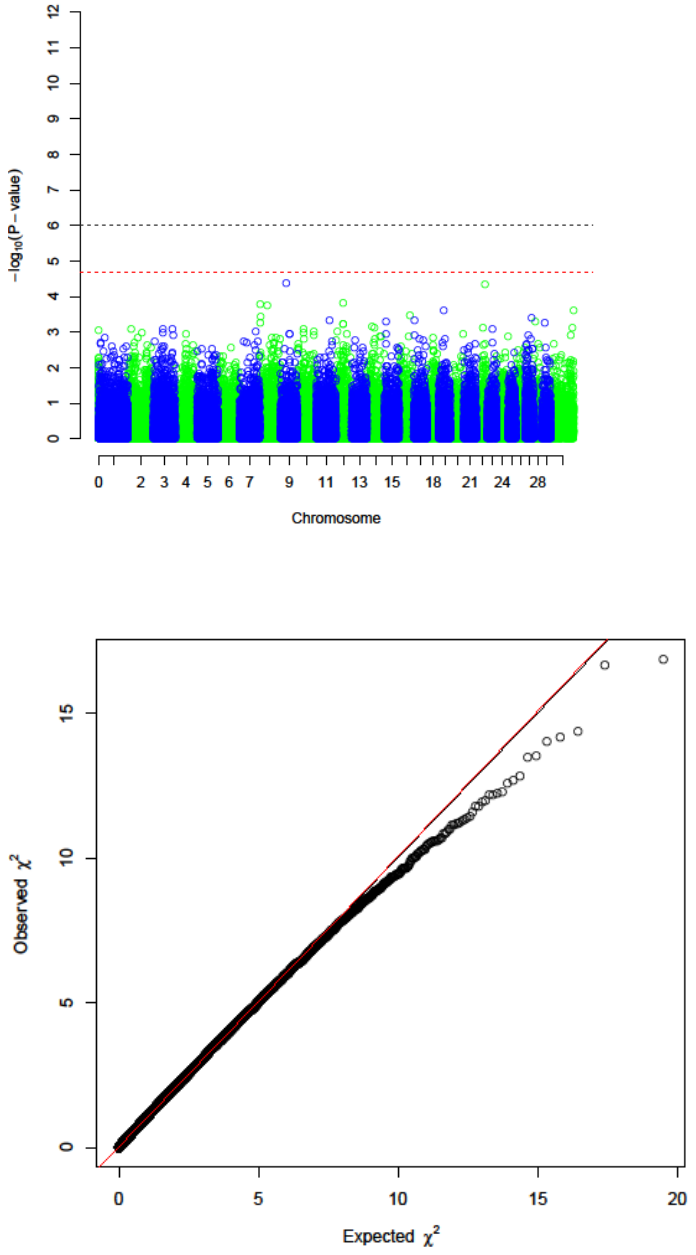

Figure 6.4: Manhattan plot displaying the GWA results (-log10 (P) of the corresponding Pc1df, P-values corrected for the genomic inflation factor λ) and Q–Q plot (below) of observed P-values against the expected P-values for body weight. Genome-wide P<0.05 (black dashed line) and suggestive (red dashed line) thresholds are shown

Table 6.4: SNP associations for *Strongyles*

Population	Chr.	SNP Marker	Position (bp)	F_A	F_U	CHISQ	log P-value (P)	Gene
Shurugwi	29	snp45158-scaffold615-95117	3968976	0.06	0.70	19.52	9.93×10^{-06}	*HRASLS5*
Chipinge	3	snp44005-scaffold595-4718582	90576796	0.67	0.13	16.00	6.34×10^{-05}	*ZFYVE9*
Matobo	14	snp35366-scaffold425-347876	73026013	0.17	0.90	21.48	3.58×10^{-06}	*NCALD*
Matobo	9	snp36947-scaffold448-1987984	76995912	0.10	0.70	17.91	2.32×10^{-05}	*OPRM1*
Binga	19	snp10639-scaffold1377-1859736	23852460	0.11	0.92	19.03	1.29×10^{-05}	*ATP2AE*
Binga	28	snp13217-scaffold1507-522594	6772317	0.11	0.83	15.65	7.63×10^{05}	-
Matobo	17	snp5993-scaffold121-2283992	58749480	0.08	0.70	20.48	6.03×10^{-06}	-
Matobo	26	snp30498-scaffold3363-112737	44763296	0.00	0.40	20.62	5.59×10^{-06}	-
Matobo	15	snp54683-scaffold837-94165	56807645	0.23	0.90	16.48	4.90×10^{05}	-
Binga	1	snp20580-scaffold2027-408470	6544523	0.06	0.92	22.25	2.40×10^{-06}	-

List of top ten SNPs associated with *Strongyle* infection identified by genome-wide association analysis within individual sub-populations. F_A and F_U- Allelic frequencies for affected and unaffected, Chr.- chromosome; (-) on associated genes refers to uncharacterized genes, *ns*- not significant

Table 6.5: SNP associations for *Eimeria*

Population	Chr.	SNP Marker	Position (bp)	F_A	F_U	CHISQ	log P-value (P)	Gene
Shurugwi	29	snp45158-scaffold615-95117	3968976	0.06	0.70	19.52	9.93×10^{-06}	*HRASLS5*
Binga	20	snp49830-scaffold711-2279114	19009631	0.69	0.00	15.2	9.68×10^{-05}	*PDE4D*
Chipinge	12	snp42761-scaffold568-3276429	64032233	0.09	0.59	15.45	8.45×10^{-05}	*GPC5*
Chipinge	23	snp31000-scaffold3423-173708	15136679	0.00	0.41	15.71	7.39×10^{-05}	*KIAA03*
Chipinge	23	snp4884-scaffold1164-70551	30426514	0.19	0.73	15.73	7.29×10^{-05}	*CCDN3*
Tsholotsho	2	snp9359-scaffold1341-265416	22981313	0.10	0.71	16.45	4.98×10^{-05}	*RAPGEF4*
Matobo	28	snp1049-scaffold1028-563238	4231554	0.09	0.58	16.64	4.51×10^{-05}	*PCNX2*
Binga	1	snp14650-scaffold1590-595886	120458177	0.69	0.00	15.2	9.68×10^{-05}	-
Tsholotsho	13	snp49123-scaffold7-6847705	25544289	0.85	0.25	15.74	7.26×10^{-05}	-
Chipinge	11	snp52883-scaffold793-1222376	47038137	0.19	0.73	15.73	7.29×10^{-05}	-

List of top ten SNPs associated with *Eimeria* infection identified by genome-wide association analysis within individual sub-populations. F_A and F_U- Allelic frequencies for affected and unaffected, Chr.- chromosome; (-) on associated genes refers to uncharacterized genes, *ns*- not significant

Table 6.6: SNP associations for *Strongyle* intensity of infection

Population	Chr.	SNP Marker	Position (bp)	F_A	F_U	CHISQ	log P-value (P)	Gene
Shurugwi	29	snp45158-scaffold615-95117	3968976	0.06	0.70	19.52	9.93×10^{-06}	*HRASLS5*
Matobo	28	snp56862-scaffold90-2142665	19248672	0.69	0.14	17.03	3.68×10^{-05}	*JMJD1C*
Chipinge	17	snp23387-scaffold2341-25639	9520754	0.54	0.03	17.94	2.28×10^{-05}	*NR3C2*
Binga	4	snp49507-scaffold706-1451989	20658229	0.83	0.04	18.8	1.45×10^{-05}	*DGKB*
Binga	4	snp49508-scaffold706-1500437	20706677	0.83	0.04	18.8	1.45×10^{-05}	*DGKB*
Binga	4	snp49510-scaffold706-1569574	20775814	0.83	0.04	18.8	1.45×10^{-05}	*DGKB*
Binga	4	snp21932-scaffold2156-19824	90363844	0.67	0.00	18.46	1.73×10^{-05}	-
Binga	11	snp46972-scaffold656-562152	53613950	0.67	0.00	18.46	1.73×10^{-05}	-
Chipinge	2	snp37699-scaffold464-2080725	100621022	0.67	0.10	18.77	1.47×10^{-05}	-
Chipinge	17	snp52353-scaffold779-851002	1165612	0.79	0.20	18.77	1.47×10^{-05}	-

List of top ten SNPs associated with *Strongyle* intensity identified by genome-wide association analysis within individual sub-populations. F_A and F_U- Allelic frequencies for high and low loads of infection, Chr.- chromosome; (-) on associated genes refers to uncharacterized genes, *ns*- not significant

Table 6.7: SNP associations for *Eimeria* intensity of infection

Population	Chr.	SNP Marker	Position (bp)	F_A	F_U	CHISQ	log P-value (P)	Gene
Shurugwi	29	snp45158-scaffold615-95117	3968976	0.06	0.70	19.52	9.93×10^{-06}	*HRASLS5*
Binga	4	snp49507-scaffold706-1451989	20658229	0.83	0.04	18.8	1.45×10^{-05}	*DGKB*
Binga	4	snp49508-scaffold706-1500437	20706677	0.83	0.04	18.8	1.45×10^{-05}	*DGKB*
Binga	4	snp49510-scaffold706-1569574	20775814	0.83	0.04	18.8	1.45×10^{-05}	*DGKB*
Chipinge	6	snp30831-scaffold340-1546957	11987875	0.59	0.09	15.45	8.45×10^{-05}	-
Tsholotsho	2	snp29701-scaffold3212-210914	23568400	0.06	0.65	15.75	7.23×10^{-05}	-
Chipinge	3	snp56222-scaffold879-1023557	40371352	0.14	0.69	15.93	6.58×10^{-05}	-
Matobo	3	snp15889-scaffold167-197764	81670173	0.47	0.00	16.44	5.02×10^{-05}	-
Binga	4	snp21932-scaffold2156-19824	90363844	0.67	0.00	18.46	1.73×10^{-05}	-
Binga	11	snp46972-scaffold656-562152	53613950	0.67	0.00	18.46	1.73×10^{-05}	-

List of top ten SNPs associated with *Eimeria* intensity identified by genome-wide association analysis within individual sub-populations. F_A and F_U- Allelic frequencies for high and low loads of infection, Chr.- chromosome; (-) on associated genes refers to uncharacterized genes, *ns*- not significant

Table 6.8: SNP associations for PCV

Population	Chr.	SNP Marker	Position (bp)	F_A	F_U	CHISQ	log P-value (P)	Gene
Shurugwi	4	snp55533-scaffold860-529654	17408877	0.13	0.75	16.38	5.19×10^{-05}	*THSD7A*
Matobo	28	snp1049-scaffold1028-563238	4231554	0.09	0.58	16.64	4.51×10^{-05}	*PCNX2*
Chipinge	17	snp23387-scaffold2341-25639	9520754	0.54	0.03	17.94	2.28×10^{-05}	*NR3C2*
Shurugwi	14	snp51276-scaffold75-5261761	35863280	0.09	0.67	15.28	9.28×10^{-05}	-
Chipinge	2	snp5777-scaffold1203-687366	68392864	0.50	0.03	15.89	6.73×10^{-05}	-
Tsholotsho	2	snp29701-scaffold3212-210914	23568400	0.06	0.67	15.94	6.53×10^{-05}	-
Binga	24	snp27305-scaffold290-2740278	35916592	1.0	0.2	17.14	3.47×10^{-05}	-
Binga	27	snp7600-scaffold1270-1052577	36860194	0.70	0	18.26	1.93×10^{-05}	-
Chipinge	2	snp37699-scaffold464-2080725	100621022	0.67	0.10	18.77	1.47×10^{-05}	-
Chipinge	17	snp52353-scaffold779-851002	1165612	0.79	0.20	18.77	1.47×10^{-05}	-

List of top ten SNPs associated with PCV identified by genome-wide association analysis within individual sub-populations. F_A and F_U- Allelic frequencies for low and normal PCV, Chr.- chromosome; (-) on associated genes refers to uncharacterized genes, *ns*- not significant

6.6 Discussion

The traits that were used for this study were FECs for both *Strongyles* and *Eimera* the egg counts were further categorised to either high, medium or low intensities, which measured the level of parasite burden in the animals. Measurement of FEC was conducted using the floatation method. Another trait that was assessed was the packed cell volumes, which gives an indication of the degree of anaemia, especially if the animal has been infested with blood-sucking internal parasites such as *Haemonchus contortus*. The study indicated that GIN resistance is a heritable trait, which can be incorporated in genetic improvement programs. Heritability (h^2) estimates for FEC traits obtained were generally higher than what has been reported in other goat studies (Vagenas et al., 2002; Mandonnet et al., 2006). Estimates computed using pedigree data for PCV obtained in this study were comparable with those reported by Mandonnet et al. (2001). Heritability estimates had high standard errors, this could be explained by low samples sizes used. The pedigree-based estimates for all traits were higher than those computed using the kinship matrix which could be attributed to the data structure.

In the previous study (chapter 5), the present study reported low levels of LD (ranging from 0.05 ± 0.09 to 0.11 ± 0.15), using SNP data, this according to Amin et al. (2007), together with missing heritability could be the reason for lower heritability using the kinship matrix. Patterns of LD are strongly linked to MAF, i.e. on average, the signals from low-frequency variants are less replicated than those from high-frequency variants. Minor allelic frequencies less that 0.05 were less than 5 % of the SNPs; hence this explains why generally there were higher heritabilities for the traits. This supports reports by Vinkhuyzen et al. (2013), where the authors explained that heritability will be too low for traits with predominantly low frequency causal variants and too high for those with predominantly high-frequency causal variants. This has

consequences when one performs genomic partitioning to investigate the frequency spectrum of causal variants for complex traits. According to Yang et al. (2011), a transformation that involves uniformly scaling the usual SNP-based kinship coefficients can improve the detection of causal variants.

Population clusters reported in this study were Chipinge, Binga, Shurugwi; Matobo and Tsholotsho. Indicator traits for GIN infections SNPs were not being detected for the respective clusters. Differences were being observed within a cluster of Matobo and Tsholotsho, where similar SNP associations could be expected. Results from the single-SNP association studies indicated six SNPs on four regions associated with *Eimeria* (chr. 4 and 25) and Strongyles resistance, on (chr. 4) (Table 6.3). Annotation of genes was conducted in the National Center for Biotechnology Information (NCBI) website. Genes annotated to be associated with *Eimeria* include the recognition complex subunit 5 (*ORC5*), which is involved in the initiation DNA replication together with mating type transcriptional silencing and the *RELN* gene which is involved in cell-cell interactions critical for cell positioning and neuronal migration during brain development. Genes associated with Strongyles resistance is the Sodium leak channel, non-selective (*NALCN*) which is responsible for the neuronal background sodium leak conductance (http://www.genecards.org/). These genes have not been implicated elsewhere for GIN resistance.

The single-SNP genome-wide analyses identified some SNPs for *Strongyles* (chr 12), which were not identified by the within-population genome-wide analyses, together with markers on chromosome 12 and 17 being uncharacterised. On the other hand, several other significant SNPs were identified by within-population genome-wide analyses especially for PCV. This supports the polygenic pattern of inheritance for GIN traits. The minimal number of SNPs

detected using the mmscore function could be explained by low levels of LD as well as low levels of F_{ST} reported. LD ranging from 0.05 ± 0.09 to 0.11 ± 0.15 and F_{ST} values ranging from 0.01 - 0.04, were reported, thus this could have an impact on the SNPs detected.

Different studies have utilized different thresholds of LD as being sufficient to achieve accuracy in genomic selection, these are $r^2 > 0.2$ in goats by Meuwissen et al. (2001), $r^2 > 0.33$ in humans by Ardlie et al. (2002) and $r^2 > 0.25$ in cattle by Qanbari et al. (2010). Low detection of SNPs could also be due low samples sizes that we used in this study, of 246 animals. Most genomewide association studies that have detected significant results using SNP-based markers had samples size of at least 2000 animals, for example, studies by Kijas et al. (2012) and Martin et al. (2016). An exception was the study by Kijas et al. (2013) where 182 goats were used for the analysis and significant results were obtained, this could be attributed to a very well defined phenotype (horn/polledness) and defined breeds. According to Kemper et al. (2011) to increase power of detection for SNPs associate to parasite resistance (Martin et al., 2016), the number markers need to be increased i.e. using a denser SNP marker panels therefore increasing LD between the marker and the polymorphism, or by increasing the number of observations for a trait, thus reducing the relative size of the experimental error.

Novel association results would be expected because some of the previous efforts have not used indigenous breeds for QTL detection. These breeds have undergone adaptation to tropical environmental conditions with much less exposure to artificial selection for production. As a consequence, indigenous animals might have developed mechanisms to tackle heavy parasite burdens over time as compared to temperate breeds. This supports results from this study, where majority of the animals had low intensities of infection despite the minimal treatment or parasite control in these farming systems.

For the case-control within-population, GWAS the most significant SNP across all parasite traits was in the Shurugwi population and the region identified was on chromosome 29 (snp45158-scaffold615-95117, P = 9.93 x 10^{-06}) for *Strongyles* and *Eimeria* infections, also for loads of infection of both parasites. The gene positioned at this location is the *HRAS* Like Suppressor Family Member 5 (*HRASLS5*), among its related pathways are phospholipases and it is also involved in transferase activity i.e. transferring acyl groups (www.ncbi.nlm.nih.gov/gene). The other common SNP positioned on chr 4 for both *Strongyles* and *Eimeria* infections was had several SNP markers (P = 1.45 x 10-05). The gene positioned at this location is the diacylglycerol kinase beta (*DGKB*), involved in glycerolipid, glycerophospholipid and phosphatidylinositol metabolic pathways. The other significant SNPs identified were for *Strongyles* in different populations. These were located on chr 14 (*NCLAD*), chr 17 (*ATXN2*) and chr 26 from Matobo and on chr 1 from Binga, with the last two being uncharacterized. Ten regions on six different chromosomes were found to be associated with PCV, however three of these were annotated (chr. 4, 17 and 28). The Thrombospondin type 1 domain containing 7A (*THSD7A*) gene on chromosome 4, codes for protein found in endothelial cells from placenta and umbilical cord, this protein interacts with alpha V beta 3 integrin and paxillin to inhibit endothelial cell migration and tube formation. The Pecanex Homolog 2 (*PCNX2*) gene on chromosome 28 plays a role in tumorigenesis of colorectal carcinomas with high microsatellite instability. Nuclear receptor subfamily 3 group C member 2 (*NR3C2*) gene on chromosome 17 encodes the mineralocorticoid receptor, which mediates aldosterone actions on salt and water balance within restricted target cells. These genes have not been EPO elsewhere for GIN resistance.

Among the regions (chr 22, 23 and 26) associated with GIN in goats reported in other studies (Bolormaa et al., 2010b; de la Chevrotière et al., 2012a). Chromosome 23 (locations 15 and 30 Mb) was reported for association with *Eimeira* in Chipinge populations. The regions identified corresponded to the cyclin D3 (*CCND3*) gene and dyslexia-associated protein (*KIAA03*). Their roles are specific to cell cycle regulation and pathways involved development of the cerebral cortex by regulating neuronal migration and cell adhesion respectively. However, a region between the two genes, houses the HLA class II histocompatibility antigen DR alpha chain (*LOC102189356*). According to Amills et al. (1998) and Ackerman and Cresswell (2004), the major histocompatibility complex (MHC) class II genes encode proteins that present processed peptides derived from extracellular antigens to helper T cells bearing the $CD4^+$ differentiation marker. An example of its function in GIN includes eliciting the development of an appropriate immune response towards trichostrongyliasis (Amills, 2014).

According to Benavides et al. (2015), differences in results obtained from studies are due to the nature of immune response, different experimental approaches, infection protocols (natural *vs*. artificial), GIN species, phenotypic traits measured, environment with which animals were raised (tropical *vs*. temperate; dry *vs*. humid climates) and statistical methods for analyses. Comparison across studies are also not easy due to that livestock populations are characterised by high levels of relatedness, that is, closely related animals with a complex population structure and an a priori unbalanced distribution of allele frequencies. This is even more challenging in this study, where animal were reared under extensive systems and animals bred indiscriminately in communal grazing area. Insight could be taken from sheep genetic studies and translated to goat studies.

In this study, of the top SNPs identified, none of them were found on genes implicated with resistance. The findings in the study made it difficult to ascertain the mechanisms giving rise to resistance in the goat populations in this study. Genes which are implicated to be associated with genetic resistance to GIN include those involved in wound healing, mounting the Th_2 immune response against GIN infections, those that are involved in MHC-mediated antigen processing and presentation among others (see review of sheep by Benavides et al. (2016)). There are generally limited studies which focused on identification of regions associated with GIN resistance in goats, with which to compare with, as there are in sheep. This makes it difficult to understand the mechanism underlying genetic resistance in goats; hence insights are obtained in sheep genetic studies.

6.7 Conclusion

Genetic parameters were estimated, indicating that parasite resistance is a heritable trait which can be included in breeding programs. Regions on chromosome 4 and 29 were identified as the top SNPs associated with parasite resistance, however, regions on chr 23 that were detected, were found close to the MHC class II gene at located at 24Mb. Conducting genome-wide association within populations identified several regions associated with GIN resistance, thereby confirming the polygenic nature of GIN resistance.

6.8 References

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Chapter 7

7 General discussion, conclusions and recommendations

7.1 General discussion

Goat production in the communal areas represents the most extensive type of farming (low input/output). In these farming systems, goats are an important source of livelihood, supplementing income in mixed crop-livestock systems, especially in the tropics and sub-tropics. Goats have an ability to survive and maintain condition in harsh environment; however, they are susceptible to gastrointestinal parasite (GIN) infections. Control of these parasites mainly revolves around the use of anthelmintics. In tropical developing countries anthelmintics are often unavailable or too costly for smallholder farmers. According to Baker (1999), breeding tropical goats for enhanced resistance to nematode parasites should lead to sustained improvements in animal health and performance. Different methods of parasite control have been reviewed in Chapter 2.

The objective of the study involved identifying SNPs that are associated with GIN and determine the mechanisms in which goats have developed the ability to fight infection in an environment where parasite control is practiced at a very low scale. A few experiments have been conducted in genetic GIN resistance to infection in goats (Mandonnet et al., 2001; Walkden-Brown et al., 2008; Estrada Reyes et al., 2016). Despite the need for more studies of goat-GI nematode interactions, limited studies exist. Mechanisms of resistance in goats are suggested to differ from those in sheep (Pomroy et al., 1986; Jallow et al., 1994). Of the four distinctive manifestations of host acquired resistance to GIN in sheep, namely poor establishment of infective larvae (L3), reduced worm development and growth, reduced worm fecundity and accelerated/rapid worm rejection, only the last two are believed to be expressed by goat breeds (Chiejina and Behnke, 2011).

Indigenous goats in Zimbabwe have no definite genetic structure, such that it was difficult to infer the breeds owned by farmers. In chapter 5, the Illumina Goat 50 k beadchip was utilized to assess population genetic structure; this information was then used in the genome-wide association analyses in chapter 6. There were high levels of genetic diversity, low levels of inbreeding across populations and low levels of population differentiation. Population structure analyses using both PCA and ADMIXTURE indicated that the research animals were in 5 clusters, and individuals in these clusters had common genetic components, thus, making it difficult to infer the breeds of these animals. The extent of genetic diversity and population substructure at polymorphic loci are critical for genotype-phenotype association studies (Periasamy et al., 2014), such as gastrointestinal parasite resistance. Population stratification has been demonstrated to result in false-positive associations in various species including humans (Helgason et al., 2005) and cattle (Zenger et al., 2007; McKay et al., 2008).

There were low levels of linkage disequilibrium reported in this study, however this made this difficult to detect genetic difference between populations in terms of resistance to parasites as well as identify loci which are associated with parasite resistance. Although several different ‘‘significant’’ SNP markers were identified using the two association approaches, none of the markers were associated to most candidate genes (those involved in innate and adaptive immune pathways) associated with parasite resistance as reviewed by (Benavides et al., 2016). Studies by Bolormaa et al. (2010b) and de la Chevrotière et al. (2012a) using microsatellites identified chr 23 to be associated with GIN resistance. One of the main determinants of the immune response elicited against pathogens is the major histocompatibility complex (MHC), which is located on chr 23, for example, the development of an appropriate immune response to trichostrongyliasis (Amills, 2014).

Using the single-SNP association, a few regions were identified to be associated with *Eimeria*, *Strongyles* resistance and PCV as compared to the within-population genome-wide analysis. According to Muchadeyi (2016, personal communication) and Hayward (2013), it is not ideal to perform genome-wide analysis in extensively raised populations due to poorly defined population structures, mixed infections and low sample sizes, hence in this study we conducted within-population analyses as an alternative method. The genetically fragmented nature of goat populations/ecotypes makes it challenging to use results on anything other than the population in which they are derived (Zvinorova et al., 2016b). In general, it is not possible to extrapolate results obtained across distantly related populations. Due to small sample size, associations with candidate genes should be treated as an indication and certainly require further research to be validated.

The use of genetic markers will make a large contribution to breeding programmes, but for low input/output farming systems, several challenges can hinder progress. In addition to that, conducting GWAS in low-input/output small-holder systems is difficult due to the requirements needed and also genotyping costs. However, nucleus breeding schemes can be incorporated in the farming systems to overcome costs as well as incorporate appropriate phenotyping and record keeping.

7.2 Conclusions

The study determined the management of GIN in low-input/output farming systems, level of prevalence, population genetic structure and biomarkers associated with GIN resistance. Low levels of parasitic control and level of knowledge warrant the need for farmers to be trained and increase their awareness. The utility of the Goat 50k SNP was demonstrated by its ability to estimate genetic parameters in populations which had no pedigree data, enable analyses of genetic diversity and population structure. There was low genetic differentiation among populations, low levels of

inbreeding and high genetic diversity. Evidence from the study indicated that goat populations shared ancestory, to a certain degree. The study was able to identify multiple SNPs that were associated with *Eimeria* and *Strongyles* at the genome-wide level. The identification of several loci in the association analyses supports the polygenic nature of GIN resistance, which explains that the traits are under the control of several genes, each having a small effect.

7.3 Recommendations

Further work in GIN resistance in goats reared under extensive systems is required to dissect the underlying genetic mechanisms. This can be explored by increasing the density of SNP markers as well as and increasing sample sizes. Futher work can be conducted in the identified genes to confirm or validate their role in mechanisms involved in eliciting immune responses towards GIN infections.

7.4 Research outputs and author contributions

7.4.1 Peer reviewed publications and manuscripts

i. **Zvinorova, P.I.**, Halimani, T.E., Muchadeyi, F.C, Matika, O., Riggio, V. and. Dzama, K. 2016. Prevalence and risk factors of gastrointestinal parasitic infections in goats in low-input low-output farming systems in Zimbabwe. Manuscript published Small ruminants Research 143: 75-83. This forms the basis of Chapter 4 and is entirely the work of Miss Zvinorova.

ii. **Zvinorova, P.I.**, Halimani, T.E., Muchadeyi, F.C., Matika, O., Riggio, V. and Dzama, K.. 2016. Breeding for resistance to gastrointestinal nematodes–the potential in low-input/output small ruminant production systems. Manuscript published in Veterinary Parasitology, 225: 19-28. This paper forms the basis of Chapter 2 and is entirely the work of Miss Zvinorova.

iii. **Zvinorova, P.I**., Halimani, T.E., Muchadeyi, F.C., Katsande, S., Gusha, J and Dzama, K.. Management and control of gastrointestinal nematodes in communal goat farms in Zimbabwe. Manuscript accepted for publication in the Tropical Animal Health and Production journal. DOI **:**10.1007/s11250-016-1200-9. This paper forms the basis of Chapter 3 and is entirely the work of Miss Zvinorova.

7.4.2 Conference outputs

i. **Zvinorova, P.I**., Halimani, T.E., Muchadeyi, F.C., Matika, O., Riggio, V. and Dzama, K., 2015. Seasonal prevalence and risk factors of gastrointestinal parasitic infections in goats in low-input low-output farming systems in Zimbabwe. 48th Congress of the South African Society for Animal Science, 3 - 6 September 2015, Durban, South Africa. This paper forms the basis of Chapter 4 and is entirely the work of Miss Zvinorova.

ii. **Zvinorova, P.I**., Halimani, T.E., Muchadeyi, F.C., Matika, O., Riggio, V. and Dzama, K., 2016. Preliminary genome-wide association study of gastrointestinal parasites resistance in Matabele goats in Zimbabwe. 49th Congress of the South African Society for Animal Science, 3-6 July 2016 (pp 49). Stellenbosch, South Africa. This paper forms the basis of Chapter 6 and is entirely the work of Miss Zvinorova.

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