Gastrointestinal (nematode) infections in small ruminants: Epidemiology, anthelmintic efficacy and the effect of wattle tannins

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General Abstract

Nematode parasites have become the biggest problem for small ruminant production in South Africa due to their resistance to the commercial anthelmintics. Notable, wattle tannin has been used as an alternative strategy for control. However, the concentration and the frequencies can likely influence its effect on the parasites control. The objective of this study was to determine the degree of pasture infestation and nematode infection in sheep and goats, as well as investigate nematode resistance to the anthelmintics, and the potential of wattle tannin in nematode control.

The first study dealt with the epidemiology of internal parasites. Eight Merino ewes and eight Nguni does averaged 7-18 months of age were observed for 1 year during the months of February 2008 to January 2009 at the University of KwaZulu-Natal Research Farm (Ukulinga). Egg count per gram (EPG) and coccidian oocysts per gram (OPG) were counted according to Mc Master Technique (Hansen & Perry, 1994) by magnifying parasitic eggs from monthly rectal faecal samples dissolved in saturated sodium chloride. Faecal samples also were cultured for 15 days to identify infective nematode larvae (L_3) using Baermann technique. Herbage samples were collected monthly from four paddocks as well to count L_3 on the pasture. Sheep live weight was also recorded monthly. Seasonal effects was significant ($P<0.05$) on the EPG, OPG, faecal culture L_3 and pasture L_3. A higher level of infection was observed in summer (wet) than in winter (dry season). *Trichostrongylus spp* larvae were the most prevalent larvae (26.5%) while *Strongyloides, Haemonchus contortus, Nematodirus* and *Cooperia spp* occurred in the faecal culture by percentage of 20.9%, 16%, 16% and 14.5%, respectively.

For parasite resistance, Ivermectin 1% (IVM), Closantel 5% (CST) and a combination of Abamectin 0.08% and Praziquantel 1.5% (CPA) were evaluated. Twenty four sheep (12 females and 12 males) aged between 7-18 months were used for 21 days. Animals were naturally infested by gastro-intestinal parasites. EPG and faecal culture L_3 were counted on day 0, 7, 14 and 21. Closantel was the most effective. *Haemonchus spp*. were least affected whilst *Trichostrongylus spp*. were the most affected by all drugs.

In the third study, wattle tannins were evaluated as an alternative nematode control drug. Three experiments (Exp.) were conducted to determine the effect of tannin concentration (Exp.1 and 2) and frequency (Exp.3) on nematode parasites. In Exp.1, 0, 0.8, 1.6 and 2.4 g tannin/kg BW were drenched for three consecutive days per sheep (16 females and 8 males, aged 8-9 months) for 21 day. In Exp.2, 30 sheep (14 males and 16 females, aged 9-18 months) were randomly allocated into three tannin treatments (0, 0.8 and 1.6 g tannin/kg BW) and drenched for a day. In Exp.3, 26 sheep (11 males and 15 females aged 9-18 months) were divided into three groups of 9, 9, and 8 sheep each. These groups were drenched with 1.6 g tannins/kg BW/day; once, twice or thrice for the 3 groups respectively. For the three experiments, EPG and L_3 larvae were counted in individual feacal samples. For all tannin treatments, EPG decreased ($P<0.05$) over time. Though the differences among tannin levels and frequencies varied ($P<0.05$) over time, EPG consistently decreased with increasing tannin level and frequency. Thus 1.6 and 2.4 g tannin /kg BW for 3 consecutive days had nearly similar effects on the EPG.

The results of this study are rather inconclusive that weather conditions such as rainfall had a direct effect on internal parasites development. Nematode parasites at Ukulinga Research Farm were resistant to the commercial anthelmintics used. Drenching with 1.6g wattle tannin/kg BW over three successive days is enough to reduce EPG and reduce the degree of pasture contamination.

Declaration

The research described in this thesis was carried out in the Discipline of Animal and Poultry Sciences, School of Agricultural Sciences and Agribusiness, Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg Campus, under the supervision of Prof. Ignatius V. Nsahlai.

This is to declare that this thesis is the result of my own investigation, except where acknowledged, and has not been presented in any previous application for a degree purpose.

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I, Prof. Ignatius V. Nsahlai the supervisor, approved the release of this thesis for examination.

Signed…… Date…….

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Conference Abstracts

1. **Mawahib.A.A. Ahmed & Nsahlai I.V. (2008).** Effectiveness of anthelmintic drugs on gastrointestinal Nematode species in sheep. World Conference on Animal Production, Capetown, South Africa. 25/11/2008.

2. **Nsahlai I.V. & Ahmed M.A.A. (2008).** Wattle tannins have the potential to control gastrointestinal nematodes in sheep. World Conference on Animal Production, Capetown, South Africa. 24/11/2008.

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Abbreviations

Ak= Acacia Karoo

BW = live body weight

CPA = Combination Abamectin and Praziquantel

CST = Closantel

CT = condensed tannins

DM = dry matter

EPG = eggs per gram

FEC = Faecal egg count

GIN = gastrointestinal nematodes

GLM = General Liner Model

HT = hydrolysable tannins

IVM = Ivermectin

kg = kilogram

L_3 = infective larvae

ml = millilitre

OPG= oocysts per gram of faeces

PCV= packed cell volume

SCC = somatic cell counts

SED = Standard Error Deviation

spp. = species

VDMI = voluntary dry matter intake

WT = wattle tannins

Chapter 1

General Introduction

1.0 Background

Gastro-intestinal nematode parasitic infection is one of the major health problems in the world. Gillian *et al*. (2004) reported that nematode infections affect the health of millions of people and animals, causing huge economic loss in livestock farming. Previously, Mulugeta *et al.* (1989) reported that infection is enormous in small domestic ruminants, causing major production loss.

Nematode infection is rampant in most developing countries where poor pastures and the quantities of nutritious food consumed do not cover the nutritional requirements of animals (Leng, 1991). In addition, there is insufficient veterinary care and the environment is conducive to nematode growth and transmission (Fikru *et al.*, 2006). Nematode infection is a serious veterinary health concern in South Africa as well (Van Wyk *et al.*, 1999). The problem is manifested especially in small ruminants (sheep and goats). The consequences of nematode infection include: reduced feed intake and weight gain, reduced immunity, lower fertility, a reduction in milk production and work capacity, treatment expenses and death in critical infections (Fikru *et al.,* 2006; Hale, 2006).

However, determination of the degree of nematode infection depends mainly upon the age of the host, the breed, the parasite species involved, and the epidemiological patterns which include husbandry practices and physiological status of the animals (Tembely *et al.*, 1997). More importantly, environmental conditions such as temperature, rainfall and humidity are conducive to the development of nematode eggs (Menkir *et al.,* 2006) and free living stages (Tembely *et al.*, 1997).

Currently, the primary control strategy for nematode infections is the use of chemical treatments (Waller, 1994; Pomroy *et al.,* 2002; Crawford *et al.,* 2006). Three classes of modern synthetic anthelmintics are known, each having a different mode of action (Gillian

et al., 2004), but the control strategies are still insufficient because of the potential resistance to the drugs.

Thus, alternative methods for control of gastrointestinal nematodes need to be developed. Knowledge of the seasonal population trends, nematode life cycle and the prevalence of larvae in sheep and goats is necessary for the developing of control programs (Menkir *et al.,* 2006). Several attempts at controlling nematodes through the use of forages containing condensed tannins have been made. Condensed tannins have anthelmintic effects and could be used to control gastrointestinal nematodes (Niezen *et al.,* 1995; Molan *et al.,* 2000).

The main objectives of this study were to:

(i) Investigate the degree of pasture infestation during the winter and summer months.
(ii) Quantify the degree of nematode infection in sheep and goats in an intensive production system.
(iii) Investigate the gastrointestinal nematode resistance to standard anthelmintics.
(iv) Investigate the potential of tannin-rich feeds in the control of gastrointestinal nematodes.

Chapter 2

Literature review

2.0 Helminths

The name ‘‘helminth” is derived from the Greek words ‘‘helmins” or ‘‘helminthos”, meaning a worm, and is usually applied only to the parasitic and non-parasitic species belonging to the phylum *Platyhelminthes* (such as flukes and tapeworms) and *Nemathelminthes* (roundworms and their relatives). The *Annelida* (earth worms, leeches) are basically different from both the *Platyhelminthes* and *Nemathelminthes* and are not regarded as helminthes, though some (e.g. leeches) may be parasitic and others (e.g. earthworms) may serve as intermediate hosts for helminths (Soulsby, 1982). The main parasites that influence livestock farms are the *Nemathelminthes* which include many superfamilies of veterinary importance. These are *Trichostrongyloidea, Strongyloidea, Metastrongyloidea, Ancylostomatoidea, Rhabditoidea, Trichuroidea, Filarioidea, Oxyuroidea, Ascaridoidea and Spiruroidea* (Sissay, 2007). However, in small ruminants, gastrointestinal nematodes are important members of the order *Strongylida*. This contains *Trichostrongyloidea, Strongyloidea, Metastrongyloidea* and *Ancylostomatoidea*, but most of them belong to the superfamily *Trichostrongyloidea*. Small ruminants are infected with a group of these *strongylid* nematodes, causing clinical effects known as parasitic gastroenteritis (Zajac, 2006). The characteristics of nematodes life cycle and control are discussed below.

2.1 Nematodes (roundworms)

In general, the nematodes are the most numerous animals on earth (Smyth, 1962; Jasmer *et al.,* 2003). Nematodes make up a large assemblage of worms of relatively simple structure with a widespread distribution, their cylindrical, non-segmented bodies distinguishing them easily from other helminthes. They occur in fresh water, in the sea, in the soil, and are amongst the most successful parasites of plants and animals (Smyth, 1962). Concerning the morphology of nematodes, the body is elongated, cylindrical and tapered at the extremities. The body is also unsegmented and covered with cuticle which is thick and continuous with the cuticular lining of the buccal cavity, the oesophagus, the rectum and the distal portions of the genital ducts (Jacobs *et al*., 2003).

Roundworms consist of an external membrane, a cortex, a matrix and fibre stratum (Soulsby, 1982), divisible into nine distinct layers. The cuticle consists of an underlying sub-cuticle layer, the hypodermis, which consists of cells in the free-living form and a syncytium containing a number of nuclei in the parasitic forms. This layer has the longitudinal lines located, dorsally, ventrally and laterally. The lateral line contains the longitudinal canals of the excretory system (Figure 2.1).

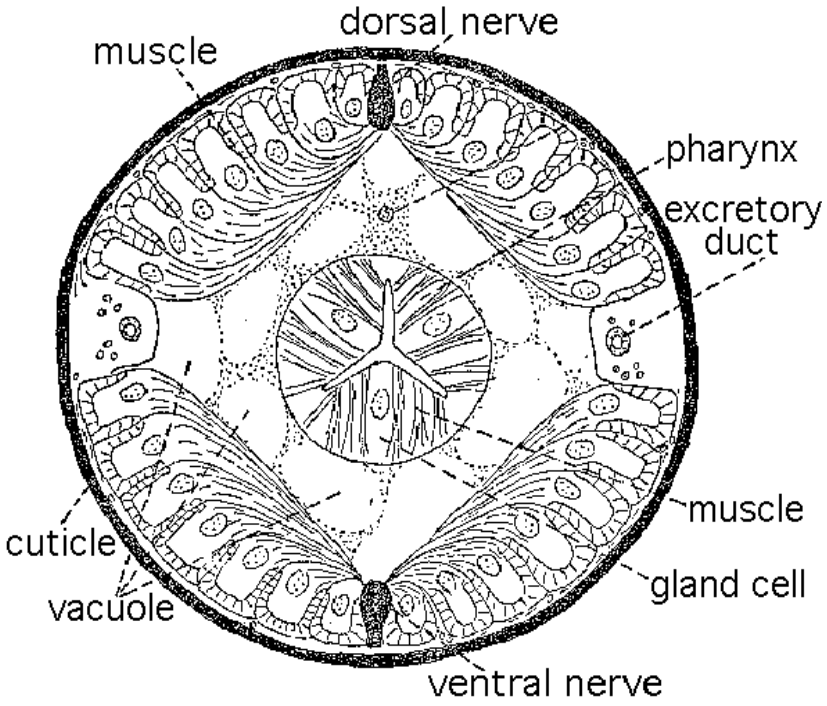

Figure 2.1 Internal anatomy of nematode. http://www.ucmp.berkeley.edu

The muscular layer which follows next and lines the body cavity consists of a number of cells having a basal contractile portion which is transversely striated and a cytoplasmic portion which contains the nucleus and is connected to the nerve trunks running in the dorsal or ventral line.

The mouth is anterior and usually surrounded by three lips, one dorsal and two ventral. In other forms there are only two lips or lips may disappear completely (*Strongyloides*). The mouth may lead into a buccal capsule which has thick cuticular walls and may contain

special teeth, or into the pharynx, which is usually cylindrical and surrounded by muscular tissue, or directly into the oesophagus.

The oesophagus of nematode parasites shows variations in structure that are used for the classification of species (Sandground, 1925). The oesophagus is a strongly muscular organ lined with a cuticle which divides the wall into one dorsal and two ventral sectors. The wall of the oesophagus contains three oesophagus glands, which secrete digestive enzymes. The intestine is a simple tube with a non-muscular wall composed of a single layer of columnar cells standing on a basal membrane. It leads to the rectum which is lined with cuticle and to the genital duct ending with a cloaca. The part of the body behind the anal or cloacal opening is called the tail (Soulsby, 1982).

The life cycle of the nematode (Figure 2.2) may be direct or include an intermediate host. The sexes are usually separated (male and female). However, all of the economically important *strongylid* (gastrointestinal) parasites of small ruminants have direct life cycles, requiring no intermediate hosts (Sissay, 2007). The mature parasites (worms) breed inside the host and lay eggs which pass through the host and are shed in the faeces. After the eggs pass out of the host, they hatch into first-stage larvae (L_1) and moult into second-stage larvae (L_2) under appropriate conditions of temperature and humidity. The larvae need moisture to develop and move. During this time the larvae feed on bacteria. (L_2) moult into infective larvae (L_3), which migrate out of the faeces and up blades of grass. When an animal (sheep or goat) grazes, they may ingest parasite larvae along with the grass. Normally L_3 can moult into fourth-stage larvae (L_4) within 2-3 days, remaining for further 10-14 days to moult into young adult parasites (Soulsby, 1982; Hale, 2006; Coffey *et al.*, 2007).

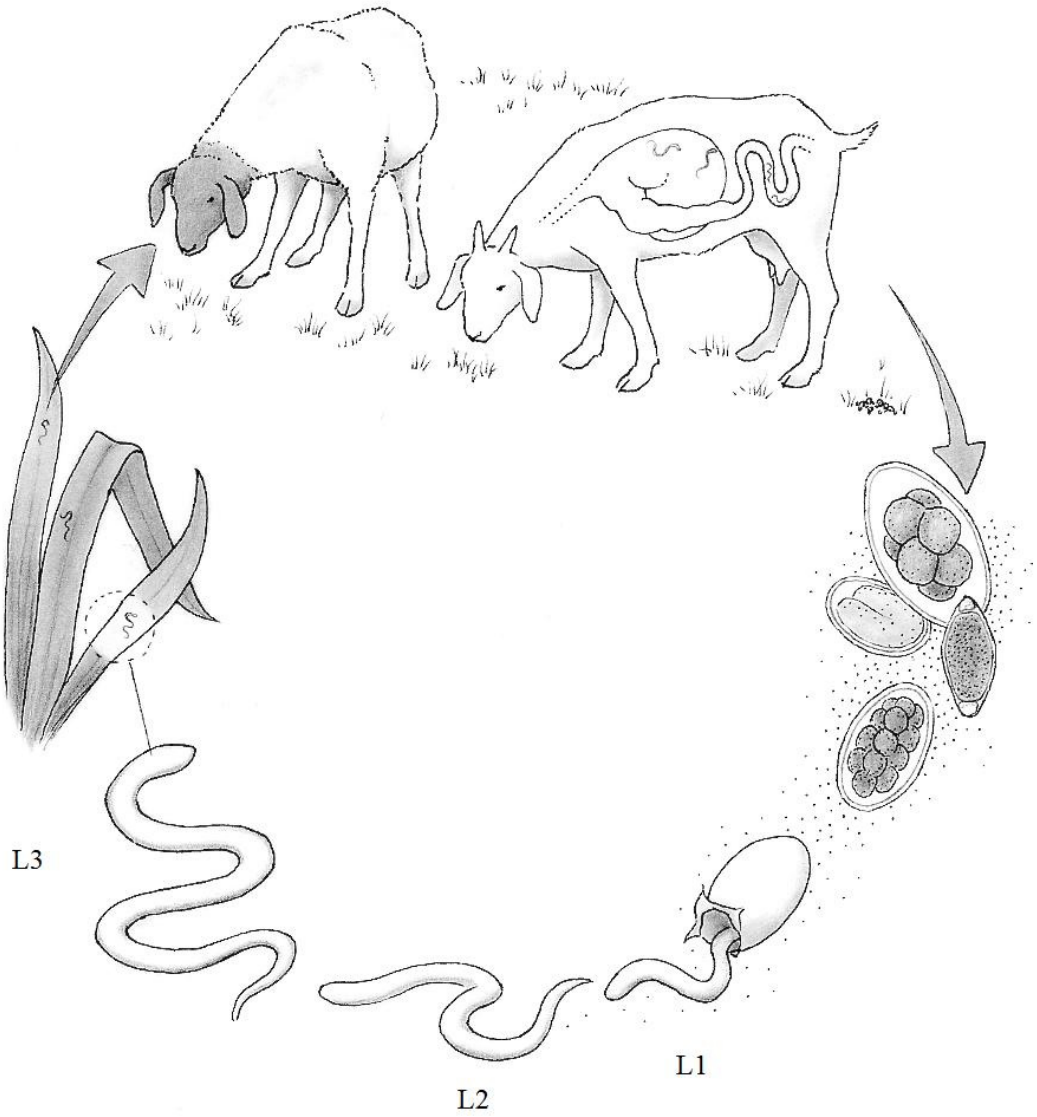

Figure 2.2 Principal life cycle of nematode parasites (after Mekonnen, 2007)
L_1, L_2 and L_3 stand for first, second and third larvae stage.

Parasite numbers rise with time when conditions are suitable and internal parasite burdens impact on the health and well-being of the animal when their numbers grow beyond what the animal can tolerate (Hale, 2006).

Nematodes are pathogenic parasites, causing disease in the host. Usually they live in the digestive system of the host. *Haemonchus contortus* attaches to the wall of the abomasum in sheep and goats, feeding on the host's blood, causing aneamia. Other nematodes usurp the nutrients eaten by the host, causing weight loss (Hale, 2006). *Teladorsagia*

circumcincta in the abomasum reduces feed intake. In the small intestine *Nematodirus battus* dehydrates the animal. *Trichostronglus colubriformis* and *Trichostronglus vitrinus* reduce feed efficiency in small ruminants. In cattle, *Haemonchus placei* in the abomasum causes anemia and *Ostertagia ostertagi* reduces feed intake. *Cooperia oncophora* and *Cooperia punctata* in the small intestine of the cattle reduce feed efficiency (Houdijk & Athanasiadou, 2003) and affect protein metabolism. Absorption and/or retention of minerals (especially phosphorus) are particularly affected (Robert & Kyriazakis, 2001). The effects of nematode parasites on the host are manifest as a loss of condition, rough hair coat, scours, diarrhoea, bottle jaw, pale mucous membranes (eyelids, gums), anaemia and death (Hale, 2006).

Holmes (1993) reported that the nematode mode of action results in the loss of considerable quantities of host protein into the nematode tract; the proteins represent plasma and frequently erythrocytes, exfoliated epithelial cells and mucus. Furthermore, Parkins & Holmes (1989) accurately reported losses of blood proteins into nematode tract using radioisotopic technigues to be about 10 % of the total blood volume per day. This is sufficient to explain the pathophysiology of nematode infections. The observation reported the infections are usually associated with hypoalbuminaemia and, in some cases, with anaemia. Nematode parasites also affect the feed digestion, energy and nitrogen utilization in the parasitized animal and reduce the performance of the host and feed intake (Holmes *et al.*, 1986). Feed intake reduction is a primary effect of parasitism. However, the basic reason(s) for that is not clear yet (Parkins & Holmes, 1989). A number of suggestions have proposed that abdominal pain from local damage at the site of infection may be responsible (Fox, 1997). Changes in pH of the gut content (Simcock *et al*., 2006)**, c**hanges in flow rate of digesta, changes in protein to energy ratio of absorbed nutrients (Holmes, 1993; Parkins & Holmes, 1989) as well as changes in secretion of the gastrointestinal hormones cholecystokinin and gastrin (Fox, 1997). There are many ways used recently to control nematode parasites in animals via chemical drugs and non chemical drugs.

2.3 Chemical drugs

The prophylactic treatment of nematode infection depends basically on the use of anthelmintics (Mickael *et al.*, 2003). Notable, the availability of safe, broad spectrum anthelmintics has helped to reduce the incidence of a great number of worm diseases (Kohler, 2001). In general, anthelmintic groups are greatly effective against the immature

and mature stages of virtually all of the important gastrointestinal nematodes as well as many extra intestinal helminth species (Kohler, 2001). The majority of modern anthelmintics exert their effects in three biochemical and physiological areas. Moreover, the known target sites are solely proteins and include ion channels, enzymes, structural proteins and transport molecules (Kohler, 2001). There are also a number of ways that the anthelmintics are administered, such as orally or injectable. The rotation of anthelmintics between the active groups is also purported to increase their efficacy, and even rotation between animal species has been reported to decrease resistance (e.g Barger, 1999).

The active ingredients represented by common anthelmintics in market place are:

- Benimidazoles group (Thiabendazoe, Mebendazole, Albendazole and Netobimine).
- Tetrhydropyrimidines (Pyrantel, Morantel).
- Salicylanilides (Colsantel).
- Imidazothiazoles (Levamisole).
- Macrocyclic lactones (Ivermectin, Moxidecttin).
- Piperazine.
- Triclabendazole.
- Praziquantel.
- Clorsulon.

Thiabendazole, in 1961, was the first successful product with lower toxicity for treatment of nematodes, but the benzimidazole anthelmintics represent the beginning of the modern chemical assault on helminth parasites (Kaplan, 2004), followed by the main anthelmintic groups Levamisole and Ivermectin. Earlier studies have shown that the anthelmintics act in different ways.

2.3.1 Benzimidazoles

Benzimidazole affects microtubules of the worm (Kohler, 2001), by binding to B-tubulin, inhibiting the polymerization of tubulin and the formation of microtubules. The lack of microtubules inhibits many cellular functions such as transport, cell division, neural transmission, and cell differentiation, ultimately leading to cell death in the worm (Prichard, 2005).

2.3.2 Imidazothiazoles

Imidazothiazoles are nicotinic drugs. They affect the nervous system of the worm; consequently, they cause sustained muscle contraction, leading to paralysis in nematodes and other parasites. Levamisole, tetrahydropyrimidines (e.g. pyrantel and morantael) and some other structurally related compounds are the main nicotinic drugs (Kohler, 2001).

2.3.3 Ivermectin

The Avermectins/milbemycins bind to glutamate and gamma-amino butyric acid (GABA)-gated causing a hyper-polarization of nerve or muscle cells, leading to paralysis and killing the parasite (Prichard, 2001).

2.3.4 Praziquantel

The action of Praziquantel is linked to an induction of calcium flux across the tegumental membrane (Prichard *et al.*, 1982) which increases tegumental calcium levels causing muscle contraction in the parasite body.

2.3.5 Closantel

Closantel has the ability to interfere with the proton gradient in the parasite mitochondria, which in turn inhibits the generation of ATP by the parasite (Van den Bossche, 1985). Recent results using nuclear magnetic resonance (^{31}P-NMR) to measure effect of closantel on fluke intrategumental pH, suggest that closantel is a membrane-active molecule that is capable of affecting a number of helminth biochemical and physiological processes (Pax & Bennett, 1989).

2.3.6 Other drugs

Exclusion of oxamniquine and related compounds, the target side and action mechanisms of other anthelmintics have been less extensively investigated compare to those discussed above (Kohler, 2001). However, nematode resistance against anthelmintics has developed and has become a problem in the control of nematodes. The use of the above chemicals independently or in combination has led to a reduction in the ability of the chemicals to reduce the parasites burden in a number of species. This resistance to the chemical drugs is discussed below, and coping strategies are reported.

2.3.7 Drug resistance

Drug resistance is the ability of worms in a population to survive drug treatment that used to be effective against the same species and stage of infection at the same dose rate (Kaplan, 2004; Coffey *et al.,* 2007). The most common anthelmintic resistance problem has occurred in gastro-intestinal nematodes of ruminants and horses (Prichard, 2005). European Medicines Agency (2006) indicated that resistance to anthelmintics is a growing problem in sheep, goats and horses worldwide and is a rising problem in cattle, but information on anthelmintic resistance in other animal species is currently more limited. In South Africa, surveys on 59 farms of mainly sheep indicated that 90% of the sheep farms in South Africa that are resistant to drugs from at least one of the anthelmintic groups; 40% of these farms show multiple resistance to compounds from the anthelmintic groups (Van Wyk & Van der Merwe, 1993).

The main factor affecting the rate of gastrointestinal nematode resistance to anthelmintics is host nutrition. A number of studies have addressed the relationship between the nutritional status of the host and its ability to regulate a parasite infection (Robert & Kyriazakis, 2001). Also the age, breed and the productive state of the animal could influence resistance development. Generally, anthelmintic resistance develops due to the extent of genetic polymorphism in the population, the initial frequency of resistance-contributing alleles, the number of genes involved and complexity of the resistance mechanisms, the biology of the nematode, whether the resistance gene(s) are dominant or recessive in expressing the resistance phenotype, treatment coverage, the relative reproductive fitness of the wild-type (susceptible) and resistant genotype in the absence and presence of the anthelmintic, treatment frequency, other management practices which may impact on parasite transmission and drug dosage (Prichard, 2005).

The mechanism of anthelmintic resistance is not completely understood. Drug resistance can happen in a limited number of ways (Wolstenholme *et al.,* 2004). There may be a change in the molecular target, so that the drug no longer recognizes the target or a change in metabolism that inactivates or removes the drug or that prevents its activation, or a change in the distribution of the drug in the target. Drug resistance research has revealed the importance of appropriate target identification for efficacy of the drug (Table 2.1).

Table 2.1 The mechanisms of anthelmintic resistance (Kohler, 2001).

Drug	Mechanisms
Benzimidazoles	Altered target (structure of B-tubulin isotypes)
Nicotinic agonists	Altered target (structure and/or arrangement of nAChR* subunits)
Macrocyclic Lactones	Altered target (structure of GluCl* channel & subunits), overexpressed target? (P-glycoprotein mediated increased drug efflux
Oxamniquine	Deficiency in drug-activating enzyme (sulfotransferrase)
Praziquantel	Unknown
Salicylanilides	Unknown
Clorsulon	Unknown
Triclabendazole	Unknown

* nAChR stands for nicotinic acetylcholine receptors.
* GluCl stands for Glutamate-gated chloride channels.

Nematode resistance has negative impact on small ruminant production. Also many small holders do not have money for increasingly expensive medicines that do not seem to work. So other means of nematode control have been explored such as biological control, which is discussed below.

2.4 Non chemical drugs

Natural or biological ways of controlling nematodes provide alternatives to chemical means of control. These include tannin rich feeds, use of fungi, use of bacteria, nutrient supplementation of the diet, use of copper wire particles, also use of plants with anthelmintic properties have been considered.

2.4.1 Fungal candidates

Nematophagous fungi were described as alternative mean to reduce pasture contamination and therefore reduce the parasite infection (Larsen, 1998; Epe *et al*., 2009)**.** A number of microfungal species are able to attract and kill the developing larval stages of parasitic nematodes in a faecal environment (Epe *et al*., 2009). However, Larsen (1998) reported that *Duddingtonia flagras* was the only fungus that able to consistently and significantly reduce the numbers of infective *Trichostrongyle* larvae and most of the economically important gastrointestinal parasites in faeces from animals feed fungal spores. The whole philosophy of using *Duddingtonia flagras* against nematode parasites is to reduce the L_3 numbers available to be picked up by grazing animals (Chandrawathani *et al.,* 2004). This reduction in L_3 stages on herbage will subsequently prevent the build-up of worm burdens in hosts. The mechanism of action of *Duddingtonia flagras* has been investigated. Larsen *et al.* (1995) suggested that the chlamydospores of this fungus germinate in faeces, forming specialized, three-dimensional networks that could trap the parasite larvae. A fungal dose of 5g of grain/sheep per day for 2 consecutive days was sufficient to reduce larval numbers from faecal culture (Waller *et al.,* 2001).

2.4.2 Use of bacteria

There have been serious attempts at using bacteria. In particular is the use of *Bacillus thuringiensis*. Previous studies showed that strains of *Bacillus thuringiensis* could probably control endoparasitic nematodes and ectoparasitic arthropods of sheep and cattle (Pinnock, 1994). Other researchers confirmed that *Bacillus thuringiensis* toxin could affect free-living stages of some parasitic nematodes (Bone *et al.,* 1987) and trematode cercariae (Horak *et al.*, 1996). Calcium can affect the bacteria activity and microbial toxin, so some change on eggs permeability or ion flux may be involved in toxicity of the bacteria (Bone *et al.,* 1987). In addition, Wei *et al.* (2003) demonstrated the toxicity of *Bacillus thuringiensis* on different free-living nematode species. The result indicated that *Bacillus thuringiensis* is active against multiple nematode species, toxicity in nematodes correlates with damage in the intestine. That puts *Bacillus thuringiensis* in a good order of alternative strategies for controlling nematode parasites.

2.4.3 Use of natural plant extracts

Preliminary investigations were carried out to determine the use of neem *(Azadirachta indica)* extract as an alternative anthelmintic drug in small ruminants. Results suggest that

neem could be successfully used as an alternative to commercial anthelmintics (Thomas *et al.,* no date). Also, plant cysteine proteinases from the fruits or latex of plants such as papaya, pineapple and fig have high proteolytic activities that are known to digest nematode cuticles, especially in humans and small ruminants (Gillian *et al.,* 2004). In addition, plants such as chicory, plantain, and wormwood have anthelmintic effects on the internal parasites, although wormwood produces toxic compounds (Hale, 2006).

2.4.4 Copper wire particles

Some recent researchers have used the copper wire particles to control internal parasites. Studies have shown copper oxide to reduce parasite load in lambs while high doses may increase the toxicity of copper oxide in sheep, however, copper treatments do not appear effective in mature sheep (Burke *et al.,* 2005). To estimate safety level of copper oxide wire in controlling nematode, study by Burke *et al.* (2007) observed that a dose of copper wire less than 0.5g/kg W was considered best possible to reduce the risk of copper toxicity. It was effective to reduce EPG in young goats, while 5g/kg W of copper oxide was effective in older goats. An important observation of this author was that packed cell volume (PCV) is equal in treated and untreated goats. This could mean copper oxide does not appear to be effective in controlling L_4 stage (preadult) larvae, which also feed on blood, leading to decreased PCV in infected goats.

2.4.5 Tannins

Tannins are defined as phenolic compounds of high molecular weight ranging from 500 to more than 2500 bound to proteins that form insoluble or soluble tannin-protein complexes. They have been closely associated with plant defence mechanisms towards insect and mammalian herbivores (Hagerman & Buther, 1991). Tannins are soluble in water with the exception of some higher molecular weight structures. Oligomeric compounds with multiple structure units with free phenolic groups can complex with proteins, starch, cellulose and minerals, and tannins are distributed in many plant parts like wood, fruit, leaves, barks and roots located basically in the tissues in the vacuoles. In the plant kingdom tannins are found in both flowering plants and non-flowering plants. They are found in many plant species such as Acacia *spp, Sericea lespedeza* as well as pasture species such as *Lotus spp*. Tannins are divided according to their chemical structure and properties, into two main groups: hydrolysable (HT) and condensed tannins (CT) (Athanasiadou *et al.,*

2001a). The characteristics of the two groups produce a different effect on the ruminant when ingested.

Hydrolysable tannins (gallotannins, ellagitannins) are molecules which contain a carbohydrate, generally D-glucose, as a central core (Min & Hart, 2003). The hydrolysable groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid (Haslem, 1989). Hydrolysable tannins are usually found in lower concentrations in plants than condensed tannins. Hydrolysable tannins are subdivided into taragallotannins (gallic and quinic acid) and caffetannins (caffeic and quinic acid). They are hydrolyzed by tanninase enzymes which engage in ester bond hydrolysis. Hydrolysable tannins can form compounds such as pyrogallol which is toxic to ruminants. Toxic compounds from more than 20% HT in the diet can cause liver necrosis, kidney damage with proximal tuberal necrosis, lesions associated with hemorrhagic gastroenteritis and high mortality, which were observed in sheep and cattle (Reed, 1995). Hydrolysable tannins can also affect monogastrics by reducing growth rates, protein utilization and causing damage to the mucosa of the digestive tract and increasing the excretion of protein and amino acids. Quantities of HT (3-7%) in poultry diet can cause depression in growth, lower egg production and death.

Condensed tannins (CT or Proanthocyanidins), on the other hand are the most common type of tannins found in forage legumes, trees and stems (Barry & McNabb, 1999). Condensed tannins are widely distributed in legume pasture species such as *Lotus corniculatus* and in several kinds of acacia and other plant species (Degen *et al.,* 1995). Condensed tannins have a variety of chemical structures affecting their physical and biological properties (Min *et al.,* 2003). Condensed tannins consist of flavanoid units (flavan-3-ol) linked by carbon-carbon bonds. The complexity of CT depends on the flavanoid units which vary among constituents and within sites for interflavan bond formation. The term proanthocyanidins (PAs) is derived from the acid-catalyzed oxidation reaction producing red anthocyanidins upon heating PAs in acidic alcohol solutions. Anthocyanidin pigment is responsible for the colors observed in flowers, leaves, fruits juices and wines. The astringent taste of some leaves, fruits and wines is due to the presence of tannin.

Generally, the effects of CT depend on its intake level and its structure (Min *et al.*, 2003). Low to moderate CT concentration may improve animal performance (Waghorn & Shelton, 1997; Athanasiadou *et al.*, 2001b; Min *et al.*, 2003). Levels of CT less than 50mg/kg BW in feed can reduce the risk of bloat; increase the uptake of essential amino acids and proteins; enhance the production of milk and wool; and be effective against gastrointestinal parasites (Athanasiadou *et al.*, 2001b). However, higher levels of CT could decrease voluntary feed intake, rumen digestibility of fiber and animal growth (Rojas *et al.*, 2006). Min *et al.* (2003) reported the concentration of CT of more than 55g CT/kg DM reduced voluntary dry matter intake, digestibility and depress rates of body weight gain. The concentration of CT in *Lotus corniculatus* diet can reduce rumen nitrogen digestibility, ammonia pool size and increase the flow of undegraded feed nitrogen to the abomasum (Min *et al.*, 2002), also CT from *Ficus infectoria* leaves at 1.5% level in the supplement could be used for improving the performance of lambs (Dey *et al.*, 2007). The mechanisms driving CT concentration dynamics are not well understood (Haring *et al.*, 2006). Condensed tannins have the ability to combine with soluble rumen proteins, reduce the degradation of protein to ammonia in the rumen and allow more dietary protein to flow to the small intestine (Min *et al.*, 2003).

The manner in which condensed tannins affect nematode parasites can be classified as direct or indirect. The direct effects of CT might be mediated through CT–nematode interactions affecting physiological functions of gastrointestinal parasites (Nguyen *et al.*, 2005). Condensed tannins can also react directly by interfering with egg hatching and development to infective stage larvae (Min & Hart, 2003). This reduces pasture contamination and infective larvae ingestion, which in itself might provide adequate control for gastrointestinal parasite. Condensed tannins also have the ability to bind with proteins and nematode walls making these inactive or killing them (Athanasiadou *et al.*, 2001a). The non-direct effects of CT appear to improve protein nutrition by binding to plant proteins in the rumen to prevent microbial degradation; this increases protein flow to the duodenum. Min & Hart (2003) have shown improved protein nutrition decreases parasite infection by enhancing host immunity. Therefore, CT might counteract parasites with one or more of the aforementioned mechanisms.

Knowledge about the degree of nematode infections depends on measuring faecal egg counts (FEC) as eggs per gram (EPG). Condensed tannins can reduce worm egg

production and inhibit larval development (Min *et al.*, 2005). Researchers have reported that lower FEC in sheep offered tannin supplemented feed, than in sheep offered the unsupplemented feeds. For example studies by Niezen *et al.* (2002 & 1998) have shown that EPG were lower in lambs grazing *Sulla*, than lambs grazing *Lucerne*; and the warm burden was also lower. Min *et al.* (2005) reported that goats grazing *Sericea lespedeza* had a lower EPG, reducing *Haemonchus contortus* by 89% and *Trichostrongylus* by 50%. Condensed tannins from Quebracho extract included in feed are responsible for a reduced parasitic burden in infected sheep (Athanasiadou *et al.*, 2000). On the other hand, a study by Kahiya *et al.* (2003) compared the effect of CT from *Acacia karoo* and *Acacia nilotica* included in the goat's diet by 40% dry matter. A. karoo decreased faecal egg counts and *Haemonchus contortus* worms while A. nilotica had no significant decreases observed. This may be related to type and concentration of tannins.

Condensed tannins also have positive nutritional effects in ruminants. Condensed tannins in forage legumes improve the sheep nutrition by reducing rumen degradation and increasing crude protein flow to the intestine (Min *et al.*, 2002). Condensed tannins can form complexes with protein by hydrogen bonding, which dissociate in the abomasum and anterior duodenum. These complexes could protect proteins from microbial digestion in the rumen, thereby increasing the availability of feed proteins for digestion and post-rumen absorption (Min *et al.*, 2003). Condensed tannins bind to soluble proteins and other entities mostly in suitable rumen pH (5.8–6.8) making them insoluble (Barry & Manley, 1984). This is influenced by the structure and molecular weights of both the CT and the proteins (Min & Hart, 2003). Subsequently, CT can improve the host performance in general by reducing the level of parasitism and increasing protein availability in the small intestine (Athanasiadou *et al.*, 2001b).

Concerning the CT effects on animal production, previous studies have examined the effect of CT on methionine and cysteine metabolism because of their significance in wool production, and on phenylalanine as an indicator of general protein metabolism. The result of these studies have shown that CT gives a greater degree of production during digestion for cysteine, methionine and phenylalanine, that means CT effects on wool production is positive (Lee *et al.*, 1995) due to the relation between the digestion of protein with the growth of the wool by animals. The concentration and the type of CT determine wool growth and quality (Min *et al.*, 2003). For examples *Lotus pedunculatus* gives a greater

degree of production of wool than *Lotus corniculatus* (Lee *et al*., 1995) and the effects on wool growth being greatest in lambs which grazed *Sulla*, and lowest in those which grazed *Plantain* and *Lotus pedunculatus* (Niezen *et al*., 1998). Also wool production was significant ($P<0.05$) for lambs grazing *Lotus* which content 32-37g/kg of CT than *Lucerne* which contains less than 2g/kg DM of CT (Douglas *et al*., 1995). In addition, Min *et al.* (2001) reported that wool production was better in lambs grazing *Lotus* than lambs grazing pasture.

In relation to milk production, does grazing forage containing CT have significantly lower milk somatic cell counts (SCC) than does grazing forage that does not contain CT. The mechanism by which the CT reduced SCC in goats milk is not known clearly (Min *et al.,* 2005), but it can increase the milk protein concentration, depending upon CT concentration (Min *et al.,* 2003). However, moderate concentrations of CT, less than 50mg/kg BW, can enhance the production of milk and wool (Athanasiadou *et al*., 2001b). Condensed tannins can affect live-weight gain negatively if the concentration of CT is high. For example Niezen *et al.* (1998) reported that lambs grazed on *Sulla* had higher weight gain than lambs grazed on *Plantain*. A study by Douglas *et al*. (1995) indicated there was no difference in live weight gain between lambs grazing *Lotus* and *Lucerne*. It is clear that a small amount of CT could improve animal production in general.

Condensed tannins include four sub-species of wattle tannins from four Acacia *spp*, namely *A. mearnsii* (black-wattle tree), *A. decurren* (green wattle), *A. dealbata* (silver wattle) and *A. pyonantha* (golden wattle). Mature wattle trees have an average of 35% tannin (Gujrathi & Babu, 2007). This amount is distributed in all plant parts, mainly concentrated in the bark and tannin extracted from the bark is available commercially (Santana *et al.,* 1997).

The wattle trees (*Acacia spp*) grow and are distributed in huge area of plantation forests in Africa, particularly available in Kenya and South Africa (in Natal and Western Cape provinces), China, America and Australia. Wattle trees have fast growth rates in the altitude range of 2000-4500 feet with minimum rainfall of 35 inches annually. The growth of these plants requires special climatic conditions in fertile soil called the mist-belt.

The chemical structure of wattle tannins formed from trihydroxyl flavan-3-ols (Roux *et al.,* 1961), also has both aliphatic and aromatic hydroxyl groups for which reason it is utilized as a polyol component in polyurethane synthesis (Ge *et al.,* 2003). Recent studies indicated that wattle tannins could be a possible alternative remedial control measure against gastro-intestinal parasites in sheep and goats. Authors reported that it does work on nematode population even *Haemonchus contortus* (Max *et al.,* 2005).

2.6 Conclusion

Gastro-intestnal nematode parasites in tropical and sub-tropical areas have been highlighted as main concern for small ruminants. Their control depends mostly on chemical compounds, which recently could not offer the ideal help to the animals due to parasite resistance. However, the review has shown that condensed tannins can be of potential benefit to the ruminants when supplemented in their diet.

The review also has shown that nematode parasites cause extensive losses of protein in the abomasum and small intestines, which affect the body functions. More importantly, several problems associated with the anti-parasitic drugs from over use/or misuse were observed worldwide. Development of resistance by the parasites to the drugs has become more pronounced. A number of studies conducted on forages containing tannin have shown that condensed tannins can potentially reduce the high infection of the gastrointestinal parasites in small ruminants. Therefore, tannins can be economically sustainable because they cost less to produce have no residual effect on the animal products, thus a reduction in the anthelmentic use.

Chapter 3
Epidemiology and seasonal dynamics of internal parasite infections in small ruminants

3.0 Abstract

An epidemiological study of internal parasite infections of ewes and does was carried out at the University of KwaZulu-Natal Research Farm in South Africa for 1 year (February 2008 to January 2009). The experimental animals consisted of a total of 16 animals, including 8 Merino ewes and 8 Nguni does. These animals grazed on kikuyu pasture together with a larger university flock. Parasitological data of EPG, faecal culture L_3 and infective larvae on pasture were recorded monthly. Coccidian oocysts and live weight were also recorded. Results showed distinct seasonal effects and annual rainfall patterns. A high level of infection occurred in December and the least level of infections occurred in July. *Trichostrongylus spp* was the most prevalent parasites, followed by *Strongyloides spp, Haemonchus contortus, Nematodirus spp Cooperia spp, and Eimeria spp* were found in the faeces of the experimental animals. Seasonal effect was significant ($P<0.05$) on EPG, infective larvae of the faecal culture, pasture L_3 and *Eimeria* oocysts count. However, similarities were observed in EPG and live weight gain during the year. Information obtained gave base data for suggesting a control program with a minimum anthelmintic use.

3.1 Introduction

Death due to *Eimeria spp* and gastrointestinal nematode has been recorded in small ruminants (Agyei *et al.,* 2004). In addition they cause lower productively due to poor growth and body weight gain (Tembly *et al.,* 1997). So understanding of the parasite epidemiology and the factors that affect parasite growth are crucial for controlling and management of internal parasites (Tembly *et al.,* 1997; Waller & Thamsborg, 2004; Sissay *et al.,* 2007).

Moreover, seasonal dynamics are also essential for parasite growth and development (Teel *et al.,* 1996). The seasonal dynamics of nematode infection are the consequence of

complex inter-relationships between the sheep, their husbandry and the prevailing climate (Viassoff *et al*., 2001). The patterns of pasture contamination by nematode eggs, then larvae and the levels of infection in ewes and does are similar (Sissay *et al.*, 2007). The degree of pasture contamination is determined by the number of infective larvae on the pasture (Hale, 2006). However, many factors such as climatic conditions, the deposition of helminth eggs in faeces and the subsequent development of the parasites as well as the effect of birds, insects, fungi and wild mammals influenced the development, survival, distribution or migratory behavior of the free-living larvae seen on pasture (Stromberg, 1997). These factors are highly changeable according to the season. Faizal *et al.* (1999), based on their experiment for assessing combined effect of *Eimeria* s*pp* and gastrointestinal nematode infections in goats, summarized that developing of appropriate control strategies for parasitic infections is prerequisite.

The present study was carried out to determine the epidemiology of *Eimeria spp* and gastrointestinal nematode parasites and their effect on small ruminants. The effect of seasonal dynamics on transmuting and development of internal parasites was also examined.

3.2 Material and Methods

3.2.1 Study area

This study was conducted in the Livestock Section, of the University of KwaZulu-Natal Research Farm at Ukulinga just outside Pietermaritzburg in KwaZulu-Natal province in subtropical hinterland which is approximately 700 m above sea level. The climate is characterized by annual rainfall of 735mm, which falls mostly in summer between October and April. The maximum and minimum mean annual temperatures are 25.7 and 8.9 °C, respectively. Light to moderate frost occurs occasionally in winter.

3.2.2 Experimental animals and animal management

Small ruminants (8 ewes and 8 does), aged 7-18 months with initial weight 39 ± 7.6kg were used from February 2008 to January 2009. The weight of the animals was taken monthly. During the experimental period the animals were allowed to graze freely on contaminated kikuyu pasture which was divided into four paddocks. Water was available *ad libitum* in troughs. Experimental animals were allowed to graze together with other animals in the farm and were managed similarly to the farm flock during the one year

study. No supplemental feed was given. Anthelmintics were given eleven times during the study period as indicated in Figure 3.2. Hoof trimming occurred every six months and the offspring were tagged when necessary.

3.3.3 Sample collection and parasitological analysis

Faecal samples were taken monthly, from the rectum of the experimental animals, and placed in plastic bags bearing the animal's identification number. Samples were then conveyed to the Animal and Poultry Science Departmental laboratory where the parasitological techniques were done.

Faecal nematode egg counts and count of coccidian oocysts were done using McMaster Technique (Hansen & Perry, 1994). Enumeration of faecal eggs and coccidian oocysts were usually complete on the day following the collection day. Fifty six ml of saturated salt solution was added to four grams of faeces in a beaker. The faecal suspension was filtered into another beaker, and the sub samples were examined under a light microscope at 100 magnifications after both wells of McMaster counting chamber were filled with the suspension and were allowed to stand for 5 minutes. The number of nematode eggs and coccidian oocysts in both wells of the McMaster chamber was multiply by 50 to get EPG and OPG.

Also the faeces were pooled and mixed per group, sub-sampled into 6 trays and incubated for 15 days at 27 °C. Samples were kept damp by watering every day at 10:00 h during the period of incubation. The Baermann technique was then used to identify infective larvae according to Hansen & Perry (1994). Nine grams of faecal culture were weighed, placed into a piece of double-layer cheesecloth, and closed by a rubber band. This was placed in a funnel (supported by a funnel stand), which was then filled with lukewarm water until the water covered the faecal material. The apparatus was left for 24 hours; then 15ml of fluid were taken from the stem of the funnel into a test tube which was left to stand for 30 minutes. The supernatant was removed with a pasteur pipette and a drop of the aliquot was transferred to a microscope slide using a pasteur pipette. A drop of iodine was then added, and the slide covered with a cover slip. Under 100 magnification, the samples were examined and the larvae were identified according to Van Wyk *et al.* (2004) and http:// www.rvc.ac.uk.

Table 3.1 Differentiation of nematode infective larvae

Nematode's spp	Total length (µm)	Head	Inside the body	Sheath tail	Other differential features
Haemonchus	650-850	Narrow rounded (bullet-shaped)	16 gut cells	Medium	Tail ending in fine point.
Trichostrongylus	560-796	Tapered head	16 gut cells	Short	Smooth larval tail without filament
Strongyloides	650-850	bullet-shaped	Oesophagus extends to ½ length of larval body	Absent	Slender body, larval tail notched
Nematodirus	752-1248	Broad, rounded	8 large intestinal cells	Extremely long	Sheath tail filamentous, larval tail notched or lobed.
Cooperia	666-956	Square with re- fractile bodies	16 gut cells	Medium	Tail of larvae rounded with filament.

3.2.4 Herbage sampling

Herbage samples were cut in the morning at 6:30-7:00 using a scissor from different locations e.g. 300-600g following W shape in each paddock based on the method of Hansen and Perry (1994). Paddocks were sampled every month and placed in plastic bags. Grass samples were placed inside gauze bags and soaked in water overnight. In the first 3-4 hours the grass bags in the water were removed and replaced 5 times then left at room temperature overnight. During the next morning, the bags were removed after running fresh tap water over them into the bucket. The bucket and contents were allowed to stand for an hour. The top of the supernatant was carefully siphoned off, leaving about 1 litre. The sediment was poured into a large funnel stand with the bottom clamp fastened and then left to stand for 1 hour after discarding any heavy debris that sedimented in the first 10 minutes. The sediments (35ml) were taken and kept in a refrigerator to cool at 4 $^{\circ}$C. The third- stage L_3 were identified under a microscope after addition of 1ml of iodine to the cool sediment and 0.2ml of sodium thiosulphate as a counter stain (Hansen and Perry,

1994). The dry matter content of the pasture grass samples was determined and results were expressed as the number of L_3 per kg of herbage dry matter (count multiply by 1000/weight of dry herbage in grams).

3.2.5 Climatic data

An automatic weather station was set by Agrometeorology Department in the experimental site at the Ukulinga research farm which was used to collect standard meteorological weather data, including maximum and minimum air temperature, relative humidity and total rainfall. The data were collected every two minutes then averaged monthly.

3.2.6 Data analysis

The data of the faecal egg counts, larvae counts of the faecal culture, L_3 counts from the grass samples and coccidian oocysts count were analyzed by Repeated Measures Analysis of Variance (MANOVA) using the General Liner Model (GLM) procedure of SAS (2000). According to the following model:

$$Y_{ijkl} = \mu + M_i + S_j + (M*S)_{ij} + G_k + W_l + e_{ijkl;}$$

Where: Y_{ijkl} = individual monthly observation; μ = overall mean; M_i = monthly effect; S_j = effect of animal species; $(M*S)_{ij}$ = interaction between month and animal species; G_k = co-variate effect of initial egg count, W_l = co-variate effect of initial live weight and e_{ijkl} = error of mean.

3.3 Results

3.3.1 Climatic conditions

Monthly climatic conditions, including (minimum and maximum air temperature, total rainfall, minimum and maximum relative humidity) during the period of study are presented in Figure 3.1. The weather conditions during the experimental period showed the highest amount of rain in summer (October to December). There was no rain and the temperature was lowest in winter (May to July).

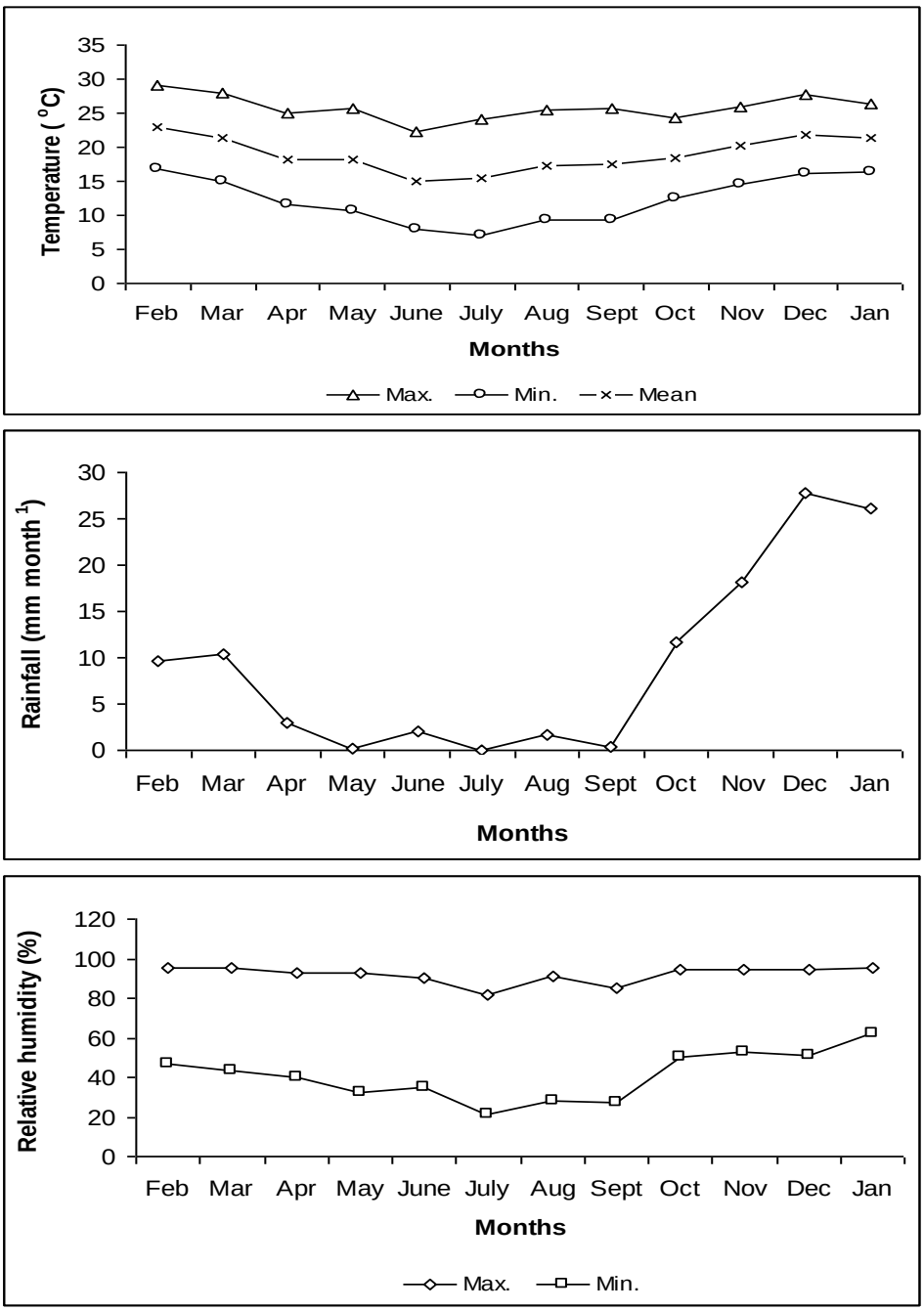

Figure 3.1 Mean maximum and minimum temperatures (˚ C), mean monthly rainfall (mm) and maximum and minimum relative humidity (%).

3.3.2 Nematode faecal egg counts and faecal culture larvae

The effect of seasons on EPG was significant ($P < 0.05$). However, initial live weight and species of animals had no significant effects on the EPG. The EPG showed seasonal variations generally in accordance with rainfall, irrespective of live weight and species of

animals during the study period (Figure 3.2). In both ewes and does the EPG started to increase in spring (August/ September) to reach the highest level when the rainfall was highest (November/ December). Subsequently, the EPG decreased during the dry period (end of May to end of July). No significant differences in the mean EPG were observed between the ewes and the does at any time of the year.

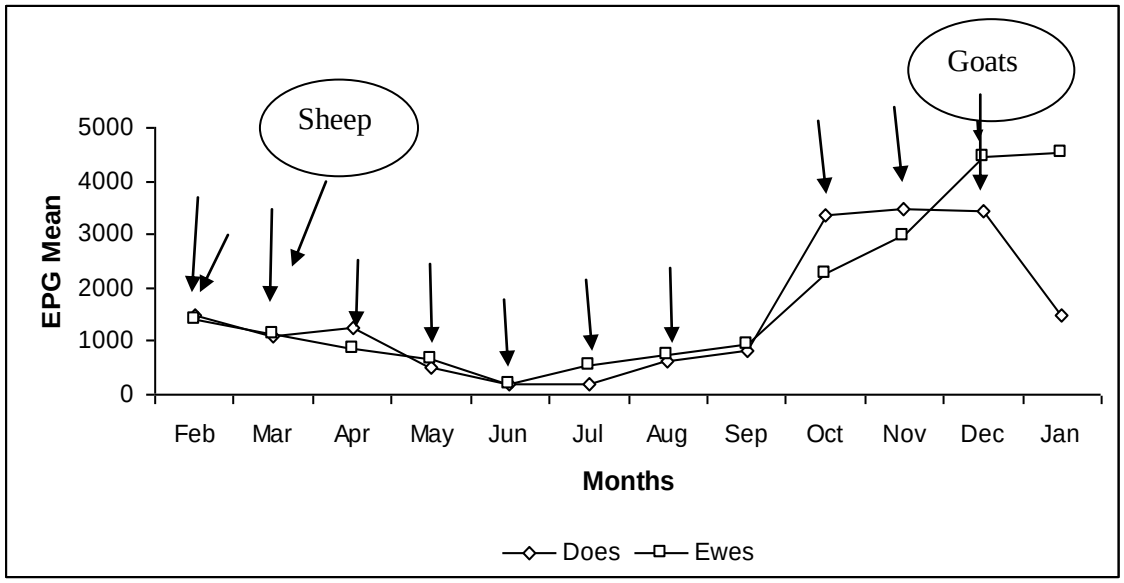

Figure 3.2 Monthly mean egg per gram of faeces (EPG) for ewes and does during a period of 12 months (February 2008- January 2009). SED value was 55. Arrows indicate time of anthelmintic treatments used. Circles indicate anthelmintic treatment for the animals mentioned in these particular months.

The average percentage of nematode (L_3) total larvae is presented in Figure 3.3. Total larvae were greatest during the time of the highest faecal egg counts. *Trichostrongylus spp* (22-24.5%) was the main species revealed in both ewes and does. *Strongyloids spp* was 19-21%, followed by the most pathogenic species, *Haemonchus contortus* (14.5-16%) and *Nematodirus spp* (13-16%) during the study period. *Cooperia spp* had lowest proportion (10-13%) of infective larvae. There was a significant ($P<0.05$) seasonal effect on total L_3 from the faecal culture.

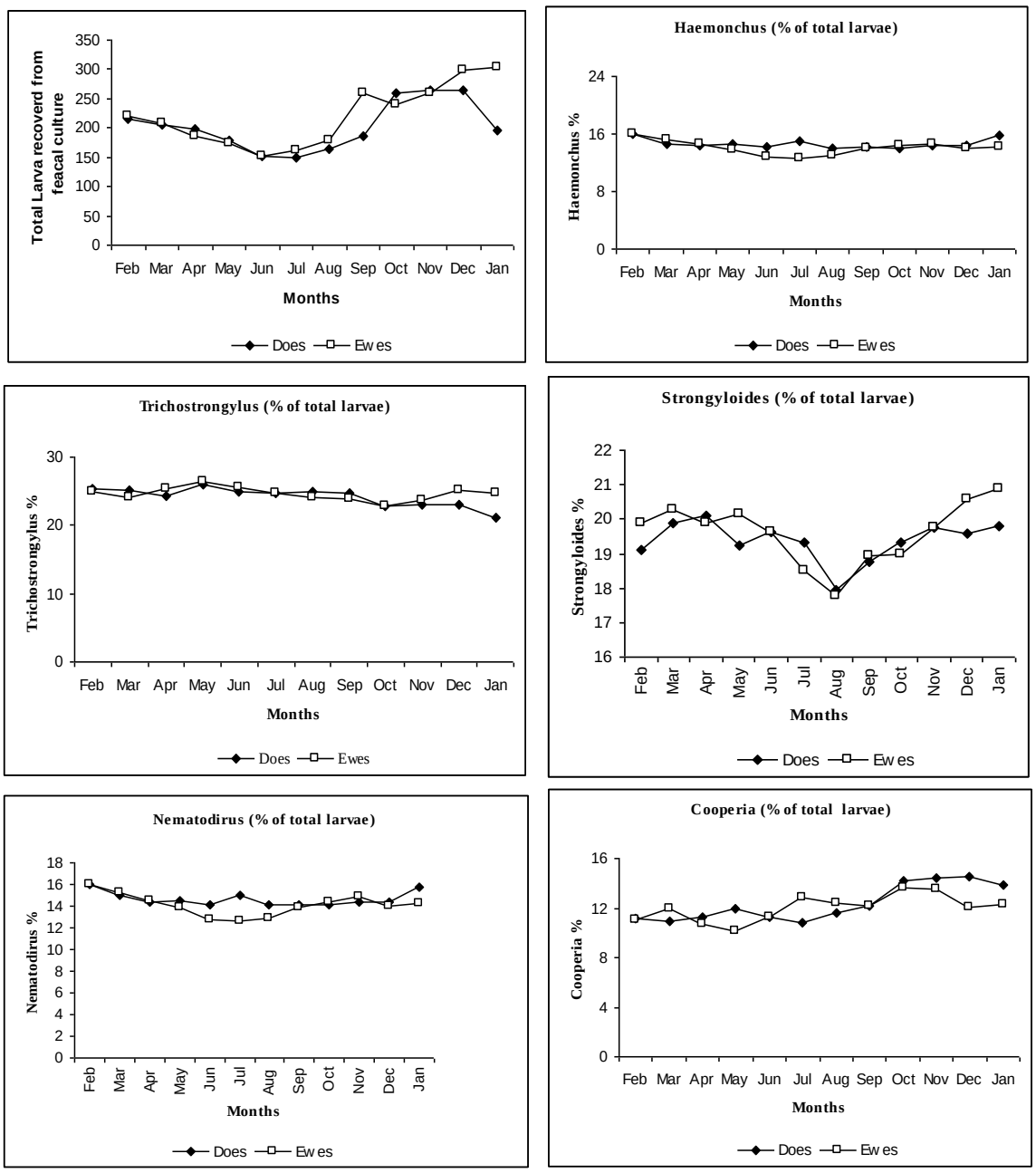

Figure 3.3 Percentage of nematode infective larvae obtained from the faecal culture. SED values were: Total larvae 0.57, *Haemonchus spp* 0.34, *Trichostrongylus spp* 0.29, *Strongyloides spp* 0.29, *Namatodirus spp* 0.28 and *Cooperia spp* 0.28.

3.3.3 Third- stage larvae on pasture

The variation in nematode L_3 larvae recovered from Kikuyu pasture at Ukulinga Research Farm is shown in Figure 3.4. The sum of L_3 on pasture was relatively similar to the pattern of EPG and rainfall pattern. Pasture recorded the highest amount of L_3 in spring (August),

whilst the lowest amount was observed in winter (July). The infestation of pasture varied significantly ($P<0.05$) with the season.

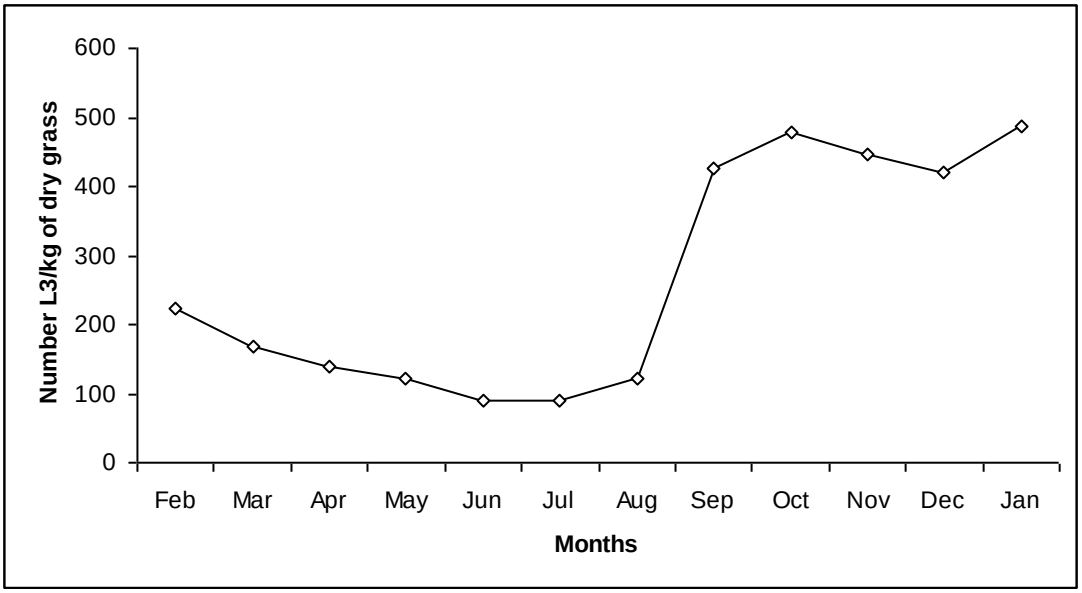

Figure 3.4 Monthly mean of nematode third stage larvae recovered from Kikuyu pasture during a period of 12 months (February 2008- January 2009). SED was 48.4

3.3.4 Coccidian oocysts count (OPG)

Group mean OPG (Figure 3.5) varied ($P < 0.05$) across seasons. At the beginning of the study OPG mean was 406.25 and 218.75 for the does and the ewes, respectively. In December, which recorded highest amount of rainfall, the OPG means were 1293.75 for does and 956.25 for ewes. In July which had no rain, the mean OPG were 712.5 for does and 537.5 for ewes. The lowest amount of OPG was observed in June; 387.5 for does and 412.5 for ewes.

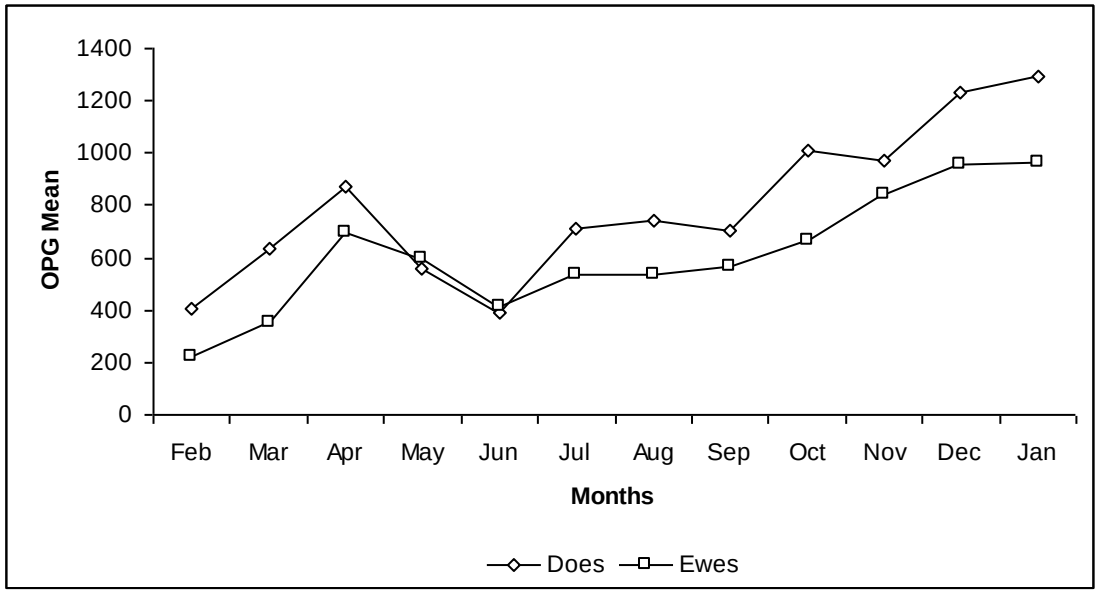

Figure 3.5 Monthly mean of coccidian oocysts per gram of faeces for ewes and does during a period of 12 months (February 2008- January 2009). SED was 112.6.

3.3.5 Live-weight variation

The season had no significant effects on the live weight of the experimental animals. The live weight of ewes and does ranged from 42 to 47kg and 27.5 to 35kg, respectively, during the entire study period. Monthly live weight means are shown in Figure 3.6.

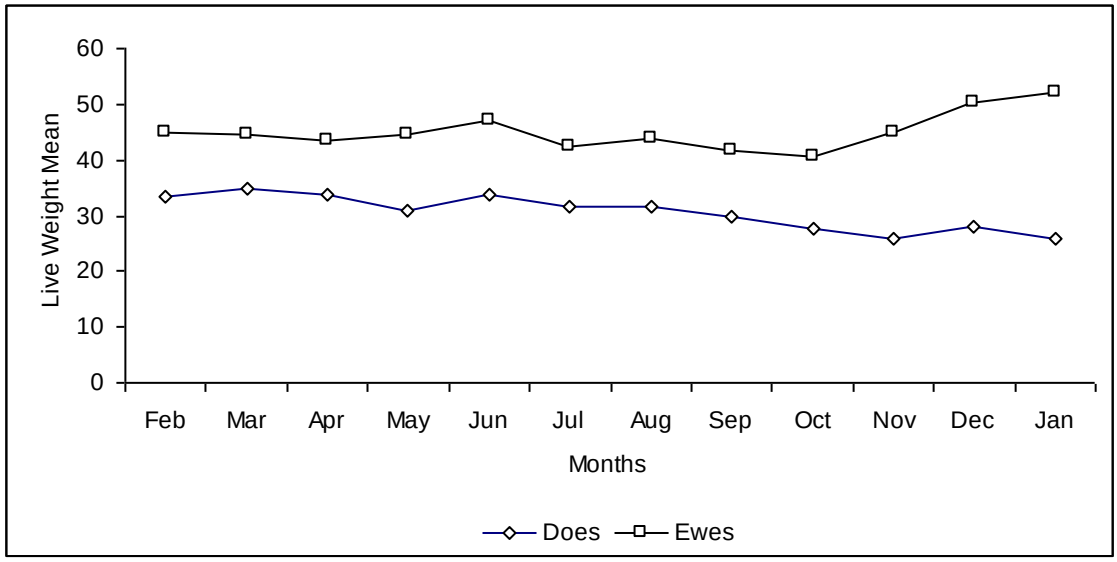

Figure 3.6 Monthly variation live weight for ewes and does during a period of 12 months (February 2008- January 2009). SED was 2.8 kg.

3.4 Discussion

This study has shown that the animals are infected with a variety of parasites such as nematode parasites and *Eimeria spp*. Similar observations were reported in Ghana (Agyei *et al.,* 2004) and Ethiopia (Fikru *et al.,* 2006). Also the EPG, larval counts from the faecal culture, L_3 from the pasture and coccidian oocysts count were highest in summer (December) and lowest in winter (July), indicating that internal parasite infections had seasonal patterns. These results are in agreement with observations made elsewhere by others (Agyei, 1997; Tembely *et al.,* 1997; Tembly, 1998; Viassoff *et al*., 2001; Waller *et al.,* 2004; Sissay *et al.,* 2007). The variation in the time to reach peak EPG among the above studies is probably due to the variation in management practice, weather, animal breed or age. December is characterized by rainy, hot and humid ecological conditions that are ideal for nematode infective larvae growth and transmittion. Teklye (1991) stated that the weather situation in Sub-Saharan Africa is suitable for the growth of infective nematode larvae.

The prevalence of pasture L_3 in this study was lower in dry months (winter) than in rainy months and is similar to the result reported by Tembely *et al.* (1997). In addition, Stromberg & Averbeck (1999) reported that larval survival varied greatly on pasture; most

of the ensheathed infective larvae can survive weeks to months on pasture, depending on the environment and species of nematode. In this study, quick recovery for the infective larvae on pasture was observed in early spring (August/ September), which agrees with Tembely *et al.* (1996) that eggs will only develop into infective stage when the weather is appropriate.

Results from the faecal culture indicated that *Trichostrongylus, Strongyloids* and *Haemonchus* were the most prevalent parasites in both ewes and does throughout the study. Southcott *et al.* (1976) considered *Haemonchus contortus* as a warm climate species. Also Sissay *et al.* (2007) reported that *Trichostrongylus circumcincta* has the ability to survive in adverse conditions within both host and pasture wherever sheep and goats are raised. However, Agyei (1997) reported that the level and number of infective *strongylate* nematode larvae on pasture were directly related to the pattern of rainfall and were also influenced by the number of rain days in the period.

The presence of both *Eimeria spp* and nematode parasites in this study is similar to that in many studies (Agyei *et al.,* 2004; Faizal *et al.,* 1999; Fikru *et al.,* 2006). *Eimeria* oocysts count followed the rainfall pattern like the EPG. Similar observations were made in Sri-Lanka by Faizal *et al.* (1999) and in Ghana by Agyei *et al. (*2004). Eggs per gram of faeces in this study had no effect on the live weight gain. This is probably due to the sheep adapting to the infection. The observation is in contrast to Broughan & Wall (2007) where there was negative relationship between live weight and EPG. However, that could be due to the fact that malnutrition and parasitism infections occur concurrently (Koski & Scott, 2001).

The results have shown the time of anthelmintic treatments used, it clearly gave the idea about the erroneous use of broad-spectrum anthelmintics which are Ivermectin, Closantel and combination of Abamectin and Praziquantel in this particular farm. Dosing frequency is one of the main factors associated with development of resistant strains (Leathwick *et al*., 2001). The goat group was treated with Albendazole the previous December. Results in January 2009 confirmed lower EPG in the goats faeces compared to the sheep group. Notable, the use of anthelmintics on this farm is in contrast to the recommended control reported in the study by Tembly *et al.* (1997), who suggested two treatments per annum, firstly treating the adult animals with an effective broad-spectrum anthelmintic at the start

of rainy season, with a second treatment recommended at the beginning of dry season. Generally this study showed the parasitological situation at the University of KwaZulu-Natal farm.

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3.5 Conclusion

Overall the study showed the effect of season on nematode parasites and *Eimeria* oocyst infections on small ruminants at the Ukulinga Research Farm. Nematode parasites also showed no effect on body weight gain. In addition the study confirmed that weather conditions had a direct effect on internal parasite development. Rainfall and humidity are likely the main factors for the growth of these parasites. However, the data obtained can be used to design an effective control program strategy for internal parasites of small ruminants on this farm. Treatment of all farm animals with an effective anthelmintic in late April should significantly reduce the number of L_3 on pasture when the weather is suitable for the development of eggs into infective larvae. Other treatment should be before and in the middle of the wet season to prevent the rise in faecal egg counts.

Chapter 4

Effectiveness of anthelmintic drugs on gastrointestinal nematode species in sheep

4.0 Abstract

The resistance of gastrointestinal nematodes (GIN) to anthelmintic drugs is affecting small ruminant production in South Africa. This study determined the effectiveness of drugs (Ivermectin 1% (IVM), Closantel 5% (CST) and the combination Abamectin 0.08% and Praziquantel 1.5% (CPA) currently being used against GIN in SA. The initial live weight of twenty-four sheep (12 females and 12 males) and egg count (EPG_0) in rectal faeces were determined. Gender, EPG_0 and initial live weight aided in blocking animals into groups, within which sheep were randomly allocated to and drenched with four drug treatments comprising: the untreated control (T0), IVM, CST, and CPA. Animals grazed throughout on infested pasture. Rectal faeces were collected on days 0, 7, 14 and 21 for determining EPG. Part of the faeces was incubated to identify and determine the abundance of larval forms of *Haemonchus*, *Trichostrongylus*, *Strongyloides*, *Nematodirus*, and *Cooperia* species. Differences among treatments changed ($P<0.05$) over time. On day7 IVM, CST, and CPA depressed EPG to 0.66, 0.37 and 0.80 of their respective starting values whilst EPG increased 1.39 times for T0. Thereafter, EPG increased consistently for all drugs; CST recorded the lowest values. *Haemonchus*, *Trichostrongylus*, *Strongyloides*, *Nematodirus* and *Cooperia* species contributed respectively 60%, 30%, 6%, 3% and 1% of the larval forms on day0; and 78%, 8%, 11%, 1% and 2% on day21. Larval forms increased for *Haemonchus spp* but decreased for *Trichostrongylus* species over time. Closantel was the most effective. *Haemonchus spp*. were least affected whilst *Trichostrongylus spp*. were the most affected by all drugs.

4.1 Introduction

Gastrointestinal nematode infection is adversely affecting sheep production and causing economic losses. The result of failure to control gastrointestinal nematodes is poor growth rates, illness and death (Min *et al*., 2004). Generally, nematode impact is higher in tropical and subtropical areas where there are ideal ecological conditions for their growth.

Anthelmintics have been used to control nematode parasitism over the last forty years (Kohler, 2001). However, nematodes are known to increasingly develop resistance to all groups of anthelmintics (Van Wyk *et al.,* 1998; Waller, 1999; Sissay *et al.,* 2006). Parasite resistance is defined as the genetically transmitted loss of sensitivity in worm populations that were previously sensitive to the same drug (Kohler, 2001).

Globally, the problem has been observed on both large and small scale farms (e.g. Van Wyk *et al.,* 1997; Waller, 1997; Fiel *et al.,* 2001). Moreover, in South Africa, Van Wyk *et al.,* (1998) reported that small ruminants are being threatened by nematode resistance and all the nematode species are involved, but *Haemonchus contortus* is the most resistant to currently available anthelmintics.

Thus, the situation has become so critical for producers to control gastrointestinal nematode on small ruminant farms. Kaplan (2004) reported there are still some farms where dewormers continue to work, while others have no effective dewormers. The problem is that there is no new dewormer currently under development for sheep and goats.

The objective of this study was to determine the prevalence of nematode resistance to chemical treatments of Ivermectin 1% (IVM), Closantel 5% (CST) and the combination Abamectin 0.08% and Praziquantel 1.5% (CPA) through the faecal egg counts in sheep. It also ascertains which nematode species has the most resistance.

4.2 Material and Methods:

4.2.1 Study area

This study was conducted in the Livestock Section, of the University of KwaZulu-Natal Research Farm in South Africa.

4.2.2 Experimental design & Sheep management

Twenty four sheep (12 males and 12 females), aged 7-18 months, with initial live weight 33.9 ± 11kg were used in this experiment. Animals were sorted by sex, initial eggs per gram of faeces and initial weight to four groups of six animals each. Each group was then randomly assigned to the four treatments; 0 treatment, Ivermectin (IVM), Closantel (CST)

and combination of Abamectin and Praziquantel (CPA) (for further details see Table 4.1). Experimental animals were allowed to graze freely on planted contaminated Kikuyu pasture (*Pennisetum clandestinum*) under the same condition with other animals on the farm to continue exposure to the infective larvae L_3. Larval contamination of pasture was determined in the four paddocks used in this study. The larval infestation ranged from 63 to 116 L_3 larvae/kg of dry herbage with a mean of 85 ± 22.5 L_3 larvae/ kg of herbage. No feed supplement was given to the experimental animals during 21 days of the study. The percentage efficacy of each compound mentioned was estimated by comparison both the arithmetric and geometric mean worm burdens of the treated (T) and control (C) groups:

% efficacy = 100 (1- 100T/C).

Table 4.1 Anthelmintic compounds used in the experiment

Active ingredient	Concentration %	Dosage rate	group	Trade name	Company
Nil	0	0	1	0	0
Ivermectin (IVM)	1	1ml/50kg	2	Virbamec LA	Virbac ANIMAL HEALTH
Closantel CLS	5	1ml/10kg	3	PRODOSE YELLOW	Virbac ANIMAL HEALTH
Combination of COB		2.5 ml/10 kg	4	VIRBAMAX	Virbac ANIMAL HEALTH
1. Abamectin ABC	0.08	0.2 mg/10kg			
2. Praziquantel PRQ	1.50	3.75 mg/kg			

IVM was injected subcutaneously in the axilla of each sheep of trial 2. CLS and the COB were drenched using hypodermic syringes.

4.2.3 Sample collection and parasitological analysis

Faecal samples were taken, in the morning, from the rectum of the experimental animals for nematode egg counts using McMaster Technique (Hansen & Perry, 1994). The samples were taken on days 0, 7, 14 and 21 post-treatment. The faeces were pooled and mixed per treatment, sub sampled into 12 trays and incubated for 15 days at 27 °C. Samples were kept damp by watering every day at 10:00 h during the period of incubation. The

Baermann technique was then used to identify infective larvae according to Hansen & Perry (1994).

4.2.4 Statistical analysis

The data of the faecal egg counts and larvae counts were analyzed using GLM procedure of SAS (2000). Following the model:

$$Y_{ijkl} = \mu + T_i + W_j + (T*W)_{ij} + G_k + W_l + e_{ijkl;}$$

where: Y_{ijkl} = individual weekly observation; μ = overall mean; T_i = effect of treatment; W_j = effect of week; $(T*W)_{ij}$ = interaction between treatment and week; G_k = co-variate effect of initial egg count, W_l = co-variate effect of initial live weight and e_{ijkl} = error of mean.

4.3 Results

4.3.1 Effect of anthelmintic on nematode eggs out put

Following drug administration, EPG increased over time for the control (T0). EPG for IVM, CST and CPA, dropped to a trough 7 days after administering these drugs; beyond day 7 EPG recovered rapidly for IVM and CPA but slowly for CST (Figure 4.1). The differences among treatments was significant ($P<0.05$) only on day 7 with CST having the lowest EPG, IVM and CPA being intermediate and T0 having the highest EPG.

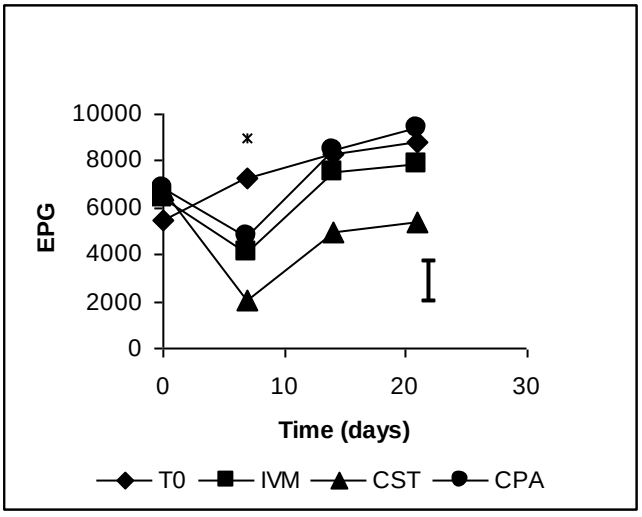

Figure 4.1 The effect of anthelmintic on total nematode eggs count T0, IVM, CST and CPA stand for 0, Ivermectin, Closantel and combination of Abamectin and Praziquantel treatment.

4.3.2 The effect of anthelmintic on nematode larvae count

Haemonchus larvae increased to day 7 for all treatments. Beyond day 7, the proportion of *Haemonchus* larvae continued to increase for T0, IVM and CST, but remained fairly stable for CPA. The overall trend is for the proportion of *Haemonchus* to increase gently. The second most abundant larvae were those of *Trichostrongylus*. The overall proportion of these larvae, even for the control, decreased consistently over time. There was, however, a rapid drop for IVM, CST and CPA from day 0 to day 7 beyond which larval proportion fluctuated only slightly. The infective larval proportion of *Nematodirus* species, though 8-6 times less numerous than *Trichostrongylus* larvae, decreased over time following a similar trend. However, for the control (T0) *Nematodirus* larvae decreased very gradually. *Strongyloides* larvae generally remained stable during the first 7 days. Beyond day 7, *Strongyloides* larvae increased to a maximum on day 21, with T0 and IVM having more larvae. Only T0 and IVM had in excess of 2% *Cooperia* larvae (Figure 4. 2).

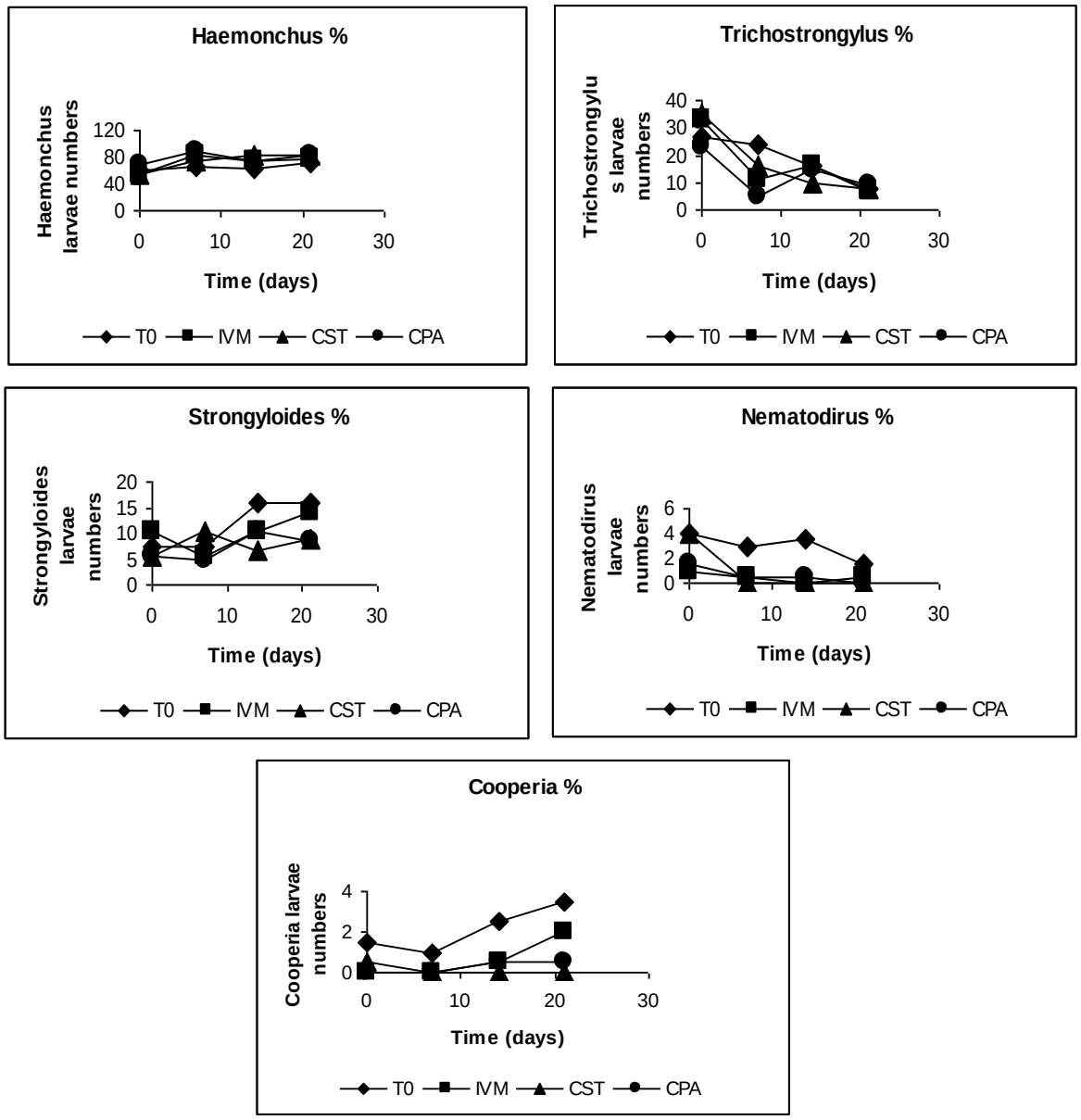

Figure 4.2 The effect of anthelmintic on nematode larvae. SED was 1.6 for *Haemonchus spp*, *Trichostrongylus spp* 1.7, *Strongyloides spp* 1.1, *Nematodirus spp* 0.8 and *Cooperia spp* 0.7. T0, IVM, CST and CPA stand for 0, Ivermectin, Closantel and combination of Abamectin and Praziquantel treatment.

4.4 Discussion

As expected there was continuous increase in faecal egg counts in the untreated group due to further infection from pastures. However, reduction in faecal egg counts on day 7 for animals treated with IVM, CST and CPA indicated quick and non-persistent treatment effects. An increase in faecal egg counts occurred on day 14 and day 21, probably due to the progress of worm resistance (Figure 4.3).

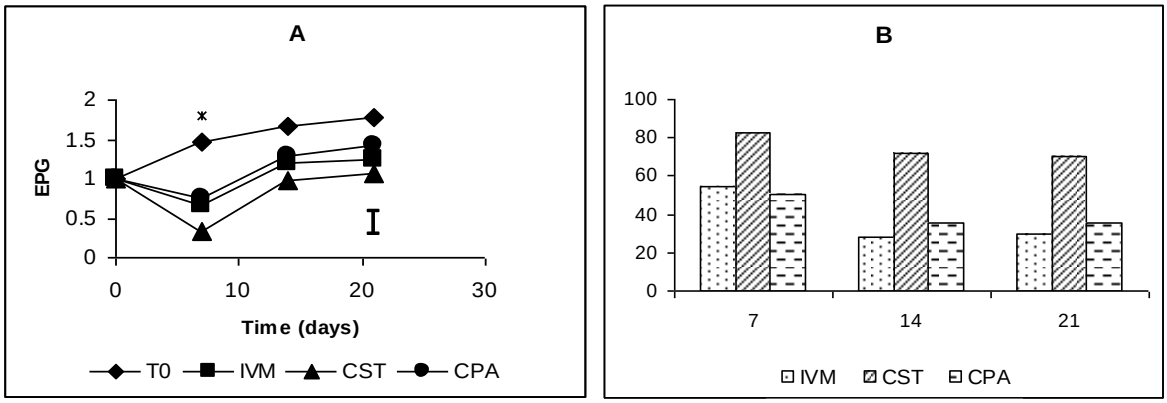

Figure 4.3 (A) Determination of nematode eggs production after treating the animals with anthelminthic T0, IVM, CST and CPA stand for 0, Ivermectin, Closantel and combination of Abamectin and Praziquantel treatment. (B) % efficacy of the above drugs.

Worm resistance in sheep and goats has been well documented in South Africa in relation to the crisis that they are causing. Surveys by Van Wyk *et al.* (1999) in Mpumalanga, KwaZulu-Natal and Lebowa area of Northern Province during summer-rainfall season of South Africa, have indicated there was anthelmintic resistance in *Haemonchus* spp in Mpumalanga and KwaZulu-Natal. In the Lebowa area anthelmintic resistance is also developing on commercial sheep and goat farms. Van Wyk *et al*. (1999) indicated that the level of *Haemonchus spp* resistance was possibly one of the highest in the world. A similar situation has been experienced in Yucatan, Mexico (Torres-Acosta *et al.*, 2003), where 38 sheep flocks were investigated for gastrointestinal nematodes resistant to benzimidazoles anthelmintic and the proportion of suspect farms was 23.7%, but all the flocks were either benzimidazole resistant or suspect and *Haemonchus spp* was the only gastrointestinal nematode genus present in the faeces of treated animals. Another study conducted by Waruiru *et al*. (1998), evaluated the efficacy of benzimidazoles (albendazole, fenbendazole and oxfendazole), levamisole, ivermectin and closantel on a farm in Kenya. The results

indicated an immediate resistance of *Haemonchus contortus* against benzimidazoles, levamisole and ivermectin, while *Trichostrongylus colubriformis* and *Oesophagostomum spp* indicated resistance against levamisole on the same farm. Ivermectin resistance developed to 47% within 15 months of the first use. Closantel was effective against *Haemonchus contortus*.

Broad-spectrum anthelmintics in sheep are divided into 2 broad types; a) Short acting products like moxidectin and Closantel (repeat the treatment after 3 weeks) and b) long acting products as albendazole or Ivermectin (repeat the treatment after 100 days) (Leathwick *et al*., 2001). The effective dose rate of anthelmintic in the market was intended to kill 95% of more susceptible parasite species (Leathwick *et al*., 2001).

At the Research Farm where both short and long acting anthelmintics have been used, none of these anthelmintics mentioned above is still as effective as would be expected. Also, more nematode strains that are resistant to anthelmintics are expected to develop and probably will not be easy to control. The reason for this expectation could be the associated factors that influence the selection of new strains such as frequency of treatment and underdosing of anthelmintic (Leathwick *et al*., 2001; Besier & Love, 2003). The experimental sheep were injected with IVM 15 days before the study, but normally the animals in this farm receive either short or long acting anthelmintics every 2-3 weeks in spring-summer. Leathwick *et al*. (2001) explained that a drench given to lambs in spring or summer is likely to select resistance more than one given in winter, because the weather conditions in spring and summer are likely to be suitable for fewer surviving eggs of the treatments to continue developing on pasture to a new generations of worms.

However, Besier & Love (2003) reported that minimizing the treatment numbers is useful to achieve acceptable results for prevention of further increase of resistance. In addition, the weight has long been considered a factor in the development of the resistance, meaning a single maximum dose of drench for all animals above a given weight (e.g. 40 kg), leading to the effective underdosing of sheep above this weight (Besier & Love, 2003). So, the resistant strains of the worms have reproductive advantage over susceptible genotypes thus contaminating pastures.

4.5 Conclusion

Gastrointestinal nematode parasites at Ukulinga Research Farm were highly resistant to commercial anthelmintics. Closantel was effective on nematode population at the farm, but not *Haemonchus spp*. Further research is necessary to find alternative strategies for the control of nematode parasites.

Chapter 5
Wattle tannins have the potential to control gastrointestinal nematodes in sheep

5.0 Abstract

Nematode resistance to anthelmintic drugs is affecting small ruminant production in South Africa. This study evaluated the effect of wattle tannins as an alternative nematode control drug. Three experiments (Exp.) were conducted to determine the effect of tannin concentration (Exp.1 and 2) and frequency (Exp.3) on nematode parasites. In each experiment gender, egg count (EPG_0) and initial live weight aided in blocking animals into groups, within which sheep were randomly allocated to and drenched with different tannin treatments. In Exp.1, 0, 0.8, 1.6 and 2.4 g tannin/kg BW were drenched for three consecutive days per sheep (16 females and 8 males, aged 8-9 months) for 21 day. In Exp.2, 30 sheep (14 males and 16 females, aged 9-18 months) were randomly allocated into three tannin treatments (0, 0.8 and 1.6 g tannin/kg BW) and drenched for a day. In Exp.3, 26 sheep (11 males and 15 females aged 9-18 months) were divided into three groups of 9, 9, and 8 sheep each. These groups were drenched with 1.6 g tannins/kg BW/day; once, twice and thrice for the 3 groups respectively. For the three experiments, EPG and L_3 larvae were counted in individual feacal samples. For all tannin treatments, EPG decreased ($P<0.05$) over time. Though the differences between tannin levels and frequencies varied ($P<0.05$) over time, EPG consistently decreased with increasing tannin level and frequency. The area under the curve decreased linearly with increasing level of tannin. 1.6 and 2.4 g tannin /kg BW for 3 consecutive days had nearly similar effects on the EPG. Thus 1.6 g tannin /kg BW for 3 consecutive days was enough to reduce EPG and reduce the degree of pasture contamination.

5.1 Introduction

Nematode parasites have been a main factor limiting small ruminant production in South Africa (Van Wyk *et al*., 1999). Despite much research into nematode parasite biology and control, they are still continued to be an critical constraint to sheep production (Viassoff *et al*., 2001). Many species of nematode were found in South Africa, e.g. *Haemonchus* and *Trichostrongylus* that are associated with production losses and clinical diseases (Van Wyk

et al., 1997). In general gastro-intestinal nematodes have been traditionally controlled using synthetic anthelmintics. However, over-dependence and even misuse of the anthelmintics has resulted in the emergence and spread of nematode populations that are resistant to most anthelmintics (Prichard, 1994). Moreover, anthelmintics are expensive for many poor farmers in the developing countries.

Thus there is a need to develop effective and/or cheaper alternative gastrointestinal nematode control strategies. Previous studies have shown that forages containing condensed tannins have anthelmintic effects and potentially could be used to control gastro-intestinal nematodes (Min *et al*., 2003), but the biological effects of condensed tannins has not been clearly defined, since the consumption of condensed tannins can lead to both detrimental and beneficial consequences in ruminants (Athanasiadou *et al*., 2001b; Waghorn, 2008). Regardless of the anthelmintic properties of condensed tannins, recent research is focused on reducing the level of parasitism and consequently improving performance in parasitised hosts (Athanasiadou *et al*., 2001b).

The aim of this study was to evaluate the effect of different concentrations of wattle tannins on egg production by gastrointestinal nematodes, hatching and development of gastrointestinal nematode eggs from sheep grazing contamination pasture.

5.2. Material and Methods

5.2.1 Study area and sheep management

The study was conducted at the Livestock Section of the Ukulinga Research Farm of the University of KwaZulu-Natal in South Africa. Experimental animals were allowed to graze naturally contaminated Kikuyu (*pennisettum clandenstinum*) pasture under the same condition with other animals on the farm.

5.2.2 Experimental design

5.2.2.1 Effect of tannin levels dosed third (Experiment 1)

Twenty four sheep (16 females and 8 males), aged 8-9 months with initial live weight 20.6 ± 9.5kg were used. The initial faecal egg counts were determined in rectal faecal samples. The sex, initial eggs per gram (EPG) of faeces and initial live weight were used to place

animals into six groups of four animals each; within a group animals were randomly assigned to four tannin treatments (0, 0.8, 1.6 and 2.4 g tannins/kg BW). Tannins were drenched (in solution) for 3 consecutive days. Larval contamination of pasture was done in four paddocks used in this study. The larval infestation ranged from 115 to 180 L_3 larvae/kg of herbage with a mean of 138 ± 28.9 L_3 larvae/kg herbage. No synthetic feed supplement was given to the experimental animals during the 21 days of the study.

5.2.2.2 Effect of tannin levels dosed once (Experiment 2)

Thirty sheep (14 males and 16 females), aged 9-18 months with initial live weight 35 ± 7.5kg were used. The sex, initial EPG and initial live weight were used to allocate animals into 3 groups of 12, 12 and 6 animals; within a group animals were randomly assigned to three tannin treatments (0, 0.8 and 1.6 g tannins/kg BW) in order. Tannins were drenched (in solution) for one day. The larval infestation which was done in 4 paddocks; ranged from 313 to 679 L_3 larvae/kg of herbage with a mean of 427 ± 172 L_3 larvae/ kg herbage. Hay was given to the experimental animals during the 15 days of the study.

5.2.2.3 Effect of dosing tannin frequency (Experiment 3)

Twenty six sheep (11 males and 15 females), aged 9-18 months and initial live weight 34 ± 7.5kg were used. Animals were placed into 3 groups (of 9, 9 and 8 sheep each) according to sex, initial EPG and initial live weight. Sheep groups were drenched with 1.6 g tannins/kg BW once, twice or thrice. Four contaminated paddocks were investigated in this study. Infective larvae were ranged from 405 to 646 L_3 larvae/kg of herbage with a mean of 478 ± 111 L_3 larvae/kg herbage. Hay was given to the experimental sheep during 28 days of the study.

5.2.3 Samples collection and parasitological analysis

Rectal faecal samples were taken in the morning on sampling days in each experiment for faecal culture and to calculate EPG. McMaster Technique (Hansen & Perry, 1994) was used to evaluate EPG. Faecal samples were pooled per treatment, thoroughly mixed, sub-sampled into trays and incubated at 27 °C for 15 days. Samples were kept damp by watering them every day at 10:00 am during the period of incubation. For larvae count 9g

of faecal culture were used to identify and enumerate nematode larvae according to the Baermann technique (Hansen & Perry, 1994).

5.2.4 Statistical analysis:

Data of EPG and nematode larvae data were analyzed using GLM procedure of SAS (2000). The effect of tannin on EPG was determined by integrating, for each animal, curves of the form: $f(x) = a + b.x + c.x^2 + d.x^3$, where f(x) is a polynomial function of EPG on time x; a, b, c and d are coefficients derived using SAS.

5.3. Results

Experiment 1

5.3.1 Nematode eggs count

Faecal egg counts remained fairly stable for the T0.0 treatment throughout the study, but decreased with time for the rest of the tannin treatments (Figure 5.1). However, the effect of tannin level only attained significance ($P< 0.05$) on days 15 and 18. Consistently, the highest level of tannin (T2.4) recorded the lowest EPG. Analysis of the area under the curve, which represents the average number of eggs produced over 21 days, confirms that T2.4 had the lowest egg production (Figure 5.1). The linear effect of tannin was significant ($P<0.05$). Figure 5.2 explains the percentage of tannin efficacy; T2.4 had over 70% efficacy for all the days of sampling.

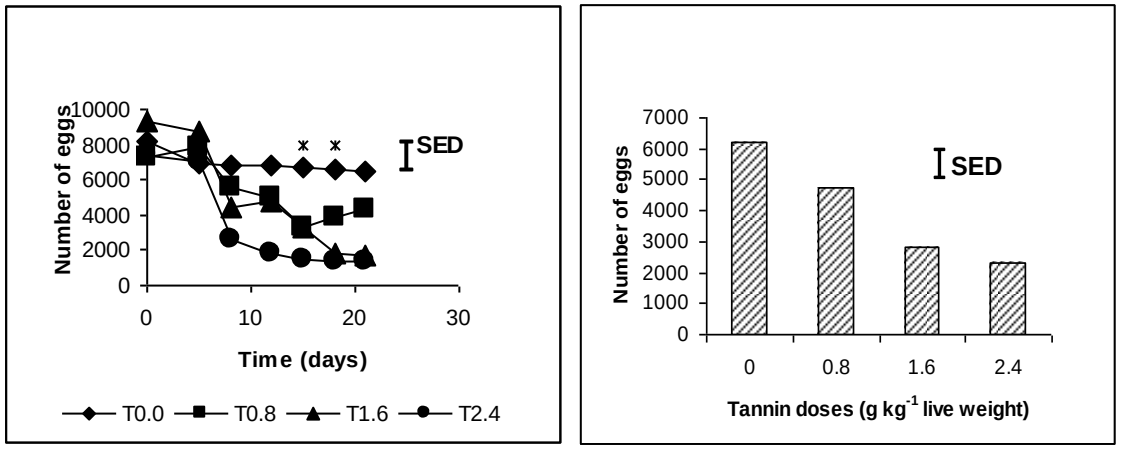

Figure 5.1 The effect of tannins level dosed three times on total nematode eggs count (T0.0, T0.8, T1.6 and 2.4 stand for dosage levels of 0.0, 0.8, 1.6 and 2.4 g tannin/kg live weight)

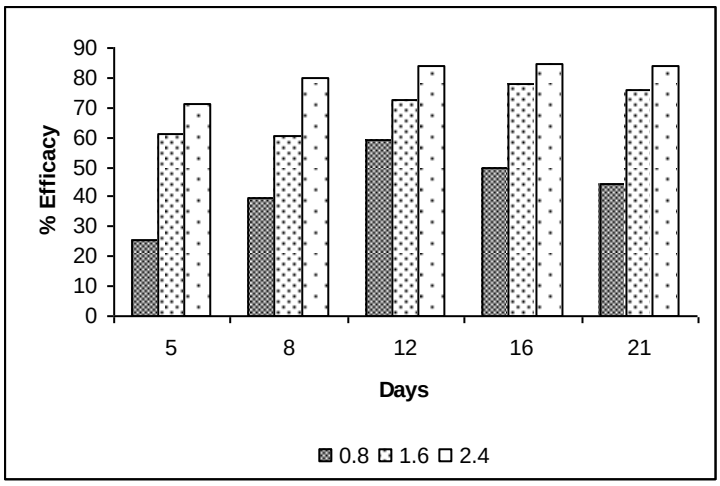

Figure 5.2 % Tannin efficacy (Experment1)
(0.8, 1.6 & 2.4 stand for dosage levels of 0.8, 1.6 and 2.4 g tannin/kg live weight)

5.3.2 Numbers of larvae recovered

Figure 5.3 shows that total number of larvae, proportions of *Haemonchus* and *Trichostrongylus* had similar trends. These larvae increase with time for T0.0, but decreased with time for T0.8, T1.6 and T2.4. The effect of tannin level was significant ($P<0.05$) on days 15, 18 and 21. The proportion of *Strongyloides* and *Nematodirus* had a trough on day 7 beyond which both increased more rapidly for the control, but rather

slowly with increasing tannin level. T0.0 had higher (P<0.05) proportions of these larvae than any of the other tannin levels. The *Cooperia* proportion decreased for all treatments but was higher (P<0.05) for T0.0 than for any of the tannin levels.

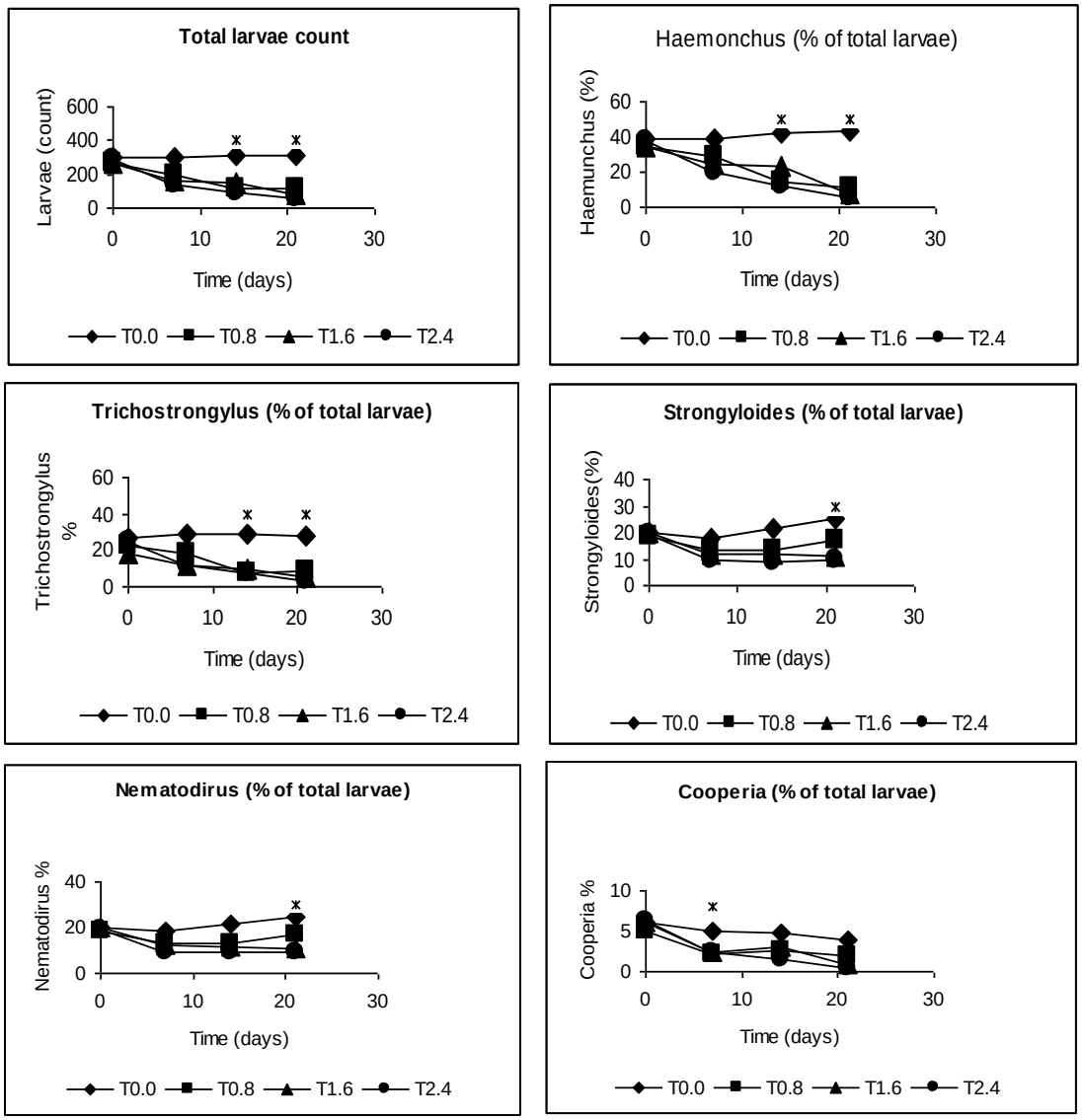

Figure 5.3 The effect of tannin level dosed thrice on nematode larvae. SED values were: Total larvae 4.5, *Haemonchus spp* 0.9, *Trichostrongylus* s*pp* 0.8, *Strongyloides* s*pp* 0.7, *Namatodirus* s*pp* 0.4 and *Cooperia* s*pp* 0.5. T0.0, T0.8, T1.6, and T2.4 stand for dosage levels of 0.0, 0.8, 1.6 and 2.4 g tannin/kg live weight.

Experiment 2

5.3.3 Nematode eggs count

Faecal egg counts remained stable for the control treatment throughout the study, but decreased with time for the two tannin treatments (Figure 5.4A). Beyond day 7, EPG remained fairly stable for 0.8 g tannin/kg W treatment but the highest level of tannin (1.6 g tannin/kg W) recoded the lowest EPG. The effect of tannin levels attained significance (P< 0.05) on day 14. The efficacy of tannin is presented in Figure 5.4B.

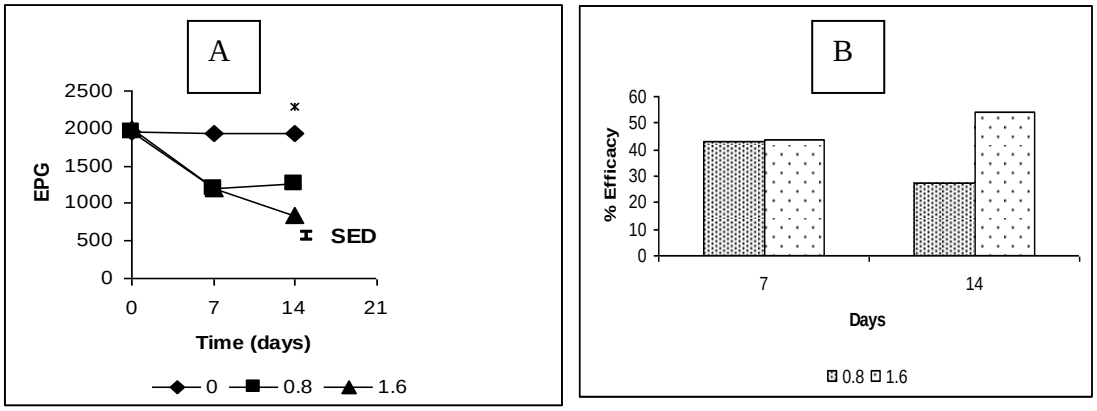

Figure 5.4 (A) The effect of tannins level dosed once on total nematode eggs count. (B) % Tannin efficacy (0, 0.8 and 1.6 stand for dosage levels of 0.0, 0.8 and 1.6 g tannin/kg live weight).

5.3.4 Numbers of larvae recovered

In Figure 5.5, total larvae are presented. The tannin levels depressed total larvae count on day 15. For the other larvae, tannin had no effect on *Haemonchus*, *Nematodirus* and *Cooperia* but increased (P<0.05) *Trichostrongylus.* It appears tannins tended to increase *Strongyloides.*

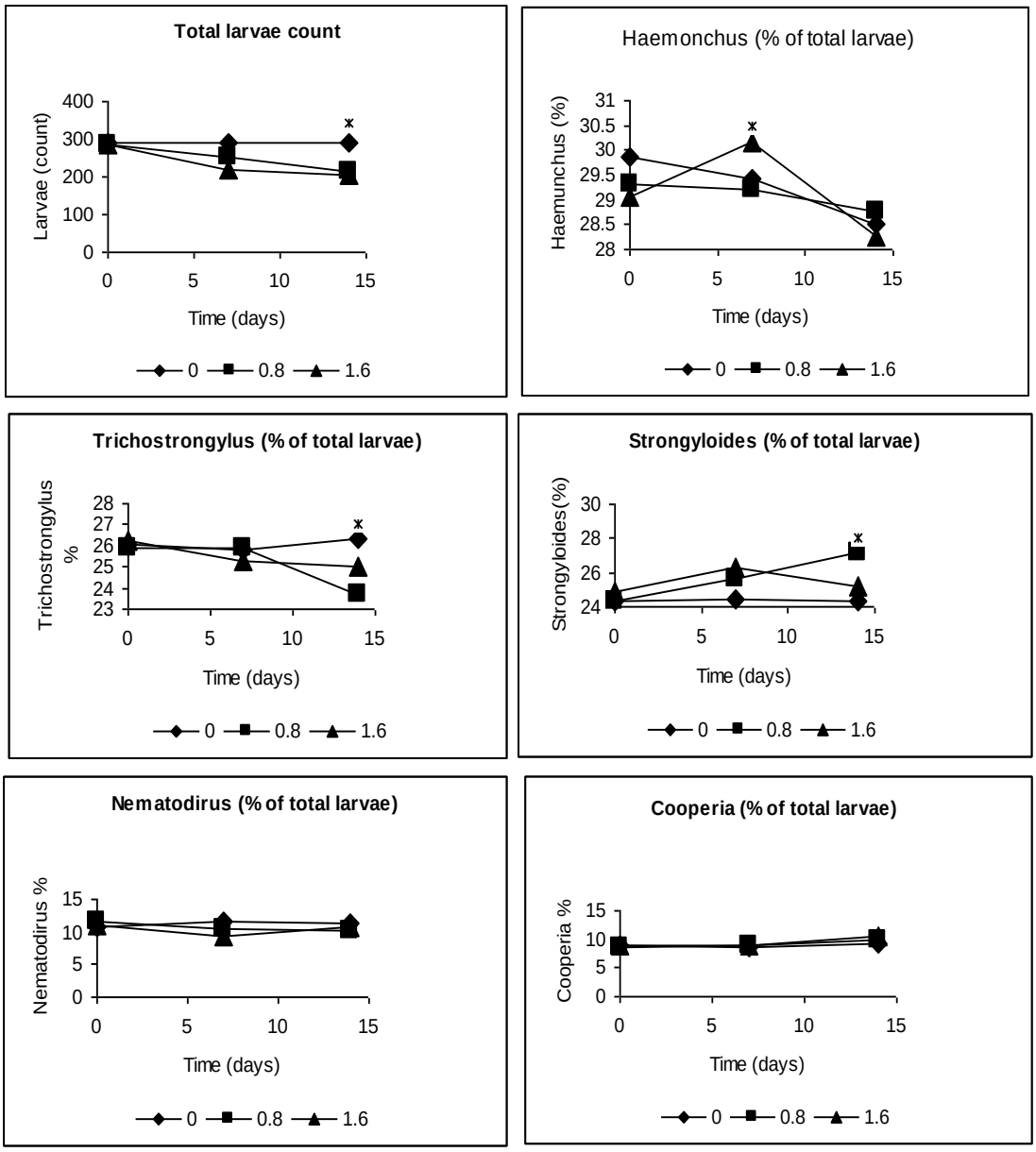

Figure 5.5 The effect of tannin levels dosed once on nematode larvae. SED values were: Total larvae 2.58, *Haemonchus spp* 0.32, *Trichostrongylus spp* 0.36, *Strongyloides spp* 0.39, *Nematodirus spp* 0.43 and *Cooperia spp* 0.35. 0, 0.8 and 1.6 stand for dosage levels of 0.0, 0.8 and 1.6 g tannin/kg live weight.

Experiment 3

5.3.5 Nematode egg count

The results demonstrate the effect of dosing tannin frequency (Figure 5.6). Dosing thrice reduced ($P<0.05$) EPG on day 15 and day 21. Figure 5.6B shows the percentage of EPG reduction relative to the starting point.

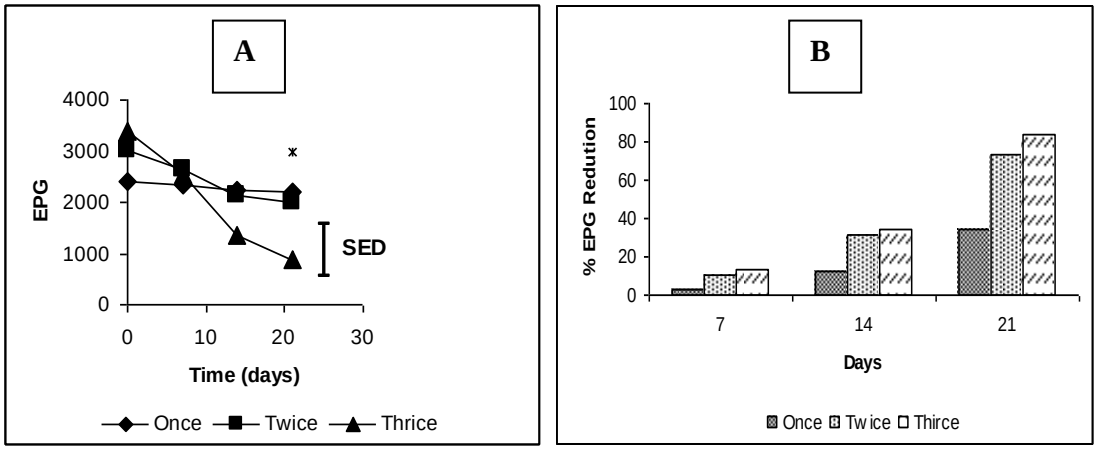

Figure 5.6 A: Effect of dosing tannin frequency on total nematode eggs count. B: % EPG Reduction (Once, twice and thrice stand for dosage once, twice and thrice using 1.6 g tannin/kg live weight)

5.3.6 Numbers of larvae recovered

Total larvae recovered are shown in Figure 5.7. The total larval count decreased with time and with the frequency of dosing. *Haemonchus* and *Trichostrongylus* proportions increased with time; on day 21, *Haemonchus* proportions followed the order: once> twice> thrice, whilst for *Trichostrongylus* the order was reversed: thrice>twice>once. The proportion of *Strongyloides* larvae had increased with time for all treatments to day 7; beyond day 7, there was a decrease with time for all treatments. *Nematodirus* and *Cooperia* were the lowest in proportions.

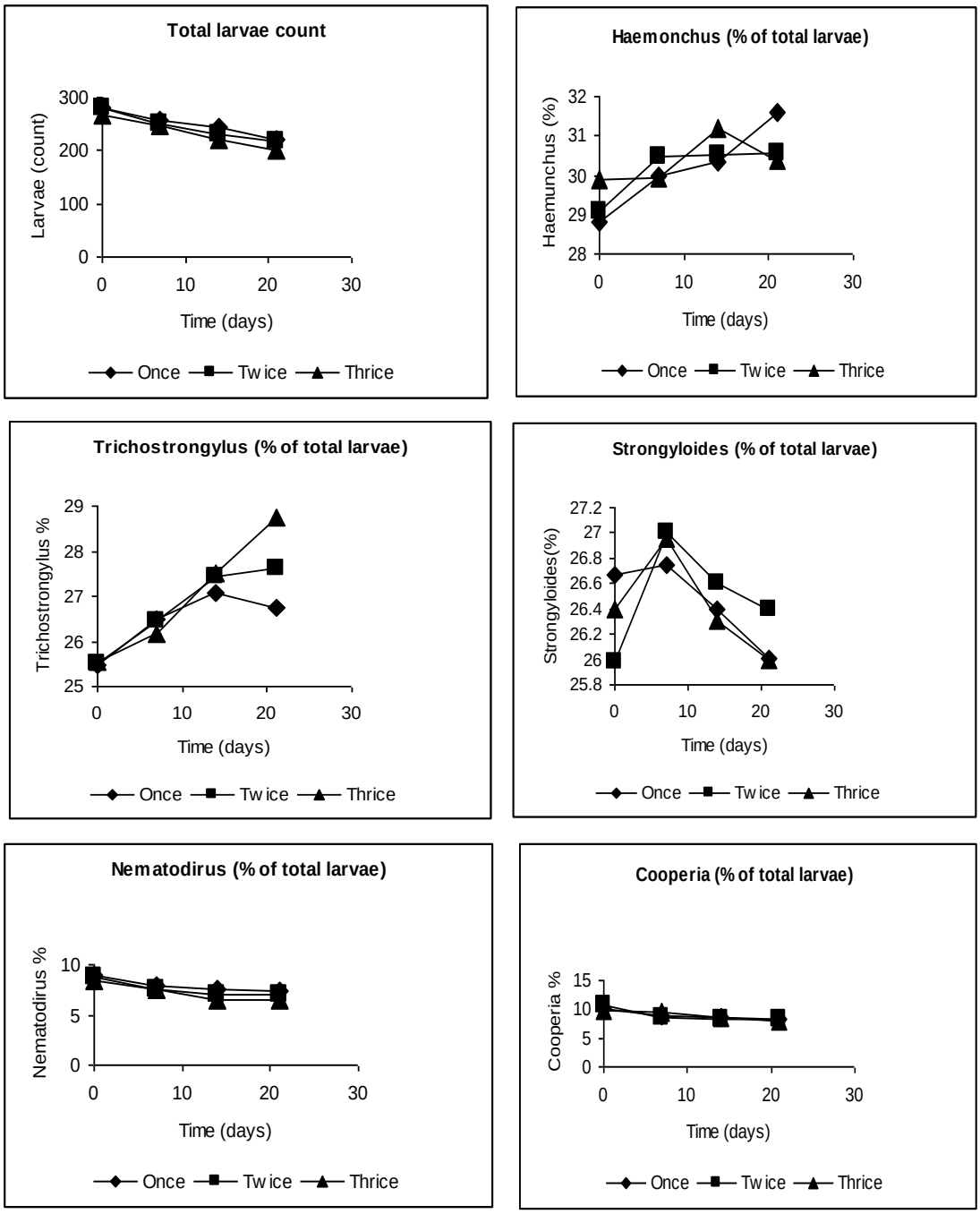

Figure 5.7 Effect of dosing tannin frequency on nematode larvae. SED values were: Total larvae 1.81, *Haemonchus spp* 0.19, *Trichostrongylus spp* 0.21, *Strongyloides spp* 0.16, *Namatodirus spp* 0.22 and *Cooperia spp* 0.15. (Once, twice and thrice stand for dosage once, twice and thrice using 1.6 g tannin/kg live weight).

5.4 Discussion

The infestation of herbage L_3 larvae ranged from 115 to 180 L_3 larvae/kg of dry herbage with mean 138 ± 28.9. This value is high enough to sustain continued re-infection of sheep over time. This could explain why T0.0 sustained high and stable EPG throughout the study, although the EPG for the tannin treatments have rapidly decreased. This could clearly indicate that tannins exert a direct and negative effect on gastro-intestinal nematodes. However, the highest reduction observed with the highest tannin level (2.4 g tannin/kg BW), and the difference between this level and 1.6 g tannin/kg BW was relatively small. A similar reduction in faecal egg counts that can be ascribed to tannins have been observed in tropical sheep infected with *Haemonchus contortus* and *Oesophagostomum,* administration of wattle tannin as an oral drench at 1.5 g tannin/kg BW for three consecutive days was shown to be effective in reducing faecal egg counts by 75% and worm burdens of infected tropical sheep with *Haemonchus contortus* by 86% and *Oesophagostomum* 28% (Max *et al.,* 2005).

Athanasiadou *et al.* (2001a) also observed that the ingestion of quebracho extract at a rate of 8% of dry matter intake (DMI) by infected sheep for 7 days caused a decrease in faecal egg counts and worm burden compared with parasites of control sheep. Other studies have shown that oral drench with quebracho extract was effective in reducing EPG and worm burdens of temperate sheep infected with *Haemonchus contortus* and *Trichostrongylus colubriformis* (Max *et al*., 2002) and in infected goats (Paolini *et al.,* 2003). In contrast, Max *et al.* (2003) revealed no significant reduction in EPG or worms burden in tropical goats drenched with wattle tannin. Since tropical goats typically browse tanniferous forages, it is possible that frequent exposure to tannin can in the long term result to a selection of nematodes that are less responsive to tannins.

Furthermore total larvae count increased with time for the control group, but decreased with time for the tannin treatments. Declining trends in the larvae yield also observed in a study (Molan *et al.,* 2000) where they reported that CT extract from several forages can inhibit hatching and larval development of *Trichostrongylus colubriformis*. Min *et al.* (2003) as well reported inhibition of egg hatching and larval development of *Haemonchus contortus* in goats grazing *Sericea Lespedeza.*

Dosing sheep with 1.6 g tannins/kg BW once, twice and thrice was shown to reduce the EPG by 34.6%, 72.9% and 83.6 % respectively on day 21 relative to the starting point. These results could mean; dosing tannin as an oral drench for three consecutive days was likely more effective than dosing once or twice. Drenching wattle tannin for three consecutive days in sheep has recently been reported in Tanzania (Max *et al.,* 2009). Administration of Black Head Persian sheep were drenched with 1.5g.tannins/kg BW, a significant reduction was observed in the EPG and warm burden. Minho *et al.* (2008) also reported that *Acacia molissima* tannin extract could control nematode parasites in lambs naturally infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*. A 60 days administration of condensed tannin extract at 1.6 g/kg BW for 2 consecutive days per month caused a reduction in EPG ($P<0.003$) and worm burden in the abomasum ($P<0.003$), but not in the small intestine.

Further notes taken in this study from the faecal culture larvae indicated a significant reduction in total larvae, but not in particular species. Therefore, no clinical sign of such parasite infection was observed e.g. edema or anemia. This could indicate their smaller infections degree and their adaptation with it. Paolini *et al.* (2003) had no significant decrease in worm population in infected goats with *Haemonchus contortus* after giving them 150ml of quebracho extract for eight days as 5% of dietary DM.

Tannin efficacy has been demonstrated in and outdoor. This study was modeling outdoor experiment. A study by Athanasiadou *et al.* (2000) resulted CT efficacy as 50% reduction of egg excretion and 30% reduction of worm burden in sheep drenched CT extract. Also a 30% reduction in the EPG has been observed in goats offered condensed tannin-containing forages (Kahiya *et al.,* 2003). Moreover, Min *et al.* (2004) indicated that 60% FEC reduction observed in goats grazing on *Sericea lespedeza* compared to those grazing on low tannin forages. The variation in the efficacy is likely due to different experimental conditions such as weather and epidemiological situation of the parasites.

Although, the mechanism by which tannins affect nematodes is yet undefined, it is possible that tannins form complexes with protein at the surface of parasites and as such disturb their metabolism, and/or that a metabolic response by nematode to tannins will divert nutrients away from reproduction, thus reducing fecundity. It is also possible that repeated drenching with tannins would numb, injure the external surface and/or kill the nematode.

In addition, Min *et al.* (2003) reported that condensed tannins might bind to faecal egg proteins, inhibiting egg hatching and larval development. Niezen *et al.* (1995) postulated that condensed tannins may bind and disturb the integrity of the parasites or affect the growth of the parasites. Moreover, Molan *et al.* (2000) explained that the manner which tannins affect the nematodes negatively could be qualified to a direct effect. However, Athanasiadou *et al.* (2001b) reported that there could be a physiological effect by increasing the host immunity. The consequence of EPG reduction and inhibition of larval development is a reduction of pastures contamination.

5.5 Conclusion

Wattle tannin reduced nematode egg production and infective larval yields. Drenching with 1.6 g wattle tannin/kg W delivered as an oral drench over three successive days is enough to reduce nematode egg production and reduce the degree of pasture contamination with infective larvae. Further studies are required to determine how wattle tannins can best be utilized in a treating system to maximize livestock production with reduced anthelmintic usage.

Chapter 6

General Discussion

Gastro-intestinal nematode parasites are currently becoming a difficult problem for small ruminant production. Synthetic anthelmentic drugs have been used for the control. However, nematode parasites are progressively developing resistance to these anthelmintics. In addition, the high cost of the drugs, their possible residual effect on animal by-products and adverse effects of the parasites seem to be pushing small scale farmers out of the industry. Treatment by dosing sheep with extract of forages containing anthelmentic properties such as wattle tannins could be an alternative strategy that is affordable by all farmers (Max *et al.,* 2005). The use of tannins in control is suitable particularly for the small farmers who do not have enough resources in terms of finance for buying expensive synthetic drugs for internal parasites. Also tannins are readily available in the tropics in many *Acacia spp*. High concentration of CT is said to have some negative effect on ruminants by reducing their voluntary intake due to the toxic effect of tannin and depression of microbial digestion in the rumen.

The internal parasites have not been investigated at Ukluinga Research Farm, University of KwaZulu-Natal, South Africa. Therefore, in this study five field experiments were conducted to determine the parasitological status of the farm. For this reason, the following questions were answered:

- What the epidemiology situation is and how can the weather conditions affect the internal parasites growth.
- Is there any resistance to the anthelmintics used at the farm?
- What else can help to impede the parasite growth?

To achieve the objectives, sheep and goats were used as models to determine the degree of internal parasite infections, herbage samples were used to investigate the pasture contamination and feacal culture to identify L_3. The parasite epidemiology and the environmental factors that affect parasite growth are considered due to the importance of these in the management of the parasites (chapter 3). Nematode parasites, *Eimieria spp* and

herbage L_3 were of higher prevalence in the wet season compared to dry season. Air temperature, rainfall and humidity seem to be having a direct affect on parasite growth. *Trichostrongylus spp, Haemonchus contortus*, *Strongyloides, Nematodirus* and *Cooperia spp* were found in the faecal culture during the investigation period.

Chapter (4) established the effectiveness of the anthelmintics used at this farm which are Ivermectin 1%, Closantel 5% and the combination Abamectin 0.08% and Praziquantel 1.5% to test the anthelmintics efficacy. Nematodes, especially *Haemonchus contortus,* had high resistance to the commercial anthelmintics tested whilst *Trichostrongylus spp* were the most affected by all drugs. However, Closantel was the most effective anthelmintic, with 70% efficacy.

The latter part of the study investigated the use of wattle tannins as alternative control strategies at Ukluinga Farm. Hence, three experiments (Chapter 5) were done to determine the effectiveness of the wattle tannins, which had a positive effect in controlling nematode parasites, and reduced pasture contamination. Numbers of nematode egg and larvae decreased as tannin levels increased between the drenches. In general tannin efficacy tended to increase with increasing tannin levels. Drenching wattle tannins for three consecutive days was the most effective compared to dosing twice or once.

Based on this study, it can be concluded that the growth rate of nematode parasites and *Eimeria* oocysts had seasonal pattern. Nematode parasites had high resistance to anthelmintics. Wattle tannins could have potential to control gastro-intestinal parasites. Small-scale farmers can incorporate tannins into the management of their small stock in different seasonal conditions. Although the results are reasonably positive, further investigation is still required to confirm the way to exploit the wattle tannins to control gastrointestinal nematode parasites in small ruminants.

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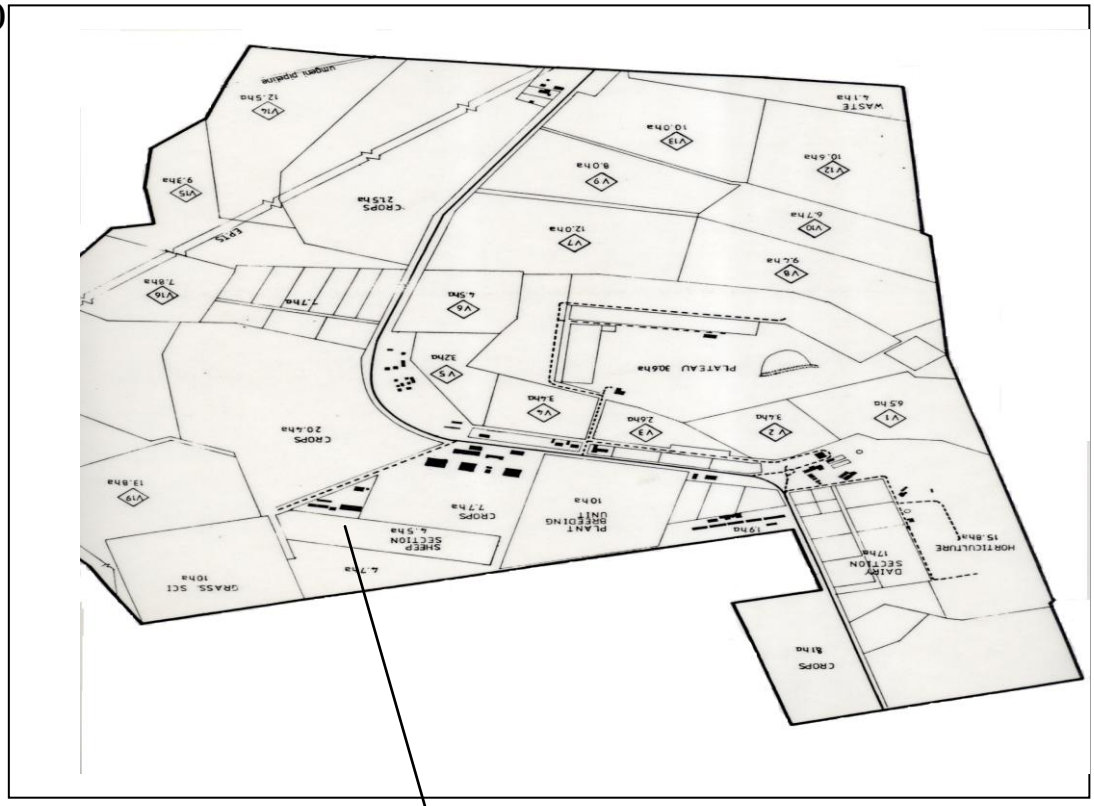
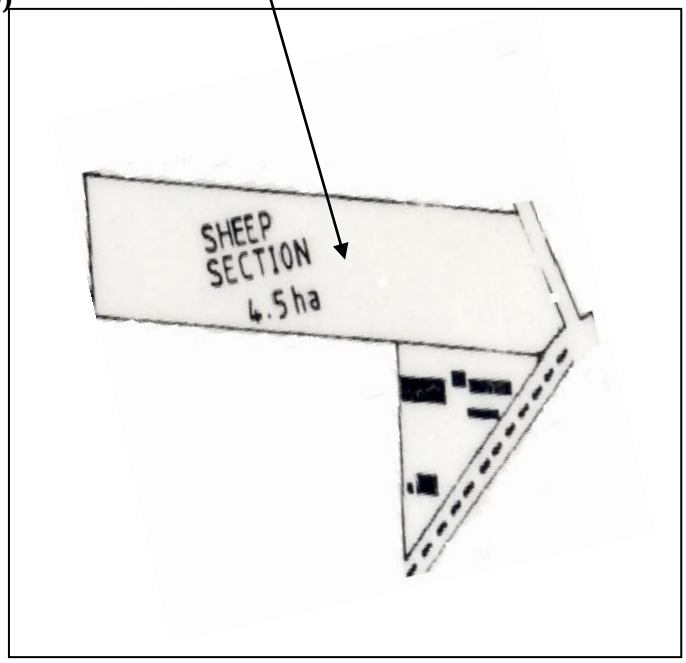
Appendix 1

(A)



(B)



Schematic diagram of the (A) Ukulinga Research Farm (B) Livestock Section (experiment site).

Appendix 2

Nguni goats grazing contaminated kikuyu (*pennisettum clandenstinum*) pasture at Ukulinga Research farm.

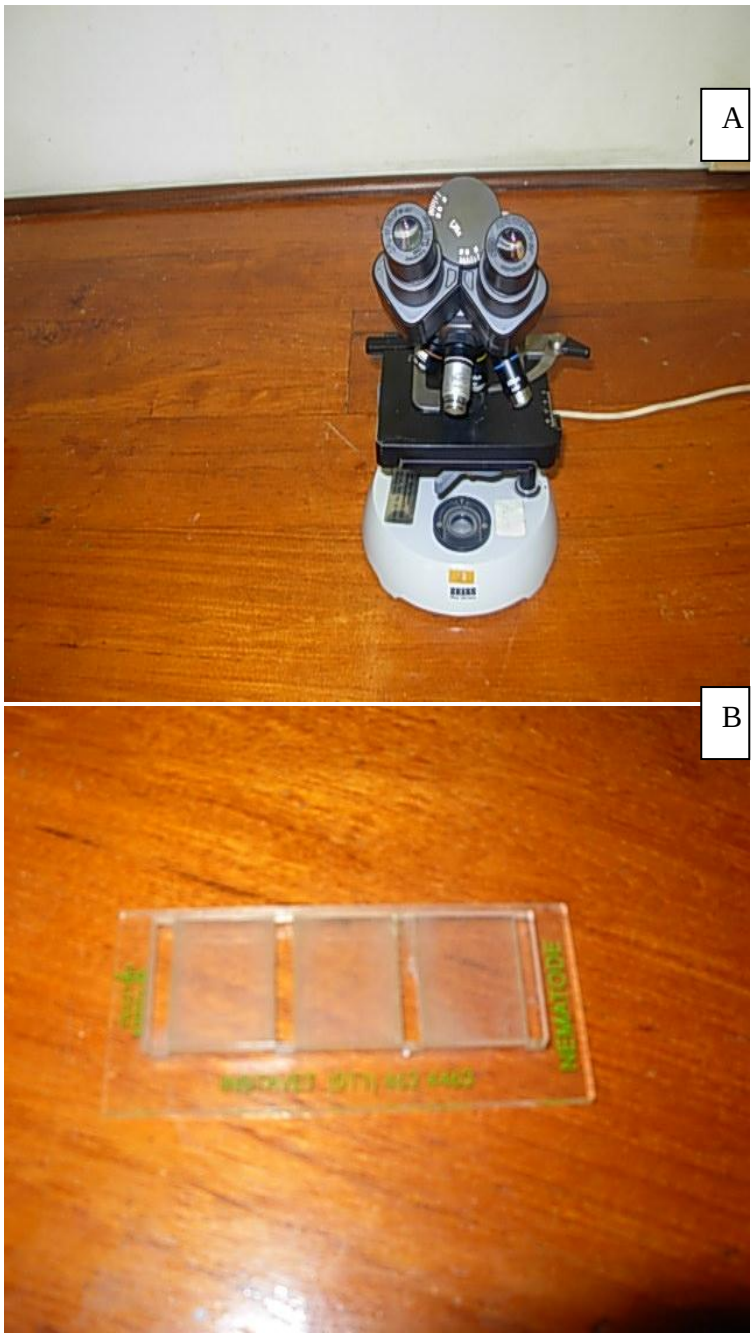
Appendix 3

Merino sheep grazing contaminated kikuyu (*pennisettum clandenstinum*) pasture at Ukulinga Research farm.

Appendix 4

Incubator used for incubated faecal culture
A. from outside
B. from inside

Appendix 5



A. A Microscope used in the lab analysis
B. Mc Master Slide

Appendix 6
Formulation of the flotation fluid

Saturated Salt Solution:

Soudium chloride (salt)	400g
Water	1000 ml

Specific gravity :1.200

Add salt until saturation, indicated by the presence of salt at the bottom of the container after stirring for 15 minutes.

Appendix 7
Formulation for other reagents used in diagnostic tests

Iodine Stain:

Iodine re-sublimed crystals	10g
Potassium iodide	50g
Water	1000ml

Dissolve the potassium iodine in the water. Then add and dissolve the iodine crystals.

Sodium Thiosulphate Stain:

Sodium thiosulphate	124.1g
Water	1000ml

Dissolve the Sodium thiosulphate crystals in the water.