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# Gastrointestinal nematodes of Ovis aries in Eastern Cape, South Africa and an evaluation of current anthelmintic procedures

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# Gastrointestinal nematodes of *Ovis aries* in Eastern Cape, South Africa and an evaluation of current anthelmintic procedures

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## ABSTRACT

Humans have known about helminth infections since ancient times. Today half of the human population is plagued by a nematode infection. Nematodes are responsible for billions of dollars in global crop damage annually and have had devastating effects on the global livestock industry. Due to the lack of a vaccine, and only a handful of approved classes of anthelmintics, nematode resistance has become a serious global phenomenon, and the South African commercial sheep industry has been one of the worst affected worldwide. The current study used fecal float parasitology to determine the nematode species found on a commercial sheep farm in Eastern Cape, South Africa, and the severity of their burden. There were five main species of nematodes found: Strongyles, *Strongyloides* spp., *Trichuris ovis*, *Nematodirus spathiger*, and *Haemonchus contortus*. The farm had two different populations of sheep – the flock population which were of acceptable wool quality and the stud population which were of superior wool quality. The two populations were compared to each other. There was no significant difference in nematode species present or severity of infection between the two populations. Although the nematode burden of this farm was not as severe as many other South African farms, a discussion of current anthelmintic treatments and possible practices that may improve animal health and wool quality are discussed.

# 1. INTRODUCTION

## 1.1 History of Parasitology

From the surviving documents of many ancient civilizations, it is clear that life, disease and death are some of humanity's oldest fascinations (Weatherall et al., 2006). In ancient cultures, disease and illness were a mystery. Some cultures believed it was a spiritual phenomenon; or that the Gods were punishing them; or in the case of the ancient Greeks, that an imbalance of the four humors of the body was the reason for illness (Weatherall et al., 2006). Although Ancient civilizations were unaware of the common microscopic culprits of disease such as bacteria and viruses, they were aware of some species of helminths or parasitic worms and documentation of some of these infections can be found in ancient texts all over the globe (Cox, 2002). The first written records of what are believed to be parasitic infections come from ancient Egypt from the years 3000 BC to 400 BC. The Egyptian Ebers papyrus of 1500 BC provides descriptions of intestinal worms which have been confirmed by the discovery of calcified helminth eggs in mummies dating back to 1200 BC (Cox, 2002). The helminth species that the ancient civilizations were aware of were likely the larger species such as Ascaris and tapeworms which can be observed with the naked eye (Cox, 2002).

As more scientific discoveries were made throughout the ages, our understanding of life and disease grew (Weatherall, et al., 2006). In the 17$^{th}$ century Robert Hooke developed the microscope, exposing a whole world that until then had been all but unknown (Weatherall, et al., 2006). In the 19$^{th}$ century Louis Pasteur and Robert Koch founded modern microbiology, furthering humanity's understanding of the invisible, microscopic world (Weatherall, et al., 2006). With these advances in understanding the microscopic world came the discovery of many other organisms and helminth species, otherwise invisible to the naked eye that could have devastating effects on humans (Weatherall, et al., 2006). Today, thanks to modern technology, humans are known to be host to nearly 300 species of helminths (Cox, 2002). But humans are not the only ones affected by helminths, livestock, crops and pets are all victims of parasitic worms, which have drastic effects on the human population as well (Taylor, et al., 2013).

## 1.2 Helminth Classification

A parasite is any organism that lives on or in another organism also known as a host, at the expense of the host (Georgi, 1985). Parasitic species can be found in virtually all of the animal kingdoms, but the parasites of interest here are parasitic worms or helminths. Helminth is a generic name derived from the Greek word for worm. They are metazoans which can be divided into two phyla: Platyhelminthes and Nematoda (Castro, 1996).

Platyhelminthes also known as flat worms get their name from the Greek root for flat (Castro, 1996). The phylum can be subdivided into three different classes: Turbellaria, Trematoda and Cestoda (Georgi, 1985). The Turbellaria or planarians are free-living, carnivorous flatworms that may occasionally be found in aquariums but are not typically parasitic (Georgi, 1985). Trematodes also known as fluke worms, are flat, leaf-shaped parasitic worms (Castro, 1996). They can vary in length from a few millimeters to 8cm. they have an oral sucker and a ventral sucker or acetabulum which is used to attach to host tissues (Castro, 1996). Trematodes lack a body cavity and their internal organs are imbedded in a type of connective tissue called parenchyma (Castro, 1996). Cestodes, are more commonly known as tapeworms. Because they are classified as Platyhelminthes they share many basic structural characteristics with trematodes (Castro, 1996). Unlike trematodes however, they are large, elongated segmented worms that can range from a few millimeters up to 10 meters (Castro, 1996). Cestodes are typically found inside the intestines of their host (Castro, 1996).

Organisms of the second phylum of helminths, Nematoda, are also known as round worms (Castro, 1996). Nematodes are worms with cylindrical bodies; a large, fluid filled body cavity or pseudocoelom; and a protective noncellular outer cuticle surrounding the body wall (Castro, 1996; Georgi, 1985). Nematodes also have a complete alimentary canal with both a mouth and anus (Castro, 1996). They are typically bisexual, reproduce sexually and males are almost always smaller than females and the developmental process of nematodes includes egg, larval and adult stages (Castro, 1996). In this paper, all species of helminths discussed belong to the nematoda phylum.

## 1.3 Anthelmintic Development and Classification

Anthelmintics are a class of antiparasitics which specifically target parasitic worms (Georgi, 1985). Two hundred years ago, treatment for parasitic infections in people and livestock was very basic if given at all and typically consisted of either metals or plant extracts (McKellar & Jackson, 2004). Over the next 120 years some small advances were made and arsenicals, nicotine sulfate, copper sulfate and carbon tetrachloride became commonly used anthelmintics (McKellar &Jackson, 2004). But although these drugs proved to be more effective than the rudimentary medicines of the past, they still had serious shortcomings in their spectrum of activity, efficacy and toxicity (McKellar & Jackson, 2004).

In 1940, phenothiazine was introduced and 14 years later piperazine was introduced as veterinary anthelmintics (McKellar & Jackson, 2004). These compounds proved to be much more effective than previous anthelmintics against target infections and were also met with acceptable tolerance in host animals (McKellar & Jackson, 2004). However, the greatest progress in anthelmintic development occurred between 1960 and 1980 with the discoveries of thiabendazole and levamisole. These compounds proved to have *in vivo* potency at much smaller doses than any other compound to date (McKellar & Jackson, 2004). Thiabendazole also led to the discovery of the benzimidazoles, a class of structural analogues with excellent broad spectrum activity and safety in host animals (McKellar & Jackson, 2004). In 1981, ivermectin was introduced for treatment of helminth infections in animals. Ivermectin proved to be effective at the microgram per kilogram dosage. It also had excellent broad spectrum efficiency and safety in host animals (McKellar & Jackson, 2004). Today there are a number of anthelmintic classes approved for veterinary use. These include: benzimidazoles and probenzimidazoles, salicylanilides and substituted phenols, imidazothiazoles, tetrahydropyrimidines, organophosphates, macrocyclic lactones and more recently, the amino-acetonitrile derivatives, the cyclic octadepsipeptides, and the spiroindoles (Vercruysse & Claerebout, 2014).

The benzimidazoles act by inhibiting nematode tubulin polymerization (Vercruysse & Claerebout, 2014). This in turn inhibits microtubule formation and therefore the transportation of secretary granules and enzymes within the cell cytoplasm (McKellar & Jackson, 2004). Additional effects of benzimidazoles on nematodes include depletion of energy reserves and the inhibition of waste excretion (Vercruysse & Claerebout, 2014). Nematode resistance to the benzimidazoles has been found to be caused by an alteration in the β-tubulin which significantly decreases the benzimidazoles binding affinity for the receptor (Vercruysse & Claerebout, 2014).

The salicylanilides and substituted phenols act by uncoupling helminth oxidative phosphorylation (Vercruysse & Claerebout, 2014). These lipophilic ionophores shuttle protons across the mitochondrial membrane affecting oxidative phosphorylation and ATP production leading to cell and organism death (Martin, 1997). The imidazothiazoles and tetrahydropyrimidines both act as cholinergic agonists at nicotinic neuromuscular junctions (McKellar & Jackson, 2004). They open and then block acetylcholine receptor-mediated ion channels leading to muscle contractions and spastic paralysis (Martin, 1997; McKellar & Jackson, 2004). One concern with these classes of anthelmintics however is the fact that they also act as nicotinic agonists in the mammal hosts leading to a much narrower therapeutic index than other classes (McKellar & Jackson, 2004).

The organophosphate class of anthelmintics acts by inhibiting the enzyme acetylcholinesterase. (Martin, 1997) This leads to a buildup of the neurotransmitter acetylcholine, leading to the phosphorylation of esterification sites, blocking the transmission of cholinergic nerve impulses (Martin, 1997; Vercruysse & Claerebout, 2014). This ultimately leads to spastic paralysis and death of the nematode (Vercruysse & Claerebout, 2014). Macrocyclic lactones were originally thought to act by inducing the release of the neurotransmitter gamma amino butyric acid (GABA) which interferes with neuronal transmission (McKellar & Jackson, 2004). Although macrocyclic lactones do in fact act in this way to some degree it is now known that their primary mechanism of action is their receptor mediated effect on glutamate-gated chloride ion channels which increases muscle cell chloride ion permeability (Martin, 1997).

The only available amino-acetonitrile derivative on the market today is Monepantel (Vercruysse & Claerebout, 2014). It acts as an agonist of the mptl-1 channel, a channel belonging to a class of nicotinic acetylcholine receptors. It causes constant fluctuation in muscle ions leading to muscle depolarization and irreversible nematode paralysis (Vercruysse & Claerebout, 2014). The spiroindoles act as antagonists of beta-subtype nicotinic acetylcholine receptors in nematode neuromuscular junctions leading to flaccid paralysis and death (Vercruysse & Claerebout, 2014). Although a number of anthelmintics have been developed with many different mechanisms of action as mentioned above, the victories have been short lived.

Resistance among target helminth species has been reported in all of the approved classes of anthelmintics (Vercruysse & Claerebout, 2014).

### 1.4 Anthelmintic Resistance

Gastrointestinal nematode infections plague 50% of the human population worldwide and lead to thousands of deaths annually (Stepek et al., 2006). But despite the advances made by modern medicine, the proportion of the human population affected by nematodes is not falling. This is the result of a combination of factors such as lack of sanitation and health education in developing countries (Stepek et al., 2006). A newer and much more serious factor is anthelmintic resistance. In the absence of vaccines, infections have been controlled by only a few classes of approved drugs which has led to nematode species rapidly developing resistance to anthelmintics, the effects of which can already be seen on a global scale (Kaminsky et al., 2008; Taylor et al., 2013). It is estimated that nematode species that feed on plants are responsible for approximately $100 billion in global crop damage annually (Taylor et al., 2013). But nematodes don't just affect humans and crops. Parasitic nematodes have led to substantial economic losses to livestock production globally (Kaminsky et al., 2008).

The resistance of nematodes to the three major classes of anthelmintics – the benzimidazoles, imidazothiazoles and the macrocyclic lactones has become a phenomenon in gastrointestinal nematodes of farm animals worldwide (Kaminsky et al., 2008). Although there is no doubt that anthelmintic resistance is of global concern, the South African commercial sheep industry has been reported as the population worst affected by anthelmintic resistance worldwide (Tsotetsi et al., 2012). In 1975 anthelmintic resistance in sheep was reported for the first time in South Africa, and the severity of resistance has increased rapidly ever since (Van Wyk et al., 1996). It was found that about 88% of South African farmers are aware of veterinary helminthosis and that 67% of them treat to prevent helminth infections (Tsotetsi et al., 2012).

The primary means of controlling nematode infections in livestock employed by South African farmers is the use of anthelmintic drugs primarily ivermectin, albendazole and levamisole (Tsotetsi et al., 2012). But despite their efforts, 90% of South African sheep farms harbor nematode strains resistant to at least one class of anthelmintics and 40% of South African farms have been found to house strains of nematodes resistant to three or more classes of anthelmintics (Van Wyk et al., 1996). Although farmers are doing their best to combat nematode infections, the severity of anthelmintic resistance has led to a decrease in their efficacy (Tsotetsi et al., 2012). Multi-drug resistant nematodes, some resistant to all classes of anthelmintics, are found worldwide (Kaminsky et al., 2008). Nematodes are becoming resistant to available anthelmintics faster than new anthelmintics are being produced and no reversion of susceptibility seems to have occurred (Van Wyk et al., 1996). This rapid development of multi-drug resistance emphasizes the need to develop new classes of anthelmintics (Kaminsky et al., 2008; McKellar & Jackson, 2004). But aside from developing new anthelmintics, new holistic solutions must also be investigated so as to avoid an overreliance on anthelmintic drugs (Bath, 2014). By doing this, nematode populations and infections may be controlled in a way that also prolongs the useful lifespan of future anthelmintics (Bath, 2014).

## 1.5 Current Study

In the current study the nematode species present and the severity of infection on a commercial sheep farm in Eastern Cape, South Africa was investigated. Although nematode infections are a problem worldwide and especially in South Africa, I wanted to learn more about the nematode species present there. The most common and significant species of nematodes found included strongyles, *Strongyloides* spp., *Trichuris ovis*, *Nematodirus spathiger*, and *Haemonchus contortus*. Also, because there is such a heavy reliance on anthelmintic drugs, I wanted to examine the current procedures that are being used to control nematode populations and infections in the hopes of finding ways to improve it.

Although the production of new anthelmintic drugs will ultimately be the most effective means of controlling the nematode population and curbing the growing issue of anthelmintic resistance, I was interested to see if a different anthelmintic class or some simple nonmedicinal practices might help improve both animal health and wool quality on this farm and potentially other farms in the area. I hope that my findings and suggestions might help the farm produce better quality wool, have happier, healthier animals, save money and control their nematode populations using both anthelmintics and nonmedicinal practices as a way to maintain the effectiveness of current anthelmintics and prolong the useful lifespan of future anthelmintics.

# 2. BACKGROUND

## 2.1 Nama Karoo Climate

The Nama Karoo is a vulnerable region of the Afrotropical ecozone. It is a vast, open and arid region whose vegetation is primarily dwarf shrubs and grass. Steep, rugged mountains and cliffs lie scattered among the sprawling flatlands of the Nama Karoo (Dean & Milton, 1999). Its lack of rich species diversity can be attributed to its harsh climatic fluctuations, but the species that do live there are well adapted to tolerating its extremes (Lovegrove, 1993). Most of the Nama Karoo is located on the central plateau of South Africa’s Cape Providence. The climate is very harsh, commonly being affected by droughts with considerable fluctuations in both seasonal and daily temperatures. Temperature variations between day and night as great as 25°C are not uncommon. The average maximum temperature in mid-summer (January) exceeds 30°C (86°F) and mean minimum winter (July) temperatures are regularly below freezing. Rainfall is seasonal, with highest rainfall occurring during summer and spring (December – March) with an average annual rainfall between 100 mm and 500 mm (Palmer & Hoffman, 1997).

## 2.2 Strongyles

The term strongyle is a nonspecific label given to a wide variety of nematodes based on the appearance of the ovum (Georgi, 1985). Strongyle eggs are characterized as ellipsoidal eggs with thin walls and an embryo in the morula stage of development that persists even after being shed in host feces (Georgi, 1985). Ova laid by female worms from the superfamilies Strongyloidea, Trichstrongyloidea and Ancylostomatoidea are all considered to be strongyle eggs (Georgi, 1985). It is often not possible to identify strongyle eggs to the genus level because eggs of the strongyloidea and thrichstrongyloidea superfamilies are usually similar in both size and appearance. If a more specific identification than "strongyle" is needed, the sample must be cultured to the third stage larvae for further examination and definitive identification (Georgi, 1985).

For the most part, the life cycles of the three nematode superfamilies that are considered strongyles are very similar (Georgi, 1985). First, the eggs are shed in the feces, where after a day or two they hatch producing the free living first stage larvae. This larvae feeds then molts into the second stage larvae which is also free living (Georgi, 1985). When the larvae molts into the infective third stage, the cuticle is temporarily retained for protection. These protected larvae migrate out of the feces after approximately one week and into water films on nearby soil and plants (Georgi, 1985). Once the third stage infective larvae is ingested by a new host, it sheds its protective cuticle and migrates to the gut. There it reproduces sexually, the female lays her eggs which are passed in the feces and the cycle continues (Georgi, 1985).

### 2.3 *Strongyloides* spp.

*Strongyloides* is a genus of obligate parasitic nematodes found in the gastrointestinal tracts of various vertebrates including mammals, birds, amphibians, and reptiles (Viney & Lok, 2007). Fifty species of *Strongyloides* have been identified, all of which are highly specific, only infecting one or at most very few different species (Viney & Lok, 2007). *Strongyloides* infections are relatively common amongst both wild and domestic animals and infections can be detected by eggs or larvae in the host's feces or by examination of the small intestines where worms are most often concentrated in the upper half to third of the small intestine. The adult female worm is the only parasitic stage of *Strongyloides* and when the intestines are observed microscopically, worms and dark trails of eggs left behind by the female can be seen in the intestinal mucosa (Viney & Lok, 2007). The examination of the small intestine unfortunately requires the sacrifice of the host (Viney & Lok, 2007). Another common characteristic of the *Strongyloides* genus is the unique presence of a free-living, dioecious adult generation, in fact, the free-living adult worm was classified as a distinct species from the intestinal parasite until it was later discovered that both were in fact different stages of the same life cycle (Viney & Lok, 2007).

The life cycle of *Strongyloides ratti*, the species of *Strongyloides* known to infect rats, is the best understood of the *Strongyloides* species (Viney & Lok, 2007). While some differences are likely to abound between the life cycles of *S. ratti*, and the *Strongyloides* species found in this study, the life cycle of *S. ratti* is discussed here due to the fact that the species of *Strongyloides* found in this study is unclear. The host first becomes infected with *S. ratti* when the infective third stage larvae penetrate the host's skin (Viney & Lok, 2007). Twenty-four hours post infection, the larvae can be found in the naso-frontal region of the host where they are believed to then be swallowed and migrate to the small intestine (Viney & Lok, 2007). Some *Strongyloides* species may migrate to the small intestine via the lung as well. While migrating to the small intestine, the worms molt to the fourth stage adult worm approximately four days post infection (Viney & Lok, 2007). Once in the small intestine, the female worms burrow into the lining of the gut and begin laying eggs. Shortly after the female begins laying her eggs they will be detectable in the feces (Viney & Lok, 2007).

Once the eggs in the feces hatch into first stage larvae, they have two potential developmental fates (Viney & Lok, 2007). They can develop and reproduce via sexual or heterogonic development, progressing via the second and third stage to fourth stage male and female rhabditiform worms. This free living stage then mate and the female lays her eggs which later hatch to release first stage larvae. These first stage larvae molt from the second stage to the third stage infective filariform which can persist in the environment and travel to find a new host (Viney & Lok, 2007). Most *Strongyloides* species typically only have one free living adult generation before developing into infective larvae, however, some species such as *S. planiceps* have been known to have multiple generations of sexually reproducing free living rhabditiform worms (Viney & Lok, 2007).

The second potential developmental fate leads to homogonic or asexual reproduction. The first stage larvae molt and develop directly into infective third stage worms via second stage development. These third stage worms can infect a new host and the cycle repeats (Viney & Lok, 2007). Whether *Strongyloides* develop via direct or indirect development depends on many factors including the sex ratios of free living worms as well as environmental conditions (Viney & Lok, 2007).

## 2.4 *Trichuris ovis*

The eggs of worms belonging to the *Trichuris* genus are brownish yellow in color and have a barrel-like shape with a thick membrane (Georgi, 1985). They also have characteristic plugs at either pole of the ovum and are approximately 40 µm by 70µm in size (Georgi, 1985). Eggs of the *Trichuris* genus are extremely resistant to both cold, frost and drought and can persist in the pastures and environment for years, making eradication extremely difficult (Junquera, 2015). The adult worms are white to yellow in color and vary from 3 to 8 cm in length with males being smaller than females (Junquera, 2015). Worms of the *Trichuris* genus are commonly referred to as whipworms due to their characteristic wipe-like shape (Georgi, 1985). They are covered in a flexible, protective cuticle, making them exceptionally durable and resistant to environmental changes (Junquera, 2015). *Trichuris* worms have a tubular digestive system with both a mouth and an anus and lack excretory organs. They have a nervous system but lack a circulatory system (Junquera, 2015).

*Trichuris* worms undergo a direct life cycle (Junquera, 2015). Female worms can produce several thousand of eggs per day which are shed in the host's feces. Once shed, the larvae begin to develop inside the eggs for 10-25 days depending on species and environmental factors such as temperature (Junquera, 2015). Hosts become infected with *Trichuris* worms when they ingest food and water contaminated with eggs (Junquera, 2015). Once inside the host, the worms hatch and travel to the small intestine where they enter the intestinal mucosa, move to the cecum and complete development to adults. The adult worms feed on the host's blood, sexually reproduce, the eggs are shed in the feces and the cycle continues (Junquera, 2015). The prepatent period for *Trichuris* infections is between 50 and 90 days depending on species and the severity of infection (Junquera, 2015).

Infections with worms belonging to the *Trichuris* genus are often benign and livestock infected are often asymptomatic except for in cases of severe infection (Georgi, 1985). Signs of livestock suffering from severe *Trichuris* infections include enteritis, ulcerations, bleeding and anemia, bloody diarrhea, dehydration, and loss of appetite and subsequent weight loss (Georgi, 1985). Fatalities from infections with *Trichuris* spp. are possible however, they are rare and typically only seen in young animals (Georgi, 1985). Prevention is extremely difficult in livestock due to the extreme resistance of eggs to environmental factors and their ability to persist for years without death (Junquera, 2015). *Trichuris* infections have been successfully managed with the use of both benzimidazole and macrocyclic lactone anthelmintics (Junquera, 2015).

## 2.5 *Nematodirus spathiger*

Eggs of *Nematodirus* worms are the largest nematode eggs found in ruminant gastrointestinal tracts (Junquera, 2014). They are ovoid ranging in size from 70-120 µm by 130-230 µm depending on species (Junquera, 2014). They are characterized as having a thick shell and a morula of 4-8 cells (Junquera, 2014). This is unlike many other gastrointestinal nematode species which typically have morulae of 16 cells (Georgi, 1985). The eggs of *Nematodirus* species are very resistant to cold, allowing them to lie dormant and persist in pastures and the environment for long periods of time (Junquera, 2014).

Worms of the *Nematodirus* genus, sometimes referred to as thin-necked or thread-necked worms get their name from their characteristic bulbous head and thin neck (Junquera, 2014).

Worms may vary in length from 1-2.5cm long depending on species. Adult worms are typically whitish in color and females are larger than males (Junquera, 2014). Adult female worms also have a spine at the tip of their tail which can be used as a quick indicator of sex (Georgi, 1985). While inside the gastrointestinal tract of a host, the worm remains inside a protective cuticle (Junquera, 2014). All *Nematodirus* worms are characterized as having a tubular digestive system with a mouth and an anus. They also have a nervous system but lack a circulatory and excretory system (Junquera, 2014).

*Nematodirus* worms have a direct life cycle (Junquera, 2014). Female worms lay eggs in the host's small intestine which are then shed in the feces. The worms develop from L1 to L2 then to infective L3 stage larvae inside the egg and feces which makes them extremely resistant to both cold and dryness. Development to infective larvae can take anywhere from a few weeks to months depending on the species and environmental conditions (Junquera, 2014). Due to the larvae's extreme resistance to cold and dryness, eggs may either hatch immediately or lie dormant in the host or the environment until spring. This ability to lie dormant for an extended period of time is called hypobiosis (Junquera, 2014). Some species, such as *N. battus* must first be subject to a cold period before they will hatch (Junquera, 2014).

Other species such as *N. spathiger* can undergo hypobiosis for several months, this can result in infections occurring late in summer or the larvae may lie dormant through the winter inside either the host or the environment (Junquera, 2014). Livestock become infected with *Nematodirus* worms when they ingest pasture contaminated with infective L3 larvae either inside or outside the egg (Junquera, 2014). After ingestion, the L3 larvae travel to the host's small intestine (Audebert et al., 2004). A few days after ingestion, the L3 larvae molt into sheathed L4 larvae. After a few days the L4 larvae develop into immature worms (Audebert et al., 2004).

These worms then develop into mature worms, reproduce sexually. The eggs are shed in the feces and the cycle repeats (Junquera, 2014).

Infections with *Nematodirus* species are not typically very pathogenic with the exception being *N. battus* infections in lambs (Junquera, 2014). Chronic infections with other *Nematodirus* species typically results in reduced weight gain or weight loss, and loss of appetite (Junquera, 2014). Infections with *Nematodirus* species can be controlled using either benzimidazole or macrocyclic lactone anthelmintics, however, animals usually develop natural resistance to *Nematodirus* worms (Junquera, 2014). Animals with natural resistance will not become sick but will still shed eggs in their feces which could infect other animals (Junquera, 2014).

### 2.6 *Haemonchus contortus*

*Haemonchus contortus* known by common names such as stomach worm, barber's pole worm, and wire worm is a parasite commonly found in small ruminants worldwide and is of concern to their health and productivity (Machen et al., 1994; Tsotetsi et al., 2012). *Haemonchus contortus* gets the name barber's pole worm due to the white egg-filled uterus that spirals around its blood-filled gut giving it a characteristic red and white striped appearance resembling a barber's pole (Georgi, 1985). They are cylindrical nematodes that are tapered at both ends with a complete digestive system (Leite-Browning, 2006). Adults can range from 10 mm to 30mm in length and female adults can produce as many as 5,000 eggs per day (Machen et al., 1994).

While grazing, the host ingests third stage infective larvae which travel to the abomasum and burrow into the lining. Here, it develops into the fourth or pre-adult stage, and then molts into the fifth stage adult worm which feeds on the blood of the host (Machen et al., 1994). These fifth stage worms mate, producing eggs which are passed in the feces and deposited on the pasture. These eggs hatch into the first stage, progressing though the second and third stages on pasture (Machen et al., 1994). Propagation is favored in warm, moist conditions whereas hot and dry or cold conditions can be detrimental to larvae on pasture (Machen et al., 1994). The third stage larvae in the pasture are ingested, burrowing into the abomasum of a new host, thus completing the cycle (Leite-Browning, 2006). This life cycle takes 17-21 days (Machen et al., 1994).

A unique aspect of the *H. contortus* life cycle is that it is able to undergo hypobiosis, remaining in a dormant third stage over the winter inside the host abomasum (Leite-Browning, 2006). The larvae begin to undergo hypobiosis in the fall during which they do not feed or lay eggs, thus not doing any harm to the host. The larvae that are left in the field over the winder will die off (Leite-Browning, 2006). When the ewe reproduces, it indicates to the dormant larvae that it is nearly spring and the worm continues to develop (Leite-Browning, 2006). Lactating ewes cannot control the heavy parasitic burden as effectively as dry ewes leading to a rapid onset of a heavy parasitic load in spring. This leads to both a high number of larvae being shed in the feces as well as an increased risk of illness caused by the heavy parasitic burden (Leite-Browning, 2006). The increase in shed larvae increases the parasite population of the pasture in turn increasing the risk of infection for other sheep in the flock (Leite-Browning, 2006).

*Haemonchus contortus* can be present in a host without any deleterious effects (Georgi, 1985). Pathogenesis only occurs as a result of infection where blood loss from the feeding nematodes exceeds the host's hematopoietic capacity to overcome it (Georgi, 1985). Poor nutrition, heavy parasitic loads, defective phenotypes and stress can all contribute to the development of pathogenic effects from *H. contortus* infection (Georgi, 1985). At peak infection, *H. contortus* can remove up to one fifth of the circulating erythrocyte volume per day from lambs and average one tenth of the circulating erythrocyte volume over the course of a two month nonfatal infection (Georgi, 1985). Signs of Haemonchosis include pallor of the skin and mucous membranes, loss of plasma protein leads to anasarca commonly manifesting as submaxillary edema or “bottle jaw”. The appetite typically remains good and noticeable weight loss may or may not occur. Diarrhea is uncommon, but if present, it usually indicates a secondary infection with another parasite (Georgi, 1985). If hematocrit levels are less than 15% and accompanied by extreme weakness and shortness of breath prognosis is typically not good as progressive anemia rapidly leads to death (Georgi, 1985). Young animals and females a month before or after parturition are most at risk of pathogenic infections of *H. contortus* (Machen et al., 1994).

# 3. METHODS

Testing was conducted on a farm centered between the towns of Graaff-Reinet, Middleburg, and Cradock in Eastern Cape, South Africa. This region is known as the Karoo, an arid region of the Eastern Cape. The farm was home to approximately 2,000 sheep and samples were collected between 21 – 25 July 2014. The pastures had very little vegetation and were largely dirt and short grass, with the primary food source being bunks that were filled by the staff with forage. There was a river that created a figure-8 shape around and through the farm property but did not run through any pastures where samples were taken.

The sheep were housed on different pastures depending on their sex and wool quality. These different classifications were the stud pasture, which housed ewes of the highest quality fleece, and the flock pasture which housed ewes of acceptable wool quality. Feces were collected from the ground and stored in plastic bags. The bags were labeled according to the respective pastures they were collected in. Due to this, it is unclear which individual sheep the samples were collected from but enough samples were taken to get an accurate representation of the population for each field.

To test for intestinal nematodes, fecal floats were performed. Approximately one gram of feces was transferred into a Vetoquinol fecalyzer. Feca-med by Vedco, a pre-mixed sodium nitrate flotation solution was filled to a designated line on the fecalyzer. The inner chamber of the fecalyzer was rotated repeatedly to remove the ova from the feces and suspend them in solution. The flotation solution was then used to fill the fecalyzer until a meniscus formed over the top. A 22mm cover slip was then placed over the meniscus and allowed to sit for 15-20 minutes. The cover slip was then removed and transferred to a slide. The slide was immediately examined in a back and forth sweeping pattern at 100× magnification. The number of ova seen for different parasite species were tallied for the entire slide and the prevalence (P) of animals harboring each nematode was calculated as P=number of infected hosts/number of examined hosts×100. Images were taken at 450× magnification for clarification and identification purposes.

The farm's current anthelmintic medications and parasite prevention procedures were then examined for their effectiveness against the species of nematodes found in the sheep. This was done to determine if the farmer was potentially over treating his animals, leading to unnecessary financial loss as well as increasing chances of anthelmintic resistance of infectious nematode species. Other treatments were also considered and recommendations based on the evaluation of current treatment procedures were made on how to potentially improve the farm's wool production and anthelmintic procedures. The evaluation of current parasite prevention procedures is discussed further in the discussion.

## 4. RESULTS

A number of different nematode species were found in the fecal specimens upon fecal float analysis. These included *Trichuris ovis*, *Nematodirus spathiger*, *Haemonchus contortus*, strongyles and *strongyloides* spp. (Fig. 1). The most prevalent nematodes seen were strongyles, being present in nearly all of samples taken from both the stud (94.1%) and the flock (100%) pastures (Fig. 2). There was a higher prevalence of all of the intestinal nematodes in the flock specimens compared to the stud specimens with the exception of *T. ovis*, There was no evidence of *T. ovis* in the flock specimens, but they were prevalent in the stud population (47.1%). Despite this, there was no significant difference (p=.387) in the prevalence of any nematode species between the flock and stud sheep populations.

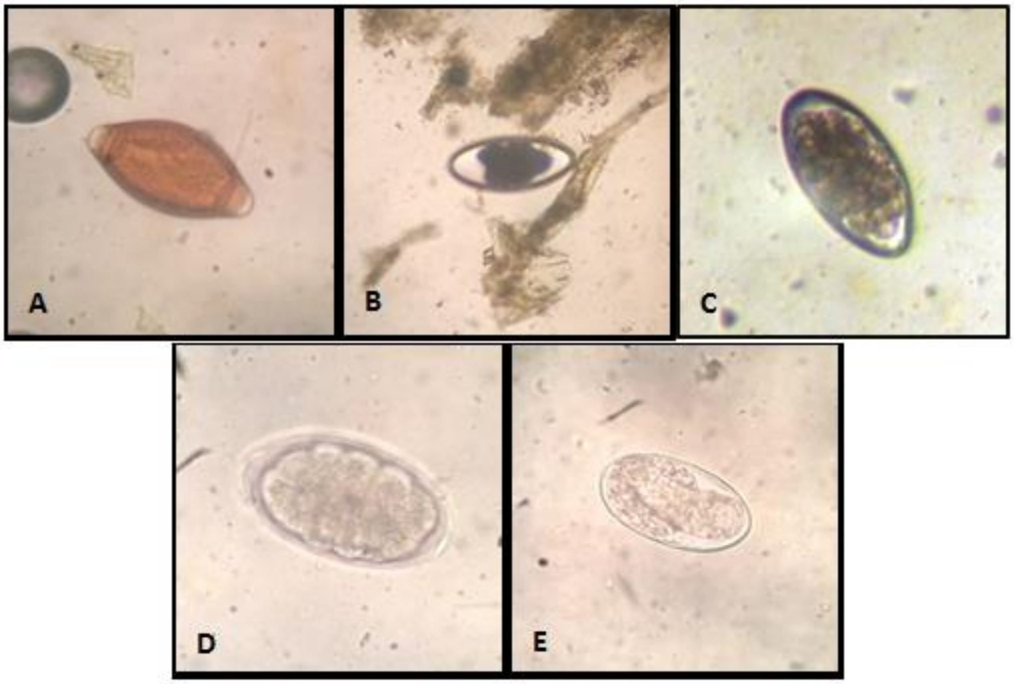


**Figure 1. Nematode species found in *O. aries* feces.**
***A.*** *Trichuris ovis* ovum at 450× magnification. *Trichuris* spp. ova are typically brown and have a characteristic lemon shape with plugs at each pole. **B.** *Nematodirus spathiger* ovum at 100× magnification. *Nematodirus* spp. ova are characteristically very large with tapered ends and 4-8 cell morulas. **C.** *Haemonchus contortus* ovum at 450× magnification. **D.** Strongyle ovum at 450× magnification. Strongyle ova are typically small, ellipsoidal, smooth walled and contain a morula. **E.** *Strongyloides* ovum at 450× magnification. *Strongyloides* are typically smaller than strongyle ova and contain a rhabditiform larva in fresh fecal specimens.

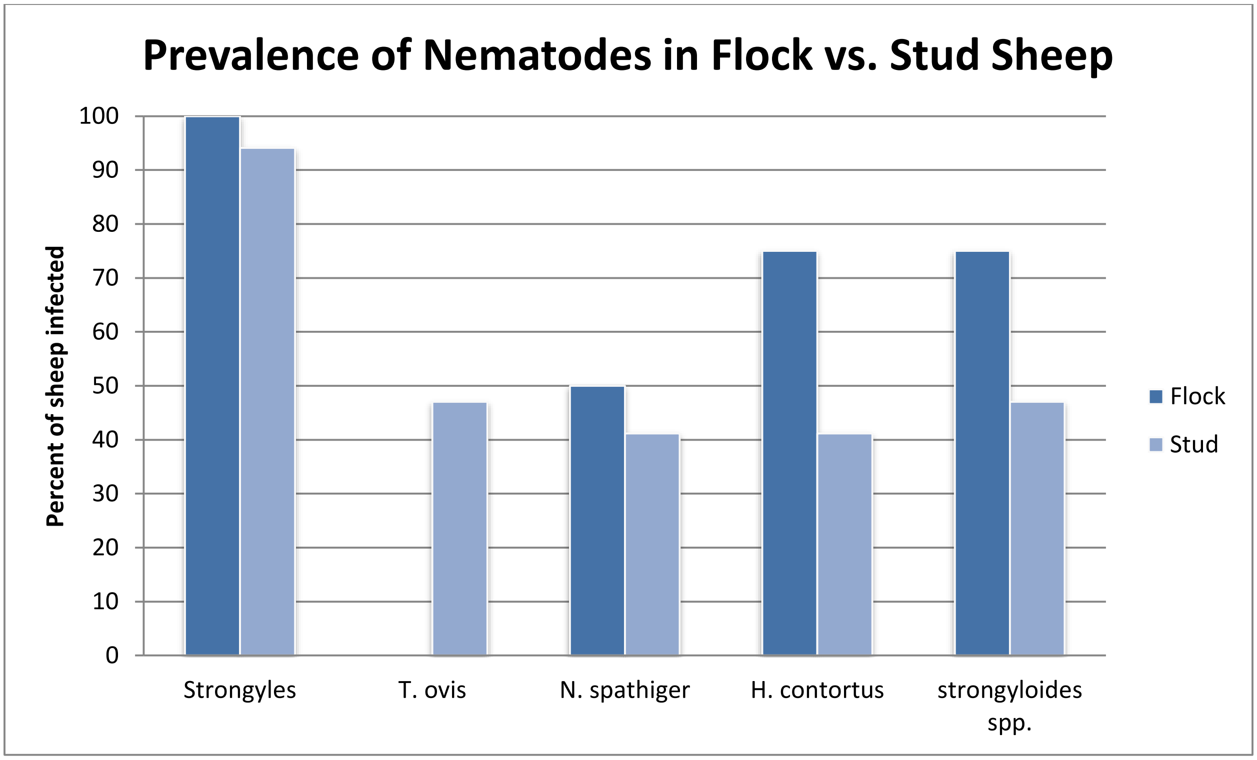


**Figure 2. Prevalence of nematode species in flock and stud sheep.** The prevalence of strongyles, *T. ovis*, *N. spathiger*, *H. contortus*, and strongyloids in flock versus stud sheep populations at a commercial sheep farm in Middleburg, South Africa.

# 5. DISCUSSION

## 5.1 Findings of this Study

The main nematode species that were present in sheep on this South African commercial farm were strongyles, *Strongyloides* spp., *Trichuris ovis*, *Nematodirus spathiger*, and *Haemonchus contortus*, with the most prevalent of all of these species being strongyles. All of the species present on this farm can have detrimental effects on the sheep in high enough numbers however, the severity of infection in the samples that were taken were not at a level that would have likely led to serious illness. These numbers are likely to fluctuate throughout the year depending on season and other environmental conditions. Therefore, although parasite levels were not high at the time of study, levels may rise at a later date. Although most of the sheep harbored at least a few of these different species, they were at levels significantly lower than those reported elsewhere in South Africa. This suggests that the current treatment procedures the farm is employing are quite successful.

## 5.2 Current Anthelmintic Procedures

Currently the farm is using a number of drugs on their sheep. These include, Cydectin, Ripercol, Brutel, Covexin and Sulmetrim NF. Cydectin is an injectable anthelmintic containing 1% m/v of Moxidectin. Moxidectin belongs to the macrocyclic lactone class of anthelmintics (Vercruysse & Claerebout, 2014). Macrocyclic lactones are effective against nematodes including hypobiotic larvae and some species of arthropods (Vercruysse & Claerebout, 2014). Ripercol is a second anthelmintic used on the farm. It is an oral drench that contains 99.997% m/m levamisole hydrochloride. Levamisole belong to the imidazothiazoles class of anthelmintics and is effective against nematodes but is not ovicidal (Vercruysse & Claerebout, 2014). The sheep are treated with these two classes of anthelmintics twice a year, once in the winter (June or July) and again in the summer (December or January). At first it may seem like over treatment to treat with two different classes of anthelmintics simultaneously but research suggests that using two different anthelmintic classes concurrently may in fact delay the development of anthelmintic resistance (Shalaby, 2013). A computer based model found that when animals are treated with two different classes simultaneously resistance didn’t develop for over 20 years (Shalaby, 2013). However, there is some debate about the effectiveness of combination drug therapy (Bath, 2006). Some studies have found that combination drugs may in fact mask anthelmintic resistance until it progresses to the point that all drugs are rendered ineffective (Bath, 2006).

Another anthelmintic used by the farm is Brutel. It is a 3.75% Praziquantel oral drench that is effective against all ages and stages of cestodes or tapeworms. Covexin is an anticlostridial the farm uses on their sheep. Sulmetrim Plus NF is a broad spectrum antimicrobial containing 4% trimethoprim m/v and 20% sulphamethoxazole m/v. It is used to treat a wide array of infections including respiratory, urogenital and alimentary infections. The farm only uses Sulmetrim Plus NF on a case to case basis with sick animals.

### 5.3 Suggested Ways to Reduce Helminth Burden

For a number of reasons, the use of anthelmintic drugs has become the primary means of controlling helminth infections and burdens. These include their cheap costs, their seemingly immediate and easy means of controlling infection and the power of the pharmaceutical companies selling the drugs (Bath, 2005). Although drugs work in the short term, unless other practices are implemented alongside the use of anthelmintic drugs, resistance will continue to occur to the point that no anthelmintic is an effective means of controlling infection (Bath, 2005). There are a number of simple practices that can be implemented that will not only improve the longevity of current anthelmintics being used by farmers, but also reduce unnecessary costs (Bath, 2005).

One easy way to improve animal health is to improve host resistance (Bath, 2014). This can be done by selective breeding and culling for natural resistance and resilience. This can be done in a number of ways including ram selection, ewe culling and adequate nutrition (Bath, 2014). Selecting rams for resistance is best achieved by performing fecal egg counts (FEC) on rams. The rams with the lowest scores are going to be the naturally more resilient rams (Bath, 2014). Because rams make up on average only 2-4% of the breeding flock but contribute 50% of the genetic material, breeding for resistance using just the rams will have a significant improvement on natural resistance (Bath, 2014). Genetic resistance can also be improved by culling ewes that are the worst at coping with infection. Not only does this improve the genetic pool but it can also reduce the frequency of blanket treatment necessary to control disease (Bath, 2014). One disadvantage to implementing this strategy is the slow development of noticeable improvement. Since this is based on breeding, it will take years before the effects are seen, but if incorporated into the anthelmintic procedures will have a significant impact over time (Bath, 2014). Providing the sheep with a diet rich in proteins and trace elements is also essential to maintaining a well-functioning immune system in any animal (Bath, 2014).

Another means of controlling helminth infections without the direct use of drugs is adopting practices that reduce the parasite load in both the animals and the pastures (Bath, 2014). One way of achieving this is by minimizing the length of stay on each pasture. It can take weeks to months for larvae to hatch and then develop into the infective stages; by reducing the length of stay it will minimize the ingestion and build-up of infective larvae in the animals (Bath, 2014). Not only is it good for the control of helminth infections, but it is also a great way of maximizing pasture utilization. High pressure, short duration grazing allows for proper grazing without overgrazing maximizing plant vigor (Bath, 2014). If the duration of time each pasture is grazed cannot be reduced, reducing the number of animals per unit area will have a similar effect (Bath, 2014).

Increasing the time of absence on each pasture can also significantly reduce the helminth burden of a given pasture; this is because without hosts, the larvae will of most helminth species will desiccate (Bath, 2014). In dry climates like that of the karoo, pastures should be left vacant for at least a few months to effectively reduce infective larvae numbers (Bath, 2014). Alternating grazing with non-susceptible species or grazing with a mixed population of sheep and non-susceptible species such as horses, cattle or ostriches will also help reduce the burden of parasitic worms on sheep (Bath, 2014). It is important to also identify “hot spots” where helminths are likely to occur in extremely high numbers. These areas are primarily wet areas especially in a dry climate (Bath, 2014). In order to reduce exposure to these hot spot areas, chronically wet or marshy areas should be fenced off, and leaking water troughs should be fixed (Bath, 2014).

Topographical features can also contribute to the significance of infective helminth burden on pasture. This includes the slope of the pasture and the direction in which it faces (Bath, 2014). Flat pastures will often have poor drainage leading to an increase in moisture and an environment more suitable for helminth survival. In the southern hemisphere, pastures that face south get less sun thus making the pasture cooler and leading to an increase in moisture retention (Bath, 2014).

Regular monitoring of FECs, weight changes, milk production and activity can help farmers determine which times of the year infections are at their peak and use this information for strategic treatment with anthelmintics (Bath, 2014). When using anthelmintics, it is important to weigh every animal that is going to be treated and set the dose for all the animals according to the heaviest animal in the flock (Bath, 2014). Ensuring the proper functioning of equipment and its accuracy and repeatability is also essential. These practices will ensure that every animal receives enough medication to control infection, minimizing the development of resistance (Bath, 2014). Targeting specific populations that are particularly susceptible to infection such as lambs and pregnant or lactating ewes will also further improve the effectiveness of anthelmintic treatment and reduce the need for excessive blanket treatments (Bath, 2014). Taking the time to research which classes of anthelmintics and brands will be most effective in the farm's region is also important. Most farmers will buy drugs based on which is cheapest however this often leads to a sacrifice in quality and effectiveness (Bath, 2014).

## 6. CONCLUSION

Despite our best efforts it cannot be denied that anthelmintic resistance has become a global problem. Heavy reliance on anthelmintic drugs, overtreatment by many farmers worldwide, and the failure of a new class of anthelmintics to reach the market in the past 25 years have further fueled the problem and rate at which resistance develops. On the pharmaceutical end, efforts need to be made to develop new classes of anthelmintics and if possible, develop a vaccine. However, the development of new drugs will not be enough to conquer the problem of anthelmintic resistance. Changes in mindset and farming practices will need to be made to ensure the longevity of future anthelmintics.

First and foremost, the way we think about helminths and their infections needs to change. Currently many people and farmers work to almost eradicate all helminth species on their farm. It is important to realize that without any exposure to infectious helminth species, animals will only become more susceptible to infection due to the inability to build up a natural immunity. Furthermore, it is essential that the mindset shift from short term control to long term sustainability. To do this, pharmaceutical companies, parasitologists and veterinarians will have to work together to convey the dangers of heavy anthelmintic reliance as well as the importance and benefits of long term, sustainable anthelmintic practices aside from drugs. Together, we can not only overcome the issue of anthelmintic resistance worldwide, but ensure that it does not become a global phenomenon again in the future.

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