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THE EFFECT OF VITAMIN E SUPPLEMENTATION ON AN EXPERIMENTAL *HAEMONCHUS CONTORTUS* INFECTION IN DORSET LAMBS

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THE EFFECT OF VITAMIN E SUPPLEMENTATION

ON AN EXPERIMENTAL *HAEMONCHUS CONTORTUS* INFECTION

IN DORSET LAMBS

BY

BRITTANY MARIE DE WOLF

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ABSTRACT

Gastrointestinal parasitism in sheep, particularly lambs, results in substantial economic losses to producers worldwide. Nutritional status has been shown to play an important role in host immune response to parasitic infections. The objective of this study was to investigate the effect of vitamin E supplementation on an artificial *Haemonchus contortus* (*H. contortus*) infection in immature lambs. Twenty Dorset lambs were stratified into two treatment groups according to parasite susceptibility. Worm-free lambs, 28 to 32 weeks of age, were supplemented with vitamin E (d-α-tocopherol) for twelve weeks following the recommendations of the National Research Council for minimal daily requirement (VE5; 5.3 IU/kg body weight (BW)/day (d), n=10) or optimal immune function (VE10; 10 IU/kg BW/d, n = 10). Five weeks after initiation of vitamin E supplementation, lambs were infected with 10,000 *H. contortus* L3 larvae. Samples were taken weekly for determination of fecal egg counts (FEC), packed cell volume (PCV), and serum α-tocopherol. After six weeks of infection, the lambs were humanely slaughtered for the determination of tissue vitamin E content, worm burden and histologic evaluation of the abomasum. Increased dietary vitamin E supplementation had no effect on liver (P=0.08), muscle (P=0.62), or lymph node (P=0.38) α-tocopherol content. There was no effect of treatment on FEC or PCV, however there was a 49% reduction in total abomasal worm burden (P=0.002) in the VE10 group. These results indicate that elevated levels of vitamin E supplementation had a beneficial effect on the abomasal worm burden; however, there was no treatment effect on PCV or FEC.

PREFACE

This thesis is written in manuscript format. Chapter 1 includes detailed information on the sheep industry, gastrointestinal nematodes in small ruminants, ruminant immunity, and current vitamin E research. Chapter 2 includes a condensed paper written in the style of the journal Veterinary Parasitology. Additional details on the methods used throughout the study are included in the appendices.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

ON THE TOPIC OF PARASITIC INFECTION AND CONTROL METHODS

IN SMALL RUMINANTS AND VITAMIN E

Introduction

Gastrointestinal nematode parasites of small ruminants are responsible for staggering economic losses to producers on nearly every continent. The abomasal nematode causing the largest detriment to production is the barber pole worm, *Haemonchus contortus,* which poses particular risk to young animals due to their naïve immune system. The problem is compounded by the recent development of parasitic resistance to all classes of chemical dewormers used for the control of gastrointestinal parasite infections. Due to this evolution of parasite resistance to chemical anthelmintics, alternative approaches for control are needed (Miller and Horohov, 2006). The control of parasitic infection in flocks and herds is heavily reliant on the integrated use of anthelmintics along with grazing management strategies.

Factors influencing the host-parasite relationship include age, genetics, and nutritional status. While past studies have demonstrated that supplementation with vitamin E, a free radical scavenging antioxidant, enhances immune responsiveness and performance in lambs (Finch and Turner, 1996), the effect of vitamin E supplementation on an immune response against parasitic nematodes is unknown. A recent increase in the recommended daily intake of vitamin E for sheep for optimum immune status based on studies showing the role of vitamin E in enhancing immune function in small ruminants

(National Research Council, 2007), creates further need to investigate the effect of vitamin E supplementation on a lamb's response to parasitic infection. A dual approach that examines the potential of nutritional modulation to enhance immunity to parasitic infection is needed. This literature review will present a comprehensive background on parasitic infection in small ruminants and current research investigating sustainable parasite control methods.

GASTROINTESTINAL PARASITES AND SHEEP

Overview

The United States sheep industry is comprised of nearly 6.2 million animals and generates an estimated $392 million annually (USDA, 2008). Although it is difficult to determine precise figures for financial losses resulting from parasites, one study quantified sheep and goat parasitic production losses to exceed $270 million each year (Baker et al., 1990). Parasitic infection of sheep and goats is a particular problem in the Southeastern region of the country, as warmer climate conditions are conducive to parasitic growth and transmission. Seventy-five percent of 467 sheep producers surveyed within the southeastern region of the United States identified stomach and intestinal nematodes as a major concern and threat to production (NAHMS, 1996). The most recent survey of sheep producers conducted by the United States Department of Agriculture indicated that 74% of producers reported the predominant disease in their flocks to be due to stomach and/or intestinal worms (USDA, 2008).

While most economic losses are due to animal mortality, production losses are also significant contributors (Barger and Cox, 1984). Clinical signs of gastrointestinal nematode (GIN) infection contributing to production losses include decreased rate of

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gain, a reduction in feed efficiency, reduced meat, wool, and milk production, and a decreased reproductive efficiency (Hartwig, 2000). Control of GIN has been complicated by an increasing parasite resistance to traditionally used anthelmintics, resulting in drastic decreases in reported efficacy for all classes of available drugs (Fleming et al., 2006, Kaplan et al., 2005, Zajac, 2010, Zajac and Gipson, 2000). The dramatic emergence of resistance to multiple drug classes presents an urgent need for the reform of current small ruminant parasite control practices. Novel methods of GIN control are needed to prevent continued losses within the sheep industry. Understanding all aspects of the host-nematode interaction is necessary to ensure the future success and survival of pasture-based sheep production.

Gastrointestinal Nematodes of Small Ruminants

Small ruminants are affected by many species of nematodes, cestodes and coccidia that ultimately lead to production losses. Prophylaxis and treatment expenses are massive burdens in animal husbandry. The most common and pathogenic gastrointestinal parasites of small ruminants are discussed below.

Trichostrongylids

The predominant species of nematodes affecting sheep belong to the Superfamily Trichostrongyloidea and include *Haemonchus contortus* and *Telodorsagia circumcincta,* located in the abomasum*,* and *Trichostrongylus colubriformis*, *Cooperia* spp., *Nematodirus* spp. and *Oesophagostumum* spp., located in the small and large intestine respectively (Miller and Horohov, 2006). Small ruminants on pasture are typically challenged with several nematode species at one time, contributing to the condition known as parasitic gastroenteritis (PGE) (Zajac, 2006). Trichostrongylids cannot be

differentiated from one another by microscopic examination of their eggs; only infective larvae and adult worms allow the determination of individual species (Zajac, 2006).

Parasite Distribution

Nematode populations thrive in warm, moist conditions, and are therefore widely distributed in tropical and subtropical regions of the world (O'Connor et al., 2006). In the United States, many studies have documented the higher incidence of production losses due to GIN occurring in the Southeast region of the country (Burke et al., 2007; Howell et al., 2008; Kaplan et al., 2004; MacGlaflin et al., 2011; Vanimisetti et al., 2004). The eastern South Central region of the United States (Alabama, Arkansas, Kentucky, Louisiana, Mississippi, and Tennessee) report the highest percentage (77.8) of sheep producers indicating their top health condition of concern to be stomach/intestinal worms (USDA, 2008).

In the temperate Northeast region of the United States, few studies have been conducted, and to our knowledge, none have been executed in recent years (Gibbs, 1977; Herd et al., 1984; MacGlaflin et al., 2011; Tritschler et al., 1989). These studies confirmed the species in the Northeast in order of predominance to be *H. contortus, Nematodirus* spp., *Teladorsagia* spp., and *Trichostrongylus colubriformis* (Gibbs, 1977; MacGlaflin et al., 2011; Tritschler et al., 1989). While all species can inhibit production, the abomasal nematode that poses the greatest threat to animal health and producer livelihood worldwide is *Haemonchus contortus.*

Haemonchus contortus

Haemonchus contortus is extremely pathogenic and the most economically

devastating parasite of sheep and goats worldwide (Mortensen et al., 2003). *Haemonchus contortus* is commonly called the barber pole worm due to a distinct red and white striped appearance, caused by the helical winding of the white egg-filled uterus of the female adult worm wrapped around the digestive tract. The female adult worms are 18-30 mm long while the male adult worms are smaller and thinner than the females, at 10-16 mm long (Zajac, 2006).

Signs and symptoms

Larval and adult stages of *H. contortus* feed on the blood of the host, inflicting severe anemia and death in heavily infected animals (Bethony, 2006). Anemia is characterized by pale mucous membranes, particularly visible in the lower eyelid. Sudden deaths occur as a result of hemorrhagic anemia caused by severe blood loss from the gut. Although animals with low levels of infection do not generally exhibit clinical signs, subclinical infections are also of great concern to producers due to weakness and significant decreases in weight, fertility, and milk yield within a herd or flock (Mir, 2007).

To feed on the host, *H. contortus* exposes an oral lancet used to slit capillaries of the abomasal mucosa open and feed on the released blood, simultaneously secreting an anticoagulant causing prolonged bleeding at the site (Johnstone, 2000). At peak levels of infection, *H. contortus* accounts for the loss of up to one fifth of the circulating erythrocyte volume per day from lambs, and one tenth of the circulating erythrocyte volume per day from adult sheep (Bowman et al., 2003).

Disease is acute in animals with heavy worm burdens. *H. contortus* is extremely prolific; females can produce up to 10,000 eggs each day at a rate of one egg every ten

seconds (Lee, 2002). Edema under the jaw, commonly called "bottle jaw", is another frequent sign of *H. contortus* infection caused by significant blood plasma and protein loss to the host (Barriga, 1997). The effects of haemonchosis are most severe in very young or old animals as well as pregnant or lactating, undernourished, diseased or stressed animals due to a compromised immune system (Kaplan, 2006). Lambs are the most affected members of a flock, but mature sheep under physiological stress also may have fatal anemia (Bethony, 2006).

Life cycle of *Haemonchus contortus*

   H. contortus is dependent on a small ruminant host to develop and complete a 21 day life cycle. Adult female *H. contortus* worms begin laying eggs which exit the body in the manure of the host sixteen to twenty-three days post-infection (Miller and Horohov, 2006). If environmental factors are favorable, eggs will hatch and molt from the first larval stage (L1) to the second stage larvae (L2). The cuticle of the L2 is retained as the larva develops into the infective L3 stage. While the time from initial egg hatching to reaching the infective L3 stage can be as little as five days, development may be delayed for up to months if environmental conditions are cooler (Miller and Horohov, 2006).   Infective L3 stage larvae leave the fecal pellet and climb up blades of moist grass on pasture to be consumed by grazing animals. After ingestion, the larvae shed their outer protective layer in the rumen and then invade the mucosa of the host abomasum, molting into the L4 stage larvae (Miller and Horohov, 2006). After a final molt, the parasite becomes an L5 stage adult worm at which point a lancet develops, allowing the parasite to pierce vessels of the mucosa and begin feeding on blood (Miller and Horohov, 2006).

Female adult worms begin laying eggs which are then released through the feces of the animal, perpetuating the life cycle of *H. contortus.*

Hypobiosis and the Periparturient Rise

A unique feature of the *H. contortus* is the ability to undergo a stage of arrested larval development, known as hypobiosis. If environmental conditions are not favorable (i.e. - too cold or dry), the L4 development into the adult stage can be delayed or paused, enabling the parasite to survive otherwise fatal environmental conditions (Urquhart, 1996). This period of hypobiosis occurs in the abomasum of the host, with maturation of the larvae resuming when favorable conditions return in the spring.

Lactating sheep are particularly susceptible to *H. contortus*, and the re-emergence of the parasite from hypobiosis can account for a significant and rapid rise in parasitic infection levels around the time of lambing (Baker et al., 1999). The susceptibility during the periparturient period is attributed to the suppression of the immune system by increased levels of hormones and metabolic demands of pregnancy on the host, which is exacerbated by suboptimal feed intake in pregnant ewes (Vlassoff et. al., 2001). Leathwick et. al. (1995) found the establishment of larvae in the abomasum to increase 30% three weeks prior to lambing in pregnant ewes. While the precise mechanisms triggering hypobiosis are poorly understood, it is proposed to be influenced by both immune and environmental cues (Miller and Horohov, 2006). It is clear that hypobiosis is an important adaptation that contributes to the immense success and survival of *H. contortus.*

Anthelmintics Used in the Control of Gastrointestinal Nematodes

Control of gastrointestinal nematodes is almost entirely based on the use of

anthelmintics. There are currently three classes of anthelmintics used in sheep, including 1) benzimidazoles (including albendazole and fenbendazole), 2) cholinergic agonists (such as levamisole) and 3) avermectins (including ivermectin and moxidectin) (Zajac, 2000). These drugs are FDA-approved for use in the treatment of gastrointestinal parasites in sheep.

Resistance of parasites to these traditionally used anthelmintics has become a serious problem in veterinary medicine, particularly in sheep husbandry (Roos, 1997). The development of widespread resistance of gastrointestinal nematodes to chemical dewormers has left veterinarians and farmers with few options to effectively control parasites within a flock.

Anthelmintic Resistance

Resistance can be defined as the failure of the majority of a parasite population to be eliminated by an anthelmintic treatment, or the need for a greater concentration of anthelmintic to reach efficacy (Prichard et al., 1980). The first resistance to commercial dewormers in the United States was recorded in 1957 to phenothiazine, followed shortly after by thiabendazole in 1964 (Fleming, 2006). The problem has only grown in recent years, as many studies have documented the increasing inability of anthelmintics to control parasitic infection. To date, parasitic resistance to anthelmintics has been reported worldwide and exists to every class of available drugs (Gopal et al., 1999; Sangster, 1999; Wolstenholme et al., 2004; Zajac and Gipson, 2000).

The increasing resistance of gastrointestinal nematodes to chemical dewormers has become an epidemic, resulting in large revenue losses for sheep producers. While

resistance to commercial dewormers had been documented in multiple parasites, the most dramatic resistance has been observed in *H. contortus* (Fleming, 2006). Once considered to be native to the southern region of the United States due to warmer climates, *H. contortus* has become one of the most significant obstacles to sheep producers in New England, particularly due to a growing resistance to routinely implemented commercial dewormers that has become evident in recent years. To date, resistance to every class of anthelmintics has been documented in the United States, with the first total failure to all available drugs documented in Arkansas in 2005 (Mortensen et al., 2003; Kaplan, 2005).

Factors Influencing the Development of Anthelmintic Resistance

The problem has only been exacerbated by previous recommendations by both parasitologists and veterinarians to rotate the anthelmintic used, to deworm all animals frequently, and to move animals to clean pastures just after deworming- practices which were once thought to control parasites, but have been recently shown to only exacerbate the problem (Zajac, 2010). Repeatedly treating a parasite population with an anthelmintic puts selective pressure on individuals that have innate or acquired resistance to the drugs. Resistance is inherited by transmission of resistant alleles; even point mutations can lead to a decrease of susceptibility to a pharmaceutical compound, enabling the resistant proportion of the parasite population to survive treatment (Prichard et al., 2007). Treatment becomes ineffective when the proportion of resistant genes increases and thereby dilutes the susceptible genes (Sangster, 1999).

The ever decreasing efficacy of anthelmintics has resulted in reduced productivity and increased mortality in the flocks of sheep producers. It is clear that past methods of parasite control are not only ineffective in managing parasites but unsustainable as well,

and many producers are looking to reduce or eliminate the use of chemical dewormers entirely.

Refugia

A key concept in sustainable parasite control is refugia, or the proportion of a parasite population that is not selected by drug treatment. Refugia is an essential factor in delaying the development of anthelmintic resistance (Van Wyk, 2001). By treating only the animals that exhibit clinical signs of parasitic infection and require treatment and leaving the rest of the animals untreated, susceptible worm strains remain in the flock and have a diluting effect on the population of resistant worms (Zajac, 2010). The goal of an integrated parasite control program is to keep refugia high by treating only affected animals and moving away from an overreliance on traditionally used chemical dewormers.

Best management practices for parasite control

Currently, the recommendation for best management practices (BMP) for sustainable parasite management incorporate a decreased reliance on commercial dewormers as well as the integration of alternatives to dewormer use in parasite control programs. An integrated parasite management program determines individual susceptibility to parasites. Deworming only those animals affected by parasites and controlling animal exposure to parasites through pasture rotation is currently recommended. Rotational grazing is a practice currently recommended to reduce infection. Moving a flock or herd to graze on different pastures periodically can substantially reduce pasture infectivity. Rotating more resistant mature animals with

susceptible younger animals may also prove beneficial, although this strategy may be impractical for producers with limited acreage (Van Wyk et al., 2006).

Selective Deworming

Research has concluded the major factor in the development of drug resistant parasites is the method of deworming all animals in a population simultaneously and frequently, a common practice implemented by most producers in the past. It is estimated that only 20-30% of the animals in a population harbor about 80% of the parasites (Kaplan, 2006). By deworming only the most susceptible animals rather than every animal as previously recommended, parasites can be more effectively controlled by lessening the evolutionary selection for resistant populations of worms. This method of selective deworming, or "smart drenching", can potentially slow the development of drug resistant parasites while reducing the number of treatments given, a clear financial benefit for producers. One of the most important aspects of smart drenching is a selective treatment approach based on the use of the FAMACHA© scoring system.

The FAMACHA© Method

A system to easily identify animals most affected within a population has been recently developed in South Africa at the Onderstepoort Veterinary Institute. This method was developed in consideration of small farms lacking resources, allowing producers to effectively and inexpensively assess animals without the use of lab equipment or diagnostics (Van Wyk, 2002). In order to identify the most susceptible animals, producers can implement the FAMACHA © (Faffa Malan Chart) system to quickly assess the presence of anemia due to *H. contortus* infection based on mucous membrane pallor in goats and sheep, assessed by examination of the lower eyelid

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(Kaplan, 2004). Treatment can then be administered selectively to animals exhibiting anemia. The FAMACHA© method is a practical tool for implementing selective deworming, a management approach that operates on the premise that only animals showing clinical symptoms or reduced productivity should be treated with anthelmintics, thereby slowing down the development of anthelmintic resistance (Mahieu et al., 2007).

While other parameters can be used to identify parasitic infections in animals, such as body condition scoring, the presence of diarrhea, and fecal egg counts, the FAMACHA© system is a valuable tool producers can use to identify animals consistently infected and in need of treatment, as well as to reduce the number of animals treated and the cost of total treatment on a farm (Burke, 2007).

Alternatives to commercial dewormers

The rapid and dramatic spread of parasitic resistance to anthelmintics as well as concern about their possible detrimental effects on the environment has encouraged researchers to initiate the development of alternative parasite control methods. Since the main method of controlling *H. contortus* has become relatively ineffective, other methods need to be investigated.

Although initial field trials have shown potential, to date no vaccine has been developed to successfully prevent haemonchosis (Bethony, 2006, Emery et al., 1993). The discovery of a fungus capable of trapping nematodes has been shown to be highly effective in reducing levels of infective larvae on pasture and may play a huge role in future pasture management methods to control nematode infection. Experiments with *Duddingtonia flagrans* have been shown to reduce the percentage of infective *H. contortus* larvae on pasture by 76.6 to 100 percent (Pena, 2002).

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The potential anthelmintic properties of condensed tannins (CT) found in some plants and grazing forages that have shown positive results for controlling gastrointestinal nematode infection are currently being examined. Supplementation with tanniferous quebracho extract from tree bark has been shown to reduce fecal egg count and female fecundity of the barber pole worm in goats (Paolini, 2003). Terrill and co-workers (2009) reported that sericea lepsideza, a CT-containing hay resulted in reduction of both fecal egg count and adult worm numbers in the abomasum of small ruminants. A significant number of other studies have shown supplementation with copper oxide wire particles (COWP) to demonstrate anthelmintic activity in experimentally infected sheep (Bang et al., 1990; Nyman, 2000).

Several other novel techniques of control, including breeding genetically resistant sheep, implementing alternative management practices such as intensive rotational grazing, and nutritional modification are also currently being studied as alternative GIN control. Current research has made some progress in the discovery of groundbreaking, non-chemical solutions to the sustainable management of *H. contortus* and other economically important gastrointestinal nematodes worldwide.

Immune Response to Gastrointestinal Nematode Parasites

Ruminant Immunity

In a 2002 study, Binns et al. reported approximately 10-35% of lambs die within the first 6 months of life, a mortality rate nearly double that of mature sheep. These losses can be attributed to an underdeveloped, naïve immune system of young ruminants. In the first 3-4 months of life, ruminant neonates are almost entirely dependent upon maternal antibodies passively transferred through colostrum. The majority of maternal

immune cells are T cells, which provide early cell-mediated immune system (Tizard, 2004). While maternally derived antibodies provide protection, they concurrently inhibit the lamb from mounting an antibody response of its own (Morein et al., 2002). Due to this inhibition, lambs are not vaccinated against infectious diseases until they are at least 3-4 months old (Tizard, 2004). Lambs are particularly vulnerable to infection when maternal antibodies are no longer present and have not yet begun to effectively mount their own immune response.

Th1/Th2 response

The immune system is comprised of widespread individual effector cells, tissues and organs with complex interactions that allow a rapid response to any foreign pathogens entering the body (Tizard, 2004). Cytokines, low weight signaling proteins involved in immune cell communication, are produced by the activation of CD4+ T helper cells and allow for immune attacks against intracellular or extracellular pathogens, respectively (Tizard, 2004). T helper lymphocytes are differentiated into two subsets, T helper type 1 (Th1) and type 2 (Th2), based on the cytokines they secrete (Mosmann et al., 1989).

Th1 type cells are responsible for the activation of cellular immunity, and a Th1 type response is typically seen in response to viral infection, intracellular pathogens and the activation of delayed-type hypersensitivity reactions. A Th1 type response typically responds to viruses and vaccinations, and is characterized by the cytokines IL-2, tumor necrosis factor, and interferon- γ which mobilize macrophages, T cells, and natural killer cells to promote proliferation and inflammation (Miller, 2006).

A Th2 response is characterized by the increased production of immunoglobulin secretion by B cells, particularly IgG and IgE and the cytokine IL-4, as well as increased eosinophils and mast cell recruitment (Shallig, 2000). A Th2 type response is associated with allergic reactions (Mosmann et al., 1989) and is also activated upon parasitic infection (LaCroux et al., 2006; Shakya et al., 2009). It is hypothesized that there is a balance between the expression of both the Th1 and Th2 responses, although challenges with different pathogens may alter the type and ratio of cytokines expressed, such as the increase of Th2 type cytokines during a parasitic infection (Kidd, 2003).

Host Response to Parasitic Infection

Inflammation during parasitic infection is important in larval and adult stage worm expulsion from the mucosa (Miller, 2006). LaCroux et al. (2006) reported a clear Th2 type of immune response in lambs repeatedly exposed to *H. contortus*. Upon parasitic infection, antibodies, lymphocytes, eosinophils, globule leucocytes and mast cells act upon larval stages of *H. contortus* embedded within the mucosa and adult worms inhabiting the lumen of the gastrointestinal tract (Miller and Horohov, 2006). Effector cells degranulate, releasing vasoactive molecules, which physically expel worms from the abomasal mucosa (Miller and Horohov, 2006).

Eosinophils are small granulocytes produced in bone marrow and are closely associated with parasitic infection, activated by Th2 cytokines IL-3 and IL-5 (Balic et al., 2000). Mediators released by eosinophils include major basic protein, peroxidases, and neurotoxins, and have been shown to have lethal effects against gastrointestinal nematodes *in vitro* (Kilon and Nutman, 2004). Mediators act as potent vasodilators, inducing smooth muscle contraction and subsequent expulsion of parasites with the

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abomasum (Miller, 2006). Buddle et al. (1992) found that sheep with low egg counts had higher numbers of circulating eosinophils, which have been also been associated with larval migration inhibition (Douch et al., 1984).

Infection with *H. contortus* is associated with the production of mucosal mast cells and globule leukocytes within the GI tract of the sheep (Miller, 1996). Sheep that are more genetically resistant to parasites have more globule leukocytes than more susceptible breeds (Bisset et al., 1996). Mucosal mast cells, recruited from bone marrow by Th2 cytokines such as IL-4, release histamine, ultimately becoming globule leukocytes after degranulation (Miller, 1996). Many studies have documented the role of mast cell degranulation in worm expulsion (Douch et al., 1986, Sykes et al., 2007), but simply quantifying mast cells numbers upon necropsy is not an accurate reflection of activation status and can therefore be misleading (Balic et al., 2000). Quantification of globule leukocytes has been shown to provide a more accurate measure of mast cell activity (Balic et al., 2002).

Resistance to parasitic infection is defined by an animal's ability to prevent the establishment of parasites mediated by immune mechanisms. Animals with a greater tolerance to infection may still harbor a parasitic infection but may not exhibit clinical signs, and are said to be resilient. While resilient animals remain productive during a parasitic infection, they also still maintain the number of worms on pasture infecting a flock (Zajac, 2010).

Factors Affecting Host Response to Parasitic Infection

Age is the most important factor in an animal's ability to effectively combat parasitic infection. The immune system matures with age; hence young lambs harbor the

most severe *H. contortus* infections and account for the largest financial losses among producers as a result of reduced weight gain and death in heavily infected hosts (Miller, 2006). The effects of haemonchosis are also more pronounced in pregnant or lactating, undernourished, diseased or stressed animals due to a compromised or underdeveloped immune system (Kaplan, 2006).

Genetic factors also play a role in an animal's resistance to GIN. Several tropical and subtropical breeds of sheep are documented to have more natural resistance to parasitic infection. Resistant breeds include St. Croix (Zajac et al., 1990), Florida Native (Amarante et al., 1999), and Gulf Coast Native (Miller et al., 1998). Resistance in these breeds is defined as reduced establishment of larvae and subsequent development into adult stages (Balic et al., 2002). The nutritional status of an animal is also a factor in the ability to respond to GIN infection.

Parasite and Nutrition Interaction

Nutritional status is a key component of the immune response of the host and the host's subsequent ability to mount an effective defense against gastrointestinal nematodes (Knox et. al., 2003). It is well documented that animals on diets deficient in protein or key vitamins have a decreased capacity to withstand parasitic pathogenesis, and concomitantly gastrointestinal nematodes significantly inhibit the host's ability to efficiently utilize nutrients (Knox et al., 2003). Lambs fed a diet high in protein harbor less severe parasitic infections due to an increased immune response (Strain, 2001). Studies have shown that supplementation with protein (Kahn et al., 2003; Louvandini et al., 2006), energy (Valderrabano et al., 2002), and trace elements (Coop and Field, 1983)

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improves host resistance and resilience to parasitic infection. Few studies have examined the role of vitamins in parasitic infection.

Vitamin E

Structure and Source

Vitamin E is the general name for eight individual tocopherol and tocotrienol isomers naturally occurring in green leafy vegetables, nuts, wheat germ, and seeds (NIH, 2007).   Based on the position and number of methyl groups attached to the aromatic ring structure, both isomers have the four forms α, β, γ, and δ (Stocker, 2004). The most biologically active form of vitamin E found in nature is α- tocopherol (Han and Meydani, 2000).

Vitamin E and Immunity

Vitamin E is a fat-soluble vitamin best known for its role as an antioxidant (Combs, 1990). *In vitro* studies have documented the role of vitamin E in enhancing membrane stability and cell communication (Meydani et al., 1990), reduction of oxidative stress (Lee and Wan, 2002), and the proliferation of T cells through the up-regulation of the cytokine interleukin 2 (IL-2) (Meydani et al., 1990).

Past studies provide evidence that the Th1/Th2 balance of an animal may be shifted to the Th1 type response during vitamin E supplementation. In studies of diseased and aging humans and mice, vitamin E has been shown to up-regulate Th1 associated cytokines and decrease the Th2 associated cytokines. The same studies provided evidence that oral supplementation with vitamin E increased the production of IL-2 and decreased IL-4 production, a key Th2 cytokine (Han et al., 2006; Adolfsson et

al, 2001; Han and Meydani, 2000). Vitamin E has been shown to inhibit inflammatory cytokine responses but its effect on the Th1 or Th2 balance is undetermined in the young, immune system of lambs (Hernandez et al., 2009).

Vitamin E and Ruminant Immunity

Research investigating the role of vitamin E in regulating the immune system of ruminants has yielded conflicting results. Studies in livestock have linked supplementation with vitamin E to increased antibody secretion and improved immune responses in ruminants (Finch and Turner, 1996). Supplementation of vitamin E during the last month of gestation resulted in a 5-20% decrease in lamb mortality (Kott et al., 1998). When ewes were supplemented with vitamin E, their lambs were shown to be born with greater IgG levels (Gentry et al., 1992), and calves supplemented with vitamin E also had higher levels of IgG in addition to higher IgM as compared to control animals (Hidiroglou et al., 1992). Despite these documented effects of vitamin E on immunoglobulin synthesis, other research on supplementation with vitamin E has yielded no effect (Hatfield et al., 2002; Rivera et al., 2002)

Recent recommendations of the National Research Council (NRC) have significantly increased previous guidelines for daily vitamin E requirements in sheep 20 times to 10 IU/kilogram of body weight daily (NRC, 2007) from 0.5 IU/kilogram of body weight daily (NRC, 1985). Recommendations were based on studies linking enhanced immune function in sheep supplemented with vitamin E (Tengerdy, 1990) and a study indicating previous recommendations of vitamin E did not prevent myopathy and hepatic lipidosis in lambs (Menzies et al., 2004).

Vitamin E and Parasitic Infection

Although it is apparent that vitamin E may enhance the immune response of the host through greater immunoglobulin production as well as the production of a stronger immune response, it may actually impede the ability of the host to mount an effective immune response to GIN infection by suppressing the Th2 type response needed to combat haemonchosis. Studies in mice confirm that oral supplementation with vitamin E has a significant effect on the Th1/Th2 balance (Han et al., 2006). Vitamin E supplementation enhances a Th1 type response, causing an increase in IL-2, while decreasing Th2 type cytokines, interleukin-4 and interleukin-5- those that would normally be induced during a parasitic infection (Han et al., 2006). A Th2 type response required to successfully combat parasitic infection is characterized by an increase in immunoglobulins IgG and IgE, as well as the abomasal infiltration of effector cells such as globular leukocytes, mast cells, and eosinophils (Miller, 1996). Vitamin E supplementation has also been shown to decrease mast cell proliferation (Kempna et al., 2004), and important gut inflammatory response required by the host to successfully expel worms from the host in a parasitic infection (Miller, 1996). This reduction of the inflammatory response induced by vitamin E supplementation may interfere with the ability of the host to combat a parasitic infection.

To date, no studies have examined the effects vitamin E supplementation on GIN infections in lambs. The antioxidant status of the host was correlated with worm expulsion in lambs and goat kids (Lightbody et al., 2001), however there were no studies specifically on supplementation with vitamin E and host response. Interestingly, lambs with lower vitamin E concentration had increased worm expulsion, while goat kids with

higher vitamin E concentration were less tolerant to infection (Lightbody et al., 2001). A 2003 study by McCarthy et al. in mice challenged with *T. gondii* found that supplementation of infected mice with vitamin E and selenium was detrimental to the host, resulting in higher numbers of tissue cysts and increased severity of meningoencephalitis. These findings suggest vitamin E may affect the ability of a lamb to effectively respond to parasitic infection.

Conclusion

Although there has been much research investigating the role of vitamin E on the cellular immune response of humans and mice, research in livestock species has been limited and conflicting. There are currently no studies evaluating how vitamin E supplementation influences gastrointestinal nematode infections in young lambs. The objective of this study was to characterize the response of the host and parasite to nutritional modulation of immunity through vitamin E supplementation. This study evaluated the possibility that current levels of vitamin E supplementation recommended by the NRC may compromise the ability of lambs to effectively combat gastrointestinal nematode parasitic infection. We hypothesized that lambs supplemented with vitamin E will have less resistance to GIN. The treatment effect was determined by the effect of vitamin E supplementation on fecal egg counts, packed cell volume, and worm burden. Data collected from this study will lay the groundwork for future studies of the mechanisms involved in vitamin E and parasitic infection

CHAPTER 2: The Effect of Vitamin E Supplementation on an Experimental

Haemonchus contortus Infection in Dorset Lambs

This thesis is written in manuscript format and includes a condensed paper written in the style and formatting of the journal Veterinary Parasitology. This manuscript is currently unpublished but is prepared for future submission to the journal of Veterinary Parasitology. Additional details on the methods used throughout the study are included in the appendices.

CHAPTER 2

The Effect of Vitamin E Supplementation on an Experimental *Haemonchus contortus*

Infection in Dorset Lambs

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ABSTRACT

Gastrointestinal parasitism in sheep, particularly lambs, results in substantial economic losses to producers worldwide. Nutritional status has been shown to play an important role in host immune response to parasitic infections. The objective of this study was to investigate the effect of vitamin E supplementation on an artificial Haemonchus contortus (*H. contortus*) infection in lambs. Twenty lambs were stratified into two treatment groups according to parasite susceptibility. Worm-free lambs, 28 to 32 weeks of age, were supplemented with vitamin E (d-α-tocopherol) for twelve weeks following the recommendations of the National Research Council for minimal daily requirement (VE5; 5.3 IU/kg body weight (BW)/day (d), n=10) or the requirement for optimal immune function (VE10; 10 IU/kg BW/d, n = 10). Five weeks after initiation of vitamin E supplementation, lambs were infected with 10,000 *H. contortus* L3 larvae. Samples were taken weekly for determination of tissue α-tocopherol content, worm burden and histologic evaluation of the abomasum for inflammation and infiltration of eosinophils, globule leukocytes and mast cells. There was a 49% reduction in total abomasal worm burden ($P = 0.002$) in the VE10 group. VE10 lambs did not differ from VE5 lambs in serum or tissue α-tocopherol, FEC, PCV or histologic parameters. Further studies are necessary to elucidate the mechanism of action of vitamin E on gastrointestinal nematode infection of lambs.

1. Introduction

Gastrointestinal nematode parasites in sheep account for staggering economic losses to producers worldwide. The problem has been compounded by the development of parasite resistance to all classes of chemical dewormers used to control gastrointestinal parasites (Fleming et al., 2006, Kaplan et al., 2005, Zajac and Gipson, 2000). The blood-feeding nematode *Haemonchus contortus,* commonly called the barber pole worm, is of particular concern due to its highly pathogenic nature. Larval and adult stages of *H. contortus* feed on the blood of the host, resulting in severe anemia and death in heavily infected animals (Bethony, 2006, Mortensen et al., 2003). The effects of haemonchosis are most pronounced in young, pregnant or lactating, undernourished, diseased or stressed animals (Kaplan, 2006).

An effective immune response mounted during parasitic infection is characterized by a T helper type 2, or Th2-type reaction (Shakya, 2009). Upon parasitic infection, antibodies, lymphocytes, eosinophils, globule leucocytes and mast cells act upon larval stages of *H. contortus* embedded within the mucosa and adult worms inhabiting the lumen of the gastrointestinal tract (Miller and Horohov, 2006). Effector cells, including mast cells, degranulate and release vasoactive molecules, acting to physically expel worms from the abomasal mucosa (Miller and Horohov, 2006).

Nutritional status has well documented effects on the host's ability to combat heavy parasitic infestation, and concomitantly gastrointestinal nematodes significantly inhibit the host's ability to efficiently utilize nutrients (Knox et al., 2003). Animals on diets deficient in protein or trace nutrients have a decreased capacity to withstand

parasitic pathogenesis, while lambs fed a diet high in protein harbor less severe parasitic infections due to an increased immune response (Strain, 2001). Studies have shown that supplementation with protein (Kahn et al., 2003; Louvandini et al., 2006), energy (Valderrabano et al., 2002), and trace elements (Coop and Field, 1983) improve host resistance and resilience to parasitic infection.

Vitamin E is a fat-soluble vitamin best known for its role as an antioxidant (Combs, 1990). *In vitro* studies have documented the role of vitamin E in enhancing membrane stability and cell communication (Meydani et al., 1990), reduction of oxidative stress (Lee and Wan, 2002), and the proliferation of T cells through the up regulation of the cytokine interleukin 2 (IL-2) (Meydani et al., 1990) .

Past studies in ruminants supplemented with vitamin E have resulted in improved immune responses and performance in sheep and lambs, as well as decreases in lamb mortality in supplemented pregnant ewes (Finch and Turner, 1996, Kott et al., 1998). Numerous studies in ruminants provide evidence that vitamin E may enhance ruminant immune response through increased antibody production (Tengerdy et al., 1983), and higher levels of immunoglobulins G and M (Hidiroglou et al., 1992). No studies have examined the effect vitamin E supplementation may have on parasitic infections in lambs.

Studies in mice and humans have demonstrated the ability of vitamin E to promote a Th1 type response through increased production of interleukin 2 and interferon-γ, Th1 associated cytokines, during a challenge with influenza (Han et al., 2000). Other studies suggest that vitamin E may simultaneously dampen a Th2 response, as evidenced by a reduction in Th2 cytokines interleukins 4 and 5 in mice challenged with asthma

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(Okamoto et al., 2006), decrease IgE levels in mice challenged with an allergen (Bando et al., 2003), and the inhibit mast cell proliferation (Kempna et al., 2004).

Vitamin E supplementation has also been shown to decrease mast cell proliferation (Kempna et al., 2004), an important gut inflammatory response required by the host to successfully expel worms from the host in a parasitic infection (Miller, 1996). A study by Lightbody et al. (2001) evaluating antioxidant status in goat kids and lambs found that goat kids had consistently higher plasma antioxidant levels than lambs as well as significantly higher total worm burdens than lambs. The higher antioxidant status in this study was correlated with decreased resilience to infection (Lightbody et al., 2001). Supplementation of vitamin E and selenium to mice challenged with *T. gondii* was resulted in higher numbers of tissue cysts and increased severity of meningoencephalitis (McCarthy et al., 2003). These findings suggest vitamin E may inhibit an animals' ability to effectively respond to parasitic infection.

The most recent recommendations of the National Research Council (NRC) significantly increased daily vitamin E requirements in sheep from 0.5 IU/kg body weight (BW) (NRC, 1985) to 10 IU/kg BW (NRC, 2007) for optimal immune function. The current NRC recommendation (2007) was based on studies indicating that the previous dietary recommendation for vitamin E intake did not prevent myopathy and hepatic lipidosis in lambs (Menzies et al., 2004) in addition to studies showing enhanced immune function in sheep supplemented with vitamin E (Tengerdy, 1990). While it is clear that vitamin E supplementation clearly affects the immune response of lambs, there are no studies indicating the effect recent NRC recommendations may have on the ability of lambs to effectively combat parasitic infection.

To date, no studies evaluated the direct effect of vitamin E supplementation on gastrointestinal nematode infections in lambs. The objective of this study was to investigate the effect of vitamin E supplementation on an experimental *Haemonchus contortus* infection in Dorset lambs supplemented with either the minimal recommendation of vitamin E (5.3 IU/kg/day) or the recommendation for optimal immune function (10 IU/kg/day) (National Research Council, 2007). The current study hypothesizes that lambs supplemented with vitamin E will have a decreased capacity to effectively combat gastrointestinal nematodes (GIN). The results will lay the groundwork for future studies of the mechanisms involved in vitamin E and parasitic infection.

2. Material and methods

2.1 Study Subjects

Twenty Dorset lambs were housed at the University of Rhode Island's Peckham Farm. Lambs were weaned between 2 and 3 months of age. During the study, lambs were group fed 0.94 kg of grain/lamb/day as well as 1.36 kg hay/lamb/day for the duration of the study. This study was conducted with the approval of the Institutional Animal Care and Use Committee of the University of Rhode Island.

2.2 Experimental Design

Twenty Dorset lambs, 2-3 months of age were dewormed and housed in an environment free of parasites. Each lamb was administered a primary artificial infection of 10,000 *Haemonchus contortus* stage L3 infective larvae to stratify lambs according to parasite susceptibility. Three weeks post-infection, the lambs were dewormed with 0.2

mg/kg ivermectin and 1.5 mg/kg praziquantel per kg of body weight (EquiMax, Pzifer Animal Health, Eaton, PA) and levamisole hydrochloride (8 mg/kg) (Pfizer Animal Health, Eaton, PA). Lambs were stratified, using fecal egg shedding rates and sequentially assigned to two treatment groups. The male: female ratio was balanced between treatment groups.

Lambs were supplemented with vitamin E (d-α-tocopherol) for 11 weeks via a commercially available oral supplement (Emcelle, Stuart Products, Bedford, TX). The VE5 group of lambs was supplemented with the minimal amount of vitamin E recommended by the National Research Council (5.3 IU/kg body weight/day, n=10), while the VE10 treatment lamb group was supplemented with the National Research Council recommendations for optimal immune function (10 IU/kg body weight/day, n=10). The vitamin E was mixed into the grain fed to the lambs during the morning feeding and was dosed according to the body weight of the heaviest animal in each group.

At week 5 of supplementation, lambs were artificially infected with 10,000 *H. contortus* L3 larvae. Body weight, feed intake, fecal egg count (FEC), packed cell volume (PCV), and serum α-tocopherol levels were determined weekly. Lambs were humanely slaughtered 6 weeks post infection. Histologic samples of the abomasum were examined for inflammation and infiltration of globule leukocytes, mast cells, and eosinophils. Sections of muscle, lymph node, and liver were collected and analyzed for α-tocopherol content. Abomasal contents were collected at necropsy for parasite recovery, quantification and identification of sex and stage of development.

2.3 Blood and tissue collection

Blood samples were collected via jugular venipuncture into sterile SST and

EDTA vacutainer tubes (Becton Dickinson Biosciences, San José, CA) for α-tocopherol analysis and PCV determination, respectively. Serum tubes were centrifuged (946 x g, 10 min, 25$^°$C) and serum was aliquotted and stored at –80$^°$C until analysis for α-tocopherol content. PCV was determined by the micro-hematocrit centrifuge method. Liver, lymph node, and muscle tissue samples were collected at necropsy for α-tocopherol analysis, snap frozen in liquid nitrogen, and stored in –80$^°$C until analysis.

2.4 Feed sampling

Grain and core samples of hay fed to the lambs were sampled weekly throughout the study. Feed samples were kept at -20$^°$C until analysis. Composite samples of hay and grain were analyzed for α-tocopherol content (Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI) as well as nutritional analysis (Dairy One Inc., Ithaca, NY).

2.5 α-tocopherol analysis

Serum, tissue and feed samples were extracted into hexane and analyzed for α-tocopherol content by high performance liquid chromatography (HPLC) using a C_{18} column (Waters Corporation, Milford, MA,) with acetonitrile, methylene chloride, and methanol (70:20:10) as the mobile phase. Samples were analyzed at 292nm with a photo diode array detector. Serum samples were analyzed at the RI-INBRE Core Facility at the University of Rhode Island with a Hitachi LaChrom Elite HPLC System and software package (Hitachi High Technologies America, Inc., Pleasanton, CA). Tissue and feed samples were analyzed at the Diagnostic Center for Population and Animal Health (Lansing, MI).

2.6 Histology

At slaughter, sections of the cardiac, fundic, and pyloric regions of the abomasum were fixed in 10% formalin buffered saline (FBS). Fixed samples were embedded in paraffin and stained with hematoxylin and eosin to enumerate eosinophils and globule leukocytes, and toluidine blue for enumeration of mast cells. Ten fields on each tissue section per animal were randomly selected and examined at 400X magnification. Mean numbers of cells per animal were expressed per 40X field. Abomasal tissue samples were assessed for overall levels of abomasal inflammation based on a subjective scale, ranging from 0 to 4. Abomasal tissue was assessed by a pathologist for infiltration of numbers and concentrations of inflammatory cells, including lymphocytes, plasma cells, eosinophils and mast cells. A score of zero correlated with no inflammation. A score of 1 indicated minimal inflammation, with few scattered lymphocytes, plasma cells, eosinophils and mast cells. A score of 2 was given to cases of mild inflammation, with formation of small lymphoid follicles, small numbers of eosinophils and mast cells. A score of 3 was indicative of moderate inflammation, with the formation of large lymphoid follicles, moderate numbers of eosinophils and mast cells. A score of 4 was indicative of severe inflammation, characterized by the presence of large lymphoid follicles and significant mucous cell hyperplasia.

2.7 Parasitology

Rectal fecal samples were collected for fecal egg count determination according to the modified McMaster technique (Whitlock, 1948). At necropsy, the abomasum was opened along its greater curvature and contents were collected. Content volume was

brought up to two liters and a 10% aliquot was taken and fixed with equal volume of 10% FBS. After washing, the abomasum was incubated overnight in saline at 37º C to obtain embedded larvae. Contents were collected and fixed with equal volume 10% FBS. All worms from the 10% aliquot were counted from each sample, with total burdens estimated by extrapolation of aliquot counts. For each animal, the first 150 worms were sent to the Virginia/Maryland Regional College of Veterinary Medicine and were identified according to species, sex, and developmental stage.

Statistical Analysis

Serum α-tocopherol, FEC and PCV data were analyzed using mixed procedure in SAS with repeated measures (SAS Inc., Cary, NC). Each multivariable model included terms treatment, week, and treatment x week. Histological and parasite recovery data were compared using a Student's t test. Significance was defined as $P \leq 0.05$ and results are reported as mean $\pm$ SE.

3. Results

3.1 Feed Analyses

Nutrient analysis of the hay and grain fed to the lambs is presented in Table 1.

3.2 α-tocopherol analysis

Serum α-tocopherol concentrations of all groups across time are shown in Figure 1. Lambs in both VE5 and VE10 treatment groups had increased serum vitamin E levels after week 0; however, there was not a significant difference in serum alpha-tocopherol concentrations between groups. There were no differences in muscle ($P = 0.62$) or lymph

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node ($P = 0.38$) tissue α -tocopherol content between VE5 and VE10, however, the difference between VE5 and VE10 approached significance in the liver ($P = 0.08$) (Figure 2).

3.3 Blood packed cell volume

The PCV of VE5 and VE10 did not differ ($P = 0.39$). The PCV decreased over time across treatment ($P < 0.0001$). There was a 21% decrease in blood PCV across treatment groups over the infection period (Figure 3).

3.4 Parasitological parameters

3.4.1 Fecal egg count

Mean FEC increased over the infection period in both treatment groups. There was a trend towards a reduced FEC in VE10 lambs versus VE5 lambs ($P = 0.10$, Figure 4). There was no treatment x week effect ($P = 0.44$).

3.4.2 Parasite Recovery

Lambs were slaughtered 6 weeks post infection. The sex ratios of adult worms recovered did not differ between groups ($P = 0.4$). VE10 lambs had a lower abomasal worm burden than VE5 lambs ($P = 0.002$, Figure 5). There was a 49% reduction in total abomasal worm burden in the VE10 group as compared to VE5 lambs (Figure 5).

3.5 Histology

3.5.1 Abomasal Inflammation

Abomasal tissue samples were assessed for overall levels of abomasal inflammation by a pathologist and based on a subjective scale, ranging from 0 to 4 (Table 2).

Inflammation scores of all study lambs ranged from 2 to 2.4 (Table 2). There were no significant differences between treatment groups in mucosal abomasal inflammation ($P = 0.389$).

3.5.2 Eosinophil and Globule Leukocyte Numbers in Abomasal Mucosa

There was a wide variation in the individual response within the VE10 lambs for all cell types evaluated. Consequently, the difference in the means for eosinophils and globule leukocytes, though greater in VE10 versus VE5, did not rise to the level of significance (Figure 6). Mucosal mast cells were unable to be quantified.

4. Discussion

The objective of this study was to determine the effect of vitamin E supplementation at 5.3 or 10 IU d-α-tocopherol/kg BW/d on the progression of an experimental *Haemonchus contortus* infection in lambs. Abomasal worm burdens were significantly lower in VE10 lambs. This finding is also reflected in the trend observed in the fecal egg counts, as the VE10 lambs had consistently lower counts overall than VE5 lambs. The PCV slowly decreased in both treatment groups throughout the duration of the study, although there were no significant differences between the two groups. There was no difference in serum and tissue α-tocopherol concentration between VE5 and VE10 however, the difference between VE5 and VE10 in the liver approached significance. There was no treatment effect of α-tocopherol on abomasal inflammation, but it is clear that there was some effect of vitamin E on eosinophil and globule leukocyte infiltration, as the most reactive animals were found in the VE10 group (Figure 1, Figure 6, Table 2).

Fecal egg counts have been shown to be an accurate indicator of parasite burdens in sheep (Douch et al., 1996). Trichostrongyle type eggs were observed in fecal samples starting at 3 weeks post exposure to oral drenching with 10,000 infective L3 *Haemonchus contortus* larvae, consistent with the prepatent time period of this abomasal nematode (Zajac, 2006). The large variation in FEC can be accounted for by the fact that parasitic infections are often over dispersed with a minority of the animals in a population shedding the majority of the eggs (Zajac, 2010). As expected, the pattern of increase in FEC over time corresponded with a gradual decrease in PCV over the duration of the 6 week infection. No lambs experienced PCV falling below the acceptable range of 25-45 % for sheep (Zajac, 2010).

Lambs used for this study were stratified by parasite susceptibility and then sequentially assigned to treatment groups. The Dorset sheep breed is considered to be susceptible to parasitic infection, but within-breed variation in susceptibility or resistance is well documented (Vanimisetti et al, 2004). Lambs experienced both a primary and secondary infection therefore the lambs may have developed some level of immunity to the parasites, resulting in less severe clinical signs than may have been seen in comparison to a single naturally acquired pasture infection (Dobson et al., 1990). Repeated challenge of the immune system with infective parasitic larvae leads to significant acquired immunity (Dobson et al., 1990). It is possible that differences between groups may therefore be less dramatic as a result of both the lack of substantial establishment of adult worms and acquired resistance in the study lambs due to multiple experimental infections.

Although serum α-tocopherol concentrations below 2.0 μg/ml have historically been considered deficient (McMurray, 1982; Maas et al., 1984), the considerably lower values of 0.60 to 1.50 μg/ml are currently considered adequate for lambs (DCPAH, Lansing, MI). The average liver α-tocopherol concentrations were 72.4 and 85.4 μg/g dry weight in VE5 and VE10, respectively, well above the 20-40 ug/g dry weight considered adequate (Diagnostic Center for Population and Animal Health, Lansing, MI, Hidiroglou et al., 1994, Menzies et al., 2003). There were no significant differences between the groups in serum or tissue α-tocopherol concentrations, although liver α-tocopherol content within VE10 approached significance (P=0.08).

The lack of significant difference in serum α-tocopherol levels in the current study could be partially explained by the finding that after blood becomes saturated with the vitamin, it is deposited into the tissues, causing a decline in serum tocopherol levels (Ochoa et al., 1992). It is possible that the two dosages of vitamin E supplementation recommended by the NRC used in this study (5.3 IU/kg body weight/day versus 10 IU/kg body weight/day) for growing lambs were not dramatically different enough to detect a biological difference when supplemented orally. Pending analyses by the Diagnostic Center for Population Animal Health in Lansing, MI will confirm serum α-tocopherol concentrations.

In this study, vitamin E supplementation did not influence the level of inflammatory cells in the abomasa of the two treatment groups of lambs. Both groups showed some degree of moderate inflammation expected of a GIN infection, but no lambs showed severe levels of abomasal inflammation. Levels of inflammation may

have been higher in the case of a naturally acquired pasture infection in opposition to the controlled artificial infection used in this study.

The overall mean number of both cell types was consistently higher, although not significantly, in the VE10 lambs. There was a large amount of variability in lamb immune response to parasitic infection, but it is clear that the most reactive lambs were contained within the VE10 group. In general, it appears that inflammatory cells in the abomasum were inversely associated with *H. contortus* worm burden and FEC. While all current literature has indicated that vitamin E acts to dampen a Th2 response, it is clear that this did not occur in the present study. While the precise mechanisms are unclear, it is possible that supplementation may have increased a general inflammatory response, allowing for increased recruitment of eosinophils to abomasal mucosa and subsequent increased expulsion of worms in comparison to lambs supplemented with lower levels.

Interestingly, total abomasal worm burden was the only parasitological variable that showed a significant difference between treatment groups. Retrieval of the nematodes from the abomasum showed that the number of *H. contortus* adult worms was lower in the group treated with higher levels of vitamin E. Although contrary to studies reporting a decreased Th2 response, in this study vitamin E supplementation did not compromise the ability of lambs to combat parasitic infection. It appears that vitamin E may have actually hindered the ability of the worms to establish in the gastrointestinal tract of supplemented sheep in this study.

Currently, there are no known studies examining the direct effect of vitamin E on gastrointestinal nematodes. Based on the reduced worm burden and FEC of the VE10 group, it appears that vitamin E supplementation may inhibit parasite establishment

within the host through unknown mechanisms. While this study examines the effect vitamin E has on the host immune response to parasitic infection, future studies may warrant examining the possible effects vitamin E may have on the parasite itself, as parasite metabolism or survival within the host may also be influenced by supplementation.

In summary, supplementation with vitamin E resulted in significantly lower worm burdens, and clearly influenced lower FEC's and higher levels of Th2 immune cell infiltration. Further research into the effect of vitamin E supplementation on immune function in neonatal ruminants as well as the direct effect of vitamin E on nematodes is warranted.

5. Conclusion

Overall, the results from this study indicate that elevated levels of vitamin E supplementation had a beneficial effect on the abomasal worm burden of Dorset lambs challenged with an experimental infection of *Haemonchus contortus*. Lambs supplemented with the vitamin E levels recommended by the National Research Council for optimal immune functions tended to have a lower overall mean FEC as well as higher overall mean numbers of eosinophils and globule leukocytes within the three sections of the abomasum. Future studies examining differing levels of vitamin E supplementation than those used in this study, as well as the specific effects of vitamin E on parasitic mechanisms could provide greater insight to the relationship of vitamin E to *Haemonchus contortus* infection.

Acknowledgments

References

Adolfsson, O., B. T. Huber. 2001. Vitamin E-enhanced IL-2 production in old mice: naive but not memory T cells show increased cell division cycling and IL-2-producing capacity. *Journal of Immunology* 167, 3809-17.

Amarante, A. F., T. M. Craig, W. S. Ramsey, S. K. Davis, F. W. Bazer. 1999. Nematode burdens and cellular responses in the abomasal mucosa and blood of Florida Native, Rambouillet and crossbreed lambs. *Veterinary Parasitology* 80, 311-324.

Baker, R.L., T.G. Watson, S.A. Bisset, A. Vlossoff. 1990. Breeding Romney sheep which are resistant to gastro-intestinal parasites. *Proceedings of the Australian Association of Animal Breeding and Genetics* 50, 417.

Balic, A., V. M. Bowles, E. N. T. Meeusen. 2002 . Mechanisms of immunity to *Haemonchus contortus* infection in sheep. *Parasite Immunology* 24, 39-46.

Bang, K.S., A.S. Familton, A.R. Sykes, 1990. Effect of *Ostertagiasis* on copper status in sheep: a study involving use of copper oxide wire particles. *Research in Veterinary Science* 49, 306-314.

Barger, I.A., Cox, H.W. 1984. Wool production of sheep chronically infected with *Haemonchus contortus. Veterinary Parasitology* 15, 169-175.

Barriga, O.O., 1997. Veterinary Parasitiology for Practitioners.2nd ed., Burgress Publishing.

Bath, G.F., H. J. Krecek. 2001. Sustainable approaches for managing haemonchosis in sheep and goats. Final report of FAO Technical Cooperation Project, South Africa. 1-129.

Bethony, J.,A. 2006. Vaccines against blood-feeding nematodes of humans and livestock. *Parasitology* 133, S63-79.

Binns, S. H., I. J. Cox. 2002. Risk factors for lamb mortality on UK sheep farms. *Preventative Veterinary Medicine* 52, 287-303.

Bisset, S.A., A. Vlassoff, P.G. Douch, W.E. Jones, C.J. West, R.S. Green. 1996. Nematode burdens and immunological responses following natural selection in Romney lambs selectively bred for low or high fecal worm count. *Veterinary Parasitology* 61, 249-263.

Bowman, D.D., R.C. Lynn, M.L. Eberhard, 2003. Georgis' Parasitology for Veterinarians. 8th ed., W.B. Saunders.

Brunet, S. 2007. The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of a tannin-rich plant extract (sainfoin) both in vitro and in vivo. *Parasitology* 134, 1253-1262.

Buddle, B.M., G. Jowett, R.S. Green, P.G. Douch. 1992. Association of blood eosinophilia with the expression of resistance in Romney lambs to nematodes. *International Journal for Parasitology* 22, 955-960.

Burke, J. M. 2004. Effect of copper oxide wire particles dosage and feed supplement level on *Haemonchus contortus* infection in lambs. *Veterinary Parasitology* 123, 235–243.

Burke, J. M. 2007. Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. *Veterinary Parasitology* 147, 89-95.

Burke, J. M. 2009. Herbal dewormer fails to control gastrointestinal nematodes in goats. *Veterinary Parasitology* 160, 168-170.

Carravagi, C.M., W. Gibbons, F. Wright. 1968. The appearance of tocopherol in the blood of sheep after intramuscular injection of alpha tocopherol acetate. *New Zealand Agricultural Research* 11, 313.

Chaka, H., T. Eguale, D. Lidetu, D. Gizaw. 2009. Evaluation of the efficacy of anthelmintics sold on Ethiopian markets against *Haemonchus contortus* in experimentally infected Sheep. *Tropical Animal Health and Production* 41.6, 973-978.

Chartier, C., E. Etter., H. Heste, I. Pors, C. Koch, B. Dellac, 2000. Efficacy of copper oxide needles for the control of nematode parasites in dairy goats. *Veterinary Research Communications* 24, 389-399.

Coop, R. L., A. C. Field. 1983. Effect of phosphorus intake on growth rate, food intake and quality of skeleton of growing lambs infected with the intestinal nematode *Trichostrongylus vitrinus*. *Research in Veterinary Science* 35, 175-181.

Dobson, R.J., Waller, P.J., Donald, A.D. 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep: The effect of infection rate on the establishment of infective larvae and parasite fecundity. International Journal of Parasitology 20, 347-352.

Douch, P.G., Green, R.S., Morris, C.A., McEewan, J.C., Windon, R.G., 1996, Phenotypic markers for selection of nematode-resistant sheep. *International Journal for Parasitology* 26, 899-911.

Emery, D.L., S.J. McClure, B.M. Wagland. 1993. Production of vaccines against gastrointestinal nematodes of livestock. *Immunology and Cell Biology* 71, 463-472.

Finch, J. M., R. J. Turner. 1996. Effects of selenium and vitamin E on the immune responses of domestic animals. *Research in Veterinary Science* 60, 97-106.

Fleming, S. A., T. Craig, R.M. Kaplan. 2006. Anthelmintic Resistance of Gastrointestinal Parasites in Small Ruminants. *Journal of Veterinary Internal Medicine* 20, 435–444.

Gibbs, H. C. 1977. Spring rise in fecal nematode egg counts in sheep in Maine. *American Journal of Veterinary Research* 38, 533-534.

Gill, H. S. 2000). Induction of T helper-1 and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. *Immunology* 99, 458-463.

Gopal, R.M., Pomroy, W.E., West, D.M. 1999. Resistance of field isolates of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* to ivermectin. *International Journal for Parasitology* 29, 781-786.

Han, S. N., S. N. Meydani. 2000. Antioxidants, cytokines, and influenza infection in aged mice and elderly humans. *Journal of Infective Disease* 182, S74-80.

Han, S. N. 2006. Age and vitamin E-induced changes in gene expression profiles of T cells. *Journal of Immunology* 177, 6052-61.

Hartwig, N. 2000. Sheep health-fact sheet no.8: Control of internal parasites of sheep. *USDA*, Iowa State University.

Hatfield, P. G., B. L. Robinson. 2002. Serum α-tocopherol and immune function in yearling ewes supplemented with zinc and vitamin E. *Journal Animal Science* 80, 1329-34.

Henriksen, S.A., K. Aagaard. 1976. En enkel flotation og McMaster metode. *Nordisk Veterinair Medicin* 28, 393-397.

Herd, R. P., C. F. Parker, K. E. McClure. 1984. Epidemiological approach to the control of sheep nematodes. *Journal of American Veterinary Medical Association* 184, 680-686.

Hernandez, J., E. Soto-Canevett. 2009. In vitro effect of vitamin E on lectin-stimulated porcine peripheral blood mononuclear cells. *Veterinary Immunology and Immunopathology* 131, 9-16.

Hidiroglou, M., and K. Karpinski. 1987.Vitamin E Kinetics in Sheep. *British Journal of Nutrition* 58, 113.

Hidiroglou, M., T. R. Batra, L. F. Laflamme, F. Markham. 1992. Possible roles of vitamin E in immune response of calves. *International Journal for Vitamin and Nutrition Research,* 62.

Hidiroglou, N., McDowell, L.R., Batra, T.R., Papas, A.M. 1994. Tissue α-tocopherol concentrations following supplementation with various forms of vitamin E in sheep. *Reproduction Nutrition Development* 34, 273-278.

Howell, S.B., Burke, J.M., Miller, J.E., Terrill, T.H., Valencia, E., Williams, M.J., Williamson, L.H., Zajac, A.M., Kaplan, R.M. 2008. Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States. *Journal of the American Veterinary Medical Association* 233, 1913-1919.

Jakhsi, N. S. A. 2006. The effect of subclinical experimental infection with haemonchosis on some productive aspects of Karadi lambs. Kurdistan 1st Conference on Biological Sciences, University of Dohuk.

Johnstone, C., 2000. Parasites and parasitic diseases of domestic animals: The nematodes – *H. contortus*. University of Pennsylvania. http://cal.nbc.upenn.edu/merial/Trichos/trich_5b.htm

Kahn, L. P., I. Kyriazakis, F. Jackson, and R. L. Coop. 2000. Temporal effects of protein nutrition on the growth and immunity of lambs infected with *Trichostrongylus colubriformis. International Journal for Parasitology* 30: 193-205.

Kaplan, R.M., Burke, J.M., Terrill, T.H., Miller, J.E., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Larsen, M., Vatta, A.F., 2004, Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. *Veterinary Parasitology* 123, 105-120.

Kaplan, R. M., J.M. Burke, S.B. Howell, J.R. Rocconi. 2005. Total anthelmintic failure on a meat goat farm in Arkansas. American Association of Veterinary Parasitologists, 50th Annual Meeting, Minneapolis, MN.

Kaplan, R. M. 2006. Update on Parasite Control in Small Ruminants 2006: Addressing the Challenges Posed By Multiple-Drug Resistant Worms. American Association of Bovine Practitioners, Saint Paul, MN.

Kaplan, R. M. 2004. Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States.*Veterinary Parasitology* 123, 105-120.

Kempna, P., E. Reiter, M. Arock, A. Azzi, and J.-M. Zingg. 2004. Inhibition of hmc-1 mast cell proliferation by vitamin E: Involvement of the protein kinase B pathway. *Journal Biology Chemical* 279, 50700-50709.

Kilon, A.D., and T.B. Nutman. 2004. The role of eosinophils in host defense against helminth parasites. *Journal of Allergy and Clinical Immunology*, 113, 30-37.

Knox, M. R., J. W. Steel, C. A. Anderson, L. L. Muir. 2003. Nutrition-parasite interactions in sheep. *Australian Journal of Experimental Agriculture* 43, 1383–1488.

Kott, R. W., V. M. Thomas, P. G. Hatfield, T. J. Evans, K. C. Davis. 1998. Effects of dietary vitamin E supplementation during late pregnancy on lamb mortality and ewe productivity. *Journal of American Veterinary Medical Association* 212: 997-1000.

Lee, D. L. 2002. The biology of nematodes. CRC Press.

Lightbody, J. H., L. M. Stevenson, F. Jackson, K. Donaldson, D. G. Jones. 2001. Comparative aspects of plasma antioxidant status in sheep and goats, and the influence of experimental abomasal nematode infection. *Journal of Comparative Pathology* 124, 192-199.

Louvandini, H. 2006. Influence of protein supplementation on the resistance and resilience on young hair sheep naturally infected with gastrointestinal nematodes during rainy and dry seasons. *Veterinary Parasitology* 137, 103-111.

MacGlaflin, C.E., A.M. Zajac, K.A. Rego, K.H. Petersson. 2011. Effect of vitamin E supplementation on naturally acquired parasitic infection in lambs. *Veterinary Parasitology* 175, 300-305.

Mahieu, M., Arquet, R., Kandassamy, T., Mandonnet, N., Hoste, H., 2007. Evaluation of targeted drenching using FAMACHA method in Creole goat: reduction of anthelmintic use, and effects on kid production and pasture contamination. *Veterinary Parasitology* 146, 135-147.

Matthews, J. B. 2006. Veterinary parasite immunology. *Parasite Immunology* 28, 271–273.

Menzies, P.I., Boermans, H., Hoff, B., Durzi, T., Langs, L. 2003. Survey of the status of copper, interacting minerals, and vitamin E levels in the livers of sheep in Ontario. *Canadian Veterinary Journal* 44, 898-906.

Menzies, P., L. Langs, H. Boermans, J. Martin, and J. McNally. 2004. Myopathy and hepatic lipidosis in weaned lambs due to vitamin E deficiency. *Canadian Veterinary Journal* 45, 244-247.

Meydani, S. N. 1990. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *American Journal of Clinical Nutrition* 52, 557-563.

Miller, J. E. 1998. Epidemiology of gastrointestinal nematode parasitism in Suffolk and Gulf coast native sheep with special emphasis on relative susceptibility to *Haemonchus Contortus* infection. *Veterinary Parasitology* 74, 55-74.

Miller, J. E., D.W. Horohov. 2006. Immunological aspects of nematode parasite control in sheep. *Journal Animal Science* 84, 124.

Min, B.R, T.N. Barry, G.T. Atwood, W.C. McNabb. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science and Technology* 106, 3–19.

Mir, R. A. 2007. Clinicopathological Changes in Sheep Experimentally Infected with *Haemonchus contortus*. *World Journal of Agricultural Sciences* 5, 562-566.

Morein, B., I. Abusugra. 2002. Immunity in neonates. *Veterinary Immunology and Immunopathology* 87, 207-13.

Mosmann, T. R, Coffman, R.L. 1989. Th1 and Th2 cells : Different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology* 7, 145-173.

NAHMS. 1996. Reference of U.S. regional sheep health and management practices. Fort Collins, CO, USDA APHIS VS.

NIH. 2007. Vitamin E (Office of Dietary Supplements). Retrieved on January 31, 2009 from ods.od.nih.gov/FactSheets/VitaminE3.asp

Njeru, C.A., L.R. McDowell, N.S. Wilkinson, S.B. Linda, L.X. Rojas, S.N. Williams.1994. Serum and tissue tocopherol in sheep after intramuscular injection and (or) dietary vitamin E supplementation. *Journal Animal Science* 72, 739-745.

NRC, 1985. *Nutrient Requirements of Sheep*, 6th rev. ed. National Academy Press, Washington, DC.

NRC. 2007. *Nutrient Requirements of Small Animals*. Washington, D.C., National Academy Press.

Ochoa, L, L.R. MacDowell, S.N. Williams, N.S. Wilkinson, J. Boucher, E.L. Lentz. 1992. Alpha-tocopherol concentrations in serum and tissues of sheep fed different sources of vitamin E. *Journal Animal Science* 70, 2568.

O'Connor, L. J., S. W. Walkden-Brown, L. P. Kahn. 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Veterinary Parasitology* 142, 1-15.

Paolini, V., J.P. Bergeaud, C. Grisez, F. Prevot, Ph. Dorchies, H. Hoste. 2003. Effects of condensed tannins on goats experimentally infected with Haemonchus contortus. *Veterinary Parasitology* 113, 253-261.

Peña, M. T., J.E. Miller, M.E. Fontenot, A. Gillespie, M. Larsen. 2002. Evaluation of Duddingtonia flagrans in reducing infective larvae of Haemonchus contortus in feces of sheep. *Veterinary Parasitology* 103, 259–265.

Prichard, R. K., C. A. Hall, J. D. Kelly, I. C. A. Martin, A. D. Donald. 1980. The problem of anthelmintic resistance in nematodes. *Australian Veterinary Journal* 56, 239-250.

Prichard R.K. and Roulet A. 2007. ABC transporters and β-tubulin in macrocyclic lactone resistance: prospects for marker development. *Parasitology* 134, 1123–1132.

Richard, R. M. 1954. The Effect of Phlebotomy versus Stomach Worm, *Haemonchus Contortus*, Infection on the Growth and Certain Blood Constituents of Lambs. *Journal of Animal Science* 13, 274-282.

Rivera, J. D., G. C. Duff, M. L. Galyean, D. A. Walker, G. A. Nunnery. 2002. Effects of supplemental vitamin e on performance, health, and humoral immune response of beef cattle. *Journal of Animal Science* 80, 933-941.

Roos, M.H.1997. The role of drugs in the control of parasitic nematode infections: must we do without? *Parasitology* 114, S137-S144.

Sangster, N.C., 1999. Anthelmintic resistance: past, present and future. *International Journal for Parasitology* 29, 115-124.

Schallig, H.D., 2000. Immunological responses of sheep to *Haemonchus contortus. Parasitology* 120, S63-S72.

Shakya, K. P., J.E. Miller, D.W. Horohov. 2009. A Th2 type of immune response is associated with increased resistance to Haemonchus contortus in naturally infected Gulf Coast Native lambs. *Veterinary Parasitology* 163, 57-66.

Strain, S. A.J., M.J. Stear. 2001. The influence of protein supplementation on the immune response to *Haemonchus contortus. Parasite Immunology* 23, 527-531.

Sykes, A.R, H.L. XIE, M. Stankiewicz, J.F. Huntley. 2007. The effect of vaccinating infection during pregnancy and dietary protein supply on the periparturient immune response of sheep to infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* larvae. *Animal* 1, 249-260.

Tengerdy, R. P. 1990. Immunity and disease resistance in farm animals fed vitamin E supplement. *Advances in Experimental Medicine and Biology* 262, 103-110.

Terefe, G. 2007. Immune response to *Haemonchus contortus* infection in susceptible (INRA 401) and resistant (Barbados Black Belly) breeds of lambs. *Parasite Immunology* 29, 415-424.

Terrill, T. H., Miller, J.E. 2005. Nematode Parasites in Small Ruminant Grazing Research: Changing Perspectives. *Conference Proceedings*. Agricultural Research Station, Fort Valley State University, Fort Valley, GA.

Terrill, T. H., G.S. Dykes, S.A. Shaik, J.E. Miller, B. Kouakou, G. Kannan, J.M. Burke, J.A. Mosjidis. 2009. Efficacy of sericea lespedeza hay as a natural dewormer in goats: Dose titration study. *Veterinary Parasitology* 163, 52-56.

Tizard, I. R. 2004. <u>Veterinary Immunology: An Introduction</u>. Philadelphia, Saunders.

Tritschler, J. P., D. J. Giordano, G. C. Coles. 1989. The epidemiological control of nematodes in New England lambs. *Cornell Veterinarian* 79, 327-338.

USDA, 1996. Reference of 1996 U.S. regional sheep health and management practices.

USDA, 2003. Highlights of NAHMS sheep 2001: parts II and III.

USDA. 2008. Animal Health Report, 2007. Retrieved June 10, 2009 from http://www.aphis.usda.gov/publications.

Valderrabano, J., R. Delfa, J. Uriarte. 2002. Effect of level of feed intake on the development of gastrointestinal parasitism in growing lambs. *Veterinary Parasitology* 104, 327-338.

Vanimisetti, H. B. 2004. Inheritance of fecal egg count and packed cell volume and their relationship with production traits in sheep infected with *Haemonchus contortus*. *Journal of Animal Science* 82 (6), 1602-1611.

Van Wyk J.A. 2001. Refugia - overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *Onderstepoort Journal of Veterinary Research* 68, 55-67.

Van Wyk, J.A., Bath, G.F.. 2002. The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Veterinary Research* 33, 509-529.

Van Wyk, J.A., Hoste, H., Kaplan, R.M., Besier, R.B. 2006. Targeted selective treatment for worm management--how do we sell rational programs to farmers? *Veterinary Parasitology* 139, 336-346.

Vlassoff, A., D. M. Leathwick, and A. C. G. Heath. 2001. The epidemiology of nematode infections of sheep. *New Zealand Veterinary Journal* 49, 213-221.

Waghorn T.S., Leathwick D.M., Miller C.M., Atkinso, D.S. 2008. Brave or gullible: testing the concept that leaving susceptible parasites in refugia will slow the development of anthelmintic resistance. *New Zealand Veterinary Journal* 56, 158-63.

Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., Sangster, N.C. 2004. Drug resistance in veterinary helminths. *Trends in Parasitology* 20, 469-476.

Wood IB, K. Bairden, J.L. Duncan, T. Kassai, J.B. Malone, J.A. Pankavich, R.K. Reinecke, O. Slocombe,S.M. Taylor. 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine. *Veterinary Parasitology* 58, 181-213.

Zajac, A. M., T.A. Gipson. 2000. Multiple anthelmintic resistance in a goat herd. *Veterinary Parasitology* 87, 163-172.

Zajac, A. M., G.A. Conboy. 2006. Veterinary clinical parasitology. Blackwell Publishing.

Zajac, A. 2010. Integrated Parasite Control in Small Ruminants, Goat and Sheep Parasite Control Workshop, Foster, Rhode Island. Oct. 10, 2010.

Figure 1. Serum α-tocopherol in lambs supplemented with either the NRC minimal

vitamin E recommendation (VE5; 5.3 IU/kg BW/day, n=10) or amount for optimal

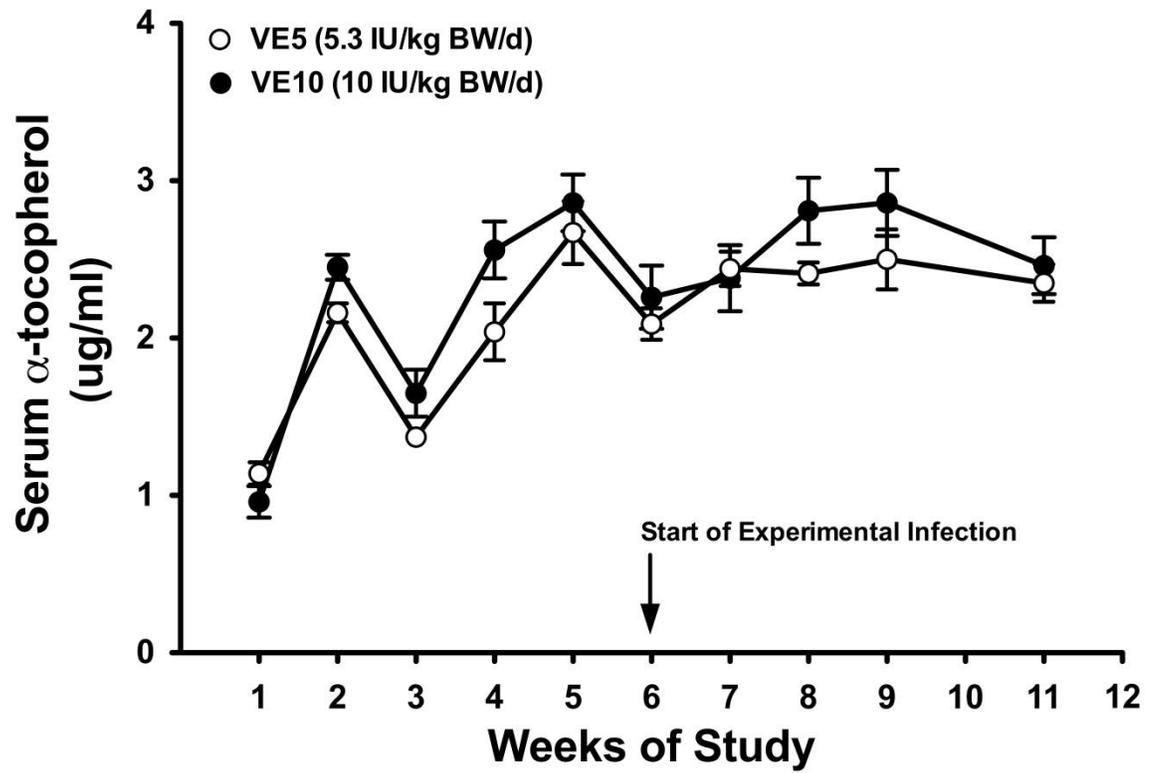immune function (VE10; 10 IU/kg BW/day, n = 10) for 11 weeks. Mean ± SE.

Figure 2. Tissue α-tocopherol in lambs supplemented with either the NRC minimal vitamin E recommendation (VE5; 5.3 IU/kg BW/day, n=10) or amount for optimal immune function (VE10; 10 IU/kg BW/day, n = 10) for 11 weeks. Mean ± SE.

*P≤ 0

* versus week 3, 4, 5 and 6 *(P=0.02)*

Figure 3. Blood packed cell volume (%) measured over time (across treatment) in lambs supplemented with either the NRC minimal vitamin E recommendation (VE5; 5.3 IU/kg BW/day, n=10) or the level for optimal immune function (VE10; 10 IU/kg BW/day, n = 10) for 11 weeks. All lambs were artificially infected with 10,000 infective *H. contortus* larvae at week 5 of supplementation and slaughtered at 6 weeks post infection. Mean ± SE.
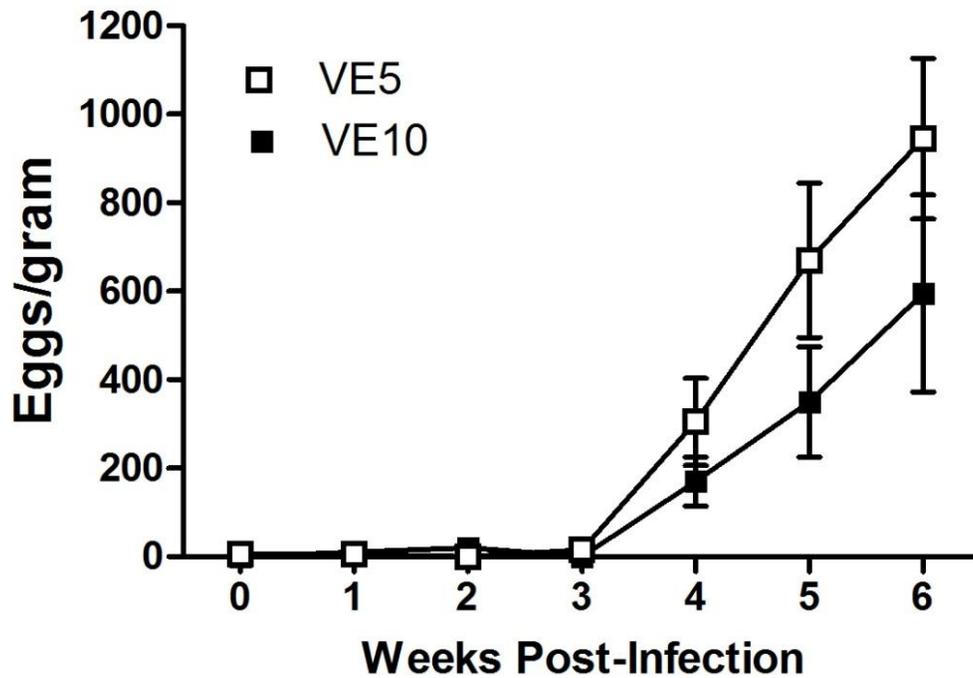
Figure 4. Fecal egg counts (eggs/gram) measured over time in lambs (n=20) challenged with experimental *H. contortus* infection supplemented with either the NRC minimal vitamin E level recommendation (VE5; 5.3 IU/kg BW/day, n=10) or the level for optimal imm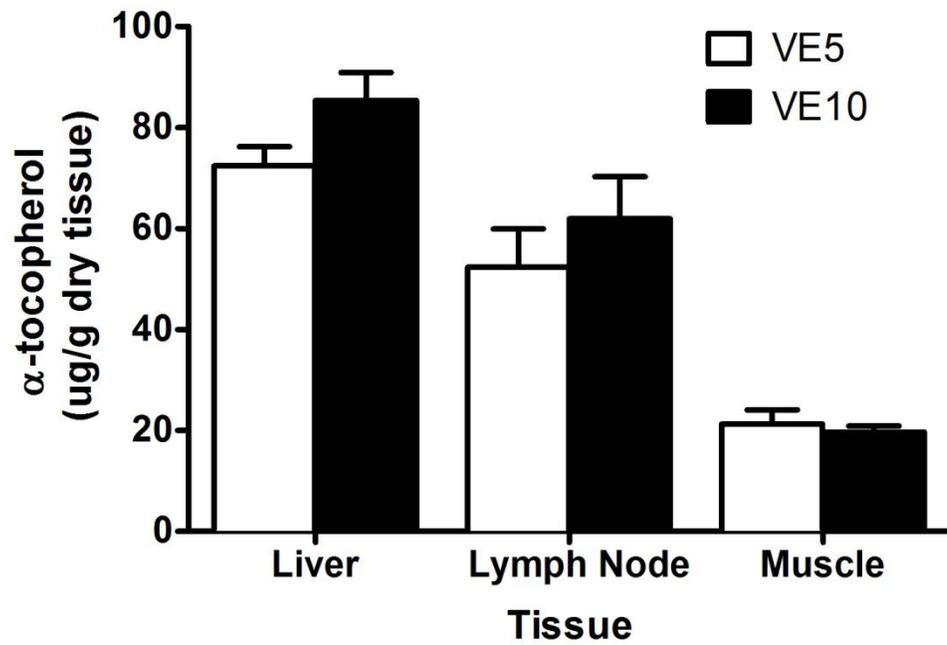une function (VE10; 10 IU/kg BW/day, n = 10) for 11 weeks. All lambs were artificially infected with 10,000 infective *H. contortus* larvae at week 5 of supplementation and slaughtered 6 weeks post infection. Mean $\pm$ SE.
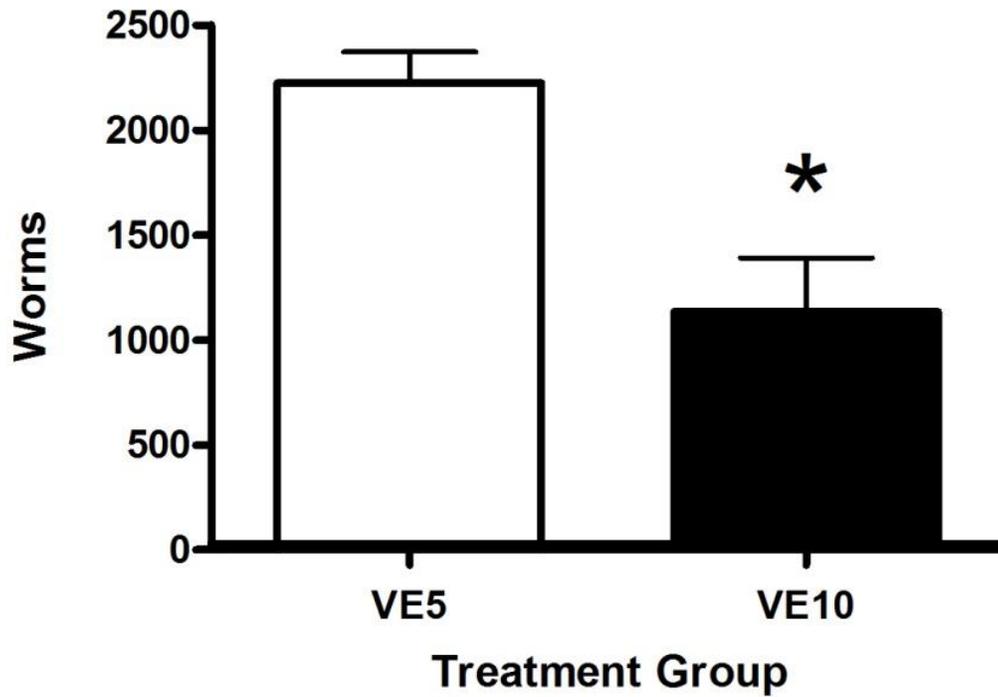
*P= 0.002

Figure 5. Total abomasal worm burden in lambs supplemented with either the NRC

minimal vitamin E recommendation (VE5; 5.3 IU/kg BW/day, n=10) or the level for

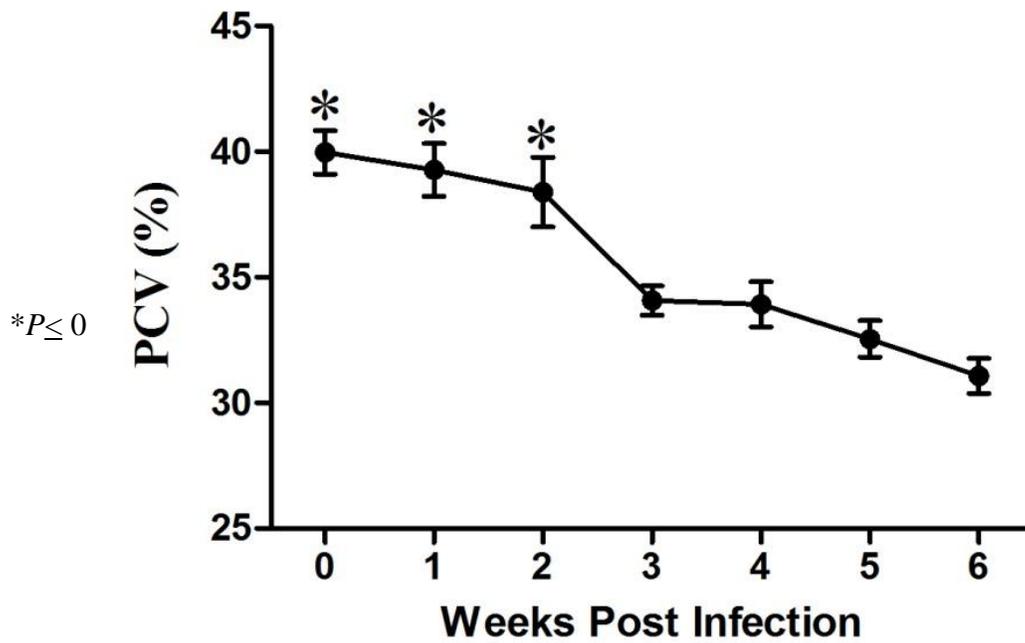optimal immune function (VE10; 10 IU/kg BW/day, n = 10) for 11 weeks. All lambs

were artificially infected with 10,000 infective *H. contortus* larvae at week 5 of

supplementation and slaughtered 6 weeks post infection. Mean ± SE.
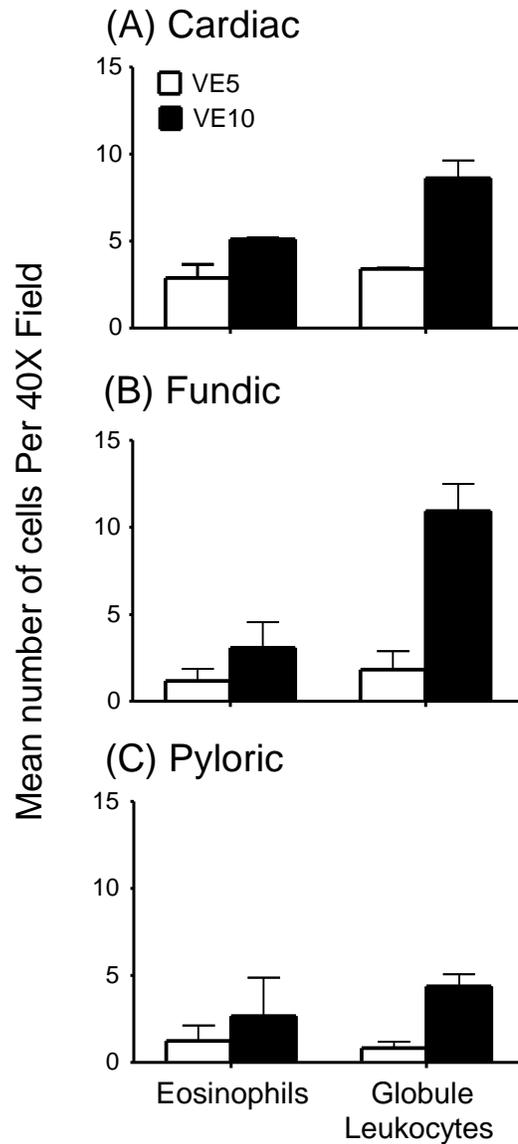
Figure 6. Eosinophil and globule leukocytes in (A) cardiac, (B) fundic, and (C) pyloric regions of abomasal mucosa in lambs supplemented with either the NRC minimal vitamin E recommendation (VE5; 5.3 IU/kg BW/day, n=10) or the level for optimal immune function (VE10; 10 IU/kg BW/day, n = 10) for 11 weeks. All lambs were artificially infected with 10,000 infective *H. contortus* larvae at week 5 of supplementation and slaughtered 6 weeks post infection. Mean ± SE.

Table 1

Nutritional content of composite hay and grain samples collected over the study period
and composited for proximate analysis and vitamin E analysis.

Components	Grain Mix-VE5	Grain Mix-VE10	Hay
DM, %	88.8	88.6	94.6
CP, % of DM	17.2	17.7	16.8
NDF, % of DM	29.4	31.7	58.5
ADF, % of DM	17.9	14.3	35.7
Calcium, % of DM	1.43	1.40	0.63
Phosphorous, % of DM	0.75	0.76	0.33
Magnesium, % of DM	0.33	0.33	0.28
Potassium, % of DM	0.99	0.98	2.17
NE_m, Mcal/kg of DM	1.90	1.87	1.31
Ne_g, Mcal/kg of DM	1.26	1.24	0.74
Total VE, IU/kg of DM	388	690	9
α-Tocopherol, µg/g of DM	353	627	8

Nutrient analysis of the feed conducted by Dairy One Cooperative, Inc. (Ithaca,
New York). The grain and hay was analyzed for vitamin E content at the
Diagnostic Center for Population & Animal Health (DCPAH, Michigan State
University, Lansing, MI).

Table 2

Inflammation scoring in abomasa of lambs artificially infected with *H. contortus* for 6 weeks and supplemented with either the NRC minimal vitamin E recommendation (VE5; 5.3 IU/kg body weight (BW)/day (d), n=10) or the level for optimal immune function (VE10; 10 IU/kg BW/d, n = 10) for 11 weeks.

Abomasal Region	VE5	VE10
Cardiac	2.4 ±0.18	2.3± 0.28
Fundic	2.0 ±0.24	2.4± 0.17
Pyloric	2.3 ±0.24	2.4± 0.24

Mean ± SE.

Standard Operating Procedure for Blood Collection

Standard Operating Procedure modified from Thesis of Caitlyn MacGlaflin, University of Rhode Island, 2010. Originally modified from Purdue University Blood Sampling in Sheep by Mitchell et al. (Retrieved on September 23, 2008 from http://www.ces.purdue.edu/extmedia/AS/AS-557-W.pdf). Blood is collected using BD blood collection equipment including a Vacutainer sleeve (REF# 364815), 21 1½ G needle (REF#367215), EDTA (REF #367863) and SST (REF #367988) tubes (Becton, Dickinson, and Company; Franklin Lakes, NJ).

Blood Collection:

1. Properly restrain animal. A blocking stand may be used or the head of the animal may be held at a 30° angle to the side.

2. If available, electric clippers may be used to prepare the neck by shaving off a patch of wool approximately 4 inches wide and 8 inches long.

3. To locate the vein, apply pressure with the thumb approximately half way down the side of the neck.

4. Secure a Vacutainer needle to the Vacutainer holder.

5. While holding off on the vein with the left hand, insert the needle into the vein making sure that bevel faces upward.

6. Once the needle is placed, push the vacutainer tube into the holder and onto the needle.

7. Gently move the needle until blood begins to flow into the tube. Blood flow will cease once the tube is full.

8. Gently remove the tube and slowly invert eight times.

9. Remove your left hand and allow the vein to relax.

10. Remove the needle from the skin, and place a finger over the area to prevent bleeding.

11. EDTA tubes should be immediately placed in ice. SST should sit upright for 30 minutes at room temperature.

APPENDIX II

Standard Operation Procedure for Blood Aliquots

<u>Serum Aliquots:</u>

1. Collect blood by jugular venipuncture into Vacutainer SST Tubes.

2. Allow tubes to sit upright for 30 minutes at room temperature.

3. Centrifuge tubes (946 x g, 10 min, 25°C).

4. With a transfer pipette, pool the serum samples for each individual animal in a 15ml Falcon tube. Place on ice.

5. With a transfer pipette, pipette serum into cryogenic vials. Cover vials with white caps, and label cap with the last 2 digits of the animal identification number.

6. Place samples in appropriate freezer box, and store in a $^-$80°C freezer.

<u>Plasma Aliquots:</u>

1. Collect blood by jugular venipuncture into Vacutainer EDTA Tubes.

2. Invert tubes eight times, then immediately place on ice.

3. Centrifuge tubes at (946 x g, 10 min, 4°C).

4. With a transfer pipette, pool the plasma samples for each individual animal in a 15ml Falcon tube and place on ice.

5. With a transfer pipette, pipette plasma into cryogenic vials. Cover vials with yellow caps, and label cap with the last 2 digits of the animal identification number.

6. Place samples in appropriate freezer box, and store in a ⁻80° C freezer.

Standard Operating Procedure for Packed Cell Volume

All samples should be run in duplicate.

Sample Preparation:

1. Collect blood by jugular venipuncture into Vacutainer EDTA Tubes, invert tube eight times, and place on ice.

2. Invert tube to mix before use.

3. Without spilling, tip tube so that blood moves toward the opening. Place capillary tube into the blood and allow tube to fill by capillary action. Only fill tube up to ¾ of the way.

4. Place fingertip at one end of the tube to prevent leaking. Insert the other end of the tube into the white sealing wax.

5. Place capillary tubes into centrifuge rotor, making sure that the wax in the end of the tube faces outward (not towards the center of the centrifuge). Keep centrifuge balanced at all times.

6. To prevent tubes from cracking, take a pin and gently push tubes against the outer edge of the rotor.

7. Centrifuge to at 15,000 RPM for 3 minutes at room temperature.

Sample Reading:

1. Spin the PCV Reader (International Equipment Company; Needham, MA) until it reaches the 100 mark.

2. Place the capillary tube into the sample groove.

3. Align the wax/blood interface with the line at the bottom of the sample groove.

4. Spin the top plate of the PCV reader until the "swirl" aligns with the plasma/air interface.

5. Spin both plates until the "swirl" aligns with the white blood cell/red blood cell interface. The value under the red line is the PCV to be read and reported as a percentage.

APPENDIX IV

Standard Operating Procedure for Fecal Egg Counts (FEC) Using the Modified McMaster's Technique

FEC were determined weekly during the experiment. FEC were determined using the established Modified McMaster technique (Whitlock, 1948). Modified McMaster slides were obtained from Chalex Corporation, Issaquah, WA.

1. Fecal samples are collected directly from the rectum and kept refrigerated until analysis. Samples should be run as promptly as possible, but within seven days from the time of collection.

2. Two grams of feces are measured on a calibrated scale and placed into a l-2 ounce cup.

3. Add 28 ml Fecasol® to feces and soak for approximately 5 minutes.

4. Gently break up fecal pellet with a tongue depressor. Set cup aside to sit for approximately 5 minutes.

5. Pour fecal solution through a 2-ply gauze into a new cup. Add 3 ml Fecasol to the first tube to clean any remaining fecal solution out. Use tongue depressor to gently press fecal solution through gauze.

6. Wet the McMaster slide with distilled water and gently pat dry top and bottom with paper towels.
.

7. Immediately pipet solution into both sides of the McMaster slide, using a 1 ml syringe, a sample of the suspension and fill one side of the chamber.

8. Place slide on microscope platform and let sit, without disturbance, for 5 minutes.

9. Focus on the top layer using the low power (10x) objective. Count all eggs inside of the grid areas (greater than ½ of egg inside grid).

10. Count only trichostrongyle/strongyle eggs (oval shaped, ~80-90 microns long)

11. Total egg count:
    (chamber 1 + chamber 2) * 50 = eggs per gram (EPG)
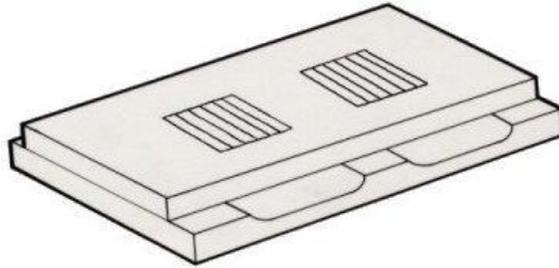
Modified McMaster slide (image from www.vetslides.com).

APPENDIX V

Standard Operating Procedure for Nematode Recovery Post Mortem

Preparation:

1. Lambs are stunned by captive bolt and exsanguinated.

2. Gastrointestinal tract is collected as soon as possible following exsanguination.

3. Mesenteric tissue is cleaned away from the abomasum and three pieces of butcher's rope are used to tie the junction of the omasum and abomasum, the junction of the abomasum and beginning of the small intestine, and at the junction of the small intestine and large intestine.

4. The rumen, reticulum, and omasum are removed and the remaining is placed in a cooler until nematode recovery begins.

Nematode Recovery:

Abomasum Wash:

1. The abomasum is removed from the tract and cleaned of excess fat and mesenteric tissue.

2. A scalpel is used to open the abomasum along the greater curvature.

3. Contents are collected into a pan by washing with warm water. The folds of the abomasum are gently rubbed to remove any debris.

4. Once completely cleaned the abomasal contents are brought up to 2 L.

5. The contents are mixed thoroughly using an inverted beaker and stirring in a figure eight motion. Immediately a 10 % aliquot of the contents, 200 ml, is taken and placed into a 500 ml jar.

6. The sample is fixed with 200 ml 10% phosphate buffered formalin (PBF) and stored until nematode quantification and identification.

Abomasum Soak:

A soak is performed to collect any larvae that may be embedded within the tissue of the abomasum.

1. Once the wash is completed, the abomasum is spread out on in a pan, covered with saline, and covered with aluminum foil.

2. The abomasum is incubated at 37ºC overnight.

3. The contents of the soak are collected and the abomasum is gently washed with water and collected.

4. Contents are allowed to settle for at least 2 hours.

5. In order to collect the settled contents a serological pipet is used to remove as much liquid without disturbing the bottom as possible.

6. The remaining sample is collected into 500 ml jars, 200 ml in each, and fixed with 200 ml 10 % PBF each.

APPENDIX VI

Standard Operating Procedure for Nematode Quantification and Identification

1. All worms from either a 10% aliquot or a 2% aliquot (in the case of high worm recovery, > 2 worms per milliliter) will be quantified.

2. When taking aliquots, samples will be stirred and aerated with a 5-15 gallon aquarium pump during quantification to insure the sample is well mixed.

3. Approximately 6 ml of sample will be pipetted onto a square petri dish with 13 mm grid (BD Bioscience) and nematodes will be counted using a dissecting microscope.

4. The first 100-150 nematodes (or as many recovered from aliquot if > 100) encountered will be separated using a bent stainless endodontic hedsrom file (Miltex, Inc, York, PA) and sent to the Virginia-Maryland Regional College of Veterinary Medicine, Department of Biomedical Sciences and Pathobiology, (Blacksburg, VA) to be identified according to species, sex, and stage of development by Dr. Ann M. Zajac D.V.M, Ph.D.

Standard Operating Procedure for Measuring the Vitamin E Content of Serum by High Performance Liquid Chromatography

Standard operation procedure modified from a protocol from the Diagnostic Center for Population and Animal Health at Michigan State University.

Reagents:

Ethanol with 0.01% BHT and 0.1% Ascorbic Acid

Mobile Phase: 70% Acetonitrile, 20% Methylene Chloride, 10% Methanol

Vitamin E Extraction:

1. Because vitamin E is light sensitive, extraction should take place under gold or red light to protect samples.

2. Place 1ml of serum in a 16 x 125 labeled test tube.

3. Add 1ml of ethanol containing 0.01% BHT and 0.1% Ascorbic Acid.

4. Add 20ul of apocarotenal internal standard.

5. Vortex for 5 minutes.

6. Add 1 ml HPLC grade hexane.

7. Vortex for 5 minutes and centrifuge at 946 x g for 5 minutes.

8. Remove 0.5ml of the top layer and dry on a 35°C heating block by nitrogen evaporation.

9. Resuspend in 0.5ml of mobile phase then transfer to HPLC vials.

10. Set up a standard curve with the α-tocopherol high standard.

HPLC:

1. Run on the Hitachi analytical HPLC system at the INBRE core facility.

2. Analyze samples using photo diode array at a wavelength of 292nm and with a C18, 3.5um, 4.6 x 75mm column and a flow rate of 1.2 ml/min.

BIBLIOGRAPHY

Adolfsson, O., B. T. Huber. 2001. Vitamin E-enhanced IL-2 production in old mice: naive but not memory T cells show increased cell division cycling and IL-2-producing capacity. *Journal of Immunology* 167, 3809-17.

Amarante, A. F., T. M. Craig, W. S. Ramsey, S. K. Davis, F. W. Bazer. 1999. Nematode burdens and cellular responses in the abomasal mucosa and blood of Florida Native, Rambouillet and crossbreed lambs. *Veterinary Parasitology* 80, 311-324.

Baker, R.L., T.G. Watson, S.A. Bisset, A. Vlossoff. 1990. Breeding Romney sheep which are resistant to gastro-intestinal parasites. *Proceedings of the Australian Association of Animal Breeding and Genetics* 50, 417.

Balic, A., V. M. Bowles, E. N. T. Meeusen. 2002 . Mechanisms of immunity to *Haemonchus contortus* infection in sheep. *Parasite Immunology* 24, 39-46.

Bang, K.S., A.S. Familton, A.R. Sykes, 1990. Effect of *Ostertagiasis* on copper status in sheep: a study involving use of copper oxide wire particles. *Research in Veterinary Science* 49, 306-314.

Barger, I.A., Cox, H.W. 1984. Wool production of sheep chronically infected with *Haemonchus contortus. Veterinary Parasitology* 15, 169-175.

Barriga, O.O., 1997. Veterinary Parasitiology for Practitioners.2nd ed., Burgress Publishing.

Bath, G.F., H. J. Krecek. 2001. Sustainable approaches for managing haemonchosis in sheep and goats. Final report of FAO Technical Cooperation Project, South Africa. 1-129.

Bethony, J.,A. 2006. Vaccines against blood-feeding nematodes of humans and livestock. *Parasitology* 133, S63-79.

Binns, S. H., I. J. Cox. 2002. Risk factors for lamb mortality on UK sheep farms. *Preventative Veterinary Medicine* 52, 287-303.

Bisset, S.A., A. Vlassoff, P.G. Douch, W.E. Jones, C.J. West, R.S. Green. 1996. Nematode burdens and immunological responses following natural selection in Romney lambs selectively bred for low or high fecal worm count. *Veterinary Parasitology* 61, 249-263.

Bowman, D.D., R.C. Lynn, M.L. Eberhard, 2003. Georgis' Parasitiology for Veterinarians. 8th ed., W.B. Saunders.

Brunet, S. 2007. The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of a tannin-rich plant extract (sainfoin) both in vitro and in vivo. *Parasitology* 134, 1253-1262.

Buddle, B.M., G. Jowett, R.S. Green, P.G. Douch. 1992. Association of blood eosinophilia with the expression of resistance in Romney lambs to nematodes. *International Journal for Parasitology* 22, 955-960.

Burke, J. M. 2004. Effect of copper oxide wire particles dosage and feed supplement level on *Haemonchus contortus* infection in lambs. *Veterinary Parasitology* 123, 235–243.

Burke, J. M. 2007. Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. *Veterinary Parasitology* 147, 89-95.

Burke, J. M. 2009. Herbal dewormer fails to control gastrointestinal nematodes in goats. *Veterinary Parasitology* 160, 168-170.

Carravagi, C.M., W. Gibbons, F. Wright. 1968. The appearance of tocopherol in the blood of sheep after intramuscular injection of alpha tocopherol acetate. *New Zealand Agricultural Research* 11, 313.

Chaka, H., T. Eguale, D. Lidetu, D. Gizaw. 2009. Evaluation of the efficacy of anthelmintics sold on Ethiopian markets against *Haemonchus contortus* in experimentally infected Sheep. *Tropical Animal Health and Production* 41.6, 973-978.

Chartier, C., E. Etter., H. Heste, I. Pors, C. Koch, B. Dellac, 2000. Efficacy of copper oxide needles for the control of nematode parasites in dairy goats. *Veterinary Research Communications* 24, 389-399.

Coop, R. L., A. C. Field. 1983. Effect of phosphorus intake on growth rate, food intake and quality of skeleton of growing lambs infected with the intestinal nematode *Trichostrongylus vitrinus*. *Research in Veterinary Science* 35, 175-181.

Dobson, R.J., Waller, P.J., Donald, A.D. 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep: The effect of infection rate on the establishment of infective larvae and parasite fecundity. International Journal of Parasitology 20, 347-352.

Douch, P.G., Green, R.S., Morris, C.A., McEewan, J.C., Windon, R.G., 1996, Phenotypic markers for selection of nematode-resistant sheep. *International Journal for Parasitology* 26, 899-911.

Emery, D.L., S.J. McClure, B.M. Wagland. 1993. Production of vaccines against gastrointestinal nematodes of livestock. *Immunology and Cell Biology* 71, 463-472.

Finch, J. M., R. J. Turner. 1996. Effects of selenium and vitamin E on the immune responses of domestic animals. *Research in Veterinary Science* 60, 97-106.

Fleming, S. A., T. Craig, R.M. Kaplan. 2006. Anthelmintic Resistance of Gastrointestinal Parasites in Small Ruminants. *Journal of Veterinary Internal Medicine* 20, 435–444.

Gibbs, H. C. 1977. Spring rise in fecal nematode egg counts in sheep in Maine. *American Journal of Veterinary Research* 38, 533-534.

Gill, H. S. 2000). Induction of T helper-1 and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. *Immunology* 99, 458-463.

Gopal, R.M., Pomroy, W.E., West, D.M. 1999. Resistance of field isolates of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* to ivermectin. *International Journal for Parasitology* 29, 781-786.

Han, S. N., S. N. Meydani. 2000. Antioxidants, cytokines, and influenza infection in aged mice and elderly humans. *Journal of Infective Disease* 182, S74-80.

Han, S. N. 2006. Age and vitamin E-induced changes in gene expression profiles of T cells. *Journal of Immunology* 177, 6052-61.

Hartwig, N. 2000. Sheep health-fact sheet no.8: Control of internal parasites of sheep. *USDA*, Iowa State University.

Hatfield, P. G., B. L. Robinson. 2002. Serum α-tocopherol and immune function in yearling ewes supplemented with zinc and vitamin E. *Journal Animal Science* 80, 1329-34.

Henriksen, S.A., K. Aagaard. 1976. En enkel flotation og McMaster metode. *Nordisk Veterinair Medicin* 28, 393-397.

Herd, R. P., C. F. Parker, K. E. McClure. 1984. Epidemiological approach to the control of sheep nematodes. *Journal of American Veterinary Medical Association* 184, 680-686.

Hernandez, J., E. Soto-Canevett. 2009. In vitro effect of vitamin E on lectin-stimulated porcine peripheral blood mononuclear cells. *Veterinary Immunology and Immunopathology* 131, 9-16.

Hidiroglou, M., and K. Karpinski. 1987.Vitamin E Kinetics in Sheep. *British Journal of Nutrition* 58, 113.

Hidiroglou, M., T. R. Batra, L. F. Laflamme, F. Markham. 1992. Possible roles of vitamin E in immune response of calves. *International Journal for Vitamin and Nutrition Research,* 62.

Hidiroglou, N., McDowell, L.R., Batra, T.R., Papas, A.M. 1994. Tissue α-tocopherol concentrations following supplementation with various forms of vitamin E in sheep. *Reproduction Nutrition Development* 34, 273-278.

Howell, S.B., Burke, J.M., Miller, J.E., Terrill, T.H., Valencia, E., Williams, M.J., Williamson, L.H., Zajac, A.M., Kaplan, R.M. 2008. Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States. *Journal of the American Veterinary Medical Association* 233, 1913-1919.

Jakhsi, N. S. A. 2006. The effect of subclinical experimental infection with haemonchosis on some productive aspects of Karadi lambs. Kurdistan 1st Conference on Biological Sciences, University of Dohuk.

Johnstone, C., 2000. Parasites and parasitic diseases of domestic animals: The nematodes – *H. contortus*. University of Pennsylvania. http://cal.nbc.upenn.edu/merial/Trichos/trich_5b.htm

Kahn, L. P., I. Kyriazakis, F. Jackson, and R. L. Coop. 2000. Temporal effects of protein nutrition on the growth and immunity of lambs infected with *Trichostrongylus colubriformis*. *International Journal for Parasitology* 30: 193-205.

Kaplan, R.M., Burke, J.M., Terrill, T.H., Miller, J.E., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Larsen, M., Vatta, A.F., 2004, Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. *Veterinary Parasitology* 123, 105-120.

Kaplan, R. M., J.M. Burke, S.B. Howell, J.R. Rocconi. 2005. Total anthelmintic failure on a meat goat farm in Arkansas. American Association of Veterinary Parasitologists, 50th Annual Meeting, Minneapolis, MN.

Kaplan, R. M. 2006. Update on Parasite Control in Small Ruminants 2006: Addressing the Challenges Posed By Multiple-Drug Resistant Worms. American Association of Bovine Practitioners, Saint Paul, MN.

Kaplan, R. M. 2004. Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States.*Veterinary Parasitology* 123, 105-120.

Kempna, P., E. Reiter, M. Arock, A. Azzi, and J.-M. Zingg. 2004. Inhibition of hmc-1 mast cell proliferation by vitamin E: Involvement of the protein kinase B pathway. *Journal Biology Chemical* 279, 50700-50709.

Kilon, A.D., and T.B. Nutman. 2004. The role of eosinophils in host defense against helminth parasites*. Journal of Allergy and Clinical Immunology*, 113, 30-37.

Knox, M. R., J. W. Steel, C. A. Anderson, L. L. Muir. 2003. Nutrition-parasite interactions in sheep. *Australian Journal of Experimental Agriculture* 43, 1383–1488.

Kott, R. W., V. M. Thomas, P. G. Hatfield, T. J. Evans, K. C. Davis. 1998. Effects of dietary vitamin E supplementation during late pregnancy on lamb mortality and ewe productivity. *Journal of American Veterinary Medical Association* 212: 997-1000.

Lee, D. L. 2002. The biology of nematodes. CRC Press.

Lightbody, J. H., L. M. Stevenson, F. Jackson, K. Donaldson, D. G. Jones. 2001. Comparative aspects of plasma antioxidant status in sheep and goats, and the influence of experimental abomasal nematode infection. *Journal of Comparative Pathology* 124, 192-199.

Louvandini, H. 2006. Influence of protein supplementation on the resistance and resilience on young hair sheep naturally infected with gastrointestinal nematodes during rainy and dry seasons. *Veterinary Parasitology* 137, 103-111.

MacGlaflin, C.E., A.M. Zajac, K.A. Rego, K.H. Petersson. 2011. Effect of vitamin E supplementation on naturally acquired parasitic infection in lambs. *Veterinary Parasitology* 175, 300-305.

Mahieu, M., Arquet, R., Kandassamy, T., Mandonnet, N., Hoste, H., 2007. Evaluation of targeted drenching using FAMACHA method in Creole goat: reduction of anthelmintic use, and effects on kid production and pasture contamination. *Veterinary Parasitology* 146, 135-147.

Matthews, J. B. 2006. Veterinary parasite immunology. *Parasite Immunology* 28, 271–273.

Menzies, P.I., Boermans, H., Hoff, B., Durzi, T., Langs, L. 2003. Survey of the status of copper, interacting minerals, and vitamin E levels in the livers of sheep in Ontario. *Canadian Veterinary Journal* 44, 898-906.

Menzies, P., L. Langs, H. Boermans, J. Martin, and J. McNally. 2004. Myopathy and hepatic lipidosis in weaned lambs due to vitamin E deficiency. *Canadian Veterinary Journal* 45, 244-247.

Meydani, S. N. 1990. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *American Journal of Clinical Nutrition* 52, 557-563.

Miller, J. E. 1998. Epidemiology of gastrointestinal nematode parasitism in Suffolk and Gulf coast native sheep with special emphasis on relative susceptibility to *Haemonchus Contortus* infection. *Veterinary Parasitology* 74, 55-74.

Miller, J. E., D.W. Horohov. 2006. Immunological aspects of nematode parasite control in sheep. *Journal Animal Science* 84, 124.

Min, B.R, T.N. Barry, G.T. Atwood, W.C. McNabb. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science and Technology* 106, 3–19.

Mir, R. A. 2007. Clinicopathological Changes in Sheep Experimentally Infected with *Haemonchus contortus*. *World Journal of Agricultural Sciences* 5, 562-566.

Morein, B., I. Abusugra. 2002. Immunity in neonates. *Veterinary Immunology and Immunopathology* 87, 207-13.

Mosmann, T. R, Coffman, R.L. 1989. Th1 and Th2 cells : Different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology* 7, 145-173.

NAHMS. 1996. Reference of U.S. regional sheep health and management practices. Fort Collins, CO, USDA APHIS VS.

NIH. 2007. Vitamin E (Office of Dietary Supplements). Retrieved on January 31, 2009 from ods.od.nih.gov/FactSheets/VitaminE3.asp

Njeru, C.A., L.R. McDowell, N.S. Wilkinson, S.B. Linda, L.X. Rojas, S.N. Williams.1994. Serum and tissue tocopherol in sheep after intramuscular injection and (or) dietary vitamin E supplementation. *Journal Animal Science* 72, 739-745.

NRC, 1985. *Nutrient Requirements of Sheep*, 6th rev. ed. National Academy Press, Washington, DC.

NRC. 2007. *Nutrient Requirements of Small Animals*. Washington, D.C., National Academy Press.

Ochoa, L, L.R. MacDowell, S.N. Williams, N.S. Wilkinson, J. Boucher, E.L. Lentz. 1992. Alpha-tocopherol concentrations in serum and tissues of sheep fed different sources of vitamin E. *Journal Animal Science* 70, 2568.

O'Connor, L. J., S. W. Walkden-Brown, L. P. Kahn. 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Veterinary Parasitology* 142, 1-15.

Paolini, V., J.P. Bergeaud, C. Grisez, F. Prevot, Ph. Dorchies, H. Hoste. 2003. Effects of condensed tannins on goats experimentally infected with Haemonchus contortus. *Veterinary Parasitology* 113, 253-261.

Peña, M. T., J.E. Miller, M.E. Fontenot, A. Gillespie, M. Larsen. 2002. Evaluation of Duddingtonia flagrans in reducing infective larvae of Haemonchus contortus in feces of sheep. *Veterinary Parasitology* 103, 259–265.

Prichard, R. K., C. A. Hall, J. D. Kelly, I. C. A. Martin, A. D. Donald. 1980. The problem of anthelmintic resistance in nematodes. *Australian Veterinary Journal* 56, 239-250.

Prichard R.K. and Roulet A. 2007. ABC transporters and β-tubulin in macrocyclic lactone resistance: prospects for marker development. *Parasitology* 134, 1123–1132.

Richard, R. M. 1954. The Effect of Phlebotomy versus Stomach Worm, *Haemonchus Contortus*, Infection on the Growth and Certain Blood Constituents of Lambs. *Journal of Animal Science* 13, 274-282.

Rivera, J. D., G. C. Duff, M. L. Galyean, D. A. Walker, G. A. Nunnery. 2002. Effects of supplemental vitamin e on performance, health, and humoral immune response of beef cattle. *Journal of Animal Science* 80, 933-941.

Roos, M.H.1997. The role of drugs in the control of parasitic nematode infections: must we do without? *Parasitology* 114, S137-S144.

Sangster, N.C., 1999. Anthelmintic resistance: past, present and future. *International Journal for Parasitology* 29, 115-124.

Schallig, H.D., 2000. Immunological responses of sheep to *Haemonchus contortus. Parasitology* 120, S63-S72.

Shakya, K. P., J.E. Miller, D.W. Horohov. 2009. A Th2 type of immune response is associated with increased resistance to Haemonchus contortus in naturally infected Gulf Coast Native lambs. *Veterinary Parasitology* 163, 57-66.

Strain, S. A.J., M.J. Stear. 2001. The influence of protein supplementation on the immune response to *Haemonchus contortus. Parasite Immunology* 23, 527-531.

Sykes, A.R, H.L. XIE, M. Stankiewicz, J.F. Huntley. 2007. The effect of vaccinating infection during pregnancy and dietary protein supply on the periparturient immune response of sheep to infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* larvae. *Animal* 1, 249-260.

Tengerdy, R. P. 1990. Immunity and disease resistance in farm animals fed vitamin E supplement. *Advances in Experimental Medicine and Biology* 262, 103-110.

Terefe, G. 2007. Immune response to *Haemonchus contortus* infection in susceptible (INRA 401) and resistant (Barbados Black Belly) breeds of lambs. *Parasite Immunology* 29, 415-424.

Terrill, T. H., Miller, J.E. 2005. Nematode Parasites in Small Ruminant Grazing Research: Changing Perspectives. *Conference Proceedings*. Agricultural Research Station, Fort Valley State University, Fort Valley, GA.

Terrill, T. H., G.S. Dykes, S.A. Shaik, J.E. Miller, B. Kouakou, G. Kannan, J.M. Burke, J.A. Mosjidis. 2009. Efficacy of sericea lespedeza hay as a natural dewormer in goats: Dose titration study. *Veterinary Parasitology* 163, 52-56.

Tizard, I. R. 2004. Veterinary Immunology: An Introduction. Philadelphia, Saunders.

Tritschler, J. P., D. J. Giordano, G. C. Coles. 1989. The epidemiological control of nematodes in New England lambs. *Cornell Veterinarian* 79, 327-338.

USDA, 1996. Reference of 1996 U.S. regional sheep health and management practices.

USDA, 2003. Highlights of NAHMS sheep 2001: parts II and III.

USDA. 2008. Animal Health Report, 2007. Retrieved June 10, 2009 from http://www.aphis.usda.gov/publications.

Valderrabano, J., R. Delfa, J. Uriarte. 2002. Effect of level of feed intake on the development of gastrointestinal parasitism in growing lambs. *Veterinary Parasitology* 104, 327-338.

Vanimisetti, H. B. 2004. Inheritance of fecal egg count and packed cell volume and their relationship with production traits in sheep infected with *Haemonchus contortus*. *Journal of Animal Science* 82 (6), 1602-1611.

Van Wyk J.A. 2001. Refugia - overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *Onderstepoort Journal of Veterinary Research* 68, 55-67.

Van Wyk, J.A., Bath, G.F.. 2002. The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Veterinary Research* 33, 509-529.

Van Wyk, J.A., Hoste, H., Kaplan, R.M., Besier, R.B. 2006. Targeted selective treatment for worm management--how do we sell rational programs to farmers? *Veterinary Parasitology* 139, 336-346.

Vlassoff, A., D. M. Leathwick, and A. C. G. Heath. 2001. The epidemiology of nematode infections of sheep. *New Zealand Veterinary Journal* 49, 213-221.

Waghorn T.S., Leathwick D.M., Miller C.M., Atkinso, D.S. 2008. Brave or gullible: testing the concept that leaving susceptible parasites in refugia will slow the development of anthelmintic resistance. *New Zealand Veterinary Journal* 56, 158-63.

Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., Sangster, N.C. 2004. Drug resistance in veterinary helminths. *Trends in Parasitology* 20, 469-476.

Wood IB, K. Bairden, J.L. Duncan, T. Kassai, J.B. Malone, J.A. Pankavich, R.K. Reinecke, O. Slocombe,S.M. Taylor. 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine. *Veterinary Parasitology* 58, 181-213.

Zajac, A. M., T.A. Gipson. 2000. Multiple anthelmintic resistance in a goat herd. *Veterinary Parasitology* 87, 163-172.

Zajac, A. M., G.A. Conboy. 2006. Veterinary clinical parasitology. Blackwell Publishing.

Zajac, A. 2010. Integrated Parasite Control in Small Ruminants, Goat and Sheep Parasite Control Workshop, Foster, Rhode Island. Oct. 10, 2010.