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The Evaluation of Eprinomectin (LONGRANGE®) on Long-Term Parasitic Infection in Nursing Calves During Summer Grazing®

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THE EVALUATION OF EPRINOMECTIN (LONGRANGE®) ON LONG-TERM
PARASITIC INFECTION IN NURSING CALVES DURING SUMMER
GRAZING®

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program in the School of Animal Science

by
Lyndsea D. Seibert
B.S., Louisiana State University, 2013
August 2016

Dedicated to

Donna Seibert
My angel in heaven John Seibert
Chelsea Seibert, Shane Seibert, Kimberly Seibert, and Nicole Callais
Robert Folsom
All of my children with four paws

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ABSTRACT

One of the most significant threats to the well-being and performance of grazing livestock are gastrointestinal nematode (GIN) parasitic infections. Allowing a large GIN burden to manifest can cause a significant cost in terms of productivity in grazing cattle. Beef producers commonly rely greatly on the efficacy of broad-spectrum anthelmintics, to not only kill resident worms but to also prevent the establishment of ingested infective stage larvae (L3) for a period following treatment. Eprinomectin (LONGRANGE[®], E-LR) is the first extended-release injectable cattle dewormer that claims to provide cattle producers season-long persistent parasite control for 100 to 150 d in a single dose, dependent on parasite species. This in turn, improves nutritional efficiency. The purpose of this study is to evaluate the efficacy of E-LR for controlling long-term GIN infections in nursing calves. E-LR was compared to the traditional anthelmintic fenbendazole (Panacur[®], F-P). Four groups of nursing calves (with their dams) grazed separate pastures and pastures were rotated after each sampling date to ensure equal parasitic pasture exposure. Two groups were treated with E-LR and two groups were treated with F-P. Calves were weighed at 28 d intervals and fecal samples were collected on d 0, 14, 28, 56, 84, and 112. Results indicated E-LR was effective in providing long term parasite control. There was no significant ($p < 0.05$) difference in the FEC between F-P and E-LR treated animals on d 14, showing that both were efficient at initially killing GIN. Significant differences in FEC between treatments started to show by d 56. On d 56, there was an increase in FEC in calves treated with F-P which continued to increase throughout the rest of the study. The E-LR treated animals had only slightly increased FEC for the rest of the study which indicated prolonged GIN control.

CHAPTER 1

INTRODUCTION

Gastrointestinal nematodes (GIN) are parasites that have caused disease and production losses in many different types of animals including ruminants. Cows, sheep, and goats are just a few of the ruminants that can become infected with GIN while grazing. The GIN parasites that affect livestock have an obligatory free-living part of the life stage where larval development to the infective larval stage (L3) takes place on pasture. The phylum Nematoda includes many parasites that are of major socio-economic importance including *Haemonchus spp.*, *Ostertagia spp.*, *Trichostrongylus spp.*, *Cooperia spp.*, and *Oesophagostomum spp.* Depending on the species and burden of these parasites, they can cause potentially fatal diseases such as parasitic gastroenteritis (PGE) (Taylor et al, 2007). Common signs of PGE include reduced weight gain or weight loss, anorexia, diarrhea, reduced production and, in the case of blood-feeding species, anemia and edema, due to the loss of blood and/or plasma proteins (Taylor et al., 2007). Every GIN present within the host has been acquired by the ingestion of L3 present on the pasture (Waller, 2006). While grazing on infective pastures, ruminants are exposed to varying numbers of L3 and can consume very high numbers leading to heavy GIN burdens.

This can constitute a significant threat to the health and welfare of grazing livestock throughout much of the world and impose a significant cost in terms of productivity in grazing cattle (Perry, 2002; Dijk et al, 2010). GIN infections in countries like the United States have accounted for losses around \$330 million USD/year (Seó, 2015). Young animals, such as calves and replacement heifers, are the most susceptible to GIN infection and the negative effects include decreased weight gain and delay in reproduction. Any delay in breeding and calving can also dramatically increase rearing costs by as much as \$50 per heifer for each month beyond 24

months of age (Van Amburgh, 2001; Looper and Bethard, 2005.). Lawrence et al. (2009), showed that the prevention and treatment of GIN can result in up to a \$201 gain per head of cattle.

There can be much variation in the level of GIN depending on the animal and its geographic location. Multiple studies have shown that the most highly parasitized ruminants in the country are located in areas where the climate is hot and humid. Heavily stocked pastures and/or limited acreage can also result in higher GIN burdens, particularly in these mild and moist conditions (Williams, 1986). Younger animals are more likely to display clinical signs of parasitism, while adult animals are more likely to have subclinical infections that may impact their performance and profitability. Age, climate, living conditions, and timing are some aspects to consider in determining an appropriate GIN management plan (Gould, 2009).

Conventional methods of controlling GIN parasites of grazing livestock have been through the use of synthetic chemotherapeutic drugs (anthelmintics). Because of the continual developments of increased anthelmintic efficacy, better safety, and broader spectrum of activity along with increased cost effectiveness, livestock producers have relied almost exclusively on their use (Morley and Donald, 1980; Waller, 1993). Though this approach has been successful, many reports of anthelmintic failure suggest that traditional deworming methods are short sighted and unsustainable. Additionally, the overuse of these anthelmintics has led to numerous reports of drug resistance that include every livestock host and every anthelmintic class. In some regions of the world, the high prevalence of multi-drug resistance (MDR) in GIN of sheep and goats threatens the viability of small-ruminant industries (Kaplan, 2004). According to Sutherland and Leathwick (2011), escalating reports since 2005 suggest that GIN anthelmintic resistance in cattle is a rapidly increasing problem. For example, benzimidazole resistance was

identified in cattle in New Zealand (Jackson et al, 2006) and South America (Suarez et al, 2007). Macrocyclic lactone (ML) resistance was found in New Zealand (Vermunt et al, 1996; Loveridge, 2003) the Americas (Gasbarre et al, 2009; Edmonds et al, 2010) and Europe (Demeler et al, 2009). However, there have only been a few detailed reports in the U.S. identifying reduced anthelmintic efficacy and increasing resistance in ruminants. Therefore, the extent of the problem in the U.S remains largely unknown and may be grossly underestimated in cattle.

Most conventional anthelmintics are short acting and do not stay at therapeutic levels in animals long enough to provide protection for an extended length of time thus requiring frequent use. The most commonly used anthelmintics remain efficacious for short periods of time ranging from 3-5 to 42 d. This shorter time length typically is not long enough to reduce the amount of GIN L3 on the pasture. In the large picture, GIN parasite programs involving frequent treatments are not convenient nor efficacious as it requires excessive handling of cattle. Excessive handling, especially during hot summer months, places more stress on the animals which can have deleterious effects on cattle health, productivity and welfare (Mader, 2006). In addition, frequent use of traditional anthelmintics contribute to the selection pressure placed on resistant GIN. Hence, with the increasing challenge of GIN control in cattle, there is an urgent need to develop a different approach for parasite control to maintain long term drug sustainability in cattle and reduce selection pressure of resistant GIN.

Merial, a multinational animal health company, has produced an extended release injectable anthelmintic that offers 100 to 150 d of GIN protection with one single dose. Eprinomectin (LONGRANGE[®], E-LR) is formulated in a polymer matrix, which aids in pulse release of the active ingredient following injection. Merial claims that a single spring treatment

works long enough to break the parasite life cycle and reduce pasture reinfection. Because of the length of protection that E-LR offers, it also aims to reduce the labor costs and stress associated with handling and treating animals.

The objectives of this study were 1) to evaluate the anthelmintic efficacy of E-LR at the recommended dose of 1 mg/kg of body weight in nursing calves during summer grazing in comparison to fendendazole (Panacur[®], F-P) and 2) to evaluate the effect of E-LR compared to F-P on weight gain in nursing calves during summer grazing.

CHAPTER 2 LITERATURE REVIEW

2.1 Trichostrongyle Type Parasites

Trichostrongyle type parasites have been found to be the most economically significant GIN in small and large ruminants (Gibbs, 1987). Heavily parasitized calves are unhealthy in appearance and usually have a dry, dull hair coat. Growth and feed efficiency are also substantially reduced. Anemia, diarrhea, weight loss and death may occur when infection is severe. Small and large ruminants are hosts to a number of GIN within the Phylum Nematoda with slight variations (Sutherland *et al*, 2009). Differences in host preference occur within GIN species. Cattle can be hosts to at least 14 GIN and the major threat to the animal's health and performance comes from those found in the abomasum and small intestines. Members of the trichostrongyle type GIN group in cattle that cause the most damage are *Haemonchus placei* (barber pole worm), *Ostertagia ostertagi* (medium or brown stomach worm), *Trichostrongylus axei* (small stomach/bankrupt worm), *Oesophagostomum radiatum* (nodular worm), and many species of *Cooperia* including *C. punctata*, *C. oncophora*, and *C. pectinata*. (Craig, 2009). *H. placei* is primarily a parasite in tropical regions, whereas *O. ostertagi* and, to a lesser extent, *T. axei* are found in more temperate climates. These nematodes are small, thread-like worms that are found in the abomasum. Species of *Cooperia* are usually found in the small intestine in cattle, while *O. radiatum* is found in the caecum and large intestine in cattle (Ballweber, 2001; Gibbs, 1986). Although some details vary, GIN of cattle have a direct life cycle and follow a similar pattern. Having a direct life cycle means that the parasite does not require an intermediate host to complete the life cycle. In other words, the parasite will only need to infect one animal to complete its life cycle.

2.1.1 Life Cycle of Trichostrongyle Type Nematodes in Cattle

Adult worms in the abomasum or intestinal tract can produce millions of microscopic-sized eggs that are passed in the feces. Under favorable environmental conditions, these eggs hatch in feces and form into the free living larval stages, 1st larval stage (L1), 2nd larval stage (L2), and 3rd larval stage (L3). The immature L1 and L2 continue to feed and develop in feces on pastures until they reach the ensheathed L3. The sheath protects the L3 stage from environmental conditions but prevents it from feeding (Taylor, 2007). When proper moisture, via rain, dew, or irrigation, is available, the L3 migrate out of the feces and onto surrounding vegetation. L3 larvae are then ingested by grazing animals. Depending on the species of the parasite, the development from L1 to L3 takes less than 14 d under optimal conditions (Craig, 2009). The L3 larvae loses its protective sheath in the rumen and invades the mucosa of the abomasum or intestinal tract where it develops to the fourth stage larvae (L4). The L4 emerge from the mucosa and eventually mature into adult worms in the lumen (Schmidt, 2013). The cycle is then repeated when the adult worms mate and produce eggs that are passed in the feces producing the next generation. Depending on the GIN present, the life cycle can take up to 6 weeks to complete (Ballweber, 2001).

2.1.2 *Haemonchus placei*

Haemonchus is a genus of parasitic roundworms that infect cattle, sheep and goats as well as other wild ruminants. Nematodes of this genus are also called the barber pole worm, twisted wireworm or large stomach worm. They are found worldwide but are more frequent and harmful in regions with tropical and subtropical humid climates. The most relevant species for livestock are *H. contortus* and *H. placei*. While both of these species can infect small and large

ruminants, *H. placei* primarily targets cattle. The adults are 20-30 mm in length (Craig, 2009). The mouth of *Haemonchus* worms have a small pointed lancet for disrupting capillaries in the mucosal tissues to allow blood flow for feeding purposes (Urquhart, 2007).

After the L3 are ingested, they move to the abomasum, penetrate the abomasal mucosa and develop to L4. L4 return to the lumen and mature to adults. L4 and adults both feed on blood flowing out of the lesions they cause to the abomasal mucosa with their lancet mouthparts (Taylor, 2007). The blood loss can be extensive enough to result in anemia. Females can produce up to 10,000 eggs daily. L4 larvae can become dormant (sometimes called hypobiosis or inhibited development) in the abomasal tissues as a means of survival in that cold and/or dry environmental conditions outside the host are not conducive for completion of the life cycle. They resume development to adults after 3-4 months when environmental conditions become more favorable. One of the clinical signs of chronic infection is edema, i.e. accumulation of liquid in the abdomen, thorax, and submandibular tissue. Submandibular edema is referred to as "bottle jaw" and is characteristic of infections with *Haemonchus* and some other GIN. Severe infections can also cause liver damage, weight loss, unthriftiness, diarrhea and dehydration (Schmidt, 2013).

The prepatent period for *H. placei* is around 21-28 d, while *H. contortus* prepatent period is approximately 18-21 d. This short life cycle allows several generations in one season and can rapidly increase pasture infectivity. Most *Haemonchus* infections are mixed with other GIN which worsen the damage caused to affected livestock (Ballweber, 2001).

2.1.3 *Trichostrongylus* spp.

Trichostrongylus is a genus of GIN belonging to the family Strongylidae that affects cattle, sheep, goats and other ruminants, as well as pigs, horses and poultry. *T. axei*, also known

as the bankrupt worm, is the predominant species of *Trichostrongylus* in cattle. It prefers to inhabit the abomasum but can occasionally be found in the small intestine (Schmidt, 2013).

Adult *Trichostrongylus* worms have a slender form and a brownish-reddish color and are 5 to 10 mm long. The prepatent period is about 20-25 d.

L3 can survive in the environment and remain infective for up to 6 months and sometimes longer. After ingestion of L3, *T. axei* complete development to adult worms in the abomasum, similar to that of *Haemonchus*. L3 of other species of *Trichostrongylus* reach and reside in the small intestines, where they enter the crypts of the mucosa, molt to L4 and emerge back into the lumen to complete development into adult worms. *Trichostrongylus* worms damage the mucosa of the abomasum and small intestine of the host leading to gastritis, enteritis, and sometimes anemia. Typical signs are diarrhea (mucous and/or hemorrhagic) or constipation, general weakness and wasting, loss of appetite, reduced weight gains or even weight loss, etc. Acute severe infections in young animals may be fatal (Ballweber, 2001).

2.1.4 *Oesophagostomum radiatum*

Oesophagostomum, belongs to the family Strongylidae. These worms are called "nodular worms" because they cause the characteristic appearance of nodules in the small and large intestine of their host (Schmidt, 2013). Adult nematodes are stout and are 15-20 mm long. The prepatent period of *Oesophagostomum* is around 41 d. After eggs are shed in feces, development from L1 to L3 takes about 1 week. After ingestion, L3 penetrate the intestinal mucosa and the host response is to form nodules or granulomas around the developing larvae (Sutherland *et al*, 2009; Ballweber, 2001). The L4 complete their life cycle to adults by emerging from the nodules and return to the lumen of the colon where they mate and females produce eggs. The eggs and developing larvae are susceptible to dry conditions and extreme temperatures, but can survive up

to 3 months, and sometimes longer, on pasture. Intestinal nodule formation disrupts the normal physiology of the gut, particularly the absorption of liquids, which leads to diarrhea, but can also have an effect on peristaltic movements. Deadly bacterial infections can result if the large intestinal nodules burst through the intestinal wall and release gastrointestinal contents into the abdominal cavity. Young animals in particular are affected most severely by this GIN.

2.1.5 *Cooperia spp.*

Several species of *Cooperia* are found in the small intestine of cattle; *C. punctata*, *C. oncophora*, and *C. pectinata* are the most common. Adult *Cooperia* have a reddish color, are up to 10 mm long, and are often coiled (Craig, 2009). The prepatent period of this nematode is 12-15 d. *Cooperia* L3 can survive up to 12 mo, and sometimes longer, on pasture, which makes it quite difficult to reduce the populations. L4 may become inhibited for up to 5 mo before completing development. This makes it possible for those larvae that infect the animal at the end of the summer to remain inside the animal during the winter and to resume development in the next spring when more favorable environmental conditions exist. In heavy infections with *C. punctata* and *C. pectinata*, there is profuse diarrhea, anorexia, and emaciation, but no anemia (Keith, 1987).

2.1.6 *Ostertagia ostertagi*

Ostertagia ostertagi is the most economically important GIN of cattle in temperate parts of the world (Craig, 2009). Adult males measure 6-8 mm, adult females are approximately 8-11 mm in length. The prepatent period of *O. ostertagi* is approximately 18-25 d. The life cycle is typical of that described for *Haemonchus*. In northern temperate cooler regions, ingested L3 typically go into hypobiosis during the winter when the temperature is too cold for development and survival outside the host. In warmer regions, hypobiosis occurs over the summer when

conditions may be too hot and dry for the development and survival outside the host. They can remain dormant for 4-5 mo (Duncan, 1987; Corwin, 1993). Large numbers of hypobiotic L4 can potentially accumulate during this time. When environmental conditions are favorable these L4 rapidly increase in size, emerge in large numbers and can cause massive destruction to the abomasum, leading to severe disruption of function. Feed conversion may become compromised and subsequently affect weight gain, body maintenance, reproductive fitness and milk production. (Vercruysse, 1997). The host can become clinically ill with anemia, edema, loss of appetite, and in severe cases, death.

Three forms of disease with this parasite are well recognized and include ostertagiasis Type I, Pre-type II, and Type II. In Type I ostertagiasis, most worms present in the animal are in the adult stage. This is due to the direct development of large numbers of L3 to adult worms over a relatively short period of time. Type I disease is seen primarily in calves 7–15 months old on pasture. It is most commonly seen during late fall and winter after calves are weaned in temperate regions and in calves during late summer and early fall in cool temperate regions. Pre-type II ostertagiasis is asymptomatic occurring when infected animals carry large populations of inhibited L4. Type II ostertagiasis is characterized by the emergence of large numbers of inhibited L4 during the spring in cool temperate regions and during the fall in warm temperate regions. Thus, in warm temperate regions, inhibition-prone L3 are acquired during spring grazing where development to adults resume in late summer and/or fall, and in cold temperate regions, inhibition-prone L3 are acquired during late autumn and mature during late winter and/or early spring (Meyers et al., 1989).

2.2 Anthelmintic Control of Gastrointestinal Nematodes

Anthelmintics are a group of antiparasitic drugs that expel worms from the body by either

paralyzing or killing them. This is done without causing damage to the host. Parasite control in livestock relies on the use of antiparasitic drugs, which comprise the largest sector of the animal pharmaceutical industry (Waller, 2006). The excellent broad spectrum efficacy, good tolerance, and low costs of available anthelmintics have accounted for the extensive use of chemical control in livestock animals over the past decades. The economic benefit of anthelmintics in the U.S. beef cattle industry was reviewed by Lawrence and Ibarburu (2007). Their conclusion over 170 published articles, according to 2005 market prices, indicated that the removal of anti-parasiticides from the U.S. beef production would result in increased production costs of nearly \$190 per head. This study review validated the importance of effective parasite control, and shows reduced anthelmintic efficacy poses a definite concern. New pharmacokinetic properties of anthelmintics are being developed which make them more reliable and convenient in terms of delivery and labor costs.

Currently, three major classes of anthelmintics are available for use in cattle including benzimidazoles (albendazole, fenbendazole, and oxfendazole), imidazothiazoles (levamisole), and macrocyclic lactones. Macrocyclic lactones are divided into two groups: first-generation avermectins (ivermectin, doramectin, eprinomectin, and abamectin), and second-generation milbemycins (moxidectin) (Edmonds, 2010).

2.2.1 Benzimidazoles

The benzimidazole class was the first anthelmintic introduced into the animal health market in the early 1960's (Campbell, 1990). Benzimidazoles are heterocyclic aromatic organic compounds produced by the synthesis of benzene and imidazole. They were formulated not only for use in farm animals but also for companion animals. Drugs in this class include thiabendazole (TBZ®), fenbendazole (Panacur®, Safeguard®), albendazole (Valbazen®), oxfendazole

(Synanthic®), mebendazole, flubendazol, oxibendazole, albendazole sulfoxide, thiophanate, febantel, netobimin, and triclabendazole (Kahn, 2005).

Benzimidazoles cause degenerative alterations in the tegument and intestinal cells of the parasite by binding to the tubulin, thus inhibiting its assembly into microtubules (Lacey, 1988). The loss of microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible worms, and depletes their glycogen stores. Due to diminished energy production, the worm is immobilized and eventually dies (Martin, 1997).

The benzimidazole class of anthelmintics rapidly grew popular amongst livestock producers because they offered major benefits over previous drugs such as breadth of spectrum, efficacy against many life stages of the parasite, and safety for the animal host (Campbell, 1990). Benzimidazoles have a huge safety margin thereby making them an ideal choice for routine use. Members of this class are effective against adult, larval stages, and inhibited larval stages of GIN (Bogan and Armour, 1987).

Benzimidazoles also exhibited some effect on reducing pasture contamination in that eggs shed on pasture failed to hatch after being exposed to drugs in this class. (Campbell, 1990; Miller and Morrison, 1992b). Some benzimidazoles offer an extended spectrum, with activity not only against GIN but also against other intestinal and liver helminths such as tapeworms and flukes (Campbell, 1990). In general, benzimidazoles are poorly effective against *Trichuris spp.* in ruminants (Campbell, 1990). Currently, thiabendazole (TBZ®), fenbendazole (Panacur®), Safeguard® albendazole (Valbazen®), and oxfendazole (Synanthic®) are approved for use in cattle by the Food and Drug Administration (FDA).

Benzimidazoles are absorbed from the GI tract and, after passage through the liver, the drugs or their metabolites are returned to the rumen and small intestine. The rate of absorption

and excretion varies widely from drug to drug, with slower absorption and prolonged recycling between enteral and parenteral tissues appearing to enhance efficacy. The relative rates of oxidation in the liver and reduction in the GI tract vary between cattle and sheep (Lanusse, 1993). Metabolism and excretion of benzimidazoles are more extensive in cattle than in sheep. Subsequently, the efficacy and length of parasite protection of most drugs in this class has shown to work better in sheep than in cattle. Albendazole, fenbendazole, oxfendazole, and febantel are said to be active against inhibited L4 of *Ostertagia* spp; however, inconsistent efficacy has been reported (Miller, 1993).

Typically, benzimidazoles have been administered in the form of a single oral drench. More recently, delivery methods have moved away from the traditional single oral drench concept. For example, benzimidazole products now include bolus, pastes, powders, feed premixes, etc. The simplest delivery method is to incorporate the drug into feed blocks for ingestion in small amounts over a prolonged grazing period. This method of drug presentation can lead to variations in the amount of drug each animal receives and is generally not recommended. More sophisticated approaches have involved the use of a special syringe to inject the drug directly into the rumen of cattle. Several devices have been developed that are introduced into the rumen by mouth and release the active benzimidazole drug in a sustained or intermittent manner for a prolonged period (Campbell, 1990; Lanusse, 1993).

The intraruminal injector is a plastic syringe in which a needle is inserted into the rumen cavity where a dose of oxfendazole is released. Other intraruminal devices deliver the drug by mouth and is retained in the rumen. One such device, delivers oxfendazole in pulses spaced approximately 3 wk apart over a period of approximately four months. This is achieved by the continuous galvanic corrosion of a magnesium alloy rod that periodically exposes an annular

oxfendazole tablet to the intraruminal fluid. There has also been a delivery system that periodically releases albendazole at monthly intervals over a 3 mo period (Campbell, 1990).

The overall margin of safety has contributed to the success of benzimidazoles worldwide over the last few decades. However, the principle concern with this class of anthelmintic is the emergence of GIN that have become resistant to the effects of treatment.

2.2.2 Nicotinic Antagonists

Another widely-used class of anthelmintics are the nicotinic antagonists which include the imidazothiazoles such as levamisole (Prohibit®, Levasol®, and Tramisol®), the tetrahydropyrimidines such as morantel tartrate (Rumatel®, and Nematel®), and pyrantel pamoate (Strongid®) (Schoenian, 2012). These compounds act selectively as agonists of synaptic and extrasynaptic nicotinic acetylcholine receptors on nematode muscle cells (Martin, 1997). Currently, levamisole is the only FDA-approved anthelmintic in this class for use in cattle and sheep, and morantel tartrate is the only FDA-approved drug in this class for use in goats (Schoenian, 2012).

Levamisole is the main drug in this class used for cattle. Its broad spectrum of activity, ease of use (being water soluble), and lack of teratogenic effects have allowed it to be used successfully. Levamisole also has an immunostimulant effect which may enhance disease control. One disadvantage is at dosage rates higher than that recommended for anthelmintic activity, animals can develop signs of neurological impairment, so its margin of safety is not as wide as the benzimidazoles or macrocyclic lactones. Levamisole acts as a nicotinic receptor agonist and eliminates GIN by causing parasite muscle paralysis. It has some efficacy against lungworms but no activity against flukes and tapeworms and is not ovicidal (Lanusse, 1993).

Levamisole can be delivered in different forms such as oral, topical, or injectable. The

absorption and excretion of levamisole is rapid and not affected by the route of administration or ruminal bypass because of its high solubility. It is effective against many parasite life stages such as adults and larval stages but lacks activity against arrested or hypobiotic larvae, such as those of *O. ostertagi*. In cattle, blood concentrations of levamisole peak less than one hour after subcutaneous administration. These concentrations decline rapidly resulting in the excretion of 90% of the total dose within 24 hours primarily through urine (Arundel, 1983). The tetrahydropyrimidine group exhibits activity against primarily adult GIN. Pyrantel tartrate is well absorbed by pigs and dogs, and less well by ruminants. The pamoate salt of Pyrantel is poorly soluble in water which causes reduced absorption in the gut and allows the drug to reach and be effective against parasites in the large intestine, which makes it useful in horses and dogs (The Merck Veterinary Manual, 2014). Pyrantel is administered by mouth as a suspension, paste, drench, or tablets. Morantel is the methyl ester analog of pyrantel pamoate and is known to be safer in ruminants than its counterpart. It is absorbed rapidly from the upper small intestine and metabolized rapidly in the liver (Arundel, 1983).

2.2.3 Macrocyclic Lactones

The macrocyclic lactones (ivermectins and milbemycins) are products or chemical derivatives of soil microorganisms belonging to the genus *Streptomyces*. The first generation ivermectins in commercial use are ivermectin (Ivomec[®] and Primectin[®]), abamectin, doramectin (Dectomax[®]), eprinomectin (Eprinex[®]), and selamectin. Second generation commercially available in the United States are milbemycins (milbemycin oxime and moxidectin, Cydectin[®]) (Schoenian, 2012). The macrocyclic lactones have a potent, broad antiparasitic spectrum at low dose levels. Macrocyclic lactones block the transmittance of electrical activity in nerves and muscle cells by stimulating the release and binding of gamma-aminobutyric acid (GABA) at

nerve endings. This causes an influx of chloride ions into the cells which leads to paralysis of the pharynx thereby inhibiting pharyngeal pumping that is vital for feeding. Additional effects include paralysis of the body wall and uterine muscles which inhibits motility and reproduction of the nematode (Kahn, 2005).

Of the macrocyclic lactones, ivermectin, eprinomectin, doramectin, and moxidectin are FDA-approved in various formulations including oral, injectable, and pour-on products for use in cattle. Abamectin (Avomec[®]) is registered only in Australia. Ivomec, Primectine and Cydectin oral are FDA approved for use in sheep. Ivermectin and moxidectin are also FDA approved as oral and subcutaneous formulations for use in small ruminants.

In cattle, the macrocyclic lactones have a very high efficacy against all GIN stages, including inhibited forms of the common GIN. They have been a more favorable class of anthelmintic in cattle in that they provide a longer protection period against new GIN infections after treatment. However, due to unfavorable dosing practices that permit the development of drug resistance, their use has become less reliable in the small ruminant industry and recent reports have demonstrated decreased efficacy in cattle (Anziani et al., 2004; Miller et al., 2007; Edmonds, 2010).

In 1998, an oil-based, long-acting injectable formulation of ivermectin was registered in Brazil and is available in most Latin American and African countries. In cattle, this formulation offers treatment and prophylactic prevention of GIN and external parasites for up 77 d. Also, an oil based long-acting injectable formulation of moxidectin was developed and is currently registered in Latin America, Australasia, and some European countries. Controlled studies using this long-acting moxidectin product showed parasite protection between 90 to 150 d against some GIN infections (Ranjan et al., 2004). In the U.S., moxidectin is licensed for use in cattle,

and comes in the form of a pour-on and injectable. This product, however, is not as long lasting, as it provides effective anthelmintic activity for up to 42 days depending on the GIN species. More recently, a long-acting injectable formulation of eprinomectin (LONGRANGE®) was registered and FDA-approved for use in cattle in the U.S. Treatment provides persistent control for 100–150 days, depending on the GIN species.

2.2.4 Amino-acetonitrile derivatives (AADs)

Amino-acetonitrile derivatives (AADs) are the most recent addition to the farm animal class of anthelmintics. Monepantel (Zolvix®), the first drug in this class, is commercially available in Australia, New Zealand, and Europe and is only intended for use in sheep. Developed by Novartis Animal Health, monepantel is an oral drench that has a unique mode of action. Monepantel causes paralysis in worms by binding to a specific receptor found only in GIN. No other species of animal has been found to possess the specific receptor for the AADs and this is believed to be the reason behind the high margin of safety in sheep as well as its high level of environmental safety. Zolvix® is effective against adult nematodes, and normal and inhibited L4. It is primarily used for the treatment and control of AAD-sensitive strains of GIN and has been shown to be effective in reducing macrocyclic lactone, benzimidazole, and imidazothiazole resistant strains in sheep (Novartis Animal Health, 2010). AADs have yet to be evaluated in cattle, but may be available at some time in the future.

2.2.5 Miscellaneous Anthelmintics

Spiroindoles is also a new class of anthelmintic that was released in 2010. The only drug in this class is derquantel. Derquantel has been approved in Australia and New Zealand as a combination anthelmintic (Startect®) in conjunction with the macrocyclic lactone drug, abamectin. By combining these two drugs, the spectrum of parasite activity is enhanced by

greater than 95%. When used together at the recommended dosage, Startect® is efficacious against the adult and L4 stages of *Trichostrongylus spp* and *Nematodirus spp*. It also shows activity against the adult stage of *Haemonchus spp*, and has demonstrated variable efficacy against *Teladorsagia circumcincta* and the L4 stages of *H. contortus*, as well as some ectoparasites. It is not approved for use in the U.S (Little, 2011).

Clorsulon is a sulfonamide given orally as a suspension and is primarily used to control immature and adult liver fluke infection in cattle and sheep. In cattle, it can also be used in conjunction with ivermectin (IvomecPlus®) as an injectable. In plasma, clorsulon is bound to protein and, when ingested by liver flukes, inhibits enzymes of the glycolytic pathway. Clorsulon has a wide safety margin and FDA-approved for use in cattle only as IvomecPlus®. The one drawback of this formulation is that the amount of clorsulon is less than the full dose and therefore only has activity against adult liver flukes. (Fetterer, 1985).

2.3 Anthelmintic Resistance in Ruminants

GIN are the most common parasites causing disease in grazing ruminants worldwide and resistance to anthelmintics has become an issue. Resistance is generally defined as a decrease in anthelmintic efficiency against GIN that are usually susceptible to the anthelmintic at the recommended dose and is an inherited trait (Sangster, 1999). Factors that play a role in resistance may include continuous and/or frequent usage of the same group of anthelmintic, incorrect or subtherapeutic dosing of the drug, and prophylactic mass treatment of all animals in the flock or herd (or lack of refugia). Excessive use of anthelmintics select for GIN in the population that have mutations that confer resistance to that drug. Repeated anthelmintic treatments of the same drug allows the resistant population to build up and they may also become resistant to other drugs in the same class. Under dosing is also an important factor in the development of

resistance. Many laboratory experiments have shown that under dosing an animal causes subtherapeutic levels of the drug in the animal and may contribute to the selection of resistant or tolerant strains. Also, treating all animals in the group can result in a large pool of resistant genes. When every animal in the group is treated with an anthelmintic, essentially all susceptible worms will be killed leaving only resistant worms to reproduce and pass down this gene to their offspring (Waller, 1994). This could potentially result in an almost completely resistant population of GIN. When a proportion of worms in a population is unexposed to an anthelmintic, those worms reproduce and can help maintain the genes for susceptibility within the population. This concept is known as refugia. By maintaining refugia, livestock producers can conserve the efficacy of anthelmintics (Van Wyk, 2001). Refugia has not been extensively studied in cattle, but considering the positive effects extrapolated from the small ruminant industry, cattle producers could potentially slow the development of resistant parasites by implementing refugia in their management practice.

The first examples of anthelmintic resistance were reported for *H. contortus* in small ruminants dating back to the 1960's (Smeal et al., 1968). Resistant strains of *H. contortus* remain as the most serious health issues in small ruminants today. The first documented case of resistance in GIN of cattle in the US was reported in an intensively grazed stocker operation that also practiced strategically-timed deworming for over 17 years (Gasbarre, 2009a). The results of that study demonstrated the presence of anthelmintic resistance to moxidectin and a benzimidazole, and the predominant surviving GIN was *C. punctata*. Since then, multiple reports have surfaced documenting an increasing problem of anthelmintic resistance in cattle (Anziani, 2004; Edmonds, 2010; Gasbarre, 2009b). To assess the extent of the issue in cattle in the U.S., a survey was initiated in 2008 by the United States Department of Agriculture, National Animal

Health Monitoring System. The study revealed that more than one-third of the cow/calf operations participating in the study had less than 90% fecal egg reduction. PCR analysis showed that *Cooperia* spp. were the most resistant GIN. These operations had used either an injectable or a pour-on formulation of either a brand name or a generic macrocyclic lactone (Gasbarre, 2014). Another study revealed that treatment with injectable ivermectin was not effective at removing adult *Cooperia* spp. and inhibited *O. ostertagi* at drug levels consistent with label claims (Edmonds, 2010).

Resistance has been well documented in small ruminants to every GIN involving many of the conventional drugs in multiple classes of anthelmintics (Shalaby, 2013). This has led the small ruminant industry to extensively research alternate methods of GIN prevention and control. Results of recent research has shown promising hope in GIN treatment and prevention in small ruminants through the use of methods that do not involve the use of anthelmintics. Such methods include the nematode trapping fungi (*Duddingtonia flagrans*), condensed tannin containing plants, vaccines, and copper oxide wire particles. Copper oxide wire particles and sericea lespedeza (condensed tannin containing plant) have both shown some activity against GIN in cattle. (J.E. Miller, personal communication).

2.4 Novel Gastrointestinal Nematode Control Strategies

Duddingtonia flagrans is also known as a nematode trapping fungi. This fungus produces thick-walled chlamydospores that are able to survive passage through the ruminant's gastrointestinal tract. The chlamydospores can be processed into a granular or dry powder supplement feed form and are mixed in with the ruminant's diet. This form of control has largely been studied in small ruminants and is effective in reducing pasture infectivity by disrupting the parasite's life cycle. The infective L3 is captured by networks of sticky traps before it migrates

out of feces and onto the forage. The fungus feeds on the L3 thereby decreasing the chance for an animal to become infected (Larsen, 2006). There have also been studies using this fungi as a biological control method in cattle in Brazil (Assis et al., 2012). Although the study showed effectiveness in controlling bovine GIN in tropical conditions, questions were raised as to whether this method can be used in various climates, different types of cattle operations, and the effectiveness and ability to expose every animal in the herd. To date, no trials in the United States have been documented using *D. flagrans* in cattle.

Several tannin-containing forages, in particular those with condensed tannins (CT), have shown anthelmintic activity against GIN of sheep and goats (Shaik et al., 2006). These plants could act through direct antiparasitic activity but they also act indirectly by increasing host resistance. Sericea lespedeza (SL), a perennial legume containing a high level of CT, can be fed to ruminants in various forms which include forage, hay, and pellets. The forage form is known to have a bitter taste, which creates a palatability issue. Problems with SL in hay formulations occurs as plant material is lost during processing. In cattle, tannin-containing plants have shown to reduce the incidence of pasture bloat (McMahon et al., 2000). Because tannin-containing plants show anti-parasitic properties in small ruminants and nutritional benefits in large ruminants, there is reason to believe they may be useful in cattle for their antiparasitic effects. The only study to date evaluating the CT as a biological control for cattle GIN was done in 2011 in an *in vitro* setting, and results were determined by a larval inhibition assay and a larval exsheathment assay (Novobilský, 2010). Results indicated that extracts inhibited *Ostertagia* and *Cooperia* larval development.

Vaccinating ruminants to control GIN infections has been a consideration. The most practical reason to vaccinate against these parasites is to reduce reliance on anthelmintics. The

use of vaccines may offer multiple benefits such as improving animal health and welfare, solving problems associated with resistance to anthelmintics, and keeping animals and the environment free of chemical residues. Vaccines have been developed to aid in the control of the lungworm, *Dictyocaulus viviparus*, in young cattle (Bovilis® Huskvac, MSD Animal Health, U.K), and tapeworms in sheep, but currently there are no vaccines commercially available for the control of GIN in ruminants. There have been two immunization studies investigating vaccine use to control *O. ostertagi* in cattle using L4 extracts (Halliday et al., 2010; Meyvis et al., 2007). These studies, performed in Europe and the U.K, have shown variable results in the reduction of FEC and L4 larvae. Further investigation is warranted to determine the exact mechanisms at work in these cases.

Copper oxide wire particles (COWP) have anti-parasitic properties when administered orally and added to the diet of small ruminants. Many studies have shown a significant relationship between COWP and a decrease in FEC of GIN, specifically *H. contortus*. The exact mechanism of how COWP control internal parasites is not yet fully understood. Copper boluses (Copasure®) are available for use for copper deficiency in cattle, but lacks research in determining if it aids in controlling GIN in cattle.

Although these novel methods of control show promising hope for the future of the small ruminant industry, they have not been extensively studied in cattle and are not yet available for commercial use in the U.S. These methods may also lack the value of convenience that cattlemen desire when choosing a strategic deworming method.

2.5 Current Gastrointestinal Nematode Control and Prevention Methods in Cattle

Current treatment of GIN in cattle today greatly relies on the macrocyclic lactones (ML) (Ballweber and Baeten, 2012). As a class, these drugs are known to be extremely effective at low

dosages, provide very high efficacy against a broad range of GIN and external parasites, achieve persistent blood levels, and can prevent reinfection from certain parasites for weeks after a single treatment. A recent survey indicated that 88% of bovine anthelmintic sales were MLs and 12% were benzimidazoles in the United States (McArthur, 2014). The MLs can be delivered in various forms such as injectable, oral, or topical routes, and all portray a wide safety margin. Use of older anthelmintics in cattle, such as the benzimidazoles and imidazothiazoles have decreased substantially. Reasons for the decline in use of those drugs is likely due to decreased efficacy as compared to MLs. When topical MLs were formulated, consumer preference narrowed even more because they were convenient and ultimately reduced stress and the labor cost associated with handling and treating herds of cattle (Gasbarre et al., 2009). Drugs in this class, such as eprinomectin and moxidectin pour-ons, have no milk withdrawal time in many countries. This feature also contributes to its popularity. Because of the potency, convenience and low cost, cattlemen may be reluctant to discontinue use of the MLs. Until other anthelmintic alternatives can be shown to consistently reduce GIN infections in real life practice, increase economic benefits, and demonstrate sustainable efficacy, it is not likely that cattle producers will stray away from current deworming practices.

Pour-on formulations have been the method of choice by many cattlemen and veterinarians because administration of the drug is easy and places less stress on the animal. Although these pour-ons seem to be convenient, there is great variability in the amount of drug each animal receives. Multiple studies have shown that grooming habits of other animals can influence the actual amount of drug that enters the body (Sallovitz et al., 2002, 2003). This drug delivery method can result in under dosing an animal, which in turn contributes to the increase in anthelmintic resistance. At this time, cattlemen are reluctant to try alternative anthelmintic

strategies due to the ease of administration and cost effectiveness of injectables and pour-ons. This stresses the importance of the development of new products and methods that can deliver extended parasite protection thereby reducing the labor intensive nature of current products.

Successful anthelmintic strategies should involve developing a program with the goal of maximizing the economic benefit while also reducing egg/larval contamination on pastures. Incorporating knowledge from the host–parasite–environment relationship in addition to developing extended pharmacological properties of anthelmintics can help to prolong efficacy of existing anthelmintics. Recently, there have been new strategies to optimize the use of existing anthelmintic drugs, as well as secure an extended use of the novel products, and to control resistant parasites from a pharmacokinetic-based enhancement of parasite exposure. The newest product to display these properties involves the use of an extended-release injectable anthelmintic that contains a unique delivery system that releases the active ingredient in two peaks resulting in extended anthelmintic activity.

2.5.1 Extended-Release Injectable

Recently, an eprinomectin extended-release injection formulation (LONGRANGE[®], E-LR) was developed by Merial to provide long-term GIN control in cattle. E-LR is composed of a 5% sterile solution of eprinomectin, and administered at a rate of 1 mg/kg of body weight. The eprinomectin is formulated in a solvent that includes a polymer matrix, which slowly releases the active ingredient following injection. E-LR is the first extended-release anthelmintic that claims to provide up to 100 to 150 days of GIN control in a single treatment depending on the species of GIN. Eprinomectin is not bound by the animal's fat cells in the tissues, instead after delivery it enters the animal's bloodstream through the trademarked breakthrough technology called Theraphase.

According to Merial, the pharmacokinetic properties of this product allow the gradual release of the active ingredient in two peaks. The first peak is immediately after the initial subcutaneous injection. Once in the body, the liquid polymers solidify into a gel to create the trademarked Theraphase matrix, which encapsulates the eprinomectin. From this gel matrix, the drug is released into the bloodstream over an extended period of time. Following the first initial peak, plasma concentrations start to decline and by d 25 the level is low and constant to approximately d 70. Following d 70, the Theraphase matrix begins to disintegrate and a second bolus of eprinomectin is released, creating a secondary peak in the blood stream around d 90-100. Thereafter, the drug gradually declines until around d 150. The gel matrix biodegrades by hydrolysis and is eliminated from the body as carbon dioxide and water. Clinical studies done by Merial have shown plasma concentrations of eprinomectin between 0.5 ng/mL and 1.0 ng/mL are the minimal drug level for nematocidal activity. While the drug is decreasing in concentration, it constantly stays at therapeutic levels with a blood concentration of 1.0 ng/mL or higher until day 150 where the concentration falls to 0.5 ng/mL. Because the Theraphase matrix allows for the controlled release of the drug into the animal's system, it can therefore exit the body quickly before lingering around at sub-therapeutic blood levels. This advanced delivery system is thought to minimize the selection for resistant parasites.

E-LR has been shown to be effective against several GIN stages including L4 and adults. Based on the results of these studies, claims were granted to control multiple GIN parasites including *C. oncophora*, *C. punctata*, *C. sumabada*, *H. placei*, *O. radiatum*, *O. lyrata*, *O. ostertagi*, *T. axei*, and *T. colubriformis*. This product also shows efficacy against *O. ostertagi* inhibited L4. Merial's confirmation studies showed control of *C. oncophora*, *C. punctata*, and *T. axei* up to 100 d, while *H. placei*, *O. radiatum*, *O. lyrata*, and *O. ostertagia* were effectively

controlled up to 120 d. The pulmonary nematode, *D. viviparous*, is the only parasite confirmed to be controlled up to 150 d.

Formulations that provide extended periods of GIN control are thought to not only treat existing infections within the host, but also to prevent the host from becoming reinfected with L3. Because L3 can overwinter, they can infect cattle as soon as they are turned out into pasture during the next grazing season. When the environmental conditions become favorable, the L3 move up grass blades and are ingested by the grazing cattle. The L3 transition to adults and millions of eggs are passed onto the pasture where the next grazing cycle begins. This process can also occur during hot and dry periods during the summer months. The L3 remain under the root mat and can move into the soil seeking moisture and cooler conditions for protection and survival. Once the environmental conditions improve, the L3 are able to migrate up the grass blades to infect the animal. To break this cycle of infection and reinfection, it takes about 100 d of continuous treatment to reduce the GIN burden in the animal and subsequent pasture infectivity. Conventional dewormers such as moxidectin (Cydectin[®]), doramectin (Dectomax[®]) and eprinomectin (Eprinex Pour-On[®]) control GIN and lungworms for up to 42 d. These anthelmintics may reduce the GIN burden in the animal short term. However, sequestered L3 on the pasture that become active again after 42 d can infect the animal with no anthelmintic activity present to kill new ingested L3. This may contribute to the lack of reduced pasture infectivity when using an unsustainable anthelmintic.

GIN infection can affect many aspects in a cow/calf operation. The physiological tasks of a heifer calf include growth, reproductive competence, gestation, lactation, and post-partum rebreeding. Post-partum rebreeding constitutes the biggest issue for heifers during development (Perry et al., 2011). The greatest factor affecting first calf heifers and young cattle's rebreeding

ability is their plane of nutrition (Lemenager et al, 1980). When GIN adversely affects the animal's nutritional efficiency and body condition, this reduces conception rates and milk production. Although peak lactation is variable from animal to animal, it generally occurs around 45-60 d post-calving. Also during this time, heifers lose weight and body energy reserves are depleted. When L3 are ingested after anthelmintic activity wanes, the animal's ability to maintain their body condition is compromised. Also, infected animal may be rendered susceptible to other infectious diseases. Because eprinomectin is released again around d 70 when treated with E-LR, this may have a positive effect on the animal's ability to maintain nutritional status, thus increasing ability to rebreed and generate profit for the producer. One study, conducted by Merial, compared heifer weight gain following treatment with doramectin as the control anthelmintic and E-LR as the treatment anthelmintic. The calves treated with E-LR showed a significant ($P < .001$) increase in mean gain per head of 11.5 kg.

There have been reports of a positive association between deworming nursing calves and an increase in weaning weight (Miller and Morrison 1990ab; 1992). However, producers might not view that cost benefit as productive especially if it means increasing stress, labor, and time by putting the herd through the chute multiple times. Because E-LR administered at the beginning of the grazing season may provide long-term control for the whole grazing season, the end result may be more acceptable. In cow-calf operations, it's very important to make sure calves are healthy which, in turn, will provide a weaning weight that other segments in the beef production system will be willing to purchase. According to a clinical field study done by Merial, the use of a long-acting anthelmintic demonstrated production benefits in young growing stocker steers. Steers were treated with either E-LR or moxidectin + fenbendazole, and the results showed that E-LR treated steers had a significant ($p < 0.001$) increase of 18.2 kg more

than control steers. One area of concern with using long-acting anthelmintics in calves in their first grazing season is the reduction of the animal's ability to develop adequate acquired immunity to GIN infection. These young calves may become more susceptible to the adverse effects of reinfection from grazing after protection from the anthelmintic wears off. One report showed that FEC was significantly ($p < 0.05$) higher in second season grazing animals that had been dewormed during their first grazing season, which suggested that the immunity to GIN may be reduced (Larsson et al., 2011). However, no differences in weight gains were observed in the second grazing season, despite the animals being subjected to different means of parasite control during their first season of grazing. This is in accordance to a similar study in which there was no observation of any negative impact of previous anthelmintic treatment of heifers despite a higher burden of adult *O. ostertagi* in those animals (Satrija et al., 1996). However, these studies used repeated administration of conventional anthelmintics. Therefore, the use of long acting anthelmintics such as E-LR may also reduce pasture infectivity to a level where reinfection is not adequate enough to maintain a strong level of immunity and subsequent infection may be worse than the initial infection.

The general idea surrounding the use of E-LR is to reduce the susceptible GIN burden for a prolonged period of time and therefore improve the nutritional status and weight gain. Fecal egg count reduction tests, larval cultures, and weight gain studies have provided evidence that E-LR is effective in doing that. Short-term products may reduce the GIN burden, but lack the ability to provide protection when it is needed the most, such as when inhibited *O. ostertagi* L4 resume development in the early fall. By using E-LR in cow-calf operations, there may be a potential for increasing profit when considering reduced labor, less stress on the animal, decrease in numbers of GIN, increase in weaning weights, and improved nutritional efficiency. It may also

be beneficial to use E-LR in an integrated control strategy with non-drug methods such as COWP, CT containing forage, nematode-trapping fungi, etc. Additionally, by treating one population of animals (either the young calves or older cows), the concept of refugia may be enhanced naturally. This clinical trial assessed E-LR in naturally infected young 2-4 m old calves as they co-grazed with their dams on pasture for effect on infection and weight gain during hot summer climatic conditions in central Louisiana

CHAPTER 3

MATERIALS AND METHODS

3.1 Location and Animals

This study was conducted at the LSU Dean Lee Research Station in Alexandria, Louisiana and consisted of 67 bulls and 53 heifer calves. All calves used in this study were spring born with birth dates ranging from February 2014 through April 2014. The average age of the calves at the beginning of treatment was approximately 4 months. Groups A and B consisted of 34 bulls and 25 heifers; 37 of which consisted of Brangus-sired breed and 22 were Simmental-sired. Groups C and D had 33 bulls and 27 heifers; 31 of those calves were Brangus-sired breed and 29 were Simmental-sired. The average birth weight of calves in all 4 groups was 40 kg. The health condition of every animal prior to and during the study was supervised by a licensed large animal veterinarian.

3.2 Source of E-LR

E-LR was provided by Merial, a global animal health company.

3.3 Experimental Design

3.3.1 Study

This study consisted of 120 resident cow/calf pairs that were randomly allocated according to breed, sex, and weight to 4 different groups (A, B, C, and D) of 30 hd each. All animals in the study were allowed to graze on pasture for the entire length of the study. The duration of the study was 112 d (6/4/14-9/29/14). Each group was rotated throughout all pastures to allow equal parasitic exposure. Calves in groups A and B were treated with the control anthelmintic, fenbendazole (F-P, Panacur® Suspension 10%, 10mg/kg, Merck Animal Health, Madison, NJ), administered orally. Calves in groups C and D were treated with E-LR (5% sterile

solution, 1 mg/kg, Merial, Duluth, GA), administered subcutaneously cranial to the left shoulder. All treatments were given by a licensed large animal veterinarian.

On d 0, calves were brought in from the pasture and processed individually through a chute to identify the animal according to its ear tag and electronic identification, and to confirm the animal's group letter. Calves were then treated with the appropriate anthelmintic, a fecal sample was obtained, and their initial weight was recorded. Following treatment, calves were designated to their corresponding location for each group, A-D. All calves were worked through the chute on d 14, 28, 56, 84, and 112 and each calf was weighed and a fecal sample was obtained. All fecal samples were stored at 4°C to be processed to determine FEC, expressed as eggs per gram (EPG) and cultured to determine GIN population distribution.

3.4 Techniques

3.4.1 Fecal Egg Count

Fecal samples were obtained directly from the rectum, placed in styrofoam cups and sealed with a lid. These samples were taken directly to the Department of Pathobiological Science lab to be analyzed for FEC using the McMaster technique (Whitlock, 1984). Two g of feces from each animal were broken up with a tongue depressor. Thirty mL of a saturated salt solution (737 g of iodized salt dissolved in 3000 mL of tap water) were added to the feces and the solution was mixed using an electric drink mixer (Drinkmaster® Drink Mixer, Hamilton Beach Brands, Inc., Glen Allen, NC). A sample of the mixture was then rapidly pipetted and transferred into one side of the McMaster chamber. The solution was again mixed using the Drinkmaster for the same amount of time and a second sample was quickly transferred into the other chamber on the McMaster slide. All trichostrongyle type eggs were counted using 100x on a microscope. This technique was performed in duplicate on each calf on d 0, 14, 28, 56, 84 and

112. The total number of eggs on both sides of the McMaster chamber was multiplied by 50 to obtain the EPG. The duplicate samples were then averaged to obtain the final EPG for each calf. Furthermore, samples with a zero McMaster FEC were processed by the double centrifugation technique to obtain a FEC less than 50 EPG.

3.4.2 Double Centrifugation (Sugar Float)

The Double Centrifugation technique is used to concentrate eggs in the sample using a highly concentrated sugar solution and is useful when FEC are low (usually less than 50 EPG). Two g of feces were broken up in individual cups with a wooden spatula. Fifteen mL of water was added to the cup and mixed extremely well, making sure to break up any clumped feces. This solution was then poured through a strainer and funnel into a 15 mL centrifuge tube; remaining fecal material left on the strainer was pressed against the strainer with a mortar stick to release as much solution as possible into the tube. Sample tubes were placed in centrifuge buckets and spun for 5 min at 1500 rpm. The supernatant was then discarded and a sugar solution (1600 mL sugar and 400 mL of water) was added to each tube until the tube was half full. The solution was stirred well with two applicator sticks to break up concentrated fecal material at the bottom of tube. Tubes were then placed back into the centrifuge where the sugar solution was poured to fill the tube to a positive meniscus. A cover slip was placed on top and tubes were spun again for 5 min at 750 rpm. Following centrifugation, the cover slips were placed on microscope slides. All trichostrongyle type eggs were counted using 100x on a microscope.

3.4.3 Fecal Larval Coprocultures (Bulk Cultures)

Fecal samples obtained from all calves in each group were mixed together in bulk and cultured to allow GIN eggs to hatch and develop to L3 for recovery, enumeration, and identification. Larval cultures were made from fecal samples obtained on d 0, 14, 28, 56, 84, and

112. Feces from each animal were combined according to group and sample date. Total weight of feces was then recorded and feces were divided into thirds (triplicate) as evenly as possible into separate plastic bins. Feces were then broken up and vermiculite was combined with feces in an approximately 50:50 by volume mixture. Water was added to all samples until a crumbly moist consistency was achieved. Each bin was covered with aluminum foil and holes were poked in foil to allow oxygen exchange. Cultures sat at room temperature (about 25°C) for 14 d, and were then processed by the Baermann technique to recover L3.

3.4.4 Baermann Technique

The Baermann technique was used to aid recovery of L3 larvae from bulk cultures. A wire screen was placed into the opening of a large funnel. A 15 mL plastic centrifuge tube was used to collect L3 beneath each funnel by attaching it to the bottom of the funnel with a short rubber hose. A piece of cheesecloth large enough to cover the screen was laid out on a flat surface and culture samples were carefully poured into the center of the cloth. The sides of the cloth were wrapped and tied around the sample. The wrapped sample was placed onto the wire screen in the funnel and warm water was added to the funnel to completely submerge the sample. The sample was allowed to sit for 24 h. A small amount of formalin was added to all samples collected in the tubes to preserve L3 for later identification.

3.4.5 Larval Counts and Identification

The supernatant in the 15 mL tube was vacuumed to 1 mL. A pipette was used to draw up a 100 uL aliquot which was placed on a microscope slide. A drop of iodine (150 g potassium iodide and 100 mL water) was mixed into the aliquot, and a cover slip was placed on top of the drop. L3 were then enumerated using 100x on a microscope. The first 100 or total (if the total was less than 100) L3 were identified to genus to estimate population percentages.

3.4.6 Fecal Egg Count Reduction Test

The FEC reduction test was used to determine the efficacy of treatments. This test compares the FEC of the treatment groups on d 0 and d 14. The formula for this calculation was:
$$\frac{\text{Pre-treatment} - \text{Post-treatment}}{\text{Pre-treatment}} \times 100.$$

3.5 Statistical Analysis

Data were analyzed using SAS® (version 9.4) as a repeated measures analysis of variance using PROC MIXED. The initial FEC values, and initial LOG FEC values (d 0) were used as a covariate. The response variables were the subsequent FEC values (for the remaining study days), LOG FEC values, and weight. Weight was analyzed independently of FEC. Fixed effects included treatment and time. Random effects include animal identification within treatment group. When overall differences were found, post hoc comparisons were conducted with pairwise t-tests of least-squares means. All differences were considered significant at $P \leq 0.05$

CHAPTER 4

RESULTS

4.1 Study 1 Results

At the beginning of the study on d 0, all calves had relatively low FEC (Figure 1). Subsequent to treatment with F-P or E-LR, the mean FEC of calves in all 4 groups decreased by d 14. On d 14, mean FEC in all 4 groups was comparatively similar and no significant difference ($P > .05$) was detected between any of the groups (Figure 1 and 2). Also on d 14, the FEC reduction test showed 95.15% and 93.64% reduction for F-P and E-LR treated calves, respectively. Between d 14 and d 28, the mean FEC for all 4 groups remained fairly constant with no significant differences ($P \geq 0.05$) (Figure 1). However, the mean logFEC did show significant differences ($P < 0.05$) on d 28 between groups A and C, and B and D (Figure 2). After d 28, the mean FEC of calves treated with F-P in groups A and B increased, while the mean FEC of calves treated with E-LR in groups C and D remained constant. On d 56, significant differences ($P < 0.05$) were found between groups B and C, and B and D (Figure 1). The mean logFEC showed significant differences on d 56 between A and C, A and D, B and C, and B and D (Figure 2). Mean FEC of calves in all groups then increased by d 84. A significant difference ($P < 0.05$) was detected again between groups A and C, A and D, B and C, and B and D (Figures 1 and 2). There was also a significant difference ($P < 0.05$) on d 84 within the two F-P groups and the two E-LR groups (Figures 1 and 2). Groups A and B continued to increase after d 84 reaching a mean FEC of 107 EPG and 157 EPG, respectively, on d 112 (Figure 1). Also between d 84 and d 112, group C remained constant with a mean FEC of 29 EPG, while group D increased to 29 EPG (Figure 1). There was a significant difference ($P < 0.05$) on d 112 between groups A and C, A and D, B and C, and B and D (Figures 1 and 2). A significant ($P < 0.05$) difference was also observed between the two F-P treated groups, while there was no significant

($P > 0.05$) difference between the two E-LR treated groups (Figures 1 and 2).

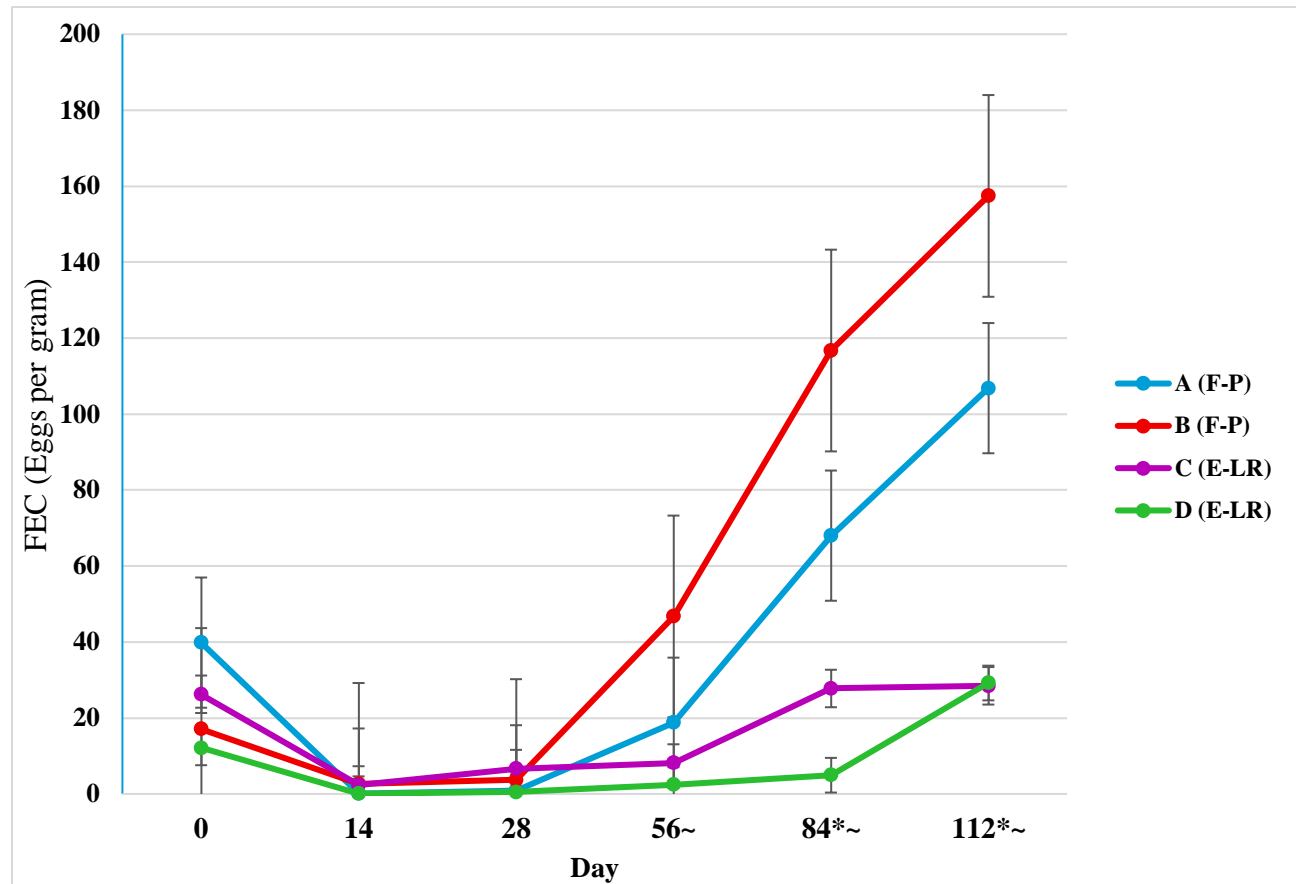


Figure 1. Mean (\pm SEM) fecal egg count (FEC) on d 0, 14, 28, 56, 84, and 112 for fenbendazole (F-P, Panacur[®], 10mg/kg, drench) and eprinomectin (E-LR, LONGRANGE[®], 1mg/kg, SQ injection) treated calves. N = 30/group. *Indicates significant difference ($P < 0.05$) between group A (F-P) and at least one or more E-LR treated groups. ~Indicates significant difference ($P < 0.05$) between group B (F-P) and at least one or more E-LR treated groups.

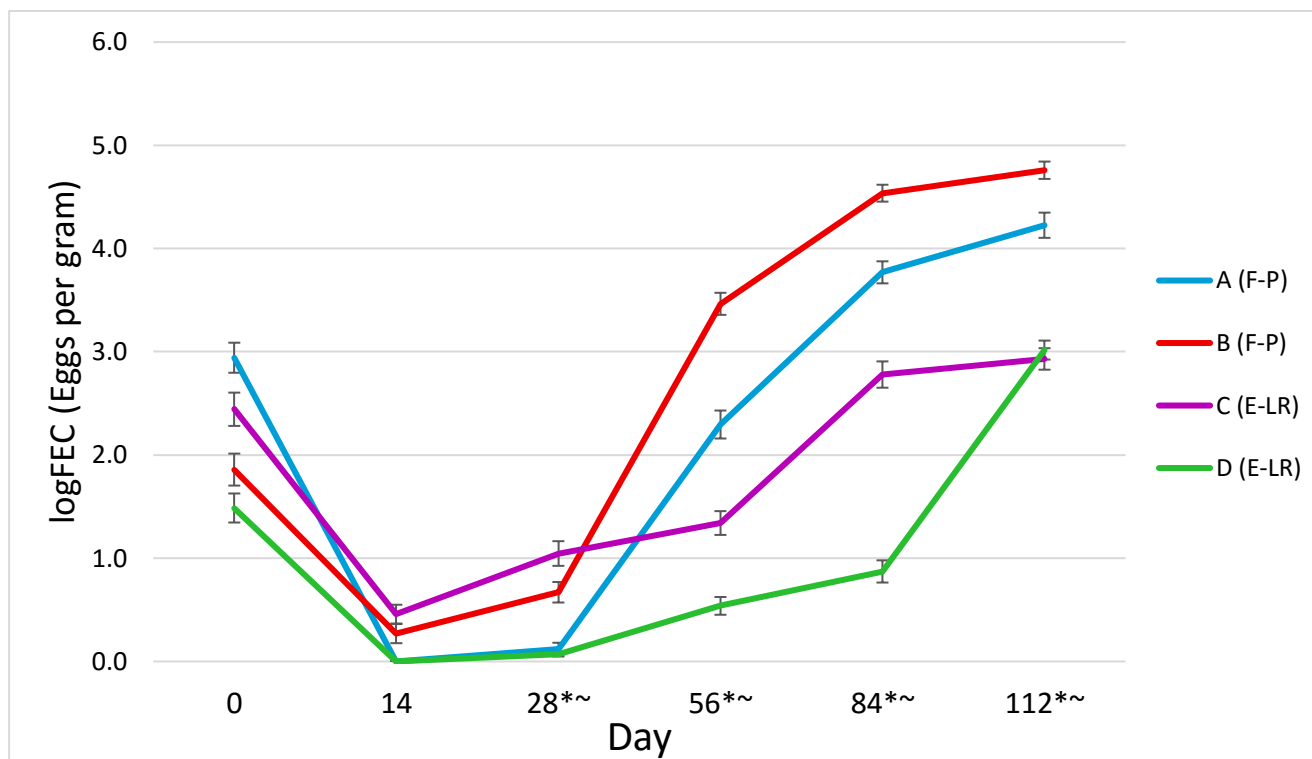


Figure 2. Mean (\pm SEM) log fecal egg count (logFEC) on d 0, 14, 28, 56, 84, and 112 for fenbendazole (F-P, Panacur[®], 10mg/kg, drench) and eprinomectin (E-LR, LONGRANGE[®], 1mg/kg, SQ injection) treated calves. N = 30/group.

*Indicates significant difference ($P < 0.05$) between group A (F-P) and at least one or more E-LR treated groups.

~Indicates significant difference ($P < 0.05$) between group B (F-P) and at least one or more E-LR treated groups.

The mean FEC of the calves that were treated with F-P were combined, and the mean FEC of the calves treated with E-LR were combined (Figure 3). The mean FEC was similar ($P > 0.05$) between F-P and E-LR treated calves on d 0. By d 14 and 28, FEC decreased and was similar ($P > 0.05$), indicating the treatment was successful. By d 56, the mean FEC of calves in both groups increased; however, the degree of increase in calves treated with E-LR was not as pronounced as the F-P treated calves. After d 56, this trend of increasing FEC continued in both groups, with the F-P treated calves having higher ($P < 0.05$) FEC. On day 84, there was an increased difference ($P < 0.05$) in FEC between calves treated with F-P and calves treated with

E-LR. The difference between groups was even more noticeable ($P < 0.05$) on d 112 as the mean FEC of F-P treated calves was approximately 4.5 times higher than E-LR treated animals.

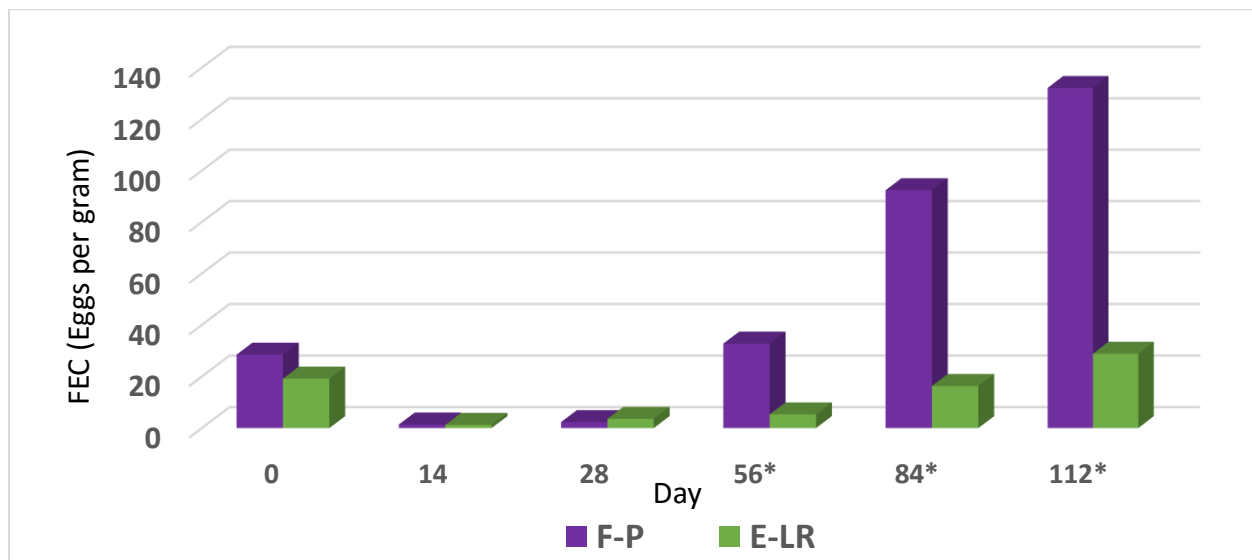


Figure 3. Mean fecal egg count (FEC) of combined fenbendazole (F-P, Panacur®, 10mg/kg) and eprinomectin (E-LR, LONGRANGE®, 1mg/kg) treated calves on d 0, 14, 28, 56, 84, 112. N = 60/group.

*Indicates significant difference ($P < 0.05$) between groups.

To determine differences in weight gain, the mean weight of both F-P and E-LR treated groups were calculated on d 0 and 112 (Figure 4). The mean weight gain for F-P treated calves (groups A&B) was 114.1 kg and 116.4 kg, respectively, and the mean weight gain for E-LR treated calves (groups C&D) was 110.9kg and 114.1 kg, respectively. There were no significant differences ($P > 0.05$) in weight gain between any of the groups at any time point in the study.

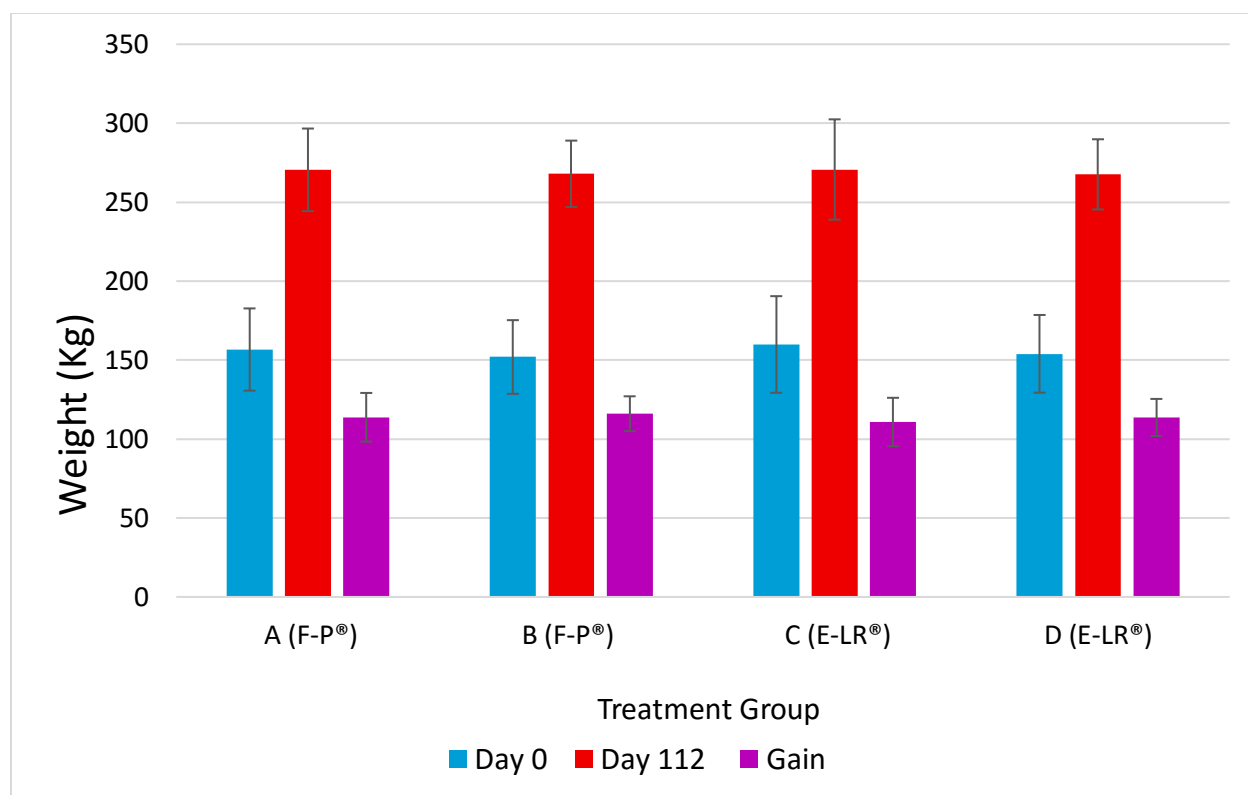


Figure 4. Weight at beginning and end of study for fenbendazole (F-P, Panacur®, 10mg/kg, (n = 59) and eprinomectin (E-LR, LONGRANGE®, 1mg/kg (n = 61) treated calves.

Data from fecal bulk cultures were used to determine the population distribution of GIN in the calves (Table 1). The predominant GIN throughout the study were *Cooperia* (46% and 49% for F-P and E-LR, respectively) and *Ostertagia* (49% and 50% for F-P and E-LR, respectively) with *Haemonchus* and *Trichostrongylus* being present in relatively low numbers. A few *Oesophagostomum* were found (<0.2%). It should be noted that the standard amount of 100 L3 used to establish a distribution population were not always able to be recovered because of such low FEC (Figure 5). On day 0, the total number of L3 recovered from feces of calves treated with F-P and E-LR was 600 and 585, respectively. After treatment, on d 14, the total number of L3 recovered was 11 and 26, respectively. These numbers continuously increased in F-P treated calves throughout the rest of the study. Whereas, the total number of L3 recovered

from feces of E-LR treated calves stayed comparatively low during the rest of the study. On d 112, the total number of L3 recovered from feces of F-P and E-LR treated calves was 600 and 64, respectively. Overall, the total number of L3 recovered from feces of E-LR treated calves on d 28, 56, 84, and 112 was considerably less than the total number recovered from F-P treated calves (Figure 4).

Table 1. Percent infective larvae recovered from fecal bulk cultures of calves treated with fenbendazole (F-P, Panacur®, 10mg/kg drench) and eprinomectin (E-LR, LONGRANGE®, 1mg/kg SQ injection). H, T, C, Oe and Os = *Haemonchus*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum* and *Ostertagia*.

	F-P				
Day	% H	%T	%C	%Oe	%Os
0	8	2	41	0	49
14	0	0	10	0	90
28	0	10	43	0	47
56	1	1	62	0	36
84	7	1	58	0	34
112	2	0	61	0	38
Total	3	2	46	0	49
	E-LR				
0	3	2	33	0	63
14	47	0	25	0	28
28	3	49	18	0	30
56	24	6	38	0	32
84	0	0	15	0	85
112	0	3	33	0	65
Total	13	10	27	0	50

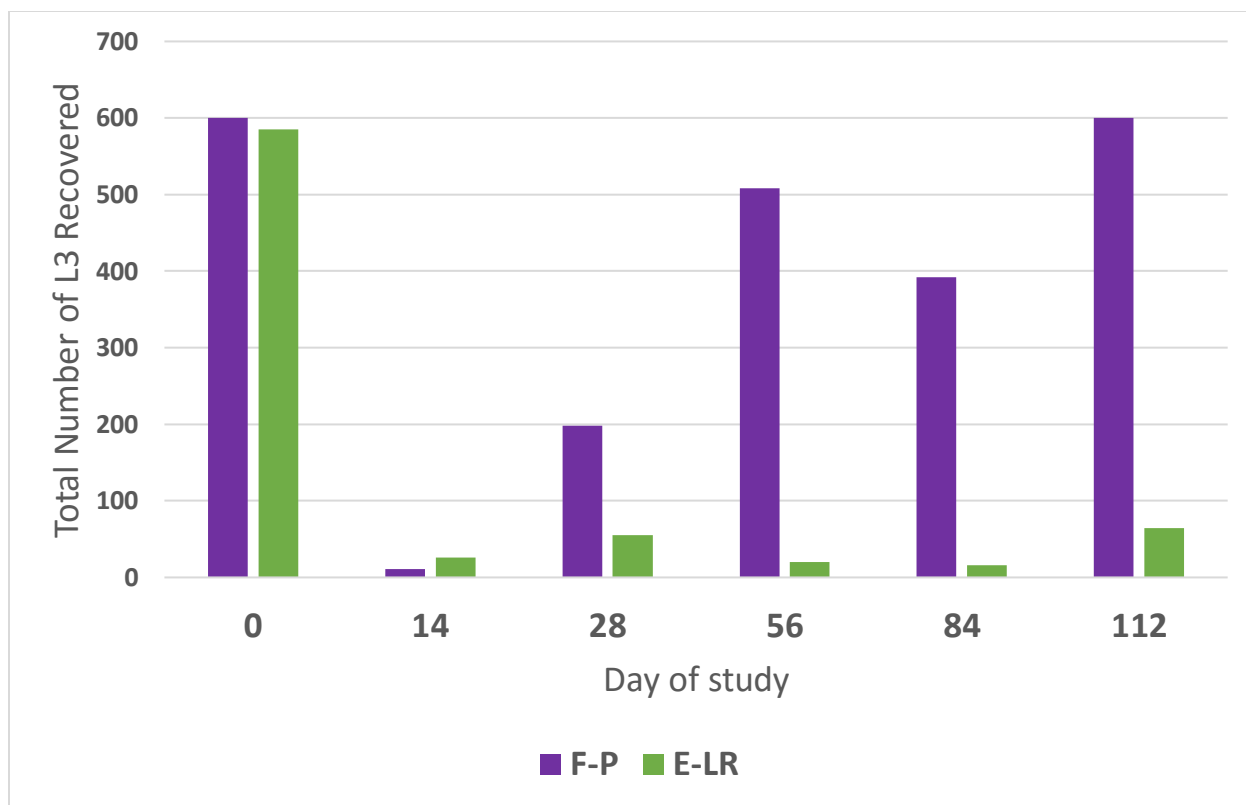


Figure 5. Total number of infective larvae (L3) recovered from feces of calves treated with fenbendazole (F-P, Panacur[®], 10mg/kg) and eprinomectin (E-LR, LONGRANGE[®], 1mg/kg).

CHAPTER 5

DISCUSSION AND CONCLUSION

With increasing reports of production losses caused by recurrent GIN infections, cattle producers are exploring new methods of long term prevention. The objective of this study was to determine the efficacy of treatment with E-LR compared to treatment with F-P for 112 days during natural pasture infection in young nursing calves. For information purposes, the degree of a mixed GIN infection in cattle is considered light with an FEC up to 200 EPG, moderate with 200-800 EPG, and heavy when higher than 800 EPG.

The present study revealed that E-LR was more efficient at providing GIN control compared to F-P. It was noted that FEC of E-LR treated calves was similar before treatment and at the end of the study. This does not concur with the claim that the product provides protection for 100-150 d post treatment. It was expected that the FEC would remain similar from d 14 to the end of the study, but reinfection was not curtailed enough to see that effect. Reinfection did occur to allow FEC to return to pre-treatment level by d 84. Therefore, under the conditions of this study long term efficacy was about 84 d.

Both F-P and E-LR treated calves started the study with very low FEC and by d 14 both treatments significantly reduced FEC and there was no significant difference between treatments. This indicated that, based on the fecal egg count reduction test, both F-P and E-LR effectively removed the GIN burden within the period of time that is used to determine efficacy (i.e., d 14). The trend after d 28 is when the differences in FEC became more prominent. By d 56, there was a distinct increase in FEC of F-P treated calves. This correlates well to the life cycle of GIN and the length of protection provided by F-P. The life cycle of these GIN is approximately 42-56 days but can vary depending on the genus. These results suggest that F-P was efficient at killing GIN initially for approximately 2-3 d, but after that reinfection is not affected. This is consistent

with published reports that the period of activity of F-P is about 2-3 days (Lanusse, 1993). E-LR treated calves, however, showed only a slight increase in FEC after d 56. This increase was noticeably minimal compared to the F-P treated calves. The mean FEC at the end of the study in groups A and B was 132, while the mean FEC in groups C and D was 29. This indicated that E-LR effectively provided long-term parasite control and was efficient in preventing reinfection. Preventing reinfection correlates to reduced pasture contamination, and the end result is calves grazing on cleaner pasture.

In this same study, weight gain was also evaluated to determine if treating with E-LR would result in calves gaining more weight than animals treated with F-P. This is important as calves with higher weaning weights could potentially benefit a cow-calf producer. The calves treated with E-LR, however, did not gain more weight compared to the calves treated with F-P. One reason for this may be due to the fact that all of the calves in the study had relatively low FEC throughout the duration of the study even though F-P treated animals did show a significant increase in FEC after d 28. A follow up study would be appropriate to compare these anthelmintic treatments on moderate and heavily infected calves as weight gain might be expected to be more of an issue under that circumstance.

Larval identification was performed to determine the trichostrongyle type GIN that were present. *Ostertagia* and *Cooperia* were the predominant L3 recovered from feces of both F-P and E-LR treated groups with more *Ostertagia* recovered than *Cooperia*. This is in contrast to a previous report where *Haemonchus* and *Cooperia* were predominant during the summer in Louisiana (Miller, 1993). As mentioned earlier, *Ostertagia* usually become inhibited within the animal during extremely hot environmental conditions, so it was expected to have more *Haemonchus* and *Cooperia* present. Why this shift in population occurred is not known. Perhaps

over the last 20 years or so, the environmental conditions have become more conducive for *Ostertagia* development outside the host. Also, the total number of L3 recovered from feces of E-LR treated calves was substantially less than that recovered with F-P treated calves. This coincides with and was expected with the relatively low FEC of E-LR treated calves. Because much fewer L3 were found in feces of E-LR treated calves, the resultant pasture infectivity would be expected to be reduced. So, essentially, treating cattle with E-LR may be an efficient means to help clean up pasture infectivity. Conversely, an opposite effect was observed in calves treated with F-P where an increase in FEC and number of L3 would most likely result in maintaining pasture infectivity at a higher level.

With the use of any anthelmintic, and especially with extended activity anthelmintics such as E-LR, the issue of resistance always poses a concern for reduced efficacy. It is just a matter of how much time it will take. The use of any extended release anthelmintic exposes the worms to the drug over a prolonged period of time, which will allow more and more resistant worms to survive and thus create an increased population of resistant worms while creating a decreased population of susceptible worms (lack of refugia). This is what would be expected, but the negative effects from selection pressures on GIN using a long acting anthelmintic treatment has not been studied. It may be expected that plasma levels fall below the optimal therapeutic level and then the drug would be present in the animal's system at subtherapeutic levels. Merial, however, has indicated this product is actually formulated to prevent this occurrence. This is done by utilizing their trademarked Theraphase matrix gel. The active component, eprinomectin, is delivered by SQ injection with the Theraphase matrix gel which releases an initial therapeutic dose of eprinomectin into the animal's bloodstream where the drug initially kills worms over a relatively short period of time and then exits the animal's system. The amount of time the

residual drug stays in the body at sub-therapeutic concentrations is short, preventing prolonged exposure. Then, after a period of about 70-80 d, there is another release of drug from the Theraphase matrix gel which provides a similar period of activity. Thus, 2 phases of relatively short activity combined to provide the extended activity. With judicious use of anthelmintics, especially extended release ones like E-LR, hopefully, resistance can be delayed as long as possible.

Another potential issue that needs to be considered with the use of this (and similar) product is treating young calves before turnout in their first grazing season may reduce the interaction between the calf's immune system and the parasites to a level which might hinder the animal's ability to develop a protective immune response upon future exposure. Therefore, when the prolonged period of activity wanes, the animal is left with reduced immunity which may result in susceptibility to subsequent GIN infection. This concern would primarily affect stocker producers, who normally purchase weaned calves right after their first grazing season. By the time these calves reach their new location, the length of anthelmintic activity will be coming to an end. If the calves are then put on a heavily infective pasture, they may not be able to immunologically combat the deleterious effects of high parasite loads. This problem may not be of much concern if calves are retained on the original operation and on the same pastures where E-LR was used as pasture infectivity level should remain low.

Despite the conditions of this study where FEC was low for the entire period, E-LR still shows promise in controlling GIN infection in nursing calves. Overall, E-LR was shown to provide a more efficient and longer protection period than F-P offered; however, there was no weight gain advantage. Further, investigations are needed to address concerns of increased resistance, lack of refugia, and decreased immunity associated with this anthelmintic.

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VITA

Lyndsea Diane Seibert was born in New Orleans, Louisiana, in 1989 to Donna and John Seibert. She has four siblings; two older sisters and the other two in which she is a triplet with. She attended elementary school in Chalmette, Louisiana and high school and Andrew Jackson Fundamental Magnet High School for her freshman year and two weeks of her sophomore year. In 2005, she then evacuated to Baton Rouge, Louisiana from Hurricane Katrina and finished her high school years at Woodlawn High. After graduating from Woodlawn in 2008, she accepted a small scholarship award and started college Louisiana State University in Baton Rouge, Louisiana in 2008. She graduated with a bachelor's degree in animal science with a focus in science and technology from LSU in the spring of 2013. Upon graduating, Lyndsea began her work on her master's degree in animal science at the LSU School of Veterinary Medicine in the spring of 2014. Under the guidance of Dr. James Miller, she did a clinical trial in partnership with Merial. She will graduate in the summer of 2016. She currently resides in Prairieville, Louisiana.