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The Effect of the Nematode Trapping Fungus Duddingtonia flagrans Against Gastronintestinal Nematodes of Exotic Ruminant Hoofstock at Disney's(R) Animal Kingdom Lodge

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THE EFFECT OF THE NEMATODE TRAPPING FUNGUS *DUDDINGTONIA FLAGRANS* AGAINST GASTROINTESTINAL NEMATODES OF EXOTIC RUMINANT HOOFSTOCK AT DISNEY'S® ANIMAL KINGDOM LODGE

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Dedicated to

My Loving and Encouraging Parents

Friends and Family

My Furry Friends

LSU's School of Veterinary Medicine Class of 2020

And My Miller Laboratory Comrades

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ABSTRACT

Gastrointestinal nematodes (GIN) can severely affect the performance of ruminant animals and may lead to an animal's death in a severe, untreated infection. Zoological parks, which have used anthelmintic drugs for treatment of GIN, are now seeing evidence of drug resistant parasites (Garretson et al., 2009). *Duddingtonia flagrans*, a nematophagous fungus, has shown a reduction in larvae of coprocultures of exotic ruminants through feed administration at Disney's® Animal Kingdom Lodge (DAKL) and has potential to biologically control forage larvae (Terry, 2013). This study evaluated the effectiveness of *Duddingtonia flagrans*, administered to exotic ruminant hoofstock at a daily dose of 30,000 chlamydospores per kg of BW with standard feed, on reducing fecal egg count (FEC), larvae development and survival in feces and level of forage larvae availability for December, 2015 – May, 2016 (peak larvae season) at DAKL. Reticulated giraffe, scimitar-horned oryx and roan antelope were kept on control savannah, Sunset (n=8), and treatment savannahs, Arusha (n=6) and Uzima (n=5). Fecal egg counts were monitored throughout the study and individual coprocultures were used to determine *in vitro* development and survival of larvae. Forage samples were collected every month to survey the larval population available to animals in each savannah. This study showed that *D. flagrans* did not significantly reduce (P>0.05) FEC over time but showed a steady decreasing trend with treatments. The percent development and survival of larvae in coprocultures were reduced (P<0.05) to less than 2% for treatments, while the control levels remained above 30%. The number of larvae collected from forages, especially *Haemonchus contortus*, were decreased in savannahs in which animals given the fungus. *Duddingtonia flagrans* shows potential as an effective means at controlling GIN for animals in zoological captivity.

CHAPTER 1
INTRODUCTION

Gastrointestinal nematodes (GIN) have a worldwide distribution and are commonly recognized for their constraints on ruminant animals, due to their host-parasitic relationship. These nematodes are parasitic to hosts by taking nutrients from hosts while residing in the hosts' gastrointestinal tract. Some common GIN found in ruminants are trichotrongylid nematodes, including *Haemonchus* spp., *Teladorsagia* spp., *Trichostrongylus* spp., *Cooperia* spp., and *Nematodirus* spp. Frequently, ruminants are known to be infected with multiple species of GIN, known as polyparasitism. Other helminth infections can concurrently be present in the host's intestines, demonstrating similar symptoms (Kassai, 1999). Nutrient loss of captive ruminants can pose a financial threat for producers and zoo facilities because of the pathology caused by these parasites. *Haemonchus contortus* is a blood-feeding GIN that causes the most impairment of ruminants. An animal with *H. contortus* infection shows symptoms such as anemia, bottlejaw (edema), diarrhea and weight loss. If the susceptible animal is left untreated then death may occur (Hansen and Perry, 1994). When compared to captive ruminants, wild ruminants have fewer problems with GIN due to the larger environments in which they live, encountering fewer larvae. Captive domestic and exotic ruminants are kept in enclosed spaces, leading to constant pasture infectivity (Ibrahim et al., 2012).

For many years, animal keepers have treated animals harboring GIN by administering anthelmintics. Drug administration showed remarkable improvement in animals but later a decline in treatment became evident. The susceptible worms were no longer available on pastures and only drug resistant worms remained. Resistant GIN quickly became an issue, thus enclosure and animal management had to be reevaluated. With knowledge that only a few animals harbored the majority of the worm population, animals with high fecal egg counts (FEC)

of trichostrongyle-type eggs were dewormed instead of the entire stock of animals. Deworming only infected animals allowed a population of susceptible larvae to remain in enclosures. Other methods, such as burning pastures, rotating animals to different pastures, and proper drug storage and administration were incorporated into practices. These practices have helped reduce the chances of drug resistance but there are fewer effective drugs available on the market.

Drug resistance is a sizeable issue worldwide that has led the search for alternative means of controlling GIN. Taylor et al. (2007) states that anthelminthic resistance has been reported in sheep and goats (*Haemonchus* spp., *Trichostrongylus* spp., and *Teladorsagia* spp.), and horses (small strongyles). Benzimidazoles were primarily found to have resistance but additional broad-spectrum classes of drugs have been identified, 1-BZ (benzimidazoles and probenzimidazoles), 2-LM (levamisole/morantel) and 3-AV (avermectins/milbenmycins), and in some cases, the narrow spectrum drug closantel. Resistance is more prevalent in the Southern hemisphere (Australia, Africa, South America and New Zealand). Resistance rates are different for these areas due to the refugia, which is the population of parasites that have not been exposed to a drug. Resistant worms are selected for with further drug administration, which makes regenerating a refugia population difficult with drug withdraw, if not permanent (Taylor et al., 2007). Research has shown the potential of substances such as inorganics, naturally occurring compounds and other living organisms to combat this parasitic problem seen in production animals. One particular agent, nematode trapping fungus *Duddingtonia flagrans*, has shown promising control properties for small ruminants but further investigations were discouraged by low commercial availability. However, recent interest has prompted further investigations for use in small ruminant production and zoological parks (Terrill et al., 2012).

Terry (2013) evaluated the efficacy of *Duddingtonia flagrans* as a biological control of drug resistant GIN of exotic ruminant hoofstock at Disney's® Animal Kingdom Lodge. A reduction in GIN larvae in coprocultures was seen for animals administered as low as 30,000 chlamydospores/kg of body weight (BW) for up to 8 weeks. As *D. flagrans* showed promise as a way to control GIN for zoo captive animals, further investigation is needed to evaluate long term control of forage GIN larval populations (Terry, 2013).

The purpose of this study was to evaluate the effects of the daily administration of 30,000 chlamydospores/kg of body weight (BW) of *D. flagrans* to exotic ruminant hoofstock on savannahs at Disney's® Animal Kingdom Lodge on FEC, the development and survivability of fecal larvae and larvae available on forage. If *D. flagrans* fed daily at a dose of 30,000 chlamydospores/kg of BW can show a reduction of FEC, a decrease of larvae in feces and a decrease the number of available on forage, then larvae available on forage should significantly decrease over time; yet, larvae available on forage should not significantly change when animals do not receive chlamydospores added to supplement feed. The information from this study can be used in the investigation of nonchemical control of GIN of ruminant hoofstock in captivity.

CHAPTER 2
LITERATURE REVIEW

2.1 Captive and Wild Ruminant Hoofstock

Ruminant livestock are used widely as a food source and human populations have continued to increase in numbers as countries develop, increasing the need for more livestock to feed families. In addition, land cultivation increases the risk of wild life endangerment. As more people become aware of exotic animals that are at risk of endangerment, the desire to preserve them also rises. Prevention of species endangerment and animal education has led to an expansion of zoos. Gastrointestinal nematode parasites are traditionally found in livestock, playing a role in reducing weight gain, increasing weight loss, and potentially causing animal loss. Similar parasites found in livestock used for food are also seen in exotic ruminant hoofstock, lending the same problems to zoos. Parasite monitoring is also important in zoological settings where mixed species are kept together due to the ability of GIN crossing species lines (Miller and Fowler, 2012). Gastrointestinal nematode parasite control may be vital to the stability of zoo operations, as monetary funding is influenced by the spectators' expectations of healthy animals.

2.2 Ruminant Trichostrongyle-Type Parasites

Helminths are "worm-like parasites" divided into three groups, Trematodes (flukes), Cestodes (tapeworms) and Nematodes (roundworms). Helminths have a life cycle of larval (juvenile), adult and egg stages, where generally most helminths' eggs are shed in a host's fecal matter (Castro, 1996). Belonging to Phylum Nematoda, Family Trichostrongyloidea is distributed worldwide and is the most collective group of helminths found in grazing ruminants. Temperate zones show more prevalence of *Teladorsagia* spp. and *Nematodirus* spp., while tropic

and subtropic areas dominantly include *Haemonchus* spp., *Mecistocirrus* spp., *Cooperia* spp., and *Trichostrongylus* spp. (Kassai, 1999).

Haemonchus contortus, the barber's pole worm, is a blood-feeding GIN that infects ruminants. These worms live in the abomasum of ruminant animals (Kassai, 1999). The adult worms cut the abomasal mucosa with their buccal lancet to feed on blood. This parasitic behavior leads to symptoms such as anemia, dehydration, edema, weight loss and reduced weight gain, and possibly death (Morgan and Hawkins, 1953). This is problematic for producers because proper weights for reproduction, wool/hair production, or meat production may be compromised. Zoo facilities may also encounter problems from ruminant animal infections by having ill-appearing animals that have to be removed from the exhibit. The ultimate worst situation is fatality of the animal, leading to major financial losses for both parties.

2.3 Trichostrongylid Nematode Life Cycle

The life cycle of trichostroylids begins with an infected ruminant shedding eggs in its feces. The oval eggs are thin-shelled, colorless and medium-sized (60-110 μm), with the exception of *Nematodirus* spp. eggs occurring twice the size as other trichostrongyle-type eggs (130-260 μm) (Kassai, 1999). Humidity and temperature are important constituents for development. Optimal temperature and humidity ranges are 18-26°C and 80-100%. Egg to L3 development will not occur below 10°C, but L3 can survive below 5°C with low metabolism. When humidity is low, feces or soil can contain enough humidity to allow egg development (Taylor et al., 2007). If environmental conditions are appropriate for development, the first stage larva (L1) will emerge from an egg, within 24 hours (Kassai, 1999). As an L1 develops, the L1 molts to become the second stage larva (L2), shedding the cuticle in the process. A second molt with cuticle retention allows an L2 to develop into the infective stage, the third stage larva (L3).

In addition to embryonated eggs, this double cuticle larval stage can survive extreme environments of freezing or desiccation, yet L1 and L2 are vulnerable. The infective L3 are stimulated by light and temperature, and requires a water droplet to move up a blade of grass to be ingested by the animal. After ingestion, an L3 exsheaths the surrounding cuticle inside the animal, based on new environmental conditions provided by the host (Taylor et al., 2007). The exsheathed L3 travels to the site of infection through the gastrointestinal tract and molts to fourth larval stage (L4). A final molt of an L4 gives rise to the sexually mature adult which then can sexually reproduce. The oviparous female will lay fertile eggs that will exit with the feces. The prepatent period, time of infection to eggs present in feces, is approximately 2-3 weeks. Larvae can arrest development in the host, called hypobiosis, when conditions outside the host are not favorable. Development will resume spontaneously when conditions are favorable. Ability to arrest and resume development in the host is epidemiologically important, as worms can survive during harsh conditions and contamination rate is increased when survival rate is highest (Kassai, 1999).

2.4 Anthelmintic Drugs

Caretakers who market ruminants rely heavily on drugs for GIN control and a large sum of funds goes toward purchasing anthelmintics. An ideal anthelmintic drug has the following standards: all life stages are affected, controls more than one genera, is safe for the host, can be readily administered, and cost effective, especially profitable. Two uses of anthelmintics are prevention and treatment. Drugs are orally administered, by drench or feed additive, or given as a subcutaneous injection. In addition, compounds that are injected into the rumen for slow dose release and water additives are available on the market (Taylor et al., 2007).

2.4.1 Benzimidazoles

The first class of broad spectrum anthelmintics was documented in 1961 as benzimidazoles (BZ) (Brown et al., 1961). Merck trademarked the first derivative of benzimidazoles from N-arylamides as thiabendazol in 1965 (Grenda et al., 1965). Additional drugs and prodrug compounds in this class are albendazole, febantel, fenbendazole, flubendazole, mebendazole, netobimin, oxfendazole, oxibendazole, parabendazole and ricobendazole. Drugs in this class have shown effectiveness against GIN adults, developing larvae and arrested larvae (Campbell, 1990). Benzimidazoles work on GIN by blocking the transport of intestinal cells' secretory granules and eliminating microtubules, without affecting microtubules of the host (Borgers et al., 1975). Routes of administration for control with benzimidazoles have evolved over time. Initially single doses were given in the form of drenches and boluses and later given in long term administration with smaller doses over time by incorporation of the drug in feed and feed blocks. Additional forms of administration were developed to introduce the drug with a syringe directly into the rumen and in time-release capsules (Campbell, 1990).

2.4.2 Imidazothiazoles

The first imidazothiazole, tetramisole, was introduced in 1966 (Thienpont et al., 1966). Later one of its isomers, levamisole, was isolated and became a widely used anthelmintic drug (Bullock et al., 1968). This group of drugs acts as an agonist to nicotinic acetylcholine receptors found on muscle cells, causing an increased conductance and depolarization, leading to the paralysis of the nematode (Harrow and Gration, 1985). This class has been reported to be effective against adults and immature stages of important GIN (Kistner and Wyse, 1975; Callinan and Barton, 1979; Armour, 1983). Levamisole has a narrow therapeutic window due to

its nicotinic action and should be carefully administered when groups of animals with varying weights are being treated (McKellar and Jackson, 2004).

2.4.3 Macrocyclic Lactones Derivatives

A group of chemical derivatives of macrocyclic lactones known as avermectins were identified to have great anthelmintic effects in 1979. These compounds are created by an actinomycete from a soil sample in Japan (Burg et al., 1979). Ivermectin, a widely used anthelmintic was introduced by Merck in 1980 (Chabala et al., 1980). The ivermectin derivative paralyzes nematodes by the disturbance of the interneuron's communication to the dendritic excitatory motorneurons (Kass et al., 1980). The pharynx is the target in nematodes for ivermectin by inhibition of γ-Aminobutyric acid (GABA) and glutamic acid, which affects their feeding (Brownlee et al., 1997). Avermectins work on inhibited stage larvae and adults (Egerton et al., 1979).

2.4.4 Amino-acetonitrile derivatives

Over time there was a focus of producing new drug components for their anthelmintic properties. Previously described as "fungicides, antibacterials, and insecticides", amino-acetonitrile derivatives (AADs) were not valued for GIN control until a study on mice showed the control of *H. contortus* (Kaminsky and Rufener, 2012). The AADs have shown activity on the fourth larval (L4) stage and adults of major GIN, including drug resistant GIN. A genetic analysis of AAD-resistant *Caenorhabditis elegans* and *H. contortus* mutants showed AADs target nematodes with the activation of a nAChR signaling pathway (Kaminsky et al., 2008). The World Health Organization (WHO) approved use of the active enantiomer AAD1566 in sheep of New Zealand in 2009 under the name monepantel. This drug is available with the tradename Zolvix©. A number of studies have shown an absence of toxicity for sheep of doses up to 200

mg/kg. Amino-acetonitrile derivatives are newer than the classical anthelmintics and are useful for controlling GIN, as there are more susceptible, unexposed GIN, but action is warranted to identify mutant worms so the drug can continue to be effective (Kaminsky and Rufener, 2012).

2.5 Anthelmintic Resistance

When administrating anthelminitics for therapeutic usage, they must work against the infective stage, if it does not affect all life stages, and it should alleviate signs caused by the parasite being treated. If the anthelmintic is being used for prophylaxis, the following should be considered: cost should be reasonable when evaluating production or preventing clinical and subclinical diseases, benefits from prevention should be evaluated against other methods such as changing production management, the anthelminitic should not interfere with acquired immunity that may save stock in the future, and persistent use of one anthelminitic is strongly discouraged as this leads to resistance (Taylor et al., 2007). Prichard et al. (1980) described that resistance occurs "when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable"(Prichard et al., 1980). According to Garretson et al. (2009), a giraffe in Florida at Lion Country Safari was diagnosed with *H. contortus* with resistance to multiple classes of anthelmintics. This young giraffe was the first case in which resistance had been a concern in a zoological setting. Lion Country Safari has seen previous death cases as a consequence of high endoparasitism (Garretson et al., 2009).

A commonly used technique to identify anthelmintic resistance is with the Fecal Egg Count Reduction Test (FECRT). Martin et al. (1989) showed that the FECRT is an effective means of identifying resistance when there is at least 25% resistant worms (Martin et al., 1989). While widely used due to lower costs, a more sensitive test, such as a controlled efficacy study should be performed and is the gold standard for determining anthelmintic resistance in a

population of GIN. Guidelines are set up by the World Association for the Advancement of Parasitology (W.A.A.V.P.) for multiple animal species and GIN to identify resistance (Wood et al., 1995). Where drug efficacy is < 95%, after a comparison of arithmetic means, then drug resistance is present. Drugs should show efficacy ≥ 99% against GIN (Martin et al., 1989; Coles et al., 2006).

Thiabendazole was the first broad spectrum on the market and GIN quickly developed resistance in sheep (Drudge et al., 1964) and horses, just 3 years after its introduction for each species (Abongwa et al., 2017).

2.6 Preventative Management Strategies to Anthelmintic Resistance

Preventative measures should be placed into practice for livestock management to possibly slow the development of anthelmintic resistance when they are needed to treat animals. Producers should start by maintaining a susceptible worm population by treating selected animals. As resistant worm populations continue to rise, caretakers should only use efficacious anthelmintics and only treat animals with high GIN infections (Dobson et al., 2011), known as "smart drenching". One way to evaluate animals with high levels of an anemia-causing GIN, *H. contortus*, is by use of the FAMACHA© scoring system, in addition to monitoring FEC. The mucous membrane color of the animal's inside lower eyelid is compared to a FAMACHA© chart to determine if the animal is anemic. Best judgement should be used when electing to use a dewormer by looking at trends of historic FAMACHA© scores with FEC values (Vatta et al., 2001).

Other management strategies include coordinating anthelmintic administration with weather, mixed animal grazing, pasture rotation, considering stocking rate, strategic deworming protocols and focusing on providing protein necessary for birth and development, which are

times of tolerating high levels of GIN (Fleming et al., 2006). Zvinorova et al. (2016) also agrees with Fleming et al. (2006) that breeding animals more resistant to GIN infections should be included in animal management (Zvinorova et al., 2016). According to Fleming et al. (2006), strategies to combat resistance will be worthless if producers continue to purchase animals harboring resistant worms, alluding to the importance of quarantining all new animals (Fleming et al., 2006). Discovering and researching anthelmintics take a lot of time and money, and therefore measures should be taken to preserve current effective anthelmintics and find alternative means of treating helminths (de Hostos and Nguyen, 2012).

2.7 Alternatives for GIN Control

The need for GIN control, with the increase in prevalence of anthelmintic resistance, has made finding alternatives a forefront. Some presently known options for integrative GIN control include: copper oxide wire particles, condensed tannin containing forages (e.g. sericea lespedeza), vaccines and nematophagous fungi (Terrill et al., 2012).

2.8 Copper Oxide Wire Particles

Copper oxide wire particles (COWP) have been evaluated as an alternative to combat anthelmintic resistance, despite possible copper toxicity that can occur in sheep. A study evaluated the efficacy of COWP at a dose up to 2 g to treat *H. contortus* in weaning lambs. This dose of COWP showed a significant decrease in FEC as compared to the lambs that received the placebo, with no signs of copper toxicity (Schweizer et al., 2016). Administration of 4 g of COWP has shown to be efficacious for up to 28 d ($P \leq 0.01$) in male goats, with reported anthelmintic resistance, after grazing on a pasture with *H. contortus* available; furthermore, doses of 4 g may be administered safely 84 d post treatment (Vatta et al., 2012). In addition, COWP can be given with current anthelmintics as an extra mean to control *H. contortus* in lambs. A study evaluated different sources of COWP, combined with albendazole, in Katahdin

lambs in the ability to reduce GIN in the feces and FEC. Significance was illustrated for the all groups of COWP in reducing the larvae in feces, and when combined with albendazole, the anthelmintic's efficacy increased leading to a decrease in multiple larval species in feces. Alternative applications with COWP in combination with current drugs could prove useful in reducing efforts against anthelmintic resistance (Burke et al., 2016).

2.9 Condensed Tannins

An additional source of alternative helminth control can be found in plants containing a compound known as condensed tannins. Sericea lespedeza (SL), *Lespedeza cuneata,* is a perennial legume that is good for improving and conversing soil, growing in sandy soils (United States Department of Agriculture, 1948). Sericea lespedeza plants may be found in some pastures and are known to contain condensed tannins. A decrease in FEC and switching the GIN population from *H. contortus* to other *Trichostrongylus spp.* may be seen when lambs graze on pastures of Bermudagrass mixed with SL or when grazing pure SL. Lambs must learn to graze SL with their dams, as they will not forage this plant on their own. Effects of grazed SL are limited when *H. contortus* is not the predominant GIN. The SL plant may be an applicable alternative when used with COWP for controlling GIN (Burke et al., 2012).

2.10 Vaccines

Vaccines have been assessed as means to control GIN infections. Helminths are known to use proteolytic enzymes when entering hosts' tissues of their destination, evading the immune response of the host while feeding on proteins. Cysteine proteases are produced by the host to hinder GIN from feeding or invading mucosa, potentially leading to parasitic elimination or death. Vaccines are aimed at containing these proteases to inhibit GIN from invading tissue by eliminating their enzymes. Some problems are seen with the vaccines, such as protein not being

folded properly, as in nature, and glycosylation that occurs after translation. More research is needed to understand genes in effect (Knox, 2012).

2.11 Nematophagous Fungi

An additional means of combatting anthelmintic resistance is the use of biological control. Biological control is defined by an organism that inherently competes with the organism of concern by keeping its population lower than if it were not present. Biological control can be either natural, where control is in the existing environment, or applied, where there is human intervention. In the environment where the competitive organism can control the other organism, there is simply not a large enough effect to control the GIN that infect the animals or the host would have control. Biological control may affect all stages of GIN control but the free-living stage appears to be the best target for control. Controlling GIN in the free-living stage involves creating an unsuitable living environment for GIN or the use of GIN as a food source. Potential sources of GIN control are nematophagous fungi, which are divided into three groups (Waller and Faedo, 1996). Barron (1977) described two categories of fungi, predacious fungi, which had nematode trapping devices, and endoparasitic fungi, which affect the nematode with spores sticking to the cuticle or after ingestion of the spores would penetrate the gut (Barron, 1977). An additional stage was identified as the egg-parasitic fungi, where the fungi intervene at GIN egg stage (Nordbring-Hertz, 1988).

Trap forming fungi, such as *D. flagrans*, have been effectively used to reduce nematode populations (Herrera-Estrella et al., 2016). *Duddingtonia flagrans* is a ubiquitous fungus that is found in low levels in the environment. The fungal spores can be incorporated in animal feed to travel through the digestive tract unchanged and concentrate in the feces in high numbers (Miller and Fowler, 2012). Larsen et al. (1991a) recovered several species of nematophagous fungi from cattle, that were fed from a successful *in vitro* study, and tested them for predacious properties

against *Ostertagia ostertagi* nematodes. *D. flagrans* was one of the isolates that survived the ruminants' gastrointestinal tract and controlled infective *O. ostertagi* larvae in the feces by 96 percent (Larsen et al., 1991b).

Administering the fungus to benefit animals must be feasible for the caretaker and receiving animal. A few ways to administer fungal spores is through feed application, supplementary feed blocks, or intra-ruminal control release devices (IRCRD) (Waller and Faedo, 1996). Long term administration of fungal chlamydospores with barley was demonstrated as a potential practical application of chlamydospores in feed (Gronvold et al., 1993). Administering fungal spores in feed blocks can be an economical way to introduce the fungus with fewer caretaker applications. According to Waller and Faedo (1996), supplementary blocks may be effective means of administering fungal spores to livestock. Lastly, an IRCRD should last preferably 60 days or more, which could be administered during periods that would address seasons highest for GIN infections (Waller and Faedo, 1996). A successful experiment using *D. flagrans* as a control for GIN larvae resulted in a large reduction (> 80%) of L3 in sheep feces at a dose of $5x10^5$ and 10^6 chlamydospores/day by introduction through an abomasal cannula and orally to normal sheep (Larsen et al., 1998). Storage of the fungus must be practical as well. Nutritional pellets were shown to be effective at reducing *H. contortus* larvae after being stored for 8 weeks in the following conditions: stored on shelves indoors, in a refrigerator at 4ºC, outside under a covering, and completely exposed outdoors. Storage of this fungus supports the use of the fungus as a feasible option for animal caretakers, as multiple scenarios do not impact fungal effect on larval reduction (Fitz-Aranda et al., 2015).

Duddingtonia flagrans has activity against infective GIN larvae such as *H. contortus*, a parasite that reduces the productiveness of ruminant animals. The nematode trapping loops may

be present by the first 9 hrs of inoculation. A “mucilaginous substance” is present at the places in contact on the larva by the trap and bacteria may be found at these sites. The cuticle may be penetrated by 48 hrs from nematode-fungal contact (Campos et al., 2008).

An important factor to take into account when introducing anything new into the environment is identifying whether long term effects will be observed. Waller and Faedo (1996) addressed the concern of this nematophagous fungi trapping other nematodes not intended to be eliminated such as native, advantageous saprophytic nematodes that help recycle fecal matter (Waller and Faedo, 1996). According to a study with soil supplemented with *D. flagrans*, native soil inhabitants were not affected by the fungal addition and *D. flagrans* was no longer present in the environment two months following pasture treatment (Saumell et al., 2016).

CHAPTER 3
MATERIALS AND METHODS

3.1 Location and Animals

This study was conducted at Disney's® Animal Kingdom Lodge in Lake Buena Vista, Florida. The animals were maintained on 3 savannahs, Sunset, Arusha and Uzima. The animals on Sunset savannah had access to 7.28 hectares for grazing, while both Arusha and Uzima savannahs each provided 4.45 hectares for grazing. The exotic ruminant species that were monitored during this study were reticulated giraffe (*Giraffa camelopardalis reticulata*), scimitar horned oryx (*Oryx dammah*) and roan antelope (*Hippotragus equinus*). These species were selected based on previous observations (Dr. James E. Miller, personal communication) that indicated they were the high-risk species most susceptible to nematode infection. Sunset savannah had 4 giraffe, 2 oryx and 2 roan antelope. Arusha had 3 giraffe and 3 roan antelope, while Uzima had 4 giraffe and an oryx. There were other animals on each savannah (Tables 1-3).

The animals were supplement fed their standard feed Mazuri® ZuLife® Wild Herbivore Diet and the pregnant female on Arusha savannah was fed Mazuri® Wild Herbivore Plus Diet (Land O'Lakes Purina Feed LLC, Richmond, IN), with acacia leaves, willow tree branches and alfalfa hay. Animals were allowed free access to water. Each morning the animals were brought into a barn to feed ad libitum from their individually measured, partial mixed ration and the remaining diet came from grazing the savannah. The forage available consisted of coastal Bermudagrass, Timothy grass and browse.

Table 1. Exotic animal species present on Sunset savannah at Disney's® Animal Kingdom Lodge (* indicates animals that were treated).

Sunset	
Common Name	Scientific Name
Ankole cattle	*Bos taurus taurus ankole*
Wildebeest	*Connochaetes tarurinus*
Impala	*Aepyceros melampus*
Hartmann's mountain zebra	*Equus zebra hartmannae*
Grey crowned crane	*Balearica regulorum gibbericeps*
Ostrich	*Struthio camelus*
Marabou stork	*Leptoptilos crumeniferus*
Reticulated giraffe* (n=4)	*Giraffa camelopardalis reticulata*
Scimitar horned oryx* (n=2)	*Oryx dammah*
Roan antelope* (n=2)	*Hippotragus equinus*

Table 2. Exotic animal species present on Arusha savannah at Disney's® Animal Kingdom Lodge (* indicates animals that were treated).

Arusha	
Common Name	Scientific Name
Waterbuck	*Kobus ellipsiprymnus ellipsiprymnus*
Wildebeest	*Connochaetes tarurinus*
Impala	*Aepyceros melampus*
Thomson's gazelle	*Eudorcas thomsonii*
Red river hog	*Potamochoerus porcus*
Plains zebra	*Equus quagga*
Grey crowned crane	*Balearica regulorum gibbericeps*
Reticulated giraffe* (n=3)	*Giraffa camelopardalis reticulata*
Roan antelope* (n=3)	*Hippotragus equinus*

Table 3. Exotic animal species present on Uzima savannah at Disney's® Animal Kingdom Lodge (* indicates animals that were treated).

Uzima	
Common Name	Scientific Name
Ankole cattle	*Bos taurus taurus ankole*
Common eland	*Taurotragus oryx*
Lesser kudu	*Tragelaphus imberbis australis*
Impala	*Aepyceros melampus*
Grey crowned crane	*Balearica regulorum gibbericeps*
African spoonbill	*Platalea alba*
Spur-winged goose	*Plectropterus gambensis*
South African shelduck	*Tadorna cana*
Reticulated giraffe* (n=4)	*Giraffa camelopardalis reticulata*
Scimitar horned oryx* (n=1)	*Oryx dammah*

3.2 Experimental Design

At Disney's® Animal Kingdom Lodge, the naturally infected exotic ruminants remained under their normal original living conditions for the duration of the study. Because Sunset savannah was the largest and almost equal to Arusha and Uzima combined, it was designated the control. Previous observations (Dr. James E. Miller, personal communication) showed that the winter months, December through March, provided the highest infection rates coupled with the highest larvae levels on forage. Sunset savannah was the control and the experimental animals were fed a daily ration of feed without chlamydospores. The experimental animals on Arusha and Uzima savannahs were treated with 30,000 chlamydospores/kg of BW of *D. flagrans* (BioWorma®, International Animal Health Products Pty Ltd, Huntingwood, Australia) mixed into their daily supplement ration. The dose of fungal spores administered was chosen based on previous studies performed at Disney's® Animal Kingdom Lodge, where a reduction of L3 was seen from *in vitro* coprocultures (Terry, 2013). Animals on each savannah were allowed to graze and water was available ad libitum. While animals were inside the barn, the animal care team observed research animals for defecation, to immediately collect samples from the tops of fresh fecal masses. Each fecal collection was implemented for multiple 3-5 day intervals, from December 8, 2015 through March 21, 2016. Fecal collections were conducted within 6 days prior to the start of chlamydospore feeding, during the 3 months of chlamydospore feeding and a month after chlamydospore feeding was removed, for a total of 30 fecal collection periods. Animals in the treatment groups were administered chlamydospores during fecal collection periods 3 – 29. All procedures prior to implementation were approved for this study's animal care and use at DAKL by two animal review processes by Disney Animal Care and Welfare Committee (DACWC). Animals' feces were monitored for FEC and used for coprocultures

(percent development and survival of L3 in feces). Savannah forages, to evaluate L3 available on forage (L3/kgDM) and percent L3 population distribution, were sampled monthly from December, 2015 through May, 2016.

All fecal and forage samples were shipped to the Louisiana State University's School of Veterinary Medicine (LSU-SVM), Baton Rouge, Louisiana by overnight express for processing.

3.3 Techniques

3.3.1 Fecal Egg Count

A modified McMaster's procedure (Whitlock, 1948) was used to determine each animal's FEC. Fecal pellets were weighed in 120 mL cups to equal 2 g of feces. Pellets were crushed and evenly mixed with a tongue depressor in 30 mL of salt solution (737 g salt mixed with 3000 mL water). The contents were mixed by an electric hand mixture (Drinkmaster® Drink Mixer, Hamilton Beach Brands, Inc., Glen Allen, NC) controlled by a rheostat switch. A transfer pipette, with a slant-trimmed tip, was used to transfer a sample of the mixture into a single chamber of a McMaster's slide (Chalex, LLC, Park City, UT). The mixing was repeated to fill the second chamber of the McMaster's slide. Each McMaster's slide was read at 100x magnification. All lanes of both chambers were visually scanned to count trichostrongyle-type eggs. The total number of eggs counted was multiplied by 50 to determined eggs per gram (EPG).

If no eggs were counted for the modified McMaster's procedure, a double centrifugation technique was performed to obtain the FEC. Two grams of feces were weighed in 120-mL cups and crushed with a tongue depressor. Fifteen mL of water were added to each cup and mixed with the feces. The mixture was poured through a small tea strainer over a funnel into a 15-mL centrifuge tube. Water was added to tubes to make 15 mL, capped, and centrifuged for 10 min at 1500 rpm. The supernatant was poured off and Sheather's sugar solution (500 g sugar with 320

mL water with a specific gravity of 1.25) was added to the 14 mL mark. Two wooden applicator sticks were used to break up the sediment in the sugar mixture. Sugar solution was added to a positive meniscus. A coverslip was placed on the top of the tube and the tube was centrifuged as per previous settings. The cover slip was removed after centrifugation and placed on a microscope slide. All trichostrongyle-type eggs were counted over the entire coverslip at 100x magnification. The number of eggs counted was extrapolated to obtain the EPG (see ch 3.4.1 for equation).

3.3.2 Coprocultures

Each individual sample of feces was weighed (between 5.0 g and 12.0 g) in 120-mL cups. The feces were crushed with a tongue depressor and vermiculite was added (approximately a 50:50 ratio). Water was added and mixed until a crumbly consistency was obtained. Cheese cloth squares, secured by rubber bands, covered the cups. Water was added to the bottom of a 250-mL tri-corner cup to about 0.5 in and the cup containing feces was inverted and suspended above the water line in the tri-corner cup. This allowed maintenance of humidity. These coprocultures remained for 14 d in an incubator at 25°C to allow complete larval development.

After the incubation period, the cups were removed from the incubator and placed on the counter top where warm water was added to cover the culture mass in each 120-mL cup. After a 12 hr period, the 120-mL cup was removed from the 250-mL cup and the supernatant in the 250-mL cup was vacuumed down to less than 15 mL and transferred to a 15-mL centrifuge tube containing approximately 1 mL of 10% formalin.

3.3.3 Forage Sampling

For forage collection, Sunset savannah consisted of 9 zones and Arusha and Uzima savannahs consisted of 5 zones each. Within each zone, an animal care team member randomly tossed a hoop (square-shaped clothes hanger – 400 cm^2) three times and grass shears were used

to cut the forage down to ground level within the hoop. Forage samples were stored in zip-top bags and packaged with cold packs to be shipped within 24 hours for overnight express.

3.3.4 Forage Processing

Forage samples were processed within 48 hrs of arrival at the LSU-SVM. A subsample was removed from the bag and weighed in a 120 mL cup. Subsamples were placed in an oven (37°C) for 10 – 14 d to obtain dry weight. Remaining forage was weighed in the zip-top bags. Samples were submerged by inverting the bags into 3.5 L labeled buckets filled with warm water and a drop of dish soap for surfactant purposes (helps L3 separate from forage). Zip-top bags were air dried for at least seven days and then weighed. Buckets remained overnight, undisturbed for 12 hrs at room temperature (approximately 25°C). A large tea strainer was used to strain forage from buckets and remaining water was left to settle for at least 12 hrs at room temperature. Water was poured off so that it could be placed in 1000 mL tri-corner cups. Contents were allowed to settle for another 12 hrs (at room temperature) and the supernatant was siphoned to approximately 400 mL and transferred to 400 mL tri-corner cups. The settling process was repeated down to less than 120 mL cups and the mixtures were transferred to 120 mL cups. Rubber bands secured Kimwipes® to the tops of the 120 mL cups, to filter remaining particulates. Cups were inverted and submerged in 250 mL cups containing warm water. After 12 hrs, the 120 mL cups were removed so debris-free water was present in the 250 mL cups. After another settling process of 12 hrs at room temperature the supernatant was vacuumed down to less than 15 mL and transferred to 15 mL tubes containing approximately 1 mL of 10% formalin.

3.3.5 Larvae Enumeration and Identification

The tubes containing sediment from fecal coprocultures were vacuumed down to concentrate the larvae in a small volume for enumeration and larval identification. The volume of remaining fluid was noted and the fluid was vortexed to get an even distribution of larvae. A 100 μL aliquot was removed and placed on a microscope slide. A wooden applicator stick was used to gently mix a drop of Lugol's iodine to stain larvae and a cover slip was placed over the drop. The complete cover slip was read at 100x magnification, where larvae were identified, up to 100 larvae, and the remaining larvae were counted on the slide. If 100 larvae were not identified for the first aliquot, additional 100 mL aliquots were taken until 100 larvae were identified and then the remaining larvae were counted for the aliquot. The values were used to calculate larvae per gram (LPG) of feces. The same procedures were done for all tubes of larvae collected from forage samples.

3.4 Calculations

3.4.1 Fecal Egg Count

When 2.0 grams of feces were weighed, the number of eggs per gram (EPG) of feces was calculated with the following equation:

$$\#\text{ of eggs counted per McMaster's slide} \times 50 = \text{EPG}$$

If the weight (wt) of the feces was less than 2.0 grams or if the number of eggs counted on the McMaster's slide was 0, a double centrifugation procedure was performed and the following equation was used to calculate the EPG:

$$\frac{2}{\text{wt of feces}} \times \#\text{ of eggs counted} \times 50 = \text{EPG}$$

3.4.2 Larvae per Gram of Feces

The number of larvae per g (LPG) recovered from coprocultures was calculated by:

$$\frac{\text{\# of L3 larvae counted per tube}}{\text{weight of feces (g)}} = \text{LPG}$$

3.4.3 Percent Larval Development and Survival

The percentage of larvae that developed and survived after hatching in coprocultures was calculated by:

$$\frac{\text{LPG}}{\text{EPG}} \times 100 = \text{\% development and survival}$$

3.4.4 L3 per Kg of Dry Matter of Forage

Forage samples were collected and used to recover L3 to determine the number of L3 that were available to the animals on the forage. To determine the number of L3 in each collected subsample, the weight of the forage was determined by:

wt of bag with collected forage – wt of empty bag = wt of collected forage (g)

A subsample of forage was removed from each bag and used to determine dry matter (DM) by:

$$\frac{\text{wt of wet subsample forage (g)}}{\text{wt of dry subsample forage (g)}} = \text{\% DM}$$

The % DM was converted into kg of DM (kgDM) by:

$$\frac{\text{wt of collected forage (g)} \times \text{\% DM}}{1000} = \text{kgDM}$$

The number of recovered L3 were divided by kgDM to get the number of L3 for each subsample on a dry matter basis (L3/kgDM) by:

$$\frac{\text{\# of L3 larvae counted per tube}}{\text{kgDM}} = \frac{\text{L3}}{\text{kgDM}}$$

3.5 Statistical Analysis

Data analyses were conducted using SAS® software (SAS Institute. 2017. SAS/STAT User's Guide, Version 9.4. Cary, NC). An n-way analysis of variance (ANOVA) PROC GLM was used to further evaluate the significance of experimental data. Data for FEC and L3/kgDM were changed with a logarithmic transformation to obtain a more normal population distribution for statistical analyses. The response variables used in the analyses were logFEC, percent larval development and survival, and logL3/kgDM. Time, savannah, treatment, and treatment x time were analyzed as fixed effects. *Post hoc* comparisons were performed with least squares means and P-values ≤ 0.05 were determined significant.

CHAPTER 4
RESULTS

4.1 Mean Fecal Egg Count

The range of mean FEC for animals on Sunset savannah, the control, was 101 to 382.6 EPG. The mean FEC of treatment animals on Arusha and Uzima savannahs ranged from 141.7 to 742 and 33.4 to 383.2 EPG, respectively (Figure 1).

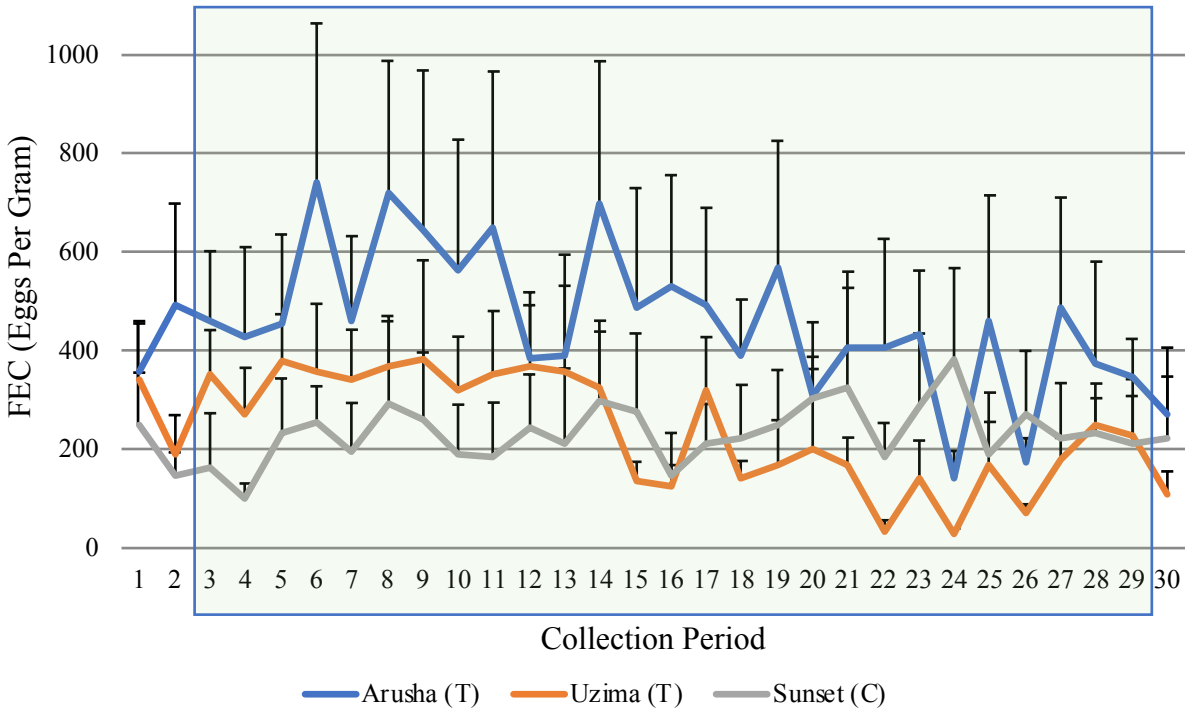

Figure 1. Mean (+SEM) fecal egg count (FEC) for captive exotic ruminant species at Disney's® Animal Kingdom Lodge on control (C) savannah (Sunset, n=8) and treatment (T) savannahs (Arusha, n=6 and Uzima, n=5) for 30 collection periods supplemented with chlamydospores of *Duddingtonia flagrans* (30,000 per kg BW). Shaded area indicates administration of chlamydospores mixed in supplement feed.

A log transformation of FEC, to obtain a normalized distribution of egg counts, showed that there was a significant difference ($P<0.001$) for treatment x time and time, but no significant difference ($P>0.05$) was observed for treatment.

During the duration of the study, there was no significant difference ($P>0.05$) observed for logFEC between treatment savannahs, Arusha and Uzima, except ($P<0.01$) for collection period 22. When comparing logFEC for Uzima and Sunset savannahs, there was no significant difference ($P>0.05$) for most collection periods, except for collection periods ($P<0.05$) 10 and 22. The logFEC comparison for Arusha and Sunset savannahs were not significantly different ($P>0.05$) except ($P<0.05$) for periods 3, 4, 8, 10, 27, and 29.

4.2 Percent Larval Development and Survival

The percent development and survival of larvae in feces for Sunset savannah animals ranged from 6.3 to 46.7%. For collection periods 1, 2, 3 and 4, percent development and survival were 33.9%, 23.3%, 11.1% and 9.2%, respectively. Subsequent to period 4, percent development and survival increased and remained oscillating around a mean of about 25% for the duration of the study.

The percent development and survival of larvae in feces for Arusha savannah animals ranged from 0.1 to 23.4%. The first three collection periods (before treatment started) were above 9% and during the treatment period, it was consistently close to 0%.

The percent development and survival of larvae in feces for Uzima savannah animals ranged from 0 to 24.7%. The first three collection periods (before treatment started) were above 21% and during the treatment period, it was consistently close to 0% (Figure 2).

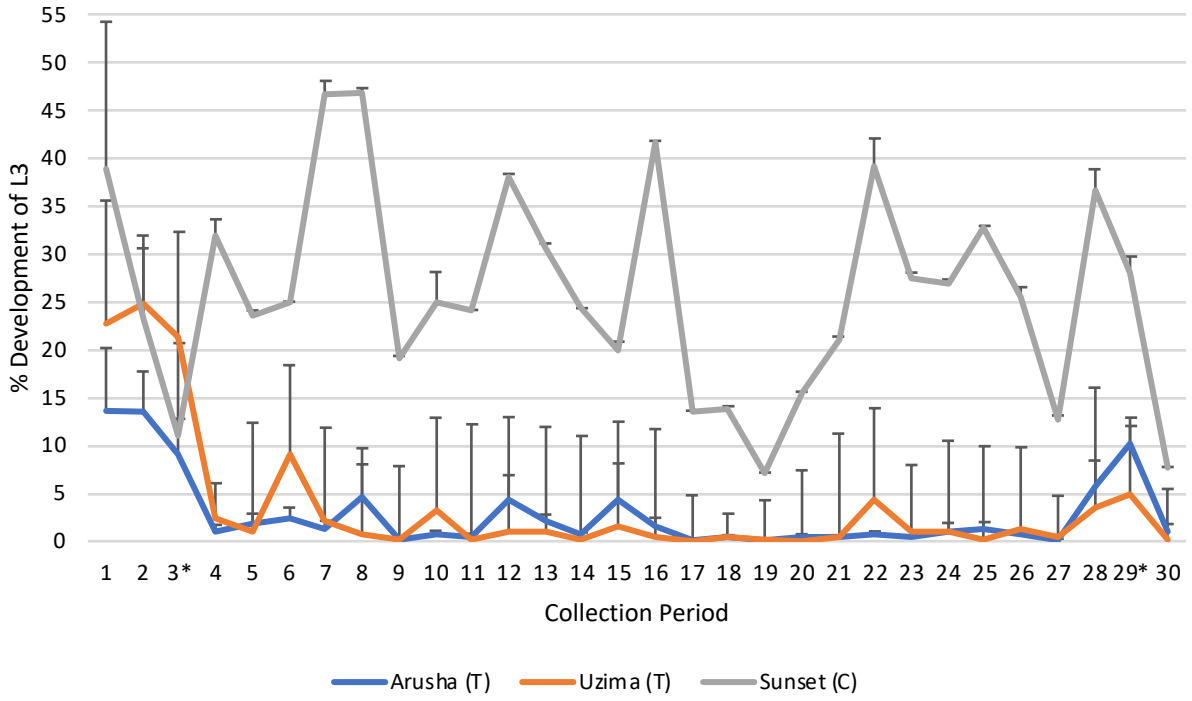

Figure 2. Mean (+SEM) percentage development and survival of infective larvae (L3) in feces of giraffe, roan antelope and oryx at Disney's® Animal Kingdom Lodge on Sunset savannah (control, n=8), Arusha (n=6) and Uzima (n=5) savannahs (treatment) for collection periods. Collection periods were at 3-5 d intervals and treatment was with *Duddingtonia flagrans* chlamydospores of (30,000 per kg BW, * indicates beginning and end of treatment) mixed in supplement feed.

A significant difference was observed for treatment x time ($P<0.05$), time and treatment ($P<0.001$). For collection period 1, animals of Arusha and Sunset savannahs showed a significant difference ($P<0.05$), but there was no significant difference ($P>0.05$) observed between animals of Sunset and Uzima savannahs and both treatment savannahs (Arusha and Uzima). For collection periods 2 – 4, there was no significant difference ($P>0.05$) between treatments in this study. Subsequent to animals being supplemented with chlamydospores, for collection periods 5 – 26, there was a significant difference ($P<0.05$) observed between animals of treatment savannahs (Arusha and Uzima) as compared to animals of the control savannah (Sunset). For collection periods 27 – 29 the difference in percent larval survival varied between savannahs. When chlamydospores administration stopped for collection period 30, there was no

significant difference (P>0.05) observed for percent larval survival and development for samples for all savannahs.

Percent development and survival for both Arusha and Uzima savannahs were similar (P>0.05), while the mean was approximately 15% higher before treatment administration and close to zero percent for each collection period during treatment. Percent development and survival of L3 for animals of Sunset savannah were similar (P>0.05) before treatment was started and significantly (P<0.05) greater than animals of both Arusha and Uzima through period 26. For periods 27 – 29, differences were mixed and at period 30 there was no difference (P>0.05) between all animals of this study.

4.3 Recovery of L3 from Forage

The mean recovery of L3 from Sunset savannah forage ranged from 323 to 12,048 L3/kgDM (Figure 3). Arusha and Uzima savannah forages had a constantly smaller range of from 214 to 2,510 and 111 to 3,142 mean L3/kgDM, respectively.

A log transformation of L3/kgDM from forage was performed to obtain a more normal distribution to compare savannahs over time. A significant difference (P<0.0001) was observed for logL3/kgDM of forage for savannah and time, but there was no significant difference (P>0.05) for savannahs x time. Overall, there was a significant difference (P<0.0001) of logL3/kgDM of forage between Arusha and Sunset savannahs and between Uzima and Sunset savannahs, while Arusha and Uzima savannahs showed no overall significant difference (P>0.05). During the month of February, the peak month for larvae, there was a significantly (P>0.0001) higher number of logL3/kgDM on forage on Sunset savannah as compared to Arusha and Uzima savannahs.

At the start of the study (DEC), the logL3/kgDM recovery was similar (P>0.05) for all 3 savannahs. Subsequent to starting treatment, the logL3/kgDM recovery remained relatively

consistent and similar (P>0.05) on Arusha and Uzima savannahs, and increased on Sunset savannah in FEB. After treatment was stopped (MAR), logL3/kgDM recovery for all savannahs returned to pre-trial values. Treatment x time was not significant (P>0.05), but in FEB, Sunset savannah larval recovery was significantly (P<0.05) greater than both Arusha and Uzima savannahs.

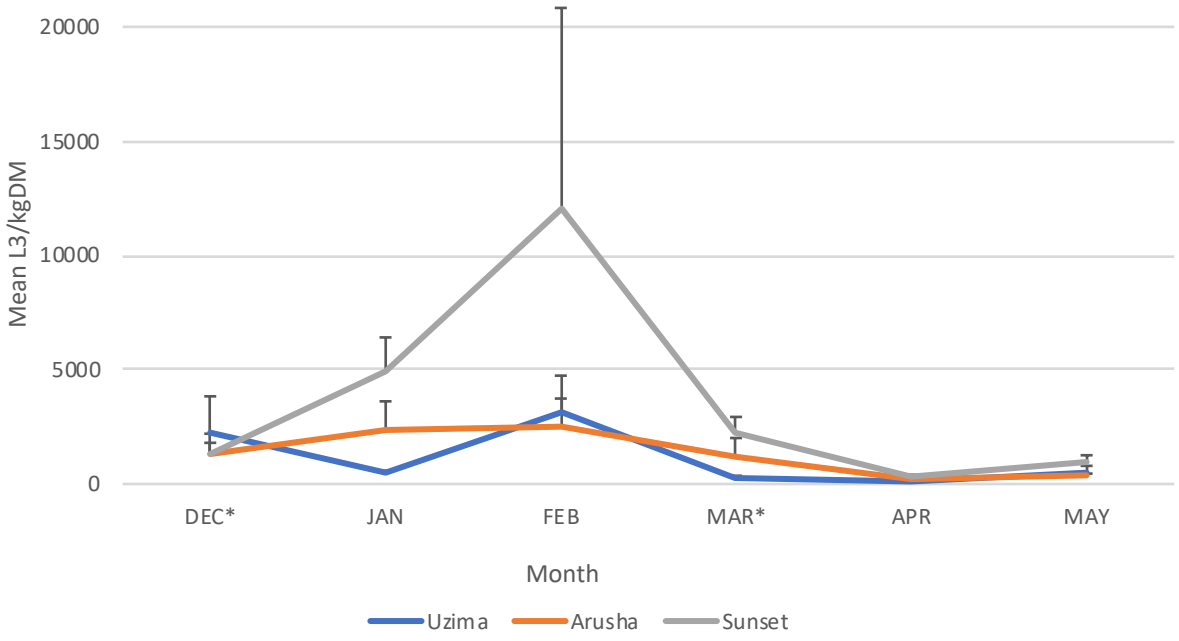

Figure 3. Mean (+SEM) infective larvae (L3) recovered from forage on a dry matter basis (kgDM) at Disney's® Animal Kingdom Lodge. Sunset savannah was the control (n=8, non-treated animals), and Arusha and Uzima were the treatment savannahs (n=6 and 5, respectively, animals treated with *Duddingtonia flagrans* chlamydospores (30,000 per kg BW) mixed in supplement feed). * indicates beginning and end of treatment.

4.4 Percent Larval Population Distribution from Forage Samples

The overall range for percent of *H. contortus* L3 recovery from forage for all months from Arusha, Uzima and Sunset savannahs was 36.3 – 93.9, 29.2 – 86.5 and 53.2 – 93.7,

respectively. Additional larval species were identified from the forage as *Trichostrongylus* spp., *Cooperia* spp., *Ostertagi* spp., and *Oesophagostomum* spp. (Figure 4).

The percent of *H. contortus* L3 for Uzima savannah started at a high 86.5% and dropped to 41.7% after a month of administration and remained less than 52% for the duration of chlamydospore administration, with an additional decrease to 29.2% the month following cease of chlamydospore administration. For the last month of forage collection, *H. contortus* percent L3 recovery when back up and was 84.2%.

Arusha savannah had an initial 93.9% *H. contortus* L3 in DEC. During the treatment period, percent *H. contortus* L3 remained relatively consistent around 90%. After treatment stopped, percent *H.* contortus L3 decreased to 39% and 36.3% for APR and MAR, respectively.

Sunset savannah had an initial 53.3% *H. contortus* L3 in DEC. Percent L3 then increased in JAN and remained relatively consistent around 80% for the duration of the study.

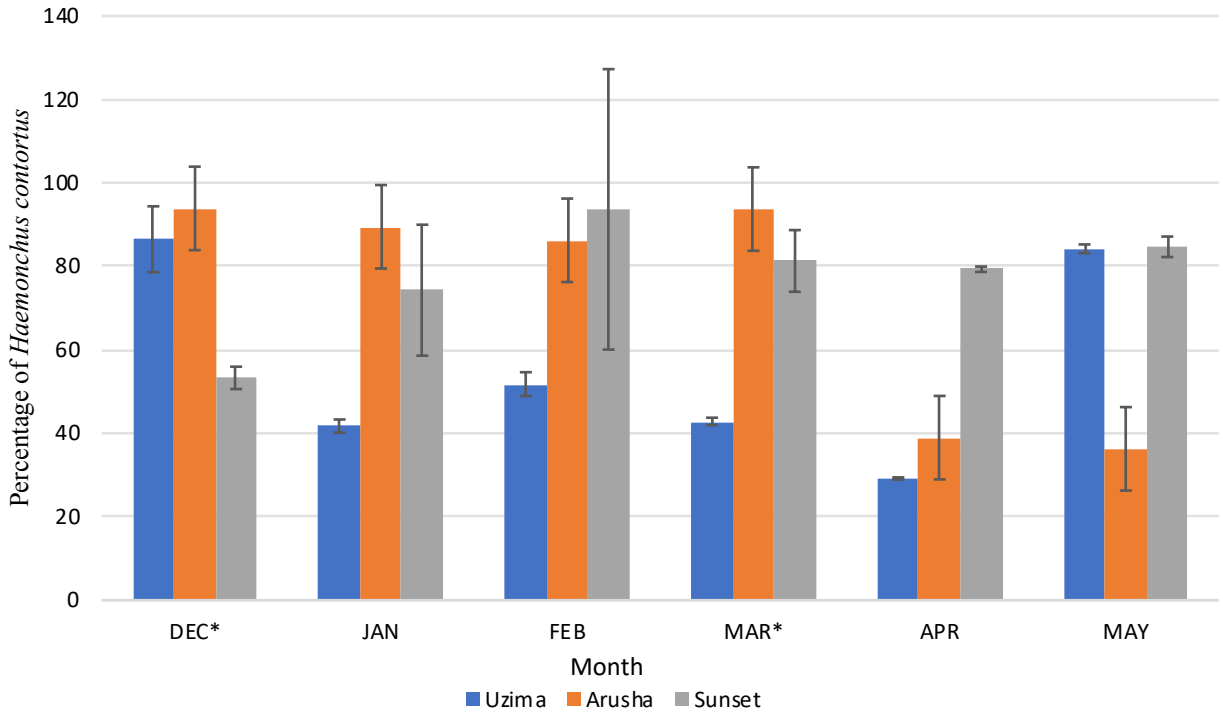

Figure 4. Mean (+/- SEM) percentage of *Haemonchus contortus* infective larvae (L3) recovered from forage samples at Disney's® Animal Kingdom Lodge. Sunset savannah was the control (n=8, non-treated animals), and Arusha and Uzima were the treatment savannahs (n=6 and 5, respectively, animals were treated with *Duddingtonia flagrans* chlamydospores (30,000 per kg BW) mixed in supplement feed). * indicates beginning and end of treatment.

The overall trend was for *H. contortus* L3 to decrease on Arusha and Uzima forage and increase on Sunset forage.

CHAPTER 5
DISCUSSIONS/CONCLUSIONS

As anthelmintic resistance awareness increases, more methods of control need to be investigated for controlling GIN in enclosures that lack refugia. According to , 7 – 10 years and up to $100 million is invested in the development of a new FDA-approved drug for an animal species. In addition to the rigorous process of drug development, when drugs are sold for human use, the costs are supplemented by sources, such as the government or insurances, but animal medication comes at the cost of the producer or animal owner. A number of studies investigated controlling GIN forage infectivity with *D. flagrans* in a livestock production type setting but few have investigated the use in zoological settings. Zoological parks are important for biodiversity, conservation and education for the public. Parasitic control is becoming of more importance as deaths are being identified as being caused by GIN (Wu et al., 2004). Previous studies at Disney's® Animal Kingdom Lodge have shown that feeding *D. flagrans* chlamydospores effectively reduced L3 in feces (Terry, 2013) but further investigations on this fungus' effects on forage infectivity in a zoological setting with captive exotic ruminants have not been performed.

Fecal egg counts were monitored during the course of the study and statistically evaluated on logFEC. *Duddingtonia flagrans* supplemented feed administered to animals on Arusha and Uzima savannahs showed a gradual decrease in FEC over the duration of the study. Animals on Sunset savannah did not receive *D. flagrans* supplemented feed and the FEC showed no significant change in FEC over time. These results were as expected as *D. flagrans* is effective against the free larval stages in the feces and does not affect the developing or adult worm population residing in the animal (Githigia et al., 1997; Dimander et al., 2003). With *D. flagrans* acting at the free larval stage, the decrease in FEC from feces of treated animals is likely due to a decrease in infective larvae available on the forage, lowering the number of larvae that

would develop to egg producing adults over time (Chandrawathani et al., 2004; Santurio et al., 2011). Although individual collection periods between treatments showed significant differences, further extrapolation on variations could not be explained as facilities nor animals were able to be observed during this study. In addition, an animal was pregnant during the duration of this study and the FEC did not seem to be affected by pregnancy.

Larval reduction in feces by nematode trapping fungi *D. flagrans* can range from 30% to more than 90% (Larsen et al., 1991b; Pena et al., 2002). During periods of treatment the percent development and survival of larvae from animals' feces was close to zero, while the animals not supplemented chlamydospores on the control savannah exhibited a higher larval development and survival. There was a corresponding reduction in number of L3 recovered from treatment savannahs' forages during a peak larval month (FEB), as compared to the high level of forage L3 from the control savannah forage. This suggested that fewer larvae developed and survived, which was most likely due to fungal predation; thus, fewer L3 were available on forage to infect grazing animals.

Results from this study are similar to another study done at Disney's® Animal Kingdom Lodge where a percent reduction was seen for L3 in feces of captive artiodactyls fed 30,000 and 500,000 chlamydospores per kg BW of *D. flagrans*. The study showed both doses were effective in reducing development and survival of larvae in feces over a short period of time in a small number of exotic hoofstock in a zoological setting (Terry, 2013). This current study used a dose of 30,000 chlamydospores per kg BW for a longer duration to evaluate the predacious nematode trapping fungi's effect on L3 available on forage for grazing. Results suggested that there may be a potential for *D. flagrans* to be used for controlling the free-living larval stages of GIN in a zoological setting when fed daily.

In addition, the trend of decreasing percent *H. contortus* L3 on treatment forage, and not on control forage, might suggest that the fungus may have a selective effect on this particular GIN. The target of *H. contortus* is important as this parasite is the main one that effects the health status and appearance of these exotic hoofstock.

Overall, this study shows the potential to reduce pasture infectivity of GIN with the long-term treatment of *D. flagrans*. There were limitations to this study such as the number of animals on each pasture and the variation in animal species that shared the environment. This however is a closer estimation to an actual zoological setting where animals need to endure the least amount of stress as possible, which could be easily influenced by changes in the environment and already established groups of animals (Wielebnowski et al., 2002). In addition, mixed species grazing can be helpful in reducing GIN populations as some animals will consume forage with L3 which then do not survive in that host (Barger and Southcott, 1978). Another factor accounting for the small sample size was the limited number of high risk animals that were available. The smaller sample size can lead to results that may be influenced by missing samples or individual animal variation. The variability could be influenced by some animals not receiving the complete dose of chlamydospores, as animals were portioned the correct dose but were allowed to eat ad libitum, possibly not consuming the full dose. A possible solution to this problem would be to find or develop a device that could be administered to the animal to provide a constant release of chlamydospores over a period of time (Waller and Faedo, 1996). Thus, daily administration would not be a limiting factor. Additional investigation should be conducted to evaluate other optional delivery methods. Further studies should also assess the effect of combining various anthelmintics with *D. flagrans* chlamydospores and/or other alternative control measures in an

integrated approach to controlling GIN in exotic zoo hoofstock and domestic ruminant production systems.

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VITA

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