TRACKING THE RECOVERY OF CYATHOSTOMIN SPECIES FOLLOWING ANTHELMINTIC TREATMENT

by

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ABSTRACT

Cyathostomin are ubiquitous gastrointestinal parasites of horses that can cause damage to the intestinal mucosa, colic, diarrhea, and host mortality. There are 50 species of nematodes in this group, and a single horse can host up to 20 different taxa. There are three classes of antiparasitic drugs (benzimidazoles, macrocyclic lactones and tetrahydropyrimidines) called anthelmintics used to treat cyathostomin infections. Cyathostomin are observed with resistance to all three classes, but levels of resistance vary. Macrocyclic lactones are more efficient in treatment than other classes of anthelmintics, but early indications suggest increased resistance is developing. The pattern of species recovery following deworming has not been fully explored and there is little understanding of how this relates to resistance. In this study, three drugs (moxidectin, ivermectin, and pyrantel) under the classes of macrocyclic lactones and tetrahydropyrimidines, were used to treat infected horses and observe their effects on the cyathostomin population. The goal of this study was to observe the patterns of recovery and presence the cyathostomin population exhibited during anthelmintic treatment. The second goal of this study was to determine which taxa are predicted to recover faster within and across treatments. The last goal of this study was to determine if there were any shifts in cyathostomin populations following anthelmintic treatment. Exploring the dynamics of cyathostomin populations in the presence of these treatments may help develop better approaches to fighting cyathostomin infections, reducing the amount of severe Cyathostominosis cases.

The primary methods of identifying cyathostomin populations were 5.8S rRNA gene sequencing of DNA extracted from equine fecal material while also monitoring the fecal egg counts of each individual. Several analyses were used to compare the cyathostomin populations following treatment including Multivariate Logistic Regression and Principle Coordinate Analysis. Multivariate Logistic Regression was used to predict the recovery rates and presence of specific taxa within and across treatments. Principle Coordinate Analysis was used to detect if there were any shifts in beta diversity following the course of anthelmintic treatment.

Logistic regression displayed significant (P-values < 0.05) differences within the cyathostomin population. *Coronocyclus coronatus* was used as the reference level in this study because it did not belong to the genus *Cylicocyclus*, which has been observed with less sensitivity to anthelmintic treatments. The interaction effect of species with treatment, showed that in the presence of moxidectin, *Cylicocyclus auriculatus* and *Cylicocylus elongatus* were less likely to be present than *C. coronatus*. In the presence of pyrantel, *C. elongatus, Cylicodontophorus bicornatus*, and *Cylicostephanus minutus* were less likely to be present than *C. coronatus*. The interaction effect of treatment with days showed that in the presence of moxidectin, with each unit increase in day, cyathostomin were less likely appear than ivermectin. Species also showed differential recovery patterns by looking at the interaction effect of treatment, species, and days. This interaction showed that in the, presence of moxidectin, *Cylicocyclus nassatus*, *Cylicocyclus radiatus*, and *Cylicostephanus longibursatus* were more likely to be present with each unit increase in day than *C. coronatus*. This interaction also showed that *C. nassatus*, in the

presence of ivermectin, was less likely to be present with each unit increase in day than *C. coronatus*.

Main effects of the regression were also significant such as species and shedding status. *C. nassatus* and *C. longibursatus* had very positive relationships indicating they were more likely to be present than *C. coronatus* regardless of treatment or time. Shedding status showed that low shedders (EPG 0-200) and moderate shedders (EPG 200-500) were less likely to have cyathostomin presence than high shedders (EPG 500 +).

Chapter 1

INTRODUCTION

1.1 Cyathostomin Lifecycle

Cyathostomin, also known as small strongyles, are a group of parasites that commonly found to infect equids. Although there have been over 50 species described within 14 genera, only 8 genera containing 40 species infect horses (Lichtenfels et al., 2008). Among these 40 species found infecting horses, 10 were found at a much higher prevalence than others (Reinemeyer et al., 1984). The number of cyathostomin species per horse was found to be up to 20 at the same time which was not dependent on host, parasitic load, or time of year (Mfitilodze et al., 1990). Cyathostomin have a direct life cycle as the parasite relies on the host without an intermediate host to be able to complete its life cycle. Cyathostomin infect the host through oral ingestion of infective third stage larvae (L_3) that typically reside in pasture. Once ingested, the L_3 pass along the host digestive tract and establish themselves within the caecum or colon which can differ depending upon species. These L_3 are also observed being able to survive chilling conditions as low as 4℃ and remain viable to infect hosts (Love et al., 1992). Once established, L_3 larvae either enter an encysted larval stage or proceed to L_4 in the mucosal wall (Proudman et al., 2000; Reviewed by Corning, 2009). Preferred site of encystment differed species to species (Collobert-Laugier et al., 2002). It is thought that larvae will encyst when temperatures cool, leading to a mass emergence in warmer conditions (Baudena et al., 2000; Reviewed by Corning 2009). After either mass emergence of encysted L_3/L_4 or

development of L_4 in the mucosal wall, they enter the lumen and proceed to their last larval stage (L_5). L_5 finally mature to adult worms in the cecum or colon where they will produce eggs that will be passed in their feces. These eggs will mature to their infective stage in 3 days if conditions are optimal (Love et al., 1992; Reviewed by Corning 2009). The rapid lifecycle of these parasites is rapid which led this study to investigate how cyathostomin species recover following control measures designed to eliminate cyathostomin infections.

1.2 Cyathostominosis

Cyathostomin are ubiquitous in equine populations worldwide and can cause a lot of problems for the host during penetration and emergence of L_3. Not only do these parasites sap nutrition from the host which can lead to weight loss, those infected may also display diarrhea, subcutaneous oedema, or pyrexia (Love et al., 1999). These clinical symptoms are combined into larval cyathostominosis syndrome. Larval cyathostominosis is mostly seen in young horses but all ages are susceptible to cyathostomes and cyathostomosis (Nielsen et al., 2017). Severe cases of larval cyathostominosis have been observed where chronic onset diarrhea occurs leading to fatality (Thamsborg et al., 1998; Reviewed by Love et al., 1999). The frequent occurrence of larval cyathostominosis has led to development of many treatments of cyathostomin infections. Cyathostominosis is still an issue in horses around the world, which led this study to investigate how cyathostomin species recover in order to combat resistant infections that can cause this syndrome.

1.3 Cyathostomin Control Methods

Pasture hygiene and chemotherapeutic strategies are some of the control methods being suggested to allow for effective control of cyathostomin populations (Proudman et al. 2000). Control of cyathostomin levels has become necessary with the emergence of larval cyathostominosis becoming a problem worldwide. Pasture hygiene relies on reducing pasture contamination by preventing individuals from coming into contact or reducing individuals contact with fecal material while grazing. Chemotherapeutic strategies rely on the effective use of anthelmintics to combat infections. Chemotherapeutic strategies are mostly used to combat cyathostomin populations within infected individuals. Before cyathostomin were more understood, treatment regimens were not very stringent, where they ignored that anthelmintic efficacy was lower in younger individuals (Herd et al., 1990), under-dosing can result in resistant populations (Mathee, 2003), and some question the importance of anthelmintic resistance (Peregrine et al., 2014). This study hopes to shed light on the recovery of cyathostomin populations following chemotherapeutic strategies to develop better methods of this strategy.

1.4 Anthelmintic Treatments

There are three main classes of anthelmintic available, benzimidazoles, tetrahydropyrimidines, and macrocyclic lactones. Anthelmintic treatments can be administered in different ways (oral paste, tablets, drench) and are sometimes given in combination in order to increases efficacy (Morris et al. 2012). Although anthelmintic drugs are all used to treat similar parasites, their mechanism of action differs by class (Gokbulut et al., 2018). Benzimidazoles affect the polymerization of microtubules of eukaryotic cells which leads to loss of cell structure and eventually death of the

organism. The next class, tetrahydropyrimidines, are some of the most widely used anthelmintics. Their efficacy varies among gastrointestinal parasites (Reinemeyer et al., 2010a; Reinemeyer et al., 2010b) and they act upon the acetylcholine receptors causing paralysis of those susceptible (Martin et al., 2007; Reviewed by Gokbulut et al., 2018). The last class, macrocyclic lactones, are highlighted by their efficacy on endoparasites and ectoparasites. Their modes of action are still under active research, but it is thought that they can act upon multiple mechanisms involving GABA and P-glycoprotein (Pouliot et al., 1997; Martin, 1997; Reviewed by Gokbulut et al., 2018).

These three drugs can be used as effective strategies to combat cyathostomin infections, but an emerging problem of anthelmintic resistance is becoming more pronounced around the world. The study observes the affects that anthelmintic treatments have on the cyathostomin population and species within them to determine if there are any differences in recovery or presence across treatments.

1.5 Anthelmintic Resistance

Without question, each class is still reliable to treat most cyathostomin infections, but studies are showing that this may not last forever as resistance is appearing around the world (Seyoum et al., 2017; Traversa et al., 2009). Resistance is usually characterized by shorter periods of cyathostomin egg reappearance following routine anthelmintic treatment (Garcia et al., 2013; Traversa et al., 2009). Cyathostomin abundance following routine anthelmintic treatments was described by Kooyman et al. (2006) and specific species are noted with altered abundance following different treatments. Kooyman et al. notes a longer reappearance period in moxidectin than ivermectin. In the presence of ivermectin, more *Cylicocyclus* species appeared earlier in the treatment than later. *Cylicostephanus longibursatus* is found to

be more abundant compared to *Cylicocyclus nassatus* following pyrantel treatment than moxidectin treatment. *C. nassatus* is noted as more abundant following moxidectin treatment than pyrantel treatment. Others used *in vitro* assays to determine resistance where they would look at the development of larvae while placed in anthelmintic treatments (Lind et al., 2005). This study observes the recovery and presence of cyathostomin following anthelmintic treatments to determine if species recovery patterns are similar to those noted in other studies to shed light of the scale of resistance amongst cyathostomin species.

1.6 Cyathostomin Identification

Cyathostomin can be identified using morphological or molecular strategies. Morphological strategies require looking at adult buccal capsules, adult cranial regions, or the intestinal cells of L_3 larvae (Lichtenfels et al., 2008; Santos et al., 2018). There are downsides to this method because most adults are only observed post-mortem. Identification of L_3 larvae only allows for the differentiation of 14 species, and morphological differentiation using eggs is impossible (Bredtmann et al., 2017). These methods are very time consuming and not very practical when trying to observe a large number of species. A more practical approach uses molecular strategies to identify species (Chilton, 2004; Hung et al., 1999). Molecular strategies allow studies to characterize the entire cyathostomin population in a much broader scope than morphological approaches. This strategy also allows fecal samples to be queried directly to give an idea of the cyathostomin population inside the horse. This study applied molecular strategies in order to observe species by using fecal samples.

1.7 Approaching Challenges of Cyathostomin Identification

Applying molecular strategies in this study was not met without problems. Amplification of low abundance samples was challenging and required optimization. Inferring abundance from the sequence data was not possible because cyathostomin are multicellular. Presence/absence was used to accommodate for these challenges. Presence/absence analysis was employed in order to eliminate the possible overestimation of abundance. Logistic regression can be used for binary outcomes in order to examine the relationship of presence/absence to the explanatory variables such as time and treatment used. Transforming the data into presence/absence data and using analyses designed for this format allowed for the study to investigate the desired questions with less bias than using absolute abundance data.

Chapter 2

METHODS

2.1 Collection of Fecal Samples

In this study, equine fecal samples were collected from the six horses of Webb Farm at the University of Delaware, six horses of Renzetti Farm, and three horses of Biddle Farm. The collections started during the first day of routine anthelmintic administration that was administered using the recommended doses for horses based on weight. The first collection course began in May, 2017 which involved the six horses at Webb Farm and the three horses at Biddle Farm treated with the macrocyclic lactone, moxidectin. The second collection course began in November, 2017 which involved the six horses at Renzetti farm treated with the macrocyclic lactone, ivermectin. The third collection course began in February, 2018 which involved the six horses at Webb Farm treated with another dose of moxidectin. The fourth collection began in June, 2018 which involved the six horses at Webb Farm and the three horses at Biddle Farm treated with the tetrahydropyrimidine, pyrantel. Fecal samples were then collected biweekly following treatment until 8 time points were obtained (~98 days). Fecal samples were collected immediately after manure deposition was observed. During collection for a given time point, fecal samples (~20g) were collected using nitrile gloves and stored at 2℃ in a polyethylene bag until all samples were collected. A portion of this sample was stored in a conical centrifuge tube (~5g) and immediately stored at -20℃ for DNA extraction.

Table 1: Source of samples

Source Farm	Horse Name	Shed Status	Drug used	Sampling Times
Webb	Darwin	Low	Moxidectin	May-Aug. 2017
Webb	Makin Magic	High	Moxidectin	May-Aug. 2017
Webb	Magic	High	Moxidectin	May-Aug. 2017
Webb	Slick	Low	Moxidectin	May-Aug. 2017
Webb	Talulah	High	Moxidectin	May-Aug. 2017
Webb	Vendetta	High	Moxidectin	May-Aug. 2017
Biddle	Caitlyn	High	Moxidectin	June-Sept. 2017
Biddle	Caleb	Low	Moxidectin	June-Sept. 2017
Biddle	Dutchman	Low	Moxidectin	June-Sept. 2017
Renzetti	Firefly	Moderate	Ivermectin	Nov. 2017-Feb. 2018
Renzetti	Gwen	High	Ivermectin	Nov. 2017-Feb. 2018
Renzetti	Harry	Moderate	Ivermectin	Nov. 2017-Feb. 2018
Renzetti	Joce	Moderate	Ivermectin	Nov. 2017-Feb. 2018
Renzetti	Luna	Low	Ivermectin	Nov. 2017-Feb. 2018
Renzetti	Molly	Moderate	Ivermectin	Nov. 2017-Feb. 2018
Webb	Darwin	Low	Moxidectin	Feb-June 2018
Webb	Makin Magic	High	Moxidectin	Feb-June 2018
Webb	Magic	High	Moxidectin	Feb-June 2018
Webb	Slick	Low	Moxidectin	Feb-June 2018
Webb	Talulah	High	Moxidectin	Feb-June 2018
Webb	Vendetta	High	Moxidectin	Feb-June 2018
Webb	Darwin	Low	Pyrantel	June-Sept. 2018
Webb	Makin Magic	High	Pyrantel	June-Sept. 2018
Webb	Magic	High	Pyrantel	June-Sept. 2018
Webb	Slick	Low	Pyrantel	June-Sept. 2018
Webb	Talulah	High	Pyrantel	June-Sept. 2018
Webb	Vendetta	High	Pyrantel	June-Sept. 2018
Biddle	Caitlyn	High	Pyrantel	June-Oct. 2018
Biddle	Caleb	Low	Pyrantel	June-Oct. 2018
Biddle	Dutchman	Low	Pyrantel	June-Oct. 2018

2.2 Fecal Egg Count (FEC)

Modified McMaster FEC Procedure (American Association of Equine Practitioners (AAEP) Infectious Disease Committee, 2019) was performed in order to examine the parasitic load of horses before and following anthelmintic treatment. In order to perform FEC, fecal material from each horse was collected. A measuring glass with a line indicating 26 ml and a line indicating displacement was then filled with Fecasol (sodium nitrate) flotation solution. Fecal material from the horse of interest is then added until the solution is displaced up to the displacement line. The solution is then mixed to homogenize the fecal material throughout, creating a slurry. The solution is then added to a McMaster slide that contains two chambers with a grid on top. Once both chambers are filled, the slide is set for ~5 minutes to allow for flotation of parasitic eggs. Once setting is done, the slide is examined under a microscope at 10x magnification, counting eggs within the grid of each chamber. After both grids are counted, the total value is multiplied by 25 in order to obtain the eggs per gram of the individual. This procedure was repeated for each individual at each time period of the study.

Individuals were then categorized by their shedding patterns which describe the typical parasitic load an individual will experience. The AAEP's Parasitic Control Guidelines categorize shedding patterns as contaminators where low contaminators carry 0-200 eggs per gram (EPG) following FEC, moderate carry 200-500 EPG, and high carry greater than 500 (AAEP Infectious Disease Committee, 2019).

2.3 DNA Extraction

After the fecal samples were collected, DNA was isolated using QIAGEN QIAmp PowerFecal DNA Kit. The isolation procedure required multiple steps in

order to isolate the DNA. These steps would allow for cell lysis, precipitation of non-DNA material, binding of DNA, washing of DNA, and elution of DNA. The first step required ~.25g of fecal material exposed to 60 µl of C1 solution and 750µl of Powerbead solution is then vortexed in order to enable cell lysis. The sample is then placed in a hot water bath of 65℃ for 10 minutes. The sample is then removed from the hot water bath and vortexed for 10 minutes increasing cell lysis within the sample. The sample is then centrifuged at 13,000 x g for 1 minute. ~500 µl of supernatant is removed from the sample and added to a clean tube which is then exposed to 250 µl of C2 solution which allowed for precipitation of non-DNA material. This sample is then vortexed and incubated at 2-8℃ for 5 minutes. After incubation, the sample is centrifuged for a second time at 13,000 x g for 1 minute. The previous step is repeated except ~600 µl of supernatant is removed and 200 µl of C3 solution is added. By this point, all non-DNA material should be isolated.

The next steps are performed in order to wash the DNA of any contaminants. No more than 750 µl is removed from the previous supernatant and 1200 µl of C4 solution is added. The solution is then vortexed for 5 seconds and 650 µl is loaded into a spin column. The spin column is centrifuged at 13,000 x g for 1 minute and any waste that passes through is disposed of. The spin column is then loaded three more times repeating the same steps. By this point, most of the contaminants should be removed but one last wash with 500 µl is performed in order to complete decontamination. Finally, the DNA sample should be relatively pure and free of all non-DNA material. 50 µl of C6 elution buffer is added to the spin column and centrifuged in order to remove the DNA from the spin column.

Each sample was extracted in duplicate and tested for quality. In order to test for quality, Qubit and Nanodrop were used in order to quantify concentration and absorbance of the DNA. When looking at the quality of a sample, the preferred ranges of concentration are between 10-100 µl and the preferred ranges of absorbance at 260/280 are between 1.8-1.85.

2.4 DNA Sequencing

In this study, the DNA samples were sent to RTL Genomics in order to be sequenced using Illumina MiSeq. Cyathostomin species were identified by 5.8S rRNA gene sequencing using custom primers designed in our lab that enabled the differentiation of 20 cyathostomin species. Custom primers targeting a 460 base pair region between the 5.8S rRNA gene and a portion of the ITS-2 region were used to produce paired amplicons.

2.5 OTU Table Generation

The amplicons from sequencing are compared to similar reads within the target area to generate Operational Taxonomic Units (OTU's) that refer to specific cyathostomin species. These OTU's are then compiled into an OTU table which is a matrix that gives the number of reads per sample per OTU. This table is needed in order to observe the number of reads for each species that is present in each sample. Before the OTU table is generated, the FASTQ file from sequencing requires manipulation by a couple of programs before the data is in interpretable form. Fast Length Adjustment of SHort reads (FLASh) is used in order to merge pair-end reads generated by sequencing whose lengths are shorter than twice the length of reads. The merged pairs result in unpaired longer reads which are more desired in genome

analysis. 150 bp was used as the maximum overlap expected in 90% of read pairs. The paired reads from FLASh were then filtered for length and quality using QIIME (Quantitative Insights Into Microbial Ecology). The resulting reads were compared to a custom database of 20 cyathostomin OTUs which contains sequences across the 5.8S rRNA gene and partial ITS-2 regions corresponding to an individual OTU. A table is then generated in the BIOM format which can then be used to generate tables that summarize taxa in either the form of relative or absolute abundance as well as attaching environmental characteristics that correspond to each sample provided by a mapping file. In this study, characteristics of interest attached were: trial name, treatment used, horse's name, the shedding patterns of the horse, farm the horse resided on, and the day the sample was collected.

2.6 Statistical Analysis

2.6.1 Presence/Absence Table Generation

The table containing species absolute abundance and environmental characteristics was converted into presence-absence data by converting any abundance less than three reads to absent and any abundance greater than three reads to present. This presence-absence table was then manipulated by R statistical language in order to perform statistical analyses. The table is in wide format but is converted into a long format for logistic regression in R using the stats package (R Core Team, 2019). Wide format was used to perform principle coordinate analysis in R using the vegan package (Oksanen et al., 2019).

2.6.2 Multivariate Logistic Regression

The first test allowed for the prediction of the odds of the presence of cyathostomin species. This was done by creating a logistic regression model that looked at the relationship between the dependent variable, presence/absence, and independent variables (predictors) that were categorical or continuous. The categorical independent variables were: treatment used, individual species, and shedding patterns. The continuous variable used in the model was the day the sample was collected on. This allowed us to look at the main effects of the model and the interactions order to determine if there was any significance. Two-way and three-way interactions were included in the model to see the interaction effects. *C. coronatus* was used as the reference category for species in the regression in which allowed us to see the odds of presence comparative to the selected reference. This reference was used because it was not within the *Cylicocyclus* genus, which was noted with altered abundance (Chapter 1.5). Comparing species to this reference allows for interpretation of altered responses following treatments. Ivermectin was used as the reference category for explanatory variable treatment and high shedding status was used as the reference for the explanatory variable of shedding status. Ivermectin was used as the reference category to compare response of species within the same class of drug (moxidectin) and outside the same class of drug (pyrantel). High shedding status was used as the reference category to compare individuals that were high contaminants (EPG > 500) to those that were low and moderate contaminants (EPG < 500). Significance was found if the P-value for the predictor was less than 0.05 and the confidence intervals did not contain 1 as this shows that changes in the predictor would influence the chances of presence in some way. A ROC curve of the logistic regression model was created in order to observe the accuracy of the model. If the

curve is close to the upper left corner (approaching 1), the model is able to distinguish between the presence/absence of cyathostomin species. If the curve is closer to the straight line (approaching 0.5), the model is not able to distinguish between the presence/absence of cyathostomin species.

2.6.3 Principle Coordinate Analysis (PCoA)

Principle Coordinate Analysis was used to compare the similarity of cyathostomin communities in various environmental conditions. To do this we used Jaccard index which compares the presence/absence species within a cyathostomin population to one another. We looked for clustering in the PCoA plot to see if samples resembled one another following anthelmintic treatment. The clustering of samples indicate less distance, which described whether or not the populations within the samples were similar or not. First, we compared the distance of samples at the beginning and end of a treatment to see if populations resembled one another. Then we looked at the overall effect of treatment. Last, we looked at spatial relations comparing treatment and farm.

Chapter 3

RESULTS

3.1 Sample Summary

In this study we had issues with low amplification samples. When this occurred samples were extracted again in order to obtain data for as many time points as possible. The tables below describe the data that was available for interpretation. Samples marked not available were not able to be amplified and samples marked as ship were out for sequencing at the time.

Table 2: Sample summary of first moxidectin trial.

Horse	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
Darwin	Data back	Data back	Data back	Data back	Ship	Ship	Ship	Ship
Makin Magic	Data back	Data back	Data back	Data back	Ship	Data back	Data back	Ship
Magic	Data back	Data back	Data back	Data back	N/A	Ship	Data back	Ship
Slick	Data back	Data back	Data back	Data back	Ship	Ship	Data back	Ship
Talulah	Data back	Data back	Data back	Data back	Data back	Ship	Data back	Ship
Vendetta	Data back	Data back	Data back	Data back	Data back	Data back	Data back	Ship
Caitlyn	Data back	Data back	Data back	Data back	Data back	Data back	N/A	N/A
Caleb	Data back	Data back	Data back	Data back	Ship	Ship	N/A	N/A
Dutchman	Data back	Data back	Data back	Data back	Data back	Ship	N/A	N/A

Table 3: Sample summary of ivermectin trial.

Horse	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
Gwen	Data back	Ship	Data back	Data back	Data back	Data back	Data back	Data back
Harry	Data back	Ship	Data back	Data back	Data back	Data back	Data back	Data back
Molly	Data back	Ship	Ship	Data back	Data back	Data back	Data back	Data back
Luna	Data back	Ship	Data back	Data back	Data back	Data back	Data back	Data back
Firefly	Data back	Data back	Ship	Data back	Data back	Data back	Data back	Data back
Joce	Data back	Ship	Data back	Data back	Data back	Data back	Data back	Data back

Table 4: Sample summary of second moxidectin trial.

Horse	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
Darwin	Data back	Ship	Ship	Data back	Data back	Ship	Ship	Data back
Makin Magic	Data back	Ship	Ship	Ship	Ship	Data back	Data back	Data back
Magic	Data back	Ship	Ship	Ship	Ship	Data back	Data back	Data back
Slick	Data back	Ship	Ship	Data back	Data back	Ship	Data back	Data back
Talulah	Data back	Ship	Ship	Ship	Ship	Data back	Data back	Data back
Vendetta	Data back	Ship	Ship	Ship	Data back	Data back	Ship	Data back

Table 5: Sample summary of pyrantel trial.

Horse	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98
Darwin	Ship	Ship	Ship	Ship	Ship	Ship	Ship	Ship
Makin Magic	Data back	Data back	Data back	Data back	Data back	Data back	Data back	Data back
Magic	Data back	Data back	Data back	Data back	Data back	Data back	Data back	Data back
Slick	Data back	Data back	Data back	Data back	Data back	Data back	Data back	Data back
Talulah	Data back	Data back	Data back	Data back	Data back	Data back	Data back	Data back
Vendetta	Data back	Data back	Data back	Data back	Data back	Data back	Data back	Data back
Caitlyn	Data back	Data back	Ship	Data back	Data back	Data back	Data back	Data back
Caleb	Ship	N/A	Ship	Ship	Data back	Ship	Ship	Ship
Dutchman	Ship	Ship	Ship	Ship	Ship	N/A	Ship	Data back

3.2 Fecal Egg Count (FEC)

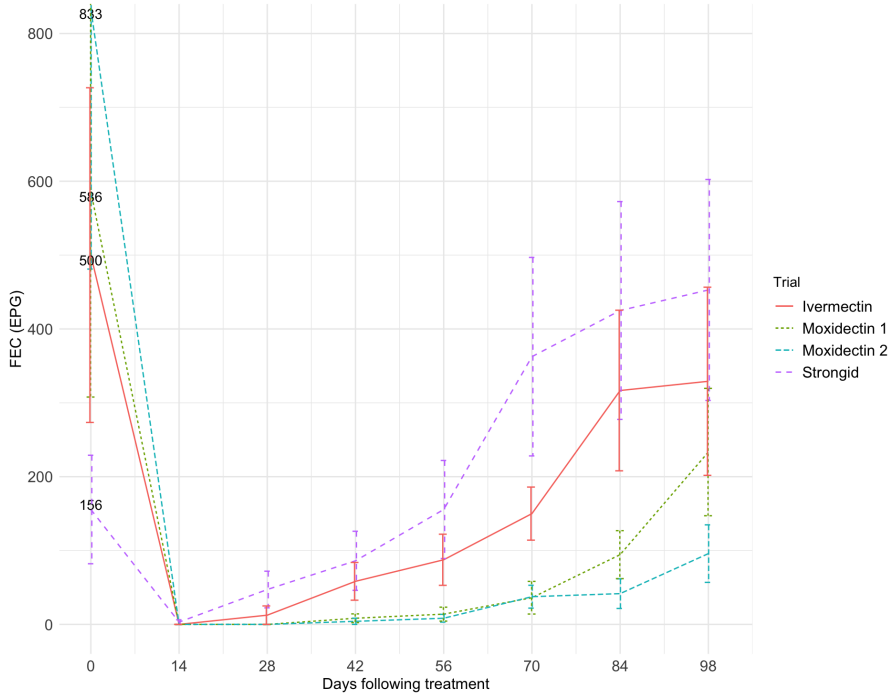

Figure 1: Average fecal egg count (FEC) in eggs per gram (EPG) over time for all the trials of anthelmintic treatment. The trials were Ivermectin (n=6), Moxidectin 1 (n=9), Moxidectin 2 (n=6), and Strongid (n=9).

The figure above displays an overview of FEC monitored over each trial allowing for general recovery rates to be displayed. Before anthelmintic treatment, FEC were all above 100 which then drop to zero two weeks after administration. Looking at general trends, Strongid (pyrantel) was shown with the highest rate of recovery. Ivermectin displayed the second highest rate, and moxidectin was the lowest for both trials.

3.3 Multivariate Logistic Regression

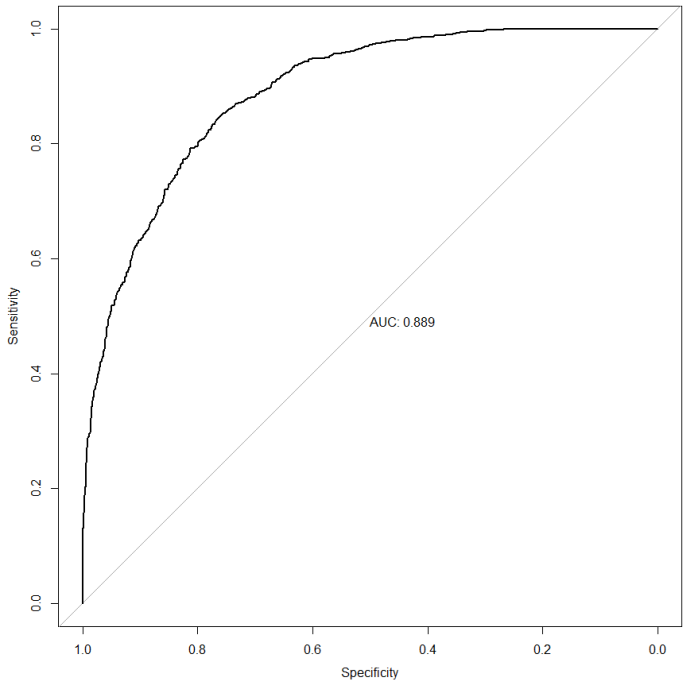

Figure 2: Receiver operating characteristic (ROC) curve showing the discrimination power of the logistic model.

The ROC curve above illustrates the diagnostic ability of the logistic model. The best prediction method would yield a curve in the upper left corner (0,1). The model displayed an AUC of 0.889 which is the probability that a classifier will rank a randomly chosen positive instance higher than a randomly chosen negative one. In other words, the AUC of 0.889 displays that there is an 88.9% chance that the model will be able to distinguish between a positive and negative class.

Table 6: Significant main effects of logistic regression predicting cyathostomin presence.

Variable	Pr (>|z|)	Odds Ratio	95% Confidence Interval	
Cylicocyclus nassatus	0.015	11.5	1.75	94.7
Cylicostephanus longibursatus	0.024	35.5	2.71	2710
Shedding Low	4.32E-11	0.218	0.138	0.341
Shedding Moderate	0.011	0.360	0.162	0.788

The table above displays significant main effects that were output by the logistic regression model. Species as main effects displayed positive relationships with extremely high odds ratios, meaning that *C. nassatus* and *C. longibursatus* were 11.5 and 35.5 times more likely to be present across all trials than *C. coronatus*. Shedding status as a main effect displayed negative relationships with odds ratios lower than 1, meaning that low shedders and moderate shedders are 0.218 and 0.360 times less likely to have cyathostomin presence across all trials than high shedders.

Table 7: Significant two-way interaction effects of logistic regression predicting cyathostomin presence.

Variable	Pr (>|z|)	Odds Ratio	95% Confidence Interval	
Moxidectin:*Cylicocyclus auriculatus*	0.036	0.017	7.89E-05	0.432
Moxidectin:*Cylicocylus elongatus*	0.037	0.07	0.005	0.797
Strongid:*Cylicocylus elongatus*	0.028	0.041	0.002	0.63
Strongid:*Cylicodontophorus bicoronatus*	0.039	0.059	0.004	0.812
Strongid:*Cylicostephanus minutus*	0.023	0.047	0.003	0.618
Moxidectin:Days	0.001	0.939	0.903	0.972

The table above displays significant two-way interaction effects that were output by the logistic regression model. Treatment:Species interactions display low odds ratios corresponding to very negative relationships. In the presence of moxidectin treatment, *C. auriculatus* and *C. elongatus* are 0.017 and 0.07 times less likely to be present than *C. coronatus*. During Strongid (pyrantel) treatment, *C. elongatus*, *C. bicornatus*, and *C. minutus* are around 0.05 times less likely to be present than *C. coronatus*. Treatment:Days interactions display how a unit increase in day will affect the presence of cyathostomin populations. With each unit increase in day, moxidectin treatment is 0.93 times less likely for cyathostomin presence.

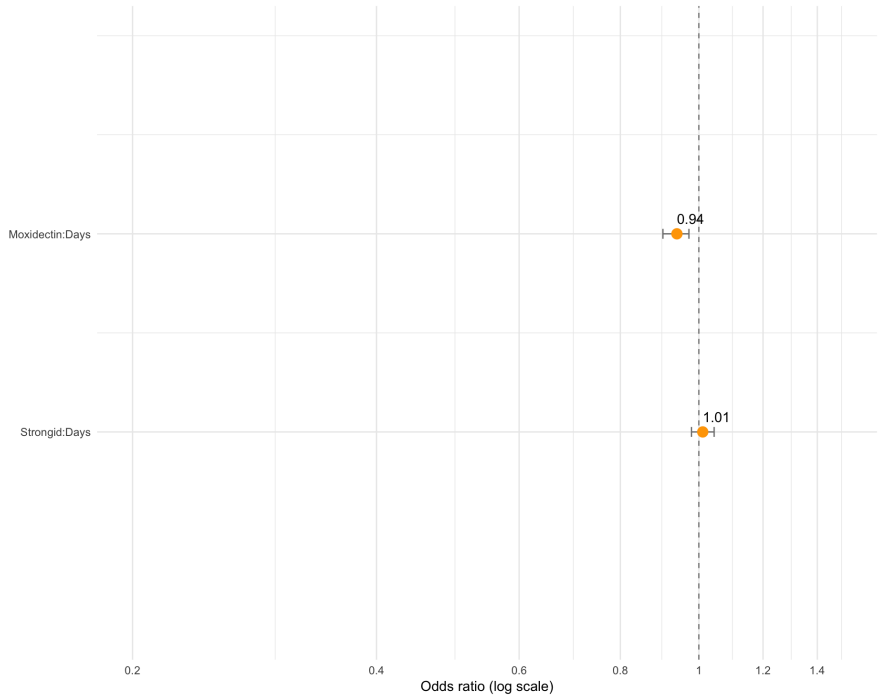

Figure 3: Odds ratios of two-way interaction effect Treatment:Days compared to ivermectin.

The figure above displays the odds ratios of the two-way interaction Treatment:Days in comparison to ivermectin. This figure displays the significant interaction Moxidectin:Days, showing that it has lower odds of cyathostomin presence with each unit increase in days than ivermectin. Strongid:Days was not significant but displayed a slightly positive relationship, meaning that it has slightly higher odds of cyathostomin presence than ivermectin with each unit increase in day.

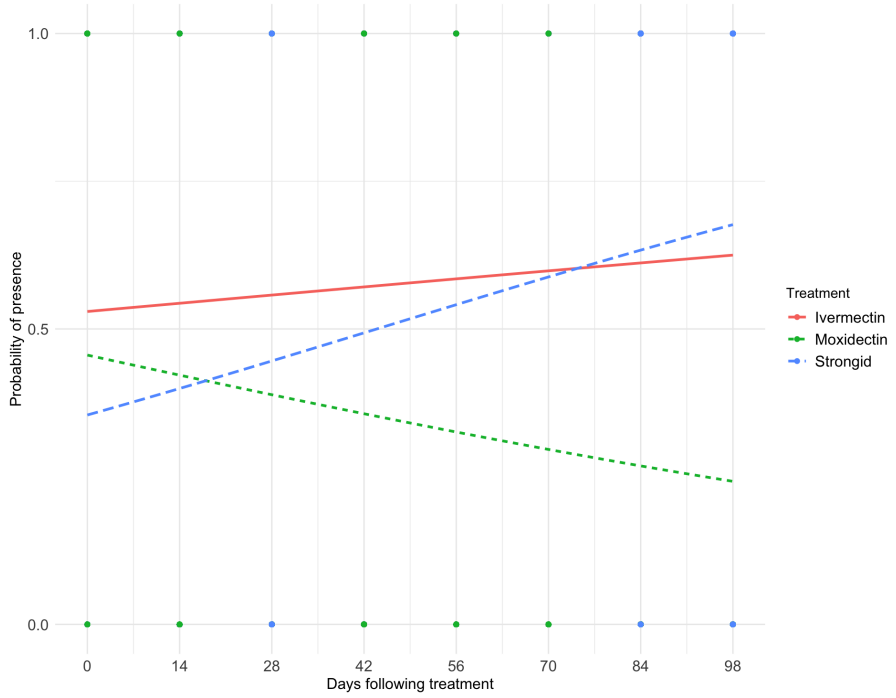

Figure 4: Logistic regression plot of two-way interaction effect Treatment:Days.

This figure is a reiteration of everything mentioned before but it allows the strength of the relationships to be better understood. For every unit increase in day, moxidectin is seen with rapid decreasing probability, strongid (pyrantel) is seen with rapid increasing probability, and ivermectin is seen with slightly increased probability. This observation resembles what was found when looking at the FEC of each trial over time. Strongid had the earliest egg reappearance period, ivermectin came next, and moxidectin had the latest.

Table 8: Significant three-way interaction effects of logistic regression predicting cyathostomin presence.

Variable	Pr (>|z|)	Odds Ratio	95% Confidence Interval	
Ivermectin:Days:*Cylicocyclus nassatus*	0.037	0.968	0.937	0.997
Moxidectin:Days:*Cylicocyclus nassatus*	0.021	1.039	1.008	1.077
Moxidectin:Days:*Cylicocyclus radiatus*	0.048	1.033	1.003	1.071
Moxidectin:Days:*Cylicostephanus longibursatus*	0.018	1.044	1.01	1.086

The table above shows the significant three-way interaction effects describing how presence relates to specific species in different treatments with each unit increase in day. For each unit change in day, *C. nassatus*, *C. radiatus*, and *C. longibursatus* are more likely to be present compared to *C. coronatus* during moxidectin treatment. During ivermectin treatment, *C. nassatus* is less likely to be present than *C. coronatus* with each unit increase in day.

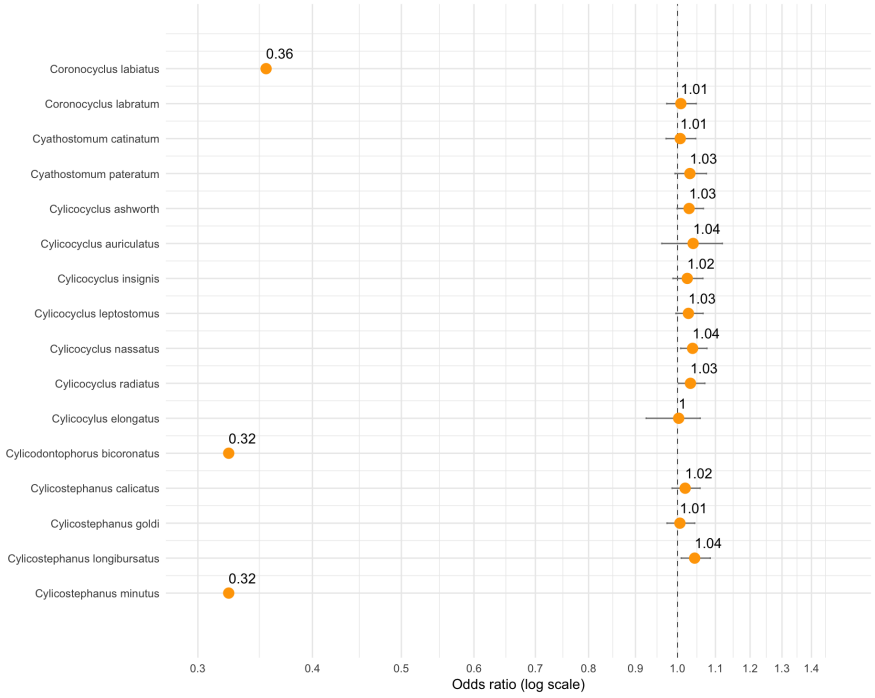

Figure 5: Odds ratios of three-way interaction effect Moxidectin:Species:Days compared to *Coronocyclus coronatus*.

The figure above displays all the odds ratios of species during moxidectin treatment for each unit increase in day. Most of the species are more likely to be present with each unit increase in day than *C. coronatus*. *C. labiatus*, *C. bicoronatus*, and *C. minutus* are less likely to be present than *C. coronatus*, but their interactions are not significant.

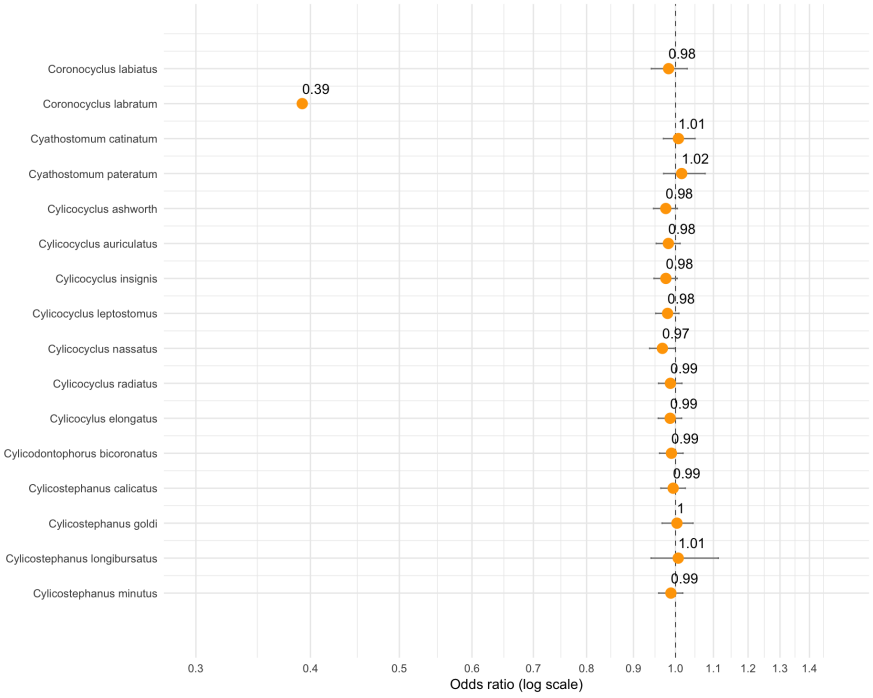

Figure 6: Odds ratios of three-way interaction effect Ivermectin:Species:Days compared to *Coronocyclus coronatus*.

The figure above displays all the odds ratios of species during ivermectin treatment for each unit increase in day. Most species are close in their odds ratios showing very little difference in recovery. *C. labratum* is the only outlier in this treatment with a strong negative relationship, but its interaction is not significant.

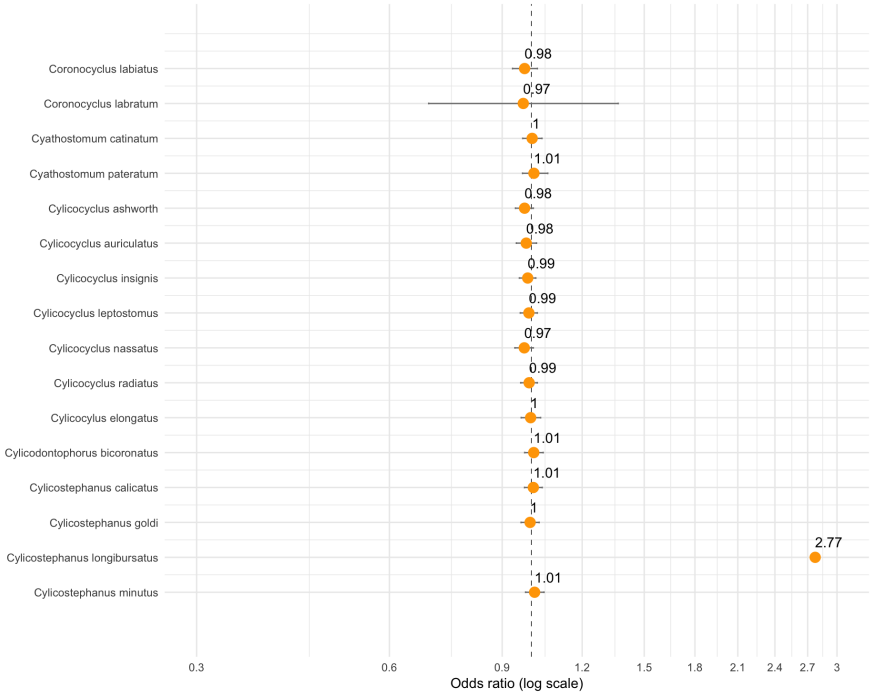

Figure 7: Odds ratios of three-way interaction effect Strongid:Species:Days compared to *Coronocyclus coronatus*.

The figure above displays all the odds ratios of species during pyrantel treatment for each unit increase in day. No interactions within this treatment are significant and most species are close in their odds ratios. *C. longibursatus* displays a strong positive relationship that isn't significant.

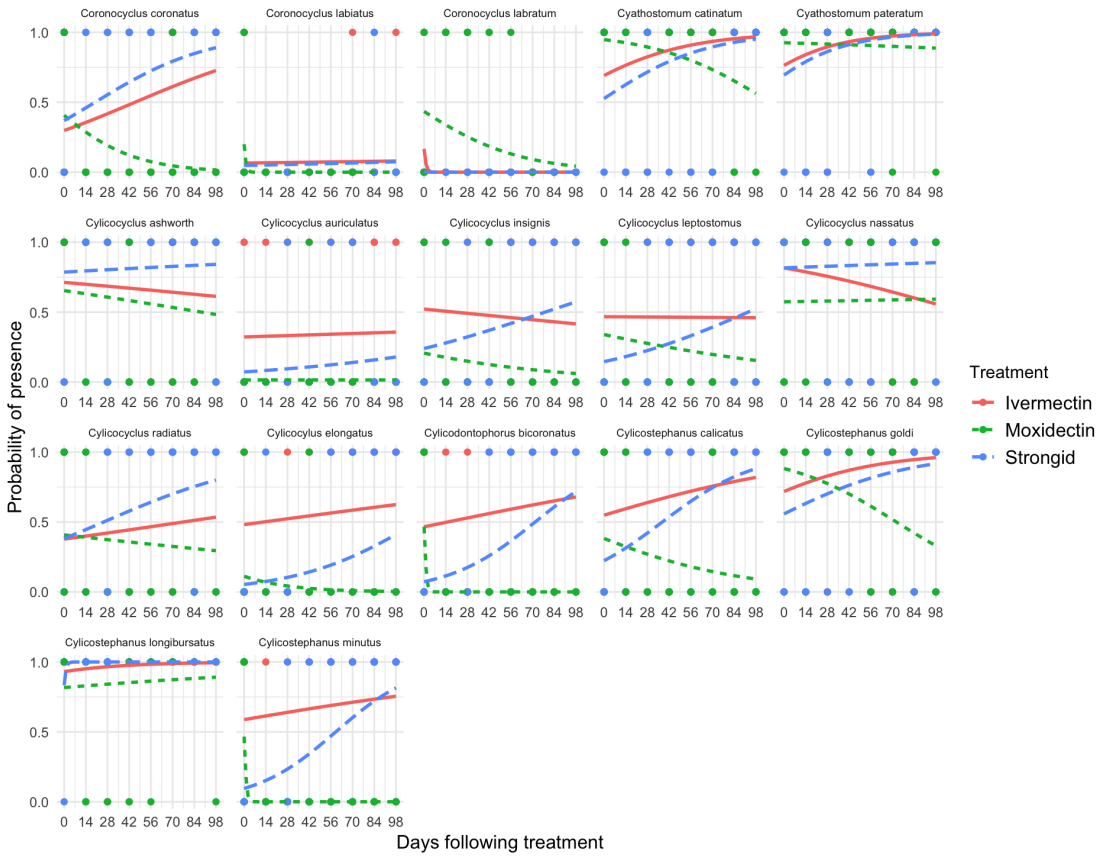

Figure 8 Logistic regression plot of three-way interaction effect Treatment:Species:Days.

The figure above describes the probability of presence for all cyathostomin species following each treatment. Significant species will be described in the next three figures below. Only three species showed significant rates of recovery for certain treatments.

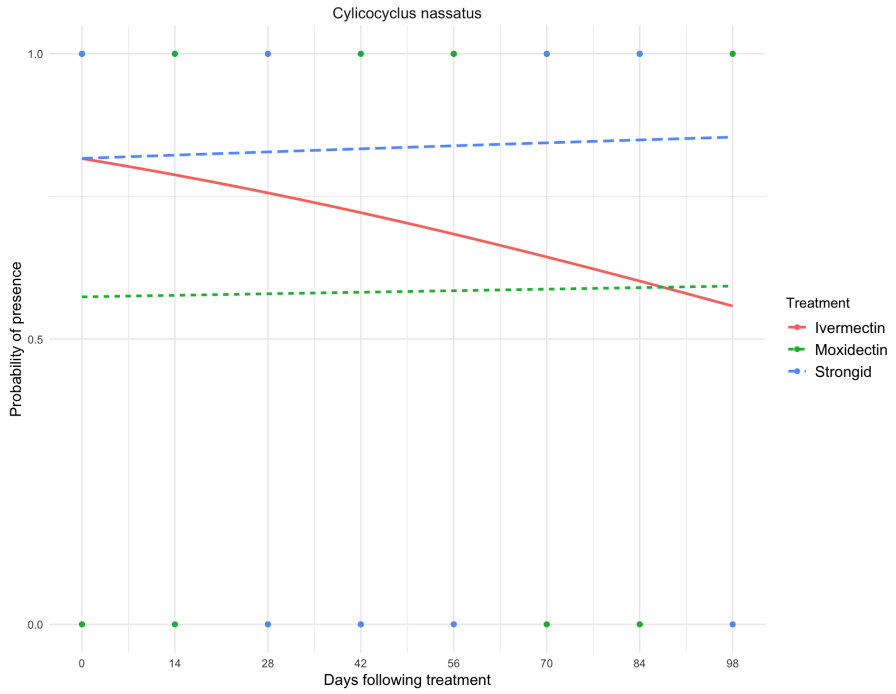

Figure 9: Logistic regression plot of three-way interaction effect Treatment:*Cylicocyclus nassatus*:Days.

This figure describes the response of *C. nassatus* in different treatments over time. *C. nassatus* has significant recovery during ivermectin and moxidectin treatments where it has a positive relationship in moxidectin and a negative relationship in ivermectin.

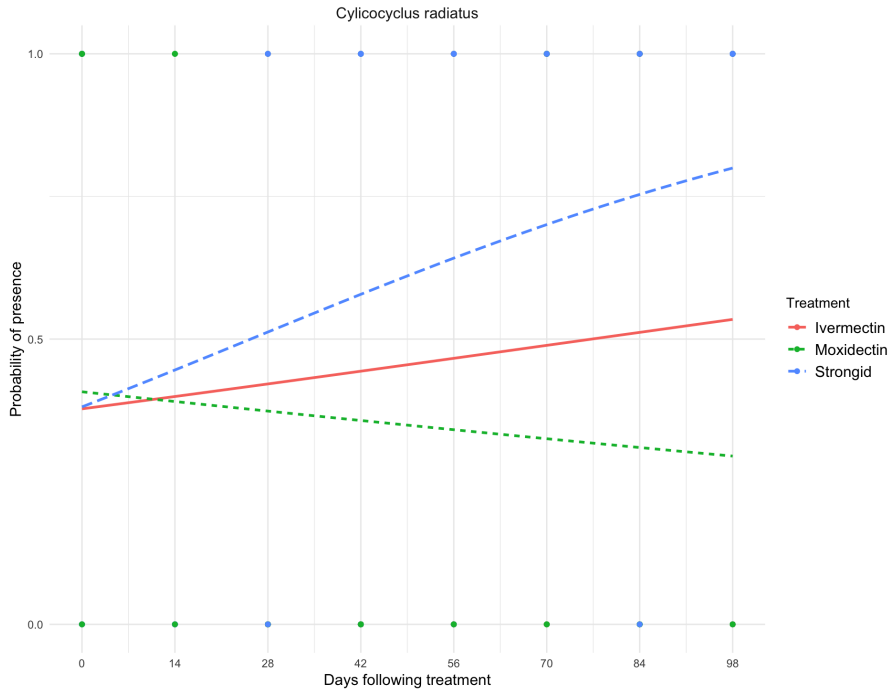

Figure 10: Logistic regression plot of three-way interaction effect Treatment:*Cylicocyclus radiatus*:Days.

The figure above describes the response of *C. radiatus* in different treatments over time. *C. radiatus* has a significant recovery during moxidectin treatment where it has a positive relationship.

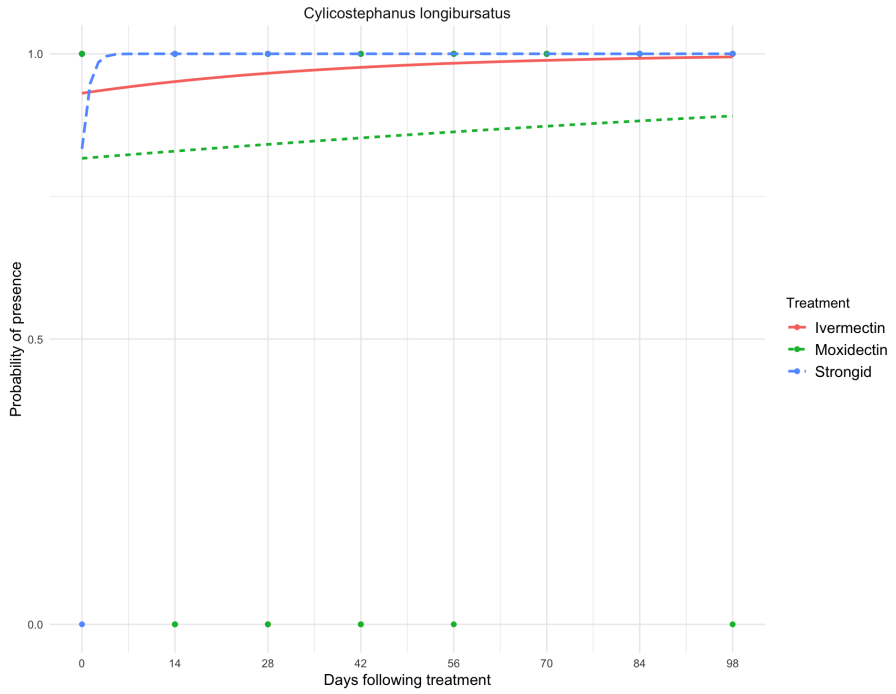

Figure 11: Logistic regression plot of three-way interaction effect Treatment:*Cylicostephanus longibursatus*:Days.

The figure above describes the response of *C. longibursatus* in different treatments over time. *C. longibursatus* has a significant recovery during moxidectin treatment where it has a positive relationship.

3.4 Principle Coordinate Analysis (PCoA)

Beta diversity was compared using Jaccard's index to observe any difference in diversity following treatment. Clustering is used to display samples resembling one another and spreading is used to display samples being different from one another based on distance.

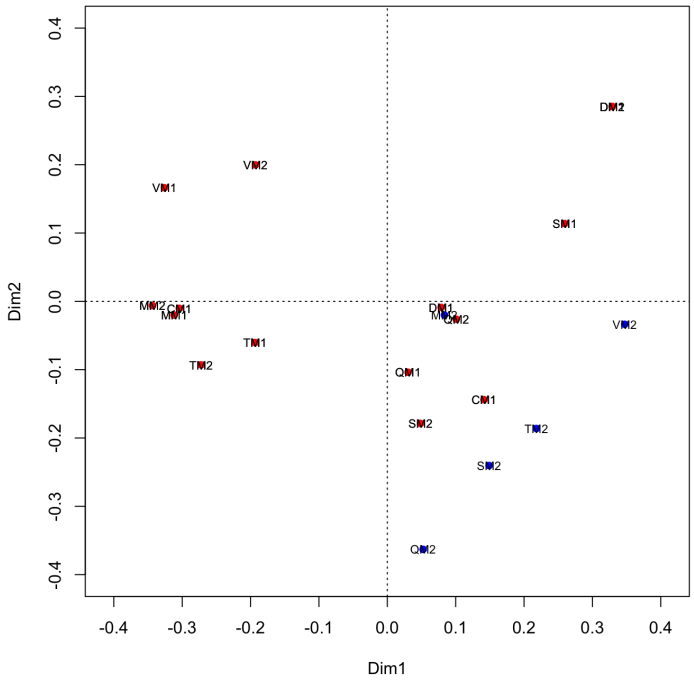

Figure 12: PCoA comparing the beta diversity of pre and post treatment. Red dots represent moxidectin pre-treatment (Day 0) and blue dots represent moxidectin post-treatment (Day 98).

The first PCoA plot compares the diversity of moxidectin pre and post treatment samples. This figure does not display clustering but there is a clear segregation in samples following treatment. This segregation describes that there is a difference in beta diversity of samples before and after moxidectin treatment.

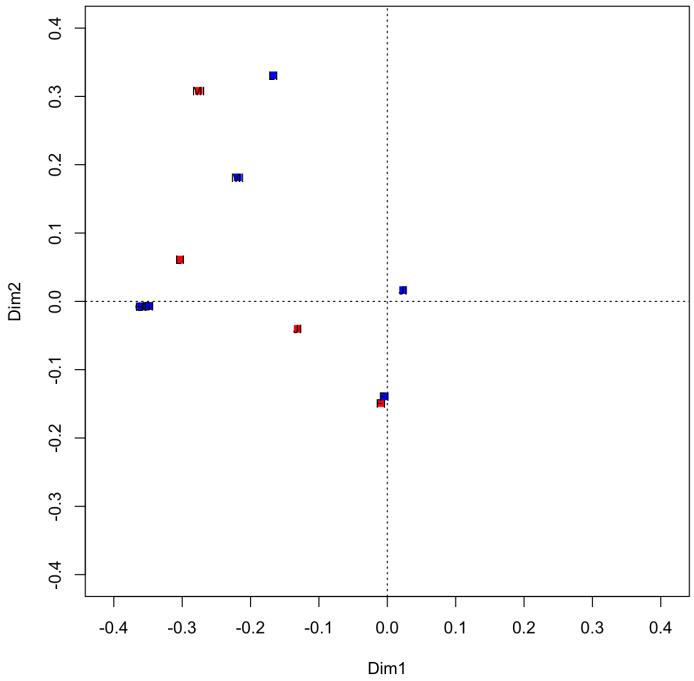

Figure 13: PCoA comparing the beta diversity of pre and post treatment. Red squares represent ivermectin pre-treatment (Day 0) and blue squares represent ivermectin post-treatment (Day 98).

The second PCoA compares the diversity of ivermectin pre and post treatment samples. This figure does not display clustering, nor is there a clear segregation of the samples. The lack of clustering and segregation describes that there is little to no difference in the beta diversity of samples before and after ivermectin treatment.

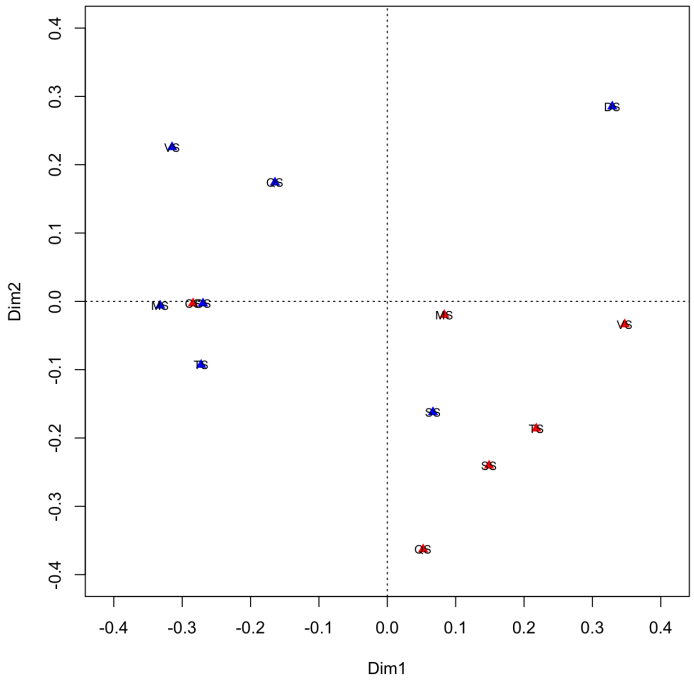

Figure 14: PCoA comparing the beta diversity of pre and post treatment. Red dots represent pyrantel pre-treatment (Day 0) and blue dots represent pyrantel post-treatment (Day 98).

The third PCoA compares the diversity of pyrantel pre and post treatment samples. This figure does not display clustering, but there is some segregation amongst the samples. The segregation describes that there is some difference in the beta diversity of samples before and after pyrantel treatment.

In the past three figures (Figures 11-13), samples were also labeled by individual to see the differences treatment has on the individual's cyathostomin population. Changes in distance are shown in some individuals but there are also individuals whose distance barely changed.

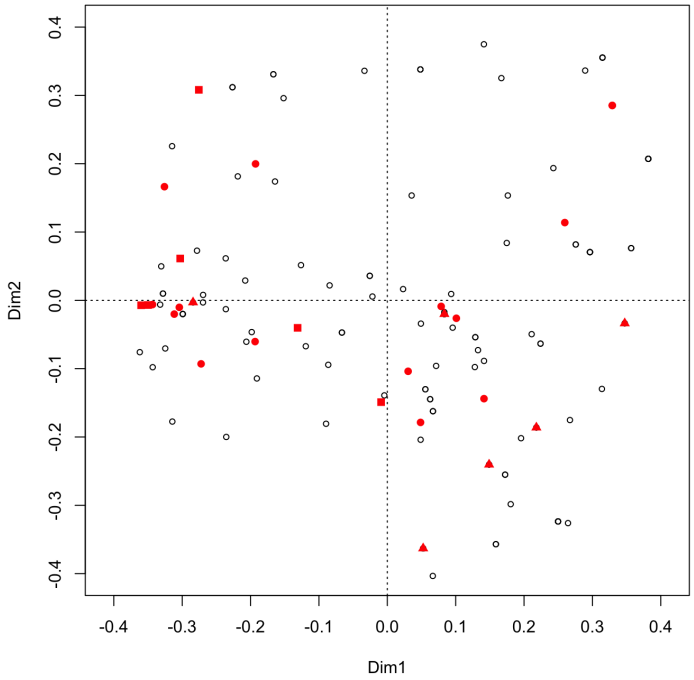

Figure 15: PCoA comparing the beta diversity of Day 0 across treatments. Circles represent moxidectin, squares represent ivermectin, and triangles represent pyrantel.

The fourth PCoA describes the beta diversities of samples pre-treatment. There was small cluster showing that the cyathostomin populations were similar to each other before going through routine anthelmintic treatment.

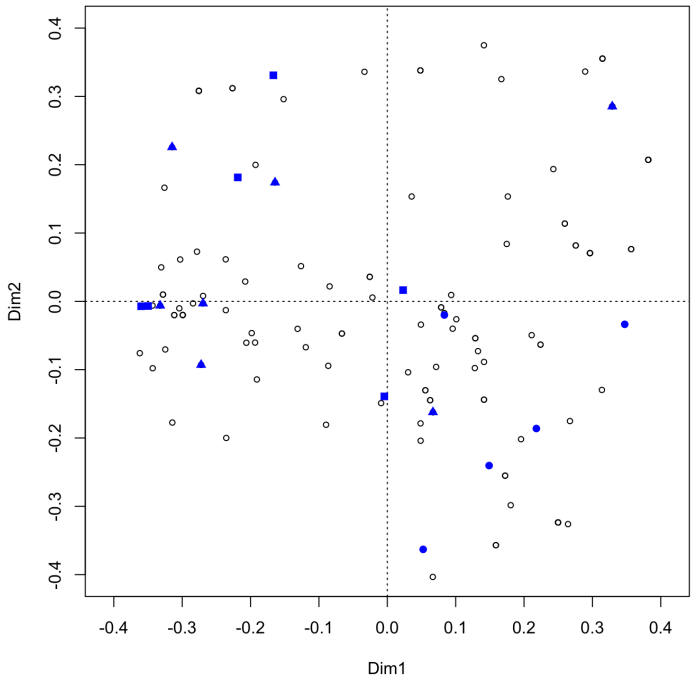

Figure 16: PCoA comparing the beta diversity of Day 98 across treatments. Circles represent moxidectin, squares represent ivermectin, and triangles represent pyrantel.

The fifth PCoA describes the aftermath of routine anthelmintic treatment. Samples had noticeable changes in distance after anthelmintic treatment, but they did not cluster based on treatment used. This relation was already described (Figures 11-13), but the goal here was to display whether or not cyathostomin populations will resemble each other based on the treatment they were exposed to.

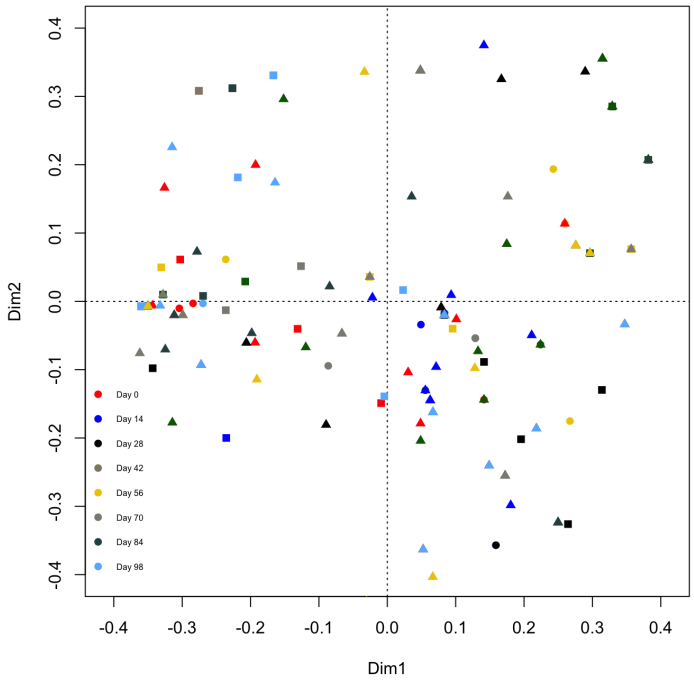

Figure 17: PCoA comparing the beta diversity across farms at all time points. Circles represent Biddle farm, squares represent Renzetti farm, and triangles represent Webb farm.

The sixth PCoA compares the beta diversity across all farm's samples. This figure does not display distinct clustering or segregation of the samples. The lack of clustering and segregation shows that cyathostomin populations located within the same space do not have similar species compositions.

Chapter 4

CONCLUSION

4.1 Interpretation of Results

Significant main effects displayed that certain species were more present than others across all treatments. *C. nassatus* and *C. longibursatus* had very positive relationships indicating that they had higher odds of occurring than other species. This observation agrees with Reinmeiyer et al. (1984) in Chapter 1.1 that characterize *C. nassatus* and *C. longibursatus* as species that make up the bulk prevalence of cyathostomin populations. Low and moderate shedders displaying strong negative relationships agrees logically that individuals with higher egg burdens contain higher levels of cyathostomin presence.

Significant two-way interactions displayed that certain species were more present in some treatments than others. *C. elongatus* and *C. auriculatus* were significantly less likely to be present in moxidectin than *C. coronatus*. This could be due to moxidectin creating an environment where they have lower fitness than others. *C. elongatus*, *C. bicornatus*, and *C. minutus* were significantly less likely to be present than *C. coronatus* during pyrantel treatment. This also does not necessarily indicate sensitivity, but it does show that they have altered presence in pyrantel treatment. During moxidectin treatment regardless of time, cyathostomin presence was significantly less likely to be present than ivermectin and pyrantel. This relationship could be due to moxidectin being more lipophilic than ivermectin, allowing for higher concentrations over time within the host leading to slower cyathostomin recovery.

Significant three-way interactions displayed that specific species have different rates of recovery. *C. nassatus*, *C. radiatus*, and *C. longibursatus* are more likely to be present than other species, with *C. coronatus* being the reference, as days go by during moxidectin treatment. These findings agree with Kooyman et al. (2006) that describes *Cylicocyclus spp*. as more present following moxidectin treatment. Moxidectin could be providing an environment where *Cylicocyclus spp*. can thrive allowing for this difference in recovery rate. During ivermectin treatment, *C. nassatus* displayed a lower recovery rate than *C. coronatus*. This finding also agrees with Kooyman et al. that describes *Cylicocyclus spp*. as less likely to be present later than earlier during ivermectin treatment. This finding shows that *C. nassatus* may be more likely to be present earlier but as days increase this is less likely.

PCoA was used to explain how the beta diversity may change following different anthelmintic treatments. Ivermectin, moxidectin, and pyrantel did not display much difference following treatment leading to the conclusion that the effect of these treatments did not influence the overall diversity. However, there were changes in distance when looking at the individual following anthelmintic treatment. This suggests that the cyathostomin populations within these individuals were different following anthelmintic treatment. There were no noticeable patterns when looking at treatment or farm alone allowing us to say that horses on the same farm will not have similar cyathostomin populations.

In conclusion this study provides evidence that cyathostomin species have altered presence and recovery rates given different anthelmintic treatments. Beta diversity was mostly unchanged following anthelmintic treatments providing evidence that these drugs are did not select for certain species causing cyathostomin populations

to resemble one another based on treatment. Shedding status displays characterization of cyathostomin presence levels that are relatively permanent throughout the horse's life. The study also provides evidence that these drugs are still effective in reducing cyathostomin infections even though there species that seem to be present during anthelmintic treatment.

4.2 Future Direction

Continued research should expand upon this model to look at how cyathostomin response changes with the addition of treatments from other classes of drugs. Addition of data without treatment would further emphasize the effects of anthelmintic treatments on cyathostomin populations and possibly increase significance of certain interactions. Data had been obtained in another study where the distributions of cyathostomin were recorded without the presence of anthelmintic treatment. Adding samples of other trials will also give a better explanation of general fluctuations of cyathostomin which will be complete when back from sequencing. These observations should be added when continuing the study. More data describing the individual should be collected to see how host factors can influence cyathostomin populations. Trials in different seasons should also be included to see if there are any effects of local climate on cyathostomin populations. The extended investigation of this topic should give better insight into managing cyathostomin infections and increase the welfare of horses everywhere.

REFERENCES

AAEP Infectious Disease Committee. (2019). AAEP Parasite Control Guidelines.

Baudena, M. A., Chapman, M. R., French, D. D., & Klei, T.R. (2000). Seasonal development and survival of equine cyathostome larvae on pasture in south Louisiana. *Vet. Parasitol*, 88(1-2), 51-60.

Bredtmann, C. M., Krücken, J., Murugaiyan, J., Kuzmina, T., & von Samson-Himmelstjerna, G. (2017). Nematode Species Identification—Current Status, Challenges and Future Perspectives for Cyathostomins. *Front Cell Infect Microbiol*, 7, 1-8.

Collobert-Laugier, C., Hoste, H., Sevin, C., & Dorchies, P. (2002). Prevalence, abundance and site distribution of equine small strongyles in Normandy, France. *Vet. Parasitol*, 110(1-2), 77-83.

Corning, S. (2009). Equine cyathostomins: a review of biology, clinical significance and therapy. *Parasit Vectors*, 2(2).

Chilton, N. B. (2004). The use of nuclear ribosomal DNA markers for the identification of bursate nematodes (order Strongylida) and for the diagnosis of infections. *Anim Health Res Rev*, 5(2), 173-87.

Garcia, A., Brady, H. A., Nichols, W. T., & Prien, S. (2013). Equine Cyathostomin Resistance to Fenbendazole in Texas Horse Facilities. *J Equine Vet Sci*, 33(4), 223-28.

Gokbulut, C., & Mckellar, Q. A. (2018). Anthelmintic drugs used in equine species. *Vet Parasitol*, 261(15), 27-52.

Herd, R. P., & Gabel, A. A. (1990). Reduced efficacy of anthelmintics in young compared with adult horses. *Equine Vet J*, 22(3), 164-9.

Hung, G. C., Gasser, R. B., Beveridge, I., & Chilton, N. B. (1999). Species-specific amplification by PCR of ribosomal DNA from some equine strongyles. *Parasitol*, 119(1), 69-80.

Kooyman, F. N., van Doorn, D. C., Geurden, T., Mughini-Gras, L., Ploeger, H. W., & Wagenaar, J. A. (2016). Species composition of larvae cultured after anthelmintic treatment indicates reduced moxidectin susceptibility of immature Cylicocyclus species in horses. *Vet Parasitol*, 227, 77-84.

Lind, E. O., Uggla, A., & Höglund, J. (2005). Larval development assay for detection of anthelmintic resistance in cyathostomins of Swedish horses. *Vet Parasitol*, 128(3-4), 261-9.

Lichtenfels, J. R., Kharchenko, V. A., & Dvojnos, G. M. (2008). Illustrated identification keys to strongylid parasites (strongylidae: Nematoda) of horses, zebras and asses (Equidae). *Vet Parasitol*, 156, 4-161.

Love, S., & Duncan, J. L. (1992). Development of cyathostome infection of helminth-naive foals. *Equine Vet J*, 24(13), 93-98.

Love, S., Murphy, D., & Mellor, D. (1999). Pathogenicity of cyathostome infection. *Vet. Parasitol*, 85(2-3), 113-122.

Martin, R. J. (1997). Modes of action of anthelmintic drugs. *Vet. J*, 154, 11-34

Martin, R. J., & Robertson A. P. (2007). Mode of action of levamisole and pyrantel, Anthelmintic Resistance, E153 and Q57. *Parasitol*, 134, 1093-1104

Matthee, S. (2003). Anthelmintic treatment in horses: the extra-label use of products and the danger of under-dosing. *J S Afr Vet Assoc*, 74(2), 53-56.

Mfitilodze, M.W., & Hutchinson, G. W. (1990). Prevalence and Abundance of Equine Strongyles (Nematoda: Strongyloidea) in Tropical Australia. *J. Parasitol*, 76(4), 487-494.

Morris, L. H., Colgan, S., Leathwick, D. M., & Nielsen, M. K. (2019). Anthelmintic efficacy of single active and combination products against commonly occurring parasites in foals. *Vet Parasitol*, 268, 46-52.

Nielsen, M. K., & Lyons, E. T. (2017). Encysted cyathostomin larvae in foals – progression of stages and the effect of seasonality. *Vet. Parasitol*, 236(15), 108-112.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H., Szoecs, E., & Wagner, H. (2019). vegan: Community Ecology Package.R package version 2.5-5. https://CRAN.R-project.org/package=vegan

Peregrine, A. S., Molento M. B., Kaplan, R. M. & Nielsen M. K. (2014). Anthelmintic resistance in important parasites of horses: does it really matter? *Vet Parasitol*, 201(1-2), 1-8.

Pouliot, J. F., LHeureux, F., Liu, Z., Prichard, R. K., & Georges, E. (1997). Reversal of P-glycoprotein-associated multidrug resistance by ivermectin. *Biochem. Pharmacol*, 53, 17-25

Proudman, C., & Matthews, J. (2000). Control of intestinal parasites in horses. *In Pract*, 22, 90-97.

Reinemeyer, C. R., Prado, J. C., Nichols, E. C., & Marchiondo, A. A. (2010a). Efficacy of pyrantel pamoate and ivermectin paste formulations against naturally acquired Oxyuris equi infections in horses. *Vet Parasitol*, 171(1-2), 106-110.

Reinemeyer, C. R., Prado, J. C., Nichols, E. C., & Marchiondo, A. A. (2010b). Efficacy of pyrantel pamoate against a macrocyclic lactone-resistant isolate of Parascaris equorum in horses. *Vet Parasitol*, 171(1-2), 111-115.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Seyoum, Z., Zewdu, A., Dagnachew, S., & Bogale, B. (2017). Anthelmintic Resistance of Strongyle Nematodes to Ivermectin and Fenbendazole on Cart Horses in Gondar, Northwest Ethiopia. *Biomed Res Int*, 5163968.

Thamsborg, S. M., Leifsson, P. S., Grondahl, C., Larsen, M., Nansen, P. (1998). Impact of mixed strongyle infections in foals after 1 month on pasture. *Equine Vet. J*, 30, 240–245.

Traversa, D., von Samson-Himmelstjerna, G., Demeler, J., Milillo, P., Schurmann, S., Barnes, H., … Cobb, R. (2009). Anthelmintic resistance in cyathostomin populations from horse yards in Italy, United Kingdom and Germany. *Parasit Vectors*, 2(2).

Appendix

RAW STATISTICAL OUTPUT

Table 9: Multivariate Logistic Regression output from R for all interactions

Variable	Odds Ratio	95% Confidence Interval		P-Value	Std.Error	Z-Value
(Intercept)	1.045	0.232	4.212	0.951	0.728	0.061
TreatmentMoxidectin	1.182	0.222	6.744	0.846	0.862	0.194
TreatmentStrongid	0.641	0.104	4.141	0.633	0.932	-0.477
Days	1.02	0.997	1.045	0.096	0.012	1.664
SpeciesCoronocyclus labiatus	0.153	0.005	1.783	0.182	1.404	-1.335
SpeciesCoronocyclus labratum	0.454	0.019	4.757	0.544	1.302	-0.606
SpeciesCyathostomum catinatum	5.703	0.833	47.331	0.086	1.015	1.716
SpeciesCyathostomum pateratum	8.321	1.086	88.619	0.053	1.096	1.934
SpeciesCylicocyclus ashworth	6.295	1.031	44.872	0.053	0.952	1.932
SpeciesCylicocyclus auriculatus	1.121	0.171	7.351	0.904	0.947	0.121
SpeciesCylicocyclus insignis	2.679	0.452	17.196	0.283	0.918	1.073
SpeciesCylicocyclus leptostomus	2.133	0.356	13.601	0.41	0.919	0.824
SpeciesCylicocyclus nassatus	11.474	1.747	94.742	0.015	1.006	2.425
SpeciesCylicocyclus radiatus	1.449	0.234	9.275	0.689	0.928	0.4
SpeciesCylicocylus elongatus	2.266	0.38	14.501	0.373	0.919	0.89
SpeciesCylicodontophorus bicoronatus	2.124	0.354	13.615	0.413	0.921	0.818
SpeciesCylicostephanus calicatus	3.018	0.498	20.1	0.237	0.933	1.184
SpeciesCylicostephanus goldi	6.512	0.945	55.629	0.067	1.023	1.832
SpeciesCylicostephanus longibursatus	35.523	2.713	2705.241	0.024	1.579	2.262
SpeciesCylicostephanus minutus	3.553	0.592	23.729	0.174	0.932	1.361
SheddingLow	0.218	0.138	0.341	0	0.231	-6.593
SheddingModerate	0.36	0.162	0.788	0.011	0.402	-2.538
Days:SheddingLow	1.002	0.994	1.011	0.598	0.004	0.528
Days:SheddingModerate	0.999	0.986	1.012	0.886	0.007	-0.143
TreatmentMoxidectin:SpeciesCoronocyclus labiatus	2.06	0.103	80.909	0.656	1.625	0.445

TreatmentStrongid:SpeciesCoronocyclus labiatus	0.55	0.01	31.629	0.758	1.942	-0.308
TreatmentMoxidectin:SpeciesCoronocyclus labratum	2.541	0.172	74.505	0.523	1.459	0.639
TreatmentStrongid:SpeciesCoronocyclus labratum	0	0	63295.701	0.997	4664.734	-0.004
TreatmentMoxidectin:SpeciesCyathostomum catinatum	7.2	0.492	109.156	0.147	1.361	1.451
TreatmentStrongid:SpeciesCyathostomum catinatum	0.35	0.024	4.556	0.427	1.324	-0.794
TreatmentMoxidectin:SpeciesCyathostomum pateratum	3.211	0.184	52.675	0.41	1.416	0.824
TreatmentStrongid:SpeciesCyathostomum pateratum	0.525	0.028	8.555	0.654	1.437	-0.449
TreatmentMoxidectin:SpeciesCylicocyclus ashworth	0.54	0.053	4.999	0.592	1.152	-0.535
TreatmentStrongid:SpeciesCylicocyclus ashworth	1.148	0.085	15.44	0.917	1.317	0.105
TreatmentMoxidectin:SpeciesCylicocyclus auriculatus	0.017	0	0.432	0.036	1.939	-2.094
TreatmentStrongid:SpeciesCylicocyclus auriculatus	0.116	0.005	1.87	0.142	1.467	-1.468
TreatmentMoxidectin:SpeciesCylicocyclus insignis	0.132	0.013	1.257	0.082	1.163	-1.74
TreatmentStrongid:SpeciesCylicocyclus insignis	0.198	0.016	2.25	0.195	1.249	-1.295
TreatmentMoxidectin:SpeciesCylicocyclus leptostomus	0.352	0.037	3.185	0.355	1.129	-0.925
TreatmentStrongid:SpeciesCylicocyclus leptostomus	0.133	0.01	1.621	0.119	1.293	-1.561
TreatmentMoxidectin:SpeciesCylicocyclus nassatus	0.201	0.018	1.965	0.179	1.193	-1.344
TreatmentStrongid:SpeciesCylicocyclus nassatus	0.773	0.05	11.592	0.852	1.375	-0.187
TreatmentMoxidectin:SpeciesCylicocyclus radiatus	0.728	0.078	6.661	0.778	1.128	-0.281
TreatmentStrongid:SpeciesCylicocyclus radiatus	0.731	0.063	8.321	0.8	1.238	-0.253
TreatmentMoxidectin:SpeciesCylicocylus elongatus	0.07	0.005	0.797	0.037	1.272	-2.085
TreatmentStrongid:SpeciesCylicocylus elongatus	0.041	0.002	0.63	0.028	1.45	-2.202

TreatmentMoxidectin:SpeciesCylicodontophorus bicoronatus	0.593	0.057	5.879	0.656	1.174	-0.446
TreatmentStrongid:SpeciesCylicodontophorus bicoronatus	0.059	0.004	0.812	0.039	1.368	-2.065
TreatmentMoxidectin:SpeciesCylicostephanus calicatus	0.304	0.031	2.783	0.296	1.14	-1.045
TreatmentStrongid:SpeciesCylicostephanus calicatus	0.157	0.012	1.845	0.145	1.27	-1.458
TreatmentMoxidectin:SpeciesCylicostephanus goldi	2.45	0.19	28.728	0.48	1.267	0.707
TreatmentStrongid:SpeciesCylicostephanus goldi	0.35	0.024	4.554	0.428	1.325	-0.792
TreatmentMoxidectin:SpeciesCylicostephanus longibursatus	0.249	0.003	5.019	0.424	1.739	-0.799
TreatmentStrongid:SpeciesCylicostephanus longibursatus	0.302	0.002	16.969	0.556	2.032	-0.589
TreatmentMoxidectin:SpeciesCylicostephanus minutus	0.354	0.033	3.529	0.38	1.183	-0.877
TreatmentStrongid:SpeciesCylicostephanus minutus	0.047	0.003	0.618	0.023	1.341	-2.275
TreatmentMoxidectin:Days	0.939	0.903	0.972	0.001	0.019	-3.361
TreatmentStrongid:Days	1.011	0.979	1.044	0.502	0.016	0.672
TreatmentIvermectin:Days:SpeciesCoronocyclus labiatus	0.983	0.941	1.03	0.435	0.022	-0.781
TreatmentMoxidectin:Days:SpeciesCoronocyclus labiatus	0.356	0	0	0.986	58.204	-0.018
TreatmentStrongid:Days:SpeciesCoronocyclus labiatus	0.976	0.934	1.022	0.259	0.022	-1.128
TreatmentIvermectin:Days:SpeciesCoronocyclus labratum	0.392	0	0	0.988	64.742	-0.014
TreatmentMoxidectin:Days:SpeciesCoronocyclus labratum	1.009	0.973	1.048	0.644	0.018	0.462
TreatmentStrongid:Days:SpeciesCoronocyclus labratum	0.971	0.691	1.366	1	76.758	0
TreatmentIvermectin:Days:SpeciesCyathostomum catinatum	1.007	0.97	1.05	0.707	0.02	0.376
TreatmentMoxidectin:Days:SpeciesCyathostomum catinatum	1.007	0.972	1.047	0.708	0.019	0.375
TreatmentStrongid:Days:SpeciesCyathostomum catinatum	1.002	0.969	1.039	0.891	0.017	0.137
TreatmentIvermectin:Days:SpeciesCyathostomum pateratum	1.016	0.971	1.077	0.534	0.025	0.622

TreatmentMoxidectin:Days:SpeciesCyathostomum pateratum	1.032	0.994	1.075	0.113	0.02	1.585
TreatmentStrongid:Days:SpeciesCyathostomum pateratum	1.009	0.968	1.06	0.696	0.022	0.391
TreatmentIvermectin:Days:SpeciesCylicocyclus ashworth	0.976	0.946	1.005	0.109	0.015	-1.603
TreatmentMoxidectin:Days:SpeciesCylicocyclus ashworth	1.03	0.999	1.067	0.077	0.016	1.769
TreatmentStrongid:Days:SpeciesCylicocyclus ashworth	0.975	0.944	1.007	0.127	0.016	-1.527
TreatmentIvermectin:Days:SpeciesCylicocyclus auriculatus	0.982	0.953	1.012	0.239	0.015	-1.177
TreatmentMoxidectin:Days:SpeciesCylicocyclus auriculatus	1.04	0.962	1.119	0.241	0.034	1.174
TreatmentStrongid:Days:SpeciesCylicocyclus auriculatus	0.981	0.947	1.018	0.301	0.018	-1.035
TreatmentIvermectin:Days:SpeciesCylicocyclus insignis	0.976	0.947	1.004	0.104	0.015	-1.627
TreatmentMoxidectin:Days:SpeciesCylicocyclus insignis	1.025	0.989	1.066	0.187	0.019	1.319
TreatmentStrongid:Days:SpeciesCylicocyclus insignis	0.987	0.957	1.016	0.364	0.015	-0.907
TreatmentIvermectin:Days:SpeciesCylicocyclus leptostomus	0.98	0.952	1.009	0.18	0.015	-1.342
TreatmentMoxidectin:Days:SpeciesCylicocyclus leptostomus	1.028	0.996	1.066	0.107	0.017	1.613
TreatmentStrongid:Days:SpeciesCylicocyclus leptostomus	0.991	0.961	1.021	0.546	0.015	-0.604
TreatmentIvermectin:Days:SpeciesCylicocyclus nassatus	0.968	0.937	0.997	0.037	0.016	-2.09
TreatmentMoxidectin:Days:SpeciesCylicocyclus nassatus	1.039	1.008	1.077	0.021	0.016	2.311
TreatmentStrongid:Days:SpeciesCylicocyclus nassatus	0.974	0.942	1.006	0.12	0.017	-1.555
TreatmentIvermectin:Days:SpeciesCylicocyclus radiatus	0.987	0.958	1.016	0.393	0.015	-0.855
TreatmentMoxidectin:Days:SpeciesCylicocyclus radiatus	1.033	1.003	1.071	0.048	0.017	1.975
TreatmentStrongid:Days:SpeciesCylicocyclus radiatus	0.992	0.962	1.021	0.578	0.015	-0.557
TreatmentIvermectin:Days:SpeciesCylicocylus elongatus	0.987	0.958	1.016	0.369	0.015	-0.899

TreatmentMoxidectin:Days:SpeciesCylicocylus elongatus	1.003	0.925	1.059	0.921	0.031	0.099
TreatmentStrongid:Days:SpeciesCylicocylus elongatus	0.997	0.964	1.033	0.863	0.018	-0.173
TreatmentIvermectin:Days:SpeciesCylicodontophorus bicoronatus	0.99	0.961	1.019	0.497	0.015	-0.679
TreatmentMoxidectin:Days:SpeciesCylicodontophorus bicoronatus	0.324	0	0	0.984	56.257	-0.02
TreatmentStrongid:Days:SpeciesCylicodontophorus bicoronatus	1.008	0.976	1.043	0.628	0.017	0.485
TreatmentIvermectin:Days:SpeciesCylicostephanus calicatus	0.994	0.964	1.025	0.71	0.016	-0.371
TreatmentMoxidectin:Days:SpeciesCylicostephanus calicatus	1.02	0.987	1.058	0.269	0.017	1.106
TreatmentStrongid:Days:SpeciesCylicostephanus calicatus	1.007	0.975	1.04	0.682	0.016	0.41
TreatmentIvermectin:Days:SpeciesCylicostephanus goldi	1.004	0.967	1.045	0.847	0.019	0.193
TreatmentMoxidectin:Days:SpeciesCylicostephanus goldi	1.006	0.974	1.044	0.724	0.017	0.353
TreatmentStrongid:Days:SpeciesCylicostephanus goldi	0.995	0.963	1.028	0.762	0.016	-0.303
TreatmentIvermectin:Days:SpeciesCylicostephanus longibursatus	1.007	0.941	1.114	0.849	0.036	0.191
TreatmentMoxidectin:Days:SpeciesCylicostephanus longibursatus	1.044	1.01	1.086	0.018	0.018	2.363
TreatmentStrongid:Days:SpeciesCylicostephanus longibursatus	2.773	2.0993E+18	1.5636E+19	0.986	59.159	0.017
TreatmentIvermectin:Days:SpeciesCylicostephanus minutus	0.989	0.959	1.018	0.452	0.015	-0.752
TreatmentMoxidectin:Days:SpeciesCylicostephanus minutus	0.324	0	0	0.984	56.257	-0.02
TreatmentStrongid:Days:SpeciesCylicostephanus minutus	1.011	0.979	1.046	0.5	0.017	0.675