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Effects of Benzyl Isothiocyanate and Papain on Parascaris Spp. Larval Embryonation

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EFFECTS OF BENZYL ISOTHIOCYANATE AND PAPAIN ON *PARASCARIS* SPP. LARVAL EMBRYONATION

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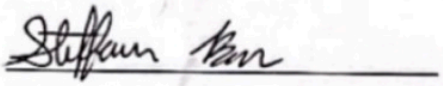
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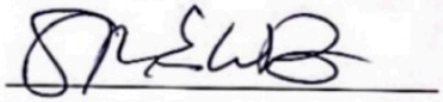
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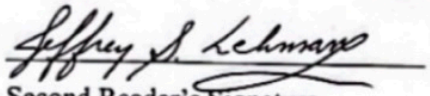
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Abstract

Ascarids (*Parascaris equorum* and *Parascaris univalens*) are a common type of intestinal parasite found in foals. By the age of one year, most horses have built a natural immunity to *Parascaris* spp. However, *Parascaris* spp. are known to be the most pathogenic type of parasite in foals and can cause poor growth, weight loss, colic, and even death. Unfortunately, *Parascaris* spp. have developed resistance to most anthelmintic drugs. Prior studies have found promising results for the use of ground papaya seeds as a short-term anthelmintic. Further research has shown similar results when determining the effectiveness of compounds found in papayas, benzyl isothiocyanate (BITC) and papain, as anthelmintics for equine strongyles. The aim of this *in vitro* study was to determine if compounds from papaya; BITC and papain, reduce *Parascaris* spp. larval embryonation. To determine whether ascarid eggs would embryonate in the presence of these compounds, eggs were incubated in different concentrations of BITC and papain in triplicate in well plates. Experimental wells were compared to control wells with either no chemical exposure or with concentrations of pyrantel (a commonly used ascarid anthelmintic). After exposure, the number of embryonated eggs were counted using an inverted microscope and were expressed as the percentage of the total and compared to each other and the control wells. A two-way completely randomized ANOVA was used to compare mean egg embryonation among treatments and chemical concentrations. Results from the two-way ANOVA test concluded that there was no statistically significant difference between the proportion of larvated ascarid eggs found in pyrantel, BITC, and papain at each chemical concentration. However, there was a trend for differences by compound and as papain concentrations increased, the proportion of larvation decreased.

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Introduction

Parascaris equorum and *Parascaris univalens*, also known as ascarids or roundworms, are yellow-white colored worms that can be as large as 15 inches long (Asaolu, 2018). Although *P. equorum* is the better-known species of ascarid that infects horses, a second species, *P. univalens* was discovered in 1883 (Nielsen et al., 2014). These species are morphologically identical, and the parasites and eggs can only be differentiated by karyotyping. Horses around 3 to 5 months are the most susceptible to ascarid infections (Clayton et al., 1979). However, by the age of one year, most horses have built a natural immunity to *Parascaris* spp. Clinical signs of an ascarid infection include ill thrift, potbelly, rough hair coat, and slowed growth. More than 5 percent of all foals with ascarid infections develop intestinal impactions, which can result in death (Southwood et al., 1998). Unfortunately, ascarids have developed widespread resistance to macrocyclic lactone parasiticide, such as ivermectin and moxidectin, and show early signs of resistance to benzimidazoles, such as fenbendazole/oxibendazole, and pyrimidines such as pyrantel (Reinemeyer, 2009). This study examines the effectiveness of benzyl isothiocyanate and papain (compounds from papaya seeds) as an anthelmintic option for treating ascarids.

Life cycle of Parascaris spp.

The life cycle of *Parascaris* spp. is a direct sexual life cycle that begins when a susceptible host consumes grass, feed, or water that has been contaminated by feces containing ascarid eggs (Shehnah, 2020). A single infected foal can shed up to 50 million eggs into the environment per day (Clayton et al., 1979). Passed in feces, ascarid begin in the environment as unicellular, L1 eggs (Fairbairn, 1961). The eggs will embryonate from the L1 stage to the

infective L2 stage after 10 days of incubation at 25-37 °C. After an infectious egg has been consumed, they begin to hatch into larvae. These L2 larvae infect the host by burrowing into the small intestine walls, migrating through portal venous circulation to the liver 7 days post infection, becoming L3 larvae (Shehnah, 2020). The L3 larvae then migrate to the lungs, travelling up to the respiratory tree until they are coughed up and swallowed, returning to the small intestines (Clayton et al., 1979). The larvae molt in the small intestines, eventually reaching reproductive maturity at the L5 stage (Fairbairn, 1961). Mature female ascarids lay many eggs in the small intestines and the host will excrete the eggs through their feces 75 to 80 days after infection (Reinemeyer, 2009). Once the eggs have been passed on to the environment, the life cycle is concluded (Shehnah, 2020). The full ascarid life cycle can be observed below in Figure 1. Ascarids eggs have a proteinaceous outer layer, which protects the eggs and allows them to stick to any surface (Fairbairn, 1957). This outer lipid layer makes the eggs resistant to extreme environmental conditions and it is how ascarids spread passively (Shehnah, 2020).

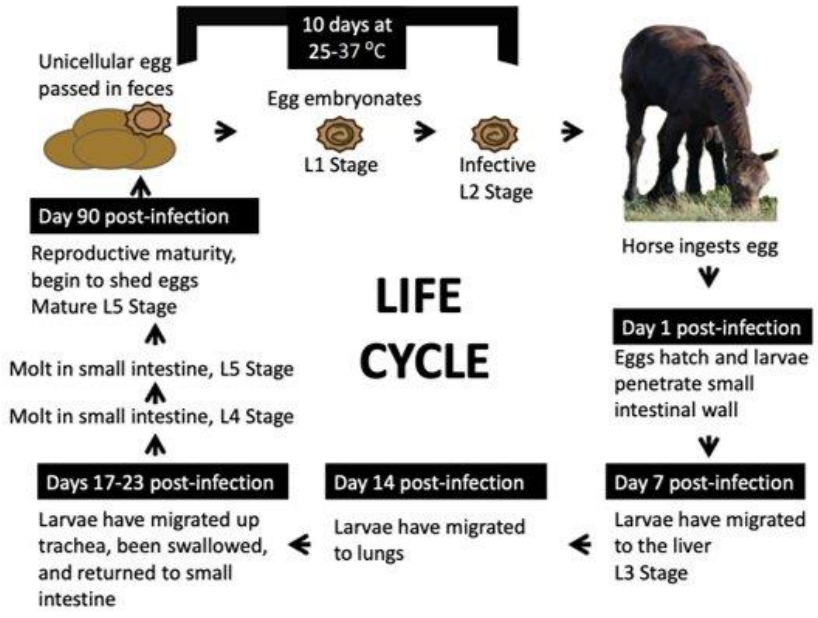

Figure 1. *Parascaris* spp. life cycle (Burk, 2013)

Pathology

Parascaris equorum is the most pathogenic parasite found in young horses (Reinemeyer, 2009). Due to the life cycle of ascarids, there are several areas where pathogenicity may occur. Inflammation or scarring of the liver, lungs, and bronchial and hepatic lymph nodes may occur after the larvae have migrated through the tissue (Srihakim et al., 1978). Large quantities of ascarids can cause intestinal blockages, colic, or even intestinal rupture (Southwood et al., 1998). Intestinal impactions can cause mild digestive upset, decreased nutrient absorption of the small intestines, or severe colic. Infected horses may also show clinical signs such as depression, anorexia, weight loss, coughing, and nasal discharge (Clayton et al., 1978). Fortunately, ascarids are one of the only nematodes to induce absolute acquired immunity, so ascarid infections are rare in horses over the age of two (Reinemeyer, 2009). However, ascarids can cause severe damage if left untreated. Because of this, it is vital to prevent and control ascarid eggs levels in foals that have not yet developed immunity to infection.

Diagnosis

All horses are expected to have some degree of internal parasitic infection. In fact, almost 100% of all horses have at least one type of internal parasitic infection (Reinemeyer et al., 1984). Therefore, it is important to conduct routine tests to maintain a low parasitic burden in both individuals and the herd. There are several methods that can be used to assess parasite egg shedding. These methods include microscopic examination for eggs in fecal samples and necropsy (Nielsen et al., 2010). However, the best method for diagnosis is through fecal egg counts (FEC) or qualitative fecal flotations. Fecal egg counts quantitatively measure the number

of eggs per gram (EPG) of feces. A high shedder, or a horse with a high ascarid burden, is identified when there are more than 200 eggs per gram (EPG) of feces (Nielsen et al., n.d.). One issue with FEC is that if the ascarid larvae are migrating or immature adults, the test may provide a false negative, meaning there is an adult ascarid infection present with no eggs in the FEC. Fecal flotations only provide a qualitative assessment of a sample. However, fecal flotations can still determine the absence or presence of ascarid eggs in a foal. Because fecal flotations cannot provide quantitative data, they cannot be used for deworming decisions or to evaluate treatment efficiency. One method used to test for anthelmintic resistance is the fecal egg count reduction test (FECRT) (Becher et al., 2010). A FECRT is conducted by doing an initial FEC before administering anthelmintic treatment followed by a second FEC fourteen days after treatment to find the mean percent reduction in FEC. This determines how many parasites survived the initial deworming. These cutoff values serve as a guide for interpretation, meaning reduced efficacy may or may not mean there is resistance present (Nielsen et al., n.d.). If the EPG after treatment is less than 80-85%, there are likely no anthelmintic-resistant parasites. Ivermectin/moxidectin has a higher cutoff value at less than 95% because small strongyle resistance has not yet been diagnosed in the US, therefore any FECRT results lower than this cutoff should be repeated before concluding resistance is present (Nielsen et al., n.d.). Strongyles, also known as cyathostomins, are considered the most prevalent and pathogenic parasites found in horses of all ages regardless of climate or management differences (Corning, 2009). When implementing anthelmintic treatment regimens, it is important to check for resistance among all parasitic species, including ascarids and strongyles, to ensure the most effective treatment method. FECs are vital for the early detection of ascarid infections and in addition, FECRTs play an important role in monitoring anthelmintic efficacy. It is important to recognize the clinical signs of a

parasitic infection to determine if a horse might have a heavy parasitic load and monitor herd parasite egg shedding using FECs. This knowledge allows for better treatment, management, and prevention of ascarids in the future.

Environmental Management

After ascarid eggs are excreted into the environment through feces, they must embryonate at 16-34 °C for three to six weeks before becoming infective (Asaolu et al., 2018). However, the infective ascarid eggs do not hatch in the environment, they hatch in the GI tract to maximize the odds of infecting the host (Fairbairn, 1961). The eggs are very durable in inclement weather, can stick to objects, and can remain in the environment for up to ten years due to their proteinaceous outer shell (Nielsen et al., 2014). Due to their persistence in the environment, there are only a few methods for controlling ascarid eggs in the environment. Ascarid eggs can remain in the environment for up to a year at temperatures of 40 °C, but the eggs become unviable at temperatures of 60 °C or higher (Naidoo et al., 2017). Composting manure often reaches these high temperatures, inactivating the ascarid eggs. A study by Gould et al., 2012 found that proper composting of manure and soiled bedding will generate a high enough internal temperature that strongyle larvae, and even ascarid eggs, are virtually eradicated within one week by exposure to temperatures over 40°C. Removing manure from stalls and pastures is one way to prevent ascarid persistence in the environment (Nielsen et al., n.d.) The main method of ascarid egg management is by using anthelmintic treatment.

Deworming Practices & Anthelmintic Resistance

It is suggested that horses only receive anthelmintic treatments known to be effective against indigenous parasitic populations and to administer treatments at the longest intervals to prevent serious environmental contamination of ascarid eggs (Reinemeyer, 2009). Decades of anthelmintic use has created resistant ascarid populations that cannot be controlled using traditional approaches (Nielsen et al., n.d.).

Common classes of anthelmintics used to treat internal parasites include benzimidazoles, pyrimidines, macrocyclic lactones, and praziquantel. Benzimidazoles, such as fenbendazole and oxibendazole, have an expected efficacy of 99% with no resistance and less than 90% if resistance is present. Unfortunately, due to overuse, ascarids show early indications of resistance and strongyles have widespread resistance to benzimidazoles. Pyrimidines such as pyrantel, have an expected efficacy of 94-99% without resistance and less than 85% within resistant populations. Ascarids present with early indications of resistance and resistance is common in strongyles populations. Macrocyclic lactones, including ivermectin and moxidectin, have a 99.99% efficacy in populations without resistance and less than 95% in resistant populations. Ascarids have the most widespread resistance to macrocyclic lactone treatments. Despite anthelmintic resistance in strongyle populations, pyrantel, fenbendazole, and oxibendazole resistance is still uncommon for ascarids. Therefore, if used responsibly in accordance with FECRT results, these anthelmintics are the best option for targeting *Parascaris* spp. With parasitic resistance to benzimidazoles, pyrimidines, and macrocyclic lactones seeming to rise, new anthelmintic options are needed.

Deworming with Papaya

Carica papaya, commonly known as pawpaws or papayas, are frequently used in folk medicine as an antimicrobial treatment (Peachy et al., 2016). These enzymes, known as cysteine proteinases (CPs), are derived from *C. papaya, Ananas comosus* (pineapple)*, and Ficus* spp. (figs) and Peachy et al., 2016, observed that papaya latex supernatant has a potent effect on the free-living stages of equine strongyles *in vitro.* In this study, Peachy found that at concentrations above 6.25 μM, strongyles did not develop. Romero et al., 2018 also examined the effect of natural food supplements on reducing intestinal worms in goats. Adding 10 grams of ground papaya seeds to a base diet was effective in reducing adult worm counts and egg counts. Romero proposed that high concentrations of certain enzymes, alkaloids, and cyanates were responsible for the anthelmintic properties.

A previous study by Samuels et al., 2015 found similar results that the use of botanical anthelmintics, such as ground papaya seeds, are effective in reducing strongyle egg counts in adult horses. Samuels administered 0.176 grams of papaya seed powder per 100 kilograms of the horse's body weight. It was found that *C. papaya* significantly decreased strongyle egg count. Papaya seeds contain the chemical compounds papain and benzyl isothiocyanate (BITC), which have been found to possess anthelmintic properties against several different parasites. However, the effectiveness of BITC and papain as an anthelmintic against equine ascarids has not been previously studied.

Glycosylate, the precursor of isothiocyanates is found in many edible species and vegetables in the Brassicaceae family (Fahey, 2001). The Brassicaceae, or the mustard family, includes broccoli, brussels sprouts, and cabbage. Benzyl isothiocyanate (BITC) is known for its fungicidal, bactericidal, nematocidal, and allelopathic properties (Fahey, 2001). Nagesh et al.,

2002 found evidence support this claim studying the effects of BITC from *Carica papaya* seeds as a potential nematicide against *Meloidogyne incognita,* the root-knot nematode. Using steam distilled oil from fresh/dry *Carica papaya* seeds, it was found that the BITC present within the seeds caused mortality within 150 to 210 minutes of exposure at 25 ppm. The concentrations used in this study were significantly smaller in parts per million compared to this study (0.01 ppm-0.5 ppm). BITC showed higher nematocidal activity *in vitro* than the commercially used nematicide, carbofuran.

The calpain family is known for the calcium dependence of the papain-like proteases, or cysteine proteinases, which can modulate the function of their substrates (Rawlings, 1994). Because they are found in lysosomes, cysteine proteinases are seen in many organisms including eubacteria, yeast, protozoa, plants, and mammals (Rawlings, 1994). The cysteine proteinases in papaya latex have been shown to have anthelmintic properties in rodents, pigs, and humans (Buttle et al. 2011). Buttle provided support that cysteine proteinases derived from papaya may work as an anthelmintic to treat intestinal nematode infections in ruminants at concentrations between 47 μmol to 234 μmol. A similar study from Stepek et al., 2007, found that cysteine proteinases from the fruit and latex of plants such as papayas, pineapples, and figs, have efficacy against nematodes within the rodent gastrointestinal tract when formulated appropriately.

Another study, conducted by Kmietsch et al., 2017, looked at the effects of chemical compounds from *C. papaya*, BITC and papain, on strongyle egg hatching and larval migration. It was observed that BITC was more effective at inhibiting strongyle egg hatching compared to papain, which could be a useful alternative for treating strongyle infections. However, both BITC and papain were more effective than the control anthelmintic, ivermectin, which is typically used to treat strongyle infections. This study will be conducted using similar methods on a different

parasite (ascarids) to determine if similar results are observed, which could possibly suggest other forms of anthelmintic treatment for *Parascaris* spp.

Material and Methods

Egg Isolation

To separate the ascarid species from other parasites in the fecal sample, methods from Andersen et al., 1973, were followed and modified as needed. First, fecal samples from quarter horse foals were collected from the Ohio State Equine Center. Samples were stored at 4°C until ascarid eggs were isolated from the stool.

A fecal egg count was conducted using the modified Stoll method to ensure that ascarid eggs were present in the samples. The samples contained 5,825 eggs per gram (EPG). The EPG was then multiplied by the total mass of the samples to estimate the total ascarid egg concentration in each sample, which estimated 1,380,175 eggs total.

To isolate the ascarid eggs, the fecal samples were mixed with 20 mL of distilled water per 1 gram of feces. The fecal suspension was poured through a tier of sieves with mesh sizes of 2000, 500, 250, and 125 µm. Since ascarid eggs are between 90-100 µm, these eggs should pass through the smallest sieve to be retrieved from the collection pan below the sieves. To isolate more eggs, the sieves were washed thoroughly with distilled water. The liquid in the collection pan below the sieves was then collected and transferred into 50 ml centrifuge tubes. The tubes were then spun down at 1000 rpm for three minutes and the supernatant was decanted, leaving only the egg pellet.

After isolating the pellet, it was suspended in a sucrose solution with a specific gravity of 1.27, or 1.3 grams of sucrose per 1 mL of distilled water. The sugar-fecal suspension was then transferred to 15 ml tubes and topped with a coverslip. The mixture was centrifuged at 1000 rpm for 3 minutes while covered with a coverslip. Centrifugal flotation allows for any eggs present to float to the top of the tube with the force of the centrifuge. After centrifugation, the coverslip was washed off into an Erlenmeyer flask using deionized water. Prior to further egg isolation, the Erlenmeyer flask containing water and eggs was stored at 4°C.

Egg De-Coating

To ensure that the eggs are fully exposed to the chemicals, it is necessary to remove the outermost acid mucopolysaccharide, or the protein uterine layer, the lipoprotein vitelline layer, and part of the thicker chitin, protein layer called the ascaroside layer (Brownell et al., 2006). *In vivo,* the outermost eggshell layers are reduced within the equine stomach, allowing for the larvae to be released from the egg (Nielsen et al., 2014). To de-coat the eggshells, methods from Burk (2013) were modified. Figure 1A below provides a visual of an ascarid egg prior to the de-coating process. First, 7.5% sodium hypochlorite (unscented bleach) was added into a tube with the egg solution at a ratio of 1 ml bleach to 1 ml of egg solution. The tube was then placed on the shaker platform, making sure to rotate the tubes so the bleach does not harm the eggs. The eggs were periodically checked on a microscope slide without a cover slip to look for de-coating of the eggshell layers.

Once the eggs were fully bleached, the chitinous layer was very thin and the egg did not appear perfectly round (Figure 1B). Bleaching took at least 8-10 minutes but varied between batches. Prolonged bleaching was avoided so as not to disrupt the chitinous layer of the shell

(Figure 1C). To decrease the bleaching action, sterile 0.855% saline solution was added to the tubes and centrifuged for 1 minute at 200xg with an acceleration of 7 and a deceleration of 4. Centrifugation allows for the eggs to pellet to the bottom, removing the bleach with the supernatant. The tubes were washed 8 times, repeating centrifugation. The eggs that were used for the experiment were stored in 0.85% saline and kept at 4°C to prevent premature embryonation.

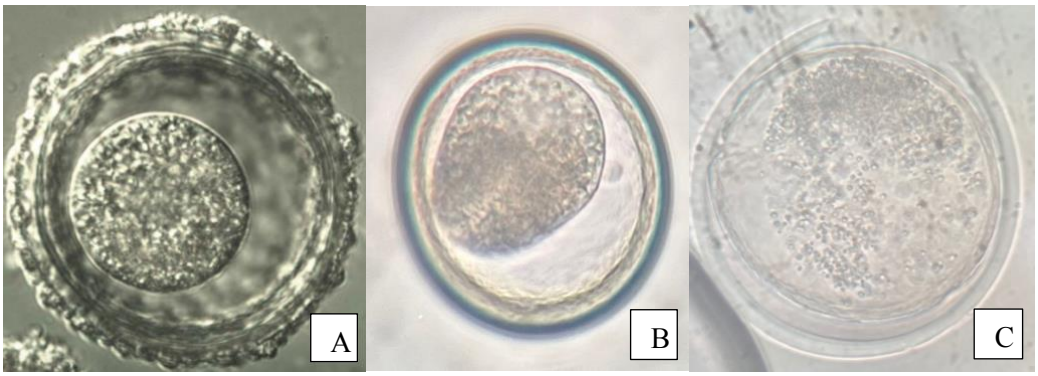

Figure 2. *Parascaris* spp. eggshell decortation (not to scale). A: *Parascaris* spp. prior to decortication (Burk, 2013). B: Bleached *Parascaris* spp. with thin chitin layer remaining. C: Mechanical disruption of chitinous layer of shell.

Chemical Exposure

To examine ascarid larval embryonation, the bleached eggs were exposed to different concentrations of chemicals in triplicate. The egg solution was adjusted to contain 100 eggs/ml of dH2O and pipetted into 24 wells per chemical, or 72 total wells. Treatments included a control group, a group exposed to pyrantel pamoate (a commonly used ascarid dewormer), and two experimental groups containing papain and benzyl isothiocyanate.

Eight different concentrations of chemicals were added to wells containing the egg solution in triplicate. The concentrations used were 0.01 μg of the chemical compound/mL egg solution, 0.025 μg/mL, 0.05 μg/mL, 0.1 μg/mL, 0.2 μg/mL, 0.3 μg/mL, 0.5 μg/mL, and 0 μg/ml. Serial dilutions were performed to achieve the eight desired concentrations listed above. Since BITC and pyrantel are insoluble in water, DMSO was used to dissolve these solid compounds while water was used to dissolve papain. The control wells had either deionized water or DMSO depending on the treatment group. The combined egg and chemical solutions were allowed to incubate for 10 days at temperatures between 25-27 °C. The wells were checked daily to ensure a consistent temperature was maintained.

Observation After Exposure

After exposure, the entire well plate was examined under an inverted microscope at 100 to 400× to count the number of motile larvae per well. Ascarid eggs in each well were identified as unicellular, multicellular, or embryonated. Embryonated eggs were defined as larvae with segmentation and slight motility (Figure 2A and 2B), multicellular ascarids were identified by the many cells within the egg (Figure 2C), and unicellular eggs had a single cell within the egg (Figure 2D). The number of embryonated eggs were expressed as a percentage of the total and compared to the control wells. The steps from trial 1 were repeated, but with modifications to the decortication process for trial 2. The eggs in trial 2 were exposed to 7.5% sodium hypochlorite for a longer period (15-20 minutes) to ensure maximal decortication of the chitin layer.

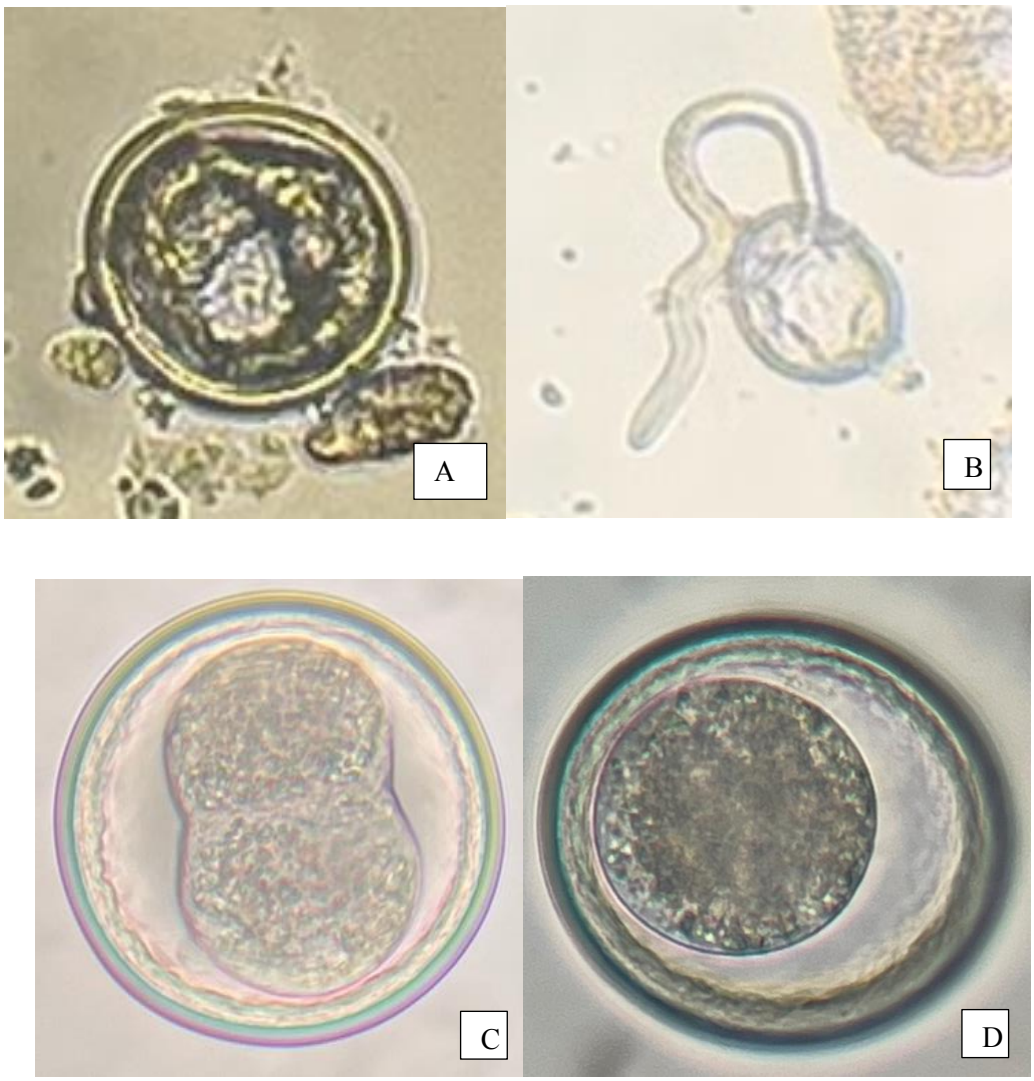

Figure 3. Comparison of unicellular, multicellular, and larvated *Parascaris* spp. eggs A: Larvated *Parascaris* spp. egg (L2). B: Hatched *Parascaris* spp. larvae (L2). C: Multicellular *Parascaris* spp. egg (L1). D: Unicellular *Parascaris* spp. egg (L1).

Statistical Analyses

After the ten-day incubation period, statistical analyses were conducted to study the results. Two trials were conducted, meaning there are two different results for each statistical test. The mean egg embryonation of each chemical concentration was recorded as a percentage of the total number of eggs in each well and compared to each other and the negative control.

Descriptive statistics and Q-Q plots were used to evaluate normality of the data. A two-way completely randomized ANOVA test with replication was used to examine ascarid larval embryonation among the treatments (BITC, papain, and pyrantel) and the concentrations of the treatments. The proportion of larvated eggs compared to the chemical concentrations for each compound were graphed in Microsoft Excel and trendlines, R^2 values, and standard error were recorded. ANOVA for regression was also calculated to provide information about the levels of variability between compounds and concentrations within a regression model.

Results

Two-Way ANOVA Results

Two-factor ANOVA test results from trial 1 indicated that there was no statistically significant difference between the effects of BITC, papain, and pyrantel and the concentrations on mean proportion of larvated eggs, $F(13, 41)=0.803$, $p=0.654$. The two-way ANOVA summary for trial 1 can be observed in Table 1.1. The two-factor ANOVA results from trial 2 indicated similar results that the interaction between compounds and concentrations had no statistically significant effect on the mean proportion of embryonation $F(14, 29)=0.579$, $p=0.859$. These results can be found in Table 1.2 below. However, there was a trend for differences by compound observed in trial 1, $F(2, 41)=3$, $p=0.061$, and trial 2, $F(2, 29)=3.137$, $p=0.058$, which suggests there could be a difference in the effectiveness of the compounds if further testing had been conducted. It appeared that as concentrations of papain increased in trial 1 and 2, the proportion of larvation decreased.

Tests of Between-Subjects Effects

Dependent Variable: ProportionLarvation

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.763[a]	22	.035	1.072	.412
Intercept	34.444	1	34.444	1064.156	<.001
Compound	.194	2	.097	3.000	.061
Concentration	.232	7	.033	1.024	.429
Compound * Concentration	.338	13	.026	.803	.654
Error	1.327	41	.032		
Total	38.811	64			
Corrected Total	2.090	63			

a. R Squared = .365 (Adjusted R Squared = .024)

Table 1.1. Trial 1 Two-Way ANOVA results calculated in SPSS Statistics

Tests of Between-Subjects Effects

Dependent Variable: ProportionLarvation

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.208[a]	23	.183	1.405	.191
Intercept	18.156	1	18.156	139.456	<.001
Compound	.817	2	.408	3.137	.058
Concentration	1.739	7	.248	1.908	.104
Compound * Concentration	1.055	14	.075	.579	.859
Error	3.775	29	.130		
Total	27.437	53			
Corrected Total	7.984	52			

a. R Squared = .527 (Adjusted R Squared = .152)

Table 1.2. Trial 2 Two-Way ANOVA results calculated in SPSS Statistics

Effects of Papain on Larval Embryonation

In trial 1 and 2, papain did not inhibit larval embryonation after incubation at concentrations between 0.01 µg/mL and 0.5 µg/mL when compared with the control of 0 µg/mL. A best fit line of y = -186.85x + 0.8238 with a R^2 value of 0.140 was plotted for trial 1 using the percentage of larvated eggs at each concentration (Figure 4.1). The best fit line for the proportion of larvated eggs per chemical concentration in trial 2 is y = -1171.5x + 0.7201 with an R^2 value of 0.552 (Figure 4.2). ANOVA for regression was also calculated to provide information about the levels of variability between compounds and concentrations within a regression model for papain. The *F* statistic from trial 1's ANOVA for regression suggests that there is no significant relationship between the chemical compounds and concentrations (F=0.361). The regression statistics are below in Appendix 1A. In trial 2, the p-value (p=0.00028) and *F* statistic (F=0.035) indicate that there is a significant difference between compounds. The regression summary for trial 2 can be found below in Appendix 1B.

Effects of BITC on Larval Embryonation

BITC did not inhibit larval embryonation in trial 1 and 2 after incubation at concentrations between 0.01 µg/mL and 0.5 µg/mL compared to the control of 0 µg/mL. For trial 1, a best fit line of y = 540.18x + 0.5613 with an R^2 value of 0.124 was plotted using the proportion of larvated eggs per concentration (Figure 4.1). Using the data from trial 2, the best fit line was y = -853.6x + 0.8265 with a R^2 value of 0.386 (Figure 4.2). ANOVA for regression was calculated for trial 1, and the *F* statistic concludes that there is no significant difference between the concentration and compounds (F=0.393). The regression summary for trial 1 can be found in

Appendix 2A. For trial 2, the *F* statistic from ANOVA regression was *F*=0.100, which also suggests that there is no significant difference between the compounds and the concentrations (Appendix 2B).

Effects of Pyrantel on Larval Embryonation

Pyrantel did not inhibit larval embryonation in trial 1 or trial 2 after incubation at concentrations between 0.01 µg/mL and 0.5 µg/mL in comparison to the control of 0 µg/mL. The best fit line of the proportion of larvated eggs per chemical concentration for trial one was y = -42.081x + 0.7557 with a R^2 value of 0.014 (Figure 4.1). In trial two, the best fit line was y = -346.72x + 0.7972 with a R^2 value of 0.068 (Figure 4.2). ANOVA regression was also calculated for trial one and two to determine the variability between the proportion of larvated eggs and concentrations of pyrantel. For trial 1, the *F* statistic was *F*=0.778, which suggests that there is no significant difference between the independent and dependent variables (Appendix 3A). The *F* statistic (*F*=0.532) for trial 2 had similar results to the previous regression, there was no significant difference observed (Appendix 3B).

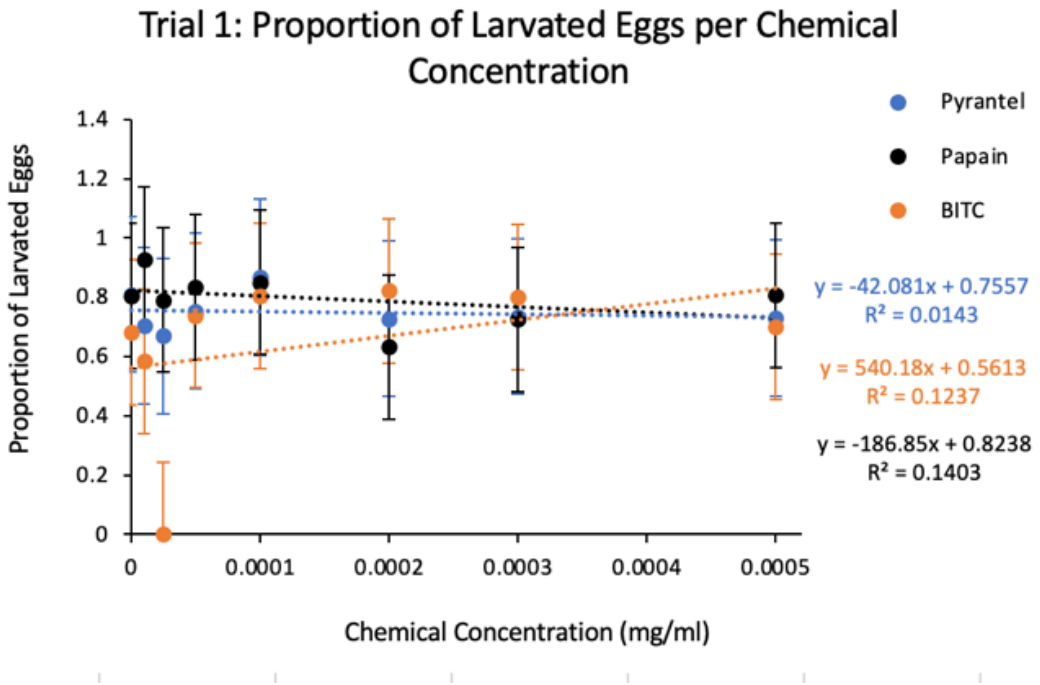

Figure 4.1. The proportion of larvated eggs compared to each chemical (pyrantel, papain, and BITC) and concentration (0.00001 mg/ml, 0.000025 mg/mL, 0.00005 mg/mL, 0.0001 mg/mL, 0.0002 mg/mL, 0.0003 mg/mL, and 0.0005 mg/mL, and 0 mg/ml) for trial one including best fit lines and standard error for each data point.

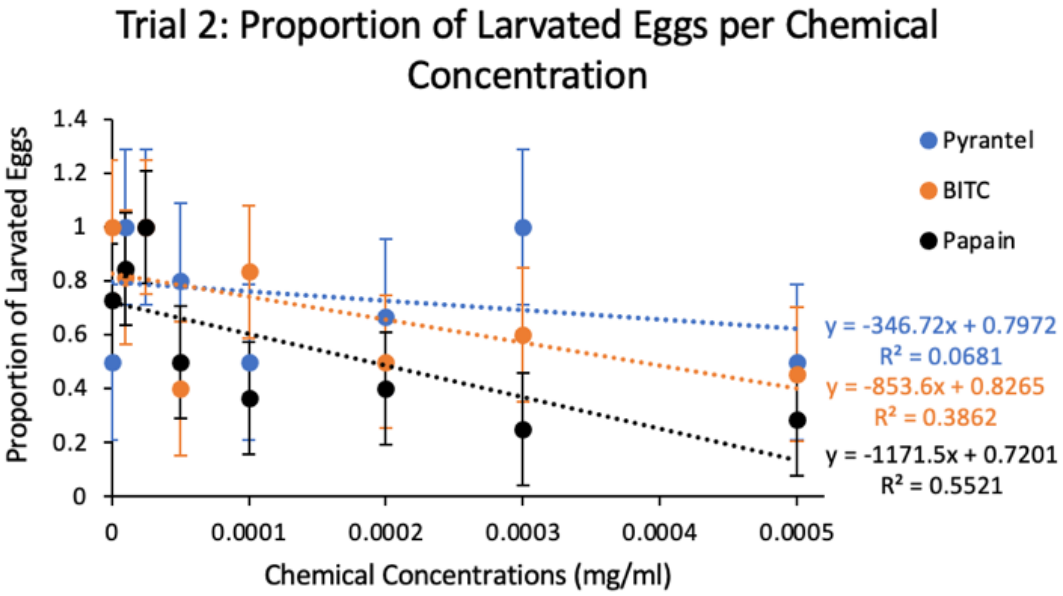

Figure 4.2. The proportion of larvated eggs compared to each chemical (pyrantel, papain, and BITC) and concentration (0.00001 mg/ml, 0.000025 mg/mL, 0.00005 mg/mL, 0.0001 mg/mL, 0.0002 mg/mL, 0.0003 mg/mL, and 0.0005 mg/mL, and 0 mg/ml) for trial two including best fit lines and standard error for each data point.

Discussion

The data does not support that papain, BITC, or pyrantel have inhibitory effects on ascarid larval embryonation. There was a trend observed for the differences by compound for papain in trial 1 and 2, which could suggest a difference in the effectiveness of compounds if further testing had been conducted. The small sample sizes in trial 2 could have negatively impacted the level of significance. The smaller the sample size of the study, the more likely it is to have random error present which could cause the true relationship to be biased towards the alternative hypothesis (Thiese et al., 2016). This phenomenon also explains the significant *F* statistic (F=0.035) observed in the trial 2 papain ANOVA for regression.

Since the control anthelmintic, pyrantel, was not significantly different than the treatment groups, it raises questions about the experimental methods and the nature of *Parascaris* spp. eggs. Even with no difference between compound, there was no difference between the negative control and the positive control. If the only finding was no difference between compounds, this could indicate that all treatments were effective. *Parascaris* spp. have a 3-4 μm thick, four-layer shell that consists of the inner ascaroside layer, a chitin/protein layer, a lipoprotein vitellin layer, and an outer acid mucopolysaccharide/protein uterine layer (Brownell et al., 2006). This impermeable shell protects eggs from strong acids, strong bases, oxidants, reductants, protein-disrupting agents, surface-active agents (Brownell et al., 2006). Despite the decortication of the outer eggshell layers with sodium hypochlorite, it is possible that the concentrations of pyrantel, BITC, and papain could not penetrate the remaining lipoprotein ascaroside layer. Partial decortication of the ascarid eggs could explain why the control anthelmintic (pyrantel) also had no effect on larval embryonation. Future studies should increase the exposure to sodium

hypochlorite to ensure only the innermost lipoprotein ascaroside layer remains without rupturing the egg.

A study by Gould et al., 2009 examined the effects of commonly used disinfectants on *Parascaris equorum* eggs. To expose the eggs to Lysol and Amphyl, Gould had a different bleaching procedure compared to the de-coating methods used for this experiment. Gould allowed for the chemical treatment groups to dry out in a 4°C refrigerator to simulate fecal material left on stall walls and floors. Once dry, the samples were left at room temperature (23°C) for four days to give the eggs the opportunity to larvate. The larvated eggs were then soaked in 10% bleach solution to easier visualize the egg development. These methods were not modified for this experiment for several reasons. This study was examining the embryonation of eggs when exposed to chemical compounds, so the eggs were not allowed to fully incubate until after they were bleached and exposed to the chemical concentrations. It also seems that Gould bleached the eggs for the sole purpose of visualization by removing the outermost shell layer, rather than bleaching the egg until only a thin chitin layer remains. However, it is interesting that Gould simulated fecal material being left in the stall by drying out the samples. Although the benefits of drying the samples out are unclear, Gould was able to yield significant results. Drying the samples prior to exposure to bleach could make a significant difference in the de-coating process. Using this bleaching method, Gould found a significant reduction in the percent of viable ascarid eggs exposed to liquid Lysol, but 92.6% of eggs were still viable. When exposing the eggs to Amphyl, there was an average of 0% viable eggs after exposure. In this experiment, Gould suggests that the combination of other parasitic management practices and disinfectant could be an effective way to reduce the incidence of ascarid infections. Future studies could observe the effects of a combination of management practices on ascarid embryonation.

Kmietsch et al., 2017, observed the effects of BITC and papain on strongyle eggs and found that both compounds show potent anthelmintic activity against cyathostome eggs and larvae *in vitro. Parascaris* spp. eggs measure around 90-100 µm in size while strongyles range from 70-90 µm and have a significantly thinner shell compared to the ascarid's four-layer eggshell (Nielsen et al., 2014). Evidence from this study and other studies such as Samuels et al., 2015 suggest the anthelmintic potential of BITC and papain against strongyle infections. Since the concentrations used in Kmietsch et al, 2017 were successful at inhibiting cyathostome egg hatch and larval migration, the same concentrations were tested on *Parascaris* spp. eggs in this study.

A study by Berger et al., 1940 tested the anthelmintic activity of papain on hatched *Ascaris lumbricoides* and found that rapid ulceration followed by digestion of the worm tissue occurred in tubes containing crystalline papain. Berger's study varies from the methods used to test papain in this study. The dried papain from Berger's study was dissolved in phosphate-phthalate buffer pH 5, while the papain in this study was dissolved in deionized water. However, in a study by Buttle et al., 2011, dried papaya latex was also dissolved in water. It would be interesting to further investigate the effectiveness of papain dissolved in water compared to papain in phosphate-phthalate buffer.

Another study looking at mature *Ascaris lumbricoides* by Dar et al., 1965 tested BITC and benzyl thiourea individually for bioactivity and found BITC to be 20 times more toxic to *Ascaris lumbricoides* than benzyl thiourea. Kermanshai et al., 2001 also found that benzyl isothiocyanate is the predominant or sole anthelmintic activity detected in papaya seed extracts. In Kermanshai's study, anthelmintic activity was present with water-soluble and non-aqueous extracts from papaya seeds, with BITC present in both extracts. Volumes of 10 to 20 µl of

aqueous papaya extract, representing 1.2-2.4 mg of seed, were sufficient to kill *Caenorhabditis elegans* in a 0.5 ml assay. These concentrations are significantly larger than the concentrations used in this study. A stronger concentration of BITC and papain could possibly be more effective at decreasing equine ascarid embryonation. Kermanshai also observed that when BITC was left at room temperature for more than 10 hours, there was a decrease in the anthelmintic activity that coincides with a decrease in BITC concentration. This study exposes equine ascarid eggs to BITC for two weeks at room temperature. Further research should be conducted to ensure the efficacy of BITC concentrations and the minimum required incubation period for equine ascarids. Kermanshai also found that papaya extract dissolved in water did not contain enough cyanogenic glucosides (highly toxic cyanide) to be toxic to *C. elegans.* In this study papain was dissolved in water, so it is possible that it did not contain enough cyanogenic glucosides to decrease equine ascarid embryonation. Since previous studies have evidence supporting the anthelmintic properties of BITC and papain against human ascarids (*A. lumbriocoides)* and other nematodes (*C. elegans*), further procedures should be modified to study *Parascaris* spp.

Ascarids are the most prevalent parasitic worms, and they are resistant to most anthelmintic drugs, so finding alternative anthelmintics is crucial (Brownell, 2006). Since BITC and papain have anthelmintic properties against strongyle infections, further research should be conducted on the effects of these compounds on hatched ascarid larvae. The equine ascarid eggs used in this study began as first stage larvae (L1) and embryonated to stage two (L2) after chemical exposure. A study by Shehnah, 2020 hatched stage three (L3) *Parascaris univalens* larvae before exposing the larvae to anthelmintics. Shehnah utilized a larval motility scoring protocol to determine the effectiveness of the anthelmintics. This scoring protocol could be a more sufficient way to evaluate the effects of BITC and papain on *Parascaris* spp. Rather than

studying the number of embryonated eggs per well, the number of motile larvae could be evaluated. Hatching the ascarid eggs would also ensure that the larvae are fully exposed to the compounds, avoiding the protective eggshell entirely.

A study by Moraes et al., 2017 examined the effects of papain and *Carica papaya* latex against *Strongyloides venezuelensis* eggs and larvae. The larvae used by Moraes were in the L3 larval stage, which differs from the L1 eggs used in this study. Moraes found that papain was effective against L3 *S. venesuelensis* eggs and larvae. Negi et al., 2020 found that extracts of papaya seeds inhibited activity against L2 *Ascaridia galli* eggs. Similarly, a study by Rew et al., 1986, found that the development of *Ascaris suum* L2 larvae were sensitive to anthelmintics *in vitro,* but not *in vivo.* However, further research should be conducted utilizing stage one (L1) ascarid eggs.

Further research could also be conducted on a separate ascarid species entirely. Burk, 2013 compared the thickness of the chitinous layers of *B. procyonis and P. equorum* using Adobe Photoshop 7.0. The chitinous layer of *P. equorum* was found to be 4.7 times thicker than the shell of *B. procyonis.* The chitinous layer of *A. lumbricoides* and *A. suum* were reported to be 2 µl or 3-4 µl in thickness (Rogers, 1956). The round shape of *P. equorum* eggs compared to other ascarid could also contribute to the strength of the shell. A different ascarid species could potentially make it easier for BITC and papain to penetrate the eggshells of L1 larvae, yielding more accurate results about the effects of these alternative anthelmintics.

Many horses in third world countries lack clean water, food, and proper veterinary care. This often results in high parasitic burdens that impact a horse's quality of life. Papayas could be an affordable alternative for deworming horses in third world countries. Samuels et al., 2015 found that papaya seeds can be fed to horses for their anthelmintic properties. Horse owners in

third world countries could be taught how to dry, crush, and mix the papaya seeds with a more palatable substance to accurately dose and administer the natural anthelmintic to their horses. This could be an affordable and effective method to improve and extend the lives of many equids. If further studies found BITC and papain to be effective anthelmintics against equine ascarids, not only would papayas benefit horses in third world countries, but it could also provide a treatment for anthelmintic-resistant ascarid infections in the United States.

Conclusion

BITC, papain, and pyrantel did not decrease ascarid egg embryonation in this study. This could be due to the high structural strength and resistance of ascarid eggshells. Future studies could include a longer decortication period prior to chemical exposure or testing the chemicals on hatched larvae to fully understand the effects of BITC and papain on ascarid embryonation.

References

Andersen, S.R., Jess Jorgensen, P. Nansen, & K. Nielsen. 1973. Experimental *Ascaris suum* infection in piglets. Acta path. Microbiol. Scand., Section B, 81, 650-656.

Asaolu, S.O., Ofoezie, I.E. 2018. Ascaris Spp. Global Water Pathogen project. Michigan State University. doi: https://doi.org/10.14321/waterpathogens.41

Becher, A., Mahling, M., Nielsen, M.K., Pfister, K., 2010. Selective anthelmintic therapy of horses in the Federal states of Bavaria (Germany) and Salzburg (Austria): An investigation into strongyle egg shedding consistency. Vet. Parasitol. 171, 116-122.

Berger, J., & Asenjo, C. F. 1940. Anthelmintic activity of crystalline papain. Science, 2364 (91), 387-388.

Brownell, S.A., Nelson, K.L. 2006. Inactivation of single-celled *Ascaris suum* eggs by low pressure UV radiation. National Library of Medicine, vol. 72(3). doi: 10.1128/AEM.72.3.2178-2184.2006

Burk, Steffanie V. 2013. Detection of antibodies against *parascaris equorum* excretory-secretory antigens, Thesis and Dissertations – Animal and Food Sciences. Vol. 21.

Buttle, D.J., Behnke, J.M., Bartley, Y., Elsheikha, H.M., Bartley D.J., Garnett, M.C., Donnan, A.A., Jackson, F.J., Lowe, A., and Duce, I.R. 2011. Oral dosing with papaya latex is an effective anthelmintic treatment for sheep infected with *Haemonchus contortus.* National Library of Medicine, vol. 4(36).

Clayton, H.M. and Duncan, J.L. 1978. Clinical signs associated with *Parascaris equorum* infection in worm-free pony foals and yearlings, Vet. Parasitology, vol. 4(1), pp. 69-78.

Clayton, H.M. and J.L. Duncan. 1979. The development of immunity to *Parascaris equorum* infection in the foal. Research in Veterinary Science, 26(3): p. 383-4.

Corning, S. 2009. Equine cyathostomins: a review of biology, clinical significance, and therapy. *Parasites Vectors* 2 (Suppl 2), S1.

Dar, R.N., Garg, L.C., Pathak, R.D., 1965. Anthelmintic activity of Carica papaya seeds. Indian Journal of Pharmacy 27, 335– 336.

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, 56(1), 5-51.

Fairbairn, D. 1957 The biochemistry of Ascaris. Experimental Parasitology, 6(5): p. 491-554.

Fairbairn, D. 1961. The in vitro hatching of *Ascaris lumbricoides* eggs. Canadian Journal of Zoology, 39: p. 153-162.

Gould, J. and M. Rossano. 2009. Efficacy of chemical disinfectants on inactivating *Parascaris equorum* egg development. Proceedings of the Equine Science Society. 29(5): p. 405-406.

Gould, J.C., Rossano, M.G., Lawrence, L.M., Burk, S.V., Ennis, R.B., Lyons, E.T., 2012. The effects of windrow composting on the viability of *Parascaris equorum* eggs. Vet. Parasitol. 191, 73-80.

Kmietsch, J., Samuels, S., Lehman, J., and S. Burk. 2017. The effects of benzyl isothiocyanate and papain on cyathostome egg hatching. Journal of Equine Veterinary Science. 52:42-43.

Morares, D., Levenhagen, M.A., Costa-Cruz, J.M., da Costa, A.P., Netto, Rodrigues, R.M. 2017. *In vitro* efficacy of latex and purified papain from *Carica papaya* against *S. venezuelensis* eggs and larvae. NIH. 59: e7 doi: 10.1590/S1678-9946201759007

Nagesh, M., Chandravadana, M.V., Sreeja, V.G., Bujji Babu, C.S. 2002. Benzyl isothiocyanate

from *Carica papaya* seeds- A potential nematicide against *Meloidogyne incognita.* Nematol. Medit. 30: 155-157

Naidoo, D. and Foutch, G.L. 2017. The time-temperature relationship for the inactivation of *Ascaris* eggs. National Library of Medicine. vol. 8(1), pp 123-126.

Negi, G.C. 2020. *Carica papaya* L. as herbal alternative to anthelmintics for the control of *Ascaridia galli* in poultry. Himachal Journal of Agriculture Research. 46(1): 100-108

Nielsen, M.K., et al. n.d. AAEP Parasite Control Guidelines. AAEP Parasite Control Subcommittee of the Infectious Disease Committee.

Nielsen, M.K., et al. 2010. Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. Veterinary Parasitology, 174(1-2): p. 77-84.

Nielsen, M.K., et al. 2014. Parascaris. Equine Infectious Diseases, Second Edition.

Nielsen, M. 2016. Evidence-based considerations for control of *Parascaris sp*p infections in horses Equine, Vet. Ed. vol. 28(4), pp. 224-231.

Peachey, L. E., Pinchbeck, G. L., Matthews, J. B., Burden, F. A., Behnke, J. M., & Hodgkinson, J. E. 2016. Papaya latex supernatant has a potent effect on the free-living stages of equid cyathostomins in vitro. Veterinary Parasitology, 228, 23-29. 10.1016/j.vetpar.2016.07.036.

Rawlings, N. D. (2015). Bacterial calpains and the evolution of the calpain (C2) family of peptidases. Biology Direct, 101-12. doi:10.1186/s13062-015-0095-0.

Reinemeyer, R.C. 2009. Diagnosis and control of anthelmintic-resistant *Parascaris equorum,* Parasites & Vectors, vol. 2(2).

Rew, R.S., Urban, J.F., Douvres, F.W. 1986. Screen for anthelmintics, using larvae of *Ascaris*

suum. NIH. 47(4):869-73.

Romero, J.J, Zarate, M.A., Ogunade, I.N., Arriola, K.G., Adesogan, A.T. 2018. Tropical plant supplementation effects on the performance and parasite burden of goats, National Library of Medicine, vol. (2)208-217. doi: 10.5713/ajas.17.0321

Samuels, S., Heskett, N., Burk, S. Efficiency of *Carica papaya* seeds for reducing equine strongyle fecal egg counts, Journal of Equine Veterinary Science, vol. 35, pp. 383-391

Shehnah, D. 2020. Egg hatching protocol and an in vitro scoring system in *Parascaris univalen*s larvae. after exposure to anthelmintic drugs. Biomedical Sciences and Veterinary Public Health, pp. 2-8.

Southwood, L.L., et al.1998. Ascarid impaction in young horses. Compendium on Continuing Education for the Practicing Veterinarian, 20(1): p. 100-106.

Srihakim, S. and T.W.1978 Swerczek, Pathologic changes and pathogenesis of *Parascaris equorum* infection in parasite-free pony foals. American Journal of Veterinary Research. 39(7): p. 1155-60.

Stepek, G., Lowe, A.E., Buttle, D.J., Duce, R., Behnke, J.M. 2007. Anthelmintic action of plant cysteine proteinases against the rodent stomach nematode, *Protospirura muricola, in vitro* and *in vivo.*

Thiese, M.S., Ronna, B., Ott, U. 2016. P value interpretations and considerations. Journal of Thoracic Disease. 8(9): E928-E931. doi: 10.21037/jtd.2016.08.16

Appendix 1A:

Trial 1 Papain ANOVA for Regression Summary Output

Trial 1 Papain
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.3746316
R Square	0.1403488
Adjusted R Square	-0.002926
Standard Error	0.0880853
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.00760056	0.00760056	0.97957543	0.36052232
Residual	6	0.04655418	0.00775903		
Total	7	0.05415474			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.82382793	0.04185556	19.682638	1.115E-06	0.72141105	0.92624481	0.72141105	0.92624481
X Variable 1	-186.8515	188.789414	-0.989735	0.36052232	-648.80255	275.099558	-648.80255	275.099558

Appendix 1B:

Trial 2 Papain ANOVA for Regression Summary Output

Trial 2 Papain
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.7430671 3
R Square	0.5521487 6
Adjusted R Square	0.4775068 9
Standard Error	0.2009728 7
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.2987778 7	0.2987778 7	7.39730 576	0.03465203
Residual	6	0.2423405 6	0.0403900 9		
Total	7	0.5411184 3			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.7201279 2	0.0954963 8	7.5408925	0.00028 201	0.4864567	0.9537991 3	0.4864567	0.9537991 3
X Variable 1	-1171.515 7	430.73615 8	-2.719798 8	0.03465 203	-2225.4891	-117.5423	-2225.489 1	-117.5423

Appendix 2A:

Trial 1 BITC ANOVA for Regression Summary Output

Trial 1 BITC
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.3516566 5
R Square	0.1236624
Adjusted R Square	-0.022393 9
Standard Error	0.2739074 2
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0635221 4	0.0635221 4	0.846676 46	0.39298553
Residual	6	0.4501516 6	0.0750252 8		
Total	7	0.5136738			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.5613097 9	0.1301527 3	4.3127009 2	0.005022 58	0.24283754	0.8797820 4	0.2428375 4	0.8797820 4
X Variable 1	540.17744 8	587.05353 3	0.9201502 4	0.392985 53	-896.2908	1976.6456 9	-896.2908	1976.6456 9

Appendix 2B:

Trial 2 BITC ANOVA for Regression Summary Output

Trial 2 BITC
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.6214774
R Square	0.3862341
Adjusted R Square	0.2839398
Standard Error	0.2049644
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.1586194	0.1586194	3.77571538	0.10000836
Residual	6	0.2520625 4	0.0420104 2		
Total	7	0.4106819 4			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.8264861 6	0.0973930 6	8.4860891 1	0.00014646	0.58817394	1.0647983 9	0.5881739 4	1.0647983 9
X Variable 1	-853.5953 8	439.29112 8	-1.94312	0.10000836	-1928.5021	221.31128 6	-1928.502 1	221.31128 6

Appendix 3A:

Trial 1 Pyrantel ANOVA for Regression Summary Output

Trial 1 Pyrantel
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.11971327
R Square	0.01433127
Adjusted R Square	-0.14994609
Standard Error	0.06647598
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.00038551	0.00038551	0.08723783	0.77767295
Residual	6	0.02651433	0.00441906		
Total	7	0.02689984			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.75572477	0.03158742	23.924863	3.502E-07	0.67843313	0.83301641	0.67843313	0.83301641
X Variable 1	-42.081485	142.47498	-0.295360	0.77767295	-390.70522	306.54225	-390.70522	306.54225

Appendix 3B:

Trial 2 Pyrantel ANOVA for Regression Summary Output

Trial 2 Pyrantel
SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.26095322
R Square	0.06809659
Adjusted R Square	-0.0872207
Standard Error	0.24431389
Observations	8

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0261699	0.0261699	0.438435	0.53247145
Residual	6	0.3581356	0.0596892		
Total	7	0.3843055			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.7971907	0.1160907	6.8669617	0.000469	0.51312692	1.0812546	0.5131269	1.0812546
X Variable 1	-346.7168	523.62703	-0.662144	0.532471	-1627.986	934.55238	-1627.986	934.55238