THE EFFICACY AND MACROSCOPIC EFFECTS OF ANTIHELMINTHICS ON THE TAPEWORM HYMENOLEPIS DIMINUTA

A Report of a Senior Thesis

by

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Spring, 2000

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Editor

ABSTRACT

The efficacy of the drugs Cestex® (epsiprantel) and Droncit® (praziquantel), developed for the Dipylidium canium tapeworm, was determined for the closely related tapeworm Hymenolepis diminuta. The cysticercoids of the H. diminuta were separated into three groups: the control; Cestex©, in a twenty-five percent concentration; and Droncit©, in a four percent concentration. The cysticercoids were examined to determine whether the length, width, or floatation had changed. This data was collected from time zero to four hours later at hour intervals. The p-value for the length change in the control group was 0.900, the Cestex© group was 0.310, and the Droncit© group was 0.600. The p-value for the width change in the control group was 0.927, the Cestex© group was 0.563, and the Droncit© group was 0.010. Thus, only Droncit© had a significant effect on the cystcercoids. However, a 20% floating rate showed that Cestex© was also effective. It is probable that both Droncit© and

Cestex© would be useful in treating other tapeworms than the D. canium for which they were developed. The expansion of these medications to more tapeworm groups could decrease the number of infestations and subsequent deaths caused by these parasites in third world countries. Future experiments should examine the mechanisms of the drug's macroscopic effects, thus validating the use of these procedures in similar culture-based systems.

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ACKNOWLEDGEMENTS

As usual, I ran into a lot of obstacles while conducting my experiment. Thankfully, I had patient and innovative supporters. Dr. Drew Crain helped in the endless rewriting of my thesis, especially the methods section. Thank you Debbie Threadgill for always knowing where everything was and having infinite resources. A special thank you to Dr. Karen Ott of Tennessee Weslyean in Athens, for helping me to, finally, identify the cysticercoids. To my mom and dad who were always ready to listen even if they did not understand a word I said. Thanks to Pam Bunde for her hours of corrections. Lastly, thank you Alan, for keeping me sane.

CHAPTER I

INTRODUCTION

An Introduction to Parasites

According to Despommier, and Karapelou (1995), over one hundred million people a year are affected by malaria; over one million die. Malaria itself is just one of thousands of parasitic diseases. Parasites are organisms that live on or dwell in other organisms and obtain nutrients from their host (Encarta, 1997). Parasites never benefit their hosts, but they do not always harm them. Parasitism is a symbiotic relationship. Symbiotic relationships consist of two organisms living in a physical coalition where one or both benefit and at least one cannot survive without the other. There are three main types of symbiosis: mutualism, commensalism, and parasitism (Smith, 1996). In mutualism, both organisms benefit from the relationship. Commensalism is where one organism benefits and the other does not benefit but is not harmed either. The final form of symbiosis, parasitism, differs from mutualism and commensalism by

one organism being negatively affected while the other is benefited.

There are two broad classes of parasites. Ectoparasites live outside of their hosts and usually attach to skin, hair, or gills; examples of ectoparasites are fleas and ticks. Endoparasites live within their hosts and can live in various areas of the body such as the intestine, heart, liver, stomach, and other tissues. Endoparasites include such organisms as hookworms, tapeworms, and heartworms. There are a few parasites that could be considered both ectoparasitic and endoparasitic. A common canine parasite referred to as “scabies” burrows into the skin and receives nutrients from the air and the dog. Therefore, scabies could be classified as both endoparasitic and ectoparasitic (Smyth, 1994).

Parasitic Classification Systems

Parasites can be classified in several different ways with regard to their relationship with their host (see Olsen, 1974, for review). Those parasites that visit their host only when they want food and leave when they are done eating are called temporary parasites. The parasites that stay in or attached to their host for long periods of time are called stationary parasites. Stationary parasites can be

further categorized as either periodic parasites or permanent parasites. Periodic parasites stay with a particular host only for a part of their development and then go to another host for the next development stage. Permanent parasites stay with a host for the majority of their lives. The only time they are not with their host is when they are being transferred to it. Parasites can also be categorized based on host fidelity: either definitive or intermediate hosts. Definitive hosts are ones in which the parasite can live in the host for its entire life. An intermediate host is one in which the parasite stays there only for some period. They depend on the host for some or all of their development. Parasites can also be categorized into microparasites and macroparasites. Microparasites are viruses and bacteria. Macroparasites are worms, flukes, mites, and fungi (Olsen).

Parasites can affect plants as well as animals. In both plants and animals, parasites show host specificity, not only for particular animals or plants but also for particular regions of that animal or plant. Plant parasites live in the roots, stems, leaves, or fruit. Animal parasites preferentially live in the heart, lungs, intestine, or blood. All

parasites have difficulty gaining entry to and exit from their hosts. A plant parasite can enter through the roots or cuts in leaves. In the United States alone there are over fifty-three million cases of parasitic diseases identified a year. The occurrence of parasites in the United States is shown in Table 1 (Spage, 1999).

Table 1.

The Occurrence of Parasites and the Diseases They Cause in Humans per Year in the United States.

Parasites	**Disease**	**Occurrence/yr.**
Entamoeba histolytica	Amoebiasis	2983
Giardia lamblia	Giardiasis	141
Cryptosporidium sp.	Cryptospiridosis	33
Plasmodium vivax	Malaria	560
Plasmodium falciparum	Malaria	350
Strongyloides stercoralis	Strongyloidiasis	<1,000,000
Trichinella spiralis	Trichinellosis	40
Enterobius vermicularis	Enterobiaisis	50,000,000
Echinococcus sp.	Hydatid disease	7100
Dirofilaria immitis	Pulminory Dirofilariasis	3
Vampirolepis nana	Rare	2,600,000

Source: (Adapted from Spage, 1999, p. 1).

An animal parasite can enter a host by penetrating the cutaneous layer or through ingestion. Once inside, the parasite can travel to its preferred destination by the circulatory system, pulmonary system, or digestive tract. Exiting can be achieved by exhalation of the host, or excretement secretion through the rectum, urinary tract, or skin. The parasite can also exit the host if the host is eaten by a predator (Raven & Johnson, 1996).

In order to enter a host, the parasite first has to be exposed to it. There are two types of transmission: direct and indirect. Direct transmission occurs when the parasite goes directly from one host to another. This can be achieved by falling onto the host, or being transferred by spores or saliva. Indirect transmission usually, but not always, includes an intermediate host. This transmission can occur through feces, water, air, or wind (Smith, 1996).

Host and Regional Specificity

Three criteria that compose host and regional specificity are: habitat, food, and reproduction. The range of habitats expanded as endoparasites evolved new systems and endoparasites now can occur in almost every tissue of the body. The most frequented places

are the digestive tract, lungs, liver, and the blood. There are also large numbers found in the nervous and excretory systems. The pH can range from 1.5 to 8.4 in these tissues, creating enormous obstacles for the adaptation of the endoparasites. However, certain aspects of these regions are highly appealing. In the digestive tract, mucus helps the endoparasites attach themselves and provides a source of nutrition. In general, endoparasites get their nutrition from the host's tissues, excretions, or the food the host eats. Another nutritional source is found in the duodenum and ileum of the host's intestinal tract. Both of these areas have numerous cells that slough off creating a nutrient-rich source of food. The blood is a source of carbohydrates, protein, and fats to endoparasites with good digestive systems. In addition to fats and carbohydrates, the blood has an excellent supply of vitamins and iron (Smyth, 1994).

Once the endoparasites obtain a source of energy, from the host, the parasites must catabolize the nutrients. Some endoparasites have digestive systems that can cause degeneration of blood and tissue, and others do not. Those that do not, such as tapeworms, are dependent on their hosts to break down these complex molecules for them. After catabolism, the

endoparasite then absorbs the products. The nutritional needs of endoparasites change with their development. Larva need a substantial amount of protein and fat. Therefore, the larva survive best in the intestinal tract and liver where there is a large amount of stored fat (Bouwens, De Bleser, Vanderkerken, Geerts, & Wisse, 1992). The diet of the host has a direct effect on the endoparasites as well. If the host does not take in a certain amount of vitamins and proteins needed by the endoparasite, the endoparasite could suffer major complications. Poor nutrition results in early death; reduced, or no, reproduction; and failure to reach maturation. Maturation can be completely dependent on the host. Often the trigger for maturation processes in the endoparasites are the sex hormone levels of the host (Hurd, Strambi, & Beckage, 1990). There are other factors that can attract and cause an endoparasite to mature. Often the pH change, the partial pressure of carbon dioxide, the presence of bile, or a change in temperature can stimulate maturation or the release of egg cysts (Smyth, 1994).

Detrimental Effects to Host

The relationship between an endoparasite and its host is extremely difficult to study. The difficulty

lies in trying to study the endoparasite in its environment. If the endoparasite is removed from the host, it is immediately detached from its life-sustaining systems and dies. An integral component to the endoparasite host relationship is the endoparasite's ability to conceal itself from the host's recognition system. Another primary concern is that oftentimes endoparasites have detrimental effects on their hosts. The problem of infesting a host and the host maintaining normal bodily functions becomes essential to the endoparasite's survival. If the host dies, the endoparasite dies. Consequently, either the host, the endoparasite, or both, have to alter their lifestyles in order to survive. Metazoan endoparasites have adapted body form to their preferred environment; they are elongated and ribbon-like (Bryant & Belm, 1990). According to Michael Sukhdeo, author of "Earth's Third Environment: The Worm's Eye View," the tapeworm Hymenolepis diminuta has adapted its feeding habitats for optimal nutrient concentrations. The H. diminuta possess four suckers, but they are not strong enough to attach the tapeworm to its host during powerful periods of intestinal peristalsis. The tapeworm has modified its feeding habits to no longer depend on its suckers. Instead,

the tapeworm generates a fixed action pattern that coincides with the peristaltic waves of the host. This fixed action pattern allows the tapeworm to travel with the nutrient source bi-directionally through the host's intestine (Sukhdeo, 1997).

There are many detrimental effects experienced by the host after parasitic infestation. Depending on the host, the endoparasite can cause decreased growth, decreased reproduction, or death. There are two types of effects that endoparasites can have on their hosts: injurious or defensive. Injurious effects include fatality and diseases. The endoparasite can destroy cells, digest tissue linings, and interfere with normal transfer of food and information from cell to cell. Endoparasites, such as hookworms, release chemicals that can have adverse effects on their hosts. Some of these chemicals are anticoagulants. Other chemicals released by the endoparasites are carcinogens. These are usually released by the endoparasite, while feeding, to maintain a steady blood flow rate. In addition, these chemicals can cause an enormous loss of blood for the host, even after the endoparasite detaches. The loss of blood directly correlates to a decrease in vitamins and nutrients for the host. In contrast to injurious

effects are defensive effects in which the host can solicit its immune system to work against the endoparasite. Antibodies have three main courses of action: to destroy the endoparasite itself, to wall off the infected tissues, and to repair damaged tissues (Olsen, 1974). Specific host functions most often affected include nutrition, metabolic processes, and muscular functioning (von Brand, 1979).

The loss of appetite is a common symptom of infection by endoparasites. In some cases, it is believed that the endoparasites absorb nutrients required by the host causing severe distensions of the host's body. These enlarged areas are usually found near the abdomen. Digestion of proteins and starches can be inhibited by the presence of endoparasites. There is some disagreement as to whether the endoparasites cause decreased digestion or interfere with absorption of these molecules. The digestion processes usually affected are those occurring in the small intestine. It has been discovered that, depending on the type of endoparasite, there can be decreased digestion of only one compound while other digestion processes and absorption remains normal. In some cases a decrease in enzyme activity has been noted but has not been directly linked to the

endoparasites. There are other factors within the host's systems that can cause loss of absorption of vitamins and a decrease in enzyme activity besides the presence of an endoparasite. The endoparasite could very well be responsible for this loss, but the host's induced inflammatory response could also be culpable. In addition, the host could have other elements acting on it such as an illness, a virus, or even environmental effects. Even if the endoparasite did take away from the host nutritionally, it is doubtful that the amount of vitamins it could consume would greatly hinder the health of the host. However, if the endoparasite were to preferentially digest certain vitamins and minerals essential to its host's survival, such as vitamin A in humans, then the endoparasite could have major repercussions on the host's system. By absorbing the host's digestive by-products as food, the endoparasite requires little energy to catabolize food sources. Consequently, the endoparasite uses fewer of its digestive enzymes and other substances (non-host) than it would use if having to digest its own food. This exchange of digestive by-products for decreased use of non-host substances creates a more stable environment for the

host because of the minimal amount of circulating non-host material (von Brand, 1979).

Coinciding with the belief that the endoparasites and their hosts have to establish an equilibrium for mutual survival, there are some endoparasites that can aid the host's digestion processes while harming other processes. This clouds the symbiont theory by suggesting that some organisms are capable of being both parasitic and mutualistic. An example of this type of endoparasite is the flagellates that live in the digestive tracts of cows and termites. The flagellates are a distinct group of organisms that can digest cellulose. Termites and cows consume large amounts of cellulose in the form of leaves and grass. As the cellulose material enters the digestive tracts of these animals, it is catabolized and utilized by the flagellates. The flagellates require very little of this material, though, and the majority of the degenerated cellulose products are used by the host animals. Without the aid of these endoparasites, the hosts would not survive; they cannot degenerate cellulose on their own (von Brand, 1979).

Endoparasitic-derived metabolic disruptions are more numerous than digestive disruptions. The type of endoparasite that causes the disruption determines the

specific effect on the host's metabolism. The alterations of carbohydrate levels due to protozoa usually decrease the host's carbohydrate level. The protozoans require a large quantity of sugars to survive. Therefore, they catabolize and use high amounts of the host's carbohydrate supply. This process has two paths it can take. First, the host could die. The decrease in available carbohydrates can result in liver failure and, ultimately, death of the host. Second, the host may live at a sub-optimal level while infected. Certain types of protozoa only partially degrade carbohydrates. The remaining carbohydrates and partially degraded by-products can still be utilized by the host (von Brand, 1979).

Compared to protozoa, the carbohydrate alteration due to helminthes is more complex. The host catabolizes carbohydrates as part of its normal functioning. Through normal metabolic pathways, the products can cause an increase in glycogen levels of the host. The glycogen builds in concentration in the host's blood and liver. The high blood sugar content often results in diabetes (Turner, Chen, & Piletsky, 1999). There have not been any definitive studies showing that arthropods have a negative or positive

effect on host carbohydrate metabolisms (von Brand, 1979).

In vertebrates, the phospholipid, triglyceride, and cholesterol levels are often altered by endoparasites (Tsuboi & Hirai, 1986). The cholesterol level is usually significantly decreased while the phospholipid and triglyceride concentrations increase. Phospholipid change varies, causing it to be difficult to predict. The changes depend on the fatty acid concentrations in the host and on the type of endoparasite. For invertebrates, the lipid concentration varies even more randomly. They can increase, decrease, or remain constant in response to endoparasites (Tsuboi & Hirai). Most of the time the concentrations will increase, especially if the lipids are acidic. The more neutral the pH of the lipid, the less likely there is to be any change in the concentration.

Loss of proteins also creates major problems for hosts. Protein loss most often occurs when there is a loss of blood, for example when a tapeworm attaches to the host and then detaches leaving holes and anticoagulant behind. Proteins can also be lost by leakage into the intestines and by being excreted. Decrease in protein concentrations can be seen in

conjunction with all types of endoparasitic infections. There are exceptions; a few helminthes can cause an increase in protein concentrations (von Brand, 1979).

Another effect the endoparasites can have on their hosts is that of altering muscular functioning. The most common result of an endoparasitic infection is that the host's muscular system tends to decline in weight and workability (Dwinell, Wise, Bass, & Oaks, 1998). Infestation of the endoparasite causes degeneration of the muscle fibers, which leads to quicker fatigue. Another explanation for muscle fatigue is that endoparasites can actually increase the potassium and sodium concentrations of the muscle. This overabundance of sodium and potassium causes overuse and overstimulation of the muscle fibers. The addition of this strenuous behavior increases the host's probability of an early death (von Brand, 1979).

Phylogenetics and Tapeworm Characteristics

The massive number and diversity of endoparasites make it necessary to choose a specific organism in order to conduct an in-depth study. This study will focus on a medically important parasite group: the helminthes. According to a press release given by the

World Health Organization on December 26, 1995, helminth infections affect more than 1.4 million people worldwide (“Study,” 1995, p. 1). The order of the phyla and classes of helminthes within the kingdom Animalia can be seen, in bold print, in Figure 1. There are three phyla with helminthic parasites: the Nematoda, the Annelida, and the Platyhelminthes. In the phylum Nematoda are the classes aphasmida and phasmidea. The nematodes are the roundworms; they are not segmented but are bilaterally symmetrical. The phylum Annelida has three classes: the hirundinea, the polychaeta, and the oligochaeta. The hirundinea are commonly known as leeches; they are flattened, external parasites, with suckers that take up the host’s blood.

Kingdom Animalia	Phylum Rhynchocoela
Subkingdom Parazoa	**Phylum Nematoda**
Subkingdom Eumetazoa	**Class asphasmida**
Phylum Cnidaria	**Class phasmida**
Phylum Ctenophora	Phylum Rotifera
Phylum Platyhelminthes	Phylum Loricifera
Class turbellaria	Phylum Bryozoa
Class trematoda	Phylum Brachiopoda
Class cestoda	Phylum Phoronida

Phylum Mollusca

Phylum Annelida

Class polychaeta

Class oligochaeta

Class hirundinea

Phylum Arthropoda

Phylum Pogonophora

Phylum Onychophora

Phylum Echinodermata

Phylum Chaetognatha

Phylum Hemichordata

Phylum Chordata

Figure 1. Phylogenetic Classification of Kingdom Animalia. (Adapted from Raven & Johnson, 1996, p. 1249-1252.)

The Platyhelminthes contain three classes, the two of interest are the trematoda and cestoda; commonly called the flatworms. They lack a circulatory system and posses one gut opening and a complex reproductive system. The trematoda are called flukes; they use more than one host and posses digestive tracts. The cestoda are the tapeworms; they do not have digestive tracts but instead absorb their nutrients through their body walls.

The specific helminth used in this study can be found in the class cestoda, the tapeworms. According to Despommier and Karapelou (1987), all tapeworms live inside their host's digestive tract. Any vertebrate can be a host for tapeworms, but there are only a few that can live in human hosts. Most tapeworms require an intermediate host. Intermediate hosts are usually small invertebrates or vertebrates such as fleas, ticks, and arthropods. The definitive hosts most

often sharing the same tapeworm infections as humans are mammalian hosts such as dogs, cats, pigs, or cows. Tapeworms can range from a few centimeters to over ten meters in length. As can be seen in Figure 2, the adult tapeworms have two main body sections: a head and a segmented body. The head, or scolex, attaches to the host itself. The scolex possesses one, or a combination, of the following: suckers, grooves, or

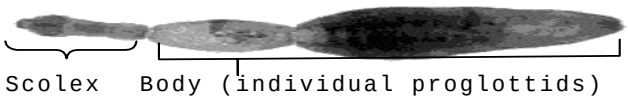


Figure 2. Tapeworm Anatomy (Adapted from Encarta, 1997).

hooks. These apparatii are the physical parts that attach into the host's flesh. The segmented body is actually a series of proglottids. The proglottids start to form next to the neck and move outward as new proglottids are added. These proglottids can be seen in Figure 3 as A and B, respectively. As the egg

developing process continues, the maturing proglottid is found more distal to the scolex (Figure 3, C). The proglottids carry both the egg and the sperm allowing self-fertilization to occur. The last proglottid segments are the mature, gravid sacks. These final proglottids detach from the body and are usually passed out the host. The proglottids are ingested by an intermediate host. Inside the host, the egg sack degenerates in the small intestine, releasing the embryo. The embryo enters the intestinal tract and matures into the larva form, termed cysticeroids. The primary host with the cysticeroids needs to then be

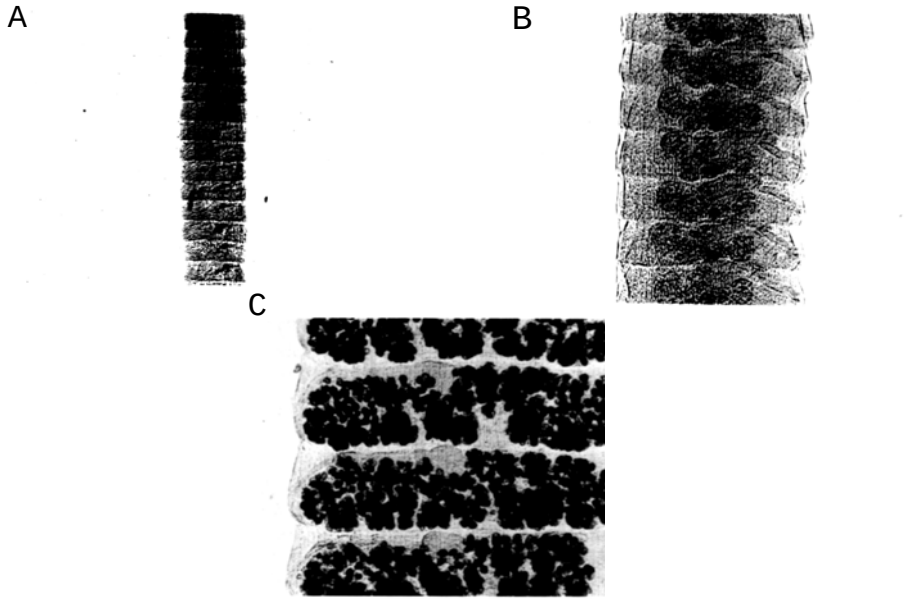


Figure 3. Immature (A), Mature (B), and Gravid (C) Proglottids of the Hymenolepis diminuta, in clockwise order from top. (Taken from Despommier, Gwadz, & Hotez, 1995, x22, p. 34).

ingested by the definitive host, where it will mature into an adult tapeworm. One factor that can prevent the cysticercoids from developing is an overabundance of tapeworms in the host. This extreme infestation prohibits the cysticercoid from reaching a stage where it can overcome the host's defensive reactions (Hopkins & Andreassen, 1991).

Tapeworms have physiologically adapted their anatomy to benefit more readily from their host. They do not have a digestive tract but receive their nutrients by absorption through the cutaneous layers. The outer covering has many small cilia-like projections that assist the tapeworm in attachment to the host and in nutritional absorption. The internal tissues of tapeworms are composed of muscular, nervous, excretory, and reproductive systems. The musculature runs laterally and transversely allowing

the tapeworm to have controlled movements. The scolex contains ganglia that may aid in locating nutrient gradients. The scolex on the H. diminuta, Figure 4, has four suckers, whereas most tapeworms have one or two suckers (Despommier et al., 1995). The excretory system

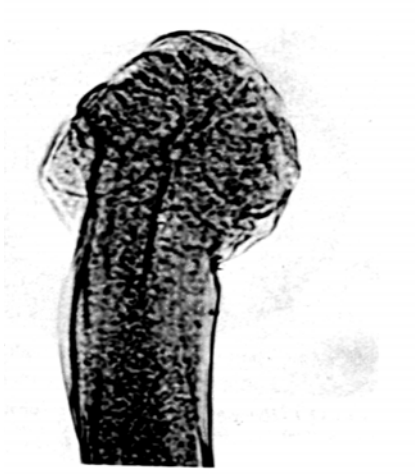


Figure 4. Hymenolepis diminuta Scolex. X 210. (Taken from Despommier et al., 1995, p. 34)

contains two tubules that run laterally and carry waste to the host's lumen. Tapeworms contain flame cells that help in the movement of fluid. Each tapeworm is hermaphroditic, as mentioned previously.

However, if two tapeworms occupy the same host, they may exchange sperm with one another. The tapeworm does not usually result in death of the host but can cause excessive bleeding, loss of energy, and improper digestive functioning. Due to the loss of blood, and the proglottid's ability to absorb low-molecular weight substances, tapeworms commonly reduce levels of vitamin B12 in the host which can lead to anemia. In general, tapeworms are easy to diagnose, as proglottids (or eggs) can usually be found in the stool of the infected host. These proglottids resemble rice grains; they are small, oval, and white (Despommier & Karapelou, 1987).

The H. diminuta is the tapeworm used in this study. It is most closely related to the Hymenolepis nana and the Dipylidium caninum. The D. caninum is found most often in dogs and cats. The H. diminuta is usually found in rats, but other definitive hosts can be mice, dogs, and humans. The intermediate host for the H. diminuta tapeworm has a large range consisting mostly of insects such as beetles and fleas. The tapeworm travels by indirect transmission, usually by the consumption of feces belonging to the definitive

host. Symptoms are usually prevalent only when there is infestation by more than ten worms. As the number of worms grows, so does the occurrence of abdominal disruptions. These disruptions include regional pain, anorexia, and irritability. They are caused by the scolex's attachment to the walls of the lumen.

The H. diminuta life cycle is characteristic of most tapeworms. It is most closely related to the lifecycles of the H. nana (tapeworms of mice) and the D. caninum (tapeworms of dogs and cats). The eggs are ingested by the intermediate host (Figure 5, 6a). The eggs hatch in the host's intestine, enter into the lumen, and transform into their cysticercoid form (Figure 5, 6b). The most common means of consumption of the intermediate host is by eating contaminated grains and flour. Once inside the definitive host, the cysticercoid is revealed as the intermediates host's tissues are digested by the definitive host's intestinal secretions (Figure 5,2). The scolex emerges from the cysticecoid first and attaches to the walls of the lumen with its four suckers (Figure 5,3a). The tapeworm reaches maturity in about twenty days (Figures 5,3b)). Its length is an average of

thirty centimeters, slightly smaller than that f the H. nanaor the D. caninum (Figure 5, 4b). The gravid proglottids contain a single genital pore from which the eggs are passed out (Figure 5, 4a, and 5a). In addition, the eggs are released when gravid segments themselves fall off the scolex into the lumen and are excreted out of the host with the feces (Figure 5, 5b). At this point, the cycle begins again with the in

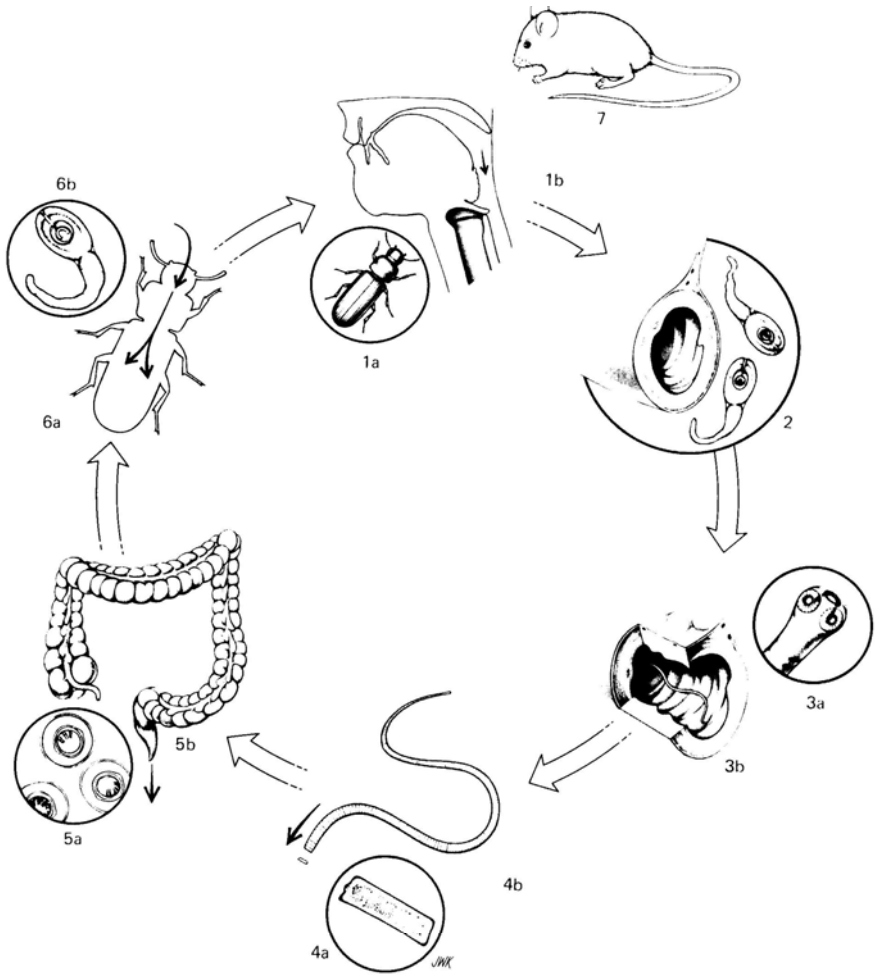


Figure 5. Life Cycle of the Hymenolepis diminuta. The intermediate host is eaten by the definitive hosts (7, 1a, 1b). After cysticercoid is revealed (2), the scolex emerges (3a). The tapeworm reaches maturity (3b) and grows to approximately thirty centimeters in length (4b). The gravid proglottids contain a genital pore (4a, and 5a) through which eggs are excreted out of the host (5b). These eggs ingested by the intermediate host (6a) and transform into cysticercoids (6b). The cycle starts over (taken from Despommier & Karapelou, 1987, p. 17).
larva ingesting the eggs (Despommier & Karapelou, 1987).

Tapeworm Treatment

Treatment of tapeworms is relatively easy as there are several medications that kill the worms and destroy their eggs. There are a number of medications used to treat dogs and cats; of these, two will be used in this study. The first drug is Cestex®. Cestex® is manufactured by Pfizer Animal Health Division (New York City, New York) and is used for

treatments in cats and dogs. It is a tablet composed of the compound epsiprantel. It is sparingly soluble and is therefore not readily absorbed by the host's intestine. Epsiprantel acts directly on the tapeworm itself by altering its calcium regulation. The tapeworm contracts violently causing damage to itself; this leaves the tapeworm vulnerable to its host's digestion processes. Digestion by the host makes it highly unlikely to observe proglottids or the worm itself in the stool after treatment. There are no known side effects of Cestex® at up to thirty-five times the recommended dose (Pfizer Animal Health, 1999).

Another drug commonly used to treat tapeworms is Droncit®, manufactured by the Bayer Corporation (Pittsburgh, Pennsylvania). Droncit® is the drug praziquantel and is safe for humans as well as small animals. It is available in a tablet or injectable form. Droncit®'s mode of operation is much the same as epsiprantel's. Droncit® causes permeability in the worm's integument resulting in calcium loss. The loss of calcium results in contractions and sporadic paralysis leaving the tapeworm accessible to digestion

by the host. Droncit®, however, has several side effects occurring in less than five percent of those treated. These side effects are reversible and vary from animal to animal. In dogs, Droncit® can cause anorexia and lethargy. In cats, the main side effect is excess salivation. Humans experience the most side effects with dizziness, headache, and nausea. Tapeworms can be controlled externally by good flea control, appropriate waste disposal, and checking grains for contamination by insects before processing (Bayer Veterinary Services, 1999).

Two important factors regarding all drugs, efficacy notwithstanding, are the cost of treatment and the resistance factor. While all organisms are capable of developing drug resistance, there has not been any evidence to suggest that tapeworms have developed resistance to either of the aforementioned drugs. The cost of treatment of tapeworms differs with the medication, dosage, and length of treatment. Cestex® is recommended at 1.25 milligrams per pound of body weight for cats and 2.5 milligrams per pound of body weight for dogs. It is a one-time dose but can be re-prescribed if the infection reoccurs. The cost

of the 12.5 mg pill, the smallest dose available, from a veterinarian averages two dollars and seventy-five cents (Pfizer Animal Health, 1999). Droncit® is also a single dose medication. The recommended amount for dogs and cats is 0.2 milliliters per five pounds of body weight. The tablet form is two dollars per pill and the liquid injectable is three dollars per milliliter (or cc) from a veterinarian (Bayer Veterinary Services, 1999).

The Experiment

Endoparasitic infestations are a concern for both animals and plants. However, these infections can be controlled through proper identification and treatment. Two tapeworms that can infect humans, as mentioned previously, are the H. diminuta and the D. caninum. There are multiple medications for the D. caninum which have been effective in controlling the endoparasite at the definitive host level. However, these medications are not regularly used on the H. diminuta. Due to the two endoparasites' extremely similar life cycles, metabolic requirements, and definitive host preferences, it seems as though a medication used for one should be effective on

another. This outcome would expand the medicinal use and, in turn, control more endoparasitic outbreaks.

Droncit® and Cestex® are offered in different phases. Droncit® is a liquid while Cestex® is solid. The difference in these forms could have an effect on how the medication affects the hosts at different times during exposure. Droncit®, being a liquid, is hypothesized to be the most effective due to the ability of a liquid to be expediently absorbed by the tapeworm. If in fact this is the case, researchers could then try to transfer other medications to a liquid form and possibly create new ones. The effects of this knowledge could influence medication forms for other endoparasites as well.

The component that will be investigated in this study is the efficacy of the medication. Efficacy of the medication will be determined, simply, by which medication kills the cysticercoid the quickest. The ova of the H. diminuta will be fed to tenebrio (mealworm) beetles. The tapeworm will develop into the cysticercoid within seven to ten days. The beetle will be opened and the cysticercoids removed. They will then be placed into RPMI medium. The medium will

be divided into three groups: control, with Cestex®, and with Droncit®. Within each of these groups, there will be differing pH measurements of 5.5, 6.5, and 7.5. The pH's are varying so as to determine whether the effectiveness of the drug is due to the acidity of the host's digestive tract pH levels. The cysticercoids will be observed at time intervals to determine their vitality. The drug that displays the fastest mortality rate on the cysticercoids will be declared the most effective.

The second study will examine histological effects of the drug on the tapeworm. There will be two groups in this study: a control and the most effective drug at the most effective pH. The total time of death will be divided into four time intervals. At each of these intervals, cysticercoids will be removed from the mediums and placed in a preservative. These preserved specimens will be cross-sectioned to determine any differences in the cysticercoids between the ones in the control group and the ones treated with the drug. Finally, the results will be combined to determine whether there is a correlation between the mortality rate and the

effects it has on the tapeworm's system. An additional outcome of this study would be to broaden the usage of drugs such as Cestex® and Droncit® to additional animals. By using H. diminuta with these medications instead of D. caninum, the final results will be able to determine whether or not these drugs would be effective against other tapeworms as well.

Chapter II

MATERIALS AND METHODS

Due to unforeseen complications in locating the cysticercoids and obtaining enough to conduct a multiple part experiment, the experimental design was changed. In the initial experimental design, I was going to determine cysticercoid viability to moniter drug efficacy. However, determining viability by methylene blue proved unsuccessful. I then tried Neutral Red and Janus Green B; Neutral Red to dye non-respiring cells (neutral bodies) red and Janus Green B to dye respiring cells green (Humason, 1979). When examined, the results were opposite from expected, making this procedure unreliable as well. As a result

of these problems, the pH-manipulation portion of the experiment was not conducted, and only the macroscopic changes between the cysticercoids of the control, Droncit© and Cestex© groups were observed. The aspects monitored were length, width, and flotation. Using these criteria, the data were assessed to determine whether the medications, Droncit© and Cestex©, had an effect on the cysticercoids in relation to the control group.

Tenebrio beetle larva were ordered from Carolina Biological Supply Company in Burlington, North Carolina. Upon arrival, the beetle larva were transported to a plastic container, about eight by twelve inches, with punctured lids. Lining the containers were slightly dampened paper towels. As the larva hatched into adult tenebrio beetles, they were transferred to another plastic container of the same size. These containers were lined with one layer of paper towels scattered with mealworm feed, covered by another layer of paper towel also scattered with mealworm feed and slightly dampened with water. When the beetles had all hatched, the ova of the Hymenolepis diminuta, in rat feces, were also ordered from Carolina Biological Supply Company (Burlington,

North Carolina). The beetles were transferred again to a new container without mealworm food in it. The beetles were kept slightly damp but without food for three days.

The ova arrived on the fourth day. The ova were mixed with one cubic centimeter of frozen egg yolk and enough water to form a sort of paste, as per package directions provided by the supply company. This mixture was placed on a six-inch diameter filter paper, placed into the container with the beetles and moistened with water. The beetles were not given any other food until the mixture had been consumed. On the third day following the introduction to the ova, the filter paper was removed and mealworm food was added. The beetles were given food and the paper towels moistened as necessary over the next eight days.

On the ninth day a beetle was chosen from the container and placed on a large plastic weigh boat. Using dissecting scissors, forceps, and pointers, the beetle was dissected. The head was removed first followed by the wings. Next, the beetle was turned dorsally and the shell was cut open. Finally, the beetle’s intestines were scraped out its shell into a

six percent saline solution. The intestines were examined for the small, translucent, tadpole-like bodies of the cysticercoids. Once the cysticercoids were identified, the remainder of the experiment was set up.

Five-milliliter petri dishes were designated either as Cestex©, Droncit©, or Control. One and a quarter milligrams of pulverized Cestex© pills were added to the Cestex© marked petri dish, giving a final concentration of twenty-five percent. Twenty microliters of liquid Droncit© were added to the Droncit© designated petri dish, yielding a four percent concentration. Fifteen milliliters of sterile RPMI-1640 medium, acquired from Sigma Chemical Company (St. Louis, Missouri), was divided equally into three sterile containers, and five milliliters was placed into each of the petri dishes.

Beetles were dissected, as described above, until there were ten cysticercoids for each petri dish. The cysticercoids were then placed into these dishes, the initial length and width were measured with a calibrated ocular micrometer at 40x, whether they were floating was assessed, and time was noted. This data was collected from time zero to four hours later. At

one-hour intervals, the cysticercoids were examined to determine whether the length, width, or flotation had changed. Statistical analysis of the data were conducted using Microsoft Excel. Length and width changes over time were assessed using simple linear regression analysis.

CHAPTER III

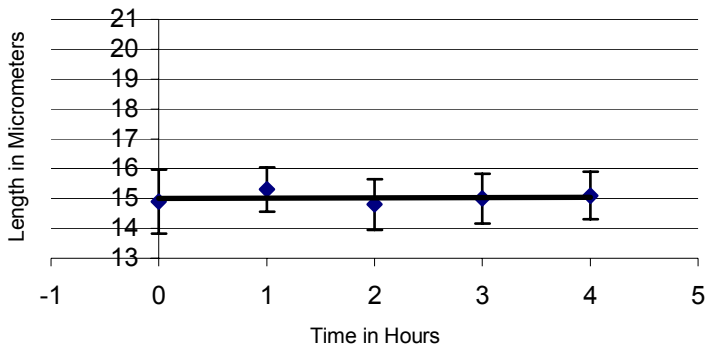
RESULTS

For cysticercoids in the experimental and control groups, observations were made on cysticercoid lengths and widths, and any recurring abnormalities were noted. The results of this experiment are presented in Figures 6-10, which will appear on the next 5 pages.

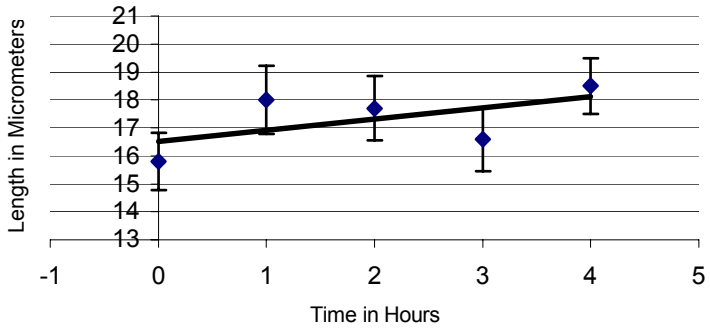
Figure 6 shows the change in the cysticercoid lengths over time. The length change is noted by using the mean length at each of the 5 time intervals. Each of the three groups (Control, Cestex©, and Droncit©) has its own graph as A, B, and C, respectively.

The change in the cysticercoid width over time is seen in Figure 7. As with the length, the width change is measured by using the mean width at each time interval. Each of the three groups (Control, Cestex©, and Droncit©) has its own graph as A, B, and C, respectively. During the experiment, some of

A



B



C

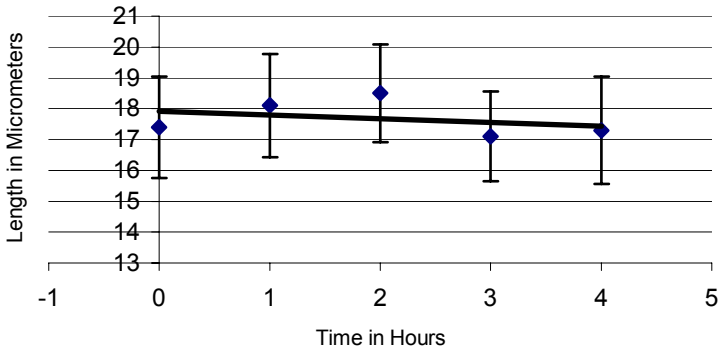
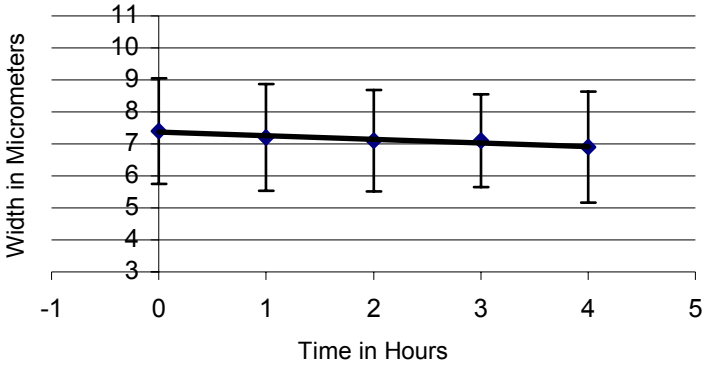
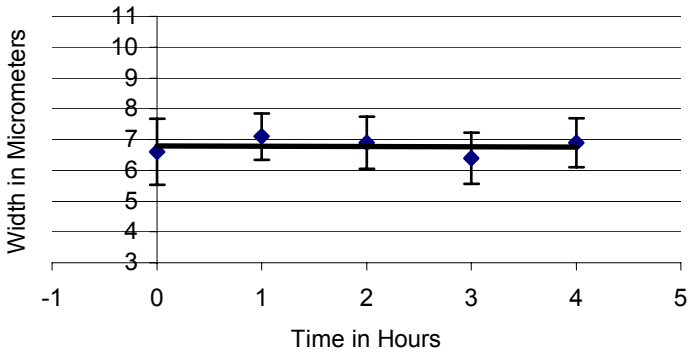


Figure 6. Mean Cysticercoid Lengths (±1SE) at Hour Intervals. The Control group is figure A, the Cestex© group is B, and the Droncit© group is C.

A



B



C

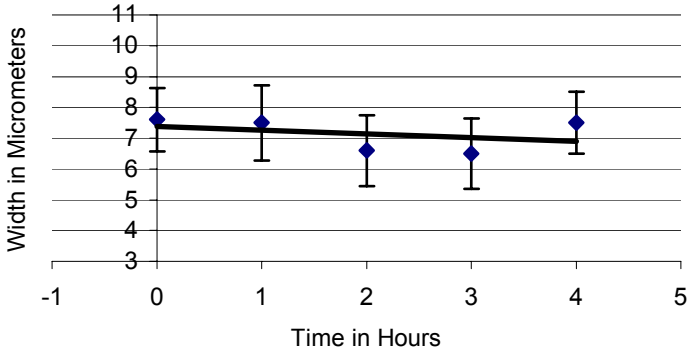


Figure 7. Mean Cysticercoid Widths (±1SE) at Hour Intervals. The Control group is figure A, the Cestex© group is B, and the Droncit© group is C.

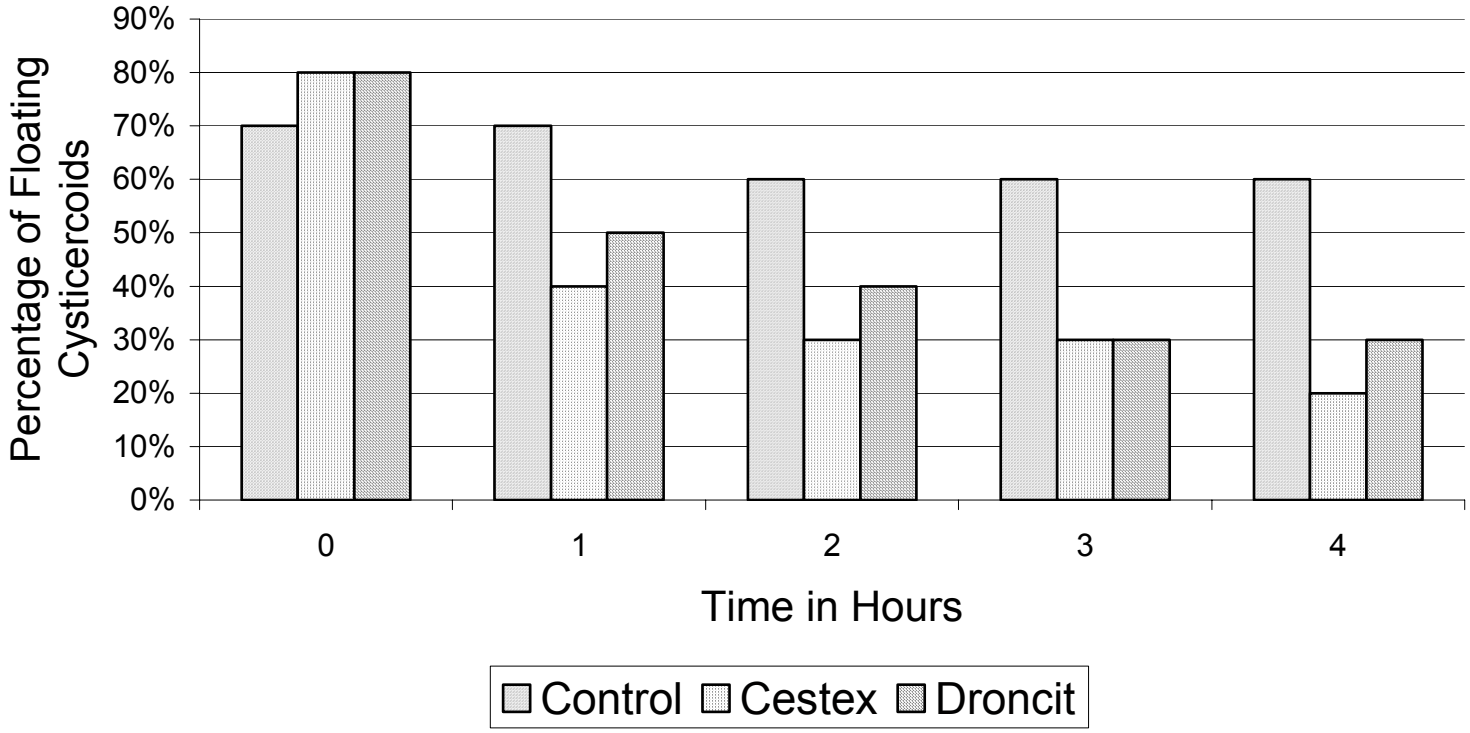
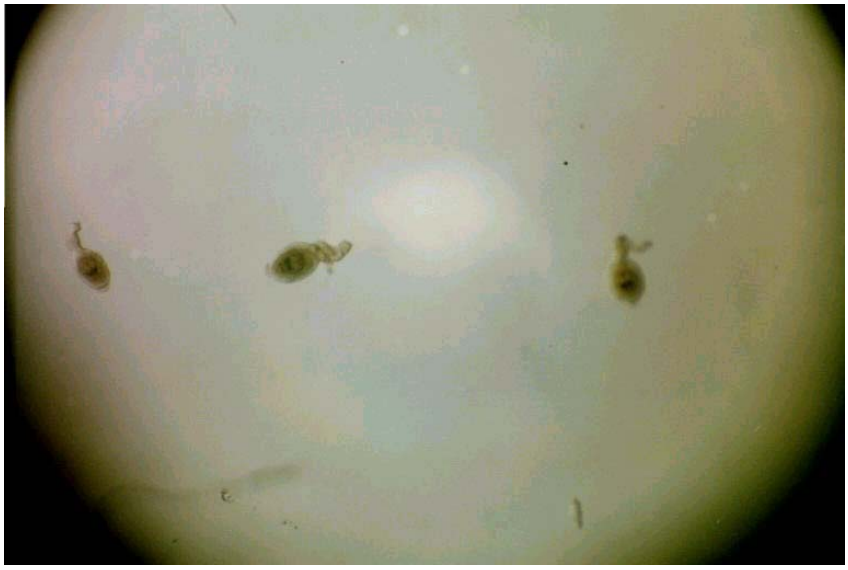


Figure 8. Cysticercoid Floating Percentages. The percentages of cysticercoids floating at each hour in the three groups.

A



B

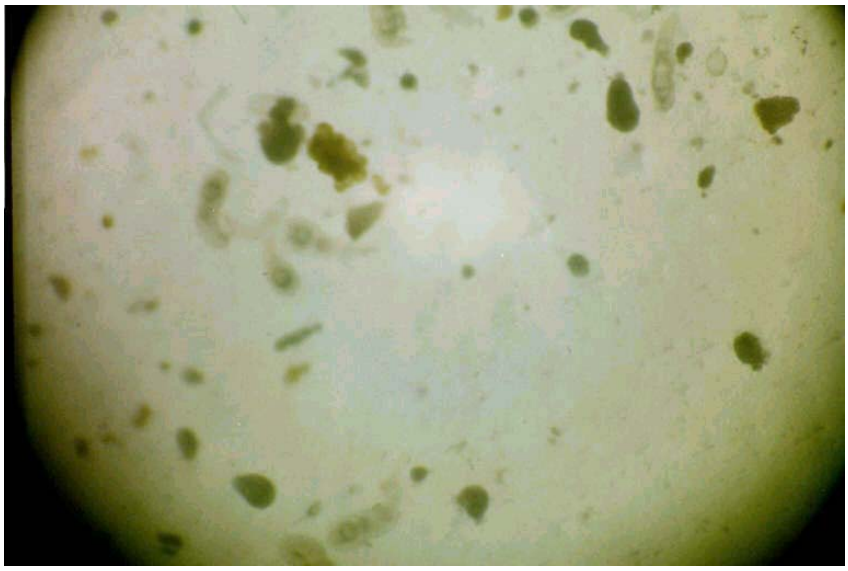
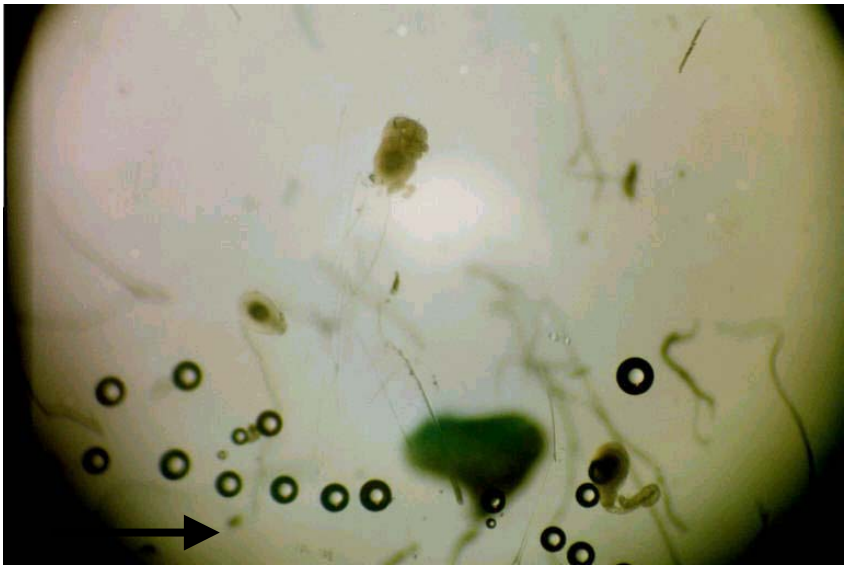


Figure 9. Floating and Sunken Cysticercoids. A. Normal floating cysticercoids, characteristic of all

cysticercoids at the onset of the experiment. B. Cysticercoids that have sunk, such as those in hour 4 the Cestex© group.

A



B

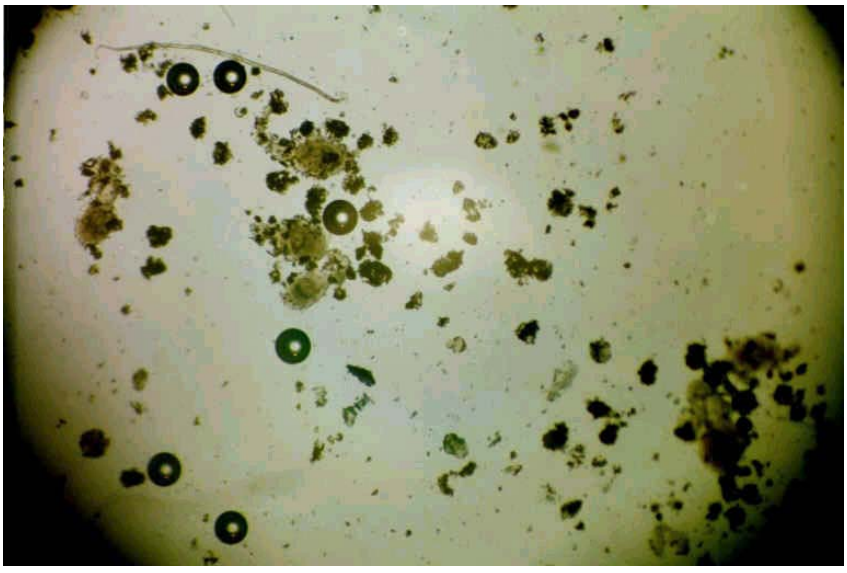


Figure 10. Cysticercoids Who Have Lost Tails and Are Surrounded by Debris. A cysticercoid, which has lost its tail, can be seen in the middle left of figure A (see arrow). Cysticercoids with attached debris are shown in B. The dark circular spots are air bubbles.

the cysticercoids, which had previously been floating, started to appear on the bottom of the petri dishes. The percentages of the number of cysticercoids floating versus the amount of time elapsed is presented in Figure 8.

Several abnormalities in cysticercoids were observed during the four-hour experiment. The first of these, previously mentioned, was that some of the cysticercoids ceased to float and sank to the bottom of the petri dishes. Figure 9 presents a photograph of floating cysticercoids (A) and cysticercoids that have settled to the bottom (B). The second of these abnormalities was less prevalent. Starting at hour two some of the cysticercoids starting losing their tails (Figure 10, A). The third irregularity also started at hour two. In Figure 10, B, the cysticercoids can be seen to have large quantities of debris attached to them.

In the statistical analyses involving linear regression, both the p-values and R^2 values were determined. The p-value is used to determine whether or not the slope of the line is different from zero, whereas the R^2 values will tell how closely the point fit the line. The p-value for the length change in the control group was 0.900, Cestex© group was 0.310, and Droncit© group was 0.600. The p-value for the width change in the control group was 0.927, the Cestex© group was 0.563, and the Droncit© group was 0.010. The R^2 values for the control length group, Cestex© length group, and Droncit© length group were 0.0067, 0.3314, and 0.1023, respectively. The R^2 value for the control group was 0.0032, the Cestex© group was 0.1229, and the Droncit© group was 0.9167.

CHAPTER IV

DISCUSSION

According to Hopkins & Andreassen (1991), it should have been simple to determine whether or not the cysticercoids were viable. In conducting the experiment, I found that not only were the cysticercoids non-mobile, but also that there was no easy determination of viability. After trying methylene blue to determine viability, I encountered two problems. First, if I were to use the dye, I would need many more cysticercoids than originally planned. The second problem was that the methylene blue occasionally dyed cysticercoids that were not viable, making it an unreliable detection method. I then tried Neutral Red and Janus Green B together. The Neutral Red was supposed to dye non-respiring cells (neutral bodies) red and the Janus Green B was supposed to dye mitochondria or respiring cells green (Humason, 1979). However, when examined, the live cysticercoids were dyed red and the nonviable

cysticercoids were green. Thus the Neutral Red and Janus Green B method was not reliable either.

Due to this complication, a new experimental approach was devised. The histological changes between the cysticercoids of the control, Droncit© and Cestex© groups were observed without regard to viability. The aspects monitored were length, width, whether the cysticercoid was floating, and any abnormalities. Using these criteria, the data were assessed to determine whether the medications, Droncit© and Cestex©, have an effect on the cysticercoids in relation to the control group.

Figure 6 shows the change in cysticercoid length over time by utilizing the mean length. The figure would suggest that the control group had little change in length over time while the Cestex© group became longer and the Droncit© group became shorter. The p-values for these three were 0.900, 0.310, and 0.600, respectively, indicating that there is no significant change. The R^2 values show a less than one percent line fit for the control group, a thirty-three percent line fit for Cestex©, and a ten percent line fit for Droncit©. The low percentage rates for line fit could explain why the data points for Cestex© and Droncit©

were not less than .05, and therefore, not significant.

The change in cysticercoid width over time, by utilizing the mean, is represented in Figure 7. The control group's width stayed relatively the same. The R^2 value is less than one percent. The p-value was 0.927, indicating there was not a sufficient change in the data. This is precisely what is expected of the control group. The Cestex© group and Droncit© group widths both decreased over the time period. In the figure they appear to decrease linearly by the same amount. However, the R^2 value shows a line accuracy of 12% for the Cestex© and 92% for Droncit©. This discrepancy can also be noted in the p-values. Cestex© is non-significant at 0.563, but Droncit© has a p-value of 0.010, making it a significant change.

Droncit© was the only group to indicate a significant change in width. This suggests that the cysticercoids are more vulnerable to Droncit© than to Cestex© within four hours of exposure. Taking the p-values for the lengths, while neither Cestex© nor Droncit© were significant, Droncit© was closer to being

significant than was Cestex©. Extended, this could mean that the Droncit© is more effective overall.

During the course of the experiment, some cysticercoids started to appear on the bottom of the petri dishes rather than floating on top of the media. While all three groups exhibited this abnormality, the percentages were different. The control group had 60% of its cysticercoids still floating by hour four. Cestex© had only 20% floating and Droncit© had 30% remaining on top of the media. Due to the control group's cysticercoids sinking, it can be assumed that exposure to the media, air, light, or other environmental factors had some effect of the sinking of the cysticercoids. However, compared to the controls, the Droncit© group had half as many floating and the Cestex© group had one third as many floating. The drugs had to have had a substantial effect on the cysticercoids because of the floating difference between the experimental and control groups.

If floating were a characteristic of vitality, then the Cestex© group would have had a higher fatality rate, with Droncit© close behind. This combined with Droncit's© significant change in width would suggest

that both drugs affect the cysticercoids but in differing ways. While Cestex© causes more cysticercoids to cease floating, Droncit© does this as well as causing them to expand outward. If there were a correlation between these two characteristics and death, the Droncit© would be more lethal than the Cestex©. In addition to floating, another abnormality was noted during the experiment; this abnormality started to occur at hour two in the Droncit© group. Some of the cysticercoids started to lose their tails. The most obvious explanation is that the Droncit© causes the cysticercoid to slowly die. In doing so the cysticercoid loses its tail as it is the narrowest part of the body and easily detached.

A third abnormality occurred in the Cestex© group, also at hour two. The cysticercoids started to have large amounts of debris attached to them. The cysticercoids themselves started appear, intermittently, as if they were mottled or cracking. This explains the debris if pieces of the cysticercoids were breaking off. Also, the Cestex©, being in pill form, had a coating. The pill itself could have clumped together in conjunction with the

coating to form dark debris floating in the medium. The attachment of the debris would be due to it containing the drug that was attracted to, and attacking the cysticercoid. Future experiments should examine the mechanisms of the drug's macroscopic effects (flotation, tail loss, and debris), thus validating the use of these procedures in similar culture-based systems.

The macroscopic changes in the cysticercoids suggest that the two drugs, Cestex© and Droncit©, had recognizable deleterious effects on the cysticercoids. The p-values indicate that only Droncit© had a significant effect on the cysticercoids. In response to these data, it is probable that both Cestex© and Droncit© would be useful in treating other tapeworms than the D. canium for which they were developed. The Droncit©, especially, has had an effect on the H. diminuta. The expansion of these medications to more tapeworm groups could decrease the number of infestations. These medications would be most beneficial studied on tapeworms infesting humans. A positive impact on these tapeworms could greatly decrease the enormous number of infestations and

deaths caused by these parasites in third world countries.

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