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Department of Pharmacology and Toxicology

Comparative Efficacy of Ivermectin, Fenbendazole, Pyrantel pamoate in the Elimination of Canine Ancylostomiasis

(Review of literature)

THESIS

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List of Abbreviations:

cSTHs- Canine Soil-Transmitted Helminths

BZ- Benzimidazoles

nAChR – Nicotinic Acetylcholine Receptor

GluCIR - Glutamate-Gated Chloride Channel Receptor

P2X – Primitive ATP-activated Receptors

MDR – Multiple drug resistance

LAMA- Larval Arrested Morphology Assay

LMA- Larval Morphology Assay

FEC – Faecal egg count

FECR – Faecal Egg Count Reduction Test

EPG – Eggs per gram

FHA – Egg hatch assay

LDA- Larval development assay

IC_{50} – Half-maximal inhibitory concentration

SC – Subcutaneous

IM – Intramuscularly

PO – Per Os

AUC – Area Under Curve

k_a – Absorption rate constant

$t_{1/2\ abs}$ – Absorption half-life

t_{max} – Time to peak drug concentration

C_{max} – Maximum concentration

MRT- Minimum residual time

1. Abstract

Gastrointestinal parasitism is one of the most common infections in dogs leading to widespread concern , notably in developing countries and economically distressed populations. The canine hookworm, *Ancylostoma caninum* is the most prevalent and important intestinal nematode parasite of dogs. However, in the past few years, cases of persistent canine hookworm infections appear to have dramatically increased, suggesting that anthelmintic resistance may have evolved in this parasite, posing an emerging threat. This study is being conducted as part of research work to compare the efficacy of three commercially available anthelmintic drugs against canine ancylostoma species with a major focus on *Ancylostoma Caninum* elimination. Fenbendazole, Ivermectin and Pyrantel Pamoate are the active substances being studied. The study takes place in order to determine their efficacy for treatment, control, and prevention. This is done by analysing each drugs pharmacokinetic properties, adverse effects & toxicity, dosage, and administration. An emphasis on the multidrug resistance is being expanded in the research as well as it poses an emerging threat nowadays. Results from a number of clinical trials testing these three active substances were used for the research.

2. Introduction

The term ‘’parasitism’’ has been defined in a variety of ways, indicating a lack of consensus on the specific meaning of the term. The definitions are divided into three categories. People who claim that a parasite injures the host and eventually kills it, those who claim that a parasite benefits from the host but does not destroy it and those who claim there is a state of equilibrium between individual hosts and parasites.(Crofton, 1971).

Canine hookworm disease is a general name for the infection caused by small intestinal nematode parasites in dogs. Among the gastrointestinal helminths, *Ancylostoma* spp. and *Toxocara* spp. are the most prevalent nematodes affecting canines, especially in neonates, posing a significant health hazard. Parasitized animals exhibit a range of symptoms depending on the density of gastrointestinal nematodes, with the most frequent and pathogenic nematode being the *Ancylostoma caninum.* (Lefkaditis, 2004).

Hookworms are hematophagous nematodes that infect many mammals, including humans. For Almost a century, scientist have speculated on the nature of the anticoagulant substances employed by these organisms to disrupt host haemostasis mechanisms. Hookworm infection is the leading cause of iron deficiency anaemia in underdeveloped nations, impacting around one billion people globally. Hookworms produce anaemia in their hosts by extracting their blood meal from lacerated capillaries in the small intestinal mucosa over time. (Stassens et al., 1996).

Ancylostoma caninum is a pathogen of increased importance in humans as well , because of its zoonotic nature it can cause Human eosinophilic enteritis. They reported having severe abdominal pain , diarrhoea , weight loss and melaena all of which were coupled with or soon followed by blood eosinophilia. (Strube and Mehlhorn, 2021)

Ivermectin, the first active substance that will be discussed, is a derivative of Avermectin BI, one of a set of naturally occurring compounds produced by *Streptomyces avermitilis* , an *actinomycete*. This antiparasitic medication has been shown to be effective against a range of nematode infections in mammals , including those caused by *Toxocara canis*, *Toxocara leonine*, *Trichuris vulpis* and *Dirofilaria immitis*. Ivermectin’s effect on hookworm infections was conflicting. (Wang et al., 1989)

Fenbendazole is a benzimidazole antiprotozoal and anthelmintic drug that is insoluble in water but soluble in dimethyl sulfoxide. Fenbendazole is effective against several Gastrointestinal and lung parasite in different animal species. In dogs and cats it has good efficacy against hookworms such as *Ascarids*, Whipworms and *Taenia*.(Panarella, 2002)

Pyrantel pamoate, a tetrahydropyridine nicotinic agonist anthelmintic, has been used to manage two key nematode families, hookworms and roundworms in companion animal medicine since the 1970s. (Kopp et al., 2008c).

In order to understand the principles of the nematode elimination, it is important to know the nematode's parasitological features such as life cycle, transmission, and symptoms.

3. Parasitological features

3.1 Human health hazard & occurrence

Humans and domesticated animals' relationship began about 15.000 years ago. This link has resulted in the dispersion of pets around the world and the transmission of their infections. (Traversa, 2012). Canine hookworm infections can be dangerous to human health as cutaneous larval migrans and eosinophilic enteritis can be a consequences of this zoonotic parasitic disease. Although *A. caninum* is not the most prevalent of the hookworm in terms of zoonotic potential, it can still pose a serious threat.

An example of an environment that is typical for infection transmission between animals and humans are shelters. Shelters provide an environment that is ideal for the spread of parasitic infections. It is established that the prevalence of most hookworms' infections is typically much higher in shelter dogs that in owned dogs. This can pose a health hazard to other animals , shelter staff and visitors. Therefore it is important that shelter workers adopt proper sanitisation, such as proper hand washing and wearing protective personal equipment to avoid risk of infection from hookworm larvae. (Raza et al., 2018).

Another ideal environment where the parasitical condition favours the spread of canine ancylostomiasis is the dog parks. This ideal urban space where dog and their owners can play and socialise is also an increased risk of expose to serious canine soil-transmitted helminths (cSTH). cSTHs are a category of nematodes infecting dogs which require a dormant stage in the environment and specific climatic conditions before becoming infective to the next host, *Ancylostoma caninum* is included into this category and the majority of cSTHs are also zoonotic. It is established that these parasites can complete its life cycle in humans. The increase prevalence of cSTH in parks was proven as part of an experiment which involved faecal sampling from different parks. 2 methods were used for the detection. The Faecal floatation test and the DNA extraction from the faeces and detected with qPCR assay. The prevalence of cSTH eggs contaminating faecal samples in park was 5.3% with hookworms being the most prevalent group of cSTH recovered from 4.5% of the faecal samples. (Massetti et al., 2022).

3.2 Life cycle & Transmission

Hookworms infections are more common in groups of dogs such as in kennels, pet shops and shelters, where the sentimental conditions favour the infection due to the large number of dogs and accumulation of faeces. (Traversa, 2012). The hatching and development processes of the hookworms thrive with high humidity and temperature, therefore, is exacerbated during the summer and wet seasons. The risk of infection increases drastically in unpaved areas where soil provides protection to hookworm larvae and diminishing sanitisation processes. (Raza et al., 2018).

In regard to human infection, the precise route has yet to be established. Percutaneous exposure may be possible in endemic regions where faeces contaminate the grass, and the soil and people frequently walk barefoot. In contrary the oral route has never been investigated or considered. An experimental human infection took place in the university of Queensland in order to investigate the possible routes for human infection by the hookworm *Ancylostoma caninum.* During the artificial infection trial experiment and monitoring of the parasite's progress it concluded that while there is no exclusion of percutaneous entry as a significant route of human infection it was observed that ingestion of *A. caninum* larvae might be more pathogenic. As it directly develops adult nematodes in the gut along with eosinophilic inflammation. Possible routes of oral infections are by drinking soil-contaminated water or eating soil-contaminated food. (Landmann and Prociv, 2003).

Four canine hookworms have been observed having zoonotic potential. *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, *Ancylostoma braziliense* and *Ancylostoma caninum*. *Ancylostoma caninum*, the most prevalent species, is found in both wild and domestic canine and feline species worldwide. Typically, the *Ancylostoma caninum* life cycle begins with mature male and female hookworm species in their host's small intestine. Females then proceed to release eggs following mating, which are shed in the hosts faeces. (Strube and Mehlhorn, 2021).

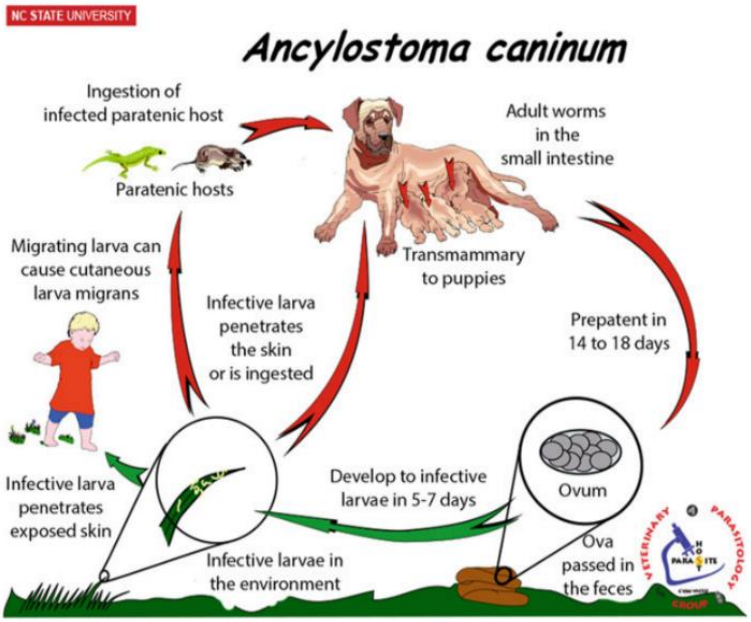

Fig.1. *Life cycle of the canine hookworm Ancylostoma caninum. Image by Strube and Mehlhorn, 2021, p.148*

Ancylostoma caninum is most commonly contracted orally by dogs by eating infected dirt, faeces, grass, grooming or drinking polluted water. (Strube and Mehlhorn, 2021).

Hookworm larvae hatch from eggs passing in the host's faeces, growing to the infective stage (filariform larva) in soil, and getting entrance to the definitive host most usually by skin penetration. (Bowman et al., 2010).

Larvae that enter the circulation through the skin are conveyed through the blood arteries to the heart and subsequently to the lungs and the larvae emerge here. Canine hookworms are ingested after exiting the pulmonary alveoli and ascending the bronchial tree to the throat. (Strube and Mehlhorn, 2021).

In response to the host signal, ingested larvae bypass migration and move directly through the stomach and into the small intestine , where they resume development. (Strube and Mehlhorn, 2021).

Worms may live everywhere in the digestive tract, including the colon, although they favour the jejunum and duodenum. Approximately 14 days after infection eggs develop in the stool. (Strube and Mehlhorn, 2021).

Hookworms are more prevalent in sub-tropical and tropical countries. Another way of transmission for puppies is nursing. When an adult dog gets infected it is established that larvae can invade different parts of the body. The larvae are dormant in the so-called resting stage for several years and are reactivated to infective stage during oestrus in the last 2-3 weeks of pregnancy. Some other factors that can trigger the infective stage is the stress, corticosteroid treatment and being subjected to a severe illness. This is the phase where they pass through the milk to the litter for a minimum 3 weeks after delivery. It is established that a bitch is in fact harbouring body larvae for three consecutive litters, although the larval output is reduced in each lactation phase. (Traversa, 2012).

3.3 Symptoms

Hookworms have been regarded as the most faithful intestinal parasites of dogs and cats ,causing developmental impairment, severe clinical signs, and high lethality rate especially in young ones. Some infections are asymptomatic and the extent of damage and appearance of clinical signs depend upon the tissues invaded, number of migrating larvae, host age and immune response of the animal. (Traversa, 2012).

The adult worms most commonly cause iron deficiency anaemia and blood loss. Young pups and kittens are most vulnerable to acute and peracute course of the disease, which can often be lethal. Therefore lethality is proven to be highest in young. (Bowman et al., 2010).

The Severity of the illness varies according on host age, resistance, worm burden–, ranging from asymptomatic infections to lethal exsanguinations. (Strube and Mehlhorn, 2021). It is a common misconception that intestinal worms are only a health hazard for puppies and kittens and that adults are mostly resilient to this kind of infection. The real truth is that pets are exposed to roundworms and hookworms throughout the year and all their lifetime. It was believed that parasitic burdens and infections rate were higher in puppies but nowadays it is proven that it can occur in dogs and cats of all ages the same way. (Traversa, 2012).

Hookworms are blood-feeding nematodes that anchor to the intestinal mucosa and submucosa with a cutting mechanism then contract their muscular oesophagus to produce negative pressure. They insert tissue into their buccal capsules which initiates mechanical and chemical injury by hydrolytic enzymes that cause capillary and arteriole rupture which both contribute to the initiation of bleeding. (Jimenez Castro et al., 2020).

Hookworms also secrete a variety of anticlotting agents to maintain blood flow. Therefore symptoms such as iron deficiency anaemia, hypoalbuminemia and enteritis characterized by diarrhoea that contain fresh (haematochezia) or digested blood are typical pathological outcomes of the infection. (Kalkofen, 1987).Canine ancylostomiasis less common symptoms is microcytic hypochromic anaemia , This is resulted in case of the moderate anaemia caused by the infection couldn't be compensated by the bone marrow hematopoietic activity. (Traversa, 2012).

A typical example of a very severe canine ancylostomiasis is accompanied with chronic iron deficiency anaemia , poor coat quality , diminishing weight , mucous presence in the stools , stunned growth , lack of stamina and poor general physical condition. (Traversa, 2012).

Juvenile *Ancylostoma caninum* species can burrow deeply and intensively into the mucosa which can in result cause life threatening consequences. After a milk borne infection puppies are more prone of being subjected to low iron reserves and therefore the infection is more crucial to them. Age is an important factor in regard to the outcome of canine ancylostomiasis infection. As the animal grows so does the resistance , regardless of if the animal had been infected several times by the infection in the past.

Other factors that play a crucial role of the severity of the disease is the state of nutrition , hematopoietic capacity and the presence of environmental stressors surrounding the animal. (Traversa, 2012)

A. caninum whole worm fractions were shown to have haemolytic activity. During an experiment that took place in the University of Queensland, Brisbane it identified a membrane-bound haemolytic protein from *A. caninum* and attribute its lytic mechanism to osmotic leaking via erythrocyte membrane pores. Particulate membrane fraction (PMF) was incubated with canine erythrocytes to identify the mechanism of haemolysis, and the effects on morphology were studied using scanning electron microscopy. (Don et al., 2004).

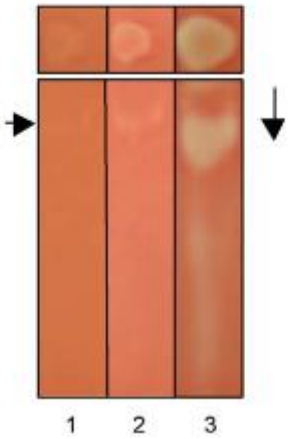

Figure 2 :Erythrocyte-agarose overlay on a native polyacrylamide gel showing zones of lysis in detergent-sobulised membranes from Ancylostoma Caninum. Overlay after 2hrs incubation time (Lane 1), after 4hrs (lane 2) and after overnight incubation (lane 3) at 37 degrees Celsius, data collected from (Don et al., 2004).

4. Resistance

The term resistance may be divided into two types: The first is the **Decreased sensitivity**, this can be described as genetically transmitted loss of sensitivity of a drug in worm population. The second one is the **True resistance** which refers to the heritable ability of some nematode parasites to survive treatment with anthelmintic drugs at the recommended therapeutic doses.

Multiple drug resistance or multidrug resistance (MDR) in *Ancylostoma caninum* species has been observed very frequently recently and this complication can be taken as an emerging threat to the pharmaceutical industries.

MDR to all anthelmintic medication classes licences for the treatment of hookworms in dogs in the U.S was found in various *Ancylostoma caninum* isolates according to a research done by (Jimenez Castro et al., 2019) using faecal samples from racing greyhounds. FECs (faecal egg counts) was performed on greyhound faeces and for the evaluation of drug response phenotypes in the experiment, EHA (egg hatch assay) and LDA (larval development assay) were used for both BZ and Macrocyclic lactones . The experiment showed a significantly high values of IC50 and IC95 from both Benzimidazoles & Macrocyclic lactones which proves the presence of MDR from the isolates. (Jimenez Castro et al., 2020).

Although greyhound's pharmacokinetic properties work differently from other breeds as they are deficient in CYP2B11 enzyme which impairs their efficiency of metabolizing some types of drugs. In addition another factor is the relative low body fat percentage which is frequent in greyhounds' breeds. This attribute could potentially influence the drug selection dynamics that may lead to the resistance in anthelmintic drugs because the lipophilic substance will have a lesser volume of distribution than expected. (Jimenez Castro et al., 2020).

The resistance of *Ancylostoma caninum* to Pyrantel has also been proven by several studies. This is considered an emerging problem in canine veterinary practice. These types of resistances in pet animals are very problematic because traditional resistance monitoring techniques such as faecal egg count reduction test, which are used in livestock animals are basically impractical for use in small animals. The use of LAMA (larval arrested morphology assay), LMA (larval motility assay), and LFIA (larval feeding inhibition assay) show promise in terms of detecting Pyrantel resistance in *A. caninum* (Kopp et al., 2008a). During this study two isolates were compared. The Pyrantel resistant (PR) isolate that show high level of Pyrantel resistant was collected from dogs in the Brisbane metropolitan area in 2005. The Northern territory (NT) isolates were collected from dog faeces from the ground of Northern territory in March 2006. After a 3-day course Fenbendazole treatment to ensure there were no other gastrointestinal parasitism , the first pair of dogs were infected orally with 300 PR isolate larvae and the second pair 300 NT isolate. (Kopp et al., 2008a).

Group	Dog	Expelled worms	Worms remaining in GIT	Efficacy (%)	Average of efficacy (%)
PR isolate	PR01	64	172	27.1	27.5
	PR02	60	155	27.9	
NT isolate	NT01	180	66	73.2	71.0
	NT02	178	81	68.8	

Table 1: In vivo efficacy of Pyrantel against the PR and NT isolates , data collected by: S.R Kopp et al

Despite the establishment of Pyrantel resistance in various helminth species during the last two decades , the mechanism of resistance remains unclear. It had been assumed that resistance may have developed as a result of changes in the relative proportions of nicotinic acetylcholine receptor subpopulations. To put this theory to test an experiment took place to put this theory to test. Two isolates of the canine hookworm *Ancylostoma caninum* with low-level resistance (isolate NT) and high-level resistance (isolate PR) to Pyrantel known to be selective to three nicotinic acetylcholine receptor subtypes. For this experiment both LAMA (Larval arrested morphology assay) and LMA (Larval morphology assay) was used for the detection of resistance to pyrantel in *A. caninum* by creating dose-response described by sigmoidal curves for all four agonist drugs. When the isolate NT was originally retrieved from the field , Pyrantel had a 71% efficacy against it while it had only 25% efficacy against the isolate PR. (Kopp et al., 2008b).

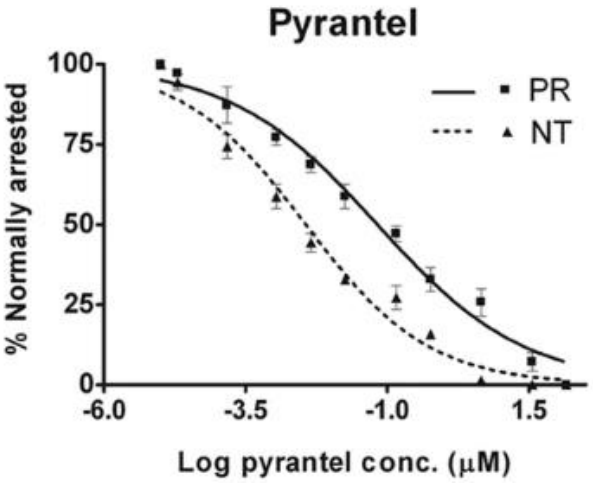

Figure 3 : Dose-response with sigmoidal curves determined by LAMA. The graphs depict the reduction in the percentage of normally arrested larvae with increasing drug concentration. Error bars represent standard errors. Data collected by Kopp et al., 2008.

According to LAMA, the Pyrantel IC50 for isolate PR was 0.066 whereas the IC50 for isolate NT was 0.0037 M giving a ratio of 18.3. The initial minimum of the response curve was around 70% for isolate PR and 50% for isolate NT , the subsequent recovery peaked around 90% for isolate PR and 70% for isolate NT. There was a significant divergence between the response curves across pyrantel concentration range of 0.001.to 0.58. (Kopp et al., 2008b).

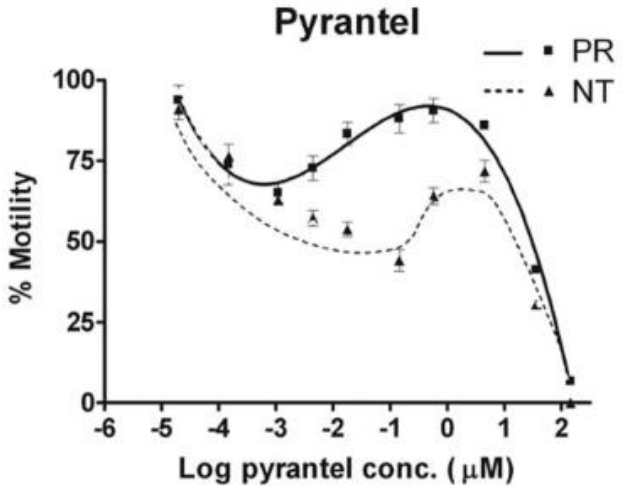

Figure 4 : Dose-response determined by LMA. Pyrantel responses describe fourth-order polynomial curves that were drawn by hand. the response to other drugs were fitted with sigmoidal curves. Error bars represent standard errors. Data collected by Kopp et al., 2008.

LAMA separates PR and NT (IC50 ratio , 7.2) compared to LMA (IC50 ratio, 2.7). However , the difference in IC50 was considerable when either test was utilized. There was no difference in responsiveness to the N-subtype against nicotine between these two isolates, suggesting that resistance to Pyrantel and Levamisole is accompanied with preserved vulnerability to nicotine at this stage of life. Despite the fact that were no differences in response to nicotine between isolates PR and NT in the larval stage , PR adults were more than twofold less sensitive to nicotine than their adult isolate NT counterparts, implying that Pyrantel and nicotine may have some overlapping effects on the nAChR subtypes in adult worms. According to LAMA and LMA tests, highly Pyrantel resistant isolate PR was nearly twice more sensitive to Bephenium than low-level Pyrantel resistant isolate NT. Despite the fact that Bephenium is a poor anthelmintic in its own right , the likelihood that sensitivity to the medication is increased in the face of Pyrantel resistance, this implies that treatments targeting the receptor subtype susceptible to Bephenium may be effective in overcoming Pyrantel resistance. (Kopp et al., 2008b)

A study conducted at the university of Georgia's school of Veterinary Sciences discovered a method to identify the rising prevalence of resistance-mutations. This was accomplished by extracting the DNA from *Ancylostoma caninum* eggs using extraction buffer from parasites treated with Fenbendazole. Afterwards real-time PCR was performed to determine whether polymorphism in codon 198 associated with drug resistance could be detected in reactions with genomic DNA. Real-time PCR was demonstrated to be particularly sensitive approach for detecting emerging mutations in the context of drug resistance in parasites and was shown that this approach can detect polymorphism in tests using genomic DNA from adult hookworms and hookworm eggs. (Schwenkenbecher and Kaplan, 2009).

Table 2 : Percentage of alleles estimated from difference in threshold cycles in real-time PCR with DNA from adult Ancylostoma caninum. The identifies of the isolates refer to the dogs they were recovered from. GA, Georgia, NC, North Carolina, f: Female , m: male, sus: Susceptible alleles , res: Resistance allele. Data collected by the experiments performed from Schwenkenbecher and Kaplan, 2009.

Isolate ID	167Sus	167res	198sus	198res	200sus	200res
GA 1208 m	99.99	0.01	99.76	0.24	99.81	0.19
GA 1208 f	100.00	0.00	99.99	0.01	100.00	0.00
GA 1210 f	100.00	0.00	99.99	0.01	99.95	0.05
GA 1213 m	100.00	0.00	100.00	0.00	100,00	0.00
GA 1213 f	100.00	0.00	99.87	0.13	99.95	0.05
GA overall	100.00	0.00	99.96	0.04	99.97	0.03
NC pool m	100.00	0.00	99.90	0.10	100.00	0.00

The weak correlation between FEC decrease and changes in adult worm load was a noteworthy finding in this investigation. While there was a substantial reduction in adult worm counts (25.73%) in the pyrantel-treated group m the faecal egg count increased in all pyrantel dogs after treatment. (Schwenkenbecher and Kaplan, 2009).

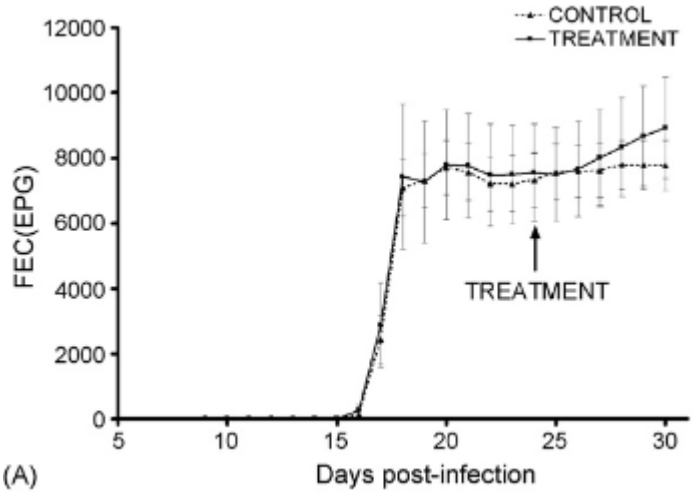

Figure 5: Mean egg count trends for the control and treatment group from infection (day 1) until euthanasia day (day 30) , data collected according to the experiment by Schwenkenbecher and Kaplan, 2009.

In this experiment we discover substantial levels of Pyrantel resistance in *A. caninum* isolates from Brisbane, with an effectiveness of just 25.7%. this is a significant decrease from an earlier report of 75.1%. Such a substantial increase in *A. caninum* resistance to Pyrantel during the last years should be a major concern to companion animal parasitologist and veterinary physicians. To reduce hazards to both dogs and people, most veterinarians suggest routine prophylactic use of anthelmintics. (Schwenkenbecher and Kaplan, 2009).

Widespread anthelmintic medication exposure can put parasitic nematodes under strong selection pressure to develop resistance. This has severely impacted benzimidazole anthelmintics for the control of livestock in many areas of the world. Understanding the mechanism of anthelmintic resistance is critical for rationally designing control mechanism to keep the present medication still viable. During an experiment that took place in university of Washington, it was exploited the first naturally occurring multidrug-resistant strain of the canine *Ancylostoma caninum.* In the research, this isolate was revealed to be resistant to Fenbendazole at the clinical dosage of 50 mg/kg for 3 days and to Ivermectin as well. Firstly a modified salt flotation approach was used to separate hookworm eggs from the infected dog faeces and then homogenized in water. After being filtered then washed by centrifugation in a buffer containing the Penicillin , Kanamycin and Ampicillin antibiotics. The eggs were allowed to hatch after incubation for 16-18 h at 27 degrees Celsius. A modified larval development assay (LDA) was used and a culture of *E. coli* strain OP50 was grown overnight at 37 degrees Celsius, the diluted medication was added in an equal volume to the corresponding well. The sigmoidal dose-response curve was used to compute the IC_{50} , and the average percentage of larvae growing to the L3 stage was plotted against the log of the drug concentration. Meanwhile after 3 days treatment courses of Fenbendazole each 3-5 weeks apart , the veterinarian was able to obtain faeces from a dog prior to the final treatment. The L3 emerging from the faeces was the resistant strain named KGR. In order to determine the phenotypic resistance of Benzimidazoles (BZ) we first determined the IC_{50} and IC_{95} of the wild type of sensitive strain named WMD, in this determination the LDA assay was used to determine these values. (Kitchen et al., 2019).

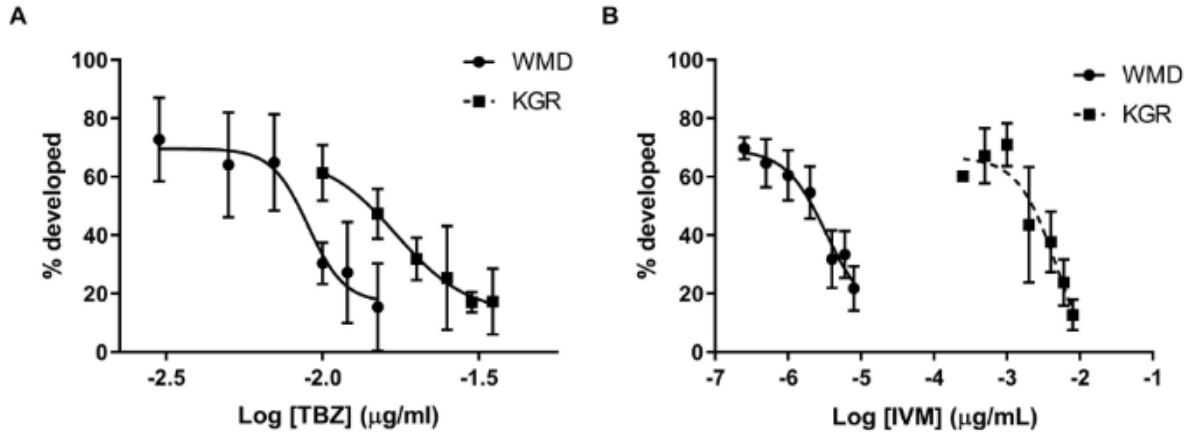

Figure 6: Dose response curve of Ancylostoma caninum susceptible strain WMD and resistant strain KGR to Thiabendazole (TBZ) and Ivermectin (IVM). Curves were analysed with the sigmoidal dose-response curve function in GraphPad Prism to generate the IC50 a

By amplifying and sequencing the tbb-iso-1 and tbb-iso-2 genes from WMD and KGR cDNA to compare them to the *A. caninum* reference genome was observed if any of these alterations were present in KGR-resistant worms. Although the efforts to identify

any of the three SNPs associated with BZ resistance in these samples were unsuccessful, it was confirmed that the KGR strain of *A. caninum* is the first confirmed report of BZ resistance in any hookworm species. Furthermore , the KGR strain is highly resistance to the macrocyclic lactone anthelmintic Ivermectin. This indicates that given the right conditions, resistance to routinely used anthelmintics can arise in wild populations of hookworms, posing a significant risk for current and future mass deworming programs.

5. Ivermectin

5.1 Mechanism of Action

Ivermectin is a combination of two chemically modified Avermectins that compromise at least 80% 22,23 – di hydroavermectin-B1a and more than 20% 22,23-dihydroavermectin-B1b. It is highly lipophilic compound that dissolves in most organic solvents but is almost completely insoluble in water. (González Canga et al., 2009)

The Macrocyclic lactones operate as unconventional agonist or allosteric modulators at a variety of vertebrate and invertebrate ion channels, Including glutamate receptors, some nicotinic acetylcholine receptors and P2X receptors at the molecular level. It is worth noting that the drug targets adult worms rather than larvae in gastro-intestinal nematodes, and there is strong evidence that inhibition of feeding via paralysis of the pharyngeal muscle may be the most important mechanism of action at this stage. (Wolstenholme et al., 2016).

Several members of this family have had their mechanism of action and analogues studied. Ivermectin, for instance it irreversibly activates GluCIR at doses ranging from 0.1 to 1μm, although at lower concentrations of 0.01μm, it induces potentiation of glutamate-gated currents. This was demonstrated in an experiment carried out by (Shan et al., 2001), his aim was to investigate the pharmacological profile of ivermectin by measuring the inhibitory potencies of strychnine, picrotoxin and zinc and measuring the percentage inhibition in a variety of dose administrations.

In addition studies carried out regarding the mechanism underlying Ivermectin potentiation shows that this chemical acts as a positive allosteric effector on the nAChR , this was studied by (Krause et al., 1998). His studied consisted of variety of experiments potentiating responses of the human a7 nAChR expressed in human embryonic kidney K-28 cells and *Xenopus laevis* human oocytes. Using a dose-response curve , it was demonstrated that ivermectin operates as a positive allosteric effector, since it enhanced efficiency on a partial agonist and had distinct effects on mutants within the M2 channel domain.

Finally regarding P2X channel receptors, these are trimeric cation channels that open in response to binding of extracellular ATP. According to an investigation it is suggested that Ivermectin interacts with regions of the channel proteins that are

entrenched in the lipid membrane to stabilize the channels open state (Silberberg et al., 2007).

5.2 Pharmacokinetic Properties

Ivermectin is perhaps one of the most extensively used antiparasitic medications in the world, and its efficacy is well documented. Drug pharmacokinetic parameters are intimately connected to pharmacological efficacy. The pharmacokinetic properties may be affected by a variety of factors such as route of administration , animal species ,body condition, age and physiological studies. Ivermectin kinetics are generally characterized by a slow absorption process, wide distribution in the organism, low metabolism, and delayed excretion. (González Canga et al., 2009).

Absorption of ivermectin was demonstrated by a variety of experiments comparing different route of administration and formulations. The drug can be given PO , IM, SC or topical depending on the species. The pharmacokinetic properties are dose-dependent, with a linear increase in the AUC as the dose increases (González Canga et al., 2009).

According to a study carried out by (Campbell and Benz, 1984) ivermectin was found to be 100% efficacious in experimentally infected dogs when given as a single dosage of 0.2 mg/kg SC against *Ancylostoma caninum,* whereas when given PO at the same dose, an effectiveness of 95% was observed. The efficacy of oral and parenteral treatment against gastrointestinal nematodes may be related to the differences in the plasma levels of drug achieved. (Gokbulut et al., 2006)

Based on the findings of another study comparing the absorption effectiveness of Ivermectin via the subcutaneous route, it was demonstrated that the absorption was very stagnant while the distribution and excretion of the medication were also fairly slow. These data findings indicate that the active substance has a lengthy residence duration in dogs. This study used 49 male crossbred dogs, comparing the different pharmacokinetic parameters such as $t_{1/2ab}$, k_a, t_{max} and C_{max} for different injectable ivermectin preparations. (Eraslan et al., 2010), (Gokbulut et al., 2006)

When taken orally, it is only 50% effective , but when given subcutaneously , it is 90% effective. A dose of 24 Mg/Kg was more than 96% effective against adults and L4 Larvae when administered through either method.(Campbell and Benz, 1984)

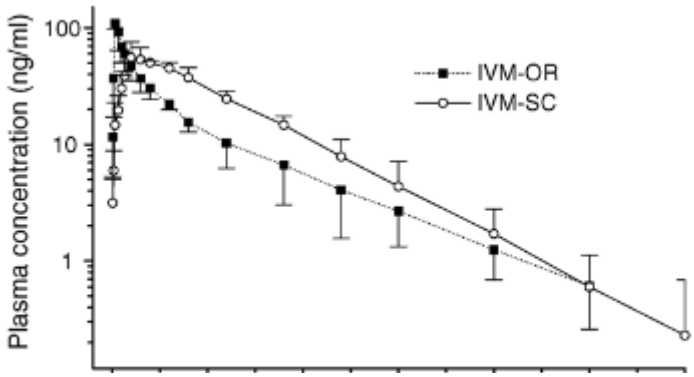

Figure 7: . Semi-log plot of mean plasma concertation of Ivermectin following oral (IVM-OR) and subcutaneous (IVM-SC) administration to dogs at a dose rate of 200µg/kg by: :Gokbulut et al., 2006.

5.3 Side effects and Toxicity

Ivermectin was the anthelmintic with the most reports of toxicoses and suspected toxicoses in dogs. If one or more of the following symptoms appear within 24hrs of an estimated exposure of more than 1000 µg/kg, then Ivermectin toxicosis should be taken into consideration in dogs (of non-collie-breed origin) or cats. In addition Acute and subacute toxicity phase documentation in non-collie breeds using a variety of formulations of ivermectin suggests a wide p.o margin of safety. (Lovell, 1990).

Ivermectin toxicoses is described with adverse effects such as tremor, mydriasis, lethargy, weakness, apparent blindness, drooling (in canine species only) and/or coma. (Lovell, 1990).

Table 3. The Estimated Dose and Onset interval in the reports to the IAPIC between January 1, 1986, and August 10,1988 , assessed as Ivermectin Toxicoses and Suspected Toxicoses in Dogs (n=150) and Cats (n=40) data collected by: Lovell, 1990.

	DOGS		CATS	
Estimated dose of Ivermectin	No. times reported	% Of all reports	No. times reported	% Of all reports
≥100 and <500 µg/kg	41	27.3	0	0.0
≥500 and <1000 µg/kg	6+	4.0	2	5.0
≥1000 and <2000 µg/kg	17	11.3	5+/-	12.5
≥2000 and <4000 µg/kg	22	14.7	5	12.5
≥4000 µg/kg	26+/-	17.3	8	20.0
Unknown	38+/-	25.3	20	50.0

Table 4. The Most commonly Described Clinical signs in the reports to the IAPIC between January 1 ,1986 and August 10 , 1988, Assessed as Ivermectin Toxicoses and Suspected Toxicoses in Dogs (n=150) and Cats (n=40) , Data collected by: Lovell,1990.

	DOGS		CATS	
Clinical signs	No. times reported	% Of all reports	No. times reported	% Of all reports
Ataxia	89	59.3	20	50.0
Mydriasis	62	41.3	10	25.0
Abnormal behaviour	54+	36.0	9+	22.5
Tremor	52	34.7	7	14.5
Weakness/recumbent	46	30.7	10	25.0
Apparent blindness	40	25.7	5	12.5
Hypersalivation/drooling	36	24.0	1	2.5
Lethargy	36	24.0	9	22.5
Coma	20	13.3	10	25.0
Seizure	19	12.7	3	7.5
Vomiting	12	8.0	0	0.0
Hyperthermia	10	6.7	0	0.0
Death	3	2.0	4	10.0

Ivermectin has a breed predisposition to the most severe side effects. The most important one is the Collie Breed with the most documented neurotoxicity linked with Ivermectin administration. Breeds such as Australian shepherds, Border collies, Shetland sheepdogs and old English sheepdogs also appear to show toxic reactions. (Nelson et al., 2003).

Collies are more vulnerable to Ivermectin exposure as a result of a mutation known as the MDRI-gene mutation. The MDRI gene encodes P-glycoprotein, a critical component of the blood brain barrier and its part of in the defence mechanism of pumping out a variety of drugs such as Ivermectin. Collies with these mutations have a faulty blood-brain barrier, resulting in more extreme neurotoxic effects from Ivermectin toxicoses. (Nelson et al., 2003).

6. Fenbendazole

6.1 Mechanism of Action

Fenbendazole and its different dosage variant forms are commonly used to treat helminthiasis in animals. The drug is used very effectively in veterinary treatment protocols and has a broad spectrum of activity, including nematodes and cestodes. (Varlamova et al., 2021).

Fenbendazole is a carbamate of 5-(phenylthiol)-2-benzimidazole. It acts by inhibiting the fumarate reductase activity, hence diminishing glycogen consumption in the intestines of helminths, impairing their microtubular function and mitochondrial metabolism. (Varlamova et al., 2021).

Fenbendazole acts by reversibly binding to the parasites tubulin and inhibiting the polymerization of microtubules in cell organelles. This binding impairs the parasites capacity to create energy at the cellular level, ultimately leading to its demise. (Panarella, 2002).

6.2 Pharmacokinetic properties

Fenbendazole is a highly effective benzimidazole that is frequently used nowadays. It is more typically employed in farm animals and horses. (McKELLAR et al., 1990).

It is often used in cats and dogs to treat parasite infestations. It is approved for use in small animals for the treatment of roundworms, hookworms, whipworms and tapeworms. (Wiebe, 2015).

All animal species thoroughly metabolize benzimidazole anthelmintic , and the parent drug is short-lived, with metabolic metabolites predominating in the plasma. Extended dosing regimens have been proven to increase Fenbendazole effectiveness against canine nematodes. (McKELLAR et al., 1990).

6.3 Side effects and toxicity

Table 5: adverse reactions , data by: Flores et al., 2016.

Percentage	Clinical signs
>10%	Few side effects at common doses
1-10%	Salivation , vomiting , diarrhoea
<1%	Fatty degeneration of the liver Nodular appearance of the gastric mucosa Lymph follicle proliferation Focal encephalomalacia Cerebrum perivascular inflammation Satelitosis Neuronophagia

Benzimidazole anthelmintics have been linked to bone marrow toxicity in a variety of animals. A 1,5-year-old Doberman pinscher developed sudden fever and malaise. Fenbendazole medication was given 12 days before the presentation of the clinical signs for a suspected lungworm infection. The findings of a full blood count assay indicated pancytopenia (characterized by decrease in the amount of white blood cells, red blood cells , and platelets in the blood) and histological examination of a bone marrow core sample revealed bone marrow hypoplasia. Due to the absence of viral, neoplastic, or autoimmune aetiologies, therefore an idiosyncratic response to Fenbendazole was hypothesized. Pancytopenia was reversible in this dog once Fenbendazole was discontinued and supportive care with fluids and bactericidal antibiotics was administered. (Gary et al., 2004)

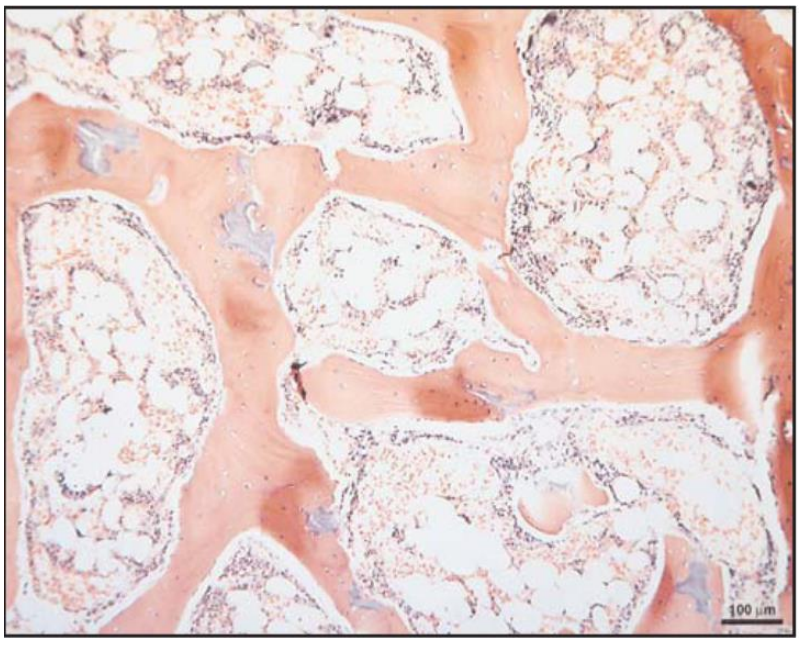

Fig.8 photomicrograph of a bone marrow biopsy specimen from a 1.5-year-old dog with pancytopenia following Fenbendazole therapy. Medullary spaces contain loose connective tissue and scant numbers of hematopoietic cells , picture by: Gary et al, 2004

7. Pyrantel Pamoate

7.1 Mechanism of action

Pyrantel has proven a reliable treatment for two crucial nematode families , the hookworms, and roundworms , since the 1970s. The mechanism of action of pyrantel is described as a nicotinic agonist anthelmintic. The nematodes nAChR's have been classified into three main pharmacological categories based on nicotinic drug affinity. Nicotine, Methyridine and Oxantel are most sensitive to the N-subtype. Levamisole and Pyrantel are most sensitive to the L-subtype. Finally, The B-subtype is the most susceptible to Bephenium. The significance of the subtypes is that Pyrantel primarily operates via the L-subtype nAChR. (Kopp et al., 2008c).

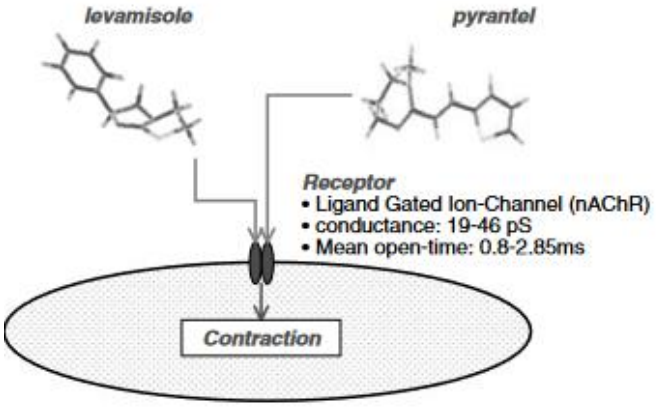

Fig.9. A simplified model of the mode of action of Levamisole and Pyrantel. These two compounds act as nicotinic acetylcholine receptor of muscle to gate these channels open, enabling calcium to enter and causing contraction and spastic paralysis. By: Martin and Robertson, 2007.

These drugs work as depolarizing neuromuscular blocking agents , resulting in an excitatory blockade by acting as an agonist at the nicotinic acetylcholine receptor (nAChR). (Kopp et al., 2008c). Thus, the parasites response will be determined by the sensitivity of nicotinic Acetylcholine receptors on muscle, as well as the amount of depolarisation and Calcium entry produced by Levamisole/Pyrantel. As well as the sensitivity of contractile proteins to calcium. (Martin and Robertson, 2007).

Pyrantels binding to the receptor is more difficult to be reversed than acetylcholine's. This causes the nematodes musculature to be stimulated, resulting in spastic spasms and paralysis. (Kopp et al., 2008c).

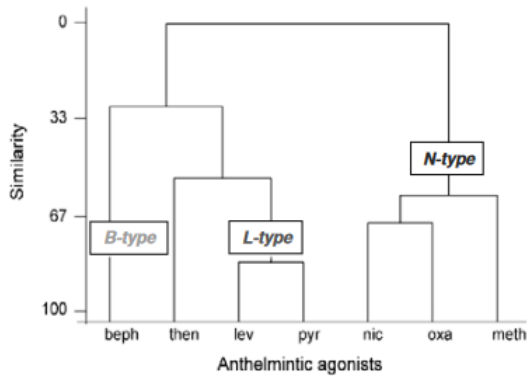

Fig 10: Dendrogram showing the similarity for different cholinergic anthelmintics when antagonised by Paraherquamide , 2-Desoxyparaherquamide and Methyllycaconitine. The three types of receptors are N-,L- and B- types by: By: Martin and Robertson, 2007.

7.2

Pharmacokinetic properties

Pyrantel pamoate is a broad spectrum anthelminthic tetrahydropyridine that its chemical structure is trans-1,4,5,6-tetrahydro-1-methyl-2-(2-(2-thienyl)vinyl)-pyrimidine hydrogen pamoate. (Pitts and Migliardi, 1974).

A variety of salts can be added to the active substance of Pyrantel, each with its own set of pharmacokinetic features and solubility. Pyrantel is available in three salts: embonate (pamoate), tartrate, and citrate. Pamoate salt is the most extensively utilized in small animal medicine. Pyrantel pamoate is extremely insoluble in water and has minimal absorption, as a result it is expelled essentially intact in the faeces and maintains high concentration throughout the gastrointestinal system. This is beneficial in terms of guaranteeing effective action against intestinal nematodes. (Kopp et al., 2008c).

7.3 Side effect and Toxicity

According to a study conducted acute toxicity data show that pyrantel pamoate is an extremely safe preparation. Also chronic toxicity studies showed likewise that is remarkably non-toxic substance. Toxic effects apart from occasional transient abdominal colic are almost non-existent. (Hatchuel, n.d.).

8. Clinical trials

Experiment 1:
A survey of intestinal parasites in stray dogs in the Madrid area took place to determine the efficacy of three anthelmintics in naturally infected dogs. The dogs were divided into 3 categories according to their treatment protocols. Group A with Mebendazole , Group B Fenbendazole and group C with a combination of Febantel-Pyrantel-Praziquantel known as *Drontal plus*. The efficacy was recorded 9 days post treatment and at 16 days post treatment. The therapeutic effectiveness of the three groups against ascarids and ancylostomiases during all the intervals (day 9-16) was extremely high (75-100%).(Miró et al., 2007)

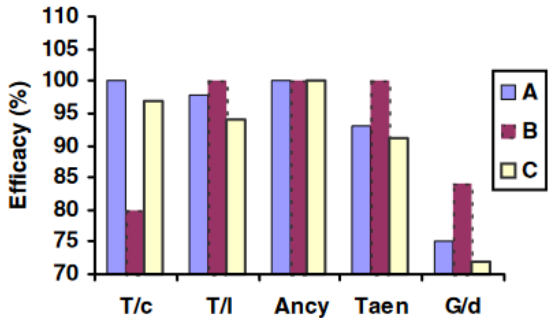

Figure 11: Efficacy at day 9 post-treatment. A Mebendazole , B Fenbendazole , C febantel-pyrantel-Praziquantel. T/c: Toxocara canis , T/l: Toxocaris leonina , Ancy: Ancylostomiases, Taen: Taenidae, G/d: Giardia duodenales data by: Miró et al.,2007.

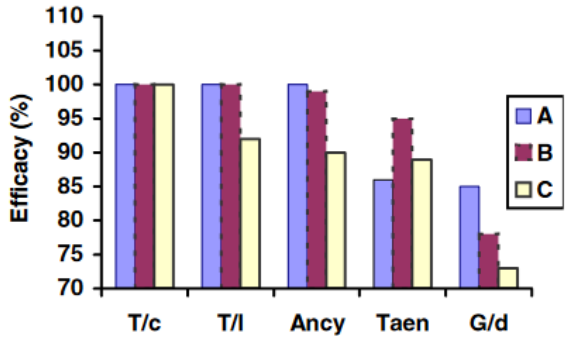

Figure 12: Efficacy at day 16 post-treatment. A Mebendazole , B Fenbendazole , C febantel-pyrantel-Praziquantel. T/c: Toxocara canis , T/l: Toxocaris leonina , Ancy: Ancylostomiases, Taen: Taenidae, G/d: Giardia duodenales. Data by Miró et al., 2007.

According to the results collected , Fenbendazole was found to be 98-100% efficient against Ancylostomiases and Mebendazole 99-100%. However the results of the study showed that the drug combination of Febantel, Pyrantel and Praziquantel was less than expected at 76-91% efficiency. Ascarids and Ancylostomids are reposted to be susceptible to Benzimidazoles and Pyrantel salts. The findings of this research support the drugs effectiveness against these intestinal nematodes. There were no statistically significant differences in the effectiveness of Fenbendazole and Pyrantel according to the results of the experiment but the diminished effectiveness of the combination Febantel, Pyrantel and Praziquantel was strongly noticeable. (Miró et al., 2007).

Experiment 2:

The goal of this study was to see how effectively Fenbendazole, Pyrantel, Milbemycin oxime and Emodepside/Praziquantel combination used to treat canine hookworms worked against *A. caninum* isolate worthy 4.1F3P. this strain was eventually identified as an *A. caninum* MDR isolate. To supply the infective larvae utilized in this investigation , two laboratory beagles were infected with *A. caninum* isolate worthy 3.1F3P. Faeces containing hookworms' eggs were cultivated by combining them with activated charcoal and then incubating them for at least five days at 76-80 degrees Fahrenheit and 56-92% relative humidity. The Baermann method was then used to collect third stage larvae. All treatments were given PO, as directed on the label. Pyrantel pamoate at a minimum of 5 mg/kg bodyweight, Fenbendazole at a minimum of 50 mg/kg bodyweight, Milbemycim oxime at a minimum of 0.5 mg/Kg bodyweight and Emodepside + Praziquantel tablets at a minimum of 1mg/kg bodyweight for three consecutive days. The adult hookworm counts at necropsy were used to assess the treatment groups effectiveness against *A. caninum*. (Jimenez Castro et al., 2020) the following formula was used to compute percent efficacy:

$$\%\ \text{Efficacy} = \left(\frac{\textit{Geometric mean control} - \textit{Geometric mean treated}}{\textit{Geometric mean control}} \right) x$$

The geometric mean (GM) worm count for the control group was 97.4 and the table below shows the FEC of the anthelmintic products used in the experiment.(Jimenez Castro et al., 2020)

Figure 13 : Number of worms recovered and percent efficacy for each treatment group. All dogs were infected with 300 A. caninum L3 on day 0, were treated on day 24, and were euthanized and worms recovered on day 34 (Jimenez Castro et al., 2020)

Fenbendazole	8	57–94	72.0[a]	26.1
Milbemycin oxime	8	55–115	88.9	8.8
Emodepside + praziquantel	8	0–1	0.4[a]	99.6
Non-treated control	8	71–132	97.4	NA

The Benzimidazoles are one of the most significant broad spectrum anthelmintic families available for controlling parasitic nematodes in both animals and humans. A noteworthy finding in this study was that after Fenbendazole therapy , there was a significant decrease in egg counts one day after completion of three-day treatment regimen (SD27) , but FEC progressively rose on SD 31 and 34. In contrast , just one dog in the Emodepside + Praziquantel group tested positive for hookworm eggs on SD 27, and all dogs tested negative for hookworm eggs on SD 31 and 34. The current investigation backs up previous finding about the MDR status of *A.Caninum* worthy 4.1F3P since treatments with Pyrantel pamoate , Fenbendazole and Milbemycin oxime were lacking efficacy. (Jimenez Castro et al., 2020).

Experiment 3:

The effectiveness of Pyrantel pamoate and Ivermectin against gastrointestinal nematodes in dogs was investigated in this study. The FECRT (faecal test reduction test) was used to evaluate the anthelmintic efficiency, and faecal floatation examinations were also done to check the concordance between coproparasitological procedures. A total of 45 naturally infected dogs in the city of Bandeirantes, Parana state were chosen and divided into three groups. Group 1, 15 animals were given Pyrantel Pamoate (145 mg) in a single dosage. Group 2, 15 animals were given Ivermectin (3 mg), and group 3 15 animals were left untreated as a control standard. Faecal test was performed at 2 and 10 days after treatment. (Jesus et al., 2015).

	EPG Nematodes (LOG X+1)			EPG *Ancylostoma* (LOG X+1)			EPG *Toxocara* (LOG X+1)		
	Day zero	Day 2	Day 10	Day zero	Day 2	Day 10	Day zero	Day 2	Day 10
G1	2.46[a]	1.80[a]	0.39[a]	0.52[a]	0.16[a]	0.12[a]	2.14[a]	1.65[a]	0.27[a]
G2	2.28[a]	0.90[b]	0.62[a]	0.50[a]	0.24[a]	0.08[a]	2.11[a]	0.82[a]	0.54[a]
G3	2.54[a]	NA*	2.63[b]	0.12[a]	NA	0.08[a]	2.54[a]	NA	2.52[b]

Figure 14 : Egg counts per gram of faeces (EPG) transformed in log(x=1) of experimental groups G1 (Pyrantel pamoate) and G2 (Ivermectin) before treatment , two and 10 days after treatment and untreated control (G3) data collected by Jesus et al., 2015.

Ancylostoma EPG fell by 98.3% two and ten days after therapy in the Pyrantel pamoate group. However, the IC was less than 90%, indicating poor drug resistance. In the current study, *Ancylostoma* received 95.2% FECRT and 79.1% confidence interval (CI), these data suggests that Ivermectin resistance is limited. These findings indicate that when these medications are administered together , they appear to have a synergistic effect and very effective against *Ancylostoma* reaching effectiveness as high as 99.6% (Jesus et al., 2015).

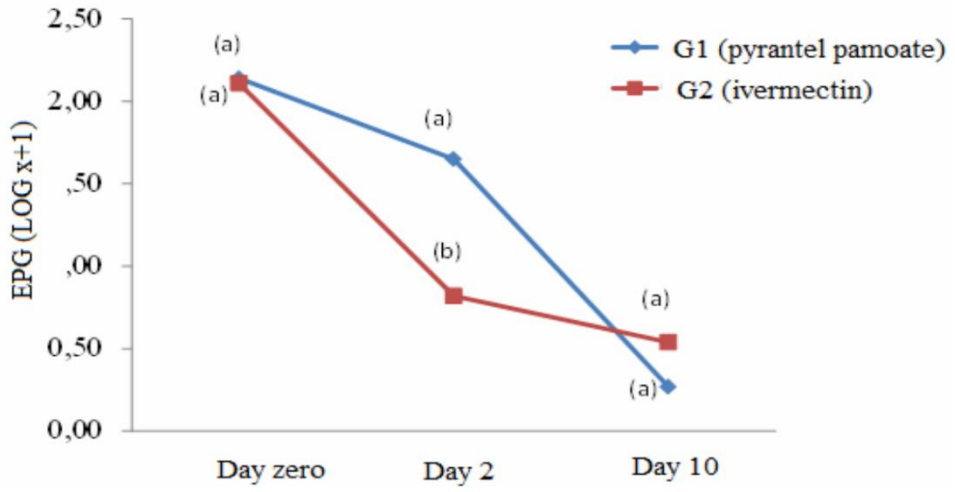

Figure 15: Changes in egg count per gram of faeces (EPG log x + 1) before , two and 10 days after treatment. Different letters represent significant differences between groups on the same day , as calculating sing unpaired t-tests . data by : Jesus et al., 2015

9. Conclusion:

The hookworm *Ancylostoma caninum* is found all around the world and is most commonly seen in dogs, where it can be transmitted orally but also by cutaneous penetration. Although this parasite does not normally infect human intestines, certain cases have shown *A. caninum* associated eosinophilic infections. Furthermore this nematode is a zoonotic agent, producing larva migrans in humans. Given the substantial morbidity associated with hookworm infection , measures for controlling the illnesses caused by these parasites in humans and animals are frequently adopted. A mass drug administration (MDA) is one of these tactics. Finding the perfect medicine by examining various pharmacological characteristics such as pharmacokinetics, mechanism of action, administration route and adverse reaction potential would provide us with greater insight into the eradication and control of canine ancylostomiasis.

As can be observed, all three drugs being evaluated in the study have been shown to be successful in the eradication of hookworm eggs and its transmission, although there were specific characteristics that each treatment stood out in its own manner. For instance Pyrantel pamoate showed that it's the safest preparation out of the three as there were little to no evidence supporting its toxic potential and adverse effects. Fenbendazole which is most effective benzimidazole used nowadays used regularly in farm animals, has shown great promise in the treatment protocols in the dog ancylostomiasis. Finally, Ivermectin underwent less clinical trials than the other two because it is mostly used as heartworm preventative in dogs and cats and not so much used in intestine nematode treatment regimens. I believe Ivermectin is one of those drugs that can be very effective in the right circumstances. Its availability in Europe is thought to be lower than expected during the last years, but with more clinical trials being performed to demonstrate its efficacy, it should become more widely used in the future. Moxidectin and Praziquantel are other drugs that I stumbled upon during my research. Moxidectin falls in the category of Avermectins as well, that through more clinical trials showed more efficacy than Ivermectin. It is now one of the most widely used drugs for Canine ancylostomiasis elimination protocols due to its promising results. Although the growing threat of MDR suggests the use of alternative drugs as the ones currently in use have been shown to be less effective over the years. In the literature, A promising new drug to be used in the future its Emodepside, a drug currently only approved as a topical flea medication for cats in the United States. Although when used in the resistant hookworm population it showed to be 100% effective, therefore it may be the last available drug for the treatment of resistant hookworm populations.

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Thank you.

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Thesis progress report for veterinary students

Name of student: Stefanos Kouroushis

Neptun code of the student: KJKPV5

Name and title of the supervisor: Prof. Dr. Csikó György

Department: DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY

Thesis title: Comparative Efficacy of Ivermectin, Fenbendazole, Pyrantel pamoate in the Elimination of Canine Ancylostomiasis

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	03.	03	Aim of thesis proposed	
2.	2022	04.	04.	How to prepare thesis	
3.	2022	04.	11	Collection of References	
4.	2022	05.	06.	Discussion on the literature data	
5.	2023	06.	18.	Schedule of the thesis topics	

Grade achieved at the end of the first semester: ....excellent (5).....

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	08.	09.	Revision of thesis parts	
2.	2022	08.	15.	Consultation on figures	
3.	2022	09.	27.	Revision of the thesis parts	
4.	2022	10.	11	Discussion on the conclusion	

5.	2022	11.	03.	Finalization of the thesis	

Grade achieved at the end of the second semester: ..... Excellent (5) .....

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: stefanos .....

Signature of the secretary of the department: .....

Date of handing the thesis in..... .....

University of Veterinary Medicine

Announcement of the chosen topic of the Thesis

Name of student (capital letters): STEFANOS KOUROUSHIS

I would like to ask for the permission of the Head of the DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY Department, to write my thesis in the following topic advertised and supervised by the Department.

Budapest, 25/06/2022 (date)

stefanos

(signature of student)

Topic of thesis:

This study is being conducted as part of research work to compare the efficacy of three commercially available anthelmintic drugs against canine ancylostoma species with a major focus on Ancylostoma Caninum elimination The study takes place in order to determine their efficacy for treatment , control, and prevention

Title of thesis:

Comparative Efficacy of Ivermectin , Fenbendazole , Pyrantel pamoate in the Elimination of Canine Ancylostomiasis

Signature of supervisor:

I approve:

Signature of Head of Department

I hereby confirm that I am familiar with the content of the thesis entitled

Comparative efficacy of Ivermectin, Fenbendazole and Pyrantel Pamoate in the Elimination of Canine Ancylostomiasis

written by Stefanos Kouroushis

(student name) which I deem suitable for submission and defence.

Date: Budapest, 07 day 11 month 2022 year

Prof. Dr. Csikó György

Supervisor name and signature

Department of Pharmacology and Toxicology

Department