INVESTIGATION OF IVERMECTIN RESISTANCE TOWARDS EQUINE GASTROINTESTINAL NEMATODES IN ENDURANCE HORSES IN TERENGGANU

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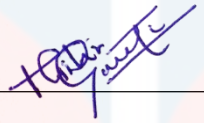
CERTIFICATION

This is to certify that we have read this research paper entitled '**Investigation of Ivermectin Resistance Towards Equine Gastrointestinal Nematodes In Endurance Horses In Terengganu'** by Nur Athirah Nabilah Binti Suhaimi, and in our opinion, it is satisfactory in scope, quality, and presentation as partial fulfillment of the requirement for the course DVT 55204 – Research Project.



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DEDICATIONS

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List of abbreviation

FEC	Fecal Egg Count
FECRT	Fecal Egg Count Reduction Test
FECR%	Fecal Egg Count Reduction Percentage
epg	Egg per gram
NaCl	Sodium chloride

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 55204 – Research Project.

Horses, notably those in Malaysia, are exposed to a diverse array of gastrointestinal nematodes. The growing resistance of parasites to anthelmintic medications in horses is one of the most troubling issues for horse owners, veterinarians, and others in the industry today. There is minimal information on the contribution of horses to clinical illness, parasite epidemiology, and anthelmintic resistance. Hence this study aimed to determine the resistance of Ivermectin in a horse stable, as well as to conclude its efficacy. Horses that fit the inclusion criteria, horses with fecal egg count (FEC) >50 and have not been dewormed for the past 6 months were chosen and treated with Ivermectin orally in proper dosage. The enrolled horse was screened with a fecal egg count reduction test (FECRT) and divided into treatment and control groups with approximately similar means of fecal egg count (FEC). Fecal samples that were collected during pre- and post-treatments were subjected to the Modified McMaster test. Then, a fecal egg count reduction test (FECRT) was performed on 28 endurance horses, from October to December 2022 to determine the resistance of nematodes towards Ivermectin in horses. Ivermectin was found to be resistant in horses enrolled for the study with an 80% of FECRT. The results concluded that there is evidence of Ivermectin resistance in equine cyathostomes in horses enrolled for the study.

Keywords: Anthelminthic resistance, Horses, Ivermectin, FECRT, FECR%, Nematodes

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Kuda, terutamanya di Malaysia, terdedah kepada pelbagai kecacingan gastrousus. Rintangan kecacingan yang semakin meningkat kepada ubat cacing dalam kuda adalah salah satu isu yang paling mengganggu bagi pemilik kuda, doktor haiwan, dan lain -lain dalam industri hari ini. Terdapat maklumat yang minimum mengenai sumbangan kuda kepada penyakit klinikal, epidemiologi parasit, dan rintangan ubat cacing. Oleh itu, kajian ini bertujuan untuk menentukan rintangan Ivermectin dalam kuda lasak, serta menyimpulkan keberkesanannya. Saringan dilakukan dan kuda lasak yang menepati kriteria kiraan telur dalm tinja (FEC)> 50 dan belum diberi ubat cacing selama 6 bulan telah dipilih dan dirawat dengan Ivermectin secara lisan dengan dos yang betul. Kuda yang menepati kriteria Ujian Penurunan Kiraan Telur Dalam Tinja (FECRT) dibahagikan kepada kumpulan rawatan dan kawalan dengan jumlah kiraan teluar cacing (FEC) yang sama nilai setiap kumpulan. Sampel tinja yang dikutip sebelum dan selepas rawatan dihantar untuk Modified McMaster. Kemudian, Ujian Penurunan Kiraan telur Dalam Tinja (FECRT) dilakukan pada 28 kuda lasak yang terpilih, dari Oktober hingga Disember 2022 untuk menentukan rintangan cacing terhadap Ivermectin dalam kuda. Pereatus penurunan kiraan telur dalam tinja (FECR%) adalah 80%. Kajian menunjukkan kerintangan ubat cacing Ivermectin sudah berlaku dalam kalangan kuda lasak.

Kata kunci: Kerintangan ubat cacing, Kuda, Ivermectin, FECRT, FECR%, kecacingan

1.0 INTRODUCTION

Parasitic infection can have persistent and even deadly impacts on the herd and individual health of horses. Frequently, the effects of horse parasite infection are not visible until internal harm has occurred. A horse that seems physically healthy may harbor >500,000 parasites in its digestive tract (Seyoum et al., 2017). The observable signs of parasite infection in horses include a poor with rough hair coat, colic, itching, coughing, weight loss, and impaired performance (Kaplan, 2002).

There are two classifications of equine parasites, which are internal and external. The external parasites are organisms that may cause harm to their host and spend most of their life cycle on the horse's exterior, typically in their hair coat. An example of an external parasite that can impact the host body is lice infestation. Internal parasite, such as nematodes usually can be exceedingly harmful in horses and spends their main lifecycle within the horse's body cavity and completes their lifecycle in the horse's gastrointestinal tract as it provides the optimum and perfect environment for the parasite reproduction and its survivability (Seyoum et al., 2017).

The majority of contemporary anthelmintics which is an oral chemical parasiticides. Every commercially available horse anthelmintic consists of one of three active ingredients: benzimidazole, tetrahydro pyrimidine, or macrocyclic lactone (Matthews, 2011). There are numerous horse owners provide their horses with anthelmintic drugs because they are affordable and easily available. Improper administration patterns of anthelmintics are one of the selective pressure for resistant horse parasites (Tyden et al., 2014). Macrocyclic lactone, specifically Ivermectin was launched to the market in the 1980s and intended to be administered via oral to horses every two months (Tyden et al., 2014). Even after treatment, horses dewormed with Ivermectin oral paste continued to display clinical signs of parasitic infection. According to Tyden et al., 2014, it was hypothesized that the misuse of Ivermectin led to the development of resistance-associated genes within the parasite population, culminating in the emergence of parasitic resistance.

In recent years, studies on Ivermectin resistance of internal parasites in horses in Malaysia is very limited. Therefore, the primary objective of the study is to obtain data and information on internal parasite resistance in horses against Ivermectin.

1.1 Research problem

It is known that the prevalence of internal parasites in horses is rising globally. The occurrence of anthelmintic resistance in horses, such as Ivermectin resistance, renders the deworming program inefficient and makes horses susceptible to chronic parasitic gastroenteritis if the deworming therapy fails. Therefore, it must be investigated to validate its use as an anthelmintic in endurance horse deworming regimens. Thus, there is a need to evaluate the current status of Ivermectin and whether resistance has been developed or remains effective.

1.2 Research question

1.2.1 Is there a presence of Ivermectin resistance against gastrointestinal nematodes in endurance horses in a horse stable?

1.2.2 What is the fecal egg count reduction (FECR%) status of endurance horses in a horse stable?

1.3 Research hypothesis

1.3.1 There is the presence of anthelmintic resistance towards Ivermectin in controlling gastrointestinal nematodes of endurance horses in a horse stable.

1.3.2 The fecal egg count reduction (FECR%) status of endurance horses in a horse stable is 80%.

1.4 Research objectives

1.4.1 To investigate the occurrence of anthelmintic resistance towards Ivermectin against gastrointestinal nematodes in endurance horses in a horse stable.

1.4.2 To investigate the fecal egg count reduction (FECR%) status of endurance horses in a horse stable.

2.0 LITERATURE REVIEW

2.1 Strongyle nematodes of the horse

Numerous species of gastrointestinal parasites inhabit the horse, but the most dangerous are nematodes of the family Strongylidae, sometimes known as strongyles. The equine large intestine is host to the adult stages of these parasites. Strongyle nematodes are also found in domesticated animals, such as *Chabertia ovina* in sheep and *Oesophagostomum* spp. in ruminants and pigs. For species identification, the shape and size of the well-developed buccal capsule of strongyle nematodes are crucial. Equine (horse, donkey, zebra) strongyle nematodes are classified into the subfamilies Strongylinae and Cyathostominae, also known as large and small strongyles, respectively (Lichtenfels et al., 2008). Within the subfamily Cyathostominae, the Cyathostominae species comprises the vast majority of horse-parasitizing species. In 2001, the World Association for the Advancement of Veterinary Parasitology accepted the proposal to refer to members of the tribe Cyathostominae as cyathostomins (Lichtenfels et al., 2002).

2.1.1 Morphology

According to Lichtenfels et al., (2008), nematode's bodies are covered with a transparent cuticle that gives them their distinctive form. Beginning with the mouth and esophagus, the digestive system proceeds to the intestinal tube, which in females terminates in an anus and in males in a cloaca. Females possess ovaries, oviducts, and a uterus, whereas males have testicles and vas deferens (Lichtenfels et al., 2008). Most notably, the spicules and the gubernaculum are male-specific accessory organs. Although these features are essential for identifying the proper horse strongyle species, they are less effective for identifying the proper ruminant trichostrongyloid species (Lichtenfels et al., 2008).

Principally important for the difference in morphology of the strongyle species of horses is the head of the worm which has a cephalic end (Figure 2.1.1). They feature a large buccal capsule surrounded by tiny teeth, a dorsal gutter, and two rounded teeth at its base (Kaplan & Nielsen, 2010). The so-called dorsal gutter is an obvious thickening that lengthen dorsally in the buccal capsule of a number of strongyles. Other than that, the mouth collar, a component of the cuticle, may include sub-medial and lateral cephalic papillae. The front edge of the mouth collar,

known as leaf crowns (Lichtenfels et al., 2008). Frequently, the internal crown is located distal to the outer crown. The external leaf crown supported by an extra-chitinous support in some species (Lichtenfels et al., 2008). Microscopically, the copulatory bursa of the male is also evident, as are other parts of the digestive and reproductive systems (Kaplan & Nielsen, 2010).

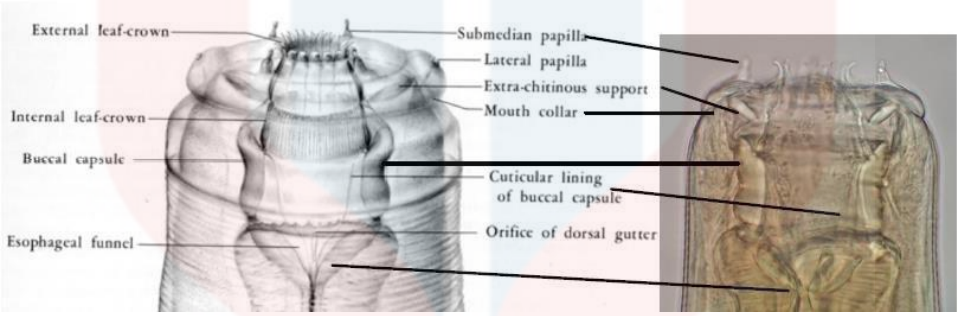

Figure2.1.1. Head of *Coronocyclus coronatus* (schematic drawing to the left; photo x 200 to the right). The schematic figure shows some of the characteristics used for species identification. The drawing is used by courtesy of Dr. R. Lichtenfels. (Photo; E. Osterman Lind).

2.1.2 Life cycle

Strongylus spp.

The life cycles of *Strongylus* species are straightforward, however, they are relatively complex due to the somatic movement of larval stages. Prepatent durations for this genus range from 6 months (*Strongylus vulgaris*) to 10-12 months (*Strongylus edentates*) (Urguhart et al., 1996).

Kaplan and Nielsen (2010) proposed for *S. vulgaris* the following developmental cycle: i) adults reside in the large intestine, and their eggs are excreted in feces; ii) each egg produces a first-stage larva in the environment, which subsequently hatches; iii) in as little as a few days, these larvae reach their third, infectious stage.

According to Duncan and Pirie (1972), infection occurs by swallowing parasitic larvae. The larvae molt and enter the mucosa of the large and small intestines within a few days. After 7 days, the larvae reach the stage four (L4) and enter the small arteriole's lumen in the gut. The L4s migrate against the blood flow for 14-21 days until they reach the cranial mesenteric artery, where they grow significantly for 3-4 months before molting into the fifth stage (L5). Young adults will then return down the mesenteric arteries to the wall of the cecum and colon, where they encapsulate in the subserosa and form nodules-like lesion. These nodules ultimately

rupture, releasing the young adults into the big intestine lumen. After 6 to 8 weeks in the lumen of the stomach, adolescent humans attain sexual maturity. Infrequently, lesions arise elsewhere in the circulatory system as a result of aberrant larval migration.

Cyathostominae

The specific life cycles of individual cyathostomin species are not yet known; thus, it is assumed that the life cycle described here applies to all members of the subfamily. Cyathostomins enter the gut during the third larval stage (L3), which develops from eggs deposited onto grazing grass through feces. Once swallowed by the horse, the eggs continue to mature and, in a "rapid" life cycle, fresh eggs may be discharged in the feces within 5 to 6 weeks. The rate of development from the first larval stage (L1) to the third larval stage (L3) is directly proportional to temperature: eggs may hatch and produce infective L3 in as little as three days during warm weather. Once they reach the L3 stage, they develop a protective membrane and can withstand cold temperatures, allowing them to stay on the pasture for an extended amount of time (Corning, 2009). L3s molt to L4s in the large intestinal wall. The L4s eventually break free from the capsules and reach the gut lumen (Fig 2), where the fourth molt occurs. Depending on the species, adult worms appear to have various location preferences in the large intestine (Ogbourne & Duncan, 1985).

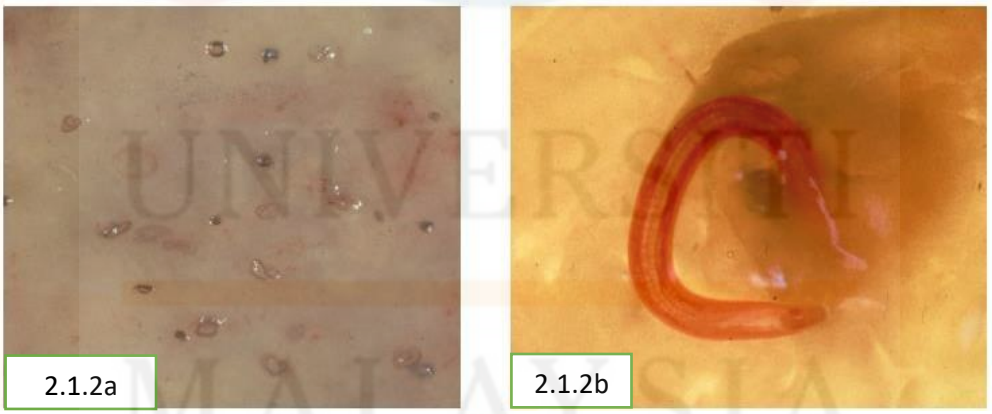

Figure.2.1.2 a) Cyathostomin fourth stage larvae (L4) encysted in the intestinal mucosa; *b)* L4 larva entering the intestinal lumen from the mucosa. (Photo: Ekberg, SVA and J. Hoglund, SLU)

2.2 Clinical signs of nematode infection

Common clinical manifestations of gastrointestinal parasite infections include diminished performance, slowed development, weight loss, colic, a rough hair coat, and debility. Due to significant weight loss, frequent diarrhea, and edema, the infections may potentially result in life-threatening diseases. Even when highly infected, cyathostomes do not cause clinical illness in the majority of horses. Infection often produces subclinical changes in gastrointestinal function that are defined by moderate inflammatory enteropathy. This may lead to changes in intestinal microcirculation and motility, resulting in protein-losing enteropathy (Kaplan, 2002).

2.3 Classes of equine anthelmintics

Current anthelmintics utilized frequently in the horse industry can be categorized into three major groups: i) Benzimidazoles (BZM) include, among others, fenbendazole (FBZ), oxfendazole, and oxibendazole (OBZ); ii) Tetrahydropyrimidines are the pyrantel salts (PRT), which include pyrantel pamoate and pyrantel tartrate, iii) Ivermectin (IVM) and Moxidectin (MOX) are macrocyclic lactones (M/L) or avermectin/milbemycins. Moxidectin is categorized as a milbemycin because it lacks the sugar groups found in avermectins. Other less popular anthelmintic classes consist of simple heterocyclics, such as piperazine, which is typically used as an additive for other anthelmintic classes. Extensive research has been conducted on the mechanisms of biological action and efficacy of currently used horse anthelmintics against specific parasites (Brady & Nichols, 2009).

2.4 Prevalence of anthelmintic resistance in equine nematodes

According to the research of Kaplan (2002), only horse cyathostomes appear to be resistant to anthelmintics. *Strongylus* spp. has been suspected of developing resistance to benzimidazole (BZ) and pyrantel for a long time, although this has never been verified. Since pyrantel and benzimidazole are less effective against big strongyles, notably *Strongylus edentates*, these occurrences likely indicate loss of efficacy rather than resistance. The state of anthelmintic resistance in horse nematodes has been evaluated numerous times over the last couple of decades (Silva et al., 2019). The first peer-reviewed report of anthelmintic resistance in horse ascarids was published by Boersema et al. (2002), but no data were offered to support or refute this assertion. In Europe, investigations undertaken in Germany (Wolf et al., 2014), the Czech Republic (Bodeček & Vavrouchová, 2013), and France (Sallé et al., 2017) have all confirmed resistance to macrocyclic lactones in *Oxyuris equi* with <50% efficacy.

2.5 Resistance to Ivermectin

In 1981, Ivermectin was launched as an anthelmintic drug for horses. Ivermectin is a macrocyclic lactone (ML) with a broad spectrum of activity against both endoparasites and ectoparasites, making it a frequent antiparasitic agent. Ivermectin is effective on nematodes and arthropods but ineffective on cestodes and trematodes. Ivermectin binds selectively and with great attraction to the glutamate-gated chloride ion channels that are present in the nerve of the invertebrate and also the muscle cells. This increases the cell membrane's permeability to chloride ions, causing hyperpolarization of the nerve cell, death, and paralysis of the parasite. This family of compounds may also interact with gamma-aminobutyric acid (GABA)-gated chloride channels. Ivermectin is administered as an oral paste at a dosage of 200 g/kg body weight, has a wide margin of safety, and is widely utilized in the horse sector. It is slowly absorbed, widely distributed, little metabolized, and excreted primarily via feces. Oral treatment results in quicker absorption than subcutaneous injection given in horses (Bazzano et al., 2020).

Even though Ivermectin has been used and administered as a horse anthelmintic medication for 20 years, there have been no reports of equine parasites developing resistance. This is the only class of anthelmintic medications used on horses against which resistance has not yet

developed. Although that many farms have a long history of using Ivermectin four to six times a year, FEC reductions at two weeks post-treatment with Ivermectin remain close to 100%. A recent controlled efficacy trial demonstrated that Ivermectin's efficacy against horse nematode parasites remained exceptionally high (99-100%); there is no evidence that Ivermectin's potency has diminished over time. Nonetheless, many parasitologists believe that resistance is inevitable as reliance on these medications grows (Kaplan, 2002).

2.6 Detection of anthelmintics efficacy and resistance

To determine the efficacy and resistance of helminths to anthelmintics, the fecal egg count reduction test (FECRT) is performed, and it remains the sole approach approved for in vivo anthelmintics medication efficacy testing in horses (Matthews et al., 2012). In general, feces samples are taken from horses 14 days before and after treatment, and the number of strongyles eggs identified between these two samples is used to calculate FECRT (WAAVP). In general, effectiveness percentages of <90% for Benzimidazole, <95% for macrocyclic lactones, and <85% to 90% for pyrantel are seen as indicative of resistance (Nielsen et al., 2015).

3.0 MATERIALS AND METHODS

3.1 Ethical considerations

This study was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan under the approval code of UMK/FPV/ACUE/FYP/023/2022.

3.2 Fecal sample collection

Horse fecal samples were collected per rectum from 28 horses that were randomly chosen in the State of Terengganu Endurance Team (STET) stable. Each horse was properly restrained before fecal sampling. In this study, sampling was done twice on day 0 pre-treatment (Dec 2022) and 14 days post-treatment (Jan 2023). A minimum of 50g fecal samples were collected per rectum using latex gloves lubricated with paraffin oil. Samples were placed in pre-labeled air-tight bags with air excluded. All the samples were stored at 4ºC until processed within 24 hours.

3.3 Fecal egg count

A modified McMaster method was performed to calculate the fecal egg count (Dauparaitė et al., 2021). Firstly, 3g of feces were weighed into a suitable container. Then, 15 ml of sodium chloride was added to soak the feces for a few minutes to one hour until the feces are soft. Next, a laboratory stirrer or shaker jar was used to homogenize the feces until all the pellets have been broken up. Later, the homogenized liquid was poured through a 100 mesh of 0.15 aperture 20cm diameter sieve into a bowl. Then, the liquid was swirled and 15 ml of it was poured into a 17 ml centrifuge tube to centrifuge for 2 minutes at about 300xg or 1500 rev/minutes, and later the supernatants were poured out or sucked off. Then, the sediment was let loose by agitating the tube before adding saturated sodium chloride solution to give the same value as before, which is 35 ml. The tube was then inverted five to six times and immediately, a sample was withdrawn using a Pasteur pipette and filled into a McMaster slide. The process of inversion was repeated to fill the second chamber. The helminth eggs were identified with the criteria of having a thin-shelled, broad ellipse and barrel-shaped side walls and containing blastomere. The eggs that were within the ruled area of the McMaster chamber were identified and counted under the microscope with ten times (10x) magnification.

$$\text{McMaster calculation: Egg per gram (epg)} = \frac{\text{Total no of eggs counted within 2 chambers}}{\text{Weight of feces (g)}} \times \frac{\text{Volume of NaCl solution}}{2(0.15)}$$

3.4 Administration of Ivermectin

All the horses enrolled in the study were divided into two groups namely the treatment group and the control group. Ivermectin (200 µg/kg) was administered to all horses in the treatment group horses per oral. To avoid drug administration errors, all treatments were administered by the same person under veterinary supervision. Fecal egg counts were performed on the day of treatment and at day 14 post-treatment. No drug was administered to the horses of the control group.

3.5 Data collection

Two weeks following treatment (day +14), the stables were revisited and fecal samples from each animal were obtained and processed as stated before (Section 3.2) to determine the post-treatment epg value. The Fecal Egg Count Reduction Test (FECRT) was performed to determine the horse's anthelmintic resistance. It was conducted following the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) to discover anthelmintic resistance in horses following Coles et al. (1992) and Coles et al. (2006). Eighteen out of 28 horses were detected with more than 50 epg of feces and were subjected to FECRT. The horses were divided into control and treatment groups Ivermectin. The second sampling was conducted and the percentage of FECRT was calculated as in FECR% formula listed below:

$$\text{FECR\%: } 100 \times \left(1 - \frac{\text{Mean epg (post treatment)}}{\text{Mean epg (post}-\text{control)}}\right)$$

Resistance was considered when FECRT is less than 95%.

In instances when an animal's FEC rose following treatment, the percentage of FECR was regarded to be zero (0). The mean percentage efficacy values for the treatment group on the farm were determined, and treatments were characterized as effective for FECR 90%, equivocal for FECR between 80% and 90%, and ineffective for FECR 80% (Kaplan, 2004).

4.0 RESULTS

4.1 Determination of mean fecal egg count before and after Ivermectin treatment in a selected horse stable

A total of 28 horses were enrolled for the study from the State of Terengganu Endurance Team (STET) stable in Terengganu (Table 4.1). Among enrolled animals 13 were geldings, 14 were mares, and one was a stallion. The horses were belonging to the breeds Arabian, Thoroughbred, Argentina Polo, and Pony. The age of the study population ranged from 3 to 24 years with a mean age of 14 years. The horses were allowed to graze in a land with mixed grasses afternoon, around the paddock in the stable. Their deworming strategy was twice a year with a drench of Albendazole. The last deworming history for all the horses enrolled for the study was in December 2021 with Albendazole.

Table 4.1: Sample details of the horses enrolled for the study

No	Sample ID	Sex	Stable	Age (Years)	Deworm
1.	Tuan Junior	Gelding	STET	19	Dec 2021
2.	Maya Luna	Mare	STET	6	Dec 2021
3.	Espia	Mare	STET	16	Dec 2021
4.	Anak Khalima	Mare	STET	3	Dec 2021
5.	Estupanda	Mare	STET	15	Dec 2021
6.	Toso	Gelding	STET	12	Dec 2021
7.	Malon	Gelding	STET	23	Dec 2021
8.	Lanin	Gelding	STET	12	Dec 2021
9.	Grieka	Mare	STET	10	Dec 2021
10.	Khalima	Mare	STET	17	Dec 2021
11.	Stavio	Stallion	STET	10	Dec 2021
12.	Blue Moon	Mare	STET	24	Dec 2021

13.	Vanidosa	Mare	STET	18	Dec 2021
14.	Franki	Mare	STET	15	Dec 2021
15.	Ibrahim	Gelding	STET	13	Dec 2021
16.	Kursina	Mare	STET	10	Dec 2021
17.	Natal	Gelding	STET	14	Dec 2021
18.	Thaylover	Gelding	STET	23	Dec 2021
19.	Malima	Gelding	STET	8	Dec 2021
20.	Juan	Gelding	STET	11	Dec 2021
21.	St.John	Gelding	STET	12	Dec 2021
22.	Wazir	Gelding	STET	20	Dec 2021
23.	Romeo	Gelding	STET	12	Dec 2021
24.	Asya	Mare	STET	17	Dec 2021
25.	Moorea	Mare	STET	12	Dec 2021
26.	Rakinda	Mare	STET	20	Dec 2021
27.	Amores	Mare	STET	18	Dec 2021
28.	Tango	Gelding	STET	13	Dec 2021

Table 4.2 shows the pre-treatment and post-treatment FEC results tabulated for each horse as well as the mean FEC during pre-treatment and post-treatment sampling of the stable. The range for FEC of the study population study is between 50 to 750 epg with a mean count of 182.14 epg. The total number of horses having 50 egg per count is 4, while the number of horses having >50 epg was 24 horses. Although there was considerable individual variation in the FECs, the average number of eggs per gram of feces (epg) was found to be notably higher in adult horses (>5 years) than in young horses (Table 4.1).

All horses with zero epg in the post-treatment FEC has been treated with Ivermectin. The range of post-treatment FEC of the study population was between 0 to 450 epg. Only one horse showed increased post-treatment FEC which was found in a young horse (<5 years) from 250epg to 450 epg.

Table 4.2: Pre and post-treatment Fecal Egg Count of sample in the stable

No.	Sample ID	Group	Fecal egg count (e.p.g) (min 50 epg)	
			Pre-treatment	Post-treatment
1.	Tuan Junior	Treatment	400	50
2.	Maya Luna	Treatment	50	0
3.	Espia	Treatment	200	0
4.	Anak Khalima	Treatment	250	450
5.	Estupanda	Treatment	150	50
6.	Toso	Treatment	150	0
7.	Malon	Treatment	300	50
8.	Lanin	Treatment	200	0
9.	Grieka	Treatment	100	0
10.	Khalima	Treatment	200	0
11.	Stavio	Treatment	100	50
12.	Blue Moon	Treatment	50	0
13.	Vanidosa	Treatment	750	150
14.	Franki	Treatment	350	100
15.	Ibrahim	Treatment	200	0
16.	Kursina	Treatment	250	0
17.	Natal	Treatment	150	0
18.	Thaylover	Treatment	150	0
19.	Malima	Control	650	700
20.	Juan	Control	50	50
21.	St.John	Control	250	400
22.	Wazir	Control	100	200
23.	Romeo	Control	50	100
24.	Asya	Control	200	50
25.	Moorea	Control	200	250
26.	Rakinda	Control	300	450
27.	Amores	Control	100	150
28.	Tango	Control	200	150
	Average		182.14	121.42

4.2 Determination of Ivermectin resistance status

Table 4.3 shows the status and the level of Ivermectin resistance in the STET horse stable. The calculation was conducted on FECRT calculation spreadsheet based on the mean of FEC post-treatment in Ivermectin groups. It showed 80% reduction in the fecal egg count from Ivermectin treatment in STET stable. Anthelmintic resistance status in STET stable was resistant with a moderate level of anthelmintic resistance as FECR% was 80%.

Table 4.3: Status and level of Ivermectin resistance with FECRT of selected horse stable

Stable	N[1]	Status	Level	Post-treatment mean FEC[2]		FECR[3](%)
				Ivermectin	Control	
STET	28	Resistant	Moderate	50	250	80

[1]No. of animals; [2]Faecal egg counts; [3]Faecal egg count reduction.

5.0 DISCUSSION

Numerous Malaysian and international research have documented anthelmintic resistance in *Parascaris equorum*, *cyathostomins*, and other significant horse parasites. Cyathostomins are the most prevalent nematode species infecting horses in Malaysia and across the world. There are more than 50 unique species within this category of parasites. Gastrointestinal nematodes (helminthiasis) caused a major negative effect on horses' health as it results in diminished performance, slowed development, weight loss, colic, a rough hair coat, and debility. Due to significant weight loss, frequent diarrhea, and edema, the infections may potentially result in life-threatening diseases (Kaplan, 2002). Hence, gastrointestinal nematode infestation is alarming and results in a serious threat to the horse industry in Malaysia, and determining the status of anthelmintics resistance among horses in Malaysia is critical.

Primarily, anthelmintic resistance was the result of the incorrect use of anthelmintic medications. It arose owing to an increase in helminths' ability to withstand a standard dose of anthelmintic medication in animals (Beleckė et al., 2021). Reproduction of surviving worms gradually increased the resistance worm population in sick animals. There are several contradicting reports on the kind of anthelmintic regimen that should be suggested to reach great levels of parasite control while also attempting to prevent resistance. The monitoring of FECR on horse farms and ranches, according to researchers, is crucial for identifying whether anthelmintic drugs are successfully controlling parasites.

In this study, the reason why FECR% from STET stable did not reach the predicted 100% may be due to the ability of worms to withstand a standard dose of anthelmintics (Ivermectin) used. However, just one horse had a significant worm burden, which affected the FECRT results. In addition, the FEC levels of all other horses in the treatment group from the STET stable reduced from 0 to 150 epg following treatment. Due to the ineffectiveness of Ivermectin against infecting gastrointestinal nematodes, it appears that a single horse contributed to the increased mean FEC relative to the other members of the herd.

Based on the results of the study (Table 4.2), it has been determined that the number of fecal eggs in a young horse was high compared to adults. It has been known for a long time that young horses are more vulnerable to strongyle infections than older animals, but the processes

behind their immunological responses remain poorly understood. Several observations suggest that immunity develops with age. Young horses (5 years) are more likely to have elevated fecal egg counts and clinical cyathostominosis. There are also age variations in the prepatent period for cyathostomins and the time necessary for strongyle eggs to return in feces following deworming. Young animals have shorter versions of these characteristics than older ones (Klei & Chapman, 1999).

The investigation of Ivermectin's efficiency against cyathostomes in equines is hence of special interest. For unknown reasons, benzimidazole resistance is the most common of the three major anthelmintic classes (benzimidazoles, tetrahydro pyrimidines, and macrocyclic lactones). Resistance to tetrahydro pyrimidines and macrocyclic lactones, on the other hand, seems to be in its infancy, according to the study. Given that no new active compounds are projected to enter the future market industry, the majority of academics feel that it is critical to developing better ways for controlling the distribution of anthelmintic medications based on need rather than prevention.

From the results, Ivermectin resistance in STET-stable has been developed (Table 4.3). Stables or horse owners must investigate alternative approaches for managing gastrointestinal nematodes in horses. In the event of "low-shedding" horses, non-chemical approaches such as herbal parasiticides are used, limiting the usage of the primary larvicides on the market. The majority of plant-based larvicide research has been undertaken in vitro; hence, in vivo study on these potential parasiticide plants is required. Mundy et al. (2016) promoted the use of natural plant-based techniques for parasite extermination and proposed that these plants be utilized as immunomodulators, which they characterized as "a biological or synthetic substance that may stimulate, inhibit, or control any immune system component". They hypothesized that using certain plants would boost the efficiency of vaccinations or parasiticides. These plant-based immunomodulators inhibit the production of free radicals, nitric oxide, and cytokines, all of which activate natural killer cells.

However, the result of anthelmintic resistance in Table 4.3 shows resistant status in STET stable may be influenced by a small number of sample animals (n=18) that meets the inclusion criteria of FECRT. The higher the number of sample animals, the higher the accuracy of the study will be. Hence, the study is approved by Animal Ethics Committee as the number of animals included in this study follows the minimum requirement.

6.0 CONCLUSION

In conclusion, Ivermectin resistance has been reported in gastrointestinal nematodes parasitizing endurance horses in Terengganu. However, the findings of reduced drug efficacy in horses beg for a comprehensive investigation to determine the extent of Ivermectin resistance in Malaysian endurance horses. Misuses of anthelmintics, such as underdosing, treating all animals at once in the same stable, continuing administration of the same anthelmintic, and frequent usage, are major factors in the development of anthelmintic resistance. Considering the cases reported of anthelmintics resistance in endurance horses in a horse stable in Terengganu and the widespread use of anthelmintics drugs for helminth control, it requires advanced knowledge and studies on the status of anthelmintics drugs at various levels, before alarming levels of drug resistance occurs. The emergence of anthelmintics resistance such as Ivermectin against gastrointestinal equine nematodes in endurance horses in Terengganu is cause for concern.

7.0 RECOMMENDATION

Instead of concentrating on a single stable, it was necessary to examine the prevalence of Ivermectin resistance in horse stables in Terengganu utilizing a larger sample size and additional study locations. There are currently no alternatives to the use of anthelmintics for the successful control of helminths, which necessitates the suggestion of a yearly or biannual gradual rotation between medication classes. In addition, it would be beneficial to research and assess the focused treatment plan under various management settings if it were to be applied in the future. It would be beneficial to do extensive, long-term field investigations on the presence of people with a high worm load.

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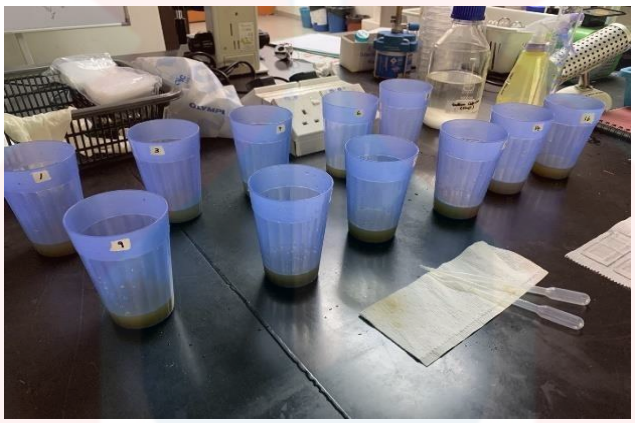
Appendix A

Appendix A.01: Manual extraction of fecal sample from horses

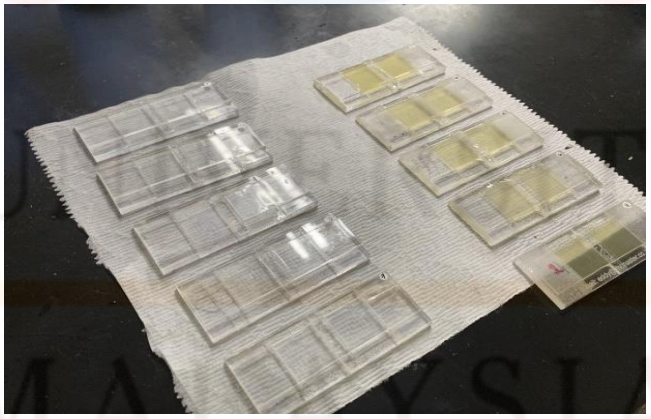
Appendix A.02: Scenario of fecal sampling in Terengganu horse stable

Appendix B

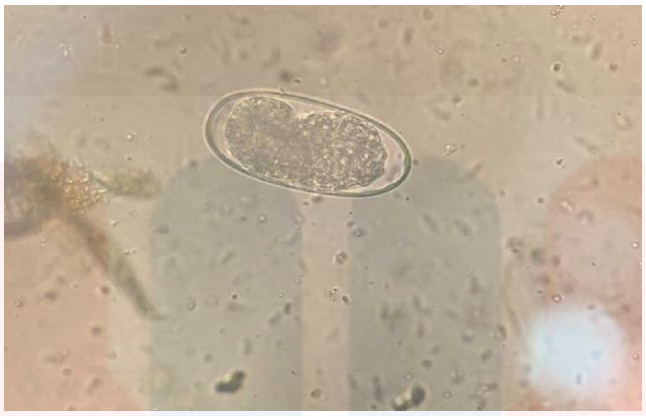
Appendix B.01: Labelled fecal samples



Appendix B.02: Process of homogenizing fecal samples with NaCl solution



Appendix B.03: Filling of Macmaster slide with fecal samples



Appendix B.04: Strongyle eggs under microscope with 40x magnification