INVESTIGATION OF BENZIMIDAZOLE RESISTANCE TOWARDS EQUINE GASTROINTESTINAL NEMATODES IN ENDURANCE HORSES

NUR RAHMAH BINTI RUSLY

(D18A0031)

A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI MALAYSIA KELANTAN

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR

THE DEGREE OF

DOCTOR OF VETERINARY MEDICINE

DECEMBER 2022

UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

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

Dr. Giguruwa Gamage Thilini Nisansala
BVSc (UOP, Sri Lanka), PhD (USJ, Sri Lanka)
Lecturer,
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Supervisor)

Dr. Mimi Armiladiana Binti Mohamad
DVM (UPM)
Senior Lecturer,
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Co-supervisor)

Dr. Tan Li Peng
DVM (UPM)
Senior Lecturer,
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Co-supervisor)

ACKNOWLEDGEMENT

Special thanks for those who have given their support, guidance, advice, and aid for the completion of this project paper:

Dr. Giguruwa Gamage Thilini Nisansala

Dr. Mimi Armiladiana Binti Mohamad

Dr. Tan Li Peng

Lab assistants of FPV UMK

My family

DVM 5 class of 2018/2023

Thank You

DEDICATIONS

This thesis is respectfully dedicated to my all-time cherished family, supportive friends, and members of the Faculty of Veterinary Medicine at Universiti Malaysia Kelantan in the name of Allah, Who is the Most Beneficent, Who is the Most Merciful, and Who is the Most Gracious. I would like to express my profound appreciation to my doting parents, Intan Nur Laily and Rusly Abdullah, whose words of motivation and encouragement keep ringing in my ears to this day. My siblings, who are incredibly dear to me and have never left my side.

By devoting this dissertation to all of my lecturers, seniors, and staff members who have assisted me along the way, I would also like to thank them for their assistance. I will be eternally thankful to them for everything they have contributed and guided me through, but especially for helping me enhance my skills as a veterinary student. I will never forget that. Dr. Thilini, Dr. Mimi, Dr. Izzati, and Dr. Tan Li Peng, deserve a particularly heartfelt thank you, as well as my FYP peers, Athirah and Judith.

My housemates, Faqihah and Najla, and my best friend, Alwafi, were always there for me during the entirety of my Doctor of Veterinary Medicine degree, and for that I am eternally grateful. They have all been my greatest supporters and confidantes. Also, to my classmates of DVM18/23 who have accompanied me through the highs and lows of completing this degree.

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List of abbreviations

FEC	Faecal Egg Count
FECRT	Faecal Egg Count Reduction Test
FECR%	Faecal Egg Count Reduction Percentage
epg	Egg per gram
NaCl	Sodium chloride
PGE	Parasitic Gastroenteritis

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.

The majority of mammalian species are affected by nematode infestation, one of the serious gastrointestinal diseases. Horses are included and are reported to be infested frequently, causing various clinical problems and affects its performance in prolonged infestation. As preventative, deworming programs are commonly implemented, using anthelmintic drugs and routines of the veterinarian's preference to curb the occurrence of gastrointestinal diseases in horses. This is a pilot study to determine the resistance of Benzimidazole in a n endurance horse stable, as well as to conclude its efficacy to be used in those horses. In a population of horses that fits the inclusion criteria in this study, horses with Fecal Egg Count (FEC) >50 and have not been dewormed for the past 6 months were chosen and treated with Benzimidazole orally in an accurately calculated dose. The enrolled horses were screened for the criteria of Faecal Egg Count Reduction Test (FECRT) and divided into control and treatment groups with approximately similar means of fecal egg count (FEC). Fecal samples that were collected during pre- and post-treatments were subjected to Modified McMaster. Then, the resistance of the nematodes towards Benzimidazole in horses were determined using Faecal Egg Count Reduction test (FECRT) that was calculated according to the recommendation of the World Association for the Advancement of Veterinary Parasitology (WAAVP) for the detection of anthelmintics resistance. The Fecal Egg Count Reduction percentage in the stable was observed to be 94%. This shows suspected resistance towards benzimidazole has been developed in the endurance horses in the stable.

Keywords: Benzimidazole, Endurance horses, Nematodes, Anthelmintic Resistance

ABSTRAK

Sebahagian besar spesies mamalia dijejaskan oleh jangkitan cacing nematoda iaitu salah satu penyakit gastrousus yang serius. Kuda ialah termasuk spesies yang kerap dilaporkan dijangkiti penyakit tersebut dan ia menjejaskan prestasi apabila dijangiti dalam jangka masa yang lama. Sebagai pencegahan, program nyahcacing biasanya dijalankan menggunakan ubat cacing antelmintik melalui rutin mengikuti pilihan veterinariawan dalam usaha mencegah penyakit gastro usus di dalam kuda. Kajian ini dijalankan untuk mengkaji kerintangan terhadap Benzimidazole di sebuah ladang kuda lasak, dalam masa yang sama mengkaji keberkesanan ubat tersebut. Dalam populasi kuda yang sesuai dengan kriteria kemasukan dalam kajian ini, kuda dengan Pengiraan Telur Najis (FEC) >50 dan tidak diberi ubat cacing sejak 6 bulan lalu telah dipilih dan dirawat dengan Benzimidazole secara oral dalam dos yang dikira dengan tepat. Kajian dijalankan di kandang kuda lasak di Terengganu (n=1). Kuda-kuda dalam kandang telah disaring untuk kriteria Ujian Pengurangan Kiraan Telur Najis (FECRT) dan dibahagikan kepada kumpulan kawalan dan rawatan dengan cara kiraan telur najis (FEC) yang hampir sama. Sampel najis yang dikumpul semasa pra dan selepas rawatan dikira menggunakan Modified McMaster. Kemudian, rintangan nematoda terhadap Benzimidazole dalam kuda ditentukan menggunakan ujian Pengurangan Kiraan Telur Najis (FECRT) yang dikira mengikut saranan World Association for the Advancement of Veterinary Parasitology (WAAVP) untuk pengesanan rintangan anthelmintik. Peratusan Pengurangan Kiraan Telur Najis dalam kandang diperhatikan ialah 94%. Ini menunjukkan rintangan yang terhadap benzimidazole telah wujud dalam kuda di kandang.

Kata kunci: Benzimidazole, Kuda lasak, Nematoda, Kerintangan Ubat Cacing

1.0 INTRODUCTION

Horses in general are predisposed to a wide range of helminth infestation which puts risk towards horses' wellbeing. Helminths consume nutrients from the host to obtain their nutritional supply while living in internal organs, bodily cavities, and tissues. Additionally, a portion of their life cycle takes place externally, generally in pastures, which oftentimes become the portal entry of the parasites into the horse body system. In a prolonged infestation, it will develop into a heavy burden of gastrointestinal parasites which in the long run will be very health threatening in horses. Horses in Malaysia are prevalent towards *Trichonem*a sp., *Ascaris* sp., *Trichostrongylus* sp., *Strongyloides* sp., *Strongylus* sp. and *Poteriostomum* sp. (Periyasamy et al., 2017). Generally, the small strongyles group of nematodes or also known as cyathostomins are predominant and highly prevalent in horses. This nematode can threaten horses' health as it can cause non-strangulating intestinal infarction leading to severe colic and was the major parasitic threat to equine health before the wide usage of broad-spectrum anthelmintics (Reinemeyer & Nielsen, 2009).

Interval treatment regimens incorporating the frequent delivery of anthelmintic drugs at intervals based on strongyle egg reappearance period (ERP) have traditionally been used to control helminths, especially nematode infestation in horses. Interval treatment programmes have had a significant impact on reducing strongyle infections and the development of substantial strongyle-associated disease. The implementation of this program, on the other hand, has made a significant contribution to the development of anthelmintic resistance in horses (Kaplan, 2004).

Benzimidazole is one of three broad-spectrum anthelmintic classes that are primarily listed for nematode control in horses besides tetrahydropyrimidines (pyrantel) and macrocyclic lactones (ivermectin, moxidectin) in the conventional deworming routine. The control approaches are based on nematode egg suppression regimens, which include using anthelmintics to all horses at intervals determined by strongyle egg reappearance periods after treatment. The extensive use of such programmes has reduced clinical disease, particularly those associated with strongyle species; but high treatment frequency has resulted in an increase in anthelmintic resistance, notably in cyathostomin species (Matthews, 2014). Therefore, this study was aimed to investigate the occurrence of equine helminths resistance towards Benzimidazole and to observe its efficacy as a deworming drug in endurance horses in Terengganu.

1.1 Research problem

Benzimidazole is one of the three main anthelmintics used in deworming programs for horses to curb helminths. Resistance of anthelmintics towards Benzimidazole will make the deworming program ineffective and make the horses predisposed to chronic parasitic gastroenteritis if the treatment fails. The status of Benzimidazole resistance in equine gastrointestinal parasites in Malaysia is unclear thus it needs to be studied in order to sustain the relevance of its usage as anthelmintics in deworming programs.

1.2 Research questions

1.2.1. What is the status of anthelmintic resistance of gastrointestinal nematodes towards Benzimidazole in the endurance horses?

1.3 Research hypothesis

1.3.1. There is presence of anthelmintic resistance towards Benzimidazole against gastrointestinal nematodes in endurance horses in the horse stable.

1.4 Objectives

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

2.0 LITERATURE REVIEW

2.1 Common intestinal parasites in horses and clinical signs

It is a general knowledge that all horses, regardless of age, are predisposed to strongyle infections and it is evident that horses are unable to mount complete immunity to these parasites by themselves which cause lifelong infestation of the endoparasites (Klei, 2000). Endoparasites that mainly affect the gastrointestinal tract consist of broad categories of nematodes such as cyathostomins and large strongyles and cestodes (or tapeworms) (Matthews, 2014). Strongylid (family Strongylidae) nematodes are further classified into two subfamilies, Strongylidae (large strongyles) and cyathostomins (small strongyles) (Lichtenfels et al., 2008). They are pervasive among grazing horses and constitute more than 75% of the total intestinal parasite biota. Due to the widespread adoption of deworming programmes, large strongyles, such the highly pathogenic *Strongylus vulgaris*, are no longer frequently detected in domestic horses. This has caused the focus of anthelmintic treatment programmes to shift to the more prevalent and ubiquitous but less harmful cyathostomin parasites (Dauparaitė et al., 2021).

The nematode infestation if left unattended in horses will cause manifestation of clinical signs including reduced performance, decreased rate of growth, weight loss, colic, rough hair coat, and debilitation. Prolonged infestation may also lead to life threatening disease which is due to severe weight loss, chronic diarrhea, and edema (Kaplan, 2002).

2.2 Benzimidazole as anthelmintic

Benzimidazole is one of the main broad-spectrum anthelmintics that is used to treat animals with gastrointestinal parasites, including nematodes. Benzimidazoles target tubulin within parasite intestinal cells, which form microtubules that are required for nutrient acquisition (Köhler, 2001). Microtubules are made up of heterodimers of the α- and β- tubulin subunits. The benzimidazoles bind to nematode β-tubulin and hinder microtubule polymerization, causing the molecule to shorten (Martin, 1997). Microtubules are essential for cellular structure, the mitotic spindle, and chemical transport across the cell membrane. Microtubule production suppression causes cellular disequilibrium and parasite death by disrupting cell structure and energy metabolism (Martin, 1997).

2.3 Resistance towards anthelmintics in horses

To control anthelmintic infestation, nematode egg suppression regimens are implemented which involves applying anthelmintics to all horses at regular intervals depending on strongyle egg reappearance periods after treatment. The anthelmintics that have been used to treat nematode infestation in horses for roughly the last three decades have almost exclusively come from one of three drug classes: benzimidazoles (such as fenbendazole and oxfendazole), tetrahydropyrimidine pyrantel, and macrocyclic lactones (ivermectin and moxidectin) (Nielsen et al., 2014).

The extensive adoption of such programs using the three primary drugs, including benzimidazoles, has significantly reduced clinical disease particularly that linked with strongyle species. Nonetheless, the high frequency of treatment has resulted in significant selection pressure causing anthelmintic resistance, especially in cyathostomin species. According to field research published in the previous decade, benzimidazole resistance is ubiquitous in cyathostomins (Matthews, 2014).

3.0 MATERIALS AND METHODS

Thirty horses with a minimum 50 egg per gram (epg) were selected for this study. The horses were divided into two groups which are the treatment group (n=10) and control group (n=10). Horses in the treatment group were treated with Oxfendazole which is a compound from the Benzimidazole family, while the control group were left untreated. All the horses were housed during the night, and spent some time in paddocks for grazing during the day. They were fed with hay, concentrates and mixed pasture. Feces and soaked bedding with urine were removed daily in all stables and new bedding were replaced based on the amount of bedding removed every day.

3.1 Sample collection

Fecal samples were collected before the treatment and 14 days post-treatment. To collect the fecal sample, horses were restrained in a horse crush for safety purposes. A minimum 50g of 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.

3.2 Parasitological examination

A modified McMaster method was used to get the fecal egg count. Firstly, 3g of feces were weighed into a suitable container. Then, 42ml of water 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 15ml of it was poured into a 17ml 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 15ml. The tube then was inverted five to six times and immediately, a sample was withdrawn using Pasteur pipette and filled into a McMaster slide. The process of inversion was repeated to fill the second chamber. Then, at 40x magnification, all eggs were counted under the two ruled grids with a total volume of 0.3ml. The total number was multiplied by 50 to have the EPG in the fecal sample. For greater sensitivity, all eggs in each chamber were counted with a total volume of 1ml and was multiplied by 15 to have the EPG.

3.3 Oxfendazole administration

Oxfendazole (10mg/kg), which is a compound from the Benzimidazole family, was administered to all horses in the treatment group horses per oral. To avoid drug administration errors, all treated animals 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.

3.4 Data Collection

Faecal Egg Count Reduction Test (FECRT) was used as a tool to detect anthelmintic resistance in the horse. It was performed according to the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) for the detection of anthelmintic resistance in horses (Coles et al., 1992). Resistance will be considered when FECRT is less than 95%. The percentage of reductions in FEC of each horse was calculated by using the formula:

$$\text{FECR\%} = 100 \left(1 - \frac{mean\ epg\ post\ treatment}{mean\ epg\ control} \right)$$

3.5 Ethical considerations

Ethical approval for the study was obtained from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (UMK/FPV/ACUE/FYP/023/2022).

4.0 **RESULTS**

4.1 Determination of mean faecal egg count before and after Benzimidazole treatment in the stable.

Table 4.1 represents the pre-treatment and post-treatment FEC of 30 animals enrolled for the study. 20 horses were randomly placed into the treatment group, which is also known as Oxfendazole group, while 10 horses were placed in the control group. For the pre-treatment fecal egg count, the epg ranges from 50 to 2050. The mean number of epg for Oxfendazole group and control group for pre-treatment were 313 and 210 respectively.

The post treatment is the epg reading that was obtained 14 days post treatment of horses in treatment group with Oxfendazole. The post treatment result shows epg ranging from 0 to 100 in the treatment group. Most horses that have been treated with Oxfendazole show significant reduction of epg after the treatment where 17 of them show reduction to 0 epg, except 3 horses having 100 epg, thereby reducing the mean of post treatment epg for Oxfendazole to 15, while the control group mean being 250.

Table 4.1: Pre and post-treatment Fecal Egg Count of sample in the stable

No.	Sample ID	Group	Fecal egg count (e.p.g)	
			Pre treatment	Post treatment
1.	Thor	Oxfendazole	250	0
2.	Typhoon	Oxfendazole	300	100
3.	Zapple	Oxfendazole	100	0
4.	Ejaz	Oxfendazole	850	0
5.	Nawan	Oxfendazole	150	0
6.	Ohara	Oxfendazole	100	0
7.	Rakinda	Oxfendazole	100	0
8.	Solstrom	Oxfendazole	50	0
9.	Vague	Oxfendazole	1100	0
10.	Jedai	Oxfendazole	50	0
11.	Ana	Oxfendazole	350	0
12.	Coco	Oxfendazole	250	0
13.	Arthus	Oxfendazole	300	100
14.	Neep	Oxfendazole	2050	100

No.	Sample ID	Group	Fecal egg count (e.p.g)	
			Pre treatment	Post treatment
15.	Regeton	Oxfendazole	150	0
16.	Medjik	Oxfendazole	350	0
17.	Gassur	Oxfendazole	200	0
18.	Sabaha	Oxfendazole	200	0
19.	Pacifica	Oxfendazole	250	0
20.	Virago	Oxfendazole	100	0
21.	Moorea	Control	200	250
22.	Rakinda	Control	300	450
23.	Amores	Control	100	150
24.	Tango	Control	200	150
25.	Malima	Control	650	700
26.	Juan	Control	50	50
27.	St. John	Control	250	400
28.	Wazir	Control	100	200
29.	Romeo	Control	50	100
30.	Asya	Control	200	50

4.2 Determination of Benzimidazole resistance status

Table 4.2 shows the status and level of Oxfendazole from the Benzimidazole family resistance in the stable. Calculations were conducted on the FECRT spreadsheet based on the mean of both FEC post-treatment in Oxfendazole and the control group. The outcome shows 94% reduction in the fecal egg count from the treatment group. The anthelmintic resistance status was low, based on the 94% fecal egg count reduction percentage (FECRT%).

Table 4.2: Status and level of Oxfendazole resistance in the stable.

Stable	N[1]	Status	Level	Post treatment mean FEC[2]		FECR[3](%)
				Oxfendazole	Control	
RTES	30	Low	-	15	250	94%

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

5.0 **DISCUSSION**

Helminths of the gastrointestinal tract can inflict damage to horses in varying degrees, depending on the species of equine, as well as their nutritional and immunological states. They either cause a loss of performance and production in the animals, most notably a decrease in body weight or an inability to gain weight, or they can even lead to an increase in mortality in acute cases (Mathewos et al, 2021). In controlling these infestations, usage of benzimidazole and Ivermectin as anthelmintics since the 1960s has revolutionized the management of equine parasites. With the introduction of these effective, secure, and broad-spectrum medications, new recommendations were made, advising horse owners to deworm all horses every eight weeks (Kaplan & Nielsen, 2010). The prevalence of these guidelines led to a considerable decrease in parasite disease-related morbidity and mortality. As a result, horse health and performance saw a substantial improvement as equine parasites were properly under control for the very first time.

Regrettably, with time, the gastrointestinal nematodes have successfully adapted to the chemical threat (Kaplan & Nielsen, 2010). With the widespread use of such programs which has substantially reduced clinical disease, injudicious use of anthelmintics in the horses and repetitive usage of the same drug for worm drenching purposes results in gastrointestinal parasite resistance towards the anthelmintic drugs in horses. The high treatment frequency has led to considerable selection pressure for anthelmintic resistance, particularly in cyathostomin species (Matthews, 2014). However, equine gastrointestinal parasites resistance towards anthelmintic, especially in the east coast region of Malaysia, has yet to be reported hence the induction of this study.

To address the issue of internal parasites resistance, a fecal egg count reduction test, or FECRT, can be carried out to evaluate the efficacy of an anthelmintic used on stables, whether it be traditional or alternative. This can be a helpful tool for veterinarians or researchers to find out the efficacy of anthelmintic drugs that are used in a deworming program, and further prevent development of anthelmintic resistance in a horse population. FECRT will facilitate in calculating the proportion of internal parasite eggs that have been reduced as a result of the anthelmintic treatment.

The result of the current study showed a decrease in the mean of FEC for the post-treatment group that was treated using Oxfendazole from the Benzimidazole family. The fecal egg reduction percentage (FECR%) reveals the level of resistance to indicate the effectiveness of an anthelmintic drug. In this case, the FECR% was 94% which is interpreted as reduced efficacy with suspected resistance. While the FECR mean for the post Benzimidazole treatment showed significant reduction, such a result for FECR% could come out due to the smaller sample size that was used in this study. This was attributed to the three horses in the post-treatment group which had an epg of 100 that contribute to the value of the mean of FEC for the post treatment result compared to other members of the herd that had a value of epg of 0 each after the treatment. In a nutshell, based on the result, the stable has developed a very mild benzimidazole resistance. This result also could be due to horses typically having lower egg-per-gram ratios than other species, including cattle and sheep (Matthews, 2014). Hence, in an especially smaller sample size like in this study, a little difference of mean for pre and post treatment would result in a huge leap in the FECR% calculation result.

A benzimidazole resistance in gastrointestinal parasites in horses is not surprising since there have been numerous reports of nematode populations around the world developing resistance to benzimidazole which are one of the earlier registered anthelmintics, and field studies frequently note resistance to this class in single populations (Kaplan, 2004). To deal with this issue, the rotational method of deworming, by switching to a different class of deworming medication every 12 months is one of the best ways to combat anthelmintic resistance in horses (Blanek et al., 2006). Another strategy for preventing anthelmintic resistance is to combine different chemical groups of anthelmintics when administering the medication. This strategy has a variety of modes of action and may even prevent the development of resistance altogether because each individual worm may be less resistant to the combined formulation than to a single anthelmintic active ingredient. (Lanusse et al., 2018).

6.0 CONCLUSION AND RECOMMENDATION

In conclusion, there is a suspected resistance of gastrointestinal nematodes towards Benzimidazole in the endurance horses in this study. It is recommended for caretakers and horse owners to implement effective methods to control nematode population and prevent development of anthelmintic resistance in horses, by practicing proper deworming programs, practice rotational usage of anthelmintic drugs and prevent repetitive usage of anthelmintic drugs.

Based on results of the current study, extensive research with more study sites and larger populations are suggested to fully investigate the status of Benzimidazole resistance in horses. A study enrolling horses from various different stables from this region, with different purpose and husbandry management will conclude a more accurate result in order to further investigate anthelmintics resistance in the east coast of Malaysia.

7.0 REFERENCES

Blanek, M., Brady, H. A., Nichols, W. T., Hutcheson, D. P., Jackson, S. P., Pond, K. R., & Wilson, K. R. (2006). Investigation of Anthelmintic Resistance and Deworming Regimens in Horses1. *The Professional Animal Scientist*, *22*(4), 346–352. https://doi.org/10.15232/s1080-7446(15)31118-9

Coles, G. C., Bauer, C., Borgsteede, F. H. M., Geerts, S., Klei, T. R., Taylor, M. A., & Waller, P. J. (1992). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, *44*(1-2), 35–44. https://doi.org/10.1016/0304-4017(92)90141-u

Dauparaitė, E., Kupčinskas, T., von Samson-Himmelstjerna, G., & Petkevičius, S. (2021). Anthelmintic resistance of horse strongyle nematodes to ivermectin and pyrantel in Lithuania. *Acta Veterinaria Scandinavica*, *63*(1). https://doi.org/10.1186/s13028-021-00569-z

Kaplan, R. M. (2002). Anthelmintic resistance in nematodes of horses. *Veterinary research*, *33*(5), 491-507. https://doi.org/10.1051/vetres:2002035

Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology*, *20*(10), 477–481. https://doi.org/10.1016/j.pt.2004.08.001

Köhler, P. (2001). The biochemical basis of anthelmintic action and resistance. *International Journal for Parasitology*, *31*(4), 336–345. https://doi.org/10.1016/s0020-7519(01)00131-x

Kaplan, R. M., & Nielsen, M. K. (2010). An evidence-based approach to equine parasite control: It ain't the 60s anymore. *Equine Veterinary Education*, *22*(6), 306–316. https://doi.org/10.1111/j.2042-3292.2010.00084.x

Klei, T. R. (2000). Equine Immunity to Parasites. *Veterinary Clinics of North America: Equine Practice*, *16*(1), 69–78. https://doi.org/10.1016/s0749-0739(17)30119-0

Lanusse, C., Canton, C., Virkel, G., Alvarez, L., Costa-Junior, L., & Lifschitz, A. (2018). Strategies to Optimize the Efficacy of Anthelmintic Drugs in Ruminants. *Trends in Parasitology*, *34*(8), 664–682. https://doi.org/10.1016/j.pt.2018.05.005

Lichtenfels, J. R., Kharchenko, V. A., & Dvojnos, G. M. (2008). Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). *Veterinary parasitology*, *156*(1-2), 4-161. https://doi.org/10.1016/j.vetpar.2008.04.026

Mathewos, M., Girma, D., Fesseha, H., Yirgalem, M., & Eshetu, E. (2021). Prevalence of gastrointestinal helminthiasis in horses and donkeys of Hawassa District, Southern Ethiopia. *Veterinary Medicine International*, *2021*. https://doi.org/10.1155/2021/6686688

Matthews, J. B. (2014). Anthelmintic resistance in equine nematodes. *International Journal for Parasitology: Drugs and Drug Resistance*, *4*(3), 310–315. https://doi.org/10.1016/j.ijpddr.2014.10.003

Martin, R. J. (1997). Modes of action of anthelmintic drugs. *The Veterinary Journal*, *154*(1), 11–34. https://doi.org/10.1016/s1090-0233(05)80005-x

Nielsen, M. K., Reinemeyer, C. R., Donecker, J. M., Leathwick, D. M., Marchiondo, A. A., & Kaplan, R. M. (2014). Anthelmintic resistance in equine parasites—Current evidence and knowledge gaps. *Veterinary Parasitology*, *204*(1-2), 55–63. https://doi.org/10.1016/j.vetpar.2013.11.030

Periyasamy, U. D., Adzahan, N. M., Isa, M. M., & Ramaiah, D. S. (2017). PREVALENCE OF GASTROINTESTINAL NEMATODES AMONG HORSES FROM VARIOUS ESTABLISHMENTS IN MALAYSIA. *VETERINARY SCIENCES*, 49. http://psasir.upm.edu.my/id/eprint/78281

Reinemeyer, C. R., & Nielsen, M. K. (2009). Parasitism and Colic. Veterinary Clinics of North America: Equine Practice, 25(2), 233–245. https://doi.org/10.1016/j.cveq.2009.04.003

Appendix A: Fecal collection

Table 1 shows the total of 30 horses from Royal Terengganu Endurance Stable (RTES) were enrolled in this study, including 14 mares and 16 geldings. The horses were estimated to be between 2-20 years old. The previous deworming on the horses were between January to February 2021 using Ivermectin.

Table 1: List of horses selected for the study.

No.	Horse ID	Sex	Stable	Previous Deworming
1.	Thor	Gelding	RTES	Jan ‘21
2.	Typhoon	Gelding	RTES	Jan ‘21
3.	Zapple	Gelding	RTES	Jan ‘21
4.	Ejaz	Mare	RTES	Jan ‘21
5.	Nawan	Mare	RTES	Jan ‘21
6.	Ohara	Gelding	RTES	Jan ‘21
7.	Rakinda	Mare	RTES	Jan ‘21
8.	Solstrom	Gelding	RTES	Jan ‘21
9.	Vague	Gelding	RTES	Jan ‘21
10.	Jedai	Mare	RTES	Jan ‘21
11.	Ana	Mare	RTES	Jan ‘21

12.	Coco	Mare	RTES	Jan ‘21
13.	Arthus	Gelding	RTES	Jan ‘21
14.	Neep	Gelding	RTES	Jan ‘21
15.	Regeton	Gelding	RTES	Jan ‘21
16.	Medjik	Mare	RTES	Jan ‘21
17.	Gassur	Mare	RTES	Jan ‘21
18.	Sabaha	Mare	RTES	Jan ‘21
19.	Pacifica	Mare	RTES	Jan ‘21
20.	Virago	Gelding	RTES	Jan ‘21
21.	Moorea	Mare	RTES	Jan ‘21
22.	Esmeralda	Mare	RTES	Jan ‘21
23.	Amores	Mare	RTES	Jan ‘21
24.	Tango	Gelding	RTES	Jan ‘21
25.	Malima	Gelding	RTES	Feb ‘21
26.	Juan	Gelding	RTES	Feb ‘21
27.	St. John	Gelding	RTES	Feb ‘21
28.	Wazir	Gelding	RTES	Feb ‘21

29.	Romeo	Gelding	RTES	Feb ‘21
30.	Asya	Mare	RTES	Feb ‘21

Figure 1: Fecal collection per rectal.

Figure 2: Restraining for fecal collection.

Appendix B: Modified McMaster Method

Figure 3: Weighing feces for McMaster method.

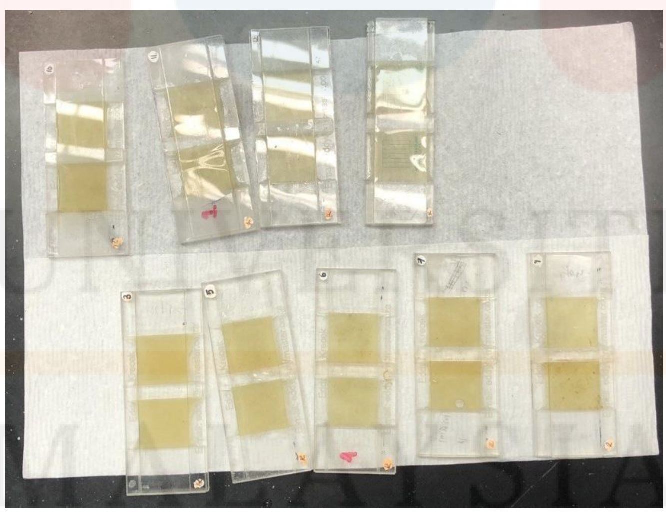

Figure 4: McMaster slides loaded with fecal solutions.

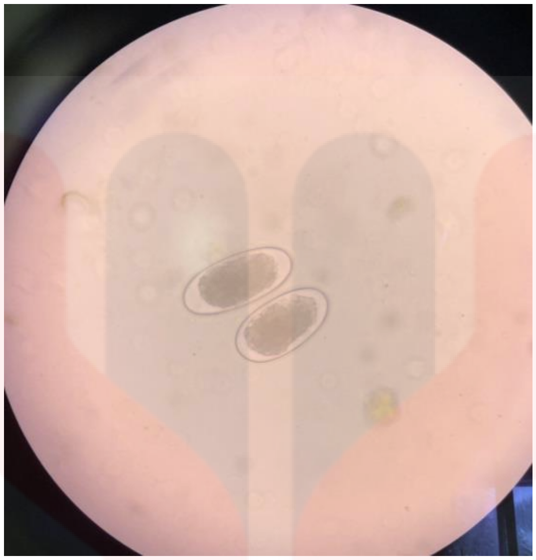

Figure 5: Example of Strongyle eggs detected.