IDENTIFICATION OF GASTROINTESTINAL NEMATODE SPECIES IN AN EQUESTRIAN PARK IN TERENGGANU

JUDITH EMBANG LIBAN

(D18B0011)

A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI MALAYSIA KELANTAN
IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR
THE DEGREE OF
DOCTOR OF VETERINARY MEDICINE

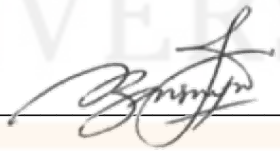
DECEMBER 2022

UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

This is to certify that we have read this research paper entitled "**Identification of Gastrointestinal Nematode Species in An Equestrian Park In Terengganu"** by Judith Embang Liban, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course DVT 55204 – Research Project.

Dr. Mimi Armiladiana Binti Mohamad
DVM (UPM)
Lecturer/Senior Lecturer
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Supervisor)



Dr. Basripuzi Nurul Hayyan Binti Hassan Basri
DVM (UPM), MVSc (UPM), PhD in Veterinary Parasitology (Glasgow)
Lecturer/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. Mimi Armiladiana Binti Mohamad

Dr. Basripuzi Nurul Hayyan Binti Hassan Basri

Dr. Nur Zul Izzati Binti Mohd Rajdi

Dr. Giguruwa Gamage Thilini Nisansala

Lab assistants of FPV UMK

Family and sisters

Apocalypse-survivor group

DVM 5 class of 2018/2023

Thank You

DEDICATIONS

I dedicate my dissertation work to my family and many close friends. Endless gratitude given to them for their endless financial and emotional support.

I also dedicate this dissertation to many of my lecturers who have supported me throughout the process of finishing this thesis. Specially mentioned Dr Nur Zul Izzati, Dr Mimi Armiladiana, Dr Nurul Hayyan and Dr Thilini for their professional guidance, selfless contribution and help throughout the years. All of their training and guidance made me a better veterinary student.

Last but not least, I dedicate this work to my friends Sin Jia, Rui Ying, Yong Yi and Izad for their wonderful companionship throughout my 5 years veterinary student journey. It would not have been possible without the mental and emotional support from them.

Table of Contents

List of Tables

List of Figures

List of Appendices

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 gastrointestinal nematode infestation is one of the most important parasitic diseases that can result in a reduction in performance and growth of horses. This study was done to identify the type of gastrointestinal nematodes in stabled horses in an equestrian park in Terengganu in 2022. A total of 31 horses of various signalment were selected for this study. Fresh faecal samples were collected from the horses rectally. The samples were then processed using modified McMaster faecal egg count test and faecal culture. Out of the 31 faecal samples collected, 26 horses (84%) showed positive for gastrointestinal nematode egg, predominantly strongyle eggs. Upon faecal culture and identification of L3, it was found that the only larvae identified was the cyathostomins.

Keywords: Gastrointestinal nematode, faecal sample, modified McMaster faecal egg count, faecal culture, stabled horses

ABSTRAK

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

Jangkitan nematoda gastro-usus adalah salah satu penyakit parasit yang paling penting yang boleh mengakibatkan penurunan prestasi dan pertumbuhan kuda. Kajian ini dilakukan untuk mengenal pasti jenis nematoda gastrousus dalam kuda di sebuah taman ekuestrian di Terengganu pada tahun 2022. Sebanyak 31 ekor kuda pelbagai latar belakang telah dipilih untuk kajian ini. Sampel najis segar dikumpulkan dari kuda kandang secara rektal. Sampel kemudiannya diproses menggunakan ujian kiraan telur najis McMaster ubah suai dan kultur najis. Daripada 31 sampel najis yang dikumpul, 26 ekor kuda (84%) menunjukkan positif telur nematod gastrousus, kebanyakannya telur strongyle. Selepas menjalani kultur najis dan mengenalpasti identiti L3, satu-satunya larva yang dikenal pasti ialah cyathostomin.

Kata kunci: Nematoda gastro-usus, sampel najis, teknik McMaster yang telah diubah suai, kultur najis, kuda kandang

1.0 INTRODUCTION

Gastrointestinal nematode infestation is a global issue in the horse rearing industry. The ubiquitous nature of nematodes, paired with grazing nature of horses both predisposed them to be continuously infected with gastrointestinal nematodes. This disease is a serious health hazard in horses, which can cause poor body condition, reduced in performance, poor reproductive performance, and stunted growth. Horses are susceptible to many species of nematode infestation, namely the large and small strongyles. According to Urquhart et al. (1996), the nematodes naturally found in horses are from the *Ascaris* sp., thread worm, pinworms, and hairworms.

Gastrointestinal nematode control in horses largely relied on benzimidazole, tetrahydropyrimidines and macrocyclic lactone anthelmintic drugs (Gokbulut & McKellar, 2018). However, the extensive usage of these drugs is leading to the development of anthelmintic resistance in equine, and such anthelmintic resistance has been detected in all of the drug family (Raza et al., 2018). Increasing anthelmintics resistance nowadays further predisposed the horses to have heavier worm burden thus, reducing their quality of life and performance.

There are insufficient studies on the occurrence and prevalence of gastrointestinal parasites (nematodes) in horses in Malaysia, particularly in the equestrian park in Terengganu. A field survey on ivermectin and mebendazole treatment against horses' gastrointestinal nematodes in Selangor found out that the prevalence of nematodes in that particular stable is of the *Cyathostominae* spp. (Rohanizal, 2011). Another study done by Periyasamy et al. (2017) in various equine establishment in Selangor and Putrajaya showed prevalence of *Trichonema* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Parascaris* sp., *Strongylus* sp., *Triodontophorus* sp., and *Poteriostomum* sp.

The understanding of epidemiology of horse gastrointestinal nematodes in the local equine industry and geographical climate conditions can help in formulating a more effective nematode control regime that is beneficial for both animal and human.

1.1 Research Problem

The worrisome rate of anthelmintic resistance development in horses' gastrointestinal nematodes occurs all over the world. There is limited research on the species of gastrointestinal nematodes in horses in Malaysia. Identification of gastrointestinal nematodes in horses is helpful in selecting suitable management and anthelmintic regime for the horses to prevent further disease.

1.2 Research Questions

What is the genus and/or species of gastrointestinal nematode in horses in an equestrian park in Terengganu?

1.3 Research Hypothesis

The genus and/or species of gastrointestinal nematode found in horses in an equestrian park in Terengganu are the cyathostomins, also known as small strongyles.

1.4 Objectives

To investigate the genus and/or species of gastrointestinal nematodes in horses in an equestrian park in Terengganu.

2.0 LITERATURE REVIEW

2.1 Horse

Horse, *Equus caballus*, is a hoofed herbivorous mammal that served many purposes in Malaysia, such as for leisure riding, equestrian sport, polo, show jumping, dressage, park patrols and hippotherapy (Hanis et al., 2020). Horses are adapted to grazing, hence exposing them to the ubiquitous helminth larvae in the pasture. They possessed a small stomach and lack the ability to vomit. They are considered as hindgut fermenter, in which they have large caecum where fermentation of feed take place. The inability to vomit in horses makes them prone to colic. This condition can be exacerbated with heavy infestation of gastrointestinal parasites.

2.2 Gastrointestinal Nematode in Horses

The most common gastrointestinal nematodes in horses in Malaysia are the cyathostomins (Periyasamy et al., 2017), which is also known to be small and non-migratory strongyles infecting the caecum and colon (Young et al., 1999). Other nematode species that are clinically important in horses are the *Strongylus vulgaris*, *Parascaris equorum*, and *Oxyuris equi* (Chapman, 2013).

2.3 Factors Leading to Gastrointestinal Nematodes Infestation in Horses

Horses are grazing animals, and horses' gastrointestinal nematode infective stages are ubiquitous in nature. This leads them to be constantly having gastrointestinal nematode infestation. Furthermore, the increasing incidence of anthelmintic resistance seen in the veterinary industry also increases the risk of infection. The main reason for the development of anthelmintics resistance in horse gastrointestinal nematodes is due to heavy reliance on anthelmintics.

The first safe and effective broad-spectrum anthelmintics that was used in the veterinary medicine against gastrointestinal parasites was the benzimidazoles (Gokbulut & McKellar, 2018). It was also the first anthelmintics that has been reported to cause anthelmintic resistance in gastrointestinal helminth. Following that, tetrahydropyrimidine class and macrocyclic lactones also shows evidence of resistance in horses.

2.4 Life Cycle of Gastrointestinal Nematodes

Gastrointestinal nematodes have 2 types of life cycle, which are direct and indirect. The most common gastrointestinal nematodes found in horses, the cyathostomins, have a direct life cycle, in which they do not require an intermediate host. The adult cyathostomins reside in the large intestine of horses, where they mature and lay eggs.

It took only 2 weeks for an egg to hatch and develop into L3, provided the temperature is optimal. The larvae will then migrate to the surrounding grass. It will be ingested by the horses when the grass is grazed. The L3 of cyathostomins will exsheath and invade the wall of ileum and large intestine, in which they will develop into L4, before they emerge into the gut lumen and moult into adult worms (Taylor et al., 2016).

2.5 Faecal Egg Count

The most widely used standard quantitative tests to estimate the amount of helminth eggs in the animal is through the McMaster method. The modified variation of McMaster technique, as recommended by the World Association for the Advancement of Veterinary Parasitology, is a straightforward method to determine parasite burden and anthelmintic efficacy in domestic species (Lester & Matthews, 2013). The modified McMaster method differs from conventional McMaster technique in which it uses centrifugation method (Coles et al., 1992).

2.6 Larval Identification

Coprological examination is useful in the identification of gastrointestinal nematode genus/species (Jota Baptista et al., 2021). Faecal culture needs to be done to differentiate the species of strongyles, by allowing the eggs to hatch and develop into L3, and then to be recognized by their morphological characteristics (Henriksen and Korsholm, 1983).

3.0 MATERIALS AND METHODS

3.1 The Study Area

This study was conducted in 2 equestrian parks in the state of Terengganu, namely the Royal Terengganu Endurance Stable (RTES) and State of Terengganu Endurance Team (STET).

3.2 Data Collection Tools

Weighing scale	Surgical gloves	Spatula
Sodium chloride solution	Beakers	Ice box
McMaster slide	Centrifuge	Tea sieve
Microscope	Centrifuge tube	Specimen container
Gauze	Paraffin oil	Measuring cylinder
Glass slide	Cover slip	Lugol's Iodine

3.3 Sample Collection

The faecal samples were collected through simple random sampling from 31 horses from an equestrian park in Terengganu. The horses subjected to this study were of various signalment, such as stallion, gelding and mare of various age groups. To collect the faecal sample, horses were restrained in a horse crush for safety purposes. A minimum of 50g of faecal samples were collected rectally using lubricated surgical gloves directly from the rectal ampulla. Once collected, the gloves were labelled with the horses' identification and are stored in room temperature until faecal culture is done in the laboratory.

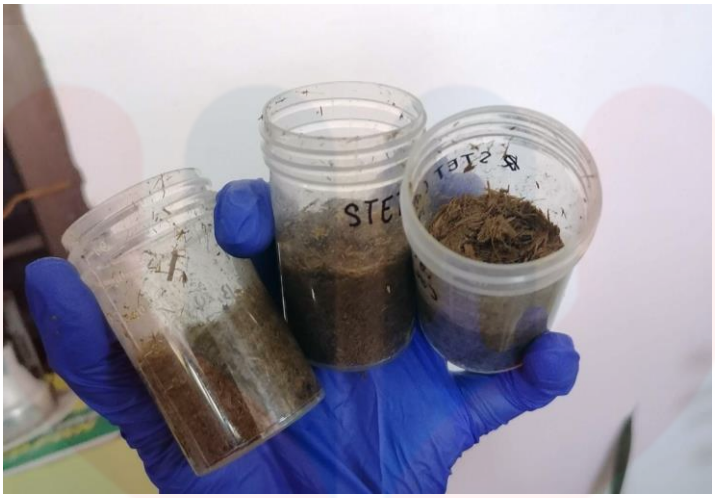
3.4 Faecal Egg Count

The faecal samples were subjected to a modified McMaster faecal egg count method to identify the presence and morphology of the nematode eggs. 3g of faeces was weighed and mixed into 15 ml of tap water. The faeces were broken down into homogenized mixture, and it was sieved through a plastic tea sieve. The filtered mixture was then poured into a centrifuge tube to be centrifuged at 800 rpm for 1 minute. The supernatants were discarded, and the sediments were mixed with 45 ml of saturated sodium chloride (NaCl). The mixture was then pipetted into McMaster chamber to be observed under the light microscope at 10x magnification for egg identification and quantification. The number of eggs calculated was multiplied by 50 to give the eggs per gram of faeces (EPG) of the faecal sample.

3.5 Faecal Culture

For larval identification, faecal culture was done. Five faecal samples were pooled into 1 sample in a specimen container. The pooled faecal samples were broken down by using mortar and pestles. The faeces were transferred into a specimen container until it filled half of the total volume of the container (Figure 1). The faeces were packed nicely with a gloved hand. Then, it was moistened by using distilled water. A piece of gauze was then used to cover the mouth of the container. The faecal culture was kept at room temperature for 7 days in shared area. Distilled water was occasionally sprayed onto the gauze to keep the faecal sample moist.

Figure 1. Faecal sample that filled half of the specimen container

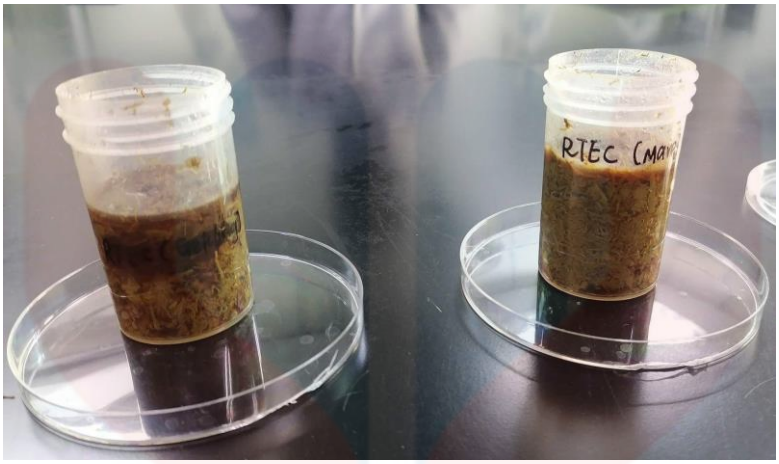


3.6 Extraction and identification

On day 7, the L3 were harvested by using Baermann procedure (Figure 2). The gauze was removed, and lukewarm water was filled into the container until a meniscus was formed over the mouth of the container. A petri dish was then placed on the meniscus over the container. Then, both the petri dish and the container were inverted. The petri dish will then be filled with more lukewarm distilled water until the bottom of the petri dish is filled as shown in. The water was allowed to stand for 30 mins. The water containing the migrated L3 was collected using pipette and transferred into a centrifuge tube and allowed to sediment. The samples were then preserved in the solution at 4 °C until further identification.

For observation of the larvae, the supernatant was discarded by using a pipette and the sediments were pipetted onto a petri dish to be observed directly under the dissecting microscope. Once the presence of the L3 were confirmed, 2% Lugol's iodine were dropped onto the petri dish to kill and stain the L3. Next, the stained L3 will be transferred onto a glass slide, covered with cover slip, and to be observed under the compound microscope for identification.

Figure 2. Specimen container containing faecal sample on a petri dish 7 days later



4.0 RESULTS

4.1 Prevalence of gastrointestinal nematodes in the horses

Out of the 31 horses examined, 27 (87%) of them showed positive FEC results, which indicates that they are infected with gastrointestinal nematodes (Table 1). The predominant egg that was found during the McMaster method was strongyles egg.

Table 1. Prevalence of gastrointestinal nematodes in the horses

No of horses examined	No. of positive samples	No. of negative samples	Prevalence
31	**27**	**4**	**87%**

4.2 Prevalence of gastrointestinal nematodes in relation to sex

Out of the total 31 horses examined, 13 are males (gelding and stallion) and 18 are female (mare). Out of all male horses, 12 horses are infested with gastrointestinal nematodes, which represent 92% of the male horses infested. Meanwhile, 15 out of 18 mares are infested with gastrointestinal nematodes, representing 83% (Table 2).

Table 2. Prevalence of gastrointestinal nematode in relation to sex

Sex of horse	Number of horses examined	No. of positive samples	Prevalence
Male (stallion / gelding)	**13**	**12**	**92%**
Female (mare)	**18**	**15**	**83%**

4.3 Nematode species found in the horses

Based on the morphological characteristics of L3 cultured, the only gastrointestinal nematodes that were able to be identified is the cyathostomin. It was identified through the elongated body (Figure 3), tapering anterior portion (Figure 4), serrated sheath (Figure 5) and long filamentous tail (Figure 5).

Figure 3. Elongated body of cyathostomin (10x magnification)

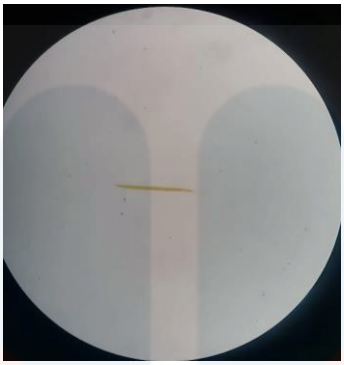


Figure 4. Tapered anterior portion (40x magnification)

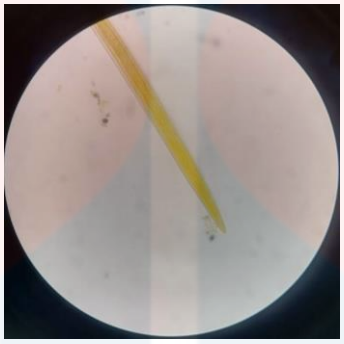


Figure 5. Serrated sheath (40x magnification)

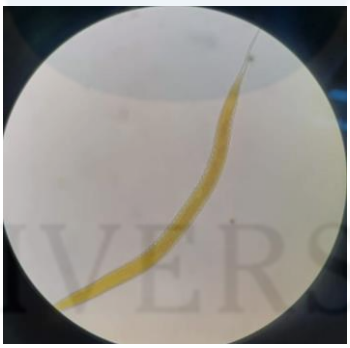
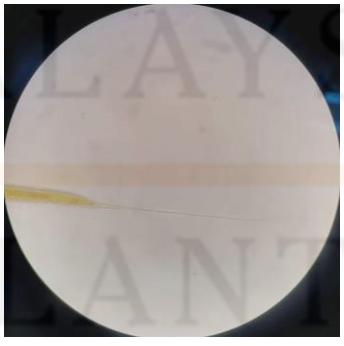


Figure 6. Long filamentous tail (40x magnification)



5.0 DISCUSSION

Gastrointestinal nematodes in horses is one of the important parasitic diseases in horses all over the world that can cause various problems in horses, especially in terms of performance in endurance horses. Determination of parasitic infection in this study was done through coprological study, namely the modified McMaster method and faecal culture method. Modified McMaster method is mainly to quantify the number of eggs present in the faeces, while the faecal culture method is mainly used for morphological identification of larvae. The modified McMaster method carried out has revealed 87% of the study sample population shows positive gastrointestinal nematode count, which is considerably higher as compared to the study carried out by Uma et al. (2017) in various establishments in Malaysia. The main reason for this is believed to be due to the long-time interval from the previous deworming schedule. The equestrian parks in this study carried out their last deworming between 8 to 10 months prior to the study, in which the suggested deworming regime was to deworm the horses quarterly, which is once every 3 months.

The result obtained was also analysed in relation to the sex of the animal. It was found that out of all of the male horses, including gelding and stallion, 92% of them showed positive gastrointestinal nematode infection; meanwhile in the female horses group, the mare, 83% of them showed positive gastrointestinal nematode infection. The reason for male horses having a higher percentage of showing positive gastrointestinal nematode infection is contributed by the activity of the horses themselves. Based on the author's observation, male horses (gelding and stallion) are more frequently allowed to graze outside at the paddock as compared to females (mare) that are usually kept in stable with the foals.

Through faecal culture, the eggs are allowed to hatch and molt into L3. It is through the morphological characteristics of the L3 that we are able to identify the genus/species of the

gastrointestinal nematodes. In our study, the cyathostomins have been shown to show the highest occurrence of horses' gastrointestinal nematode infection. According to Chapman (2013) and Wood et al. (2012), the cyathostomins were reported to be resistant to benzimidazole, ivermectin and pyrantel. In Malaysia, the most commonly used anthelmintic for deworming regime are from the class of macrocyclic lactone, pyrimidines and benzimidazole, which is used as a rotation method given quarterly, usually per oral. The anthelmintics commonly used in Malaysia are the type of anthelmintic that has been previously reported to show evidence of resistance. This possibly explains why the presence of other nematodes was not detected. It can be that the other type of gastrointestinal nematode has been reduced in the environment due to good deworming regime done over the years, thus reducing the presence of the infective larvae stage in the environment.

6.0 CONCLUSION AND RECOMMENDATION

Based on this study titled “Identification of Gastrointestinal Nematode Species in an Equestrian Park in Terengganu”, it was concluded that the main type of gastrointestinal nematode identified from the park was cyathostomin, and it shows as high as 87% positive gastrointestinal nematode infection in the studied equestrian park. Despite the regular and scheduled deworming regime carried out by the farmer, the infection remains considerably high. This can be attributed to the development of anthelmintic resistance, especially in the cyathostomin which has been reported frequently in the recent years.

Thus, is it advisable to carry out anthelmintic resistance study on the horses in the equestrian park to confirm the presence of anthelmintic resistance. Through this, farmers and veterinarians can choose a better deworming regime and drugs to control gastrointestinal nematode infection in the horses.

REFERENCES

Chapman, S. (2013). Control of gastrointestinal nematode species in horses: An evidence-based approach. Livestock, 18(5), 195–200. https://doi.org/10.12968/live.2013.18.5.195

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A. and 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. Vet. Parasitol., 44: 35-44.

Gokbulut, C., & McKellar, Q. A. (2018). Anthelmintic drugs used in equine species. Veterinary Parasitology, 261, 27–52. https://doi.org/10.1016/j.vetpar.2018.08.002

Hanis, F., Chung, E. L., Kamalludin, M. H., & Idrus, Z. (2020). The influence of stable management and feeding practices on the abnormal behaviors among stabled horses in Malaysia. *Journal of Equine Veterinary Science*, *94*, 103230. https://doi.org/10.1016/j.jevs.2020.103230

Henriksen, S. A., & Korsholm, H. (1983). A method for culture and recovery of gastrointestinal strongyle larvae. Luno).

Jota Baptista, C., Sós, E., Szabados, T., Kerekes, V., & Madeira de Carvalho, L. (2021). Intestinal parasites in przewalski's horses (equus ferus przewalskii): A field survey at the Hortobágy National Park, Hungary. Journal of Helminthology, 95. https://doi.org/10.1017/s0022149x21000274

Lester, H. E., & Matthews, J. B. (2013). Faecal worm egg count analysis for targeting anthelmintic treatment in horses: Points to consider. Equine Veterinary Journal, 46(2), 139–145. https://doi.org/10.1111/evj.12199

Periyasamy, U. D. (2017). Prevalence of gastrointestinal nematodes among horses from various establishments in Malaysia. [Unpublished bachelor's thesis]. Universiti Putra Malaysia.

Raza, A., Qamar, A. G., Hayat, K., Ashraf, S., & Williams, A. R. (2018). Anthelmintic resistance and novel control options in equine gastrointestinal nematodes. Parasitology, 146(4), 425–437. https://doi.org/10.1017/s0031182018001786

Rohanizal (2011). Field Evaluation of Ivermectin and Mebendazole Treatment Against Gastrointestinal Parasites in Stable Horses in Selangor. *6th Proceeding of the Seminar on Veterinary Service.*

Taylor, M. A., Coop, R. L., & Wall, R. (2016). Veterinary parasitology. Wiley Blackwell.

Wood, E. L., Matthews, J. B., Stephenson, S., Slote , M., & Nussey , D. H. (2012). Variation in fecal egg counts in horses managed for conservation purposes: Individual egg shedding consistency, age effects and seasonal variation. *Parasitology,* 140(1), 115–128. https://doi.org/10.1017/s003118201200128x

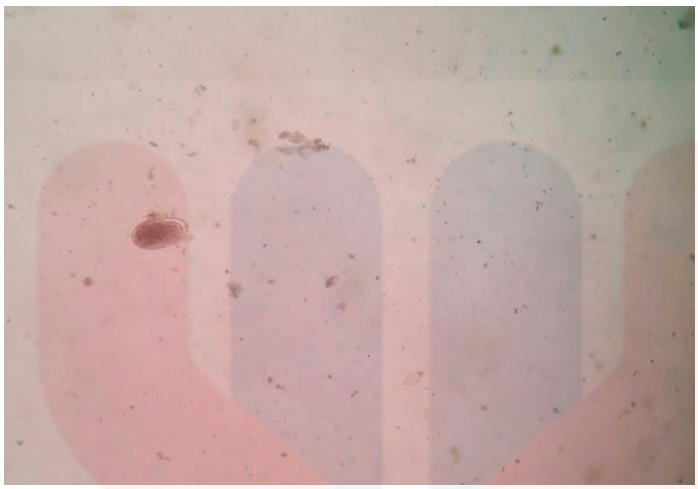
Young, K. E., Garza, V., Snowden, K., Dobson, R. J., Powell, D., & Craig, T. M. (1999). Parasite diversity and anthelmintic resistance in two herds of horses. Veterinary Parasitology, 85(2-3), 205–214. https://doi.org/10.1016/s0304-4017(99)00100-4

APPENDICES

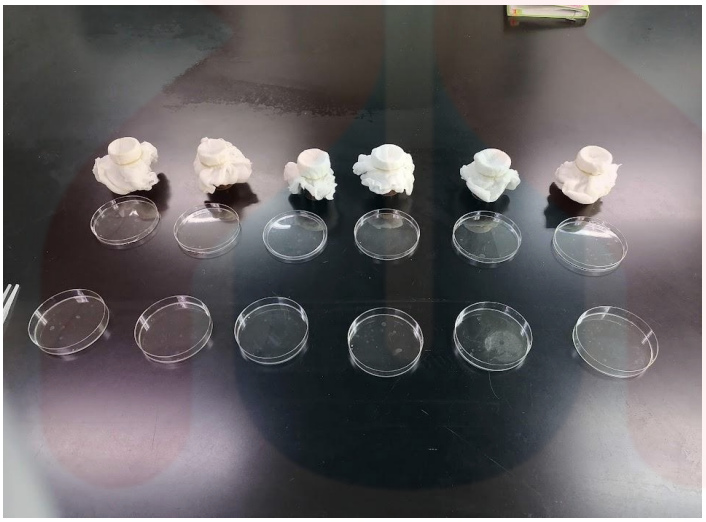
No.	ID	Sex	Stable	Previous deworming	FEC (4 Nov 22)
1.	Coco	Mare	RTES	Jan ‘21	250
2.	Arthus	Gelding	RTES	Jan ‘21	300
3.	Neep	Gelding	RTES	Jan ‘21	2050
4.	Reggaeton	Gelding	RTES	Jan ‘21	150
5.	Medjik	Mare	RTES	Jan ‘21	350
6.	Gassur	Mare	RTES	Jan ‘21	200
7.	Sabaha	Mare	RTES	Jan ‘21	200
8.	Pacifica	Mare	RTES	Jan ‘21	250
9.	Virago	Gelding	RTES	Jan ‘21	100
10.	Moorea	Mare	RTES	Jan ‘21	0
11.	Tuan Junior	Gelding	STET	Dec ‘21	400
12.	Maya Luna	Mare	STET	Dec ‘21	50
13.	Espia	Mare	STET	Dec ‘21	200
14.	Anak Khalima	Mare	STET	Dec ‘21	250
15.	Estupenda	Mare	STET	Dec ‘21	150
16.	Toso	Gelding	STET	Dec ‘21	150
17.	Malon	Gelding	STET	Dec ‘21	300
18.	Lanin	Gelding	STET	Dec ‘21	200
19.	Grieka	Mare	STET	Dec ‘21	100
20.	Khalima	Mare	STET	Dec ‘21	200
21.	Stavio	Stallion	STET	Dec ‘21	100
22.	Amores	Mare	STET	Dec ‘21	0
23.	Blue Moon	Mare	STET	Dec ‘21	50
24.	Vanidosa	Mare	STET	Dec ‘21	750
25.	Tango	Gelding	STET	Dec ‘21	0

26.	Franki	Mare	STET	Dec ‘21	350
27.	Ibrahim	Gelding	STET	Dec ‘21	200
28.	Kursina	Mare	STET	Dec ‘21	250
29.	Natal	Gelding	STET	Dec ‘21	150
30.	Thaylover	Gelding	STET	Dec ‘21	150
31.	Esmeralda	Mare	STET	Dec ‘21	0

Appendix A: Faecal egg count result of the horses

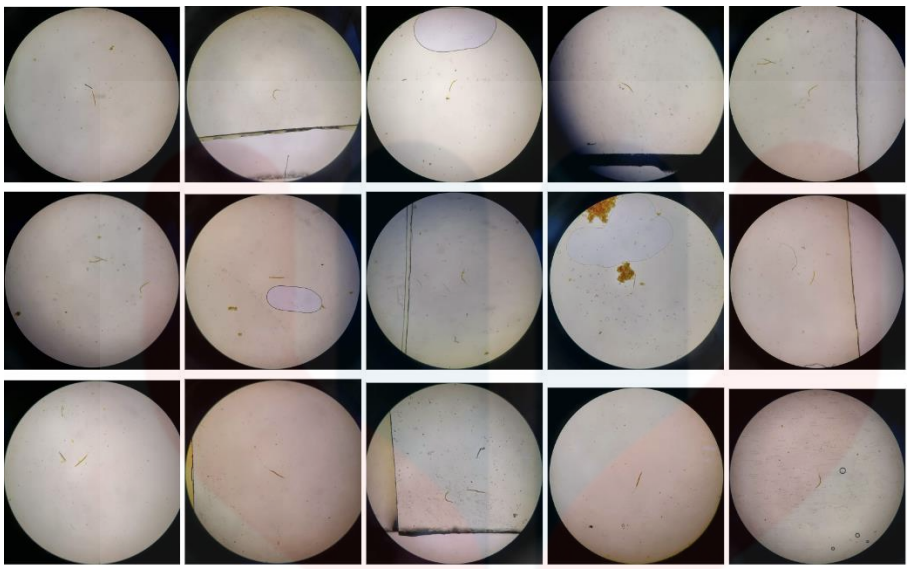


Appendix B: Faecal egg count result of the horses

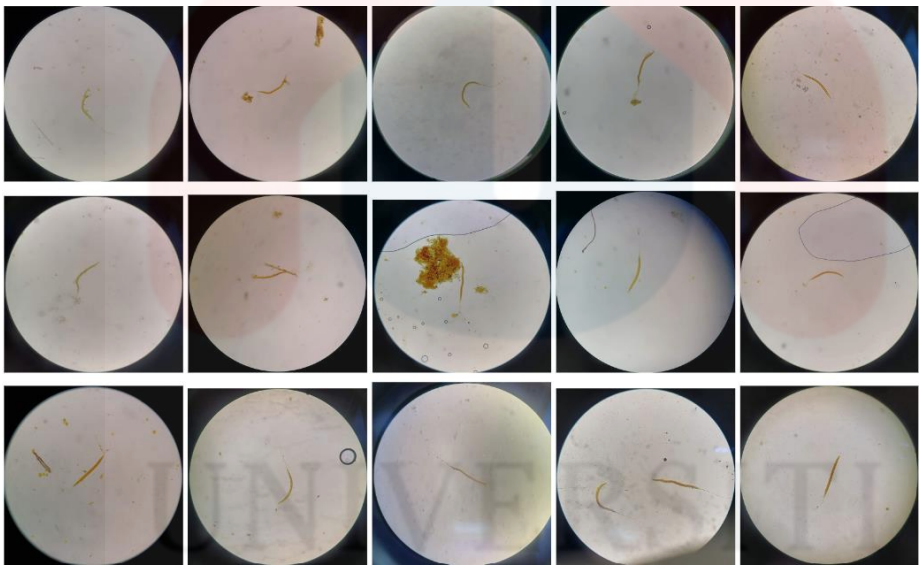


Appendix C: Harvesting the L3 on day-7 of faecal culture

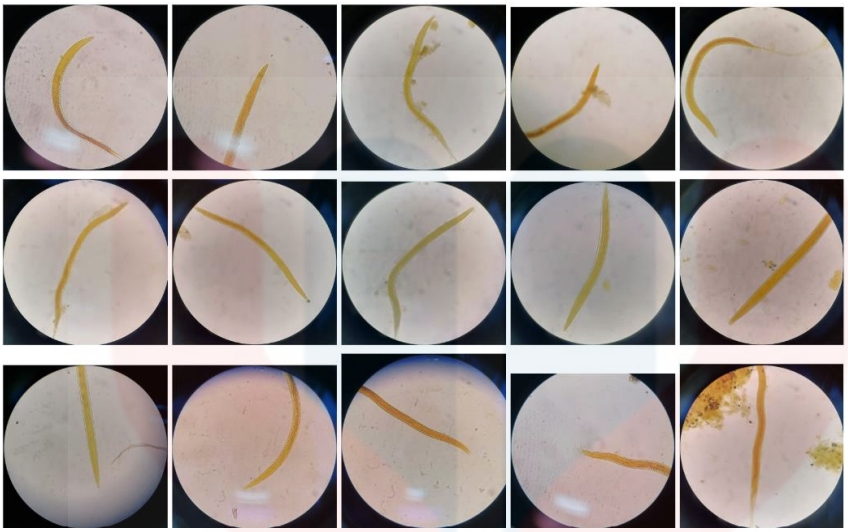
Appendix D: L3 stored in chiller before identification



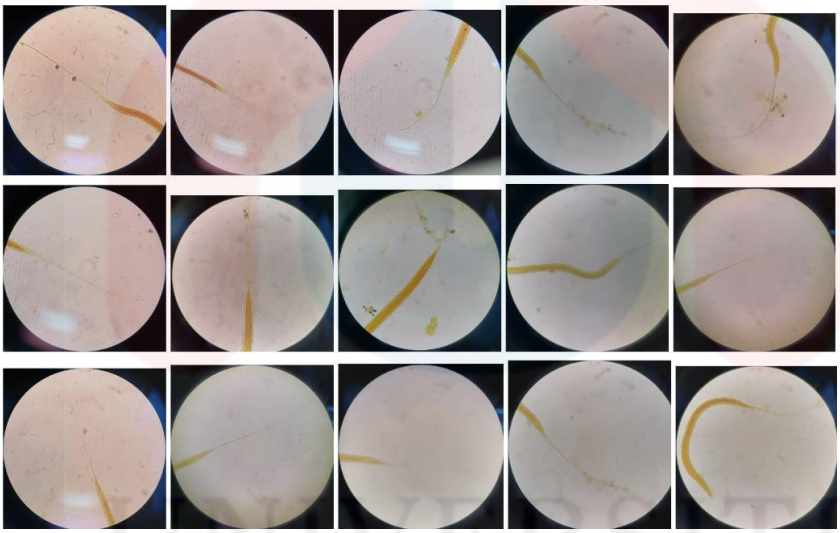
Appendix E: 4x magnification of cyathostomins



Appendix F: 10x magnification of cyathostomins



Appendix G: 40x view of anterior end and sheathed body of cyathostomins



Appendix H: 40x magnification of posterior end of cyathostomin with long filamentous tail