UNIVERSITI MALAYSIA KELANTAN

DETECTION OF SETARIA SPP. AMONG HORSES IN KELANTAN, MALAYSIA USING MICROFILARIA CONCENTRATION METHODS.

By

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ABSTRACT

The equine industry in Malaysia, especially in Kelantan, has gained interest due to activities such as horse racing and endurance riding. However, horses in this region are susceptible to a range of parasitic infections, among which *Setaria* spp., particularly *Setaria digitata*, is of significant concern. *Setaria digitata* is the most commonly reported species in Asia, making it a primary focus for detection in the region. While it is typically found in cattle as its definitive host, horses are considered aberrant hosts, where the parasite may migrate to abnormal locations, causing a range of health issues. In horses, the most common manifestation is ocular setariasis, where a thread-like worm can be seen moving in the anterior chamber of the eye. This can lead to symptoms such as corneal opacity, excessive eye discharge, inflammation, and potentially blindness if untreated. This study aimed to detect *Setaria* spp. in horses in Kelantan using two microfilaria concentration techniques, which are the buffy coat method and the acetone test. A total of 30 blood samples were collected, but no significant findings were observed, possibly due to the method's sensitivity. One sample showed a suspicious result potentially indicating *Setaria* spp., but identification could not be definitively confirmed. These results suggest that while the acetone test may offer more potential for species detection compared to the buffy coat method, further investigation is needed to improve diagnostic accuracy for *Setaria* spp. in the Kelantan equine population. This study contributes to the ongoing efforts to better understand the distribution and detection of *Setaria* spp. in Kelantan, Malaysia.

ABSTRAK

Industri kuda di Malaysia, terutamanya di Kelantan, mendapat perhatian disebabkan oleh aktiviti seperti lumba kuda dan tunggangan daya tahan. Walau bagaimanapun, kuda-kuda di kawasan ini mudah terdedah kepada pelbagai jangkitan parasit, antaranya *Setaria* spp., terutamanya *Setaria digitata*. *Setaria digitata* merupakan spesies yang paling kerap dilaporkan di Asia, menjadikannya fokus utama untuk pengesanan di rantau ini. Walaupun ia biasanya ditemui pada lembu sebagai perumah definitifnya, kuda dianggap sebagai perumah yang tidak normal, di mana parasit ini boleh berhijrah ke lokasi yang tidak normal dan menyebabkan pelbagai masalah kesihatan. Pada kuda, manifestasi yang paling biasa adalah setariasis okular, di mana cacing yang menyerupai benang dapat dilihat bergerak di ruang anterior mata. Ini boleh menyebabkan gejala seperti kelegapan kornea, rembesan mata berlebihan, keradangan, dan berpotensi menjadi buta jika tidak dirawat. Kajian ini bertujuan untuk mengesan *Setaria* spp. pada kuda di Kelantan menggunakan dua teknik penumpuan mikrofilaria, iaitu kaedah lapisan buf and ujian aseton. Sejumlah 30 sampel darah telah dikumpulkan, tetapi tiada penemuan signifikan diperhatikan, berkemungkinan disebabkan oleh sensitiviti kaedah tersebut. Satu sampel menunjukkan keputusan yang mencurigakan yang berpotensi menunjukkan *Setaria* spp., tetapi pengenalpastian tidak dapat disahkan secara muktamad. Keputusan ini mencadangkan bahawa walaupun ujian Aseton mungkin menawarkan lebih banyak potensi untuk pengesanan spesies berbanding dengan kaedah lapisan buf, penyelidikan lanjut diperlukan untuk meningkatkan ketepatan diagnostik bagi *Setaria* spp. dalam populasi kuda Kelantan. Kajian ini menyumbang kepada usaha berterusan untuk lebih memahami dengan lebih baik taburan dan pengesanan *Setaria* spp. di Kelantan, Malaysia.

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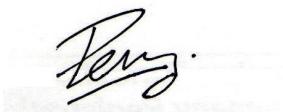

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CHAPTER 1

INTRODUCTION

In Malaysia, the equine sector primarily includes horse racing, equestrian sports, and breeding. However, they are mainly concentrated in urban areas as it requires a high cost of horses, equipment, stable facilities, and management and veterinary care, which often limits participation to wealthier individuals (Darmansah et al., 2017). In Kelantan, horses are used for a variety of purposes with racing and endurance riding being the most common, which have become part of their culture (Khan, 2016). Other than that, owning and rearing horses is a popular recreational hobby where the people in Kelantan enjoy it as a leisure activity. Recently in 2021, a horseback archery training center was established that offers structured lessons and instruction and training in the sport (HarakahDaily, 2021).

Setaria spp. is a filarial nematode parasite belonging to the family *Setariidae* and the order Spirurida. (Helmi et al., 2025). Different species have different definitive hosts, such as horses for *Setaria equina* and ruminants for *S. digitata, S. marshalli,* and *S. labiatopapillosa* (Davoodi 2014). Setarial parasites are commonly found in the peritoneal cavity of ungulates. They are virtually non-pathogenic but may be associated with mild fibrinous peritonitis (Kaur et al., 2013). However, larvae of *Setaria* spp. often invade other organs such as the brain and spinal cords, and frequently migrate to the eye, resulting in severe intraocular inflammation, photophobia, corneal opacity, blepharospasm, and lacrimation. (Gilger, 2016). Among the species, *Setaria equina* is one of the most commonly encountered, particularly in tropical climates, where its mosquito vectors thrive. (Healy et al., 2025). However, *Setaria digitata* has been more frequently reported in ocular diseases, particularly in Asia, where it causes similar clinical signs to those observed with *Setaria equina*.

Setaria spp. can be detected using various diagnostic techniques, including microscopy, molecular methods such as polymerase chain reaction (PCR). Other than that, microfilaria concentration methods are one of the methods that can be used in resource-limited conditions. There are two examples of microfilaria concentration methods that can be utilized, which are the buffy coat method and the acetone test. The buffy coat method involves centrifugation of blood to separate the buffy coat that contains mostly white blood cells, platelets and microfilariae in cases of parasitic infections like *S. digitata* to can be detected under microscopic examination (Chagas et al., 2020). Whereas acetone test involves acetone to lyse red blood cells which clears the background and facilitates visualization of microfilariae in sediment (Watanabe et al., 2004).

The purpose of this study is to detect the occurrence of *Setaria* spp. infection among horses in Kelantan, Malaysia,using microfilaria concentration method, which is the buffy coat method and Acetone test. This method aims to provide a reliable and efficient means of detecting the presence of microfilariae in the blood of horses, thereby facilitating the identification of infected individuals. By investigating the distribution and occurrence of *Setaria* spp. in this region, the study seeks to contribute valuable insights into the epidemiology of equine setariasis, which may guide the development of effective prevention and management measures.

1.1 Research problem statement

Ocular and neurological setariasis in horses caused by migration of *Setaria* spp. to unusual sites within the body significantly impacts the health, performance, and welfare of horses, particularly those used for recreational and sports purposes. It may lead to economic losses for owners due to treatment costs, loss of function, or long-term disability. Despite increasing reports of severe clinical outcomes associated with *S. digitata* in horses in other countries, there is a notable lack of epidemiological data on the prevalence of this parasite among equine populations in Kelantan, Malaysia. Additionally, there is lack of information on the sensitivity of microfilaria concentration methods, such as the buffy coat method and acetone test, in detecting *Setaria* spp. in horses within this region. It limits our understanding of the burden of the infection, especially in horses that may carry the parasite without showing obvious clinical signs. By focusing on detection methods, this study aims to bridge the knowledge gap and provide a foundation for future efforts in parasite control and prevention.

1.2 Research questions

1. What is the occurrence of *Setaria* spp. infection among horses in Kelantan as detected by the microfilaria concentration method?
2. What is the comparison of detection using the buffy coat method and the acetone test in detecting *Setaria* spp. microfilariae in horses in Kelantan?

1.3 Research hypothesis

1. There is a detectable occurrence of *Setaria* spp. infection among horses in Kelantan using microfilaria concentration methods.

2. There are significant differences in the effectiveness of microfilaria concentration methods in detection of *Setaria* spp. microfilaria between buffy coat technique and acetone test in horses.

1.4 Research Objectives

1. To determine the occurrence of *Setaria* spp. infection among horses in Kelantan using the microfilaria concentration method (buffy coat technique and Acetone test).
2. To determine the effectiveness of methods of detection of the buffy coat technique and acetone test in identifying *Setaria* spp. microfilariae in horses in Kelantan.

CHAPTER 2
LITERATURE REVIEW

2.1 Overview of *Setaria* spp.

Setaria spp. is a genus of filarial nematodes belonging to the family *Setariidae,* which primarily infects ungulates, such as cattle, deer and horses. They are transmitted through mosquitoe vectors, including *Aedes, Culex,* and *Anopheles* species. (Akinniyi & Vaughan, 2024). The larvae develop in mosquitoes and then transmitted to new hosts during the mosquito's blood-feeding process. (Bain & Babayan, 2003). While *Setaria* spp. primarily infects ruminants like cattle, horses are considered non-natural hosts for this parasite, leading to ectopic migration of *S. digitata* larvae. These larvae can migrate to abnormal locations, causing pathological conditions such as ocular setariasis and neurological setariasis in horses (Lee et al., 2021). Ocular setariasis, which results from larvae invading the eye, is a significant cause of vision impairment in equines, leading to symptoms like corneal opacity and excessive discharge, and potentially resulting in blindness. (Yu et al., 2021). Similarly, neurological setariasis or cerebrospinal nematodiasis, where larvae migrate to the central nervous system, results in severe neuropathological signs such as hindlimb ataxia and gait disturbances (Lee et al., 2021).

Setaria equina is a species that is spread widely through vectors and primarily affects horses, especially in tropical climates (Akinniyi & Vaughan, 2024). It is well adapted to equine hosts and is considered one of the most prevalent *Setaria* species in horses (Akinniyi & Vaughan, 2024). Whereas *S. digitata, S. marshalli,* and *S. labiatopapillosa* typically infects cattle as its definitive host, but it can also infect horses, which they serve as aberrant hosts (Kim et al., 2010). *Setaria digitata* has been the subject of several studies, particularly in countries like India and South Korea, where it has caused outbreaks of ocular and neurological setariasis in horses (Yu et

al., 2021). In Malaysia, there is only one published case of *S. digitata* infection in a horse in Malaysia, specifically involving ocular setariasis in Kelantan (Peng et al., 2019).

Another species that is primarily found in ruminants but occasionally infects horses is *Setaria marshalli* (Kim et al., 2018). *Setaria marshalli,* just like other species it can cause significant neurological disorders when larvae fail to migrate to their typical infection sites in non-natural hosts, leading to cerebrospinal setariasis. (Kitajima et al., 2022). The next species of Setaria is *Setaria labiatopapillosa.* The migration of larvae from *S. labiatopapillosa* to the eye or central nervous system in horses can result in conditions similar to those caused by *S. equina* and *S. digitata*, such as ocular setariasis and neurological disorders (Jayasinghe & Wijesundera, 2003).

While all can cause both ocular and neurological issues in horses, *S. digitata* is most frequently associated with ocular setariasis in Asian horses, whereas others species are known causes of the serious condition known as cerebrospinal nematodiasis (lumbar paralysis) when larvae reach the central nervous system. (Wijesundera et al., 1999). As for geographic distribution. *S. equina* is found more globally in horses, while *S. digitata* is more concentrated in Asia, particularly in horses in countries like India, South Korea, and Malaysia (Rahman, 2020).

2.2 Lifecycle and Transmission of *Setaria* spp.

As a mosquito-borne filarial parasite, *Setaria* spp. thrives in tropical and subtropical climates, which provide ideal conditions for the lifecycle of both the parasite and its insect vectors (Akinniyi & Vaughan, 2024). The life cycle of *Setaria* spp. involves both definitive and intermediate hosts, which primarily mosquitoes of the genera *Culex, Aedes,* and *Anopheles*. In the definitive host, such as cattle or horses, adult *Setaria* worms reside primarily in the peritoneal cavity. (Kumar & Kumar, 2018). Female adult worms release microfilariae (larval stages) into the bloodstream, which circulate throughout the host's body. These microfilariae are then ingested by mosquitoes when they feed on the host. (Marchiondo, 2019).

Once ingested by mosquitoes, the microfilariae undergo development within the mosquito's body. In mosquitoes, microfilariae develop into infective L3 larvae over a period of 11-13 days, depending on environmental conditions (Kumar & Kumar, 2018). Transmission of *Setaria* spp. occurs when infective third-stage larvae (L3) are introduced into the definitive host during the blood-feeding process of an infected mosquito. Following entry, the larvae will migrate through host tissues and ultimately mature into adult worms, usually residing in the peritoneal cavity. Mature females subsequently release microfilariae into the host's bloodstream, which are then ingested by mosquitoes during later feedings, completing the lifecycle.

2.3 Clinical Manifestations of *Setaria* spp. infections in horses

The clinical manifestations of *Setaria* spp. infections in horses. The clinical signs of *Setaria* spp. infections in horses are mainly caused by the migration of larvae to abnormal locations within the body, such as the eyes, brain, and spinal cord. While *Setaria* spp. are generally non-pathogenic in their natural hosts, such as cattle for *S. digitata* and *S. equina*, horses act as aberrant hosts, and the larvae's migration can lead to significant health problems.

One of the most common manifestations of *Setaria* spp. infection in horses is ocular setariasis, which occurs when the larvae migrate to the eye. (Dabas, 2021). This condition is particularly associated with *Setaria equina*, *Setaria digitata,* and *Setaria marshalli.* Horses infected with *Setaria* spp. typically display clinical signs such as photophobia and lacrimation. As the larvae move through the intraocular tissues, they cause mechanical trauma and inflammation, resulting in corneal opacity, corneal edema, and potential blindness if left untreated. (Shin et al., 2017). The serrated cuticle of the worm and its lashing movements within the anterior chamber cause severe trauma to the cornea, which often leads to inflammation (Rafee & Amarpal, 2016). The consequences can include synechia (adhesion of the iris to the cornea or lens), cataract formation, and retinal detachment, which can severely impair vision. (Paglia et al., 2004).

Another significant manifestation, particularly for *Setaria equina* and *Setaria marshalli*, is neurological setariasis. This condition occurs when larvae migrate to the central nervous system (CNS), including the brain and spinal cord. (Hwang et al., 2022). It often leads to acute hindlimb ataxia and other neurological symptoms. (Lee et al., 2021). Affected horses often exhibit signs such as a staggering gait, severe ataxia and difficulty standing, which can progress to paralysis in extreme cases. (Lee et al., 2021) The condition is most commonly observed in late summer and autumn, coinciding with increased mosquito activity, which facilitates the transmission of infective larvae. Histopathological analysis reveals microcavitation, axonal degeneration, and infiltration of eosinophils and macrophages in the affected spinal cord and brain tissues. (Lee et al., 2021).

2.4 Diagnostic Methods for *Setaria* spp.

There are several diagnostic methods for detecting *Setaria* spp. in horses, including Modified Knott's Test, Membrane Filtration, and direct blood smears with staining. (Hanafiah et al., 2023). These methods are widely used in veterinary parasitology due to their ability to concentrate and visualize microfilariae in blood samples. While these methods are effective, they can be time-consuming, require specific reagents and are more suitable for well-equipped laboratories. Alternatively, Buffy Coat Technique and Acetone Test are other techniques that offer rapid and effective methods of detecting microfilariae. Buffy Coat Technique involves centrifuging blood to separate the buffy coat and examining it under the microscope. (Hanafiah et al., 2023). This technique, without the need for staining, allows for quick results, making it ideal for field studies. Acetone Test, on the other hand, involves using acetone to lyse red blood cells, clearing the background and concentrating the microfilariae for easier detection. (Watanabe et al., 2004). This test has been found to be more efficient than the Modified Knott's Test for detecting *Setaria digitata* microfilariae in cattle (Watanabe et al., 2004).

2.5 Microfilaria concentration method (Buffy Coat Method)

The buffy coat method involves centrifugation of blood to separate the buffy coat which can be detected under microscopic examination (Chagas et al., 2020). Centrifugation of anticoagulated whole blood is done in a capillary microhematocrit tube, allowing for the separation of blood components based on density. The white thin layer is known as the buffy coat. It contains white blood cells, platelets and any microfilariae, in this case, *Setaria* spp. present in the sample. This layer is then carefully extracted, placed onto a microscope slide, stained and examined under a microscope. The procedure enhances visibility of microfilariae by concentrating them in a small volume, which is especially useful when parasitemia is low or intermittent, as is often the case in equine hosts harboring *Setaria* spp.

2.6 Microfilaria concentration method (Acetone Test)

Acetone test is another microfilaria concentration method that can be used to detect microfilaria of *Setaria* spp. It uses Acetone to lyse red blood cells in a blood sample thus clearing the background to allow concentration of microfilariae in the sediment after centrifugation. The sediment is can then be examined under a microscope for the presence of any microfilariae. This method is useful to detect low levels of microfilariae that might be missed by direct smear examination. (Watanabe et al., 2004). It is suitable to use in cattle, other ruminants, and horses whose blood is less prone to hemolysis compared to carnivores like dogs. Additionally, the Acetone test is simple and cost effective, therefore it is practical for use in a laboratory.

CHAPTER 3

MATERIALS AND METHODS

3.1 Ethical Considerations

Ethical approvals for using animals in the current study was obtained from the Animal Ethics Committee, Faculty of Veterinary Medicine of Universiti Malaysia Kelantan under the approval code of UMK/FPV/ACUE/FYP/012/2025 (Appendix A).

3.2 Study Area and Target Population

The study area involved three horse stables in Kelantan, Malaysia. The target population for this study consisted of horses in Kelantan, Malaysia. A total of 30 horses were selected from stables located in the Bachok and Machang districts of Kelantan. 22 horses were selected from stables in Bachok, including 1 in Teaching Farm, UMK, 6 from JHJ Stable and 15 from Wakaf Aik. 8 horses were selected from those in Kota Bharu, including 8 from Kok Lanas. The stables were chosen based on their accessibility and the presence of horses suitable for the study. Horses were selected regardless of their deworming history. However, demographic information such as age, breed, and sex were recorded for each horse to ensure a diverse representation of the equine population.

No.	District	Number of samples collected
1.	Bachok	22
2.	Machang	8
	Total	30

Table 3.1 Samples collected according to Districts in Kelantan.

3.3 Sample Collection

A total of 30 blood samples were collected from horses in Kelantan throughout July to October. Blood was aseptically collected from the jugular vein of each horse, with approximately 3 to 5 ml of blood drawn and stored in Ethylenediaminetetraacetic acid (EDTA) tube. The samples were collected using a 1.0 inch 21G vacutainer needle and each blood tube was labelled accordingly. At the laboratory, the samples will be stored in a chiller at 2 to 8 °C and processed within7 days.

3.4 Sample Processing for Buffy Coat Method.

The blood samples were processed using the Buffy Coat Method to detect the presence of *Setaria* spp. Microfilaria. First, the blood was drawn into microhematocrit tubes and centrifuged at 1300-1500 rpm for 5 minutes to separate the blood components. After centrifugation, the buffy coat layer, which contained the white blood cells and microfilaria, was identified between the red blood cells and plasma. A diamond pen was used to mark the microhematorit tube at the level of the buffy coat. Then, the tube was carefully snapped at the marked line to extract the buffy coat layer along with a small amount of plasma. The mixture was transferred to a glass slide, where a drop of saline and a drop of methylene blue were added. A coverslip was placed over the sample and the slide was examined under a microscope. The staining process helped break down red blood cells, enhancing the visibility of microfilaria under the microscope and allowing easier detection even in cases of low parasite burden.

3.5 Sample Processing for Acetone Test.

Then, the blood samples were processed using the Acetone test. Firstly, acetone hemolytic solution was prepared by mixing 5 ml of acetone, 5 ml of 0.5% methylene blue

aqueous solution and 90 ml of distilled water. Then, 1 ml of whole blood was added and mixed in centrifuge tube contain 9 ml of acetone hemolytic solution. The mixture is centrifuged at 160 x g for 5 minutes, then the supernatant was discarded. 9 ml of distilled water was added to the 1 ml of the residual fluid containing the microfilaria. The centrifuge tube was centrifuged again at 160 x g for 5 min. The supernatant was decanted, and the sediment was spread onto a microscope slide, covered with a coverslip. Then the sediment was examined under a light microscope for the detection of microfilariae of *Setaria digitata* (Watanabe et al., 2004).

3.6 Data Analysis

Data were analyzed using descriptive analysis methods. The occurrence of *Setaria* spp. infection was summarized based on the number of positive cases detected by both the Buffy Coat Method and the Acetone Test. For categorical variables such as sex, breed, and diagnostic test results, frequencies and percentages were calculated. The effectiveness of each detection method was compared descriptively, evaluating the number of positive detections to assess the sensitivity and reliability of the Buffy Coat Method and the Acetone Test in detecting *Setaria* spp. in horses.

CHAPTER 4
RESULT

4.1 Demographic Data of Selected Horses

Table 4.1 presents the demographic data of the horses enrolled in the study. Of the 30 horses sampled, 60.00% were male and 40.00% were female. The majority of the horses were adults, ranging in age from 2 to 20 years (80.00%), while 20.00% were young horses under 2 years old. Regarding breed, 53.33% of the horses were of Arabian crossed breed, 26.67% were Warmblood, 13.33% were Thoroughbred, and 6.67% were Pony. Based on the physical examination findings, 93.33% of the horses were healthy, while 6.67% exhibited suspected signs of infection. Specifically, only 1 horse showed an opaque lesion in the left eye, suggesting a potential ocular infection, while the rest of the horses were physically healthy.

Table 4.1 Demographic Data of Horses Sampled in Kelantan (n = 30)

Category		No of horses	Percentage (%)
Sex	Male	12	40.00
	Female	18	60.00
Age	Undetermined	5	16.67
	Young (<5 years old)	6	20.00
	Adult (≥ years old)	19	63.33
	Undetermined	5	16.67
Breed	Polo	1	3.33
	Thoroughbred	3	10.00
	KKB/Pony	3	10.00
	Warmblood	6	20.00

	Arabian Crossed	17	56.67
Ocular clinical signs		1	6.67

4.2 The Occurrence of *Setaria* spp. Microfilariae

Based on the results obtained (Appendix B), the occurrence of *Setaria* spp. microfilariae was determined using both the Buffy Coat Method and the Acetone Test. For the Buffy Coat Method, all 30 horses tested negative for *Setaria* spp. microfilariae, indicating no detectable presence of the parasite. For Acetone Test Only 1 horse exhibited a suspected positive result (Figure 4.2), while the remaining 29 horses tested negative for the parasite.

Table 4.2 Occurrence of *Setaria* spp. Microfilariae in Kelantan using Microfilaria Concentration Methods

Method	Suspected Positive Cases (Frequency)	Negative Cases (Frequency)	Total Horses	Percentage of Positives (%)
Buffy Coat Method	0	30	30	0.00
Acetone Test	1	29		3.33

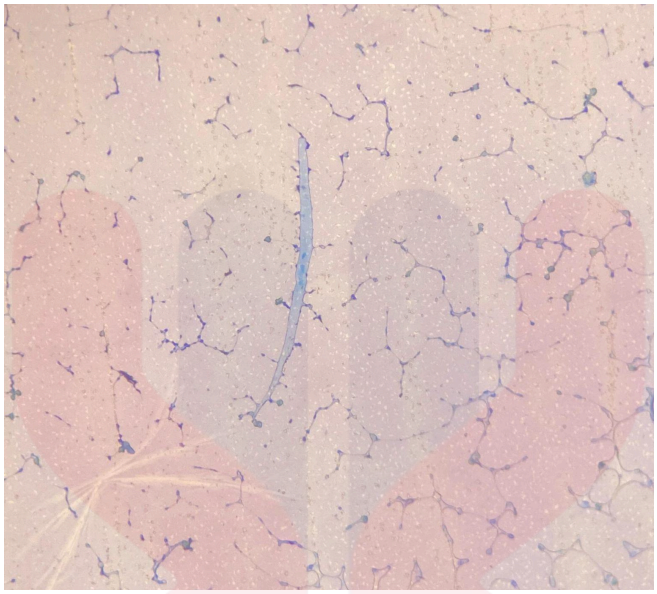

Figure 4.2 Acetone test suspected *Setaria* spp. microfilaria positive result (10x)

4.3 Comparison of Buffy Coat Method and Acetone Test

The Buffy Coat Method and the Acetone Test were compared in terms of their effectiveness in detecting *Setaria* spp microfilariae in horses. For the Buffy Coat Method, this method showed no positive results for *Setaria* spp. microfilariae, with all 30 horses testing negative for the parasite. Whereas for Acetone Test, only 1 horse exhibited a suspected positive result, while the remaining 29 horses tested negative. The Acetone Test demonstrated greater sensitivity in detecting *Setaria* spp. microfilariae compared to the Buffy Coat Method, which showed no detectable presence of the parasite. However, the Acetone Test still had a relatively low occurrence rate of suspected positives (3.33%).

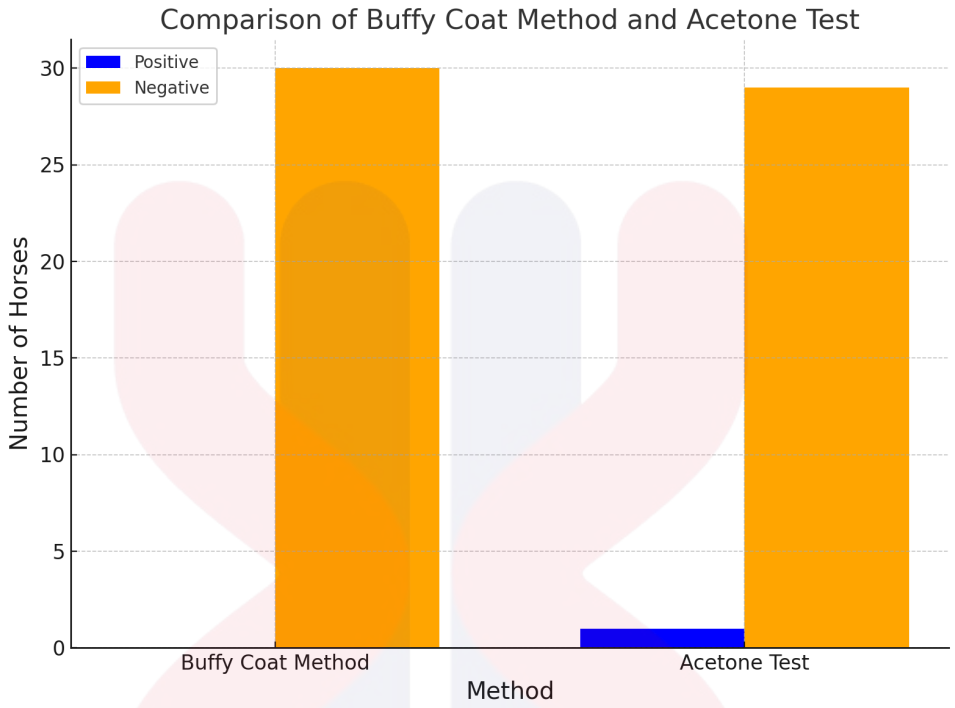

Figure 4.3 : Comparison of the Buffy Coat Method and Acetone Test for the Detection of *Setaria* spp Microfilariae

CHAPTER 5
DISCUSSION

The study aimed to detect the presence of *Setaria* spp. in horses in Kelantan, Malaysia, using two microfilaria concentration methods, the Buffy Coat Method and the Acetone Test. The primary objective was to assess the occurrence of *Setaria* spp, a parasitic nematode that can cause ocular and neurological issues in horses, especially in tropical climates. By employing these methods, the study aimed to identify any detectable occurrences of *Setaria* spp. microfilariae in blood samples collected from horses in Kelantan, a region where limited data on this parasite exists. Furthermore, the study aimed to evaluate and compare the effectiveness of the two detection techniques, with the expectation that the Acetone Test might offer greater sensitivity in identifying microfilariae compared to the Buffy Coat Method. This research contributes to filling the knowledge gap in equine parasitology, which can aid in the development of more effective diagnostic and management strategies for *Setaria* spp. in Malaysia.

The findings from the study revealed that the occurrence of *Setaria* spp. microfilariae in horses in Kelantan were possibly low. Specifically, only one documented case of ocular setarisis caused by *Setaria digitata* in a horse in Kelantan, Malaysia. (Peng et al., 2019). The Buffy Coat Method showed no positive results, with all 30 horses testing negative for *Setaria* spp. microfilariae. In contrast, the Acetone test yielded a single suspected positive result, translating to a occurrence rate of 3.33%. From 30 horses sampled, there was only one horse exhibited clinical signs of infection, such as ocular opacity but it did not test positive in either the Buffy Coat Method or Acetone Test. While this finding suggests that *Setaria* spp may be present in the local equine population, the low number of positives implies that either the parasite's occurrence

is low in the studied region or the diagnostic methods used were not sensitive enough to detect microfilariae in all infected individuals. The low occurrence of Setaria *spp*. in Kelantan could also reflect the management of the stables such as the mosquito control measures andproper veterinary care. For example, covering stables with fine wire mesh or destruction of mosquito breeding sites could significantly reduce vector exposure (Peng et al., 2019), which in turn would lower the risk of *Setaria* spp. transmission to horses.

For the Buffy Coat Method, although widely used for detecting microfilariae in various parasitic infections, it did not yield significant findings in this study. All 30 horses tested negative for *Setaria* spp. microfilariae using this method. A potential reason for this lack of positive results could be methodological limitations. One possible explanation is the difficulty in accurately snapping the microhematocrit tube at the buffy coat layer, which could result in a failure to isolate the optimal layer containing the microfilariae. Moreover, the samples were collected from distant locations, and since microfilariae are motile, they could have migrated to other blood layers during transportation or storage before examination. (Chagas et al., 2020). Besides, deworming status of the horses should have been noted as treatment with macrocyclic lactone such as ivermectin may have impacted the results because they are highly effective at eliminating the circulating microfilaria in the peripheral blood. (Martin et al., 2020). These factors could have contributed to the low sensitivity of the Buffy Coat Method in this particular study.

However, despite these challenges, the Buffy Coat Method remains an important screening tool in resource-limited settings, as it is relatively simple and cost-effective. It provides a quick, low-cost alternative that can be particularly useful in field studies, even if it may miss some infections, particularly those with low parasitemia. Thus, while the method did not show

significant results here, it should still be considered a valuable tool for initial screenings, especially in settings where more advanced techniques are not accessible.

In contrast, the Acetone Test showed more promising results, detecting suspected microfilariae in 1 horse. The suspected positive microfilaria observed in the sample appeared elongated, thread-like and often wavy in shape, which is characteristic of microfilariae in general including *Setaria* spp. (Mathison et al., 2019). In addition, the methylene blue used in the Acetone Test solution during preparation stains the microfilariae blue, a common feature for many types of filarial larvae, depending on the type of solution used. (Watanabe et al., 2004). While the exact species could not be confirmed, these microfilaria exhibited key characteristics typically seen in *Setaria* spp., such as rounded anterior (blunt head) and tapered, pointed posterior (tail) which can further suspect it to be *Setaria* spp. (Rahman, 2020). The result was considered suspected because only one microfilaria was found and it could either be an artifact or represent the presence of a very low number of *Setaria* spp. microfilaria in the sample. (Figure 4.2). To confirm the result, further diagnostic techniques should be performed such as molecular methods like Polymerase Chain Reaction (PCR) for species-specific identification, which would definitively confirm the microfilaria belong to *Setaria* spp. (Wijesundera et al., 1999).

Although the Acetone Test only detected suspected microfilariae in one horse, it still demonstrated a clear advantage over the Buffy Coat Method in terms of visualization and clarity. The acetone test's ability to lyse red blood cells and concentrate the microfilariae makes it a more effective method for visualizing low-level infections that might be missed by other techniques. Even if the acetone test had shown negative results, it would still offer the advantage of providing clearer visualization and a simpler methodology compared to the Buffy Coat

Method, which could be advantageous in confirming negative cases. Overall, the acetone test may offer a better balance between sensitivity and clarity in the field, especially in detecting suspected low-level infections.

Additionally, early detection of *Setaria* spp. Infection in horses can be achieved through several diagnostic methods. Firstly, is by conducting PCR, which is one of the most sensitive and specific techniques. It allows detection of *Setaria* spp. at an early stage, even before clinical signs appear, by targeting species-specific DNA (Verocai et al., 2024). In addition to molecular methods, wet blood examination can be done, where a small amount of the blood is placed on a microscope slide, usually mixed with a drop of saline and covered with a coverslip and examined immediately while still wet without drying or staining. (Sivajothi et al., 2018). Another widely used technique is the Modified Knott's Test, which concentrates microfilaria by lysing red blood cells, followed by microscopic examination. This method is effective for detecting *Setaria* spp. but can be less sensitive in cases of low parasitemia (Hanafiah et al., 2023).

CHAPTER 6
CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In conclusion, this study aimed to detect the occurrence of *Setaria* spp. in horses in Kelantan using two microfilaria concentration methods which are the Buffy Coat Method and the Acetone Test. Although the Buffy Coat Method yielded no positive results, the Acetone Test detected a suspected case in one horse, indicating that the parasite might be present in the region at low concentrations. The findings indicate a low occurrence of *Setaria* spp. infection among the sampled horses, however, limitations in the detection methods may have influenced the results. However, it would be beneficial to incorporate antibody testing against filarial infections which could offer a more accurate picture of the occurrence. Further research incorporating larger sample sizes and advanced molecular techniques is recommended to enhance diagnostic accuracy and better understand the epidemiology of *Setaria* spp. in the equine population of Kelantan.

6.2 Recommendations

It is recommended that future studies expand the sample size to enhance the reliability of the findings and conduct longitudinal studies to monitor seasonal variations in prevalence and assess the role of environmental factors, such as mosquito populations and proximity to cattle, in transmission. To improve diagnostic accuracy, future research should also focus on refining the Buffy Coat Method by ensuring accurate centrifugation and immediate sample handling. Additionally, to improve the accuracy of the Buffy Coat Method, it is essential to handle the sample with care when break microhematocrit tube. The microhematocrit tube should be broken just below the buffy coat layer approximately 1 mm below using a sharp glass slide. This method

ensures a clean break, allowing the buffy coat layer to be transferred to the slide without contamination from other blood layers. Furthermore, to provide greater sensitivity and specificity in detecting *Setaria* spp., molecular techniques such as PCR or serology testing such as ELISA can be performed.

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APPENDIX

APPENDIX A

UNIVERSITI MALAYSIA KELANTAN
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FAKULTI PERUBATAN VETERINAR
Faculty of Veterinary Medicine

Ruj. Kami (*Our Ref.*) : UMK.A06.800-1/2/8 (12)
Tarikh (*Date*) : 1st SEPTEMBER 2025

DR. TAN LI PENG
Main Supervisor
Faculty of Veterinary Medicine

Dear Dr.,

APPROVAL OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) TO CONDUCT RESEARCH INVOLVING ANIMALS

We are pleased to inform you that your application for approval to conduct research from Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University Malaysia Kelantan has approved. Please refer the table below for approval code:

Approval Code	***UMK/FPV/ACUE/FYP/012/2025***
Title	**Molecular Prevalence of Setaria Digitata and its Associated Risk Factors Among Horses in Kelantan**
Duration of Research	**04 September 2025 – 10 January 2026**
Number of Sample/Strain/Gender	**50/ Equus Ferus caballus (Domesticated Horse)/**
Name of Student	**ISABEL SIO HWEE LING – D21B0111** **NUR AMIRA SYAFIEQA BINTI YA'AKOB – D21A0129**

You are advised to always follow "3R" (REDUCE, REFINE, & REPLACE) and all animal ethics and animal welfare principles to reduce suffering in animal.

Thank you.

"ISLAM MEMBIMBING, RAJA MEMIMPIN, NEGERI DIBERKATI"
"MALAYSIA MADANI"
"BERKHIDMAT UNTUK NEGARA"

Yours sincerely,

(DR. MOHAMMED DAUDA GONI)
Chairman
Institutional Animal Care and Use Committee
Faculty of Veterinary Medicine

ENTREPRENEURSHIP IS OUR THRUST

Table of Demographic data of horses sampled in Kelantan, Malaysia (n = 30)

No.	ID Number	Sex	Age	Breed	Stable location	Health status
1.	Mira	Female	Unable to determine	Arabian Crossed	Teaching Farm UMK	Healthy
2.	Cassandrra	Female	15	Anglo	JHJ Stable	
3.	Rizkikul	Male	7	Unable to determine		
4.	Joker	Male	Unable to determine	KKB/Pony		
5.	Wawa	Female		Anglo-Arab		
6.	Felia	Female		Anglo		
7.	Puteri	Female		Pony		
8.	Mirzan	Male	8	Arabian Crossed	Bukit Ajil Stable	
9.	Black Putra	Male	8			
10.	KY Putra	Male	8			
11.	Black Boy	Male	3			
12.	Marie	Female	7			
13.	Angela	Female	8			
14.	KJ Putra	Male	7			
15.	Dubai	Male	2			Mild ocular signs
16.	Bobby	Male	4			
17.	G	Female	4			
18.	Flower	Female	19	Warmblood		
19.	Ikarus	Male	14	Warmblood		

20.	King	Male	14	Arabian Crossed	Wakaf Aik	Healthy
21.	Pokchek	Male	11	Thoroughbred		
22.	Bespoke	Female	11	Arabian Crossed		
23.	Lady	Female	13	Thoroughbred		
24.	W1	Female	4	Warmblood		
25.	Kabir	Female	17			
26.	Angel	Female	20			
27.	TM	Female	8	Thoroughbred		
28.	Makmo	Female	30	Pony		
29.	Yatim	Female	4	Warmblood		
30.	Kuku Buruk	Female	20	Polo		