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DETECTION OF *ANCYLOSTOMA SPP.* IN PET DOGS IN IPOH WITH THE AID OF ARTIFICIAL INTELLIGENCE MICROSCOPY AND ITS ASSOCIATED RISK FACTORS

By

DANIEL GOH ZHU ERN

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ABSTRACT

Hookworm (*Ancylostoma spp.*) is a significant parasitic infection that affects canines and has zoonotic potential. This study aims to detect the presence of hookworm in pet dogs in Ipoh, Perak, Malaysia with the aid of artificial intelligence microscopy (Element AIM™) and to identify any associated factors. A total of 31 faecal samples were collected and analysed. The result revealed a hookworm prevalence of 12.9% (n=31). Questionnaires were conducted for the participating pet owners and analysed using Fisher's Exact Test to determine any associated factors. The statistical test showed that only multi-dog households (p=0.043) showed significant association. Associations regarding the age (p=0.112), sex (p-0.101), deworming within the past year (p=1.000) and whether the pet dogs were taking out for walks (p=1.000) did not show any statistical significance. Furthermore, the questionnaire showed that all participating pet dog owners were aware of deworming their pet dogs and hookworm's zoonotic potential. The study showed that artificial intelligence microscopy can be used to detect for presence of hookworm in pet dogs. However, the study was limited by its small sample size and focuses on a single geographical area. Therefore, further studies with larger and diverse samples should be conducted to validate the findings. This study highlights the potential of AI-based tools in veterinary diagnostics and its assistance in parasitic detection in animals, in particular hookworm in pet dogs.

Keywords:

Hookworm, *Ancylostoma spp,* artificial intelligence microscopy, zoonotic potential, veterinary parasitology.

ABSTRAK

Cacing kait (*Ancylostoma spp.*) adalah jangkitan parasit yang signifikan yang menjejaskan kesihatan anjing dan mempunyai potensi zoonotik. Kajian ini bertujuan untuk mengesan kehadiran cacing kait dalam anjing peliharaan di Ipoh, Perak, Malaysia dengan bantuan mikroskopi kecerdasan buatan (Element AIM™) dan mengenal pasti sebarang faktor yang berkaitan. Sebanyak 31 sampel najis telah dikumpulkan dan dianalisis. Hasil kajian mendedahkan prevalens cacing kait sebanyak 12.9% (n=31). Soal selidik telah dijalankan terhadap pemilik anjing peliharaan yang terlibat dan dianalisis menggunakan Ujian Tepat Fisher untuk menentukan sebarang faktor yang berkaitan. Ujian statistik menunjukkan bahawa hanya isi rumah dengan lebih daripada satu anjing (p=0.043) mempunyai hubungan signifikan. Hubungan berkaitan umur (p=0.112), jantina (p=0.101), penyahcacingan dalam setahun yang lalu (p=1.000), dan sama ada anjing dibawa berjalan (p=1.000) tidak menunjukkan sebarang kepentingan statistik. Tambahan pula, soal selidik menunjukkan bahawa semua pemilik anjing peliharaan yang terlibat sedar akan amalan penyahcacingan dan potensi zoonotik cacing kait. Kajian ini menunjukkan bahawa mikroskopi kecerdasan buatan boleh digunakan untuk mengesan kehadiran cacing kait dalam anjing peliharaan. Walau bagaimanapun, kajian ini terhad oleh saiz sampel yang kecil dan tertumpu pada satu kawasan geografi. Oleh itu, kajian lanjut dengan sampel yang lebih besar dan pelbagai harus dijalankan untuk mengesahkan penemuan ini. Kajian ini menekankan potensi alat berasaskan kecerdasan buatan dalam diagnostik veterinar dan bantuannya dalam pengesanan parasit dalam haiwan, khususnya cacing kait dalam anjing peliharaan.

Kata Kunci: Cacing kait, *Ancylostoma spp.*, mikroskopi kecerdasan buatan, potensi zoonotik, parasitologi veterinar.

CERTIFICATION

This is to certify that we have read this research paper entitled **'Detection of *Ancylostoma spp.* in Pet Dogs in Ipoh with the aid of Artificial Intelligence Microscopy and its Associated Factors'** by **Daniel Goh Zhu Ern**, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirements for the course DVT 55204 – Research Project.

Dr Norhidayah binti Noordin

DVM (UMK), MVSc. (UPM)
Lecturer
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Supervisor)

Dr Basripuzi Nurul Hayyan binti Hassan Basri
Paraclinical Head of Department
Senior Lecturer
DVM (UPM), PhD in Veterinary Parasitology (UoG)
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Co-supervisor)

Dr Mohammed Dauda Goni
Senior Lecturer
DVM (UNIMAID), PhD in Public Health and Epidemiology (USM)
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Co-supervisor)

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DEDICATIONS

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LIST OF ABBREVIATIONS

AI	-	Artificial Intelligence
AIM	-	Artificial Intelligence Microscopy
CAPC	-	Companion Animal Parasite Council
CDC	-	Centers for Disease Control and Prevention
CI	-	Confidence Interval
CLM	-	Cutaneous Larva Migrans
DVM	-	Doctor of Veterinary Medicine
KAP	-	Knowledge, Attitude, and Practice
TroCCAP	-	Tropical Council for Companion Animal Parasites
UMK	-	Universiti Malaysia Kelantan

CHAPTER 1

1.0 INTRODUCTION

Hookworm infections are caused by species of the genus *Ancylostoma* and *Unicinaria stenocephala* (Zajac et al., 2021) and carries significant importance in the veterinary field and also human health. According to the Centers for Disease Control and Prevention (2017), the hookworm species commonly associated with cutaneous larva migrans (CLM) has been associated with *Ancylostoma caninum*, *A. braziliense* and *Uncinaria stenocephala* which can be found in dogs. According to the Tropical Council for Companion Aniamal Parasites (2019), these hookworm species can be found in tropical and subtropical regions. Based on Climate-Data.org (2022), Ipoh has a humid and warm climate which is ideal for hookworm to thrive in this environment. Therefore, the detection of hookworm is crucial.

Traditionally, the diagnosis for hookworm infections in dogs involved microscopic examination of faecal samples, which, although effective, are labour-intensive and requires significant expertise (Foreyt, 2001). The availability of artificial intelligence (AI) offers promising solutions in enhancing the accuracy and efficiency for parasitic detection (HESKA,2024). AI microscopy has the potential to revolutionize the diagnostic process by automating the identification of *Ancylostoma* spp. in dog faecal samples in a sanitary and fast manner, providing good imagery within minutes which allows for easy storage for medical record keeping and also imagery sharing with clients and specialists (Valéria Inácio et al., 2020; Zoetis, 2023). This revolutionary technology would definitely contribute to the point-of-care for hookworm infection in dogs, allowing implementation of tailored preventive measures thus enhancing the dog's quality of life (Celeritas Digital, 2024 & HESKA,2024). Hence, this study is essential for the integration of AI to support clinical diagnosis of hookworm infection in pet dogs.

In parallel with the availability of improved diagnostic techniques which in this case AI microscopy, this study aims to identify pet owners' awareness towards the importance of regular deworming and the zoonotic potential of hookworm infections whilst identifying any other associated factors through the simple questionnaire.

1.2 RESEARCH PROBLEM STATEMENT

There is currently lacking of studies performed specifically for the detection of hookworm in pet dogs in Ipoh. The most recent parasite study conducted in Ipoh dates back to 2014 where ectoparasites and endoparasites were identified in stray and pet dogs in 2012 and 2013 (Chandrawathani et al., 2014). The endoparasites identified were *Ancylostoma* spp, *Toxocara canis* worm, *Giardia* sp and *Ascaris* sp whereas the ectoparasites identified were *Demodex canis, Rhipicephalus microplus, Rhipicephalus sanguineus* and *Ctenocephalides canis.* However there appears to be limited research conducted to assess pet owner's awareness towards the importance of deworming and the zoonotic potential of hookworm. Similarly, studies done for the detection of hookworm in dogs with the aid of artificial intelligence microscopy remains sparse. According to Suwannaphong et al.(2013), the conventional method requires professional technicians in microscopic examination of faecal samples because one has to acquire certain level of expertise and training in order to detect parasitic eggs among the abundance of impurities or morphologically similar parasite in the faecal samples. Therefore, with the aid of artificial microscopic examination, this will allow lesser time required for examination and also reduces chances for human errors.

1.3 RESEARCH QUESTIONS

- Can hookworm be detected in pet dogs in Ipoh with the aid of AI microscopy?

- What are the associated risk factors for hookworm infection in pet dogs in Ipoh?
- Are pet dog owners aware of the importance of deworming their dogs?
- Are pet dog owners aware of the zoonotic potential of hookworms?

1.4 RESEARCH HYPOTHESIS

- Hookworms can be detected in pet dogs in Ipoh with the aid of AI microscopy.
- There are associated risk factors that contributes to hookworm infections in pet dogs in Ipoh.
- Awareness on the importance of deworming in pet dogs among pet dog owners in Ipoh is low.
- Awareness on the zoonotic potentials of hookworm among pet dog owners in Ipoh is low.

1.5 RESEARCH OBJECTIVES

- To detect the presence of hookworm in pet dogs in Ipoh with the aid of AI microscopy.
- To identify the associated risk factors contributing to hookworm infections in pet dogs in Ipoh.
- To investigate the level of awareness of pet dog owners in Ipoh towards the importance of deworming their pet dogs.
- To determine the level of awareness among pet dog owners in Ipoh on the zoonotic potential of hookworm in pet dogs.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF *ANCYLOSTOMA* SPECIES IN DOGS

Hookworms in dogs are common in Southeast Asia with a prevalence ranging from 70% to 100% (Mahdy et al, 2012). The prevalence level of hookworm in pet dogs is between 0.8% to 39% (Chandrawathani et al, 2014). Although there is limited data available on the prevalence of canine hookworm in pet dogs in Malaysia, specifically in Ipoh, hookworm infection is endemic in Malaysia based on its prevalence in Southeast Asia (Traub et al., 2004; Zain et al., 2015).

The hookworm species that can be found in dogs are *Ancylostoma caninium* which is also known as the canine hookworm*, Ancylostoma braziliense, Ancylostoma ceylanicum* and *Unicaria stenocephala.* All hookworms fall under the phylum Nematoda and their superfamily is known as Anylostomatoidea. According to Taylor et al (2015), the hookworm can be found worldwide in the tropics and warm temperature areas. Therefore, Malaysia being a tropical country would be an ideal environment for hookworm to strive.

The predilection site is in the small intestine. Hookworms are known to cause haemorrhagic anaemia in dogs, especially young puppies that are less than 1 year old. Dogs that are chronically infected are presented with poor body condition such as poor coat and underweight. In severe cases, infected dogs have diarrhoea with dark faeces (Peregrine, 2023). Infected dogs also display abnormal behaviours such as pica and even anorexia. The presence of a one hookworm egg in the faeces is confirmatory evidence of infection. This can be further supported by considering the life cycle of hookworm whereby the adult hookworm which attaches itself at the mucosa of the small intestine will shed eggs which will then be released into the environment via the faeces (Companion Animal Parasite Council,2024).

The diagnosis for hookworm infection is through the detection of eggs upon performing faecal flotation (Foreyt, 2001). There is slight variation in size between

Ancylostoma spp and *Uncinaria stenocephala*. Hookworm eggs are thin shelled and colourless. According to CAPC (2023), the size of *Ancylostoma* spp egg is 52-79µm by 28-58µm while the larger *Uncinaria stenocephala* has an egg size of 71-92µm by 35-58µm. Upon the detection of eggs, anthelminthics treatment can be provided such as mebendazole, fenbendazole, pyrantel or nitroscanate which kills the adults and any developing intestinal stages (Taylor et al,2015).

2.2 ZOONOTIC POTENTIALS OF *ANCYLOSTOMA*

Hookworm can cause human enteric infection and cutaneous larva migrans (Bowman, 2020). *Ancylostoma caninum* can develop in the human intestine and cause inflammation of the intestine or eosinophilic enteritis. Humans infected are often presented with abdominal pain (CDC, 2019). The hookworm species that penetrates the human skin upon contact on contaminated environments causing cutaneous larva migrans or 'creeping eruption' are *Ancylostoma braziliense*, *Uncinaria stenocephala* and even *Ancylostoma caninum* (Bowmann,2020; CDC 2019). Since these hookworm species are host specific which in this case is found in dogs, it does not reach reproductive maturity in humans (Bowman, 2020). 'Creeping eruption' occurs in humans because the hookworms are unable to penetrate into the dermis and migrates at the epidermis thus leaving pruritic and serpiginous tracks (Nabarro et al., 2019).

Ancylostoma ceylanicum which can be found in dogs is the only animal hookworm species that can cause patent infection in humans because humans are the principal host (Bauerfeind,2014; CDC,2019). This also means that *Ancylostoma ceylanicum* is able to complete its life cycle in humans (Taylor,2014). *Ancylostoma ceylanicum* is highly endemic in Southeast Asia and is found in Malaysia (Baueffeind, 2015; CDC, 2019), Baueffeind (2015) also stated that *Ancylostoma ceylanicum*'s reservoir host is dogs and have a

prevalence of more than 90% in Asia-Pacific which includes stray dogs in Malaysia.

Therefore, hookworms possess zoonotic potentials as they can cause enteric infection and cutaneous larva migrans. Hookworm also has the potential to develop its lifecycle in humans, specifically *Ancylostoma ceylancium*.

2.3 OVERVIEW OF AI MICROSCOPY IN THE VETERINARY FIELD

Artificial intelligence (AI) is a special field under computer science that focuses on creating a system that is able to gather information and make decisions or solve problems. Artificial intelligence encompasses machine learning and deep learning (Remli,2024). Artificial intelligence microscopy is a microscope which integrates artificial intelligence to acquire microscopic images, analyse and interpret the data (Zeiss, 2024).

Today, there are artificial intelligence microscope diagnostic instruments available in the veterinary market. Examples of these diagnostic instruments are VETSCAN IMAGYST ™ by Zoetis and Element AIM by Heska (HESKA, 2024; Zoetis, 2021). Both devices have slight differences but they both use an algorithm which is integrated by deep learning.

The Companion Animal Parasite Council recommends faecal flotation for diagnosis of hookworm infections in dogs. This method of diagnosis requires microscopic examination of the faecal samples which can be labour intensive and requires significant expertise. According to Nagamori et al (2020), the diagnostic accuracy and sensitivity of faecal examinations requires trained personnel who have experience in preparing the faecal samples and also performing microscopical examination of the faecal slides. It was also noted that various veterinary practices will perform different preparation methodologies thus leading to incidence of missing diagnosis of dogs with hookworms due to technician error or the limitations attributed by passive flotation technique. Generally, the diagnostic machines compose of three elements which is to prepare the sample and apply centrifugal flotation,

placing the sample under a microscopic scanner and the data will be analysed by deep neural networks. Therefore, such diagnostic tools were developed to ease and provide systematic faecal examination.

CHAPTER 3

3.0 RESEARCH METHODOLOGY

3.1 ETHICAL CONSIDERATIONS

Animal ethics application is required for this study, as to ensure that although only faecal samples are collected from defecation sites of the pet dogs, the welfare of the pet dogs needs to be adhered to at all times. The approval code from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University Malaysia Kelantan : UMK/FPV/ACUE/FYP/007/2024.

3.2 SAMPLE COLLECTION AND PREPARATION

A cross-sectional study was conducted using the data collected to assess the prevalence of hookworm and its associated risk factors. The pet dog owners who visited the veterinary clinic containing the artificial intelligence microscopy were asked if they would like to participate in the study. The sampling was not limited to a veterinary clinic, and anyone who owned a pet dog that lived in Ipoh throughout its life could participate upon the owner's consent. Once the pet dog owners agreed to participate in the study, they had to fill out the questionnaires for the final selection and also provide consent. The questionnaire comprised four sections. The first section pertained to the pet owner's information, the second section was regarding the dog's information, the third section was related to the health and management of the dog, and the fourth section related to the owner's knowledge of hookworm and its zoonotic potential. The questionnaires are shown in the appendix.

Upon consent, the participants were provided with a pair of gloves, a sterile urine container, and a zip lock bag. Participants used the pair of gloves to collect their dog's fecal sample in the morning and placed the sample into the sterile urine container. The sterile urine container containing the fecal sample was then placed into a zip lock bag to prevent exposure

to the environment. The fecal sample was then transported by the owner to the veterinary clinic equipped with artificial intelligence microscopy to process the sample within 24 hours of receipt. The sample drop-off dates were also provided to the owners to ensure a streamlined flow in the collection and processing of fecal samples. Participants were advised to send the fecal samples fresh. However, if they were unable to do so, the fecal sample needed to be stored in the refrigerator at 4°C for no longer than 48hours.

3.3 HOOKWORM DETECTION BY ELEMENT AIM™

Element AIM™ is an automated closed system faecal flotation and urine sediment analyser with built in artificial intelligence microscopy. This analyser was developed by HESKA, which is a part of Antech Company. The company produces a wide range of diagnostic solutions for the veterinary field. The Element AIM™ is able to detect presence of *Giardia* spp., coccidia, roundworm, hookworm, whipworm and tapeworm in faeces. A faecal prep kit is provided in a Faecal Prep Kit Box by the company to conduct the test. The preparation kit consists of the 10 ml sample tube which contains faecal and liquid volume indicator, the faecal sample transfer device, filter, cartridge and also the faecal flotation solution which is zinc sulphate with specific gravity of 1.18.

3.4 SAMPLE PREPARATION AND PROCEDURE

Upon receiving the faecal sample, 2 ml of fresh faeces from the pet dog were placed into the 10 ml sample tube using 2 wooden applicator sticks. The zinc sulphate flotation fluid was used to fill the tube to the liquid fill line indicated on the sample tube. The faeces were thoroughly mixed with the wooden applicator sticks until homogenized. The filter was inserted into the sample tube with the pointed side facing downwards. The filled tube was placed into a tabletop high-speed horizontal centrifuge (TG18A) with a counterbalance and spun at 1500 rpm for 5 minutes.

After 5 minutes, the sample tube was removed from the centrifuge. The cartridge was removed from its packaging, the Faecal Prep Device Tubing was attached to the cartridge port, and the Faecal Prep Device Cap was attached to the sample tube. The cap was twisted slowly to dispense the sample into the cartridge until the cartridge window was filled and the filter changed colour. The tube was slowly removed from the port, and the cartridge was secured by pressing down on the cap until a click was felt.

The protective film was removed before the cartridge was loaded into the analyser. Care was taken to ensure the windows on the top and bottom of the cartridge were not touched, and the cartridge was loaded by holding its handle. The preparation procedures for faecal samples were shown in the appendix.

3.5 INTERPRETATION

Once the result is generated by the analyser which is less than 15 minutes, the digital images captured by the microscope are displayed numerically. The images were manually screened to tally with the artificial intelligence result before proceeding with data recording.

3.6 STATISTICAL ANALYSIS

The data was entered and analysed using Microsoft Excel (Version 16.91). The descriptive and statistical statistics were calculated for hookworm prevalence and five factors (pet dog ownership, age, sex, deworming management and pet dogs taken for walk) that could contribute to the presence of hookworm in pet dogs in Ipoh. These factors are obtained from the questionnaires conducted on the participating pet dog owners. Fisher's Exact Test was used to assess for any association between the prevalence and risk factors for hookworm in pet dogs based on the questionnaire. The significance level was set at 5% ($p<0.05$).

CHAPTER 4

4.0 RESULTS

4.1 Descriptive & Statistical Statistics

A total of 31 faecal samples were collected in Ipoh, Perak, Malaysia. Out of these, 4 samples (12.9%, 95% CI) were tested positive for hookworm. Figure 1 illustrates a microscopic image taken by the Element AIM^{TM} analyser of *Ancylostoma spp.* eggs detected during analysis.

Figure 1: Microscopic image showing hookworm (*Ancylostoma spp.)* eggs detected in a faecal sample.

Additionally, one sample, although negative for hookworm, showed positive for coccidia while two other samples showed presence of yeast despite being hookworm negative. Although these findings were not the primary focus of the study, these findings showed the importance of comprehensive faecal diagnosis in assessing the gastrointestinal health of pet dogs.

Five associated risk factors were evaluated to see if there is possible association for presence of hookworm in the pet dogs. These factors are also referred to as variables include pet dog ownership, age of the dog, sex of the dog, deworming practices and whether the dog is taken out for walks. The Fischer's Exact Test revealed significant association between multidog household and hookworm infection (p=0.043). There was no significant association with the other factors. The compiled data is tabulated in table 1 below.

Table 1 : Analysis of associated risk factors using Fisher's Exact test with 95% confidence interval.

Variables		**Tested (n)**	**Positive (n)**	**Prevalence %**	**Odds Ratio**	**P-Value (Fisher)**	**95% CI**
Pet dog ownership	Own one dog	16	0	0	0.00	0.043	(0,0.392)
	More than one dog	15	4	26.7			
Age	Puppy	2	26	7.69	0.125	0.112	(0,0.392)
	Adult	2	5	40.0			
Sex	Male	0	15	0	0.00	0.101	(0,0.413)
	Female	4	16	25.0			

Deworm within a year	Yes	3	21	14.3	1.50	1.000	(0,0.403)
	No	1	10	10.0			
Walks the dog	Yes	3	22	13.5	1.26	1.000	(0.484,1)
	No	1	9	11.1			

4.2 Questionnaire findings on owner awareness

In addition to faecal sample analysis, the questionnaire conducted to evaluate pet dog owner's awareness regarding the importance of deworming and zoonotic potential of hookworm infections revealed that all respondents (100%) were aware of the need for regular deworming. Similarly, 100% of the participants demonstrated awareness towards the zoonotic risks of hookworm infection which can be transmitted to humans. These findings indicate a high level of awareness among the participating pet dog owners.

CHAPTER 5

5.0 DISCUSSION

This study confirms the presence of hookworm (*Ancylostoma spp.*) in pet dogs in Ipoh with a prevalence rate of 12.9% with confidence interval of 95%. This prevalence falls within the reported range of 0.8% to 39% for pet dogs (Chandrawathani et al., 2014) and aligns with the endemic nature of hookworm in tropical regions as mentioned by Mahdy (2012). The humid and warm climate of Ipoh definitely provides an optimal environment for the survival and propagation of hookworm (Climate-Data.org, 2022; TroCCAP, 2019). The species such as *Ancylostoma caninum, Ancylostoma braziliense* and *Ancylostoma ceylanicum* are likely contributors to the observed prevalence. It is important to note that as introduced earlier, *Ancylostoma ceylanicum* is capable of completing its lifecycle in humans and is endemic in Southeast Asia therefore highlighting its zoonotic potential (Bauerfeind, 2014). It is promising to note that upon providing questionnaires to the participants to see if participants are aware of the importance of deworming and hookworm's potential zoonotic risk, all participants (100%) are aware.

The study has addressed the research question, hypothesis and objectives. The prevalence rate of 12.9% for hookworm detection in pet dogs in Ipoh supports the hypothesis that with the aid of AI microscopy, hookworm can be detected in pet dogs in Ipoh. The third and fourth question, hypothesis and objectives, which assessed pet dog owners' awareness of deworming importance and hookworms zoonotic potential revealed 100% awareness for both. These findings reject the hypothesis that awareness levels would be low. The regular veterinary interactions significantly contributed to owner education on deworming and zoonotic risks.

The study was able to address the second question, hypothesis and objective as the associated risk factor identified was pet dogs from multihousehold (p=0.043). Shared living environments are known to cause an increased risk of infection (Mahdy et al.,2012). In multi-dog households, the shared spaces such as kennels can facilitate the spread of hookworm through the faecal-oral route especially if one of the dogs is infected. Therefore, it is recommended that all pet dogs in a multi-dog household should be dewormed simultaneously and owners should practice good hygiene practices by removing the faeces promptly to avoid hookworm eggs from hatching and larvae from developing and dispersing into the environment.

It was found that adult dogs exhibited a higher prevalence of 40% when compared to puppies which was at 7.69%. However, this association was not statistically significant (p=0.112). This could indicate that there could be possibility of cumulative exposure to contaminated environments over time. Peregrine (2023), noted that adult dogs have prolonged exposure to contaminated soil or faeces and therefore have higher risk of hookworm infection. Puppies however tend to receive intensive attention such as regular deworming which could explain their lower prevalence. It is important to note that the participants indicated that their puppy was dewormed together with the vaccination schedule which was a monthly interval for the first 3 doses prior to annual booster.

The study showed that female dogs had a significantly higher prevalence of hookworm infection which is at 25% when compared to male dogs which was at 0% but this is not statistically significant (p=0.101). It is important to note that this observed higher prevalence of hookworm in female dogs compared to males may be influenced by the sample size and population dynamics. Based on the questionnaire results, it was found that all 4 of the female pet dogs came from a multi-dog household. With the exception of one female puppy, the other 3 female pet dogs are kept outdoors. In a multiple dog setting and possibility

of exposure to a contaminated environment, this was likely the cause of hookworm infections in these female pet dogs.

Although it was found that all the participants are aware of deworming, the lack of significant association between deworming practice and hookworm prevalence (p=1.000) suggests that other factors such as environmental contamination or improper deworming schedule play a larger role. According to Sunwannaphong (2013), reinfection can occur in contaminated environments even when deworming is given. Therefore, pet dog owners should be educated by veterinarians about the importance of correct timing and frequency of deworming and also educated on the use of effective anthelmintics (Taylor et al., 2015).

Lastly, dogs that are taken for walks showed a higher prevalence of 13.5% compared to those not taken out for walks which is at 11.1%. While this difference was not statistically significant (p= 1.000), there is further need for future studies and exploration in this potential trend. Walking the pet dogs in public spaces, especially contaminated areas, increases the exposure. Public spaces include parks, streets whereby stray dogs potentially infected with hookworm defecates and contaminates the environment. According to a study, stray dogs contribute significantly to environmental reservoirs of hookworm (Lim et al., 2013).

The adoption of AI microscopy in the analyser, as used in this study, Element AIM can significantly enhance diagnostic efficiency especially in high volume clinics. Throughout this study, the total time taken to run a test for each faecal sample including utilising the tabletop high speed horizontal centrifuge (TG18A) and generating a result, the turnaround time is less than 20 minutes whereas when using the conventional method, it takes a minimum of 20 minutes for the faecal suspension when dog faeces is used and this does not include the time taken to observe under compound microscope (Chandrawathani, Permaalatha, Omar, & Che Mamat,2019). Conventional faecal flotation although is the gold

standard for detecting hookworm eggs (Foreyt,2001), this method is labour-intensive, time-consuming and prone to human error due to technician variability. For instances, the use of passive flotation have a lower sensitivity when compared to centrifugal flotation (Nagamori et al., 2020). The morphological similarities between parasite eggs may lead to misidentification or overlooked cases and this depends on the skill of the observer.

All four pet dogs positive for hookworm had diarrhoea which is one of the clinical signs of hookworm infection as stated by Peregrine (2023). Apart from hookworm, one sample tested positive for coccidia and two samples were positive for yeast. Although these findings were not part of the main study which is to detect presence of hookworm, this finding shows the importance of faecal analysis in veterinary practice. Coccidia was is a protozoan parasite that affects the intestinal tracts of animals and the common species found in canines are *Isospora spp* (Bowman,2020). According to Foreyt (2001), infection occurs through ingestion of sporulated oocyst from contaminated environments. This particular pet dog had shown signs of diarrhoea which is one of the clinical signs of Coccidia infection.

The presence of yeast in the faecal sample of two pet dogs suggest possible microbial dysbiosis. However, yeast finding can be considered common in faeces samples. According to a study by Mandigers et al (2014), yeast can be a common normal inhabitant in the gastrointestinal tract and there have been no significant studies done on the pathogenic role of yeast in dogs. Furthermore, both pet dogs did not show any clinical signs of diarrhoea and are healthy.

There were limitations throughout this study. Firstly, is the sample size. The small sample size of 31 with further restrictions to pet dogs in Ipoh limits the statistical power and generalizability of findings. For future studies aimed to analyse subgroups such as age and

sex, the sample sizes should be further improved to ensure statistical reliability (Lwanga & Lemeshow, 1991).

The second limitation is that the study was limited to Ipoh. Hookworm prevalence may vary across Malaysia and therefore further studies should include a more diverse geographical region in Malaysia which includes other states to better represent the national distribution of hookworm infections. According to Chandrawathani (2014), rural areas which have a higher stray dog population can exhibit increased transmission risks. Therefore, future studies can also include rural, urban and semi-urban settings.

Although 100% awareness was found amongst pet dog owners in this study regarding the importance of deworming and hookworm's zoonotic potential, the question does not directly evaluate if pet owners are aware of the importance of deworming schedules. Further studies should be conducted which includes surveys which assess the participants knowledge, attitude and practice (KAP) regarding deworming for hookworm prevention and hookwomr's zoonotic potential.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

This study confirmed the presence of hookworm (*Ancylostoma spp.*) in pet dogs in Ipoh with a prevalence of 12.9% and significant association was identified for multi-dog ownership. The high level of owner awareness is promising however further efforts are required to ensure that owners are aware of the right deworming schedule. The usage of AI microscopy can revolutionise diagnostic support and support the development of tailored interventions to reduce hookworm prevalence in Malaysia. This study showed how artificial intelligence can assist in detecting presence of ectoparasite in pet dogs and in this case, the detection of hookworm in pet dog in Ipoh. However, its implementation requires further validation in diverse settings as well as strategies to address cost-related barriers for smaller veterinary clinics.

The survey revealed that all participants were aware of the importance of deworming and the zoonotic risks of hookworm. Although this awareness is promising, the lack of significant association between deworming practices and hookworm prevalence indicates a need for greater emphasis on proper deworming schedules. Further studies should be employed such as implementation of KAP surveys to evaluate behaviours and practices that influence deworming adherence and zoonotic risk prevention.

Despite the study's limitations including small sample size and focus on a single geographical area, the findings contribute valuable insights into the prevalence and associated risk factors of hookworm infections in pet dogs in Malaysia. These results provide a foundation for further studies especially those involving larger and more diverse sampling. Future studies with increased sample size and study region expanding to rural areas will better represent Malaysia's hookworm prevalence. Further study is needed to better

understand the presence of yeast in a dog's faecal sample and to see if there is any evidence that supports yeast's role in causing pathogenicity in the gastrointestinal tract.

This study underscores the need for routine deworming, continued public health education to mitigate risk of hookworm infection in both humans and dogs. The integration of AI-based diagnostic tools into veterinary practices represents a significant step forward in improving point of care diagnosis especially in parasite detection and management of infection which ultimately enhances animal health, improves animal welfare and also reduces zoonotic risks.

7.0 APPENDIX

1. General pictures taken during the procedure for faecal analysis using the Element AIM analyser.

Figure 1: Faecal collection set containing sterile container, a spoon and gloves in a zipper bag provided to participants.

Figure 2 : The cartridge and faecal sample preparation kit for faecal analysis.

Figure 3 : A compact scale (A&D HT-500) was used for counterbalance.

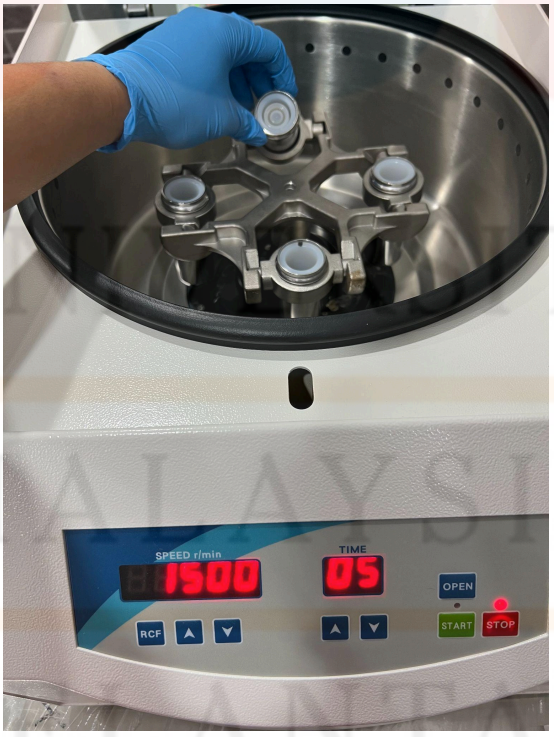


Figure 4 : A tabletop high speed horizontal centrifuge (TG18A) was used to centrifuge the sample.

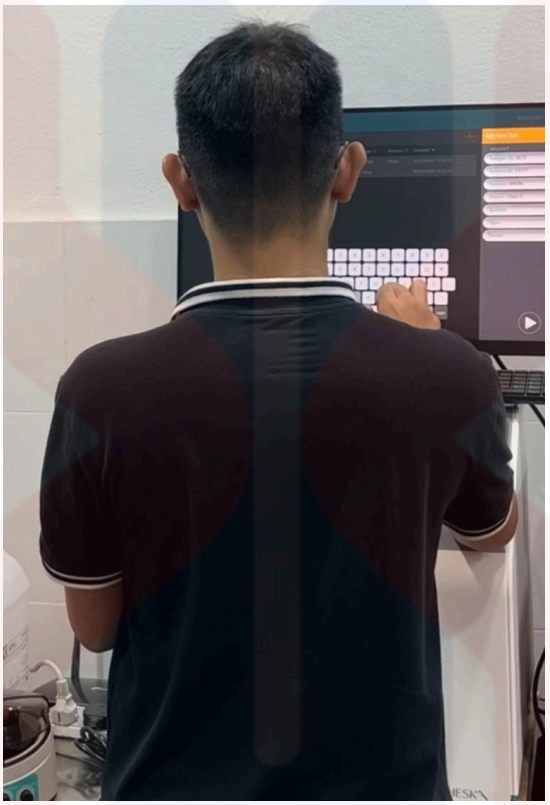


Figure 5: Entering the patient particulars into the Element AIM analyser.

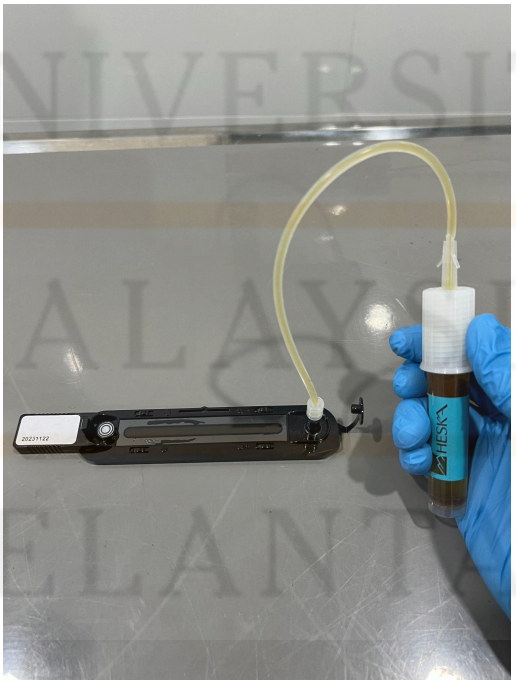


Figure 6: Transferring the faecal solution into the cartridge for analysis.

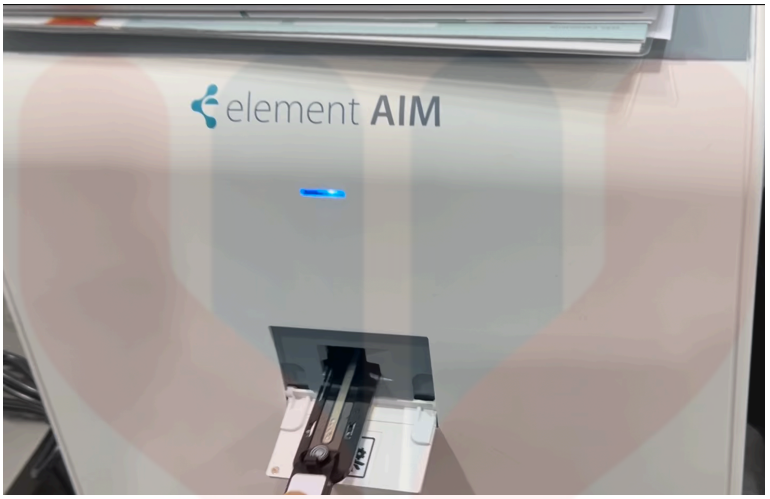


Figure 7: Placing the loaded cartridge into the analyser for hookworm egg detection.

Figure 8: The time taken to analyse a sample and for the results to be generated indicated in the figure is 11 minutes 40 seconds.

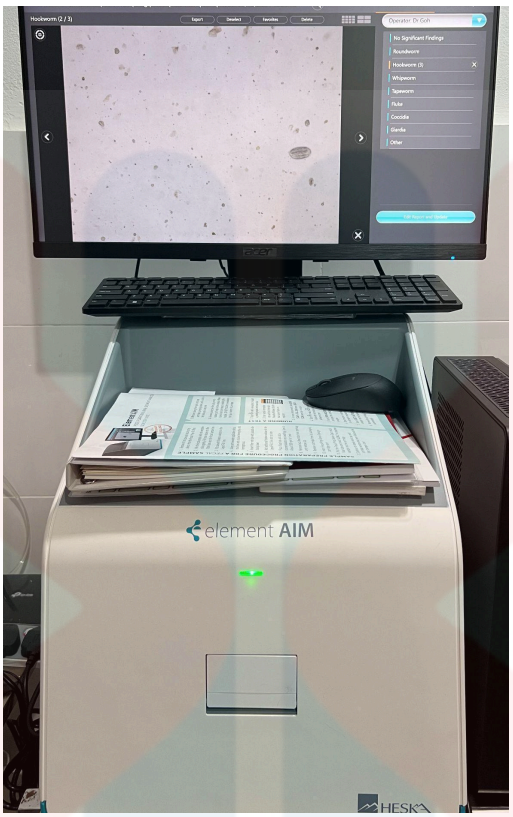
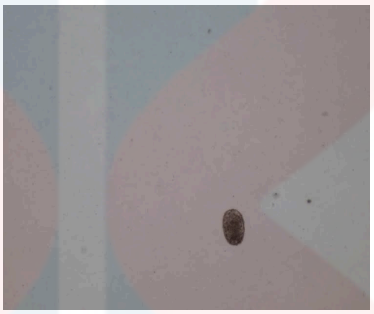
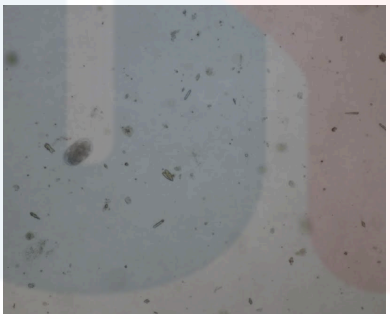
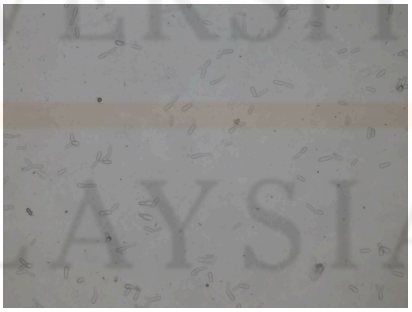


Figure 9: Results will be displayed on the screen upon completion.

2. Positive results for hookworm with other findings identified from negative result pet dogs

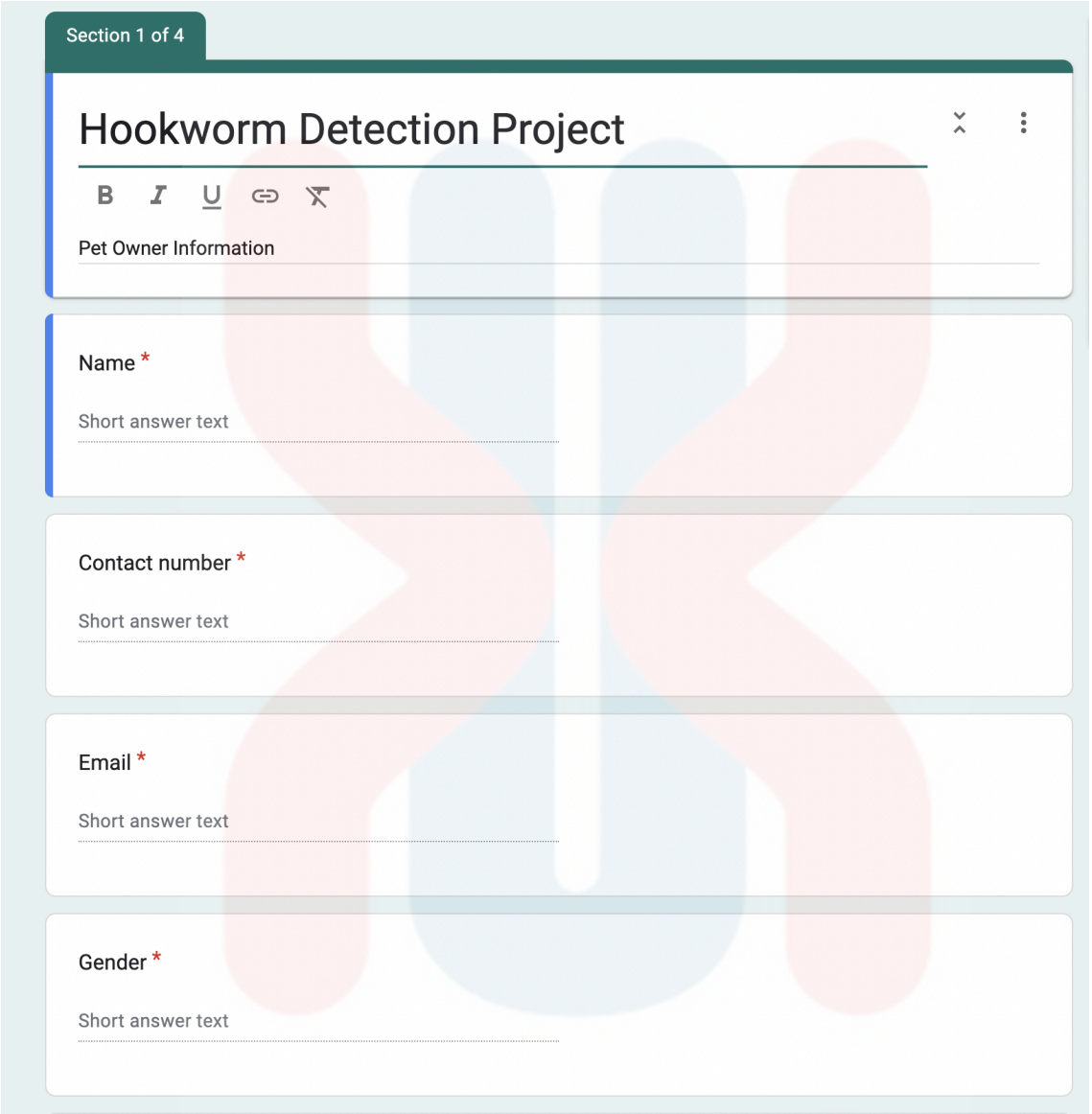
Table 4: Significant result findings from faecal sample.

Positive for hookworm		
No	Sample ID	Image
1	006	

2	024	
3	029	
4	031	
Presence for yeast but hookworm negative		
1	013	

2	015	
Presence for coccidia		
1	005	

3. Screenshots of the questionnaire where Google Form was used as the platform for data collection



Age of owner *

Short answer text

Have been residing in Ipoh for at least 1 year? *

- Yes
- No

How many pet dogs owned currently *

- One dog only
- More than one dog

If more than one pet dog, please list number of pet dogs.

Short answer text

Section 2 of 4

Pet Dog's Information

Description (optional)

Pet dog's name *

Short answer text

Age Range

- Puppy (0-1 year)
- Adult (1-7 years)
- Geriatric (8+ years)

What is your pet dog's breed *

Short answer text

Gender *

- Castrated male
- Intact male
- Spayed female
- Intact female

After section 2 Continue to next section

Section 3 of 4

Health and Management

Description (optional)

Deworming status: *
Has your pet dog been dewormed in the past year?

- Yes
- No

If yes, how frequently do you deworm your pet dog?

- ○ Every 3 months
- ○ Every 6 months
- ○ Others

If others, please state how frequent you deworm your pet dog.

Short answer text

Vaccination status: *
Is your pet dog up-to-date with vaccinations?

- ○ Yes
- ○ No

If no, please specify last vaccination date

Short answer text

Do you take your dog for walks? *

- Yes
- No

After section 3 Continue to next section

Section 4 of 4

Awareness and knowledge

Description (optional)

Are you aware of the importance of regular deworming for your pet dog? *

- Yes
- No

How did you learn about deworming? *

- [ ] Veterinarian
- [ ] Internet
- [ ] Friends
- [ ] Family
- [ ] Pet shops
- [ ] Others

For others, please specify on how did you learn about deworming

Short answer text

Are you aware that hookworms (*Ancylostoma spp.*) can be transmitted to humans? *

- ( ) Yes
- ( ) No

Diet: *

What type of diet do you provide for your pet dog?

- ○ Commercial dog food
- ○ Home-cooked meals
- ○ Raw diet
- ○ Mixed
- ○ Others

If diet management is others please specify

Short answer text

Housing management: *

Where is your pet dog primarily kept?

- ○ Indoor
- ○ Outdoor
- ○ Both

How did you learn that hookworm has zoonotic potential? *

- [ ] Veterinarian
- [ ] Internet
- [ ] Friends
- [ ] Family
- [ ] Pet shops
- [ ] Others

For others, please state how did you learn about hookworm's zoonotic potential.

Short answer text

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