IDENTIFICATION OF *TRICHINELLA SPIRALIS* IN PORK MEAT IN KELANTAN, MALAYSIA

ABDUL MUHAIMIN BIN SIMUNIR

(D18A0001)

A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITY MALAYSIA KELANTAN IN PARTIAL FULFILMENT OF THE REQUIRENMENT 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 *Trichinella Spiralis* in Pork Meat in Kelantan, Malaysia'** by Abdul Muhaimin Bin Simunir, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirenment for the course DVT55204 – Research Project

Dr Intan Noor Aina Kamaruzaman

DVM (UMK), PhD in Infection and Global Health (Liverpool)

Senior Lecturer,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Supervisor)

Dr Mohammad Sabri Bin Abdul Rahman

DVM, MVSc (UPM)

Lecturer,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Co-supervisor)

ACKNOWLEDGEMENT

Special thanks for those who has given their support, encouragement, guidance, advise and aid for the completion of this project paper:

Dr. Intan Noor Aina Binti Kamaruzaman

Dr. Mohammad Sabri Bin Abdul Rahman

Lab assistants of FPV UMK

Family

DVM 5 Class of 2018/2023

Thank you

DEDICATIONS

I would like to dedicate this body of work to my family and friends who have stuck by me throughout the way. Their endless support and encouragement has always been my source of motivation and comfort to get me through the challenges I face.

I would also like to dedicate this body of work to first and foremost my supervisor Dr. Intan Noor Aina and co-supervisor Dr. Mohammad Sabri who was there to monitor my progress physically and remotely from the beginning to the end until I manage to complete this small project.

I would also like to dedicate this project to the faculty lab assistants from Ms. C.W. Salma Binti C.W Zalati redirecting me to the proper lab to where I can begin this project to Madam Nora Faten Afifah binti Mohamad in helping me get the chemicals that I need and not to forget Ms. Hasimah Binti Hassan who was there to guide me and accompany me until this project had been accomplished.

I would also like to give a special thanks to my project mates Lak Ong ‘A and Kirthekka whom I went sampling with because without them I would have no medium of transport to get to the sampling site. Special thanks to my close friends who were there to always motivate me and give support when the road gets though. Thank you

Table of Contents

List of Tables

List of Figures

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, University Malaysia Kelantan, in partial requirement on the course DVT55204 – Research Project.

Trichinellosis is a disease caused by *Trichinella* sp. This is an emerging and, in some regions, re-emerging parasitic disease with socio-economic consequences in some developing countries. The parasitic nematode *Trichinella* sp. are also found to be the causative agent of human trichinellosis which is a disease that not only raise alarms in the public health aspect but also can result in a detrimental negative impact to the economy of the porcine animal production of the country due to swine being the intermediate host. This is an identification study to determine the presence of these parasitic nematodes within the commercial pork meat in Kota Bharu, Kelantan. A total of 30 pork meat were collected from the local pork meat market here in Kota Bharu, Kelantan. The meat sample collected was inspected by the digestion method to separate the larvae if they are present in the meat. At the end of this study, no *Trichinella* sp. was detected under the stereomicroscope after the meat digestion process.

Keywords: Commercial pork meat, Digestion Method, Kelantan, Kota Bharu, Nematode, *Trichinella* sp., Trichinellosis

ABSTRAK

Abstrak kertas penyelidikan yang dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, dalam keperluan sebahagian daripada kursus DVT55204 – Projek Penyelidikan.

Trichinellosis adalah penyakit yang disebabkan oleh *Trichinella* sp. Ini adalah penyakit parasit yang muncul oleh akibat sosio-ekonomi di beberapa negara membangun. Nematod parasit *Trichinella* sp. juga didapati menjadi agen penyebab trichinellosis manusia yang merupakan penyakit yang bukan sahaja menimbulkan kebimbangan dalam aspek kesihatan awam malah boleh mengakibatkan kesan negatif kepada ekonomi pengeluaran haiwan khinzir negara kerana babi adalah hos pengantaraan. Ini adalah kajian pengenalpastian untuk menentukan kehadiran nematod parasit ini dalam daging babi komersial di Kota Bharu, Kelantan. Sebanyak 30 daging babi dikutip dari pasar daging babi tempatan di Kota Bharu, Kelantan. Sampel daging yang dikumpul telah diperiksa dengan kaedah penghadaman untuk mengasingkan larva jika terdapat dalam daging. Pada akhir kajian ini, tiada *Trichinella* sp. dikesan di bawah stereomikroskop.

Kata kunci: Daging babi komersial, Kaedah Pencernaan, Kelantan, Kota Bharu, Nematod, *Trichinella* sp., Trichinellosis

1.0 Introduction

It is widely accepted that *Trichinella* spp. is the primary culprit behind the bulk of human trichinellosis infections (Gottstein et al., 2009). Trichinellosis is a disease that not only raises worries about the general health of the human population but also has the ability to negatively affect the production of porcine animals and food security on an economic level. Due to the relevance of the zoonotic potential of infection, the majority of the world's regions have concentrated on the control of trichinellosis and their efforts to remove it from the food chain. According to a report by Murrel and Pozio (2009) from 1986-2009 that, there were 65,818 cases and 42 deaths from 41 countries due to trichenellosis. The World Health Organization European Region accounted for 87% of cases and 50% of those occurred in Romania, mainly during 1990-1999. Farm pigs are the primary human infection source in the world. Outbreaks in Europe over the past three decades have been reported blamed on meat from horses and wild boars. Ingestion of *Trichinella* larvae that are encysted in the muscular tissue of meat from domestic or wild animals causes the infection in humans (Gottstein et al., 2009). Dupouy-Camet and Bruschi (2007) reported that an estimated amount of 100–300 *Trichinella spiralis* larvae will result in the beginning of the disease and that an intake of 1000–3000 or more larvae will cause severe disease. However, this is estimate data and is not based on any practical value and minimum infectious dose that cause the disease have yet to be known.

The human zoonotic disease trichinellosis, also called trichinosis or trichiasis, is referred to by this word. It is brought on by a family of worms from the *Trichinella* genus according

to Pozio & Murrell (2006). The most frequent sources of human trichinellosis are by ingesting larvae in the muscle from domestic and wild pigs. (Yera et al., 2022)

The infected animal's muscle will contain the encysted larvae, which will continue to develop inside the muscles, particularly those of the diaphragm, tongue, liver, pancreas, and kidney. *T. spiralis* can produce significant clinical indications in humans, such as fever, stomach pain, diarrhea, nausea, vomiting, and myalgias, despite having a relatively low pathogenicity for swine (Rawla & Sharma, 2022).

1.1 Research Problem

As of the year 2022, only one study done on the detection of *Trichinella* sp. in Malaysia. Insufficient reports of the presence of *T. spiralis* in Malaysia constitute the stated research concern here. *T. spiralis* presence in Kelantan's commercial swine meat markets has never been investigated. Moreover, the zoonotic potential of *T. spiralis* towards consumers of commercial swine meat raises public health concerns.

1.2 **Research questions**

1.2.1 Is *Trichinella* spp. present within the commercial pork meat market in Kota Bharu, Kelantan?

1.2.2 What is the prevalence of *T. spiralis* in pork meat in Kota Bharu, Kelantan?

1.3 **Research Hypothesis**

1.3.1 There is presence of *Trichinella* spp. within the commercial pork meat market within Kota Bharu, Kelantan.

1.3.2 There is low prevalence of *T. spiralis* within the commercial pork meat in Kelantan.

1.4 **Research Objectives**

1.4.1 To determine whether *T. spiralis* is present in the Kota Bharu, Kelantan pork meat market.

1.4.2 To determine the prevalence of T. *spiralis* in pork meat in Kota Bharu, Kelantan

2.0 Literature Review

2.1 *Trichinella spiralis*

In recognition of its importance in understanding the root causes of human disease and its value as a model for fundamental biological research, *T.spiralis* was the first species to be found and is also the most well-characterized (Pozio, 2019). Due to its widespread occurrence in domestic and sylvan animals as well as its high infectivity for laboratory animals, this is the case. This species' genetic diversity is higher than isolates from other places, and it is thought to have originated in East Asia. It is thought that human migration throughout the world disseminated it to brown rats (*Rattus norvegicus*) and domestic pigs. The colonization of North, Central, and South America, New Zealand, Hawaii, and Egypt by Europeans between the 16th and 20th centuries served as a catalyst for the expansion of this parasite and its hosts. (Pozio & Zarlenga, 2013). It is believed to have spread among wildlife animals in cold climate regions and has a low resilience to low environmental temperatures.(Pozio & Murrell, 2006)

Figure 1. World map showing the distribution area of *Trichinella spiralis*. The distribution is strongly influenced by human activity, which introduced the zoonotic pathogen into North, Central and South America, New Zealand and Egypt.

2.2 Epidemiology of Trichinellosis in Humans

Case numbers are extremely low because human infections only occur inadvertently upon the illegal and legal importation of Trichinella-infected meat from abroad in about 20% of the world's countries, mostly small islands, or city-states, where *Trichinella* sp. infections cannot develop due to the lack of potential reservoirs. A data compiled by Pozio (2007) from the 55 countries where trichinellosis is most common, researchers calculated that there are roughly 10,000 cases of clinical trichinellosis worldwide each year, with 200 fatalities.

Human trichinosis is closely linked to eating raw or undercooked meat; this is a culturally contextualized element, as in some cases traditional cuisines centre on raw or undercooked meat or products derived from livestock. Trichinellosis is uncommon or nonexistent in regions where people regularly consume meat that has been well cooked, even though it is still transmitted by wildlife. Human infections with *Trichinella* spp. mostly by eating domestic pork and related products, particularly those from pigs that were kept in free-range or backyard circumstances.

2.3 Life Cycle of *Trichinella* spp.

With adult males measuring on average 1.2 mm in length and bigger females measuring on average 2.2 mm, *Trichinella* spp. worms are among the tiniest species of nematodes (Diaz et al., 2020). During the initial enteral phase of infection, adult worms have an average life span of 4 to 6 weeks and mate in the small intestinal mucosa when they are mature. Up to 1500 larvae can be produced by female worms before they die and are eliminated by faeces. (Diaz et al., 2020)

The enteral phase, parenteral phase, and encysting phase are the three successive stages of *Trichinella* spp. life cycle in humans and its host animals. The life cycle of the nematode begins during the enteral phase in the stomach and small intestines, when gastric acid and pepsin chemically dissolve and release the larvae encysted in the animal muscle that was consumed raw or undercooked. Within a week after being released, larvae enter the small intestinal mucosa, develop, and mate. For circulation to striated muscles to encyst within another week, with or without a surrounding capsule, gravid females release larvae that enter lymphatic channels Diaz et al., 2020).

Circulating larvae could encyst everywhere in the body, including the heart and extraocular muscles, although they oftenly choose large, highly vascularized striated muscles like the tongue, diaphragm, psoas, pectoralis major, and gluteus maximus (Gottstein et al., 2009). Encapsulated cysts can form nurse-cell complexes after entering striated muscle fibres through capillaries and being activated by cytokines to release vascular endothelial growth factor. In addition to further enclosing the cyst, the nurse-cell complexes include materials that resemble collagen that shield it from immune attack and subfreezing temperatures and allow it to stay trapped in striated muscle for years. Usually, the parasite's life cycle is finished in two weeks (Diaz et al., 2020)

2.4 Clinical effects of *T. spiralis* to humans

Clinical illness may occur if a human consumes 500 or more larvae. Many *T. spiralis* infections may go undiagnosed because they are mild or because they are attributed to other disease, like the flu. According to Diaz et al. (2020), disease manifestations might be visible in the form of gastrointestinal symptoms such nausea, stomach discomfort, and diarrhoea in cases of early infections. Due to muscle invasion by the newborn larvae, the parasite's life cycle is marked by muscular discomfort, facial edoema, fever, and eosinophilia. The failure of the larvae to infiltrate the cardiac muscle is a common cause of cardiomyopathy (Murrell, 2014).

2.5 Trichinellosis in Malaysia

As of the year 2022, there has been no cases of trichinellosis documented in Malaysia. However, trichinellosis has recently spread to neighbouring nations like Thailand, Cambodia, Laos, and Vietnam from Malaysia. No cases of trichinellosis in either humans or animals have been reported in the literature, as stated by Pozio (2007). On the other hand, 84 Singaporean students and instructors contracted trichinellosis in 1998 after visiting a Malaysian island (Pozio, 2001), indicating that the parasite is spreading among local pigs.

The seroprevalence of trichinellosis in Pigs in Northern States of Malaysia was then reported in the Malaysian Journal of Veterinary Research. To conduct their study, scientists gathered 442 serum samples from 20 different pig farms in the states of Perak and Penang.

The 115 sow and 109 porker samples came from 10 farms in Perak, while the remaining 218 came from 10 farms in Penang. To identify anti-Trichinella antibodies in serum samples, they employed a commercial ELISA kit based on T. spiralis excretory antigens. (Chandrawathani et al., 2010)

Table 1.0: Seroprevalence of *Trichinella* spp. infection in pigs in two northern states of Malaysia as determined by ELISA :

State	Number of Samples Examined	Number of Positives	Percentage
Penang	218	0	0%
Perak	224	9	4.0%
Total	442	9	2.0%

(Chandrawathani et al., 2010)

In the same study, nine out of 442 serum samples proved positive for antibodies to *Trichinella* spp. The 224 samples were collected in the state of Perak, where nine of them tested positive. This considers the first discovery of trichinellosis in pigs in Malaysia. Food safety, human health, and Trichinella-free swine production are all affected by the occurrence of trichinellosis in South-East Asia, according to a study done by Takahashi et al (2000). The percentage is low, but the finding is important because it was uncovered in samples from only two of Malaysia's thirteen states. It follows that screening for the parasite's presence in other pig farms throughout other states in Malaysia are warranted. The fact that Perak borders with Thailand may explain why only samples from that state tested positive and not those from Penang. Human and animal cases of *T. spiralis* infection are most common among the people of Thailand's northern mountain minority tribes

(Pozio, 2007). Although few cases have been reported, it is estimated that between 200 and 600 human illnesses occur each year in northern Thailand during Thai New Year celebrations. (Pozio, 2007). Based on the investigation Chandrawathani et al (2010), study of trichinellosis in pigs in the states of Kedah, Kelantan, and Perlis is warranted because of Perak's proximity to the Thailand border and the potential spread of sick pigs from that region.

2.6 Control of *Trichinella* spp. infections in Pigs

According to Gottstein et al. (2009), pigs grown on small farms with poor biosecurity measures are more likely to contract *Trichinella* than larger farms. The "backyard-pigs" have access to a variety of rodents and wild animals and

are often fed leftovers or other waste products that include meat. The concerns are exacerbated by the fact that pigs grown in this way are rarely marketed through traditional lines of distribution and are thus not submitted to rigorous veterinary inspection. Not only that, but pigs that are allowed to roam free can also contract the worm.

If pig farmers are aware of the ways in which the nematodes can be spread to domestic pigs, they can implement measures to either eliminate the danger of infection or greatly minimize it. Effective good management practices can guarantee the safety of pork without the need for a slaughter inspection or additional processing steps. Architectural and environmental barriers, rodent management, clean feed and storage areas, safe disposal of deceased animals, and only accepting piglets from farms with monitored conditions are all vital in preventing the spread of *Trichinella* spp. in pig farms (Gottstein et al., 2009).

2.7 Methods of identification of *Trichinella* spp.

These are only a few examples of the many techniques that have been developed over time for identifying *Trichinella* spp. According to the FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of Trichinellosis, the first identification technique called Trichinelloscopy, which is used to look for *Trichinella* spp. larvae, may determine how severe an infection is, and can be used to collect individual larvae for further study at the species or genotype level. Infection severity is proportional to the larvae per gramme value (lpg). A small sample of muscle is crushed between two thick slides, fastened with two screws, and then studied at magnifications of 30-40x using a

trichinelloscope or a dissection microscope. Muscle samples can also be examined with a light microscope at magnifications between 50x and 100x by compressing them between two microscopy slides. At a later stage of infection, when the larvae have formed a completely mature capsule, they are more easily detected. If there is a lack of larvae or if the larvae have not yet encapsulated, this procedure may not work. (Dupouy-Camet & Murrell, 2007).

Histology is another approach that can be taken (Van De et al., 2015). The collagen capsule, or fragments thereof, basophilic transformation of muscle cells, and the type and composition of cellular infiltrates are all characteristics that can be detected by a histological approach for detecting *Trichinella* spp. Even if no larvae are found, the basophilic change of muscle cells meets the requirements for *Trichinella* spp. invasion. When larvae are very small and cannot be easily separated from the muscle fibers as they are in the early stages of muscle invasion, this technology is more sensitive than trichinelloscopy (Wranicz et al., 1998).

Multiplex-PCR analysis is used for the molecular identification of individual *Trichinella* spp. larvae, allowing the identification of nine of the 12 identified taxa (*T. spiralis, T. nativa, T. britovi, T. pseudospiralis, T. murrelli, T. nelsoni, T. papuae, T. zimbabwenzis, T. patagoniensis*) (Karadjian et al., 2017). This test is based on the use of five primer pairs amplifying the internal transcribed spacers ITS1 and ITS2 and the expansion segment V region (ESV) of the large subunit ribosomal DNA. (Zarlenga et al., 2001)

3.0 Materials and Methods

3.3 Muscle sample preparation

Thirty samples of pork muscle meats that were specific to the predilection sites for *Trichinella* spp. were collected and kept in an -80°C freezer. Each of the samples cut and weigh about 1g which in total results about 30g of samples were used (Appendix A.1). The 30g of samples were divided into 3 groups to conduct the muscle digestion process (Appendix A.2, Appendix A.3, Appendix A.4). After dividing the muscle into 3 groups, the muscle meat is grinded.

3.4 Digestion fluid preparation

Digestion fluid was prepared by pre-heating 500ml of water in a 1000ml lab bottle to 50°C. 5g of pepsin was weighed on a digital weighing scale (Appendix A.6) and 8ml of concentrated hydrochloric acid (HCL) was measured using a 10ml measuring cylinder. This step was done in a biosafety cabinet. 8ml of measured hydrochloric acid (HCL) is then poured into a 100ml lab bottle. Then, 5g of pepsin power was then added into the 100ml lab bottle. The 100ml lab bottle containing the solution of HCL and pepsin powder was then poured into the 1000ml lab bottle containing pre-heated 500ml tap water.

3.3 Digestion method

Digestion method was carried out with 100ml of pre-heated water at 50°C is poured into a beaker. Then the meat to be digested is added into the beaker containing pre-heated water. The meat and pre-heated water in the beaker were stirred with a fork until the meat is

homogenized. The meat and water were transferred into the lab bottle containing the digestion fluid. The beaker was rinsed thoroughly with remaining water. The closed bottle was placed on the hot plate at 40°C for 30 minutes until the meat particles disappear (Appendix A.7). The digestion fluid was poured through a sieve and glass funnel into a 500ml measuring cylinder (Appendix A.8). The bottle of digestion fluid and meat was rinsed with warm water.

Using a pipette, the digestion fluid in the measuring cylinder was pipetted out. Until only 100ml of the digestion fluid remained in the measuring cylinder. Caution was taken so that the amount of aspirated digestion fluid does not exceed less than 100ml. Then the remaining 100ml was transferred into a 250ml beaker and rinsed well with water. The rinsing step was done three times with the amount of distilled water used makes up the remaining 100ml of the 250ml beaker. The fluid was then aspirated again leaving only 30ml in the 250ml beaker. Caution was taken to ensure the aspirated fluid does not exceed less than 30ml. The fluid in the beaker was then swirled and transferred onto a petri dish. The beaker was rinsed well with distilled water. The larvae then will be observed under the stereomicroscope.

A positive result indicating the presence of *Trichinella* spp. will have a morphological structure of tightly coiled or uncoiled larvae (Mayer-Scholl et al., 2017).

4.0 Results

Table 4.1: Parts and Number of Pork Meat sample collected

Meat region	Tongue	Masseter Muscles
Total number	20	10

Table 4.2: Result of muscle digestion of samples divided into 3 groups weighing 10g each.

Group A	Group B	Group C
Negative	Negative	Negative

To further elaborate the results shown in (Table 4.2). The result of the 30 pork meat sample collected from the pork meat market in Kota Bharu, Kelantan is negative as it is clearly seen in (Appendix A.9) for group B and (Appendix (A.10) for group C that the observation under the stereomicroscope was the clear digestion fluid only.

5.0 Discussion

Trichinellosis can be the result of the parasitic infection by many different types of *Trichinella* spp., This is a disease that not only is a public health food safety concern but at the same time presents as an economic threat to the pork consuming community in Malaysia. In this study the aim is to detect the presence of *T. spiralis*. The reason behind this specific species is because of it has a worldwide distribution (Figure 1). *T. spiralis* also

has a well-documented history being the main result of most human trichinellosis infections in the Southeast Asian regions (Murrell & Pozio, 2011).

Studies and investigations on trichinellosis have been conducted throughout the world and even within the Southeast Asian region. However little to no report on studies and investigations has been done within Malaysia. Which arises the question of is there any parasitic infection surveillance program being done in pork meat abattoirs in Malaysia. This can be due to the pork consuming community being a minor community living within this country. This however does not leave them out of the equation of them having assurance on food safety.

This study on *Trichinella* spp. identification is the first being done in Malaysia within the state of Kelantan. Furthermore, this *Trichinell*a spp. study was conducted by using the meat digestion method. This method that has never been done in pork meat within the pork meat inspection in Malaysia. The meat digestion method procedure will allow the muscle larvae to be released after the digestion of the muscle tissue by the means of artificial digestion fluid which composes of 1% of pepsin (1:10,000), and hydrochloric acid, followed by a sequence of selective screening, filtration, sedimentation and a final microscopic examination for the presence of the larvae (Pozio et al., 2009).

Since the digestion method does not have any internal control to monitor the effectiveness of the detection system, other factors must be measured to ensure an optimum result. The quality and accuracy of the digestion method is dependent on the performance of the

digestion method, the appropriate sample collection based on predilection sites, appropriate facilities, equipment and consumables and accurate verification findings. Hence the minimum quality assurance standards should include these main components such as muscle sample collection and preparation for testing, minimum requirements for equipment and consumables, performance of the digestion method, verification of findings and documentation.

To elaborate more on the minimum quality assurance standards for meat inspection, it's crucial to make sure the test is sensitive enough to pick up the minimum number larvae that could produce clinical signs in people (Dupouy-Carmet & Bruschi, 2007). Results from digestion method studies in pork revealed that 1g sample size allows the detection of ≥3 larvae per g (lpg) in muscle tissue whereas 3 and 5 g sample sizes can reliably detect ≥1.5 lpg and ≥1 lpg, respectively (Forbes & Gajadhar, 1999).

To get optimum results muscle samples should be labelled at the time of collection and examined as soon as possible, or they should be kept cool (between 2 and 8 °C) to prevent freezing and slow decomposition. Samples that must wait a while before being inspected, such as those used for wildlife surveillance should be kept refrigerated in clearly labelled plastic bags until testing can be done. It is feasible to store items for a longer period of time by freezing them, but doing so may hinder digestion and prevent the recovery of larvae that are not freeze-resistant; the sample weight of frozen samples should be increased to make up for the decreased test sensitivity (Gajadhar et al., 2019).

To elaborate more on ensuring the efficacy and accuracy of the digestion method the single or pooled meat samples should be chopped by a blender in order to increase the surface area of the sample for enzymatic degradation. The blending procedure must be adjusted to maximize the digestion efficacy which means the blending process should continue until there is no visible pieces of meat remaining within the blender. During this procedure full attention must be given because too little blending may result in incomplete meat digestion and over blending could result in damaging any muscle larvae present in the samples (Gamble et al., 2000).

A maximum meat-to-digestible-fluid ratio of 1:20 and a consistent temperature of 44–46 °C should be used throughout the process to encourage efficient and rapid digestion. Beakers and fluid can be pre-heated to a temperature between 46 and 48 °C to keep an initial temperature within the required range. To keep a constant temperature and lessen evaporation during digestion, the glass beaker should be covered with aluminum foil or a similar material, and the temperature should be monitored frequently with a thermometer. It is recommended to use an electric thermostat. An incubator with glass doors might make it simpler to monitor and maintain the required digestive temperature. In order to create a deep vortex without splashing, the digestion fluid must be stirred quickly enough.

Following digestion, the liquid should be carefully poured into a separatory funnel through a sieve in order to prevent overflow. Since sieves are used to collect undigested matter and are not meant for measurement, they do not need to be calibrated. However, sieves should be well cleaned after use and do not scratch the sieve surface to prevent changing the mesh size. Before using, sieves must be clear of particles to allow digestion fluid to pass through to prevent larval loss due to larvae sticking to the surfaces of the glass beaker or to leftover tissue on the sieve, an additional

volume of tap water (minimum of 100 ml) should be added to the separatory funnel after the digest fluid has been poured into it.

According to the European Commision (2015), artificial digestion is known to be more sensitive and is the recommended reference method by the European Union. This microscopic examination method comprises the process of digesting muscle tissues by combining the action of heat, hydrochloric acid and pepsin. (Yera et al, 2022). The function of pepsin in the muscle digestion procedure is to digests proteins that is contained within meat, eggs, seeds and even dairy products. In this project it acts as an enzyme to breakdown the pork meat.

Hydrochloric acid (HCL) assists in the protein digestion by supplying hydrogen ions (H+) which will activate pepsinogen which is a precursor of pepsin. Hydrochloric acid (HCL) in the stomach begins protein digestion by denaturing the protein which will result in the loss of protein function. The hydrochloric acid (HCL) also acts in converting inactive pepsinogen into its active form which is pepsin. Thus, pepsin begins to breakdown the peptide bonds between amino acids. Utilizing acidified pepsin, the process involves the enzymatic breakdown of muscle fibers to liberate muscle larvae for isolation and identification. Both individual and pooled muscle samples can be used in this approach (Nöckler & Kapel, 2007).

A contributing factor to the result of this study that sample used is from commercial pork meat market. Pork meat being sold in the commercial market gets their supply from a proper channel which is a farm and not a black market farm. These farms must have implemented a deworming program as *Trichinella* spp. can only be passed on to pigs through a few ways, such as by feeding them raw waste or animal carcasses or letting them encounter infected rodents and other wildlife

such as wild boars. *Trichinella* spp. infections in pigs are less likely or nonexistent in modern pig farms (Gamble et al., 2000). The farm management implementations that modern day swine farms have are first from the architectural standpoint where the swine farm enclosure is built to prevent entry of rodents into the farm such as openings for air ventilation or water pipes are covered with wires and the feed storage is maintained in closed silos to prevent from rodents to enter.

Since the result of this study is negative it is safe to say the pork market that is supplying the pork meat within Kota Bharu is safe to consume and free from any *Trichinella* spp. nematodes. However, this does not mean that consumers should neglect the proper meat preparing technique that is to ensure the meat is well cooked before serving it on the table. This is a fact as reported by Diaz et al. (2020), Trichinellosis is a result of the consumption of raw and undercooked pork of domesticated pigs continues to occur throughout Southeast Asia.

6.0 **Conclusion**

In conclusion, this study did not find any evidence of *Trichinella* spp in all samples collected. Further studies are warranted to maximize the sampling to detect and to determine the prevalence of the parasite in Kota Bharu, Kelantan.

7.0 Recommendations and future work

A recommendation and future work for this study would be to expand the sampling site covering the entire state of Kelantan because this is region in the country is in line with bordering Thailand and this would increase the probability of yielding a significant result.

Secondly the amount of tissue samples collected should cover all of the predilection site of *Trichinella* spp. such as the diaphragm and intercoastal muscle. This will enhance the probability of detecting the nematode as those are it's predilection sites. In terms of the digestion method procedure, it is best if it can be done based on the recommendation of the International Commision on Trichinellosis (ITC) where the among of muscle to be used is 5g per endemic area with the addition of expanding the sample site to a wider state area. Hence this can increase the total weight of pooled sample to be digested to increase the probability of finding a positive result. Another recommendation in terms of diversifying sample collection is to be able to collect muscle samples from wild boar meat as well because they are at a higher probability to be infected with the nematode compared to the commercial pork meats. Lastly, it is recommended to process the sample as soon as possible to observe viable larvae to ensure optimal results.

Reference

Candrawathani P. Preemaalatha B., Zaini M.Z., Adnan M., Nurul Aini R., Zawida Z., Jamnah O, Premila M., Gajadhar A. and Ramlan M. Malaysia Journal Of Veterinary Research. Report on Seroprevalence of Trichinellosis in Pigs In Northern States of Malaysia, Volume 1, No. 1, July 2010, Pages 33-36

Diaz, J. H., Warren, R. J., & Oster, M. J. (2020, June). The Disease Ecology, Epidemiology, Clinical Manifestations, and Management of Trichinellosis Linked to Consumption of Wild Animal Meat. *Wilderness & Environmental Medicine*, *31*(2), 235–244. https://doi.org/10.1016/j.wem.2019.12.003

Dupouy-Camet, J., and F. Bruschi. (2007). Management and diagnosis of human trichinellosis, p. 37-68. In J. Dupouy-Camet and K. D. Murrell (ed.), FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis. World Organisation for Animal Health Press, Paris, France.

Forbes, L. B., & Gajadhar, A. A. (1999, November). A Validated Trichinella Digestion Assay and an Associated Sampling and Quality Assurance System for Use in Testing Pork and Horse Meat. Journal of Food Protection, 62(11), 1308–1313. https://doi.org/10.4315/0362-028x-62.11.1308

Gamble, H., Bessonov, A., Cuperlovic, K., Gajadhar, A., van Knapen, F., Noeckler, K., Schenone, H., & Zhu, X. (2000, December). International Commission on Trichinellosis: Recommendations on methods for the control of Trichinella in domestic and wild animals

intended for human consumption. Veterinary Parasitology, 93(3–4), 393–408. https://doi.org/10.1016/s0304-4017(00)00354-x

Gajadhar, A. A., Noeckler, K., Boireau, P., Rossi, P., Scandrett, B., & Gamble, H. R. (2019, September). International Commission on Trichinellosis: Recommendations for quality assurance in digestion testing programs for Trichinella. *Food and Waterborne Parasitology*, *16*, e00059. https://doi.org/10.1016/j.fawpar.2019.e00059

Gottstein, B., Pozio, E., & Nöckler, K. (2009, January). Epidemiology, Diagnosis, Treatment, and Control of Trichinellosis. *Clinical Microbiology Reviews*, *22*(1), 127–145. https://doi.org/10.1128/cmr.00026-08

Karadjian, G., Heckmann, A., Rosa, G. L., Pozio, E., Boireau, P., & Vallée, I. (2017). Molecular identification of trichinella species by multiplex PCR: New insight for trichinella murrelli. Parasite, 24, 52. https://doi.org/10.1051/parasite/2017053

Mayer-Scholl, A., Pozio, E., Gayda, J., Thaben, N., Bahn, P., & Nöckler, K. (2017, March 3). Magnetic Stirrer Method for the Detection of Trichinella Larvae in Muscle Samples. *Journal of Visualized Experiments*, *121*. https://doi.org/10.3791/55354

Murrell, K. (2014). Helminths. *Encyclopedia of Food Microbiology*, 200–205. https://doi.org/10.1016/b978-0-12-384730-0.00163-4

Murrell, K. D., & Pozio, E. (2011, December). Worldwide Occurrence and Impact of Human Trichinellosis, 1986–2009. *Emerging Infectious Diseases*, *17*(12), 2194–2202. https://doi.org/10.3201/eid1712.110896

Nöckler, K., & Kapel, C. M. O. (2007). Detection and surveillance for Trichinella: meat inspection and hygiene, and legislation.

Encyclopædia Britannica, inc. (n.d.). Pepsin. Encyclopædia Britannica. Retrieved December 15, 2022, from https://www.britannica.com/science/pepsin

Pozio, E., & Zarlenga, D. S. (2013, November). New pieces of the Trichinella puzzle. *International Journal for Parasitology*, *43*(12–13), 983–997. https://doi.org/10.1016/j.ijpara.2013.05.010

Pozio, E. (2019). Trichinella and trichinellosis in Europe. *Veterinarski Glasnik*, *73*(2), 65–84. https://doi.org/10.2298/vetgl190411017p

Pozio, E., (2001). Taxonomy of Trichinella and the epidemiology of the infection in the south-east Asia and Australian regions. In: Proceedings of the 3rd Seminar on Food-borne Parasitic Zoonoses: Food- and Water-borne Parasitic Zoonoses in the 21st Century. South East Asian J. Trop. Med. Public Health 32 (S2), 129–132

Pozio, E. (2007, October). World distribution of *Trichinella* spp. infections in animals and humans. *Veterinary Parasitology*, *149*(1–2), 3–21. https://doi.org/10.1016/j.vetpar.2007.07.002

Pozio, E., & Darwin Murrell, K. (2006). Systematics and Epidemiology of Trichinella. *Advances in Parasitology*, 367–439. https://doi.org/10.1016/s0065-308x(06)63005-4

Pozio, E., Owen, I., La Rosa, G., Sacchi, L., Rossi, P., & Corona, S. (1999, November). *Trichinella papuae n.sp*. (Nematoda), a new non-encapsulated species from domestic and

sylvatic swine of Papua New Guinea. *International Journal for Parasitology*, *29*(11), 1825–1839. https://doi.org/10.1016/s0020-7519(99)00135-6

Rawla, P., & Sharma, S. (2022, October 9). *Trichinella Spiralis - StatPearls - NCBI Bookshelf*. Trichinella Spiralis - StatPearls - NCBI Bookshelf. Retrieved December 12, 2022, from https://www.ncbi.nlm.nih.gov/books/NBK538511/

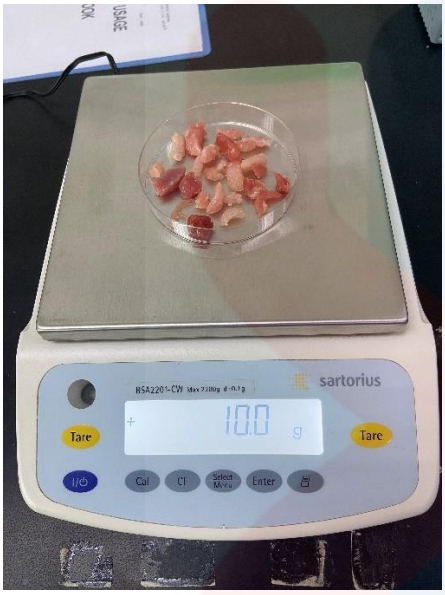
Takahashi, Y., Mingyuan, L., & Waikagul, J. (2000, December). Epidemiology of trichinellosis in Asia and the Pacific Rim. *Veterinary Parasitology*, *93*(3–4), 227–239. https://doi.org/10.1016/s0304-4017(00)00343-5

Van De, N., Thi Nga, V., Ngoc Minh, P., Vu Trung, N., Pozio, E., Trung Dung, D., & Dorny, P. (2015, June 3). Trichinellosis in Vietnam. *The American Journal of Tropical Medicine and Hygiene*, *92*(6), 1265–1270. https://doi.org/10.4269/ajtmh.14-0570

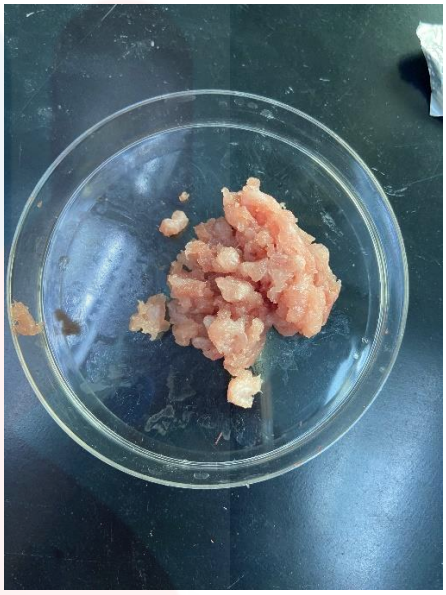
Yera, H., Bory, S., Khieu, V., & Caron, Y. (2022, September). Human trichinellosis in Southeast Asia, 2001–2021. Food and Waterborne Parasitology, 28, e00171. https://doi.org/10.1016/j.fawpar.2022.e00171

Zarlenga, D., Chute, M., Martin, A., & Kapel, C. (2001, June). A single, multiplex PCR for differentiating all species of Trichinella. *Parasite*, *8*, S24–S26. https://doi.org/10.1051/parasite/200108s2024

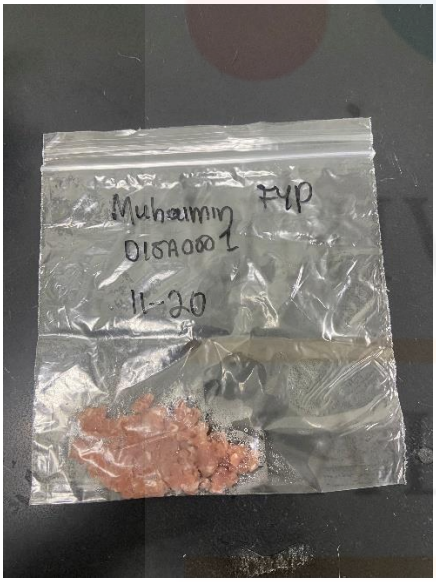
Appendix A



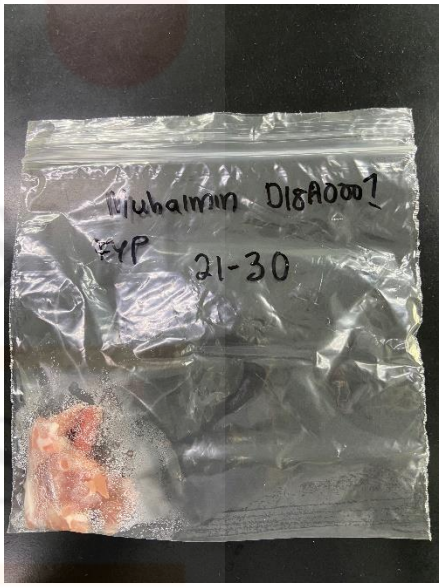
Appendix A.1: Weighing muscles samples to make 10g per group



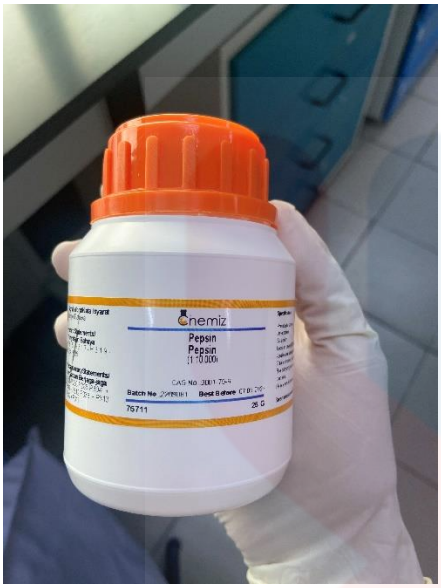
Appendix A.2: Sample group A.



Appendix A.3: Sample group B

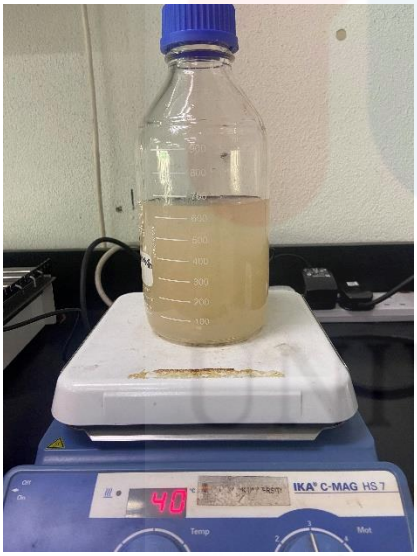


Appendix A.4: Sample group C



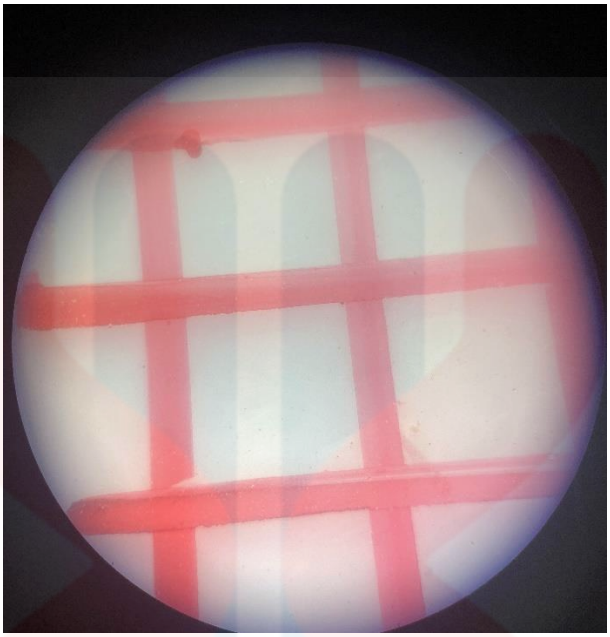
Appendix A.5: Pepsin Bottle

Appendix A.6: Pepsin weigh 5g used

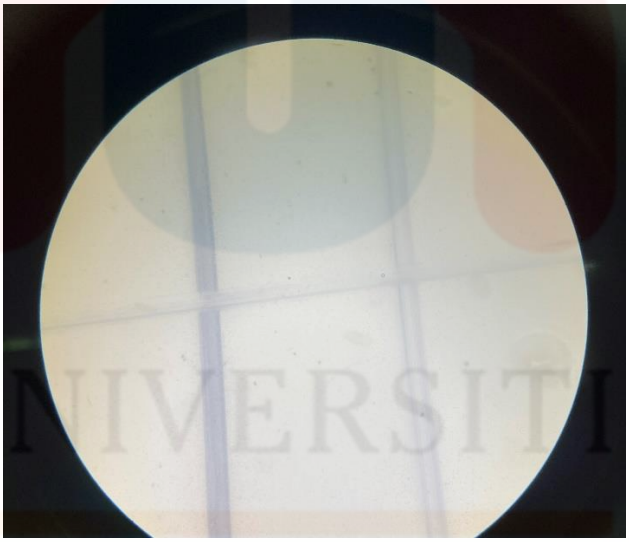


Appendix A.7: Digestion fluid with grinded meat on hot plate

Appendix A.8: Filtration of digestion fluid



Appendix A.9: Result of sample group B



Appendix A.10: Result of sample group C