EFFICACY OF COMMERCIAL GAMAT EXTRACT (*Stichopus variegatus)* PREPARATION ON STRAY CATS WITH GASTROINTESTINAL HELMINTHIASIS

SYAMIMI BINTI A. JALALAL

(D17A0037)

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

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR

THE DEGREE OF

DOCTOR OF VETERINARY MEDICINE

JUNE 2022

UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

This is to certify that we have read this research paper entitled **'Investigation of commercial Gamat extract (*Stichopus variegatus*) preparation on stray cats with gastrointestinal helminthiasis'** by Syamimi Binti A. Jalalal, and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 5436 – Research Project.

Prof. Dr. Jasni Bin Sabri

DVM (UPM), PhD in Veterinary Medicine (University of Edinburgh, Scotland)

Professor,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Supervisor)

Dr. Abd Rahman Bin Aziz

DVM (University of Agriculture, Pakistan), M. Sc. in Veterinary Medicine (University of Melbourne, Australia)

Senior Fellow,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Co-supervisor)

Dr. Mohd Farhan Hanif Bin Reduan

DVM (UMK), PhD in Clinical Pathology (UPM)

Senior Lecturer,

Faculty of Veterinary Medicine

Universiti Malaysia Kelantan

(Co-supervisor)

ACKNOWLEDGEMENT

First and foremost, I would like to express my gratitude to the divine grace, The Almighty, for enabling me to complete this research titled **"Efficacy of commercial Gamat extract (*Stichopus Variegatus*) preparation on stray cats with gastrointestinal helminthiasis"**. All of the hardships and struggle that I went through before, during, and after the completion of this research project and thesis are all made ease with the help from Him.

Completion of the final year project thesis requires help from several persons. By that, the most important person that I want to express my deepest gratitude to is no one other than my supervisor, Prof. Dr. Jasni Bin Sabri, for his never-ending reassurance, support, guidance, and encouragement to persevere through and improve my research study. It is truly an honour to be under his counsel. In addition, I would like to also express my gratitude to my co-supervisors, Dr. Abd Rahman Bin Aziz and Dr. Mohd Farhan Hanif Bin Reduan who were willing to share their expertise, guidance and moral support throughout this study.

I would also like to extend my gratitude to all laboratory assistants of FPV UMK especially Cik Hasimah Binti Hassan and Puan Nur Eizzati Binti Badrul Hisham for their guidance and assistance during laboratory work process. Huge thanks also to Dr Nur Zulaikha, Dr Siti Nor Azizah and Dr Fathin Faahimaah for their assistance during my research.

A very special thanks to Hidayat for his help and support throughout the research period, physically and emotionally. Thank you to Madeline, Jayden, Farhan and Nana and all my classmates for always welcoming and for the bond that we shared along these five years.

DEDICATIONS

My younger siblings; Siti and Eman, and Hidayat.

Table of Contents

List of tables

List of figures

List of appendices

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 5436 – Research Project

Due to the numerous nutritional and medicinal characteristics of sea cucumbers, or Gamat, and their products are frequently used in traditional medicine in Malaysia and other parts of Asia. However, no study about antiparasitic properties of Gamat was made to date. While gastrointestinal helminthiasis is one of the common and important diseases affecting cats, this pilot study aims to determine the efficacy of a commercial Gamat (*Stichopus* variegatus) jelly preparation against gastrointestinal helminthiasis in cats through per os administration. Thus, a total of six cats across the vicinity of Pengkalan Chepa and Kota Bharu were taken as samples which then were divided into two groups (treatment and control group) with 14 days of study period. Samples that were taken were faecal and blood samples. Although statistical analysis shows no significance findings ($p>0.05$), there was a gradual decrease of the helminth ova (EPG) and haematological improvement of cats in the treatment group. In short, Gamat (*Stichopus variegatus*) extract shows to be effective in reducing the gastrointestinal worm burden on stray cats.

Keywords: *Gastrointestinal helminthiasis, Gamat/sea cucumber (Stichopus variegatus), Helminth ova, Faecal examination, Antiparasitic*

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 – Projek Penyelidikan.

Oleh kerana banyak ciri khasiat dan perubatannya- Gamat dan produknya kerap digunakan dalam perubatan tradisional di Malaysia dan negara lain di Asia. Walau bagaimanapun, tiada kajian tentang sifat antiparasit Gamat dibuat sehingga kini. Walaupun helmintiasis gastrousus adalah salah satu penyakit biasa dan penting yang menjejaskan kucing, kajian rintis ini bertujuan untuk menentukan keberkesanan penyediaan jeli Gamat (*Stichopus variegatus*) komersial terhadap helmintiasis gastrousus dalam kucing melalui pemberian per os. Justeru, sebanyak enam ekor kucing di sekitar Pengkalan Chepa dan Kota Bharu telah diambil sebagai sampel yang kemudiannya dibahagikan kepada dua kumpulan (kumpulan rawatan dan kawalan) dengan tempoh kajian selama 14 hari. Sampel yang diambil ialah sampel najis dan darah. Walaupun analisis statistik tidak menunjukkan signifikan ($p>0.05$), terdapat penurunan beransur telur helmint (EPG) dan peningkatan hematologi kucing dalam kumpulan rawatan. Ringkasnya, ekstrak Gamat (*Stichopus variegatus*) menunjukkan keberkesanan dalam mengurangkan beban cacing gastrousus pada kucing terbiar.

Kata kunci: *Helmintiasis gastrousus, Gamat (Stichopus variegatus), Telur helmint, Pemeriksaan najis, Antiparasit*

1.0 Introduction

Cats, particularly the stray cats because they are not dewormed, are reservoir hosts for various species of gastrointestinal helminths that have zoonotic potential (Rojekittikhun et al., 2014). Important gastrointestinal helminths in cats are divided into three categories (nematodes, cestodes and trematodes). In this study, nematodes and cestodes will be focused as they are common gastrointestinal helminth among cats in Malaysia. Nematodes or roundworms or ascarids have cylindrical body shapes with tapering ends with various sizes. In companion animals, ascarids are large nematodes with adults sizing up to 3-18 cm and harbour in the small intestines. All tapeworms or its other name; cestodes has a scolex and neck, and can have mature, immature or gravid proglottids and the length and size are also varied. Cestodes are large with size approximately 50-60cm long but it causes only minimal harm to the host. Infected cats usually show no signs of infection (Holyoake & Sciences, 2008). Some of these helminths are host specific, meaning they only infect one type of host, whereas others are not and exist in both dogs and cat (Shapiro, 2005). In cats, these parasites include *Toxocara cati, Ancylostoma tuberforme, Ancylostoma braziliense, Strongyloides planiceps, Strongyloides felis, Strongyloides stercolis, Dipylidium caninum* and *Taenia taeniaformis*. The parasite *Toxocara cati* was discovered to be the most frequent, followed by *A. tuberforme* and *D. caninum* (Holyoake & Sciences, 2008).

Gastrointestinal helminths can be transmitted and infect the cats when they accidentally ingest contaminated soil, water faeces or food containing the parasites ova and even the intermediate hosts such as infected lice and rodents (Shapiro, 2005). Cats of any age, sex and breeds can become affected with this disease. Cats that are infected may or may not overt any clinical signs. Clinical signs that may be seen

include weight loss inappetence, diarrhoea, vomiting and intermittent abdominal pain. Additionally, colonic ulcers and iron deficiency anaemia are common in patients with a high worm burden. If the condition is left without treatment, it will continue to progress and in severe cases, it may lead to emaciation, anorexia and finally resulted in debilitation and death of the cat.

To diagnose gastrointestinal helminth, mainly nematodes and cestodes, an assessment of the faeces called faecal floatation can be conducted. It is used to test concentrated eggs and oocysts found in the stool into a drop of solution for easy identification and enumeration of the parasitic stages (Foreyt, 2001). Prophylactic treatment using various commercial product includes praziquantel, fenbendazole, pyrantel pamoate and selamectin have been proven effective, however, it is not vastly used by communities especially those that are lack of awareness and those that lived in rural area where veterinary clinics are few. Accordingly, study on alternative prophylactic and treatment against gastrointestinal helminthiasis is necessary to benefit the community as well as impose less risk of commercial drugs toxicity and resistance.

Sea cucumbers, or Gamat or its scientific name *Stichopus variegatus* also known as *Stichopus hermanni* and their product are commonly utilised in traditional medicine in Malaysia and other regions of Asia due to their diverse nutritional and therapeutic properties (Mariana et al., 2009). According to Chen (2003), the presence of sulphated glycosaminoglycans (GAGs) in sea cucumbers is the explanation for their valuable medicinal properties. Sea cucumbers belong to the Holothurioidea class of aquatic animals. They are echinoderms with long, flexible arms and legs. The bodies are gelatinous, and the skin is leathery. Chen (2003) also stated that *S. variegatus* and *S. vastus* are two sea cucumber species found in Malaysia's coastal waters that belong to the Stichopodidae family. The dorsal body part of *S. variegatus* are yellow-orange in

colour, with many conical warts organised in eight rows (Kim, 2008). Sea cucumbers are traditionally prepared as a topical oil to treat skin discomfort caused by insect bites, minor cuts and burns, stimulate wound healing, and improve overall health (Sabuan, 2016).

Gamat or sea cucumbers are nutrient-dense, including essential nutrients, minerals, fatty acids, bioactive peptides, and other useful elements. Sea cucumbers have been studied for their pharmacological actions, and it has been discovered that they have antimicrobial, anti-inflammatory, anti-fungal, anti-cancer, and wound healing qualities (Bordbar et al., 2011). Nonetheless, reports on anthelminthic effects of sea cucumbers have not been found. On that account, this study is planned to assess if *S. variegatus* has any anthelmintic potential in the treatment of naturally infected gastrointestinal helminth in cats.

2.0 Research problem

Gastrointestinal helminthiasis is a serious disease among cats and if left untreated would cause weight loss inappetence, diarrhoea, vomiting and intermittent abdominal pain. In chronic cases, it may lead to emaciation, anorexia and finally resulted in death of the cat. Treatment protocol for gastrointestinal helminthiasis in cats include Zentel (i.e., albendezole), Drontal (i.e., praziquantel, pyrantel pamoate and febantel) and Revolution (i.e., selamectin). However, there is a possibility of an adverse effect reaction from the drugs indicated, such as hypersalivation, diarrhoea, vomiting, inappetence, toxicity, and drug resistance, which are serious concerns among cat owners and veterinarians. Sea cucumber, on the other hand, had no reports or cases of cytotoxicity, nor have there been any research on sea cucumber adversity.

Numerous studies concerning Gamat on its effectiveness to various conditions including usage as antibacterial, antifungal and wound healing properties were done, however, those studies were focused among humans. Moreover, no research has been made in regard to investigating the anthelmintic properties of *S. variegatus* in both humans and animals, to date.

For those reason, to study for an alternative treatment for gastrointestinal helminthiasis using Gamat jelly (*S. variegatus* extract) is a need since it is easily obtained in the local market, vastly produced in the country as well as cheaper compared to the typically used imported products and more importantly have potential that could heal the disease thus reduce the spread of gastrointestinal helminth among cats and the causative parasites of zoonotic significance in Malaysia.

3.0 Research question

3.1 Will the cats with gastrointestinal helminth infection respond to the per os administration of commercial Gamat jelly preparation?

3.2 What would be the effects of per os administration of commercial Gamat jelly preparation?

4.0 Research hypothesis

4.1 Cats infected with gastrointestinal helminth will respond to the per os administration of commercial Gamat jelly preparation.

4.2 The reduction in helminths ova count in the faeces, improvement in CBC results and physical appearance including body weight would be the effects of per os administration of commercial Gamat jelly preparation.

5.0 Research objective

5.1 To investigate the efficacy of a commercial Gamat jelly preparation against gastrointestinal helminthiasis in cats through per os administration.

5.2 To examine and analyse the helminths ova count in faecal sample, improvement in CBC results and physical appearance including body weight of the cat in response to per os administration of commercial Gamat jelly preparation.

6.0 Literature review

6.1 Common gastrointestinal helminth in cats

6.1.1. Nematodes

In small animals, nematodes, often known as roundworms, are microscopic wormlike animals with a tough skin called cuticle. It is a multicellular, unsegmented, elongated, rounded on both ends, circular in cross section and bilaterally symmetric organism with a variety of sizes ranging between 3-18 cm (Shapiro, 2005). The cuticle, as mentioned, covers the nematode's external body surface and extends into all of the nematode's body openings, including the mouth, esophagus, rectal, and genital openings. The common ascarids in cats are *Toxocara cati, Toxascaris leonina, Ancylostoma tuberformae, Strongyloides planiceps, Strongyloides felis* and *Strongyloides stercolis.*

6.1.2. Cestodes

Cestodes are flatworms with a scolex, neck area, and repeated segments that are hermaphroditic. It is a parasitic worm that lacks a mouth, intestine, and body cavity. Dogs and cats are most typically definitive hosts (i.e., they carry adult tapeworms in the small intestine), although they can also be intermediate hosts (i.e., they carry immature metacestode stages in diverse tissues). Adult tapeworms in the small intestine of dogs and cats are frequently well tolerated, with little or no clinical symptoms of disease. The most common symptom of such

illnesses is the shedding of eggs and proglottids. Metacestode, if present in various tissues, could lead to life-threatening disease in both cats and humans (Conboy, 2009). The common tapeworm in cats includes *D. caninum*, and *T. taeniaeformis*.

6.2 Lifecycle & transmission

6.2.1. Toxocara sp.

The cat will be infected when they ingest a *Toxocara* egg that contains second-stage larva. A first-stage larva is not infective, and it will not become infective until it is outside the host for four weeks. It makes its way outside through the faeces of the host. A parasitic ovum that is eaten before it develops into second-stage larva will not develop. After ingestion, the infective eggs will hatch in the small intestine. Larvae in the second stage burrow through the intestinal wall and into a vein. The larvae are carried through the liver and into the lungs via blood. The larvae moult in the lungs and progress to the third stage. The third-stage larvae crawl up the trachea from the lungs. The larvae reach the host's throat and are swallowed. *Toxocara* migrate back to the small intestine and remain there. They moult twice more before becoming adults (Shapiro, 2005).

6.2.2 *Ancylostoma tuberformae*

The life cycle of the hookworm begins when an egg containing an embryo falls into the faeces and hatches. The first and second stages of the larvae are free-living, but the third stage larvae are infectious. These

larvae are capable of penetrating the host's skin. The third-stage larvae enter a blood vessel in the skin and travel to the lungs, where they moult and transform into fourth-stage larvae that migrate up the trachea. The larvae attach themselves to the lining of the small intestine and suck blood once the cat swallows them. The larvae moult into adults, who also suck on blood (Shapiro, 2005).

6.2.3. Dipylidium caninum

The larval double pore tapeworm emerges from the digested insect (intermediate host, e.g., fleas and lice) and matures in the small intestine of the cat after ingestion of the intermediate host. The adult tapeworm uses hooks and suckers on its scolex to adhere to the intestinal wall. The tapeworm absorbs its host's digested food through its skin. The tapeworm's end segments fill with eggs and break away from the worm as it grows. *Dipylidium caninum* eggs are packaged in packets of approximately 20 to 30 eggs. The proglottids that encase them may burst, allowing the egg packets to escape into the colon. The proglottids will flow out with the excrement if they do not split. The proglottids are free to manoeuvre under their own strength and escape the faeces. They eventually die and release their egg packages, which are eaten by larval fleas (Shapiro, 2005).

6.2.4. Taenia taeniaeformis

Life cycle of *Taenia taeniaeformis* in cats is somewhat similar to *Dipylidium caninum.* The only difference is that the intermediate host for this cestode are mice and other rodents.

6.3 Clinical signs

Weight loss, inappetence, diarrhoea, vomiting, anaemia and intermittent abdominal pain are some of the clinical indications that might be commonly noted in all gastrointestinal helminthiasis. Moderate to light *Toxocara* sp. infections have a low impact on the host. In severe infections, larvae moving through the lungs may cause pneumonia in the cat. One of the clinical signs of this stage is coughing. Adult worms can cause diarrhoea when it irritates the mucosa lining of the intestine. With heavy infections, kittens may appear potbellied, and they may not gain weight as quickly as they should. On the other hand, in cats, hookworm infections are rare and not as serious as in dogs. In both kittens and pups, a significant infection can induce severe anaemia, which can be fatal. Hookworms may also produce bloody or mucus-filled diarrhoea. For *Strongyloides sp.*, threadworm infections are mild in cats over the age of six months, although autoinfection is rare as the worm will be killed by the host's immune system in three to four months. Many adult worms develop and cause damage to the intestinal wall, resulting in diarrhoea. If the illness is not treated, it will worsen, eventually leading to emaciation, anorexia, and eventually debilitation and death of the cat (Shapiro, 2005).

6.4 Treatment

Locally, gastrointestinal helminthiasis is mainly treated medically with the use of commercially imported deworming products such as Zentel, Drontal and Revolution. The active ingredients are albendazole, praziquantel and pyrantel pamoate and febantel, and lastly selamectin, respectively.

6.5 *Stichopus variegatus* as an alternative treatment

6.5.1. History of *Stichopus spp.* usage in traditional medicine

Sea cucumbers belong to the Holothurioidae family of marine animals. Sea cucumbers come in a variety of species, including *S. variegatus*, *Holothuria atra*, and *Thelenota ananas* (Sabuan, 2016). Sea cucumbers, or Gamat and their product are commonly used in traditional medicine in Malaysia and other regions of Asia due to their diverse nutritional and therapeutic properties (Mariana et al., 2009). Gamat has long been used as a topical oil to relieve skin irritation from insect bites, small cuts, and burns, promote wound healing, and enhance general health. The most frequent species harvested and used in commercial sea cucumber extract manufacturing is *Stichopus* species. Gamat products were traditionally used by mothers after childbirth for their therapeutic effects just as mentioned above (Sabuan, 2016).

6.5.2. Functional properties of *Stichopus variegatus*

Sea cucumbers are nutrient-dense and high in value-added chemicals, making them ideal for use as functional additives. Bioactive peptides,

vitamins, minerals, fatty acids, saponins, carotenoids, collagens, gelatins, chondroitin sulphates, and amino acids are all functional features of sea cucumbers. *S. variegatus* is an edible species with therapeutic properties and a low toxicity level. On top of that, there have been no reports or cases of sea cucumber cytotoxicity, nor have there been any studies on the adversity of sea cucumber. Essential amino acids, as well as rich polyunsaturated and monounsaturated fatty acids, may aid in tissue healing (Bordbar et al., 2011).

Gamat oil, Gamat ointment, and Gamat gel are all traditional medical preparations made from *S. variegatus* in Indonesia and Malaysia. *S. variegatus* has a high protein content (47%) and a low-fat content (12%) (Pangestuti & Arifin, 2018). They also have a lot of sulphated glycosaminoglycan, especially in the body wall of the integument. The use of sulphated glycosaminoglycan isolated from *S. variegatus* in speeding wound healing in Wistar albino rats (*Rattus norvegicus*) has been studied (Subramaniam et al., 2013). Subramaniam (2013) also stated that wound contraction was reported to be increased and enhanced during the wound healing phase I.

Moreover, in a study using the body wall extract, *S. variegatus* also demonstrated antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Proteus vulgaris*. Similarly, *S. variegatus* body wall extract showed antifungal activity against *Candida albicans* and *Aspergillus niger* (Shakouri et al., 2017).

6.5.3. *Stichopus variegatus* in treatment for gastrointestinal helminth in cats

Sea cucumber or *S. variegatus* has a variety of therapeutic properties and can be easily found in Malaysian traditional medicine products like Gamat oil or Gamat Jeli and even Gamat Jel commercially (Sabuan, 2016). There have been numerous studies on *S. variegatus* extracts efficacy against inhibition of bacterial and fungal growth as well as in and accelerates wound healing (Subramaniam et al., 2013; Shakouri et al., 2017). Unfortunately, no study has been made to assess the anthelmintic properties of the *S. variegatus* up to this day. As a result, the goal of this research would be to see if *S. variegatus* has any anthelmintic potential in the treatment of cats with naturally infected gastrointestinal helminths.

7.0 Methodology

This study was conducted as per scheduled upon UMK/FPV/ACUE/FYP/002/2022 Final Year Project Research Committee and Animal Ethics Committee 2022, Faculty of Veterinary Medicine, University of Malaysia Kelantan.

7.1 Study animals

Six stray domestic shorthair (DSH) cats aged 1 to 2 years old, of both sexes in the vicinity of Pengkalan Chepa, Kelantan that appeared healthy were used for this study. The cats were kept individually in a 60cm × 43cm × 50cm metal cages. They were placed comfortably with each cage equipped with a towel to ease the cats' movements, a litter box, a food bowl and a water bowl. Three of

the cages were placed on the rack while the rest of the cages were placed on a separate rack further away from the first mentioned. The animal holding room was maintained within a standard environmental condition. The cats were fed with standard commercial dry food and clean water with frequency of changing the food and water twice per day.

Physical examination was conducted on all cats consisting of body weight, temperature, heart rate, respiratory rate and general examination and observation. Defecation, urination, ocular and nasal discharge as well as behavioural assessment were taken. This was to ensure all of the cats appeared to be bright, alert, responsive and possessed the appearance of a healthy cat upon external examination.

In conducting the experiment, the six stray cats were randomly and equally divided into the Gamat-treated group (Group 1) and Control group (Group 2). The cats were labelled as C1, C2, C3, C4, C5 and C6. Cats in Group 1 (C4, C5, C6) were treated with commercial Gamat jelly preparation while cats in Group 2 (C1, C2, C3) did not receive any treatment. Gamat jelly preparations were given twice a day for Group 1 which ended on Day 14 of the experiment.

7.2 Usage of commercial Gamat Jelly

The commercial sea cucumber jelly also known as Gamat jelly that was used in this is registered under National Pharmaceutical Regulatory Agency (NPRA), Ministry of Health, Malaysia.

Each bottle contains 350 mL of Gamat jelly with every 10 mL of the jelly contains 3.48 grams of *S. variegatus* extract, 1.1 mg of potassium sorbate and

6.6 mg of Sodium benzoate with the two latter ingredients functions as preservatives to prevent the jelly from spoilage.

7.3 Experimental design

The six stray cats were equally and randomly divided into two groups, namely the Gamat-treated group (Group 1) and control group (Group 2). Group 2 did not receive any treatment while Group 1 was treated with per os administration of 5 ml Gamat jelly, frequency of twice a day for 14 days. Samples were taken three times throughout the experiment which were on Day 0, Day 7 and Day 15.

7.3.1. Faecal examination

The faecal examination was done on each cat and aimed to assess for the severity and their extent. It is a simple, non-invasive and effective in detecting parasitic ova. The assessment of the gastrointestinal helminthiasis per faecal examination, was done prior to the experiment, during the course of the experiment and after the experiment. The two common diagnostic workups for assessing the mentioned subject are faecal floatation and McMaster technique.

7.3.1.1. Faecal floatation

Faecal floatation was conducted to identify helminth eggs and oocysts present in the faeces into a drop of solution for easy identification of parasitic stages. A fresh stool sample with 1g of weight was mixed with 40 mL concentrated sodium chloride (NaCl) solution in a plastic cup labelled A and stirred until faeces are in suspension using a spatula. The suspension was

then poured through a strainer or a filter paper into another plastic cup labelled B. The materials were pressed in the strainer with a spatula and the materials in the strainer then discarded. The filtrate from cup B then was poured into a 15 mL centrifuge tube and using a dropper to form a meniscus at the top of the centrifuge tube. A coverslip was carefully placed on top of the centrifuge tube then will be set aside for 30 minutes. The coverslip was then removed by carefully lifting it straight upward, and placed it on the glass slide for microscopic examination at 10× magnification. (Foreyt, 2001).

7.3.1.2. Faecal McMaster

A stool sample weighing 3 grams was freshly taken and mixed with 10 mL concentrated sodium chloride (NaCl) solution in a plastic cup labelled A. The suspension was stirred using a spatula and let to emulsify. Another 35 mL of concentrated salt solution was added into the same cup and stirred using a spatula to further homogenize the solution. Next, the solution was poured into another plastic cup labelled B through a strainer. The filtrate in cup B was stirred and aliquot was withdrawn using a pipette. The aliquot was then pipetted to fill a chamber of McMaster slide. The filtrate was filtered again before pipetting an aliquot to fill another chamber of McMaster slide. The slide then was left to stand for 2-3 minutes before it was observed under a light microscope. (Foreyt, 2001).

7.3.2. Blood sampling for complete blood count (CBC)

Blood was taken from all six cats on Day 0 as baseline reference. The blood was sampled at the cephalic vein or saphenous vein using a 23G needle and a 1 mL syringe. The blood samples were collected into EDTA microtubes for CBC. This was conducted as to evaluate the blood parameters, such as red blood cells (RBC), hematocrit (HCT), haemoglobin (HGB), red cell distribution width (RDW), white blood cells (WBC), lymphocytes (LYM), monocytes (MON) and granulocytes (GRA).

7.3.3. Physical assessment of post Gamat administration

The six cats were observed for any abnormalities or signs of toxicity during and after administration of Gamat jelly preparation. The body weight of each cat was recorded pre-treatment and post-treatment. Other parameters such as food and water intake, bowel movement including the faecal score, urination as well as behaviour were assessed during pre- and post-experiment.

7.3.4. Statistical analysis

The data were all expressed as mean ± SD and the value expressed represents three numbers of cats from each group. The data of weight of animal and the number of helminth ova before and after treatment period were analysed using Mann Whitney U test. Whereas, results from complete blood count before and after treatment period were analysed using Independent Sample T-test. Independent Sample T-test is a parametric form of statistics whereas Mann Whitney U test is a non-

parametric form of statistics used. The result is considered as statistically significant when $p < 0.05$.

8.0 Results

8.1 General behavioural and signs analysis

Using Mann Whitney test, the result showed that there is no significant difference in body weight changes between the Gamat jelly treated and control groups ($p>0.05$). In fact, at the end of the study, all cats of both groups showed an increase in body weight. Table 8.1 summarizes the results. Nevertheless, Group 1 experienced greater gain in body weight compared to Group 2.

Any changes in expression of behaviour of cats in both groups before and after the per os administration of commercial Gamat jelly were observed throughout the 14 days of treatment period. All six cats were observed to be less active before the treatment intervention. The first 30 minutes post administration were spent observing the behavioural pattern and signs on all six cats, followed by a 15-day assessment. Cats in Group 1 (treated group) appeared normal, with no notable changes in behaviour, food intake, water consumption, postural abnormalities, nervous and gastrointestinal signs; except one of the cats defecated on its food bowl starting from Day 6 to Day 14. Group 1's general appearance and behaviour were tabulated in table 8.2. Throughout the 15-days research, all cats in Group 1 had normal appetite and water consumption. Cats were able to take 30 grams of dry food and 150ml of water per day. Following the delivery of the Gamat oil, all three cats remain lively and receptive.

Table 8.1: The body weight of Group 1; Treatment, and Group 2; Control, before and after the experiment period (Mean ± SD)

Day	Body weight (Mean ± SD)	
	Group 1	Group 2
0	2.4 ± 0.51	2.50 ± 0.70
15	3.13 ± 0.38	2.90 ± 0.32

Table 8.2: General appearance and behavioural analyses of Gamat-treated group (Group 1)

Observation	Day 1 - 2			Day 3 - 4			Day 5 - 6			Day 7 - 8			Day 9 - 10			Day 11 - 12			Day 13 - 14		
Cat/ID	**4**	**5**	**6**	**4**	**5**	**6**	**4**	**5**	**6**	**4**	**5**	**6**	**4**	**5**	**6**	**4**	**5**	**6**	**4**	**5**	**6**
General appearance	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR
Appetite	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Bowel	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Vomiting	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Salivation	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Lethargy	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB

Note: BAR= Bright, alert, responsive, N= Normal, AB= Absent.

Table 8.3: General appearance and behavioural analyses of Control group (Group 2)

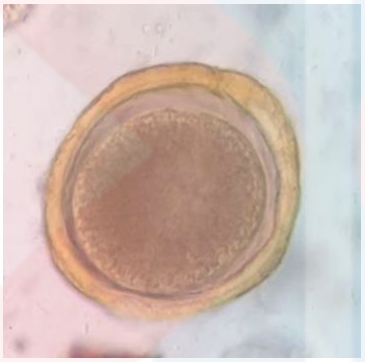
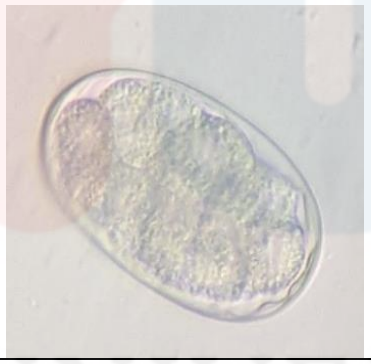
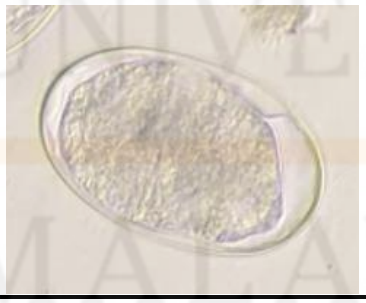
Observation	Day 1 - 2			Day 3 - 4			Day 5 - 6			Day 7 - 8			Day 9 - 10			Day 11 - 12			Day 13 - 14		
Cat/ID	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
General appearance	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR	BAR
Appetite	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Bowel	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Vomiting	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Salivation	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Lethargy	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB

Note: BAR= Bright, alert, responsive, N= Normal, AB= Absent.

8.2 Faecal examination

Table 8.4 shows the result of the microscopic examinations of the identified eggs that were detected using the floatation method and observed under the light microscope at 100× magnification.

Table 8.4: Detected ova from faecal samples

The appearance of ova under light microscope	Suspected species*
	*Toxocara cati*
	*Ancylostoma caninum*
	*Ancylostoma tubaeformae*

**Suspected ova species were identified and compared with other studies and reference books*

In Figure 8.1, Gamat-treated group (Group 1) shows decreased egg per gram (EPG) of faeces through the sampling days (Day 0, Day 7 and Day 15) indicating there is

subclinical improvement of the condition. However, one cat (C4) from the Gamat-treated group showed an increase in helminth ova EPG. On the other hand, in Figure 8.2 the Control group (Group 2) showed gradual increase of EPG throughout the sampling days.

Figure 8.1: The number of helminth ova (EPG) detected in Group 1 during sampling days

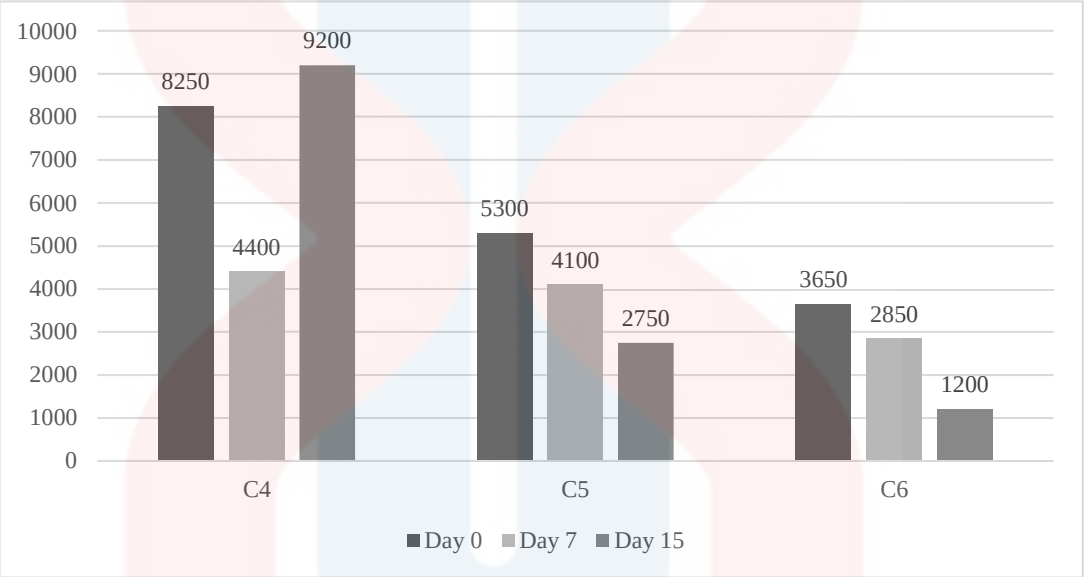
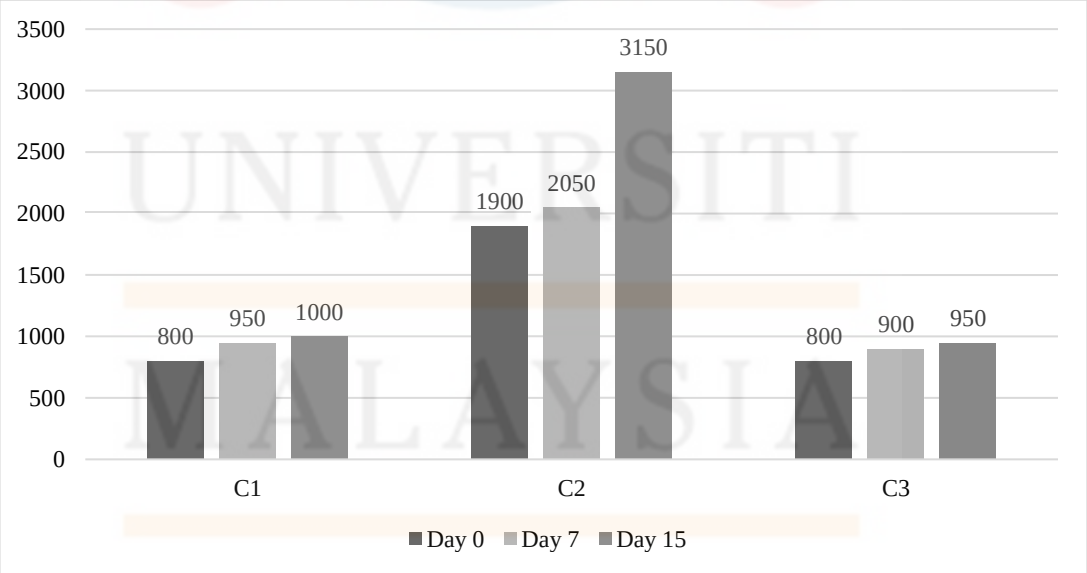


Figure 8.2: The number of helminth ova (EPG) detected in Group 2 during sampling days



8.3 Complete blood count analyses

According to the result in Table 8.7, there were no significant differences ($p>0.05$) for the haematological parameters in the control and Gamat-treated group. Similarly, there were also no significant differences ($p>0.05$) in the total white blood cells counts and differential leukocyte counts parameters in both groups.

Initially, on day 0, the mean ± SD for erythrocytes parameters of the Control group (Group 2) and Gamat-treated group (Group 1) were 0.57 ± 0.42 10^6/µl and 1.16 ± 1.66 10^6/µl, respectively. The value mentioned was below the normal range, indicative of anaemia for both groups. Likewise, the haemoglobin for Group 1 and Group 2 were 9.03 ± 2.49 g/dl and 8.23 ± 3.75 g/dl, respectively, which were below the reference range furthermore supporting that all cats were in fact anaemic pre-experimentally. Haematocrit counts for both groups following the same order were 5.67 ± 5.33% and 5.60 ± 7.56%, sequentially. These values were also below the normal range, indicative of anaemia. Nevertheless, the red cell distribution width (RDW) for both groups were above the reference level (17.26 ± 2.90% and 20.87 ± 4.92%) , indicating that the anaemia was regenerative. The total white blood cell counts and the other leukocytes parameters (lymphocytes, monocytes and granulocytes) were all slightly above the reference range for all six cats on day 0, indicative of leucocytosis and ongoing infection.

On day 7, the second sampling day, the Control group showed a slight increase in the erythrocyte parameter (2.79 ± 0.39 10^6/µl), however the value was still under the reference range. The haemoglobin value decreased but there was a slight increase for the haematocrit which are 8.83 ± 2.80 g/dl and 7.40 ± 0.62%, respectively, suggestive that the cats in Group 2 is still anaemic but is regenerative (RDW: 65.30±2.71%). Although the total white blood cell count is in the normal range, the monocytes (1.40

± 0.31) and granulocytes (11.23 ± 1.15 10^3/µl) values were still over the normal range indicative that the infection persists. Gamat-treated group, on the other hand, also shows slight increase of erythrocytes (2.89 ± 0.80 10^6/µl), haemoglobin (8.43±2.42 g/dl) and haematocrit (11.36 ± 1.14%) despite still below the normal range, indicative of anaemia. Despite that, the red cell distribution width for Group 1 has increased in two-fold (63.03 ± 2.57%) suggestive that the regenerative anaemia is more active indicating positive response towards the anaemia condition. The total white blood cell (2.94 ± 0.50 10^3/µl) counts including lymphocytes (1.96 ± 0.63) and monocytes (0.46 ± 0.13) were already in the normal range could be an indication that the infection was receding, however, the granulocytes level was slightly above the reference range (9.70 ± 0.60 10^3/µl).

Lastly, on day 15, the post experiment sampling of Group 2 shows slightly more increase of erythrocytes (5.67 ± 0.45 10^6/µl) and haematocrit (14.87 ± 2.08%) compared to the previous sampling day, although they were still below the reference values. The anaemia suggested to be regenerative since the red cell distribution width was above the normal range (32.60 ± 3.68%) despite decreases in values compared to day 7. The total white blood cell count including lymphocytes (2.04 ± 0.58), monocytes (1.50 ± 0.61) and granulocytes (11.70 ± 1.25 10^3/µl) shows increased in values suggestive of leucocytosis and indicating persistent infection. Meanwhile for Group 1, the Gamat-treated group, the haemoglobin parameter shows to be normal (11.17 ± 0.52 g/dl) while erythrocytes and haematocrit were just slightly below the reference range, which are 5.70 ± 0.70 10^6/µl and 24.33 ± 3.55%, respectively. From that information, however, the cats were still anaemic but with red cell distribution width above the normal range (35.90 ± 13.51%) still suggestive that the anaemia is regenerative. The total white blood cell (8.67.00 ± 4.80 10^3/µl) and monocytes (1.63

± 0.22) parameters shows level just slightly above normal range, but lymphocytes (1.47 ± 0.70) and granulocytes (3.87 ± 2.13 10^3/µl) levels were shown to be normal.

Table 8.5: The haematology parameters (Mean ± SD) of Gamat-treated group and control group on Day 0, Day 7 and Day 15 of the experiment

Group	Treatment			Control			Reference range
Parameters	**Day 0**	**Day 7**	**Day 15**	**Day 0**	**Day 7**	**Day 15**	
RBC (10^6/µl)	1.16 ± 1.66	2.89 ± 0.80	5.70 ± 0.70	0.57 ± 0.42	2.79 ± 0.39	5.67 ± 0.45	6.5-10.0
HGB (g/dl)	8.23 ± 3.75	8.43 ± 2.42	11.17 ± 0.52	9.03 ± 2.49	8.83 ± 2.80	8.17 ± 0.25	10.0-15.0
HCT (%)	5.60 ± 7.56	11.36 ± 1.14	24.33 ± 3.55	5.67 ± 5.33	7.40 ± 0.62	14.87 ± 2.08	30.0-45.0
RDW (%)	20.87 ± 4.92	63.03 ± 2.57	35.90 ± 13.51	17.26 ± 2.90	65.30 ± 2.71	32.60 ± 3.68	10.0-16.0
WBC (10^3/µl)	11.23 ± 8.29	2.94 ± 0.50	8.67.00 ± 4.80	12.97 ± 6.97	7.60 ± 3.70	9.33 ± 2.32	2.0-8.0
LYM	10.73 ± 5.05	1.96 ± 0.63	1.47 ± 0.70	5.67 ± 1.56	2.05 ± 0.54	2.04 ± 0.58	0.1-5.0
MON	3.17 ± 0.91	0.46 ± 0.13	1.63 ± 0.22	2.33 ± 0.91	1.40 ± 0.31	1.50 ± 0.61	0.1-1.0
GRA (10^3/µl)	11.17 ± 1.29	9.70 ± 0.60	3.87 ± 2.13	10.27 ± 1.05	11.23 ± 1.15	11.70 ± 1.25	2.0-8.0

Note: RBC=red blood cells, HGB=haemoglobin, HCT=haematocrit, RDW=red cell distribution width, WBC=white blood cells, LYM=lymphocytes, MON=monocytes, GRA=granulocyte

9.0 Discussion

Gamat (*S. variegatus*) is a common and well-known marine species in Malaysia that is utilised for traditional medicine. Gamat has been widely utilised for generations, prompting numerous investigations to investigate its medical properties. This includes the investigation of nutritional components in Gamat that may aid in weight loss, wound healing, antimicrobial capabilities, as well as antifungal activities (Shakouri et al., 2017). Though, lack of study regarding the potential anti-parasitic properties of Gamat led to this study using *S. variegatus* extract to evaluate the efficacy of the Gamat jelly preparation against gastrointestinal helminthiasis as to investigate macro and micro healing response towards the disease mentioned upon per os administration of the prepared Gamat-extracted jelly.

Based on the 14 days observation on six cats grouped equally into control group and treatment group, all of the cats show no notable abnormalities in terms of behaviours, food and water intake, as well as insignificant weight increase. No adverse reactions such as vomiting, lethargy, and hypersalivation was observed with 5mL of per os Gamat jelly administered twice a day. The cats also exhibited normal behaviour patterns such as grooming and occasional vocalizing throughout the study, however one of the cats from the Gamat-treated group had defecated inside its food bowl on Day 6 and progressed until Day 15.

It is wise to note that the change in body weight is a good measure of animal health, especially in gastrointestinal helminth infected animals (Little et al., 2015). An animal's body weight might fluctuate from day to day depending on the environment, water and food consumption, and the presence of underlying illnesses. Throughout the study period, cats in both groups experienced increased body weight as compared to

their starting body weight before on Day 0. This may be related to the cats being given an unrestricted amount of commercial dry cat food without taking into account their daily calorie requirements. Moreover, because the cats were kept in cages throughout the duration of this study, they may have had less daily activity, which may have contributed to their weight increase. However, when comparing Gamat-treated group (Group 1) to the control group (Group 2), the increase in body weight was not significant ($p>0.05$).

The species of gastrointestinal helminth that were found on three days of sampling (Day 0, Day 7, and Day 15) using faecal McMaster technique are *Toxocara cati*, *Ancylostoma caninum*, and *Ancylostoma tubaeforme*, which are all nematodes. There was a gradual increase of worm burden in all three cats from the Control group (Group 2) as the study period progressed until Day 15. Meanwhile, for cats in Gamat-treated group (Group 1), two out of three cats showed gradual decrease of worm burden upon the end of the treatment. However, one of the cats have increase of worm burden upon the third sampling day. The sudden increase of the helminth burden might be due to this particular cat defecating in its food bowl which started on Day 6 until the end of the study. Thus, it can be inferred that there might be ingestion of the helminth ova through faecal-oral route though the food bowl, hence led to the increased of EPG in the affected cat. To conclude, there was significant difference of Gamat-treated group and Control group in terms of EPG difference by which it decreases in Group 1 while increases in Group 2.

Pre-experimentally on Day 0, all six cats showed to be having regenerative iron-deficiency anaemia, leucocytosis, lymphocytosis, monocytosis and granulocytosis. This is suggestive of a pathological condition resulting from the infection of the gastrointestinal helminthiasis which is indicative of, but not diagnostic of, an ongoing

inflammatory condition. Unfortunately, due to this study limitation of lacking absolute eosinophils counts, the result is less accurate in determining the cause of elevated granulocytes due to the helminth infection. It is stated by Huang & Appleton (2016) that the host's reaction to helminth infection is characterised by eosinophilia. In the presence of particular antibodies or complement, eosinophils will be recruited more to destroy larval stages of parasitic worms in vitro (Huang & Appleton, 2016). The anaemia condition could also be suggestive of endoparasitic infection especially caused by species *A. tubaeforme* (Little et al., 2015). To add, iron-deficiency anaemia accounts for most of the anaemia which arise due to parasitic infections and hookworms particularly, induces iron deficiency by chronic intestinal blood loss which then contribute to anaemia (Osazuwa et al., 2011). As the study progressed until Day 15, the anaemia condition for the Gamat-treated group improved although the parameters were still below the reference range. Other factors of underlying disease that can lead to the same haematological condition must be considered because this study was conducted on stray cats with unknown health history.

10.0 Conclusion

Per os administration of 5 mL commercial Gamat extract preparation twice a day for a period of 14 days shows to be effective in reducing the worm burden on stray cats. This statement is evidenced by the decrease in EPG on treated cats, as well slight haematological improvement. This study affirms that Gamat extract does have anti-parasitic properties apart from its known anti-inflammatory, antibacterial and antifungal effects. This also ensures that Gamat extract would have a positive and promising prospect as a medical protocol in treating gastrointestinal helminthiasis in

domestic cats. To add, since Gamat is readily accessible and is one of the pride natural resources in Malaysia, this study would help the local Gamat industry as well as contributing to the development of an anthelmintic drug using Gamat extract.

11.0 Recommendation and future work

Several limitations were observed in this study. More in-depth research should be conducted, with a longer treatment term and a larger study size (e.g., n=100). Larger study group would to help for better analyzations of the treatment as well as providing more accurate mean values, identifying outliers as well as providing a smaller margin of error. Also, strict disinfection protocol should be implemented during the study by using 50% dilution of sodium hypochlorite with one hour contact time to inactivate the eggs (Naidoo et al., 2016). Next, absolute eosinophil counts should be carried out prior to experiment, during treatment, and after treatment in cats with gastrointestinal helminthiasis to investigate the importance of eosinophil levels in relation to healing responses. Next recommendation is to use alternative floatation methods for detecting helminth eggs. This is owing to the varying egg densities, which could be present but were not taken into account in this research. Using different floatation techniques may maximize the possibilities of detecting helminth eggs from other species in the faeces. Additionally, diagnostic workup using larval culture and molecular methods such as Polymerase Chain Reaction (PCR) to identify and confirm the helminth species would be useful to produce a more comprehensive result. Lastly, dose-finding study can be conducted to determine the no-effect dose, as well as the mean and maximum effective doses of the Gamat extract against gastrointestinal helminthiasis in cats.

APPENDICES

Appendix A

ANIMAL DATA																													
ID:			Colour:										Place of capture:																
Sex:			Temperament:																										
Age:			Group:																										
DAY	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**		**13**		**14**			
Body weight																													
GENERAL APPEARANCE																													
a.m. (a) /p.m. (p)	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	a	p	
Respiratory rate(bpm)																													
Heart rate (bpm)																													
Temperature (°C)																													
Dehydration (%)																													
Mentation																													
Mucous membrane																													
GASTROINTESTINAL																													
Appetite (food/water)																													
Urination																													
Bowel movement																													
Faecal score (1-7)																													
Vomiting																													

Appendix A- Daily assessment form

Appendix B

Fakulti Perubatan Veterinar

UNIVERSITI MALAYSIA KELANTAN

RUJ. KAMI *(Our Ref.)* : UMK/FPV/ACUE/FYP/002/2022
TARIKH *(Date)* : 20 FEBRUARY 2022

PROF. DR. JASNI BIN SABRI
Main Supervisor
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan

Dear Prof,

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, Universiti Malaysia Kelantan has been approved. Please refer the table below for approval code:

APPROVAL CODE	***UMK/FPV/ACUE/FYP/002/2022***
TITLE	***INVESTIGATION OF COMMERCIAL GAMAT EXTRACT (Stichopus variegatus) PREPARATION ON STRAY CATS WITH GASTROINTESTINAL HELMINTHIASIS***

2. Please be noted for the Final Year Project, you are responsible to supervise your student to conduct all animal-related procedures as stated during ethic application. The co-supervisor(s) for the project are encouraged to help with the procedures as well.

3. 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.

"RAJA BERDAULAT, RAKYAT MUAFAKAT, NEGERI BERKAT"
"WAWASAN KEMAKMURAN BERSAMA 2030"
"BERKHIDMAT UNTUK NEGARA"

Yours sincerely,

DR. NOR FADHILAH BINTI KAMARUZZAMAN
Chairman
Institutional Animal Care and Use Committee
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan

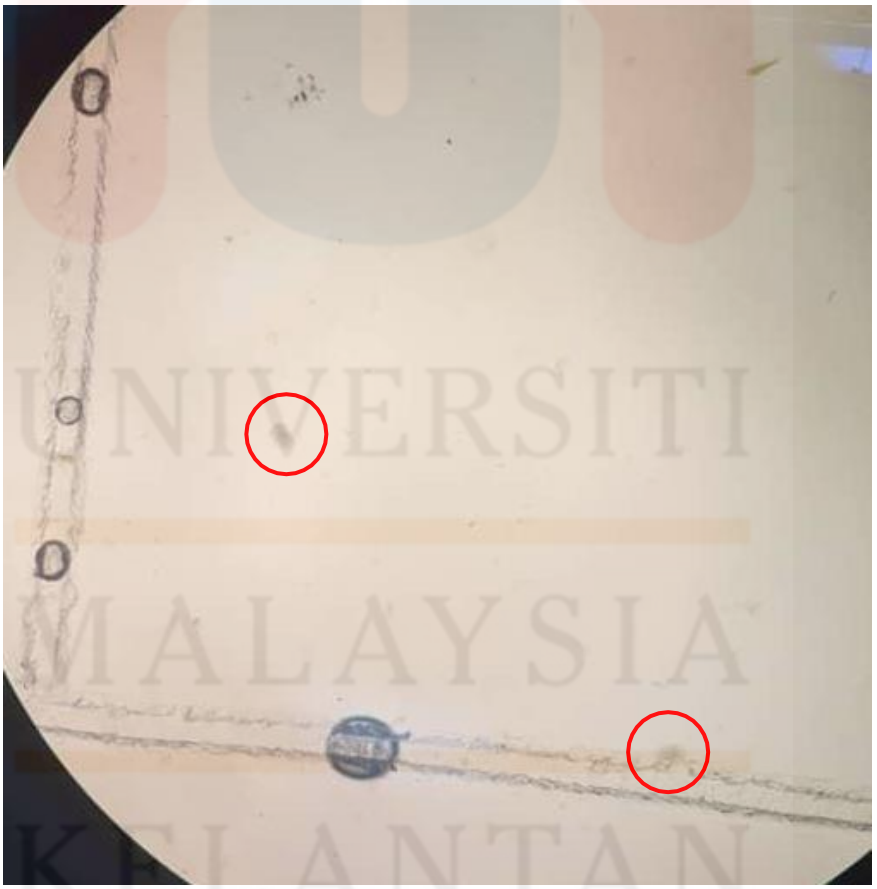
UNIVERSITI MALAYSIA KELANTAN اونيۏرسيتي مليسيا كلنتن | Karung Berkunci *(Locked Bag)* 36, Pengkalan Chepa, 16100 Kota Bharu, KELANTAN, MALAYSIA | Tel :609 771 7277 Fax :609 771 7282

Appendix B- Approval form from Final Year Research Committee and Animal Ethics Committee 2022

Appendix C

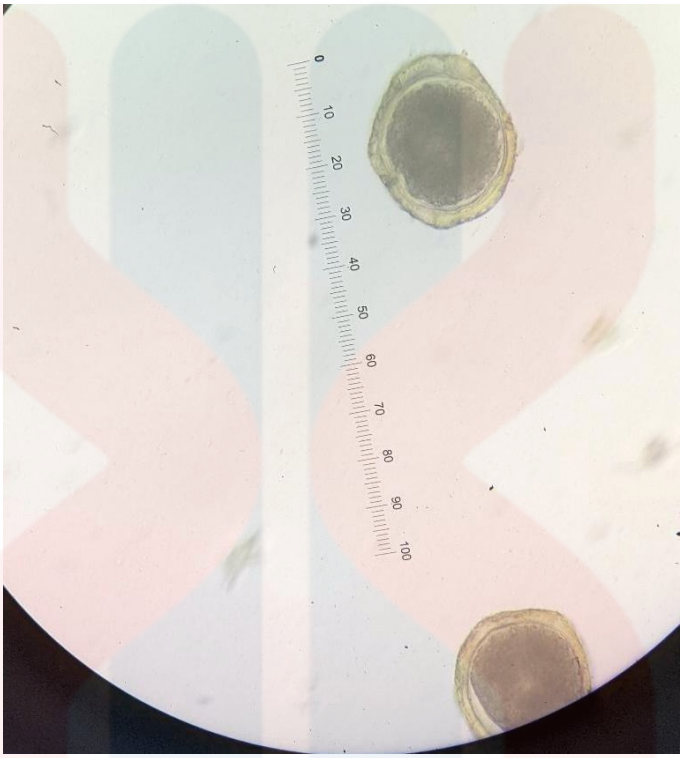
Appendix C- Materials and apparatus used for faecal examination

Appendix D

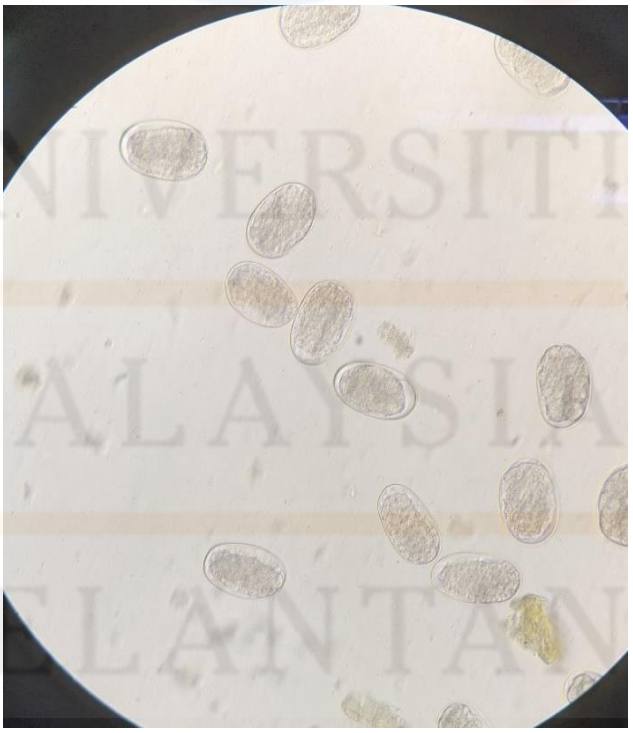


Appendix D- The process of calculating helminth egg in the McMaster chamber under light microscope with 10×magnification. Red circles show helminth eggs.

Appendix E



Appendix E.1- Toxocara cati under 100× magnification



Appendix E.2- Ancylostoma sp. under 100× magnification

REFERENCES

Bordbar, S., Anwar, F., & Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods - A review. *Marine Drugs*, *9*(10), 1761–1805. https://doi.org/10.3390/md9101761

Chen, J. (2003). Overview of sea cucumber farming and sea ranching practices in China. *SPC Beche-de-Mer Information 18 Bulletin, May 2003*, 18–23.

Conboy, G. (2009). Cestodes of Dogs and Cats in North America. *Veterinary Clinics of North America - Small Animal Practice*, *39*(6), 1075–1090. https://doi.org/10.1016/j.cvsm.2009.06.005

Foreyt William J. (2001). *Veterinary Parasitology Reference Manual* (5th ed.). Blackwell Publishing Professional.

Holyoake, C. S., & Sciences, B. (2008). *A National Study of Gastrointestinal Parasites Infecting Dogs and Cats in Australia Carlysle Sian Holyoake Bachelor of Science (Veterinary Biology) Murdoch University Bachelor of Veterinary Medicine and Surgery Murdoch University Division of Health Scie*.

Huang, L., & Appleton, J. A. (2016). Eosinophils in Helminth Infection: Defenders and Dupes. *Trends in Parasitology*, *32*(10), 798–807. https://doi.org/10.1016/j.pt.2016.05.004

Kim, F. (2008). *Pacific sea cucumber dataset from the Secretariat of the Pacific Community Reef Fisheries Observatory*. SPC Beche de Mer Information Bulletin.

Little, S., Adolph, C., Downie, K., Snider, T., & Reichard, M. (2015). High prevalence of covert infection with gastrointestinal helminths in cats. *Journal of*

the American Animal Hospital Association, *51*(6), 359–364. https://doi.org/10.5326/JAAHA-MS-6221

Mariana. (2009). Evaluating the antibacterial activity and in vivo assay of methanolic extract of Stichopus badionotus. *International Journal of Pharmacology*, *5*, 228–231.

Naidoo, D., Archer, C., Louton, B., & Rodda, N. (2016). Testing household disinfectants for the inactivation of helminth eggs on surfaces and in spills during pit latrine emptying. *Water SA*, *42*(4), 560–570. https://doi.org/10.4314/wsa.v42i4.06

Osazuwa, F., Ayo, O. M., & Imade, P. (2011). A significant association between intestinal helminth infection and anaemia burden in children in rural communities of Edo state, Nigeria. *North American Journal of Medical Sciences*, *3*(1), 30–34. https://doi.org/10.4297/najms.2011.330

Pangestuti, R., & Arifin, Z. (2018). Medicinal and health benefit effects of functional sea cucumbers. *Journal of Traditional and Complementary Medicine*, *8*(3), 341–351. https://doi.org/10.1016/j.jtcme.2017.06.007

Rojekittikhun, W., Chaisiri, K., Mahittikorn, A., Pubampen, S., Sa-nguankiat, S., Kusolsuk, T., Maipanich, W., Udonsom, R., & Mori, H. (2014). Gastrointestinal parasites of dogs and cats in a refuge in Nakhon Nayok, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *45*(1), 31–39.

S. Shapiro, L. (2005). *Pathology & Parasitology For Veterinary Technicians.*

Sabuan N. (2016). *Gamat*. http://www.myhealth.gov.my/en/gamat/

Shakouri, A., Shoushizadeh, M. R., & Nematpour, F. (2017). Antimicrobial activity of sea cucumber (Stichopus variegatus) body wall extract in Chabahar Bay, Oman sea. *Jundishapur Journal of Natural Pharmaceutical Products, 12*(1), 1–14. https://doi.org/10.5812/jjnpp.32422

Subramaniam, B. S., Amuthan, A., D'Almeida, P. M., & Arunkumar, H. D. (2013). Efficacy of gamat extract in wound healing in albino wistar rats. *International Journal of Pharmaceutical Sciences Review and Research, 20*(1), 142–145.