



Faculty of Resource Science and Technology

**A SURVEY OF GASTROINTESTINAL PARASITES OF DOMESTIC  
CATS (*Felis catus*) IN KUCHING, SARAWAK**

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(36805)

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Final Year Project Report

Masters

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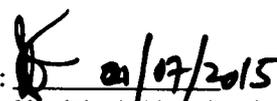
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UNIVERSITI MALAYSIA SARAWAK**

**A SURVEY OF GASTROINTESTINAL PARASITES OF DOMESTIC CATS  
(*FELIS CATUS*) IN KUCHING, SARAWAK.**

**LOW SUET EE**

This project is submitted in partial fulfilment of  
the requirement for the degree of Bachelor of Science with Honours  
(Animal Resources Science and Management)

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UNIVERSITI MALAYSIA SARAWAK

2015

## DECLARATION

No portion of the work referred to this thesis has been submitted in support of an application for another degree of qualification of this or any other university of institution of higher learning.

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## LIST OF ABBREVIATIONS

%	percentage
°C	degree celcius
µm	micrometer
D	Simpson diversity index
ddH <sub>2</sub> O	double distilled water
ELISA	Enzyme-linked immunosorbent assay
g	gram
H	Shannon diversity index
IFAT	Indirect immunofluorescence antibodies test
MAT	Modified agglutination test
ml	milliliter
n	number of units
N	total number of units
P	calculated probability
PAST	Palaeontological Statistics
PCR	Polymerase Chain Reaction
spp.	two or more species of the genus
SSPCA	Sarawak Society for the Prevention of Cruelty to Animals
x	Times

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# A Survey of Gastrointestinal Parasites of Domestic Cats (*Felis catus*) in Kuching, Sarawak.

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## ABSTRACT

Domestic cats (*Felis catus*) are known to be commonly infected with parasites because of their habit of roaming freely, especially stray cats. Up until now, there are still lack of information and published papers regarding parasites of cat in Sarawak. Main objective of this study was to investigate and compare the gastrointestinal parasites of house-owend and stray cats. A total of 60 faecal samples were collected from domestic cats in Kuching, in which 30 samples from house-owned and stray cats each. Concentration technique such as centrifugal floatation and sedimentation were used for the examination of gastrointestinal parasites. Overall prevalence of gastrointestinal parasites in all cats was 46.67% (28/60) in this study, where gastrointestinal parasite prevalence of house-owned cats was 33.33% (10/30) and stray cats was 60% (18/30). Detected nematode species includes *Ancylostoma* spp. (30%), *Toxocara* spp. (10%) and *Toxascaris leonina* (5%) while recovered flatworm (18.83%) includes 17 different types of morphologies. *Isoospora* spp. was the only protozoan parasite species recovered in this study with the prevalence of 6.67%. There was no significant difference on species diversity of gastrointestinal parasite between house-owned and stray cats.

Keyword: domestic cats, gastrointestinal parasite, prevalence

## ABSTRAK

Kucing domestik (*Felis catus*) yang diketahui biasanya dijangkiti parasit disebabkan tabiat bebas merayau mereka, terutamanya kucing liar. Sehingga kini, maklumat dan kajian mengenai parasit kucing masih kekurangan di Sarawak. Objektif utama kajian ini adalah untuk menyelidik dan membandingkan parasit gastrousus kucing peliharaan dan kucing liar. Sebanyak 60 sampel najis dikumpulkan dari kucing domestik di Kuching, di mana 30 sampel masing-masing daripada kucing peliharaan dan kucing liar. Teknik kepekatan seperti pengapungan dan pemendapan empar digunakan untuk pemeriksaan parasit gastrousus. Kelaziman keseluruhan parasit gastrousus dalam semua kucing adalah 46.67% (28/60) dalam kajian ini, di mana kelaziman parasit gastrousus untuk kucing peliharaan adalah 33.33% (10/30) dan kucing liar adalah 60% (18/30). Spesies nematod dikesan termasuk *Ancylostoma* spp. (30%), *Toxocara* spp. (10%) dan *Toxascaris leonina* (5%) manakala cacing pipih (18.83%) dikesan dengan 17 jenis morfologi. *Isoospora* spp. adalah satu-satunya spesies protozoa parasit dalam kajian ini dengan kelazimannya 6.67%. Tiada perbezaan yang signifikan terhadap kepelbagaian spesies parasit gastrousus antara kucing peliharaan dan kucing liar.

Kata kunci: kucing domestik, parasit gastrousus, kelaziman

## 1.0 Introduction

Pets such as cat, dog, hamster, rabbit, guinea pig, ferret, and chinchilla are animals kept by human for personal companion. However, these pets are prone to the infestation of parasite due to their daily activities and interactions with other animals. Cats are carnivorous animals categorised under Family Felidae. It is also one of the most common pet kept by Malaysian. However, most of the cats are commonly infected with parasites due to their habit of roaming freely, especially the stray cats. From the standpoint of veterinary, most of the stray cats, especially from the urban areas, have been act as potential role as internal parasites reservoirs. These parasites usually lead to most of the gastrointestinal diseases to domestic cats.

Gastrointestinal parasites are living organisms that inhabit within intestinal tract of another living organisms (host) which consist of protozoan parasite and helminthes. Helminthic parasites are parasitic worms while protozoan parasites are organisms consist of single cell. Helminthic parasites that normally found in domestic cats are categorised under two main phylum which are Phylum Nematoda and Phylum Platyhelminthes. Parasitic worms under Phylum Nematoda are known as roundworm while Phylum Platyhelminthes are known as flatworms. Two classes are further divided in Phylum Platyhelminthes which are the Class Cestoda and Class Trematoda with the respective general name of tapeworm and flukes. Domestic cats are usually being detected with higher infestation of nematode helminthes species.

Most of the cats harbor various internal parasites. Previous studies had reported that *Ancylostoma braziliense*, *A. ceylanicum*, *Ascaris* spp., *Dipylidium caninum*, *Joyeuxiella pasqualei*, *Physaloptera praeputialis*, *Platynosomum fastosum*, *Spirometra* spp., *Taenia*

*taeniaeformis*, *Toxocara cati*, *T. malaysiensis*, *Toxascaris leonina* and *Trichuris vulpis*, were the helminthes species found infecting domestic cats in Peninsular Malaysia (Shanta *et al.*, 1980; Mohd Zain *et al.*, 2013, Ngui *et al.*, 2014). Meanwhile, protozoan reported in Peninsular Malaysia are comprising of *Toxoplasma gondii*, *Isospora* spp., *Entamoeba* spp., *Giardia duodenalis*, *Cryptosporidium* spp., *Balantidium coli*. and *Eimeria* spp. (Shanta *et al.*, 1980; Ngui *et al.*, 2014).

Detection of the gastrointestinal parasites of domestic cats are usually done by using necropsy or coproscopy method. Necropsy is done by humanely euthanised the cats while coproscopy is done by examining the collected cat faeces. Prevalence of the gastrointestinal parasites harbor in cats varies among the studies. Factors that influencing the infestation and prevalence of gastrointestinal parasites include the geographical location, types of diagnostic technique used, occurrence and regularity of veterinary care, season change and their living condition such as stray, household or sheltered (Calvete *et al.*, 1998; Abu-Madi *et al.*, 2010; Mircean *et al.*, 2010).

In recent times, there is a rise of concerns on the risk of public health that caused by cats and various potential zoonotic organisms that related with cats; especially on the pathogens inhabiting the gastrointestinal part (Hill *et al.*, 2000; McGlade *et al.*, 2003). This is because some of the parasites species on cats have potential in infecting humans, and may cause some zoonotic diseases to human. The most significant helminthes that normally infected to the human are the ascarids and hookworms as they can cause larva migrans to human (Kazaco, 2002). However, there is still no reports of zoonotic infections that was initiated by stray cats in Peninsular Malaysia up until now. In addition, there are still lack of information and published papers regarding endoparasite of cats in Sarawak.

## **1.1 Problem Statement**

Since long time ago, there are many published papers have been reported on the condition of endoparasite of the cats in different areas of all over the world. In Malaysia, there are a few endoparasite studies on the domestic cats was done since 1978. However, most of the studies was done in Peninsular Malaysia where there are still lack of information and published papers on the study of endoparasite of domestic cats in Sarawak. Parasite infestation varies with the geographical locations and the living condition of the cats, thus investigation on the parasites of domestic cats was done in Sarawak.

## **1.2 Hypothesis**

$H_0$ : There is no significant difference on the species diversity of gastrointestinal parasites in stray and house-owned cats.

$H_A$ : There is a significant difference on the species diversity of gastrointestinal helminthes in stray and house-owned cats.

## **1.3 Objectives**

- i. To investigate gastrointestinal parasites composition of stray and house-owned cats.
- ii. To determine the prevalence of the gastrointestinal parasites of stray and house-owned cats.

## **2.0 LITERATURE REVIEW**

### **2.1 The Host**

All kinds of cats including big cats or wild cats belong to family Felidae. According to Wilson *et al.* (2009), family Felidae comprise of 37 species. The most common felid species found in the human populated areas is the domestic cats, *Felis catus*. Although there are many breed found in domestic cats, all of them are under the same species, *F. catus*. All domestic cats are descended from African wild cats.

Domestic cats have been known as the most popular pet worldwide (Driscoll *et al.*, 2009) and there are about 30% of households are owning cats throughout the whole nation (Coleman *et al.*, 1997). They have formed a long relationship with human since 4,000 years ago when the ancient Egyptians domesticated the first cat due to their agile skills in capturing and killing the rodents (Clutton-Brock, 1981; Mason, 1984; Davis, 1987; Turner & Bateson, 1988). Stray cats are known as cats that normally wander around in the open air and can be seen usually appear in the human concentrated area where food is easily found such as market, food stall or streets. They do not have a home but seek for their own shelter. Conversely, house-owned cats are cats that have home and fostered by human. Another difference between household cats and stray cats is that household cats can survive up to the age in the range of 15 to 17 years as pets but stray cats normally can survive to the age between four to five years only (Ogan & Jurek, 1997).

## **2.2 Gastrointestinal Helminthes**

Gastrointestinal helminthes are parasitic worms that inhabit within the intestinal tract of another living organisms (host) which also is known as one of the endoparasites. They feed on the blood of the host to obtain food, nourishment and nutrients. Parasitic worm involves in both immature and mature form of life cycle and undergoes three life stages: the eggs, larvae and adults. Some of the parasitic worms have their immature stages developed partially in the same or different species of animals. Those animals are known as the intermediate host. Definitive hosts are animals that harbor the sexually matured parasitic worms.

Parasites bring harms to the host in which the infected organism might undergo symptoms such as loss of desire for food, declination of body weight, extreme thinness, contain mucous membranes in pale to yellowish pale colour, diarrhea, swollen at the lower part of the chin, weakness and reduced growth. Besides, endoparasite infection might also lead to death cause by anemia or organs malfunction if severe infestation occurred with no treatment (Wanamaker & Massey, 2009). Invasion of the gastrointestinal helminthes to the host can take place in a few ways, which include consumption on food contaminated with infective larvae or eggs, accidental ingestion of intermediate host harboring infective stage parasite, feeding on a paratenic host, intermediate host transmission, maternal transmission, or direct penetration. Two main groups are categorised under parasitic helminthes, which are Phylum Nematoda and Phylum Platyhelminthes.

Most of the cats are vulnerable to the infestation of gastrointestinal parasite. Prevalence of the gastrointestinal parasite was high among the cats examined in most of the studies (Borkataki *et al.*, 2013; Capári *et al.*, 2013).

### 2.2.1 Nematode Infestation of Domestic Cats

Parasitic worms under Phylum Nematoda are known as roundworm. Among all the published studies had been done, it is reported that the most common helminthes parasite found in the intestine of the cats is nematode species. Worldwide, most of the previous studies had revealed that *T. cati* is the most prominent nematode among the examined cats. The infection of *T. cati* was the highest among the other nematodes especially in Belgium, Argentina, and Denmark which the prevalence range between 60% to 79% (Engbaek *et al.*, 1984; Vanparijs *et al.*, 1991; Sommerfelt *et al.*, 2006). Similarly in Peninsular Malaysia, a parasitological study of domestic cats was conducted in the rural areas of Selangor and Pahang had reported *Toxocara* spp. as the highest prevalence helminthes species (Ngui *et al.*, 2014).

However, different result was obtained on the most prevalent nematode species in a study done in Ipoh for eight years and a study done in the urban areas of Peninsular Malaysia on the parasite of cats (Shanta *et al.*, 1980; Mohd Zain *et al.*, 2013). Study conducted by Shanta *et al.* (1980) had recovered three nematodes species comprising of *Ancylostoma* spp., *P. praeputialis* and *T. cati* but *Ancylostoma* spp. (92.5%) was the most prominent nematodes found instead of *T. cati* (14.5%). Similarly, study conducted by Mohd Zain *et al.* (2013) had revealed the most dominant helminthes of *A. braziliense* and *A. ceylanicum* with respective prevalence of 30.8% and 29.5%. Most of the nematode species obtained from both study done by Shanta *et al.* (1980) and Mohd Zain *et al.* (2013) shows almost similar finding except for the two additional species, *Strongyloides* sp. and *T. malaysiensis*, detected in study of urban areas (Kuala Lumpur, Kuantan, Georgetown and Malacca) in Peninsular Malaysia (Mohd Zain *et al.*, 2013). Meanwhile, other countries such as Thailand, Indonesia, Brazil, and Qatar, were also obtained *Ancylostoma* spp. as their highest number of nematode species (Margono *et*

al., 1979; Labarthe *et al.*, 2004; Jittapalapong *et al.*, 2007; Schuster *et al.*, 2009; Abu-Madi *et al.*, 2010). Currently, *A. tubaeforme*, *A. braziliense* and *A. ceylanicum* were the three species of genus *Ancylostoma* found in domestic cats in Peninsular Malaysia. Domestic cats that infected with these hookworm species may possess symptom of regenerative anaemia.

In the study of Shiraz, Iran, it showed that *Physaloptera* sp. (44.6%) had the highest prevalence among the other nematode species (Zibaei *et al.*, 2007). This may be due to the difference of method applied in the study (Robertson *et al.*, 2000) as the cats were being necropsied and *Physaloptera* sp. was mostly detected on the pyloric part of the cats' stomach instead of the intestine. Other less dominant nematodes recovered from the cats' faeces includes *Aelurostrongylus abstrusus*, *Ascaris* spp., *Capillaria* spp., *Ollulanus tricuspis*, *Pterygodermatites affinis*, *Strongyloides* spp., *T. mystax*, *T. leonina*, and *Trichuris* spp. (Martínez-Barbabosa *et al.*, 2003; Sommerfelt *et al.*, 2006; Schuster *et al.*, 2009; Krecek *et al.*, 2010; Mircean *et al.*, 2010; Barutzki & Schaper, 2011; Khalafalla, 2011; Borkataki *et al.*, 2013; Mohd Zain *et al.*, 2013; Ngui *et al.*, 2014).

### **2.2.2 Flatworm Infestation of Domestic Cats**

Parasitic worms of Phylum Platyhelminthes are known as flatworms. Two classes are further divided in Phylum Platyhelminthes which are the Class Cestoda and Class Trematoda with the respective general name of tapeworm and flukes.

The second most detected helminthes species on cats is the cestode which also known as the tapeworm. Most of the studies have reported that domestic cats are normally infected with *D. caninum* and *T. taeniaeformis* (Nichol *et al.*, 1981; Engbaek *et al.*, 1984; Calvete *et al.*, 1998;

Zibaei *et al.*, 2007, Cantó *et al.*, 2013). Same goes to the study conducted in Ipoh, Malaysia, *D. caninum* (15%) was the most prominent cestode species detected, following by three other cestodes species including *J. pasqualei*, *Spirometra mansoni* and *T. taeniaeformis* (Shanta *et al.*, 1980). However, study in urban areas (Mohd Zain *et al.*, 2013) and rural areas domestic cats in Peninsular Malaysia had obtained higher infection of *T. taeniaeformis* and *Spirometra* spp. (9.5%) compared to *D. caninum*. Meanwhile, *Joyeuxiella* spp. was also one of the cestodes that reported to be the highest prevalence in some previous studies (Calvete *et al.*, 1998; Zibaei *et al.*, 2007; Schuster *et al.*, 2009), while *Diplopylidium acanthotetra*, *D. noelleri*, *Hydatigera taeniaeformis*, *Mesocestoides* spp., and *T. hydatigena* were some of the less dominant tapeworms can be found in cats.

In comparison of nematode and cestode, cases involved with the infection of trematode and acanthocephalan species are relatively lesser in cats. *Heterophyes heterophyes* (Schuster *et al.*, 2009; Khalafalla, 2011), *Heterophyopsis continua* (Schuster *et al.*, 2009) and *Platynosomum* spp. (Krecek *et al.*, 2010) are tapeworms detected in previous studies. Parasitological studies of Peninsular Malaysia were commonly reported with the presence of *P. fastosum* in domestic cats (Shanta *et al.*, 1980; Mohd Zain *et al.*, 2013; Ngui *et al.*, 2014). Acanthocephalan infection cases are rare in cats. *Centrorhynchus aluconis* is the only acanthocephalan recovered during the endoparasite study of cats in Dubai (Schuster *et al.*, 2009).

### 2.3 Protozoan Parasite Infestation of Cats

Several common types of protozoan parasites were being detected in domestic cats includes *T. gondii*, *Giardia* spp., *Isospora* spp. and *Cryptosporidium*. In Malaysia, *Cryptosporidium* spp., *Entamoeba* spp., *G. duodenalis*, *Isospora* spp. and *T. gondii* were being recovered in several previous studies (Shanta *et al.*, 1980; Ngui *et al.*, 2014).

Several previous studies had reported that *T. gondii* was the most dominant protozoan parasite in the cats in countries such as Mexico City (Martínez-Barbabosa *et al.*, 2003), Egypt (Khalafalla, 2011), and Jammu, India (Borkataki *et al.*, 2013). Low prevalence of *T. gondii* were reported in Ipoh of Malaysia (Shanta *et al.*, 1980), Mexico City (Martínez-Barbabosa *et al.*, 2003), Dubai (Schuster *et al.*, 2009), Transylvania (Mircean *et al.*, 2010), Germany (Barutzki & Schaper, 2011), and Egypt (Khalafalla, 2011), ranging from 0.8% to 9%. However, study in Jammu, India, had reported with extremely high *T. gondii* infection (88%) (Borkataki *et al.*, 2013). Presence of *T. gondii* were normally detected by collecting the serum of the animal and testing on several serological diagnostic techniques such as modified agglutination test (MAT), indirect immunofluorescence antibodies test (IFAT) or latex agglutination test. In recent times, toxoplasmosis has turn out to be a major health risk in the public as *T. gondii* were capable in infecting warm blooded animals including human. *T. gondii* can damage central nervous system permanently and cause serious sickness to its host. Attribution to the contamination of environments cause by the excretion of *T. gondii* oocysts were only done by cats, cats are known as the major reservoir of this protozoan parasite (Dubey, 2008).

Another commonly found protozoan parasite in cats was the *Isoospora* spp.. It was the only protozoan parasite recovered in cats in England (Nichol *et al.*, 1981), Belgium (Vanparijs *et al.*, 1991), Argentina (Sommerfelt *et al.*, 2006), Northern Germany (Becker *et al.*, 2012) and western Hungary (Capári *et al.*, 2013). Besides, *Isoospora* spp. was recovered as the most prominent protozoan parasite in several studies (Vanparijs *et al.*, 1991; Schuster *et al.*, 2009; Mircean *et al.*, 2010). According to the study of Borkataki *et al.* (2013), high *Isoospora* spp. infection is detected in Jammu, India (80%). *Isoospora felis* and *Isoospora rivolta* were the common species of genus *Isoospora* found in domestic cats. Normally, *Isoospora* spp. can only be detected by using microscopy method on the faecal samples. Kitten were usually infected with this protozoan parasite but no serious diseases may occur upon the infection (Bowman, 2002).

Recovery of *Giardia* spp. and *Cryptosporidium* spp. were usually done by using polymerase chain reaction (PCR) procedure. Immunoassay were initially used on the faecal samples to detect the presence of *Giardia* spp. and *Cryptosporidium* spp., and following by PCR method for genotyping in the study of central Italy (Riggio *et al.*, 2013). Presence of *Giardia* spp. were reported in Perth (McGlade *et al.* 2003), Transylvania (Mircean *et al.*, 2010), Egypt (Khalafalla, 2011), Germany (Barutzki & Schaper, 2011) and central Italy (Riggio *et al.*, 2013) in range of 0.7% to 2%. In Germany, *Giardia* spp. was the most prominent parasite compared to the other protozoan detected in cats (Barutzki & Schaper, 2011). Meanwhile, presence of *Cryptosporidium* spp. were reported in Jammu, India, with the prevalence of 4%. Giardiasis and cryptosporidiosis infection may cause diarrhea to the infected cats.

## 2.4 Factors Influencing Prevalence of Gastrointestinal Parasite

Several studies had also compared and studied the factors influencing the gastrointestinal parasite prevalence of domestic cats. According to Robertson *et al.* (2000), factors influencing the prevalence of parasites includes the geographical location, age of the cats, gender of the cats, living conditions of the cats, diagnostic techniques used, and frequency of the usage of anthelmintic. Relationship of the prevalence of infestation of gastrointestinal parasites and their living conditions were being studied between breeders' cats, boarding cats, pet shops cats, privately owned cats, and refuge cats in Perth (McGlade *et al.*, 2003), apartment and house cats in Mexico city (Martínez-Barbabosa *et al.*, 2003), feral and shelter cats in Rio de Janeiro (Labarthe *et al.*, 2004), and domestic and stray cats in Central Mexico (Cantó *et al.*, 2013). Meanwhile, lots of studies in worldwide such as Germany (Barutzki & Schaper, 2003; Barutzki & Schaper, 2011), Bangkok (Jittapalapong *et al.*, 2007), Rio Grande do Sul of Brazil (Lorenzini *et al.*, 2007), Mashhad (Borji *et al.*, 2011), Dubai (Schuster *et al.*, 2009), Veterinary Hospital of the University of Pennsylvania (Gates & Nolan, 2009), Transylvania (Mircean *et al.*, 2010), Northern Germany (Becker *et al.*, 2012), central Italy (Riggio *et al.*, 2013), central Mexico (Cantó *et al.*, 2013), western Hungary (Capári *et al.*, 2013) and Europe (Beugnet *et al.*, 2014) had compared the parasite prevalence across the age of the cats, in which most of the studies emphasised the importance of age as a factor in affecting the parasite prevalence. A number of studies were also done on studying the association of the parasite prevalence between sex (Jittapalapong *et al.*, 2007; Zibaei *et al.*, 2007; Borji *et al.*, 2011; Schuster *et al.*, 2009; Mircean *et al.*, 2010; Riggio *et al.*, 2013; Cantó *et al.*, 2013). Parasite prevalence were also reported to be related with other potential factors including geographical location (Martínez-Barbabosa *et al.*, 2003; Schuster *et al.*, 2009; Mircean *et al.*,

2010; Mohd Zain *et al.*, 2013; Beugnet *et al.*, 2014), seasonal changes (Calvete *et al.*, 1998; Lorenzini *et al.*, 2007; Abu-Madi *et al.*, 2010; Barutzki & Schaper, 2011; Cantó *et al.*, 2013), diagnostic techniques applied (Lillis, 1967; Nichol *et al.*, 1981; Vanparijs *et al.*, 1991; Krecek *et al.*, 2010), potential outdoor access of the cats (Mircean *et al.*, 2010; Beugnet *et al.*, 2014), frequency of anthelmintic treatment (Beugnet *et al.*, 2014) and interaction with other animals (McGlade *et al.*, 2003; Beugnet *et al.*, 2014).

## **2.5 Faecal Analysis Test**

In this study, both floatation and sedimentation method was selected to maximise the findings of the parasite eggs. Study had reported that centrifugation has the ability in obtaining higher yield of parasite eggs recovery compared to other methods (Dryden *et al.*, 2005). Floatation method demonstrates the theory of specific gravity by using a higher density solution to create an accumulation of the parasite above the solution while deposit the faecal particles below the solution (Vanhorn & Clark, 2010; Leventhal & Cheadle, 2012). There are few types of floatation solution were normally use for faecal floatation method in veterinary parasitological field such as the solution of Sheather's sucrose (specific gravity: 1.27), zinc sulphate (specific gravity: 1.18 - 1.20), sodium nitrate (specific gravity: 1.18 - 1.20), saturated sodium chloride (specific gravity: 1.18 - 1.20) and magnesium sulphate (specific gravity: 1.20). For routine laboratory usage, it would be best if the specific gravity of floatation solutions lies in the range between 1.22 to 1.35 (O'Grady & Slocombe, 1980). Due to the advantages in recovering different types of parasites, Sheather's sugar were selected among all the floatation solution to recover the parasites in this study as it has the highest specific gravity (1.27) among other floatation solution and a higher density than most of the parasite eggs. Generally, most of the