



**UNIVERSITI PUTRA MALAYSIA**

***MICROSCOPIC AND MOLECULAR DETECTION OF GIARDIA SPP.  
IN FECAL SAMPLES AMONG SHELTER DOG POPULATION IN  
SELANGOR***

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**FPV 2017 18**

**MICROSCOPIC AND MOLECULAR DETECTION OF *GIARDIA* SPP. IN  
FECAL SAMPLES AMONG SHELTER DOG POPULATION IN SELANGOR**

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## CERTIFICATION

It is hereby certified that we have read this project paper entitled “Microscopic and Molecular Detection of *Giardia* spp. in Fecal Sample among Shelter Dog Population in Selangor”, by Stephanie Magdalene and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 - Project

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## DEDICATIONS

This project paper is dedicated to Almighty God

To my family

Grandfather

Grandmother

Father

Mother

Brother, Sister

&

My furry family

And to all my lecturers who have committed themselves towards the noble cause of  
education.

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**LIST OF ABBREVIATIONS**

%	Percent
μL	Micro liter
nm	Nanometer
°C	Degree Celsius
<i>μm</i>	<i>Micrometer</i>
BID	Twice a day
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
gDNA	Genomic Deoxyribonucleic Acid
IFA	<i>Immunofluorescence assay</i>
ME	Microscopic examination
PCR	Polymerase chain reaction
SDS	Sodium Dodecyl Sulphate
SID	Once a day
TAE	Mixture of Tris base, acetic acid and EDTA

**ABSTRACT**

**MICROSCOPIC AND MOLECULAR DETECTION OF *GIARDIA* SPP. IN  
FECAL SAMPLE AMONG SHELTER DOGS POPULATION IN SELANGOR**

**By**

**Stephanie Magdalene**

**2017**

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**Co-Supervisor: Dr. Reuben Sunil Kumar Sharma**

*Giardia* spp. is a protozoan that can be found in feces of human and animals and is considered to have zoonotic potential. In vertebrates, including mammals, birds, reptiles and fishes, clinical signs like diarrhea, vomiting, weight loss and lethargy are frequently observed while in humans, various clinical manifestations have been reported ranging from asymptomatic to acute, intermittent or chronic non-bloody diarrhea. This study aimed to determine the occurrence of *Giardia* spp. in fecal samples of dogs from various canine shelters in Selangor by using microscopic and polymerase chain reaction (PCR) detection methods. Secondly, the occurrence of *Giardia* spp. was

investigated for its association with occurrence of diarrhea. This project was approved by the UPM animal ethics committee and consent received from the five shelters. A total of 130 dogs were randomly selected based on convenience sampling. Fecal swabs were obtained and rolled onto glass slides, air-dried and stained with both Giemsa and Ziehl-Neelson and microscopically examined. Seventy fecal samples were sufficient for nested-PCR assay where primers specific for *Giardia* were used, and subsequent gel electrophoresis to determine the specific bands corresponding to the PCR product band size. Statistical analysis was done using SPSS where  $P\text{-value} < 0.05$  was considered significant. *Giardia* spp. was detected from four dogs out of 130 dogs (3.1%) on microscopic evaluation which was confirmed on PCR detection. Other parasites detected by microscopic examination include *Cryptosporidium* and coccidia. A total of 17.1% (n=12/70) of the samples were positive for *Giardia* spp. by nested-PCR detection method. There was four times higher positive detection of *Giardia* spp. in dogs with diarrhea and it was statistically significant through Pearson's chi-squared analysis ( $P\text{-value} = 0.044$ ). In conclusion, this study reports for the first time molecular detection of *Giardia* spp. in 17.1% of shelter dogs in Malaysia using molecular detection method. It is recommended that all shelter dogs should be periodically dewormed to prevent transmission of *Giardia* spp. among the shelter animals within the same enclosures and avoid potential zoonotic transmission to care takers of the shelter. Phylogenetic characterization of *Giardia* spp. in dogs in Malaysia merit further studies.

Keywords: *Giardia* spp., Zoonotic, Dog, Microscopy, PCR

**ABSTRAK**

**PENGESANAN *GIARDIA* SPP. DARI SAMPEL TINJA DI KALANGAN  
ANJING PERLINDUNGAN DI SELANGOR DENGAN MENGGUNAKAN  
KAEDAH MIKROSKOPIK DAN MOLEKUL**

**Oleh**

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**2017**

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*Giardia* spp. adalah protozoa yang boleh didapati dalam tinja manusia dan haiwan dan dianggap mempunyai potensi zoonotik. Di kalangan vertebrata, termasuk mamalia, burung, reptilia dan ikan, tanda-tanda klinikal seperti cirit-birit, muntah, susut berat badan dan kelesuan sering dapat diperhatikan manakala pada manusia, pelbagai manifestasi klinikal dapat dilihat, dari asimptomatik kepada akut, berselangan atau kronik. Kajian ini bertujuan untuk mengesan *Giardia* spp. pada tinja anjing di pusat perlindungan haiwan di sekitar negeri Selangor dengan menggunakan kaedah mikroskopi dan polymerase chain reaction (PCR). Kedua, kaitan penemuan *Giardia*

spp. telah dikaji dengan jenis tinja dalam bentuk cirit-birit. Projek ini telah diluluskan oleh jawatankuasa UPM IACUC dengan persetujuan daripada lima pusat perlindungan haiwan tersebut. Sebanyak 130 ekor anjing telah dipilih secara rawak berdasarkan persampelan mudah. Calitan tinja diperolehi, digolek ke slaid kaca, dikeringkan dan diwarnakan menggunakan kedua-dua Giemsa dan Ziehl-Neelson dan diperiksa menggunakan kaedah mikroskopi. Tujuh puluh sampel tinja adalah memadai untuk digunakan bagi kaedah PCR dan seterusnya gel elektroforesis dilakukan untuk menentukan saiz produk PCR sepadan dengan *Giardia* spp. Analisa statistik dilakukan dengan menggunakan SPSS dengan  $P\text{-value} < 0.05$  dianggap signifikan. *Giardia* spp. dikesan daripada empat anjing (3.1%) pada penilaian mikroskopik yang disahkan dengan pengesanan PCR. Parasit lain yang dikesan melalui pemeriksaan mikroskopik termasuk *Cryptosporidium* dan coccidia. Sebanyak 17.1% ( $n=12/70$ ) sampel adalah positif untuk *Giardia* spp. melalui kaedah pengesanan PCR bersarang. Terdapat empat kali pengesanan positif yang lebih tinggi untuk *Giardia* spp. pada anjing yang cirit-birit jika dibandingkan dengan anjing yang tidak mengalami cirit-birit dan ia adalah signifikan secara statistik melalui analisis Pearson chi-squared ( $P\text{-value} = 0.044$ ). Ia adalah disyorkan bahawa semua anjing perlindungan harus diberi ubat cacing secara berkala untuk mencegah jangkitan *Giardia* spp. antara haiwan perlindungan dalam kurungan sama dan mengelakkan potensi jangkitan zoonotik kepada penjaga tempat perlindungan. Pencirian filogenetik untuk *Giardia* spp. pada anjing di Malaysia memerlukan kajian yang lebih.

## 1.0 INTRODUCTION

*Giardia* is one of the zoonotic gastrointestinal parasites known that can infect man and animals. The *Giardia* genus belongs to the type Sarcomastigophora, class Zoomastigophorea, order Diplomonadida, family Hexamitidae (Thompson, 2002). *Giardia* spp. is encountered in two forms which are trophozoite and cyst. The motile trophozoite is pear-shaped to oval in shape with bilateral symmetry with a size of 9µm by 5µm to 21µm by 15µm. The extracellular trophozoite of the parasite attaches to upper small intestine enterocytes using a highly specialized ventral adhesive disc (House *et al.*, 2011). Cysts on the other hand are oval-shaped with a thin hyaline wall with a size of 8-10µm by 7-10µm. The mature cyst has four nuclei, curved median bodies and longitudinal axonemes whereas immature cyst usually consists of only two nuclei (Ivanov, 2010).

Giardiasis is a chronic, intestinal protozoal infection seen worldwide. Infection is common in dogs, cats, ruminants and pigs. Most dogs and cats harbor subclinical infections; in some, acute watery diarrhea that contains mucus can be noticed. Besides that, steatorrhea can also be observed. Chronic or intermittent diarrhea and weight loss may be noted in animals with concurrent infections or immunocompromise (Lappin, 2014). Although the presence of *Giardia* in the gut may cause diarrhea, many hosts still remain asymptomatic in spite of shedding resistant cysts into the environment (Tysnes *et al.*, 2014).



*Giardia* spp. have been reported in 0.44%-39% of fecal samples from pet and shelter dogs and cats, with higher rates of infection in younger animals (Kahn, 2010). Animal-to-animal and animal-to-human transmission are major concerns (Olson, 2010). *Giardia* assemblages that infect dogs are assemblage C and D; whereas, cats are assemblage F. When animals share human environment like contaminated water, human assemblages A and B can also be found in feces of dogs and cats (Lappin, 2014). They are usually not associated with clinical disease in humans but they can be detected in a immunocompromised person's feces.

Risk factors for giardiasis in human are such as swimming in contaminated pools, in contact or travel to areas with low hygiene and certain sexual practices (Furness *et al.*, 2000). Risk of people being infected from pets has been conflicting reports. An increased risk of infection in relation with exposure to pigs, dogs and cats was found from a case-control study done in England (Warburton *et al.*, 1994).

Despite the large population of shelter dogs in Malaysia, not many studies have been carried out in Malaysia on *Giardia* spp. detection in dogs. The most recent study was done by Ngui *et al.*, 2014 in Malaysia on the prevalence of various gastrointestinal protozoa including *Giardia* in rural stray dog population in Selangor and Pahang. The prevalence of *Giardia* spp. in rural stray dogs was 13%. Besides that, there is also another study done by Rahman, 1990 in Malaysia on the prevalence of *Giardia* spp. among pet dogs in Penang. An amount of 21.9% of the pet dogs in Penang was detected positive for *Giardia* spp. Most of the studies have shown that there is higher prevalence

of *Giardia* spp. in younger animals compared to older animals, diarrheatic animals and animals that were kept in high stocking density (Rambozzi, 2007). The insufficiency of current data on the prevalence of *Giardia* spp. in dogs in Malaysia and the lack of prevalence of *Giardia* spp. in shelter dogs warranted this study.



The objectives of this study were to:-

1. Determine the prevalence of *Giardia* spp. among shelter dogs in Selangor using microscopy and molecular detection techniques.
2. Ascertain the occurrence of infection with *Giardia* spp. in relation to age, gender and fecal consistency among shelter dogs.

The hypothesis of this study is:-

1. There is positive detection of *Giardia* spp. in dogs from shelter population.
2. Dogs with diarrhea have higher positive detection for *Giardia* spp. compared to dogs without diarrhea.

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