



UNIVERSITI PUTRA MALAYSIA

**OCCURRENCE OF GASTROINTESTINAL PARASITES INFECTION IN
LARGE FELINES**

(Puma concolor, Panthera leo, Panthera pardus, Panthera onca)

IN ZOO NEGARA, ULU KELANG.

MARIA SYAFIQAH GHAZALI

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FPV 2018 51**



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MARIA SYAFIQAH GHAZALI

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Occurrence of gastrointestinal parasites infection in large felines (*Puma concolor*, *Panthera leo*, *Panthera pardus*, *Panthera onca*) in Zoo Negara, Ulu Kelang”, by Maria Syafiqah binti Ghazali 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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DEDICATION

I devote this dissertation to

My mother, who experienced a lot of ups and downs in her life, yet she is still the strongest person I know. During my losses, frustrations and disappointments, my mother is the one who will find a way to push through all my worries and tell me to carry on. She is the one who encourages me to be my best in chasing the dream of my life – becoming a veterinarian. She is my strength, and my everything.

My furry friend, Domod, who has been the most helpful, as a companion. A family-pet who left us in January 20th on the second week of the final year project. His existence has sparked the will in me to be my best to help those furry friends that I may encounter in the future.

My father and my brother, who taught me about life. The ones who offer me the greatest escape even for a moment, to detach myself a little while from the medical world I am dealing with.

My practical-mate; Nur Shuhada Samad and all my laboratory-mates who were there, supporting and encouraging me to finish this project excellently and telling me to not give up.

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Yours sincerely,

Maria Syafiqah Ghazali,

Final year student; Doctor of Veterinary Medicine.

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LIST OF ABBREVIATIONS

% Percentage

n Sample size

spp. Species

S. G Specific gravity



Abstrak

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan
Veterinar untuk memenuhi sebahagian daripada keperluan kursus

VPD 4999 – Projek.

KEJADIAN JANGKITAN PARASIT GASTROUSUS DALAM KALANGAN FELIN BESAR

(Puma concolor, Panthera leo, Panthera pardus, Panthera onca)

DI ZOO NEGARA, ULU KELANG.

Oleh

Maria Syafiqah Ghazali

2018

Penyelia: Dr. Nor Azlina Abdul Aziz

Penyelia Bersama: Dr. Azlan Che' Amat dan Dr. Nur Mahiza Md Isa

Kajian ini dilakukan bagi memperhatikan sejauh mana berlakunya parasit gastrousus dalam kalangan felin besar di Zoo Negara Malaysia yang terletak di Ulu Kelang, Daerah Gombak Selangor yang dijalankan selama dua minggu pada bulan Januari 2018. Sepuluh spesimen telah diambil dari puma (*Puma concolor*, n = 5) dan juga

singa Afrika (*Panthera leo*, n = 4), harimau bintang (*Panthera pardus*, n = 1), harimau kumbang (*Panthera onca*, n = 1). Kesemua spesimen najis ini diambil secara individu pada waktu pagi kemudian dimasukkan ke dalam polibag dan diletakkan ke dalam kotak yang berisi ketulan ais untuk dibawa ke makmal parasitologi di Fakulti Perubatan Veterinar UPM Serdang. Seluruh spesimen diteliti samada terdapat telur parasit, larva dan oosis dengan teknik apungan larutan garam (sodium nitrate, SG = 1.3) dan formalin – ether teknik mendapan. Kesemua felin besar di Zoo Negara telah dijangkiti parasit gastrousus. Sejumlah enam jenis parasit gastrointestinal ditemui termasuklah nematod (*Toxocara cati*, *Ancylostoma* spp, *Toxascaris leonina*, dan *Oxyuris* spp), satu cestod (*Spirometra* spp.) dan satu protozoa (spesis yang tidak dikenali). Kajian ini menunjukkan diantara jangkitan helmin, kejadian jangkitan nematod adalah lebih tinggi (100 %; n = 10/10) berbanding jangkitan cestod (10 %; n = 1/10) dan protozoa (10 %; n = 1/10). Majoriti (n = 5/10) felin besar mengalami jangkitan bercampur iaitu parasit *Toxocara cati* dan *Ancylostoma* spp.

Keywords: felin besar, Zoo Negara, parasit gastrousus, formalin – ether teknik mendapan, teknik apungan larutan garam

Abstract

Abstract of the project paper presented to the Faculty of Veterinary Medicine in
partial requirement for the course

VPD 4999 – Project.

OCCURRENCE OF GASTROINTESTINAL PARASITES INFECTION IN LARGE FELINES

(Puma concolor, Panthera leo, Panthera pardus, Panthera onca)

IN ZOO NEGARA, ULU KELANG.

By

Maria Syafiqah Ghazali

2018

Supervisor: Dr. Nor Azlina Abdul Aziz

Co-Supervisors: Dr. Azlan Che' Amat and Dr. Nur Mahiza Md Isa

The present study was conducted to observe the occurrence of gastrointestinal parasites in large felines in the National Zoo of Malaysia located in Ulu Kelang, Gombak District, Selangor, which was undertaken for two weeks in January 2018. Ten fecal samples were collected from pumas (*Puma concolor*, n = 5), African lions

(*Panthera leo*, n = 4), spotted leopard (*Panthera pardus*, n = 1) and black panther (*Panthera onca*, n = 1). All fecal samples were collected in the morning according to individual animals and kept in an ice box for transportation to Parasitology Laboratory in Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang. All samples were examined for parasite eggs, larvae and oocysts by simple floatation technique by using salt solution and formalin – ether sedimentation technique. All large feline members in Zoo Negara were infected with gastrointestinal parasite. A total of six species of gastrointestinal parasites recovered including four nematodes (*Toxocara cati*, *Ancylostoma* spp., *Toxascaris leonina*, and *Oxyuris* spp.), a cestode (*Spirometra* spp.) and a protozoa (unknown). This study suggests that among different helminth infections, the occurrence of nematode infections was higher (100 %; n=10/10) than cestode infection (10 %; n=1/10) and protozoa infection (10 %; n=1/10). Majority (n=5/10) of the large felines had mixed infections of *Toxocara* spp. and *Ancylostoma* spp.

Keywords: large felines, Zoo Negara, gastrointestinal parasites, formalin – ether sedimentation, simple floatation.

1.0 INTRODUCTION

“Zoo” is referring to any area where the wildlife animals are kept with the purposes of conservation, education, research or recreation, and open to the public as stated in The Wildlife Conservation (Operation of Zoo) Regulations 2012 under the Wildlife Conservation Act 2010. In the nature, wild animals live in a big area and usually have consequently very low genetic resistance against parasitic infection mainly due to the low exposure towards the parasites itself. However, when herds of these wild animals are kept in captivity, or in zoological gardens, parasitic infections might become worse and able to pose a serious threat to the endangered species by causing mortality as mentioned by Muoria et al. (2005). Infectivity by the gastrointestinal parasites may affect the host survivability both directly and indirectly by reducing the hosts’ immunity and affecting the physical condition with pathological effects such as blood loss, tissue damage and spontaneous abortion (Thawait, 2014).

This present study is conducted because there have been few detailed and comprehensive research available concerning the gastrointestinal parasites in wild animals maintained in zoological garden. Therefore, this project would be an attempt to study the actual status of gastrointestinal parasites infecting the large felines maintained under captivity in Zoo Negara. Other than that, the results obtained would be essential to expand the understanding on parasitic occurrence in these large felines.

The objective of this study is to investigate the occurrence of gastrointestinal parasites in large felines in Zoo Negara. The null hypothesis is, there is no occurrence of gastrointestinal parasites in large felines in Zoo Negara and the alternate hypothesis is there is occurrence of gastrointestinal parasites in large felines in Zoo Negara.

2.0 LITERATURE REVIEW

2.1 Captive animals are usually prone to gastrointestinal parasitic infections.

Parsani et al. (2001) stated that zoos have been protecting the endangered species by playing a major role in promoting the animals' biodiversity in the enclosure in the past few decades. Unfortunately, despite proper care and routine management offered, the zoo animals are under constant stress mainly due to captivity. Stress can be one of the predisposing factors for these captive animals to be infected with gastrointestinal parasites (Duszynski and Upton, 2008). Duszynski and Upton (2008) also mentioned that it is nearly impossible for the zoo management to recreate the abiotic environmental condition that mimics their native habitat such as temperature and humidity extremes, photoperiod and space requirements. Other than that, the biotic conditions such as co-evolved vertebrates and invertebrates, and seasonal dietary needs are nearly impossible to be duplicated in the captive area. Zoological garden is where the animals are frequently exposed to humans in distant and therefore proximity of massive numbers of people are unnatural and can cause various levels of stress (Duszynski and Upton, 2008).

2.2 Zoonotic gastrointestinal parasites in the zoo animals

Captive animals have the potential to be vectors of zoonoses (Duszynski and Upton, 2008). In 2008, a survey was undertaken in Zoo Negara, Ulu Kelang by a group of parasitologists from Universiti Malaya, investigated the prevalence of intestinal parasites from different groups of mammals. A total of 28 felines members, 4 of them – Jungle cat (*Felis chaus*), Sumatran tiger (*Panthera tigris sumatrae*), Indochinese

tiger (*P.t. corbetti*) and Malayan tiger (*P.t. jacksonii*) which accounted a percentage of 14.3% infectivity towards *Cryptosporidium* spp. protozoa that contribute to gastrointestinal disease in the humans (Lim et al., 2008). *Toxocara* spp and *Ancylostoma* spp. are underestimated zoonotic potential which will be further discussed in discussion section.

2.3 Findings of gastrointestinal parasites in the large feline members in Malaysia

According to Lim et al. (2008) in Zoo Negara there were mainly three species of gastrointestinal parasites infecting the large feline members (Sumatran tiger, Indochinese tigers, Malayan tigers, Gir lion, African lions, pumas and black panthers) which were the *Toxocara cati*, *Cryptosporidium* spp., and hookworm. A total of 62% of all large felines except for the black panthers were positive with *Toxocara cati*. *Cryptosporidium* spp. was 14.2 % out of all samples, detected in Sumatran tigers, Indochinese tigers and Malayan tigers. Hookworm, however only found in Gir lion which accounted 4.8 % occurrence.

3.0 Materials and Methods

3.1 Study site

The study was conducted in Zoo Negara, Ulu Kelang which situated in north – east of Kuala Lumpur. This zoological garden covered on 110 acres of land, occupied with a total of more than 5,317 specimens from 476 species of mammals, amphibians, fishes, birds and reptiles.

3.2 Animals

This study involved 10 large felines species which consist of five pumas (*Puma concolor*), three African lions (*Panthera leo*), a spotted leopard (*Panthera pardus*) and a black panther (*Panthera onca*). All animals are adults, aged more than one year and above, and individual data is recorded in **Table 1**. All animals do not show any clinical sign associated with parasitic infections such as diarrhea and having good body condition scores. In Zoo Negara, there are nine Malayan tigers however they are not taken as subject due to these species are considered as totally protected wildlife species according to Wildlife Conservation Act 716. Any studies to be conducted that involved obtaining biological samples need a special permit which only be approved by Minister of Natural Resources and Environment. For this study, the permit is not yet approved by the Minister at the time of the study.

Table 1: Individual records of the large felines in Zoo Negara involved in the study

Species	Name	Sex	Age	Origin	Last deworming date
<i>Puma concolor</i>	Anuia	Female	16 years	From Singapore zoo in 2014	19 th April 2013
<i>Puma concolor</i>	Atilia	Female	5 years	Parent raised in Zoo Negara	-
<i>Puma concolor</i>	Amelia	Female	5 years	Parent raised in Zoo Negara	-
<i>Puma concolor</i>	Agong	Male	11 years	Parent raised in Zoo Negara	19 th April 2013
<i>Puma concolor</i>	Akila	Female	11 years	Parent raised	-
<i>Panthera leo</i>	Manjaka ni	Male	13 years	Transferred from Zoo Johor in 2007	12 th July 2012 19 th April 2013 21 st June 2015
<i>Panthera leo</i>	Samba	Male	4 years	Parent raised in Zoo Negara	22 nd June 2015
<i>Panthera leo</i>	Simba	Male	4 years	Parent raised in Zoo Negara	22 nd June 2015
<i>Panthera pardus</i>	Panjang	Male	Unknown	Transferred from Zoo Melaka in 2012	-
<i>Panthera onca</i>	Apow	Male	Unknown	Acquired from Zoo Johor captured in the wild in 2010	-

- = No deworming regime stated in the health record.

3.3 Housing and husbandry

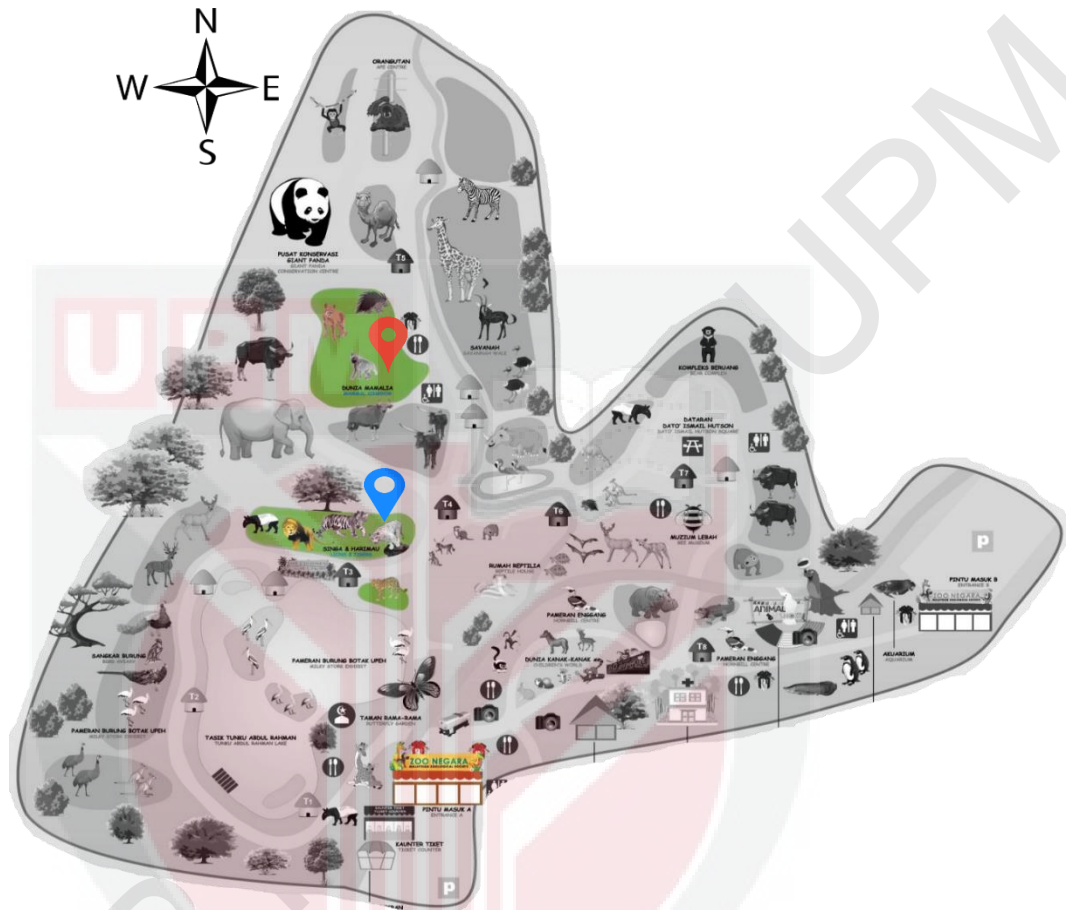


Figure 1: Map of Zoo Negara. Highlighted in greens are area of interest in this study. Pumas in Mammalian Kingdom 1 section (red sign) while lions, spotted leopard and black panther in Carnivore Section (blue sign).

The animals were mainly housed in two different location inside Zoo Negara. The pumas were housed in Mammalian Kingdom section which located further northern compared to the other species in this study which were housed in Carnivores section and located more in the center on the map (**Figure 1**).

On daily basis, after the animals were being released to the exhibition area, the night holding facilities were usually cleaned with pressure pipe water. However, the

exhibition area which covered by soils and sands were not cleaned with water, but the feces will be removed once every two days.

3.4 Diets

The large felines in Zoo Negara are fed once daily in the evening when the animals need to be kept inside in the holding facilities after the visiting hour is over at 1630 hours. The diet consists of mainly poultry meat and beef. The zoo does have own livestock reared for feeding purpose to the carnivorous animals in the zoo. Other than that, the pumas are sometimes fed with white rats once every week, that purchased in the normal animal pet store.

3.5 Fecal samples collection, sample processing and examination

A total of 10 fecal samples (one feces per animal) were collected according to individual animals listed in **Table 1**. Some of the animals are kept together in one cage, therefore before sample collections, with the help from zoo management team, the animals – pumas and African lions were kept separately for this purpose. Sample collections were carried out in the morning at 0830 hours, after they were released to the exhibition area and before the cages were cleaned. All samples were kept in zip-locked polythene bag and stored momentarily in an ice box filled with ice packs momentarily until brought to parasitology laboratory in Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang which took approximately one-hour journey. All samples were weighed until 1.00 g and proceeded with simple floatation technique by using salt solution (Sodium Nitrate, specific gravity = 1.30) and formalin – ether sedimentation technique.

Briefly, for simple floatation technique, 1.00 g fecal material was mixed together with 40 ml NaNO_3 thoroughly, filtered and transferred to 40 ml graduated cylinder. Cover slip was put on top and left for 30 minutes before it was examined under the microscope.

As for formalin – ether sedimentation, 1.00 g fecal material was put into 20 ml centrifuge tube and added with 10 ml of 5 % acid – alcohol and 2 ml diethyl ether, the centrifuge tube then placed into the centrifuge machine and spun for 5 minutes with 2000 rpm. Formalin can preserve the feces, halts and slows the developments of most parasites beside reducing the odor of samples. While, diethyl – ether can remove fats, pigments, and other substances that can obscure the view under the microscope (Bowman, 1999).

3.6 Data analysis

Data from the occurrence of gastrointestinal parasite infections in large felines in Zoo Negara were represented by means of proportion of large felines infected with gastrointestinal parasites, a descriptive statistic as shown in **Table 2**.

4.0 Result

Table 2: Proportion of large felines infected with gastrointestinal parasites.

Species	n	<i>Toxocara cati</i> n (%)	<i>Toxascaris leonina</i> n (%)	<i>Ancylostoma</i> spp. n (%)	<i>Oxyuris</i> spp. n (%)	Cestode egg n (%)	Protozoan oocyst n (%)
Puma	5	5 (100 %)	-	1 (20 %)	1 (20 %)	-	1 (20 %)
African lion	3	-	3 (100 %)	3 (100 %)	-	-	-
Spotted leopard	1	1 (100 %)	-	-	-	-	-
Black panther	1	1 (100 %)	-	-	-	1 (100 %)	-
Total	10	7 (70 %)	3 (30 %)	4 (40%)	1 (10 %)	1 (10 %)	1 (10 %)

All five pumas are infected with *Toxocara cati* (n = 5/5, 100%) and one of the pumas is having mixed infection with *Ancylostoma* spp. (n = 1/4, 20%), *Oxyuris* spp. (n = 1/4, 20%) and protozoan oocyst (n = 1/4, 20%).

The African lions, all three of them are having mixed infection with *Toxascaris leonina* (n = 3/3, 100%) and *Ancylostoma* spp. (n = 3/3, 100%).

Spotted leopard is having single infection with *Toxocara cati* (n = 1/1, 100%) while the black panther is having mixed infection with *Toxocara cati* (n = 1/1, 100%) and a cestode egg (n = 1/1, 100%) that most likely to be a *Spirometra* spp.

5.0 DISCUSSION

This is the second known study to be conducted concerning the occurrence of gastrointestinal parasite infection in large felines maintained in captivity, in Zoo Negara, Ulu Kelang.

The most common reason for these large felines to harbor gastrointestinal parasite infection may be due to high environmental contamination as they are kept intensively which make possible for the parasitic infection to escalate in such confined area. Despite proper attention to feeding and maintenance of hygiene, animals that kept in captivity or in confined areas are prone to different parasitic infections (Lim et al., 2008). In Zoo Negara, as mentioned earlier in materials and methodology section (**Refer part 3.4**), the holding cages are cleaned daily while feces in the exhibition area will be removed once every two days. However, during site visit in mammalian kingdom I section where the pumas are allocated, feces identified in the exhibition area appeared to be longer than two days which already decomposed. This situation may contribute to the occurrence of gastrointestinal parasite infection that may persist in the environment and constantly infecting those animals. In view of this, parasitic disease often represents a major concern in zoo animals for the high environmental contamination and constitutes one of major problems causing even mortality in these animals (Fagiolini et al., 2010). Mortality in these animals might be from changes in integrity of the host enterocytes (Sheppard, 1974) by the gastrointestinal parasites, causing interference with both intestinal digestion and absorption (Stein et al., 1973) which leads to changing in the architecture of the intestinal villi (Fernando et al., 1973) which allows increased flow of tissue fluid and blood into intestinal lumen (Bailey,

1994) thus the clinical sign of diarrhea, and making the host become susceptible to secondary bacterial invasion (Li et al., 1996).

Other factors besides environmental contamination, diets given to these animals might contribute to the occurrence of gastrointestinal parasite. In this case, pumas were fed with rodent which are known to be paratenic host for *Toxocara* spp. However, in this study these animals were specifically fed with white rats which were purchased from animal shop. It is possible for the white rats purchased from normal pet shop to be infected with gastrointestinal parasite infection since there are no established biosecurity measurements as practiced in laboratory animals, however, this statement is warranted for further investigation. These animals were infected once they consumed the rodents' tissues with migrated larvae of ascarids especially *Toxocara* spp. (Okulewics et al. 2012).

From the findings obtained in this study, they are mainly two gastrointestinal parasites species identified as zoonotic potential and can raise public health awareness to the zookeepers especially. The gastrointestinal parasites with zoonotic potential are *Toxocara* spp. and *Ancylostoma* spp. Generally, *Toxocara* spp. known to cause visceral larval migrans and ocular larval migrans (Magnaval, 2001) while *Ancylostoma* spp. causes cutaneous larval migrans in humans (Bowman, 2010).

In contrast with the present study, the pumas' status differs from single infection in 2008 to mixed infection ($n = 1/5$; 20 %) with *Ancylostoma* spp., *Oxyuris* spp., and a protozoan egg. While as for African lion, in contrast with Lim et al. (2008) as shown in **Table 3**, the gastrointestinal parasite infection differs, from single infection with *Toxocara cati* ($n = 2/3$, 66.7 %) to having mixed infection ($n = 3/3$; 100%) with new

occurrence of *Toxascaris leonina* and *Ancylostoma* spp. Other than that, the black panther had no gastrointestinal parasite detection on 10 years earlier, but at present the black panther is having mixed infection (n = 1/1; 100%) with *Toxocara cati* and *Spirometra* spp. In 2008 there was no spotted leopard in the Zoo Negara because it was only then acquired from Melaka in 2012, and the actual status for the presence of gastrointestinal parasite would be only *Toxocara cati* (n = 1/1; 100%).

Table 3: Result from former study conducted in 2008 (Lim et al. 2008).

Occurrence (%) of intestinal parasites in felines, primates and hoofed mammals at Zoo Negara

Species	N	<i>Toxocara cati</i> , n (%)	<i>Cryptosporidium</i> spp., n (%)	<i>Spirometra</i> spp., n (%)	Hookworm, n (%)
Feline					
Jungle cat ² (<i>Felis chaus</i>)	3	3 (100)	1 (33.3)	–	–
Sumatran tiger ^{1/2} (<i>P.t. sumatrae</i>)	3	3 (100)	1 (33.3)	–	–
Indochinese tiger ^{1/2} <i>Panthera tigris corbetti</i>	3	2 (66.7)	1 (33.3)	–	–
Malayan tiger ^{1/2} (<i>P.t. jacksoni</i>)	3	2 (66.7)	1 (33.3)	–	–
Gir lion ^{1/2} (<i>P. leo persica</i>)	3	2 (66.7)	–	–	1 (33.3)
Africa lion ^{1/2} (<i>P. leo leo</i>)	3	2 (66.7)	–	–	–
Leopard cat ² (<i>Felis bengalensis</i>)	4	2 (50.0)	–	2 (50.0)	–
Puma ^{1/2} (<i>Panthera concolor</i>)	4	2 (50.0)	–	–	–
Black panther ² (<i>Panthera onca</i>)	2	–	–	–	–
Total for feline	28	18 (64.3)	4 (14.3)	2 (7.1)	1 (3.6)

Such difference in findings might be due to time factor, different animals (animals then and now are from different batches), and difference in diagnostic fecal examination, in study done in 2008, they did fecal smear and formalin – ether sedimentation. Direct smear is known to be less efficient because of the technique of using normal saline rather than using salt solution that plays with gravitational forces hence offers much more accurate findings. That is why negative findings in study done in 2008 might be inconclusive or false negative. However, positive results obtained are just as valid as fecal floatation technique.

6.0 CONCLUSION

In conclusion, results in this study showed that even with regular fecal examination done in Zoo Negara, there remain detectable level of parasitic infection in these large felines and it is felt that if the standards were lowered or the husbandry or examination levels were relaxed, the evidence of gastrointestinal parasite may spike.

Other than that, most intestinal parasites recorded in this study are known to be human pathogenic source of zoonotic transmission which extra prudence need to be applied in Zoo Negara to prevent transmission to the humans especially, the zookeepers.

7.0 RECOMMENDATIONS

It is highly recommended for future researcher to increase the sample size to have statistically significant result. Other than that, the study should be expanded to other zoos exist in Malaysia to see the correlation between different management with the occurrence of gastrointestinal parasites in large felines kept in captivity. Moreover, it would be interesting to screen the meats given to these large felines. In this study the oocysts were not sporulated and therefore it is highly proposed to sporulate any oocyst found to get specific species identification.

Finally, polymerase chain reaction (PCR) is highly urged in order to confirm the parasite species rather than depending only based on morphological findings under the microscope.

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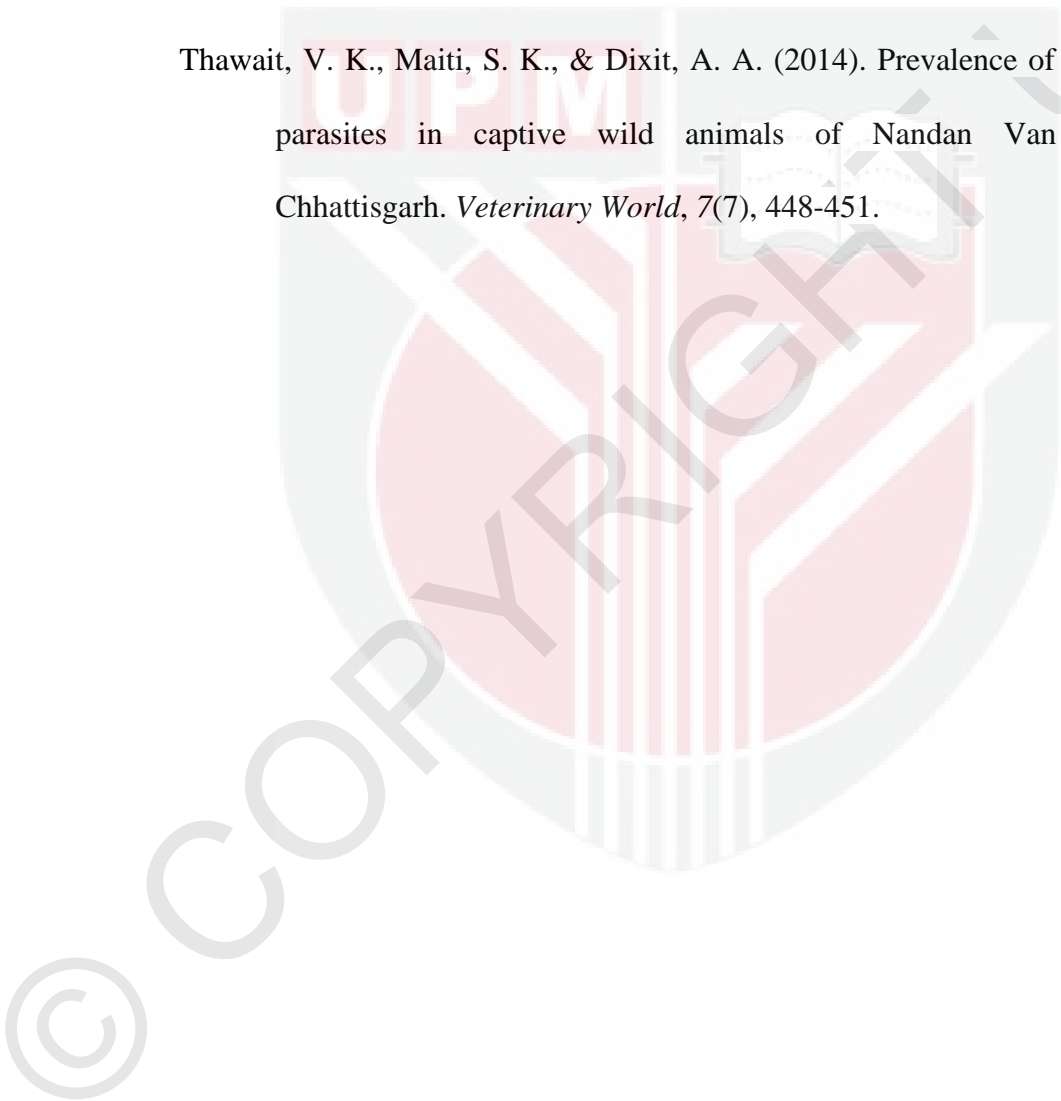
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9.0 APPENDICES

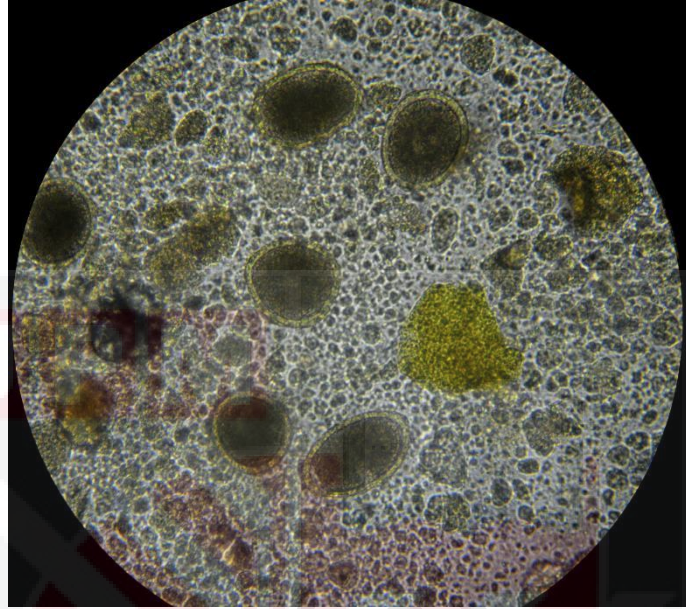


Figure 2: *Toxocara cati*

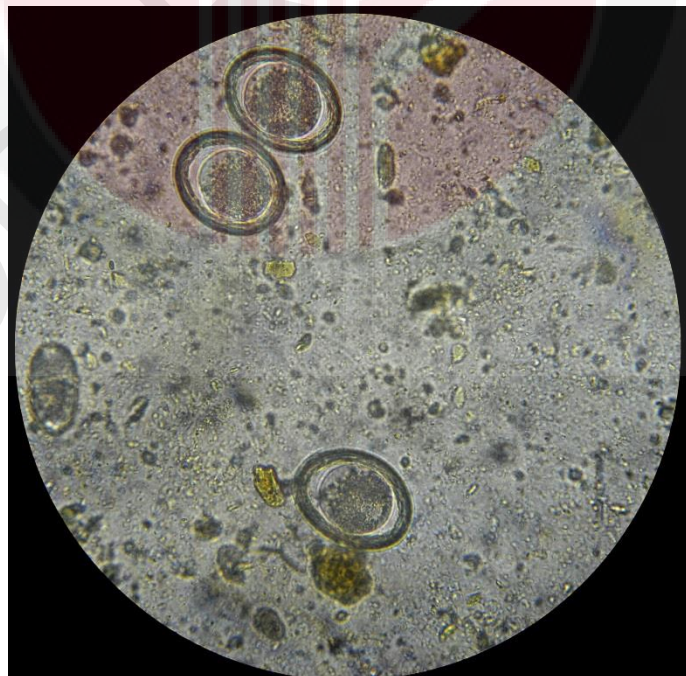


Figure 3: *Toxascaris leonina*

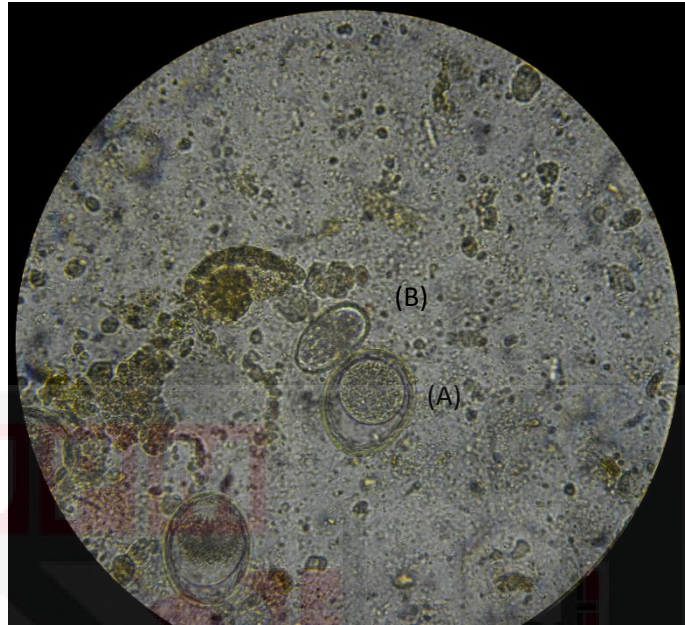


Figure 4: *Toxascaris leonina* (A) and *Ancylostoma* spp. (B)

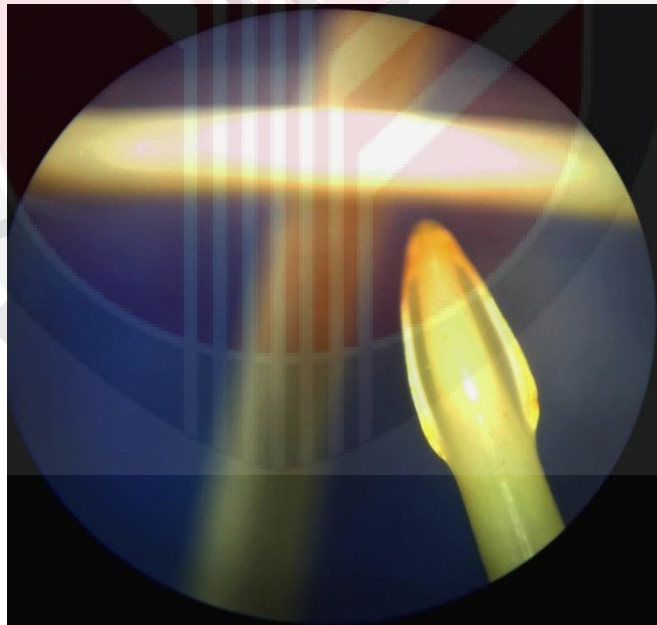


Figure 5: Head of *Toxocara cati* found in one of the Puma (Agong)

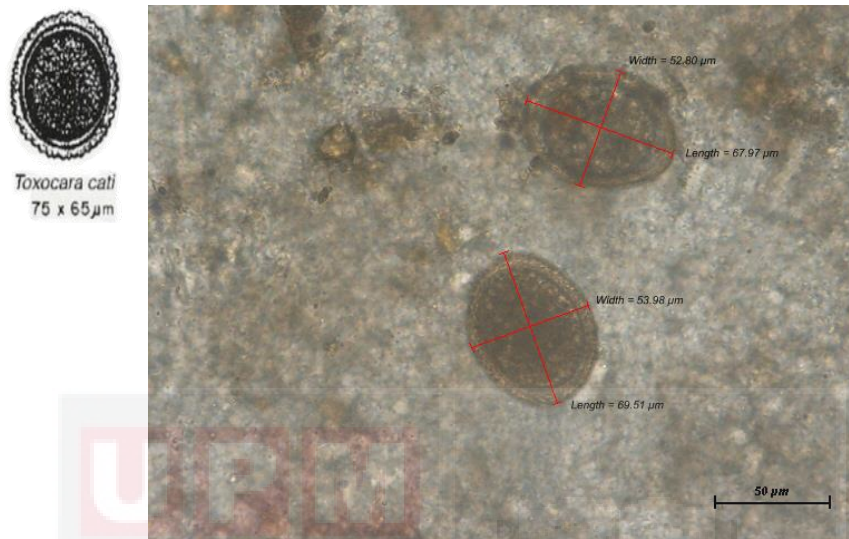


Figure 6: Measurement of *Toxocara cati*, 70 x 54 μm under 40X

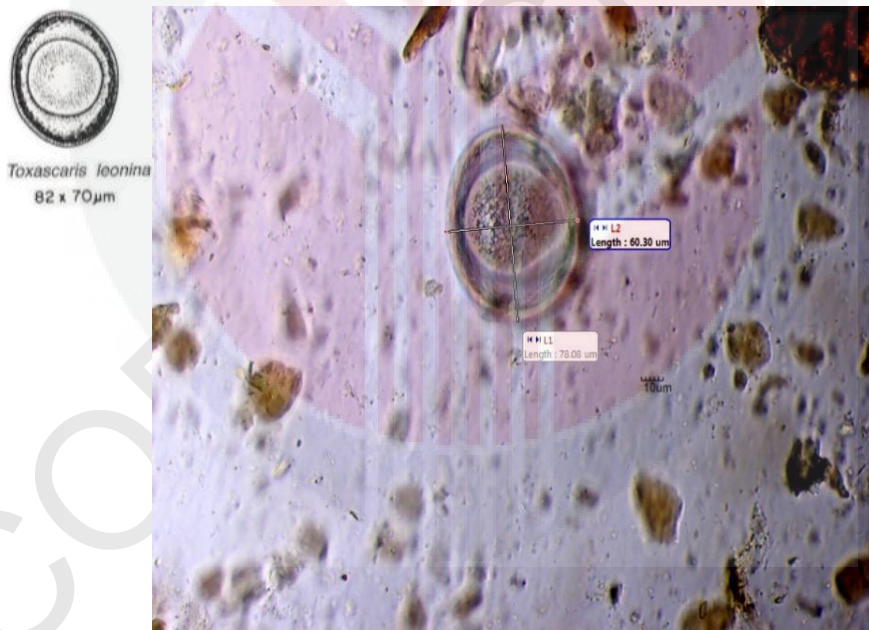


Figure 7: Measurement of *Toxascaris leonina*, 80 x 60 μm under 40X

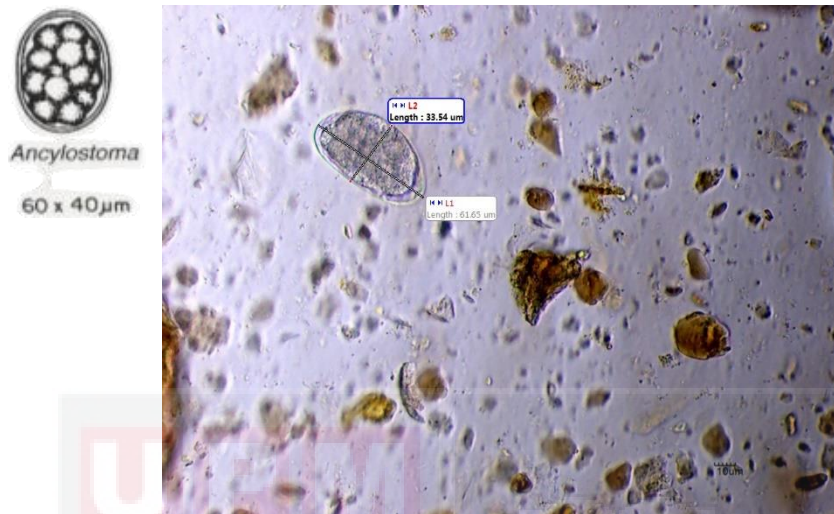


Figure 8: Measurement of *Ancylostoma* spp., 60 x 33 μm under 40X

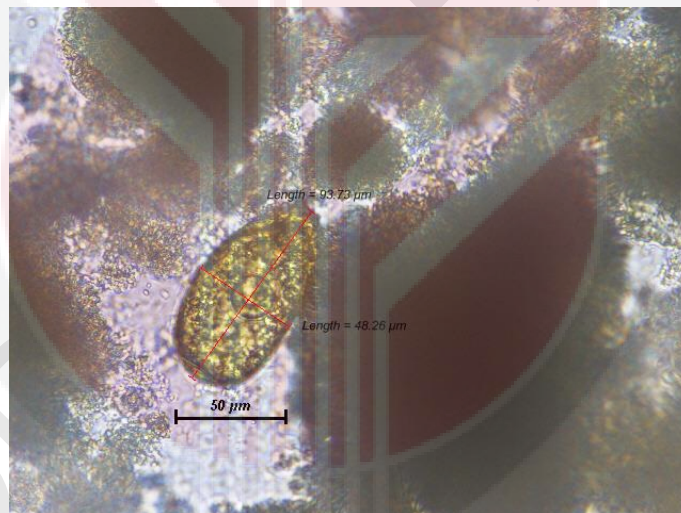


Figure 9: Measurement for Cestode egg (likely to be *Spirometra* spp.), 93 x 48 μm under 40X

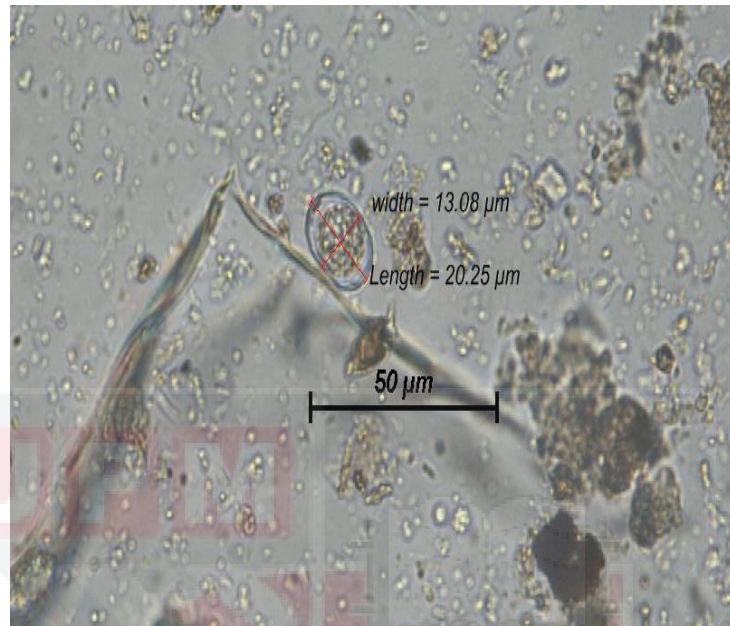


Figure 10: Measurement for protozoan oocyst, 20 x 13 μm under 40X

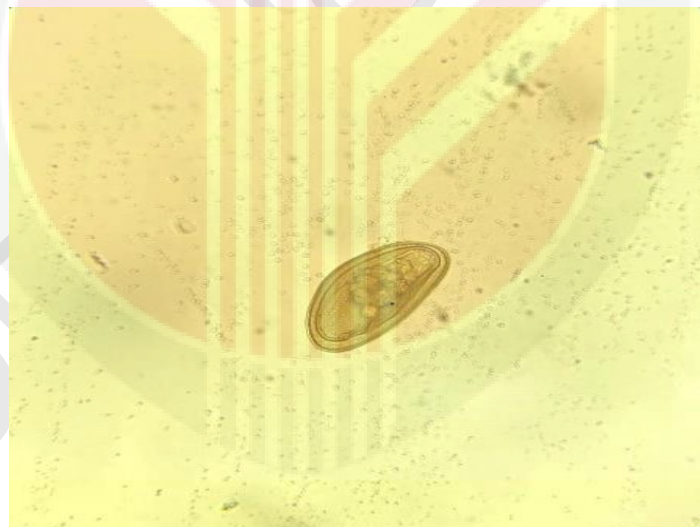


Figure 11: *Spirometra* spp.