INTESTINAL PROTOZOAN PARASITES IN SHELTER AND STRAY CAT (*Felis catus*) POPULATION IN PENANG ISLAND, MALAYSIA

JALILATUL NIKDAR M.[1], SOPHIE JADE D.[2] AND FARAH HAZIQAH M. T.[1]*

[1] School of Biological Sciences, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia
[2] School of Life Sciences, Keele University, Newcastle, Keele, Staffordshire, ST5 5BG, United Kingdom
* *Corresponding author*: farahhaziqah@usm.my

ABSTRACT. A study was conducted to determine the occurrence and species diversity of protozoan parasites that was shed by domestic cats into the environment in the northern region of peninsular Malaysia. A total of 91 fresh cat faecal samples were collected randomly from 32 stray cats and 59 shelter-owned cats around Penang island, Malaysia. The collected faecal samples were then examined for the presence of intestinal protozoan parasites by microscopic examination prior to faecal floatation method and *in vitro* cultivation technique. The prevalence of cats shedding intestinal protozoan parasites was generally low with 14.3% (13/91) namely, *Cystoisospora* spp. (syn. *Isospora* spp.). Two species of *Cystoisospora* spp. were identified as *C. felis* (6.6%) and *C. rivolta* (7.7%). However, none of the faecal samples were found to be positive for zoonotic protozoan parasite, *Blastocystis* sp., prior to the cultivation method. Determining the status of intestinal parasitic diseases in the cat population is crucial not only to formulate appropriate control strategies but also to envisage the risk to considered groups.

Keywords: Blastocystis sp., cat, *Cystoisopsora* sp., intestinal protozoan parasites.

INTRODUCTION

The relationship between human and domestic cats (*Felis catus*) started as early as 4,000 years ago when ancient Egyptians domesticated cats to help in killing rodents and venomous snakes. Cats are popular as pets worldwide because they are easy to care for and provide companionship that enriches the lives of human beings by relieving stress and loneliness (McMillan, 2019). However, keeping pets in the house in close contact with humans not only brings benefits but also some alarming concerns such as the risks cats pose as hosts for zoonotic diseases (McMillan, 2019). According to Moskvina *et al.* (2018), the prevalence rates of parasites were significantly higher in stray cats than shelter cats. Thus, cats play an important role as the host for zoonotic diseases as they live in close contact with humans either as pets or stray cats.

Protozoans are unicellular organisms in which the body consists of the cytoplasm with at least one nucleus. There are only four phyla that are implicated as pathogens of the cat from around 35 phyla and myriad species of organisms in the protozoal kingdom (Bowman *et al.,* 2008). Most of the protozoans are free living organisms and only some of them are parasitic. *Toxoplasma gondii*, *Giardia duodenalis* (syn. *G. lamblia, G. intestinalis*), *Cryptosporidium* and *Cystoisospora* spp. (syn. *Isospora* spp.) are the most common gastrointestinal protozoan parasites in cats. Besides, several studies have also suggested the role of *T. gondii* in the causation of abortions (Vogel *et al.*, 1996; Saki *et al.*, 2015).

In Malaysia, studies on parasites of domestic cats were previously conducted by Mohd Zain *et al.* (2013) and Ngui *et al.* (2014). Mohd Zain *et al.* (2013) focused on the macroparasites communities in stray cat populations from four urban cities in peninsular Malaysia whereas Ngui *et al.* (2014) focused on the gastrointestinal parasites in rural dogs and cats in Selangor and Pahang. Studies on the prevalence of intestinal protozoan infection in cats in Malaysia is still lacking and needs to be elucidated, especially in the northern region of peninsular Malaysia. Therefore, this study was conducted to determine the intestinal protozoan parasites that are frequently being shed by cats to the environment in view of creating a clearer picture of feline intestinal protozoan parasitism in Penang island, Malaysia.

MATERIALS AND METHOD

Location of Study and Faecal Sampling

A study using shelter cats and stray cats was conducted from September 2018 and March 2019 in Penang island, Malaysia. Faecal samples of shelter cats were obtained from four locations: Jabatan Perkhidmatan Veterinar Daerah Timur Laut, Society for the Prevention Cruelty to Animals (SPCA) Penang, Cat Beach Sanctuary, and Unique Cat House whereas stray cats were caught from Sungai Dua, Gelugor, Teluk Bahang and Georgetown area. The stray cats were individually caged until defeacation and were then released or returned to their original surroundings. Meanwhile, the shelter cats were caged individually by the owner until defeacation. A total of 91 faecal samples which consists of 32 faecal samples of stray cats and 59 faecal samples of shelter-owned cats were examined in this study.

Laboratory Diagnosis

The faecal floatation method was conducted according to the Manual of Tropical Veterinary Parasitology (Technical Centre for Agricultural and Rural Cooperation, 1989). Approximately 3 g of faeces were added into a beaker that contained 50 ml saturated sodium chloride solution. The faecal suspension was stirred thoroughly and was poured through a tea strainer into another beaker. The residual faecal debris was discarded whereas the strained faecal suspension was transferred into a test tube which was placed in a vertical position. A cover slip was placed on top of the test tube for at least 20 minutes. Next, the cover slip was examined using a compound microscope for the presence of protozoan oocysts. Identification was conducted based on the oocysts morphological characters according to Soulsby (1986).

In vitro cultivation method was also conducted in which a pea size amount of each faecal sample was inoculated into a sterile plastic culture tube with snap caps containing 3 ml of Jones medium supplemented with 10% heat-activated horse serum (Suresh and Smith, 2004). Each sample was incubated vertically at 37 °C for 48 hours to 72 hours before examination. A drop of the sediment was examined at 400× magnification for the detection of protozoan. The parasites were maintained subsequently after isolation by sub-culturing

once every 3 to 4 days. When no growth was detected, the sediment would be further re-suspended in a fresh culture medium and maintained for another additional 48 hours at 37 °C incubation (Chandrasekaran *et al.,* 2014). Positive results would be confirmed by observation of the vacuolar form present in the medium (Bergoma do Bomfilm and Machado do Couto, 2013).

Statistical analysis

The data obtained from the laboratory examination of the samples were collected and the prevalence rate was determined using IBM SPSS Statistics for Windows, Version 24.0.

RESULTS AND DISCUSSION

Few studies have been conducted on intestinal parasites in cats from Malaysia. However, this study focused on only the occurrence of protozoan parasites in the cat population in Penang primarily on *in vitro* cultivation and faecal examinations. The results of this study indicate that cats shedding intestinal protozoan parasites was generally low with 14.3% (13/91) namely, *Cystoisospora* spp. (syn. *Isospora* spp.) in which two species of *Cystoisospora* spp. were identified as *C. felis* (6.6%) and *C. rivolta* (7.7%) (Table 1). This study provides the first data on protozoan parasites in the cat population from the northern region of peninsular Malaysia. Most of the previous studies such as Mohd Zain *et al.* (2013) focused only on helminths as well as ectoparasites whereas Nazeh *et al*. (2015) studied the blood filarial parasites in domestic and stray cat populations.

Only two strays (6.3%) were positive for *C. felis* whereas four (12.5%) stray cats were positive with *C. rivolta*. However, in the shelter cat population, five (8.5%) were positive for *C. felis* and only two (3.4%) of shelter cats were positive with *C. rivolta*. There was no significant difference between the prevalence of *Cystoisospora* spp. and cat populations in Penang island. (Table 1.)

Coccidiosis caused by *Cystoisospora* spp. is not thought to be a common problem and it appears to be highly host-specific in cats (Dubey *et al*., 2009). It can be found in the small intestine (asexual and sexual stages) and extra-intestinal tissues (asexual stages). The infection may occur by eating a cyst, a thick-walled, egg-like stage that is passed in the faeces and matures in the soil or by ingesting monozoic cysts that are present in the musculature of their preys (Pereira *et al*., 2017). They were identified to

Table 1: Prevalence of *Cystoisospora* spp. in cat population in stray and shelter cat population from Penang Island.

Cat population	*C. felis (%)*	*C. rivolta (%)*	Total tested (n)
Stray	2 (6.3)	4 (12.5)	32
Shelter/owned	5 (8.5)	2 (3.4)	59
Total	7 (7.7)	6 (6.6)	91

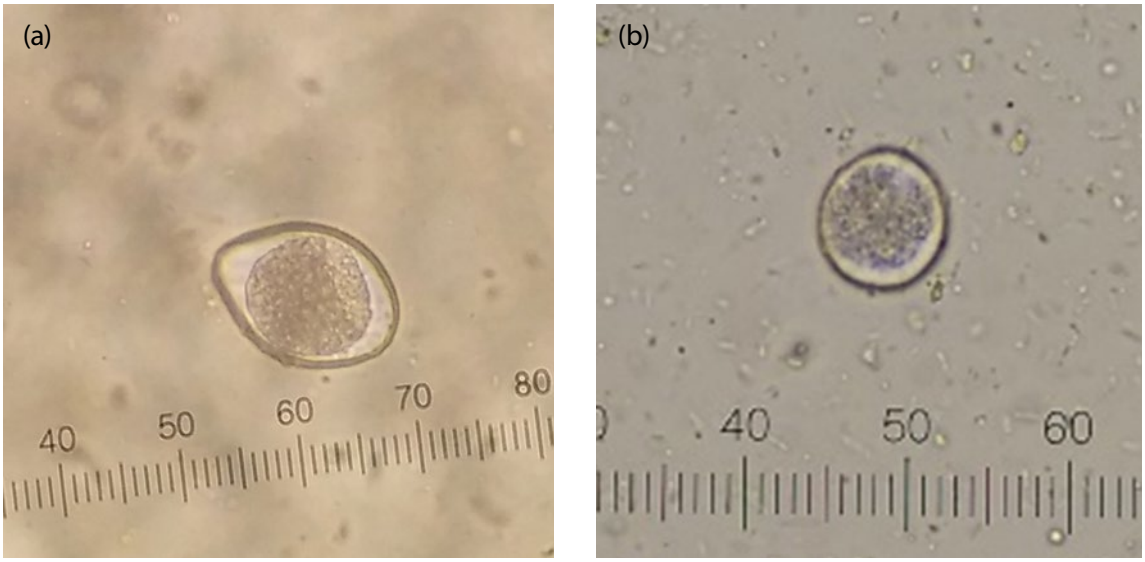

Figure 1. The oocysts of (a) *C. felis* and (b) *C. rivolta*.

the species level based on the structure of their sporulated oocyst stage. *C. felis* is the parasite with large oocysts with the size approximately between 40 µm followed by *C. rivolta*, the medium sized oocysts with the measurement between 25 µm long, and *C. bigemina*, the small sized oocysts with the measurement between 14 µm or less. The pathogenesis of *C. felis* and *C. rivolta* are mild and the primary goal of treatment of this protozoan infection is to resolve diarrhoea in which severe infection can lead to dehydration and death in younger animals (Dubey *et al.*, 2009).

Blastocystis sp. is a common protozoan parasite frequently found in the gastrointestinal tract of humans and a large variety of animal hosts worldwide, including cats. The first on the occurrence of *Blastocystis* sp. from domestic cats was reported on by Knowles and Das Gupta (1924). After many years, Duda *et al.* (1998) reported high infections in domestic cats from faecal smears under light microscopy in Brisbane, Australia with the prevalence of 67.3%. Unfortunately, Duda *et al.* (1998) failed to cultivate this parasite in the inspissated egg slant culture medium.

None of the faecal samples were found to be positive for *Blastocystis* sp. prior to the cultivation method. The results are consistent with findings from a handful of previous studies on *Blastocystis* sp. infection among cats reported in Germany (König and Müller, 1997), Malaysia (Chuong *et al.*, 1996; Farah Haziqah *et al.*, 2018) and Japan (Abe *et al.*, 2002). Most of these studies used the direct and/or cultivation method for the detection of the parasite. Interestingly, PCR assays such as DNA barcoding had successfully detected the presence of this protozoan in cat faecal samples with quite low prevalence rate (Wang *et al.*, 2013; Ruaux and Stang, 2014; Farah Haziqah *et al.*, 2018).

The previous studies posit that feline do not represent a significant reservoir and this animal were unlikely to be natural hosts of *Blastocystis* sp. This could be attributed to the extreme acidity in the gastrointestinal tract of feline which creates an unsuitable environment for the growth of *Blastocystis* sp. (Farah Haziqah *et al.,* 2018).

This study provides the first data on protozoan parasites in the cat population from the northern region of peninsular Malaysia. The only available study on protozoan parasites in cat found six species of protozoan parasites in infected cat population from the rural villages in Selangor and Pahang namely, *Entamoeba* spp., *Giardia duodenalis*, *Cryptosporidium* spp., *Balantidium coli*, *Eimeria* spp. and *Isospora* spp. with the prevalence of 34.3% which could be due to the lack of veterinary awareness or understanding of the owners on the prevention measures by Ngui *et al.* (2015). Other studies focused only on helminths as well as ectoparasites (Mohd Zain *et al.*, 2013). Nazeh *et al.* (2015) studied the blood parasites in domestic and stray cat populations.

This data is critical for veterinarians and animal caretakers who typically work with cats in animal shelters, as well as cat owners because infection was not only reported in the stray population but also in shelter-owned cats. Therefore, integrated approaches (cleaning, disinfection, proper husbandry practices, reducing environmental contamination and periodic fecal examination) should be accompanied with appropriate antiprotozoan treatment to control and prevent intestinal protozoan parasites in shelter-owned cats mainly, *Cystoisospora* spp. which was recognised as potential pathogens in cats for years.

CONCLUSION

The intimate association between humans and companion animals, which could facilitate transmission, led us to investigate the current information on the prevalence of protozoan parasites in stray and shelter cat population in the northern region of peninsular Malaysia. Generally, it was found that the prevalence of cats shedding intestinal protozoan parasites in Penang island was very low with no zoonotic protozoan infection reported. Further study is therefore important to determine the parasitic infection of protozoan infection in cats from other regions of Malaysia as stray cat populations are increasing in the urban areas. Moreover, awareness campaigns and preventive measures need to be highlighted in order to raise awareness of pet owners about animal care and animal health.

REFERENCES

1. Abe N., Nagoshi M., Takami K., Sawano Y. and Yoshikawa H. (2002). A survey of *Blastocystis* sp. in livestock, pets, and zoo animals in Japan. *Vet. Parasitol.* **106:** 203-212.
2. Bergamo do Bomfilm T.C. and Machado do Couto M.C. (2013). Morphological diagnosis and occurrence of *Blastocystis* spp. obtained from the stool samples of domestic bird species commercialised in municipal markets. *J Parasitol Vector Biol.* **5:** 20-26.
3. Bowman D.D., Hendrix C.M., Lindsay D.S. and Barr S.C. (2008). Feline clinical parasitology. Iowa: John Wiley & Sons. pp 3-354
4. Chandrasekaran H., Govind S.K., Panchadcharam C., Bathmanaban P., Raman K. and Thergarajan G. (2014). High lipid storage in vacuolar forms of subtype 6 *Blastocystis* sp. in ostrich. *Parasites & Vectors* **7:** 469-475.

5. Chuong L.S., Suresh K., Mak J.W., Init I. and Kathijah O. (1996). Prevalence of *Blastocystis* in animals from domesticated surroundings. *SE Asian J Trop Med* **27:** 850-852.
6. Dubey J.P., Lindsay D.S. and Lappin R.L (2009). Toxoplasmosis and other intestinal coccidial infections in cats and dogs. *Vet Clin Small Ani Pract.* **39:** 1009-1034.
7. Duda A., Stenzel D.J. and Boreham P.F.L. (1998). Detection of *Blastocystis* sp. in domestic dogs and cats. *Vet. Parasitol.* **76:** 9-17.
8. Farah Haziqah M.T., Chandrawathani P., Douadi B., Suresh K., Wilson J.J., Mohd Khalid M.K.N., Rajamanikam A., Lewis J.W. and Mohd Zain S.N. (2018). Impact of pH on the viability and morphology of *Blastocystis* isolates. *Trop Biomed* **35(2):** 501-510.
9. Knowles R. and Das Gupta B.M. (1924). On the nature of *Blastocystis hominis*. *Indian J Med Res* **12:** 31-38.
10. König G. and Müller H.E. (1997). *Blastocystis hominis* in animals: incidence of four serogroups. *Zentralbl Bakteriol* **286:** 435-440.
11. McMillan F.D. (2019). *Mental health and well-being in animals*, 2nd Edition. Best Friends Animal Society, USA.
12. Mohd Zain S.N., Sahimin N., Pal P. and Lewis J.W. (2013). Macroparasite communities in stray cat populations from urban cities in peninsular Malaysia. *Vet Parasit* **196(3-4)**: 469-477.
13. Moskvina T.V., Izrailskaia Kharitonova A.V. and Tsybulsky A.V. (2018). Parasites of stray and client-owned domestic cats in urban areas in Russia during 2000-2015 years. *Trop Biomed* **35(1):** 267–279.
14. Nazeh M.A., Mohamed Nor Z., Kassim M., Mansor M., Al-Adhroey A.H., Ngui R. and Sivanandam S. (2015). Prevalence of filarial parasites in domestic and stray cats in Selangor State, Malaysia. *Asian Pac J Trop Med* **8(9):** 705-709. https://doi.org/10.1016/j.apjtm.2015.07.034
15. Ngui R., Lee S.C., Yap N.J., Tan T.K., Aidil R.M., Chua K.H., Aziz S., Sulaiman W.Y.W., Ahmad A.F., Mahmud R. and Lian Y.L.A. (2014). Gastrointestinal parasites in rural dogs and cats in Selangor and Pahang states in peninsular Malaysia. *Acta Parasit* **59(4):** 737-744.
16. Pereira P.F., Barbosa A. da S., De Moura A.P.P., Vasconcellos M.L., Uchôa C.M.A., Bastos O.M.P. and Amendoeira M.R.R. (2017). Gastrointestinal parasites in stray and shelter cats in the municipality of Rio de Janeiro, Brazil. *Revista Brasileira de Parasitologia Veterinaria* **26(3):**383-388.
17. Ruaux C.G. and Stang B.V. (2014). Prevalence of *Blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. *Plos One* **9(9):** 1-5.
18. Saki J., Mohammadpour N., Moramezi F. and Khademvatan S. (2015). Seroprevalence of *Toxoplasma gondii* in women who have aborted in comparison with the women with normal delivery in Ahvaz, Southwest of Iran. *The Scientific World Journal 2015*, **2015**:764369. https://doi.org/10.1155/2015/764369
19. Soulsby E.J.L. (1986). *Helminths, arthropods & protozoa of domesticated animals*, 7th Edition, Bailliere Tindall, London.
20. Suresh K. and Smith H.V. (2004). Comparison of methods for detecting *Blastocystis hominis*. *Eur J Clin Microbial Infect Dis* **23:** 509-511.
21. Technical Centre of Agricultural and Rural Co-operation (1989). *Manual of Tropical Veterinary Parasitology.* Shah-Fisher M. and Say R.R. (Trans.). CAB International.
22. Harrison Wein (Ed.) (2018)The Power of Pets: Health Benefits of Human-Animal Interactions. In: *NIH News in Health, Feb 2018*. National Institutes of Health, US Dept o fHealth and Human Services. https://newsinhealth.nih.gov/2018/02
23. Vogel M.N., Kirisits M., Michael E., Bach H., Hostetter M., Boyer K., Simpson R., Holfels E., Hopkins J., Mack D., Mets M.B., Swisher C.N., Patel D., Roizen N., Stein L., Stein M., Withers S., Mui E., Egwuagu C., Remington J., Dorfman R. and McLeod R. (1996). Congenital toxoplasmosis transmitted from an immunologically competent mother infected before conception. *Clin Infect Dis* **23(5):** 1055-1060. https://doi.org/10.1093/clinids/23.5.1055
24. Wang W., Cuttell L., Bielefeldt-Ohmann H., Inpankaew T., Owen H. and Traub R.J. (2013). Diversity of *Blastocystis* subtypes in dogs in different geographical settings. *Parasites & Vectors* **6:** 215.

ACKNOWLEDGEMENT. The authors would like to thank Universiti Sains Malaysia (304/PBIOLOGI/6315156) for funding this study and the team from Veterinary Parasitology Laboratory USM for providing tremendous support and assistance on this study.