RE# MJVR - 0008-2016

DETECTION OF *Toxoplasma gondii* OOCYST IN CATS USING MODIFIED KATO-KATZ AND SHEATHER'S SUGAR METHODS

NORHAMIZAH A.H.[1], NORINA L.[1], RASHIDAH C.M.[1], NORSHARINA A.[1], HANAFI H.[1] AND SAIPUL BAHARI A.R.[2]

1 Regional Veterinary Laboratory Johore Bahru, Lot PTB 11098, Jalan Taruka Off Jalan Datin Halimah, 80350, Johore Bahru, Malaysia.

2 Aras 1, Lot 4G1, Podium 1A, No. 28, Persiaran Perdana, Presint 4. Pusat Pentadbiran Kerajaan Persekutuan, Putrajaya, 62624 Putrajaya, Malaysia.

* Corresponding authors: norhamizah@dvs.gov.my

ABSTRACT. *Toxoplasma gondii* is a protozoan parasite that causes toxoplasmosis in humans and animals. It belongs to the phylum Apicomplexa, subclass Coccidiasina and family Sarcocystidae. The Felidae family is the definitive host for this disease where the parasites undergo a sexual cycle of replication (oocysts). In this study, cat faeces were collected from private clinics around Johore Bahru, Peninsular Malaysia. A total of 61 samples were tested using microscopy to detect for presence of *T. gondii* oocyst via two methods; namely the Modified Kato-Katz with Kinyoun staining and Sheather's sugar floatation methods. The results showed that 40.98% of the faecal samples tested were positive for *T. gondii* oocysts. These two methods were successfully used in diagnosing toxoplasmosis in cats in this study. Morphological approaches for *Toxoplasma* oocysts identification have been neglected in recent years, due to the upsurge of more precise technologies. This study suggests that this modified technique could be introduced for screening and detection of oocysts excreted in faeces of suspected animals of the Felidae family.

INTRODUCTION

Toxoplasma gondii is a protozoan parasite that causes toxoplasmosis belonging to the phylum Apicomplexa, subclass Coccidiasina and family Sarcocystidae (Hill *et al.*, 2007). It is a common parasitic infection in humans and other warm blooded animals, with approximately a third of the world's population estimated to have been exposed to the parasite. It has been shown that up to 95% of some human populations have been infected with toxoplasmosis. Infection is often highest in areas of the world that have hot, humid climates and lower altitudes (CDC, 2008). In Malaysia, the infection is usually in a chronic form estimated to vary from

10-50% of the population (Tan *et al*., 1973; Nissapatorn *et al.,* 2003).

Humans become infected with *T. gondii* mainly by ingesting uncooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts from faeces of infected cats (Dubey *et al*., 1988). *T. gondii* oocysts are shed in large numbers by domestic cats and other members of Felidae after ingesting prey or contaminated water (Hill *et al*., 2002). These oocysts mature in the environment and are disseminated through rain and surface water, resulting in widespread contamination of the environment (Dubey *et al*., 1972; Dubey, 2001).

Toxoplasmosis has a high prevalence in Malaysians and it is highest among Malays compared to other races such as Chinese and Indians (Partono *et al*., 1975; Gandahusada, 1978; Wong *et al*., 2000). This could be explained by the fact that Malays have a habit of keeping cats in their house which leads to close contact where they will be more likely exposed to contaminated cat faeces (Tan *et al*., 1973; Thomas *et al*., 1980). Thus, the information so far suggests the importance of being able to detect the presence of *T. gondii* oocysts in cats by using a simple, cheap and fast method so as to prevent the spread of infection to humans. Information on the current status of toxoplasmosis in local cats is lacking especially due to the shortage of reliable and cheap diagnostic tools to identify the occysts in faecal samples. Thus, the objective of this study is to detect the presence of *T. gondii* oocysts in the faecal samples of cats in Johore by using the Modified Kato-Katz with Kinyoun staining and Sheather's sugar floatation methods. The information gathered from this study will enable the quick and cheap diagnosis of toxoplasmosis in regional laboratories, where faecal samples from stray and pet cats are submitted on a routine basis for screening of various pathogens. A database of this information will aid in further controlling the spread of the disease in pets and humans as well as give an overall view of the disease prevalence so that control measures can be instituted such as treatment and management of strays in accordance to the Malaysian Animal Welfare regulations.

MATERIALS AND METHODS

A total of 61 fresh faeces from domestic cats were collected from three Veterinary District Clinics in Johore (Johore Bahru, Kulai Jaya and Kota Tinggi). The identification of the cat was recorded and approximately 5 gm of faecal samples were collected by the attending veterinarian or animal caregivers; placed in an air tight container and kept at 4 °C. The samples were collected from November 2014 to August 2015. The faecal samples were then dispatched to the Regional Veterinary Laboratory at Johore Bahru where two methods for the identification of *T. gondii* occysts was conducted namely the Modified Kato-Katz with Kinyoun staining and Sheather's sugar floatation methods.

Modified Kato-Katz Method With Kinyoun Staining

Briefly, a small amount of faecal material was placed on a clean filter paper and a nylon sieve was pressed on top of it so that some of the faeces sieves through the screen and accumulates on top of the 180 mesh nylon sieve. A spatula was used to scrape across the upper surface of the screen to collect the sieved faeces. By using a wired loop, a small amount of faeces was scooped and placed on the glass slide. A thin smear was made on the glass slide. The smear was air dried, and stained with Kinyoun. (Amanto Neto *et al.*,1996). On examining under the microscope (1000×), *T. gondii* oocysts appear as red cystic structures, 10 µm in diameter.

Sheather's Sugar Floatation Technique.

Sugar floatation technique (Sheather, 1923) was performed using 5 g of faeces mixed with 45 ml of sugar solution (density 1.208) and centrifuged at 1,000×*g* for 10 minutes, and the suspension was transferred to a slide and observed for presence of 10 µm thick walled unstained oocysts under microscope (1000×).

RESULTS

A total of 25 out 61 (40.98%) faecal samples were positive for *T. gondii* by modified Kato-katz and Sheather's sugar methods. The appearance of the oocysts are shown in Figure 1 for the unstained oocysts and Figure 2 for oocysts stained by Kinyoun. The oocysts were described as spherical to sub-spherical 11-13 µm by 9-11 µm (mean 12 µm by 10 µm). The sporulated oocysts

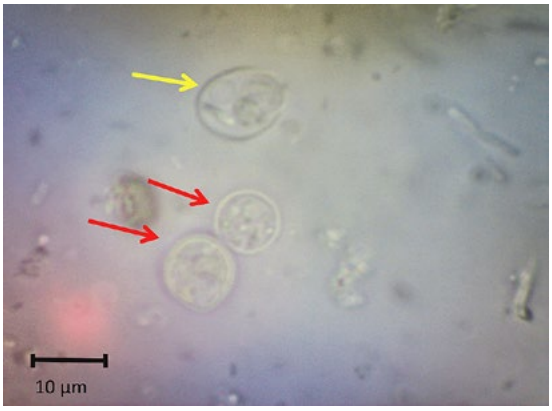

Figure 1. Sheather's sugar, magnification at 1000×. Legend: Red arrow shows *T. gondii* (unsporulated), Yellow arrow show *T. gondii* (sporulated).

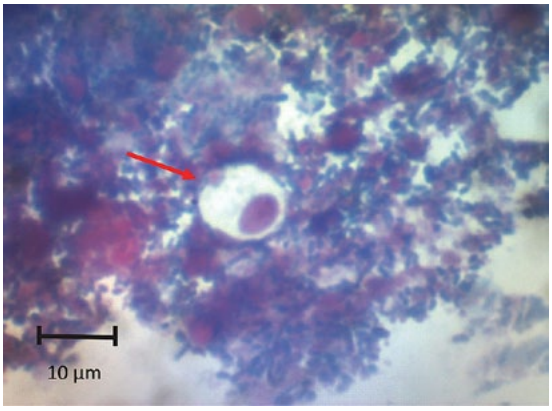

Figure 2. Modified Kato-Katz with Kinyoun, magnification at 1000×. Legend: Red arrow shows *Toxoplasma gondii* oocyst stained red

had the following dimensions; 12-15 µm by 10-13 µm (mean 13 µm by 12 µm) in size (Soulsby, 1982).

It was observed that by the Modified Kato-Katz with Kinyoun stain, the *T. gondii* oocysts appeared as red cystic structures with a 10 µm diameter, with preservation of internal details (Luciana *et al.*, 2008). This characteristic makes it easier for identification especially for an inexperienced laboratory personnel.

DISCUSSION

This study shows that 40.98% of the faecal samples collected from the three Small Animal District Veterinary Clinics in Johore were positive for presence of *T. gondii*. Up to the present moment, there is no published data in Malaysia or in neighbouring countries on detection for *T. gondii* oocyst in cats using the above methods. However, for seroprevalence of *T. gondii* in cats in Malaysia, studies by Chandarawathani *et al.* (2008) showed 14.55% of cats were serologically positive. A study conducted in the United States by Lily and Wortham (2013) using the PCR method for oocyst detection showed that 6% of cats were shedding *T. gondii* oocysts. This difference could be due to differences in techniques used as well as regional and cultural differences in managing pet cats.

The advantage of the Modified Kato-Katz by Kinyoun staining is that there is lesser handling of infective faecal material compared with Sheather's Sugar method and this will decrease the possibility of environmental and operator contamination. This method will also destroy all the viable oocysts through the staining process. The other advantages of the Modified Kato-Katz by Kinyoun is that only a small amount of faeces is required to perform the test and it is cheap, requiring hardly any equipment such as the centrifuge. This will allow field personnel to perform this test with minimal amenities. The Modified Kato-Katz by Kinyoun staining however, allows for gross genera identification, due to preservation of oocysts diameter, but more accurate studies other than morphology must be performed for adequate speciation (Scharesa *et al.*, 2005). As it is stained in red, an inexperienced technical staff may also be able to identify and diagnose the oocysts present in faecal samples.

CONCLUSION

Morphological approaches on *Toxoplasma* oocysts identification have been neglected in recent years, due to upsurge of more precise technologies. We suggest that this modified technique could be introduced for screening and detection of oocysts excretion in faeces of suspected animals. In Malaysia, pet cats and strays are common and the need for a quick and reliable method to identify oocysts is necessary for the safety of humans.

REFERENCES

1. Amato Neto V., Braz L.M.A., Di Pietro A.O. and Modolo J.R. (1996). Pesquisa de oocistos de Cryptosporidium sp em fezes: comparação entre os métodos de Kinyoun modificado e de Heine. *Rev. Soc. Bras. Med. Trop*. **29:**575-578
2. CDC (2008). Parasites – Toxoplasmosis (*Toxoplasma* infection) http://www.cdc.gov/parasites/toxoplasmosis/index.html
3. Dubey J.P. and Frenkel J.K (1972). Cyst-induced toxoplasmosis in cats. *J. Protozool.* **19:**155-177.
4. Dubey J.P. (2001). Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. *J. Parasit.* **87:** 215-219.
5. Dubey J.P. and Beattie C.P. (1988). *Toxoplasmosis of animals and man.* Boca Raton, CRC Press.
6. Emily L. Lilly and Caroline D. Wortham (2013). High prevalence of *Toxoplasma gondii* oocyst shedding in stray and pet cats (Feliscatus) in Virginia, United States, *Parasit Vectors* **6:**266
7. Hill D. and Dubey J. P. (2002). *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clin. Microbiol. Infect.* **8:**634-640
8. Luciana R.M., Vinicius S.T., Claudia V.C, Andres J.G., Roberto M.H., Henrique C.K.T. and Heitor F.A. Quantitative *Toxoplasma gondii* oocyst detection by Modified Kato-Katz test using Kinyoun Staining (KKK) in ME49 strain experimentally infected cats (2008). *Rev. Inst. Med. Trop. S. Paulo* **50(3):** 187-190.
9. Nissapatorn V., Lee C.K.C., Cho S.M., Rohela M., Anuar A.K., Quek K.F. and Latt H M. (2003) Toxoplasmosis in HIV/AIDS patients in Malaysia. *Southeast Asian J Trop Med Public Health* **34 (Suppl 2):** 80-85.
10. Scharesa G., Pantchevb N., Barutzkic D., Heydornd A.O., Bauere C. and Conrathsa F.J. (2005). Oocysts of *Neospora caninum, Hammondia heydorni, Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany. *International Journal for Parasitology,* **35(14):**1525-1537
11. Sheather A.L. (1923). The detection of intestinal protozoa and mange parasites by a floatation technique. *J. Comp. Path. Therap.* **36:** 266-275.
12. Soulsby E.J.L. (1982). *Helminths, Arthropods and Protozoa of Domesticated Animals,* 7th Edition, Bailliere Tindall.
13. Tan D.S.K. and Zaman V. (1973). Toxoplasma antibody survey in West Malaysia. *Med J Malaysia* **17:**188-91