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Seroprevalence of *Toxoplasma gondii* in household cats in Myanmar and molecular identification of parasites using feline faecal oocysts

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ABSTRACT

Felids play an important role in the transmission of *Toxoplasma gondii* to humans and other animals since they can excrete millions of oocysts into the environment as definitive hosts. In the present study, seroprevalence and risk factors of feline *Toxoplasma* infection were investigated, and molecular identification was conducted for *T. gondii* oocysts isolated from faecal samples of seropositive cats. A total of 276 cat serum samples collected from the Yangon, Myanmar were tested for *T. gondii* antibodies by ELISA. The overall seroprevalence of *T. gondii* infection was 41.30% (114 seropositive cats). Age between 1 and 6 years (OR = 3.284; 95% CI = 1.462–7.375), age > 6 years (OR = 4.560; 95% CI = 1.588–13.100) and sex (OR = 1.725; 95% CI = 1.026–2.899) were found to be significant ($P < 0.05$) factors associated with *T. gondii* infection. DNA samples extracted from a single oocyst of seropositive cats were employed in three PCR assays amplifying parasite TOX-element and mitochondrial COI, and SAG2 locus. The obtained sequences of TOX-elements (n = 6) and COI (n = 5) were identical to those of *T. gondii* previously deposited in Genbank. SAG2 PCR yielded three different sequences, all of which were clustered with Type I *T. gondii* isolates in a phylogenetic tree. This study reported the seroprevalence and risk factors for *T. gondii* infection in cats and provided the molecular information on the parasite in Myanmar.

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite capable of infecting virtually any warm-blooded animal. Felids are the only definitive hosts for this parasite, and they can excrete millions of oocysts in the environment (Gotteland et al., 2014; Salant et al., 2007). All vertebrates, such as birds and mammals, including humans, can act as intermediate hosts. Contamination of soil and water with oocysts that survive for a long time in the environment (up to 18 months) plays an important role in both animals and human toxoplasmosis (Gotteland et al., 2014; VanWormer et al., 2013). When sporulated oocysts are orally taken by hosts, sporozoites are transformed into an invasive tachyzoite stage. After repeated intravacuolar replication, host cells are disrupted and tachyzoites invade neighboring cells. The tachyzoites form causes tissue destruction and is therefore responsible

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for the clinical manifestations of the disease. The resulting immune response is accompanied by the transformation of tachyzoites into slowly replicating intracellular bradyzoites that form persistent cysts. Tissue cysts found in the retina, brain, skeletal and heart muscles are the infective stages for intermediate and definitive hosts through the consumption of muscle or brain tissue. Infective tachyzoites develop from bradyzoites that are released from lysed cysts in the intestine (Schlüter et al., 2014). There are various routes that can lead to infection in human beings, directly or indirectly, with the contamination of *T. gondii* in food and the environment. Humans can be infected by the ingestion of oocysts from contaminated water, soil, vegetables and fruits, intake of undercooked or uncooked meat containing tissue cysts, unpasteurized goat's milk and by the congenital transmission of tachyzoites from a non-immune mother to her foetus (Dubey et al., 2014; Dubey et al., 2020; VanWormer et al., 2013).

Felines, including domestic cats, can carry and shed a variety of infectious agents, including the oocysts of *T. gondii* in their faeces. *T. gondii* oocysts can be identified by faecal examination. However, the microscopic examination of *T. gondii* is hindered by the fact that oocysts of other coccidians such as *Hammondia hammondi* cannot be morphologically distinguished from that of *T. gondii* (Schares et al., 2008). Serological investigation is important not only for detecting infection in cats but also for determining the risk to human and animal health posed by *T. gondii*. Furthermore, it is essential to understand the region-specific prevalence and population structure of *T. gondii* in the definitive host and the factors that increase the likelihood of exposure to the parasite (Brennan et al., 2020).

Present-day molecular methods such as PCR targeting TOX-element or mitochondrial genes have been used for identification and discrimination of the parasites and have also provided clearer phylogenetic resolution. The 529-bp repeat element (TOX-element), 200–300 times copied in the *T. gondii* genome (Homan et al., 2000), has been used as a target for molecular detection of *T. gondii* and its high detection sensitivity compared to other genes such as 35-copied *B1* gene was reported elsewhere (Calderaro et al., 2006; Fallahi et al., 2014). Although sequences obtained from the mitochondrial cytochrome *c* oxidase subunit I (COI) gene are short, COI-PCR can provide sufficient sequence divergence to differentiate closely related coccidian taxa (Ogedengbe et al., 2011; Ogedengbe et al., 2016). Among the developed genetic markers used for *T. gondii* genotyping, the Surface Antigen 2 (SAG2) marker has been extensively used for strain identification into three clonal lineages and atypical strains (Sibley and Boothroyd, 1992; Howe et al., 1997; Dubey et al., 2005; Wang et al., 2013). It has also been used for serological diagnosis (Huang et al., 2004) and vaccine development (Cong et al., 2005).

Several studies reported the seroprevalence of *Toxoplasma* infection in humans. Seroprevalence in healthy individuals and pregnant women were reported as 31.8% and 30.7%, respectively (Nyunt, 2005; Andiappan et al., 2014), while that of school children ranged between 23.5% and 43.8% (Thái et al., 2019; Nissapatorn, 2007). However, to the authors' knowledge, no study has addressed the seroprevalence of *T. gondii* infection in cats and the genotypes of the parasites prevalent in Myanmar. Therefore, we aimed to determine the seroprevalence and risk factors for *T. gondii* infection in cats in Myanmar and detect the parasites by molecular techniques.

2. Materials and methods

2.1. Study area

The study was performed in Yangon, the largest city in Myanmar, situated between 16° 51′ 0″ N and 96° 11′ 0″ E. Yangon city is formerly serving as the capital of Myanmar, and the city's 2020 population is approximately 5.3 million. The average annual temperature in Yangon is 27.4 °C and the annual rainfall is 2681 mm (yangon.climatemps.com). The study protocol was approved by the Ethical Review Board, University of Veterinary Science and the Ministry of Livestock, Fisheries and Rural Development, Myanmar with an approval number of 1/4000/mlfrd(6976/2015).

2.2. Sample collection

Serum samples taken from 276 cats (124 males and 152 females) who visited to two veterinary clinics in Yangon City were selected by convenience sampling method. Blood were collected from each cat via a femoral vein or cephalic vein. The sample collection period was from June to September 2015. The owners were explained the objectives of the study and requested to allow their pets to be sampled. They answered a questionnaire sheet that included the address and phone number, type of cat such as household/pet or stray, if pet, pet's name and owner's name, animal's age, sex, breed, type of feed, hunting habit, place of living such as indoor or outdoor, vaccination, and veterinary care, etc. This study employed only household cats.

2.3. Determination of antibodies to T. gondii

Serum samples were tested for anti-*Toxoplasma* IgG antibodies using ELISA kits (IVD Technologies, USA) according to the manufacturer's instructions. Positive and negative control sera were provided in the kit. Briefly, *Toxoplasma* IgG specific antibodies in diluted samples (1:25) were allowed to bind to microwell-bound *Toxoplasma* antigen and incubated the well for 30 min at room temperature and washed. After washing unbound materials, HRP-conjugate (HRP-Horseradish peroxidase) was allowed to bind to the *Toxoplasma* IgGAb-Ag complex. Unbound HRP-conjugate was washed away and TMB (tetramethylbenzidine) was allowed to react with bound HRP-conjugate. The intensity of the colour produced in the HRP-TMB reaction is proportional to the amount of IgG-specific Ab in the sample. The HRP-TMB reaction was stopped by stop solution (SS) and read the optical density (OD) values

of the wells at 450 nm by ELISA plate reader (Stat Fax 3200, Awareness Technology INC, USA). The sera with OD values of <1.200 were considered as negative, while those with >1.20 were positive. The tests were performed twice.

2.4. Faecal sample collection and coprological examination

In order to collect oocysts for molecular analysis, 30 cats were randomly selected from seropositive group and their faeces were collected 8–9 weeks after serum samples were taken for serological study. The samples were stored on ice or in a refrigerator (4 °C) until microscopic examination. Description of clinical, serological, PCR, microscopic, and epidemiological features of the selected *T. gondii* seropositive household cats are shown in Table 1. The presence of oocysts in the faecal samples was examined by the flotation method using Sheather's sugar solution (SG = 1.26) as described by Salant et al. (2007). The oocysts were then collected from the coverslip on the top of the centrifuge tubes. The collected oocysts were preserved in 70% ethanol and transferred to Laboratory of Parasitology, Faculty of Veterinary Medicine, Hokkaido University, Japan in accordance with the "Guidance on regulations for the Transport of Infectious Substances 2007–2008" under Material Transfer Agreement between University of Veterinary Science, Myanmar and Hokkaido University.

2.5. DNA isolation from single oocyst

Single oocyst isolation was carried out according to Ikarashi et al. (2013) (per communication to Professor Yutaka Nakai, Tohoku University, Japan) with minor modifications. It was conducted under microscopic observation with fine-tipped micropipette tips, and each single isolated oocyst was used for subsequent molecular analysis. Each isolated single oocyst was washed with water and then transferred to a PCR tube. After brief centrifugation, the tube was exposed to five cycles of freeze-thaw, frozen at −80 °C for 5 min, and thawed at 60 °C for 5 min in a water bath to disrupt the oocyst wall. Then, the tube was filled with 10 μl of reaction buffer (containing 10 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl and 0.01% SDS). After the addition of 0.05% proteinase K, the tube was incubated at 37 °C for 1 h, 60 °C for 3 h, and then at 95 °C for 10 min.

2.6. PCR amplification of T. gondii DNA, sequencing, and phylogenetic analysis

PCR was carried out with a total volume of 25 μl solution containing 2 μl of DNA template, 0.5 μl of TksGflex DNA polymerase (1.25 U/μl) (TaKaRa Bio Inc., Shiga, Japan), 12.5 μl of 2× Gflex PCR Buffer (Mg^{2+}, dNTP plus, TaKaRa Bio Inc.), and 10 μl of nuclease-free water (Promega, Madison, WI, USA). *T. gondii* DNA of the RH strain, kindly provided by Professor Kisaburo

Table 1
Description of the 30 *T. gondii* seropositive household cats from Yangon, including clinical, serological, PCR, microscopic and epidemiological features.

Sample ID	Sex	Age (years)	Type of food	Outdoor access	Diarrhoea	OD	Microscopic (*T. gondii*-like oocyst)	PCR		
								TOX	COI	GRA2
2	F	2.0	Cat food	No	No	1.762	+	−	−	−
3	M	1.1	Kitchen food	No	No	1.751	+	−	−	−
12	F	3.3	Kitchen food	Yes	No	1.486	−	−	−	−
13	M	0.4	Kitchen food	Yes	No	1.304	+	−	−	−
16	F	3.0	Kitchen food	Yes	No	2.013	+	−	−	−
17	M	5.5	Kitchen food	No	No	1.233	−	−	−	−
21	M	8.0	Cat food	No	No	1.819	−	−	−	−
22	F	0.7	Cat food	No	No	1.820	−	−	−	−
31	F	8.3	Kitchen food	No	Yes	1.482	+	−	−	−
40	F	5.0	Cat food	No	No	1.815	−	−	−	−
41	M	0.6	Cat food	Yes	No	1.382	+	−	−	−
47	F	3.0	Kitchen food	Yes	No	1.508	−	−	−	−
48	F	4.0	Kitchen food	No	No	1.538	−	−	−	−
56	F	17.0	Kitchen food	No	No	1.367	−	−	−	−
57	M	4.0	Kitchen food	Yes	No	1.976	+	−	−	−
81	F	1.0	Cat food	Yes	No	1.469	−	−	−	−
94	F	13.0	Cat food	Yes	No	1.956	−	−	−	−
102	F	10.6	Kitchen food	No	Yes	1.676	−	−	−	−
103	M	8.1	Kitchen food	No	Yes	1.392	−	−	−	−
107	F	8.0	Cat food	No	No	1.731	−	−	−	−
108	F	3.0	Cat food	Yes	No	2.013	−	−	−	−
116	F	3.0	Kitchen food	No	Yes	1.580	+	−	−	−
128	M	5.0	Cat food	No	Yes	1.807	+	+	+	+
129	M	1.5	Kitchen food	No	Yes	1.613	+	+	+	+
130	M	2.0	Cat food	No	Yes	1.677	+	+	+	+
131	F	2.6	Kitchen food	No	Yes	1.391	+	+	−	−
132	F	3.0	Kitchen food	No	Yes	1.775	+	+	+	−
160	M	2.0	Kitchen food	No	Yes	1.613	+	−	−	−
184	F	3.0	Kitchen food	Yes	Yes	1.849	+	+	+	−
259	F	3.0	Cat food	Yes	No	1.765	−	−	−	−

Table 2
Primer sequences used in this study.

Primers	Sequences (5′-3′)	References
TOX4	CGCTGCAGGGAGGAAGACGAAAGTTG	Homan et al. (2000)
TOX5	CGCTGCAGACACAGTGCATCTGGATT	
Toxo_COI_For	GGAGGAGGTGTAGGTTGGAC	Gjerde (2013)
COIRm	CCCAGAGATAATACAAAATGGAA	
SAG2-F	GAAATGTTTCAGGTTGCTGC	Howe and Sibley (1995)
SAG2-R	AACGTTTCACGAAGGCACAC	

Nagamune, University of Tsukuba, Japan, was used as a positive control. To detect *T. gondii* DNA, specimens were amplified using primers TOX4/TOX5 targeting a non-coding 529 bp repetitive DNA fragment (Homan et al., 2000). The PCR conditions were started with an initial denaturation cycle at 94 °C for 5 min, followed by an amplification profile for 35 cycles of denaturation at 98 °C for 30 s, annealing at 60 °C for 30 s, and extension at 68 °C for 2 min, and a final extension for 7 min at 68 °C. To distinguish *T. gondii* nucleotides with nucleotides from other oocysts, we used COI primer set, Toxo_COI_For/COIRm (Gjerde, 2013) to amplify an amplicon of approximately 790 bp from the DNA specimens. Thermocycling for the target gene was done with an initial denaturation step for 5 min at 94 °C, 45 cycles of denaturation for 30 s at 98 °C, annealing for 30 s at 55 °C, and extension for 2 min at 68 °C, and a final extension for 7 min at 68 °C. Additionally, the coding region of GRA2 was amplified using the primer pair GRA2 for and GRA2 Rev (Howe and Sibley, 1995). The PCR condition was done with 5 min initial denaturation at 94 °C followed by 35 cycles of 98 °C for 30 s, 60 °C for 30 s, 68 °C for 2 min and was ended by one cycle of final extension at 68 °C for 7 min. The oligonucleotide primers used in this study are shown in Table 2.

The PCR products were examined by 1% (for COI gene) and 2% (for TOX4/TOX5 and GRA2 genes) Tris-acetate-EDTA (TAE) agarose gel electrophoresis, and stained with Red Safe Nucleic Acid Staining Solution (iNtRON Biotechnology Inc., Sungnum, Korea).

The amplified products were purified using the NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel AG, Düren, Germany) and submitted to direct sequencing using an Applied Biosystems 3130 Genetic Analyzer with a BigDye v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Carlsbad, CA, USA) and CleanSEQ (Beckman Coulter Inc., Tokyo, Japan). Multiple sequence alignment was performed using the sequence analysis software package ATGC version 7 (GENETYX Corporation, Tokyo, Japan). BLAST analysis for the obtained sequences was performed on the GenBank database at the National Centre for Biotechnology Information (NCBI) on the website www.ncbi.nlm.nih.gov/BLAST. The phylogenetic trees were constructed using the Maximum Likelihood (ML) method using MEGA X (Kumar et al., 2018). Bootstrap analyses (using 1000 replicates) were carried out to define the robustness of the findings. Gaps were treated as missing data.

2.7. Statistical analysis

All the serological data were analysed using the multivariate logistic regression to determine the association between the hypothesized risk variables and positive serology with SPSS software (version 16.0). A value of $P < 0.05$ was considered significant.

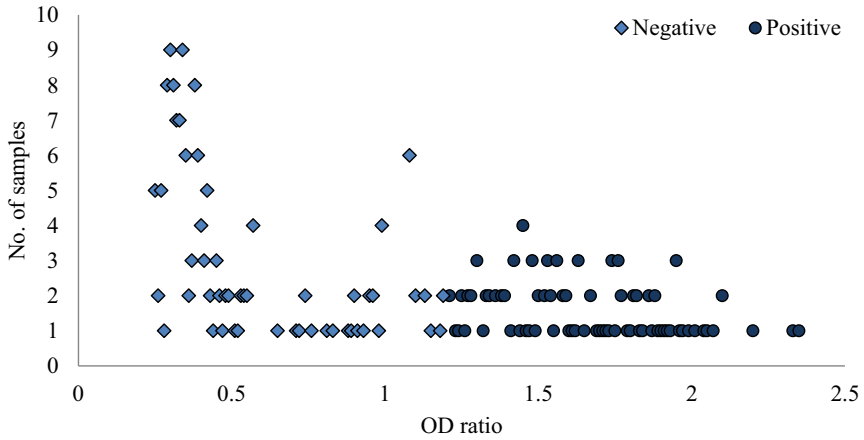

Fig. 1. Scatter graph showing the OD ratio obtained by ELISA on cat sera samples.

3. Results

3.1. Seroprevalence

Among the examined serum samples, the overall prevalence of *T. gondii* infection was 41.30% (114/276) (Fig. 1). The age of the examined cats varied from 4 months to 17 years. There was a significant ($P < 0.05$) positive association with *T. gondii* infection and factors such as age between 1 and 6 years ($P = 0.004$, odds ratio [OR]: 3.284; 95% confidence interval [CI]: 1.462–7.375), age > 6 years ($P = 0.005$, OR: 4.560; 95% CI: 1.588–13.100) and female ($P = 0.04$, OR: 1.725; 95% CI: 1.026–2.899). Other factors, including type of feed, outdoor access, the presence of litter box, and hunting habit were not significantly associated with *T. gondii* infection (Table 3).

3.2. T. gondii DNA detection

Among the 30 selected seropositive cats, oocysts were detected in 15 cats' faecal samples. Out of 15 DNA samples extracted from single oocysts, only six were positive by TOX PCR. All PCR products amplified with this primer (529 bp) were sequenced to identify species of the parasite, and the BLAST results showed that the obtained sequences were *T. gondii*. The sequences obtained were submitted to GenBank (accession numbers LC547463 to LC547468).

Out of six samples positive by TOX PCR, five were also positive by COI PCR and their sequences (~625 bp) were deposited in GenBank under the accession numbers LC547469 to LC547473. In the phylogenetic tree based on COI sequences (Fig. 2), sequence comparisons using BLAST revealed that these sequences were completely identical to *T. gondii* (KM657810, JX473253, HM771689, and KT363924) and clustered together with all sequences from this study. Moreover, species of typically heteroxenous parasites infecting dogs and cats (species in the genera *Toxoplasma*, *Neospora*, and *Hammondia*) were all found in a monophyletic clade. However, species in the genus *Hammondia* remained paraphyletic. The canid-infecting *Hammondia* spp., *H. heydorni* was monophyletic and formed a sister clade to *N. caninum*, whereas the felid-infecting *H. hammondi* was sister to *T. gondii*.

Out of six samples positive by TOX PCR, only three samples were positive by SAG2 PCR (accession numbers LC547474 to LC547476). ClustalW analysis and then bootstrapping were performed to find genotypes comparison of our isolates from cat faecal samples with published strains of *T. gondii* in GenBank. The three isolates of *Toxoplasma* were clustered with *T. gondii* Type I isolates (EU258520, LC406342, and MH606167) (Fig. 3).

4. Discussion

Although most of Myanmar's culinary habits are based on thorough cooking, new food styles such as barbecue and raw or undercooked meat-based dishes are being popular. In previous studies conducted in Myanmar, *T. gondii* infection has been recorded in domestic goats (11.4%) (Bawm et al., 2016), domestic pigs (18.4%) (Thaw, 2017) and wild insectivorous bats (29.3%) (Sun et al., 2013) indicating that this infection has been widespread in both domestic and wild animals in the country. The ingestion of raw or undercooked meat could be one of the potential causes of infection in human in Myanmar. Since domestic cats are an important reservoir of *T. gondii*, and it has been reported that *Toxoplasma* seroprevalence of humans is highly associated with living in close proximity to *Toxoplasma* seropositive cats (Pereira et al., 1992). Therefore, it is important to address the overall

Table 3
Seroprevalence and risk factors for *T. gondii* infection in household cats in Yangon, Myanmar.

Variable	No. of examined	No. of positive (%)	OR (95% CI)	*P*-value
Age				
<1 year	51	9 (17.65)	–	–
1–6 years	197	90 (45.69)	3.284 (1.462–7.375)	0.004*
>6 years	28	15 (53.57)	4.560 (1.588–13.100)	0.005*
Sex				
Male	124	42 (33.87)	–	–
Female	152	72 (47.37)	1.725 (1.026–2.899)	0.040*
Outdoor access				
Yes	104	47 (45.19)	1.265 (0.702–2.277)	0.434
No	172	67 (38.45)	–	–
Type of feed				
Kitchen food	219	97 (44.29)	1.753 (0.912–3.371)	0.092
Cat food	57	17 (29.83)	–	–
Presence of litter box				
Yes	130	51 (39.23)	–	–
No	146	63 (43.15)	1.036 (0.607–1.768)	0.897
Hunting habit				
Yes	190	77 (40.53)	–	–
No	86	37 (43.02)	0.883 (0.498–1.566)	0.671

CI, confidence interval; OR, odds ratio.
* Significant statistical findings ($P < 0.05$).

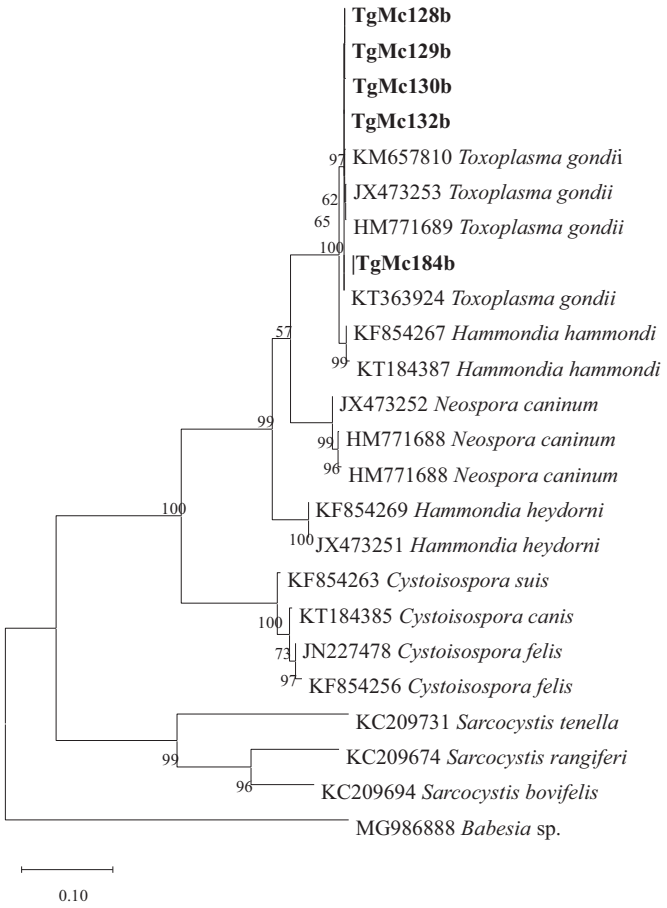

Fig. 2. Phylogenetic relationships among Toxoplasmatinae and other apicomplexan taxa as inferred from COI sequences using the Maximum Likelihood method based on the Tamura-Nei model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. TgMc128b, TgMc129b, TgMc130b, TgMc132b, and TgMc184b are our isolates from oocysts of cat faeces. GenBank accession number of each sequence is given before the taxon name. The COI sequence of *Babesia* sp. was used as the out-group.

seroprevalence and molecular information of *T. gondii* infection in cats, the definitive hosts, from Yangon, a city with a large population in Myanmar.

In this study, the seroprevalence of *Toxoplasma* infection in household cats from Yangon city was observed as 41.3%. The seroprevalence of *T. gondii* in domestic cats worldwide has been estimated to be approximately 30–40% (Dubey, 2010). Comparing with other Asian countries, the prevalence observed in our study was higher than Japan (6–16%) (Nogami et al., 1998; Salman et al., 2018), Thailand (11.0%) (Jittapalapong et al., 2007), Malaysia (14.5%) (Chandrawathani et al., 2008), China (25.2%) (Zhang et al., 2009), and Singapore (30.7%) (Chong et al., 1993), while lower than Indonesia (59.4%) (Durfee et al., 1976), Iran (63%) (Haddadzadeh et al., 2006), and Vietnam (72.3%) (Hosono et al., 2009). However, the different methodologies used, different sample sizes and sample populations in the regions surveyed may have contributed to these differences; therefore, it is difficult to compare the reported prevalence. Apart from household cats, large populations of stray cats roaming countrywide as in other countries, which may have more prevalence and spread among stray cats.

The current findings revealed that the sex of the cats exhibited significantly associated with *T. gondii* infection. Female cats were found to be at a higher risk than males. Similar findings were reported by Jittapalapong et al. (2007) and Besné-Mérida et al. (2008), while most serological surveys found no relation to sex (Sumner and Ackland, 1999; Salant and Spira, 2004). The reason is not clear, as discussed in Besné-Mérida et al. (2008), genetic or endocrine reasons of animals could be considered as causative factors.

The frequency of specific antibodies has increased with age for cats from 1 year of age. The age is the most common risk factor as identified worldwide among household cats (Salant and Spira, 2004; Opsteegh et al., 2012). The seroprevalence in older animals was generally higher than that in younger animals. Our study showed that <1 year old cats had a significantly lower prevalence than that of older cats (>1 year old). The higher rate of seroprevalence in older cats might be due to the consistent ingestion of encysted bradyzoites in the tissues of intermediate hosts (Haddadzadeh et al., 2006; Must et al., 2015; Salman et al., 2018). Furthermore, past infection with *T. gondii* in older cats might be one of the causes of increased frequency of specific antibodies.

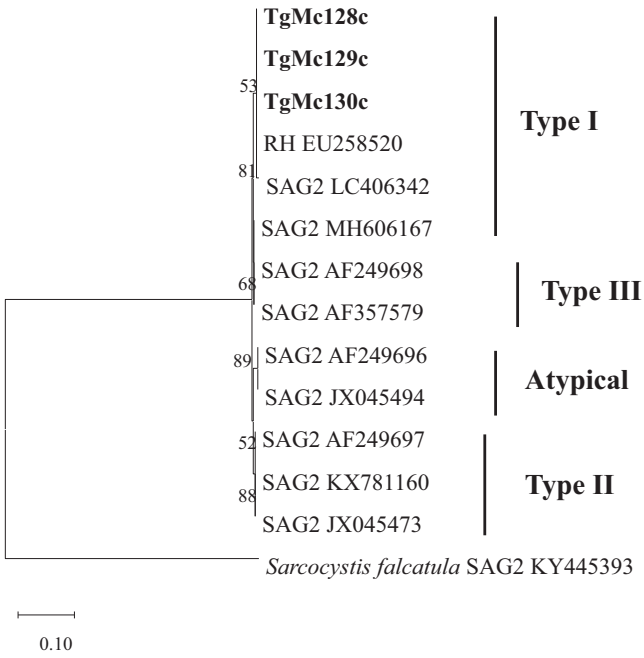

Fig. 3. The phylogenetic tree was constructed by the Maximum Likelihood method using the SAG2 sequence of reference strains and our isolates (TgMc128c, TgMc129c, and TgMc130c). GenBank accession number of each sequence is given. The SAG2 sequence of *Sarcocystis falcatula* (KY445393) served as the out-group.

Although there was not significant ($P = 0.092$) association with *T. gondii* infection, higher seroprevalence was found in cats with feeding kitchen food, which might be explained by the contamination of the parasites in raw or undercooked meat in the kitchen food. Higher seroprevalence has been referred in cats that are fed on diets including raw or undercooked viscera and/or meat in comparison with animals receiving only commercial canned or dried food (Cook et al., 2000; Brennan et al., 2020). As described above, the prevalence of *T. gondii* infection has been reported from livestock and wild animals in Myanmar (Bawm et al., 2016; Thaw, 2017; Sun et al., 2013), the higher frequency of infection might be most likely related to the ingestion of viable *T. gondii* cysts in uncooked or undercooked infected meat. Other hypothesized risk factors such as outdoor access, presence of litter box, and hunting habit were not significantly associated with *Toxoplasma* infection in this study. However, the presence of outdoor access (Lopes et al., 2008) and hunting habits have been previously reported as risk factors for *T. gondii* infection (Opsteegh et al., 2012; Brennan et al., 2020).

Cats shed *T. gondii* oocysts for only a short time (<1 week) and less than 1% of oocysts were shed at any given time. Then, cats usually develop antibodies to *T. gondii* 1–2 weeks after they have shed oocysts (Dubey, 2004). This may be the reason why oocysts were not detected in the faeces of all the 30 selected seropositive cats examined in the present study. The detection of oocysts in 15 cats even at 8–9 weeks after serological testing may indicate that these cats were re-infected with the parasites and shed oocysts at the time of faecal sampling. To understand the frequency of parasite oocysts in the faeces of tested cat group, the study should have also included seronegative cats.

PCR did not give amplicons in some of the DNA samples extracted from oocysts, indicating that some oocysts might not be *T. gondii* but other parasites such as *H. hammondi* or *Cystoisospora* spp. (Fig. S1). The diagnosis of *T. gondii* infection by faecal microscopy is complicated, as other similar coccidian oocysts such as *Cystoisospora* spp., *Sarcocystis* spp., and *H. hammondi*, are often present in the same faecal specimen. The PCR method can easily differentiate *T. gondii* from other cyst forming eukaryotic parasites, and is highly sensitive (Salant et al., 2010). In this study, to identify and confirm *T. gondii*, DNA extracted from single oocysts was tested by two molecular techniques based on PCR amplifying parasite TOX-elements and COI genes. Among the examined samples, six and five DNA samples were respectively positive by TOX and COI PCRs. Parasite mitochondrial COI gene has been suggested as a suitable for molecular species identification and delimitation of apicomplexan parasites, and as a marker for the identification of coccidian taxa (Ogedengbe et al., 2011; Martinsen et al., 2008; Feng et al., 2017). By phylogenetic analyses using the Maximum Likelihood method, partial sequences of COI of *T. gondii* from this study were confirmed that those were separate from the other similar coccidian species.

In this study, a PCR assay based on the polymorphic SAG2 locus was conducted. Three sequences obtained were closely related to *T. gondii* Type I isolates. In a previous study, clonal Type I has been identified in bats from Myanmar (Sun et al., 2013). Type I is the second most common genotype and is widely distributed in Asian countries and has been reported in the most Eastern parts of Asia (South Korea, and the Jilin province of China), South East Asia (Peninsular Malaysia and Myanmar), and Tibetan Plateau (Chaichan et al., 2017). The SAG2 gene encodes two separate forms of the surface tachyzoite protein p22, which are recognized by strain-specific monoclonal antibodies and allows for adequate genotyping of *T. gondii* (Howe et al., 1997; Fuentes et al., 2001).

Three major *Toxoplasma* genotypes have been characterized, including Type I, consisting of mice-virulent isolates, Type II, containing avirulent isolates, and Type III, comprising both avirulent and intermediately virulent isolates. Moreover, the highest proportion of atypical strains that have been associated with a number of severe cases of toxoplasmosis in immunocompetent individuals (Carme et al., 2009) were recorded in tropical South Asia (Malaysia, Myanmar, and Sri Lanka) (Chaichan et al., 2017). Although the differences between these three genotypes are less than 1%, they have strikingly different virulence phenotypes in mice (Sibley and Boothroyd, 1992; Howe and Sibley, 1995). Since DNA sequencing is the technique of choice to infer the real genetic diversity and population structure of *T. gondii* strains in cats of Myanmar, we sequenced all SAG2 PCR products in this study. In Myanmar, besides Type I, other genotypes might be circulating in animals and humans. In the future, as in other reports (Sun et al., 2013), genotyping should be tried with conventional methods such as PCR–RFLP using various genetic markers to isolate *T. gondii* into three predominant clonal lineages (Types I-III) in both animals and humans extensively in Myanmar.

Since household cats are one of the most intimate companions of humans, as a consequence, frequent contact with cats, people might have increased risk of acquiring *T. gondii* infection (Pereira et al., 1992; Elmore et al., 2010; Chomel and Sun, 2011; Wei et al., 2016). In the Yangon area, Andiappan et al. (2014) reported that the seroprevalence in pregnant women was 30.7% and that these women might become infected rather through direct contact with cats, might infect through indirect from the environment and ingestion of oocysts contaminating food and water. It is also noted that *T. gondii* infection is increasingly recognized as a problem in non-pregnant patients, such as immunocompetent adults, may lead to serious infection. According to our findings, *T. gondii* antibodies were common in household cats in Yangon. Cat owners should protect their cats from *T. gondii* infections better and reduce the further spread of the parasite in the environment. Pregnant women in particular should avoid contact with cats, soil and raw meat. Pet cats should only be fed with dry, canned, or cooked food. The cat litter box should be emptied every day. Gloves should be worn while gardening. Vegetables should be washed thoroughly before eating because they might have been contaminated with cat faeces (Foulon et al., 1994). Veterinarians should also educate cat owners on the importance of controlling the size of the cat population and encouraging them to keep cats indoors and collect faeces in litter boxes.

In conclusion, this study provides the first molecular evidence that *T. gondii* infection is widespread among household cats in Yangon area, Myanmar. Further investigation should expand the study areas to understand the overall prevalence of *T. gondii* in this country. Other common farm animals such as cattle and chicken should be tested for parasite infection as potential source for human infections. Collectively, the public health significance of *T. gondii* infection in cats could be understood more clearly in the future.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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