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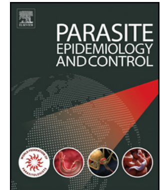
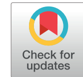


Shedding proportion of *Toxoplasma gondii*-like oocysts in feral cats and soil contamination in Oyo State, Nigeria



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Toxoplasmosis, a disease caused by the intracellular protozoan parasite *Toxoplasma gondii*, is transmitted through several hosts with cats serving as its definitive host. Oocysts are released with cat faeces into the environment (*e.g.* soil); an important medium in its transmission. The level of soil contamination with oocysts is an indicator of the level of on- going transmission. However, a dearth of information exists on the relationship between the presence of oocysts shedding cats and soil, and its importance in the transmission of *T. gondii* in Nigeria. In this study, the shedding proportion of *T. gondii*-like oocysts in cats and soil contamination levels were investigated in three communities in Ibadan, Nigeria. Soil ($n = 204$) and feral cat faecal samples ($n = 14$) were examined for the presence of oocysts using a modified sucrose flotation technique. Cat sera ($n = 15$) were also analysed for IgG antibodies to *T. gondii* by ELISA. *T. gondii*-like oocysts were identified in 21.4% (95% CI: 4.6–50.8) of the total cat faecal samples. The prevalence was 50% (95% CI: 6.7–93.3), 0% and 10% (95% CI: 0.3–44.5) in Akinyele, Laniba and Ajibode communities respectively. *T. gondii* IgG antibody was present in 86.7% of the screened cat sera (including the coppropositive cats). The seroprevalence in cats was 75% in Akinyele, 0% Laniba and 90.9% for Ajibode community ($P > 0.05$). Oocysts were recovered from 1.5% (95% CI: 0.50–4.23) of the soil samples screened and were identified from 3.8% (95% CI: 0.13–10.58) of the soil collected in Akinyele community. Akinyele also recorded the highest number of infected cats. Oocysts were identified in soil from dumpsites 2.6% (95% CI: 0.4–13.2) and residential areas 1.9% (95% CI: 0.5–6.8). Soil contaminated with *T. gondii*-like oocysts and cats shedding oocysts were found in areas with high human activities within the communities. The presence of *T. gondii*-like oocysts in the soil and the presence of cats that tested positive to antibodies specific to *T. gondii* MIC 3 Protein suggested the possibility of *T. gondii* transmission in these communities and places emphasis on its public health importance in a susceptible population.

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1. Introduction

Toxoplasma gondii is a cosmopolitan parasite that causes toxoplasmosis and is one of the most successful parasites worldwide. It is a heteroxenous coccidian parasite infecting a broad spectrum of vertebrate hosts, including humans, making it a parasite of

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zoonotic importance. Felids (domestic and wild-living cats) are essentially the only definitive hosts, excreting millions of oocysts into the environment (Dubey, 2010). The oocysts are the obligatory stage for the completion of the parasite's life cycle (Dubey, 2010). Sporulated oocysts may survive several years and may disperse through water, soil movements and microfauna. Ingesting a single sporulated oocyst may be sufficient to infect an intermediate host and begin the asexual reproduction phase (Dubey, 2010). Felids are infected by eating infected prey. The life cycle thus relies on a predator-prey relationship and environmental contamination.

The sporulated oocysts of *T. gondii* are resistant to harsh climatic conditions (Cook et al., 2002) and moist conditions are known to prolong the survival time of the oocysts to more than a year (Dubey, 2010). Ingestion of soil, food or water contaminated with sporulated *T. gondii* oocysts are significant routes of transmission to humans and animals (Aramini et al., 1999). Infections by ingestion of oocysts have been widely reported in some countries (Dubey and Jones, 2008; Zhou et al., 2011; Nasiru Wana et al., 2020) and exposure to contaminated soil is a strong risk factor particularly for children (Jones et al., 2008; dos Santos et al., 2010). The contaminated soil may also transfer oocysts to vegetables and fruits consumed by humans, thus increasing risks of primary infection (Berger et al., 2009). Varying levels of oocyst prevalence in soil have been reported (Wang et al., 2014; Solymane et al., 2014). A high oocyst dose from the environment may also be infectious for definitive hosts (Dubey, 2006), where the parasite may bypass the intermediate hosts and use a definitive host-environment cycle (Dubey, 2010); though the re-infectivity of such oocysts in cats is relatively low.

T. gondii infection presents with different clinical manifestations and can lead to severe disease or even death in immunocompromised hosts such as AIDS patients, organ transplant recipients and individuals with malignancy. It can result in abortion, stillbirth in pregnant women, or other serious morbidities in newborns. Several studies have reported *T. gondii* infection in pregnant women, immunocompromised individuals, and those with mental disorders, and in animals from different parts of Nigeria (Akanmu et al., 2010; Ayinmode and Dubey, 2012; Alayande et al., 2012; James et al., 2013; Awobode and Olubi, 2014; Ayinmode et al., 2015).

Lass et al. (2009) reported that soil contamination and the role of cats are important in the transmission dynamics of *T. gondii* and in maintaining this parasite in a contaminated environment. Infections have been linked to soil highly contaminated with oocysts and the availability of cats to maintain or release the infective stage (oocyst) into the environment (Wang et al., 2014). The presence of feral cats in the environment is of ecological and public health importance. Cats used as companion animals tend to stray from owners and become feral. They lurk around residences at night thus littering the environment with faeces (usually buried in soil) containing oocysts. Despite the available information on the prevalence of *T. gondii* infections, information on soil contamination and its direct role in enhancing *T. gondii* transmission in Nigeria remains scarce. In this study, the prevalence of *T. gondii*-like oocysts in soil and the proportion of feral cats shedding *T. gondii*-like oocysts were determined in three communities in Ibadan, one of the largest cities in Nigeria.

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2. Methods and materials

2.1. Study area

The study was carried out in three communities (Akinyele, Laniba, and Ajibode) in Akinyele Local Government Area (L.G.A.), Oyo State. Akinyele L.G.A. which is located in the northern part of the state lies on 30°45′N and 70°50′E on an elevation of 120 m above sea level and is about 20 km northwest of Ibadan city, the state capital. It is one of the 11 L.G.A. that makeup Ibadan metropolis with its headquarters situated at Moniya. The area experiences seasonal weather variation characterized by the West African monsoon climate, with distinct seasonal shift in the wind pattern. The inhabitants of the communities are predominantly traders, farmers, with few students and civil servants.

2.2. Soil collection

A total of 204 soil samples were collected from the three communities; Akinyele ($n = 79$), Laniba ($n = 37$), and Ajibode ($n = 88$). Soil collection points were around residential areas, dumpsites, community playgrounds, schools, water sources, drainage areas, and hospitals. Sampling was done on the surface layer to a depth of 5 cm. A composite sample (300 g) was made from soil collected from 3 to 4 points, 2 m apart at each collection point. The coordinates of each sample collection point were taken with an Etrex GPS for spatial analysis. Soil temperature and pH were taken *in situ* using a 4 in 1 soil survey instrument (AMT-300, AMTAST, China). The collected soil samples were transported to the laboratory, dried for 48 h at room temperature and sieved through a 150 μm mesh sieve (Matsuo et al., 2004; Mizgajska-Wiktor, 2005).

2.3. Cat blood and faecal collection

A direct overnight live trapping method was used to trap cats (Fredebaugh, 2010). Cat traps were set at dusk using baits and checked for cats at dawn. Trapped cats were immobilized using (0.3 ml) Ketamine intramuscularly (IM) (Dubey, 2010). Blood (2 ml) was drawn from the jugular vein of each cat into non-heparinised tubes and allowed to stand at room temperature for about 1 h. Separated serum was stored at -20 °C until used. Faecal samples in the traps were collected in wide-mouthed bottles for analysis. In the absence of faeces upon trapping, cats were transported to the Animal House Facility of the Department of Zoology, University of Ibadan, Ibadan where they were housed until faeces was available for collection. Afterward, trapped cats

were marked and released to avoid rescreening within the study period. Blood samples were collected by trained veterinarians at the University of Ibadan Veterinary Hospital and all trapped cats sampled in this study were handled in strict accordance with good animal practice and guidelines (Use of Animals in Research and Education: Terrestrial Animal Health Code- OIE).

2.4. Isolation of T. gondii oocysts from soil

Using a modified sucrose floatation technique (Matsuo et al., 2004), 100 g of the composite soil sample from each of the collection points was washed through 150 μm sieves and the solution collected was allowed to settle for 10–15 min. The supernatant was discarded, and the top layer of the sediment poured into a disposable cup (about 100 ml) and left to stand for 5–10 min. This was carefully decanted; the sediment was transferred into a centrifuge tube and centrifuged at 2000 rpm for 5 min. The supernatant was again carefully discarded leaving the sediment. This sediment was gently mixed with Sheathers (sucrose) solution (specific gravity of 1.21) and centrifuged. After centrifugation, more sheathers solution was added up to the brim of the tube until a convex meniscus was formed, and then a coverslip was gently placed over the tube. This was allowed to stand for another 5 min to allow sufficient time for the oocysts to float and adhere to the coverslip. The coverslip was transferred onto a slide and examined under a microscope.

2.5. Oocysts recovery from cat faecal matter

Faeces (1 g) was homogenized in 13 ml of distilled water in a disposable plastic cup and poured into a 15 ml centrifuge tube before centrifugation at 1500 rpm for 5 min (Dryden et al., 2005). The supernatant was discarded and sucrose solution (specific gravity 1.21) was added to the sediment and again centrifuged. The content of the centrifuge tube was topped to the brim with more sucrose solution using a Pasteur pipette to form a convex meniscus and a coverslip was gently placed on it and allowed to stand for 5 min. The coverslip was transferred to a slide and examined as described by Dryden et al. (2005). *T. gondii*-like oocysts found in soil and cat faecal samples were morphologically identified (Georgi, 1985; Bowman et al., 2002; Kouassi et al., 2015).

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2.6. Enzyme-linked Immunosorbent Assay (ELISA)

Specific antibodies to *T. gondii* in cat serum were detected by ELISA. Optimal working dilutions and concentrations for the various reagents and solutions were first determined by chequerboard titrations. Immulon 96-well flat-bottom ELISA plates were coated (100 μl per well) with the antigen, *T. gondii* MIC 3 Protein, (CD biosciences, New York, USA ®) at 2.5 μg/ml concentration and incubated overnight at 4 °C. The well contents were aspirated, and the plate washed 5 times for 5 min each with phosphate-buffered saline and 0.3% Tween 20 (PBST). The unreactive surfaces in the wells were subsequently blocked using blocking buffer (5% non-fat dried milk in PBS) at 100 μl per well and incubated for 2 h at room temperature. After blocking, serum samples diluted in blocking buffer (1:160) were added to the wells (100 μl per well) and then incubated overnight at 4 °C. Plate were then washed 5 times each for 5 min with PBST. Horse Radish Peroxidase (HRP) anti-cat IgG conjugate (Sigma®) diluted 1:2000 in blocking buffer was added to each well at 100 μl per well and incubated for 1 h at room temperature. The plate was washed again thoroughly before O-Phenylenediamine (OPD) substrate solution was added to each well (100 μl per well); the enzyme-substrate reaction was allowed to develop in the dark for 30 min at room temperature. The reaction was terminated with 2% sulphuric acid (100 μl/well) and plates were read at 492 nm. All samples were run in duplicate. Known positive (serum from a previously identified cat shedding *T. gondii* oocysts), negative (serum from laboratory-bred mice), and blank (water) samples were included in each plate. The cut-off point was determined as the mean optical density (O.D) readings of negative controls +2 x Standard deviation (Wonsit et al., 1992).

2.7. Spatial analysis

The distribution map was produced from the GPS coordinates obtained during sampling using Google Earth Software® and ArcGIS 10.1®.

2.8. Statistical analysis

Data obtained from the study were analysed using Quantitative Parasitology Software version 3.0 (Reiczigel and Rozsa , 2005) to determine parasite prevalence and mean intensity. Also, SPSS version 21 and Graphpad Prism version 5 were used to determine significant differences between communities. *P*-value <0.05 was considered significant.

3. Results

3.1. Proportion of Toxoplasma gondii-like oocyst shedding in feral cat faeces

T. gondii-like oocysts were identified in 3 (1.5%, 95% CI: 0.50–4.23) of the 204 soil samples, Oocysts were isolated only from soil samples from Akinyele community and constituted 3.8% (95% CI: 0.1–10.5) of the soil samples collected from the community (Table 1). The mean intensity of *T. gondii*-like oocysts was 33.3 oocysts per 100 g of soil. Soil samples contaminated with

Table 1
Prevalence and mean intensity of *Toxoplasma gondii*-like oocysts in soil samples within study communities.

Study communities	Number examined	Number positive (Prev. %; 95% CI)	Mean intensity
Akinyele	79	3 (3.8; 0.13–10.58)	33.3
Laniba	37	0	0
Ajibode-UI	88	0	0
Total	204	3 (1.5; 0.50–4.23)	33.3

T. gondii-like oocysts were from dumpsites: 2.6% (95% CI: 0.4–13.2) of 39 soil samples, and residential areas: 1.9% (95% CI: 0.54–6.87) of 102 soil samples. The recorded soil temperature in the study locations ranged from 24 to 36 °C (29.9 ± 0.18) with no significant differences ($P = 0.38$) between locations. Meanwhile, the soil pH in Akinyele community where all the contaminated soil samples were found, was more acidic (5.88 ± 0.07, $P = 0.00$) than in the other communities (Laniba 6.80 ± 0.19 and Akinyele 6.51 ± 0.12).

3.2. Proportion of *T. gondii*-like oocyst shedding in feral cat faeces

T. gondii-like oocysts were identified in 3/14 (21.4% 95% CI: 4.6–50.8) of cat faecal samples examined by microscopy. The proportion of trapped cats shedding *T. gondii*-like oocysts was 50% (95% CI: 6.7–93.2), 0% and 10% (95% CI: 0.2–44.5) in Akinyele, Laniba and Ajibode communities respectively. The mean parasite intensity recorded was 280 oocysts per gram of faeces. The intensities for Akinyele and Ajibode were 360 and 120 oocysts per gram of faeces, respectively.

3.3. Seroprevalence of *Toxoplasma gondii* specific IgG

Of the 15 cats, 86.7% (95% CI: 62.1–96.3) tested positive to *T. gondii* antibody (IgG) (Table 2). All three cats with microscopy positive faecal samples were also positive by ELISA. There were 75% (95% CI: 30.1–95.4) and 90.9% (95% CI: 62.3–98.4) cats positive for IgG antibodies in Akinyele and Ajibode communities respectively with no significant differences ($P = 0.56$) (Table 2).

3.4. Spatial distribution

In Akinyele community, all three soil samples positive for *T. gondii*-like oocysts were collected within the same sampling area approximately 3 m apart from each other. Indeed, feral cats copropositive for *T. gondii*-like oocyst and seropositive to *T. gondii* antibody were also trapped in the same area. In Ajibode, *T. gondii* oocysts were not identified in the soil but seropositive cats were found in three of the trap points. Cats were not trapped in Laniba and no oocysts were identified in the soil from the community (Fig. 1).

4. Discussion

Soil is a potential environmental source of *T. gondii* and other parasitic infections in animals and humans (Dabritz and Conrad, 2010). In the natural environment, oocysts and other parasite ova can remain infective for several months (Dumètre and Dardé, 2003). Environmental contamination with infective stages of disease causative agents contributes significantly to the transmission and maintenance of diseases in a given community as well as hampers control efforts.

In this study, we examined soil samples for contamination with *T. gondii*-like oocysts; and the low prevalence of oocysts found was similar to soil contamination reported in Lyon, France (Afonso et al., 2008). However, Lass et al. (2009) and Du et al. (2012) recorded higher prevalences in Poland and China, respectively. Our prevalence value is also lower than the prevalence of oocysts of *T. gondii* reported in the city of Ahvaz, Iran, where 9% of 200 soil samples collected across sand pits, playgrounds, public parks and areas around rubbish dump sites (Saki et al., 2017) were contaminated. Low prevalence has sometimes been attributed to the small proportion of the total land area used by cats for defecation (Afonso et al., 2008). Cats often defecate in places where the faeces are used as scent marks and several cats may use a single location especially when cat density is high (Uga et al., 1996; Turner and Bateson, 2000). This supports the observation of low prevalence of *T. gondii* oocysts in soil sampled across multiple sites as shed oocysts may be unevenly distributed unless factors like rainfall help in the spread during runoffs. In this study, oocysts identified in soil collected from dumpsites and around residential areas were likely from faeces deposited in the soil by cats, because they were frequently seen in these areas at dusk in search of food and/or water. The presence of *T. gondii*-like oocysts in soil from the studied communities could potentially increase the risk of human infection since high soil contact is common in rural agrarian communities. This is also supported by a study that suggested that in rural environments with low oocyst count, there is a higher risk of human exposure to infection when human soil contact is high (Afonso et al., 2008).

Dumètre and Dardé (2003) and Lass et al. (2009) suggested that the common places for human sources of infection in both rural and urban communities are likely to include public enclosures, playgrounds and drinking water systems, especially for children, who may accidentally swallow *T. gondii* oocysts during play hours (Ajmal et al., 2013). Bowie et al. (1997) also suggested that the transmission rate of *T. gondii* increases if it accidentally finds its way into water bodies. No positive soil from playgrounds was detected in this study; the uneven distribution of *T. gondii*-like oocysts in soil due to cat defecation behavior may explain the

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Table 2
Seroprevalence of *T. gondii* IgG in feral cats within study communities, Oyo State.

Communities	No examined	IgG positive	% Positive	95% (CI)
Akinyele	4	3	75	(30.1–95.4)
Laniba	–	–	–	–
Ajibode-UI	11	10	90.9	(62.3–98.4)
Total	15	13	86.7	(62.1–96.3)

"–" no trapped cats.

absence of oocysts in soil samples from other sites in the communities. In a study in central California, soil contamination in urban public places like parks, public playgrounds and community gardens was found to show large seasonal and spatial variation and *T. gondii* was only detected during fall and only in coastal sites (de Wit et al., 2020). The variation in oocyst distribution as has been reported in many studies may explain the low prevalence of oocysts found in this study. Oocyst recovery or detection in soil or other environmental matrices maybe affected by the lack of techniques with sufficient sensitivity to detect and identify oocysts (Dabritz and Conrad, 2010; Dumètre and Dardé, 2003). Nevertheless, the detection of oocysts around residences demonstrates a significant public health concern especially for children and immunosuppressed or immunocompromised individuals.

Temperature is another factor that affects the survival and infectivity of *T. gondii* oocysts, as well as the population density of the intermediate and mechanical host (Gilot-Fromont et al., 2012). The generally warm temperature recorded in this study supports the survival and development of *T. gondii* oocysts. However, other factors such as the densities of intermediate and definitive hosts are also important dynamics in the life cycle, which may also influence the parasite population. The low number of cats trapped in the study is suggestive of a low feral cat population in these communities and therefore explains the low proportion of oocysts encountered. Du et al. (2012) stated that cat densities are indicative of the level of soil contamination with *T. gondii*.

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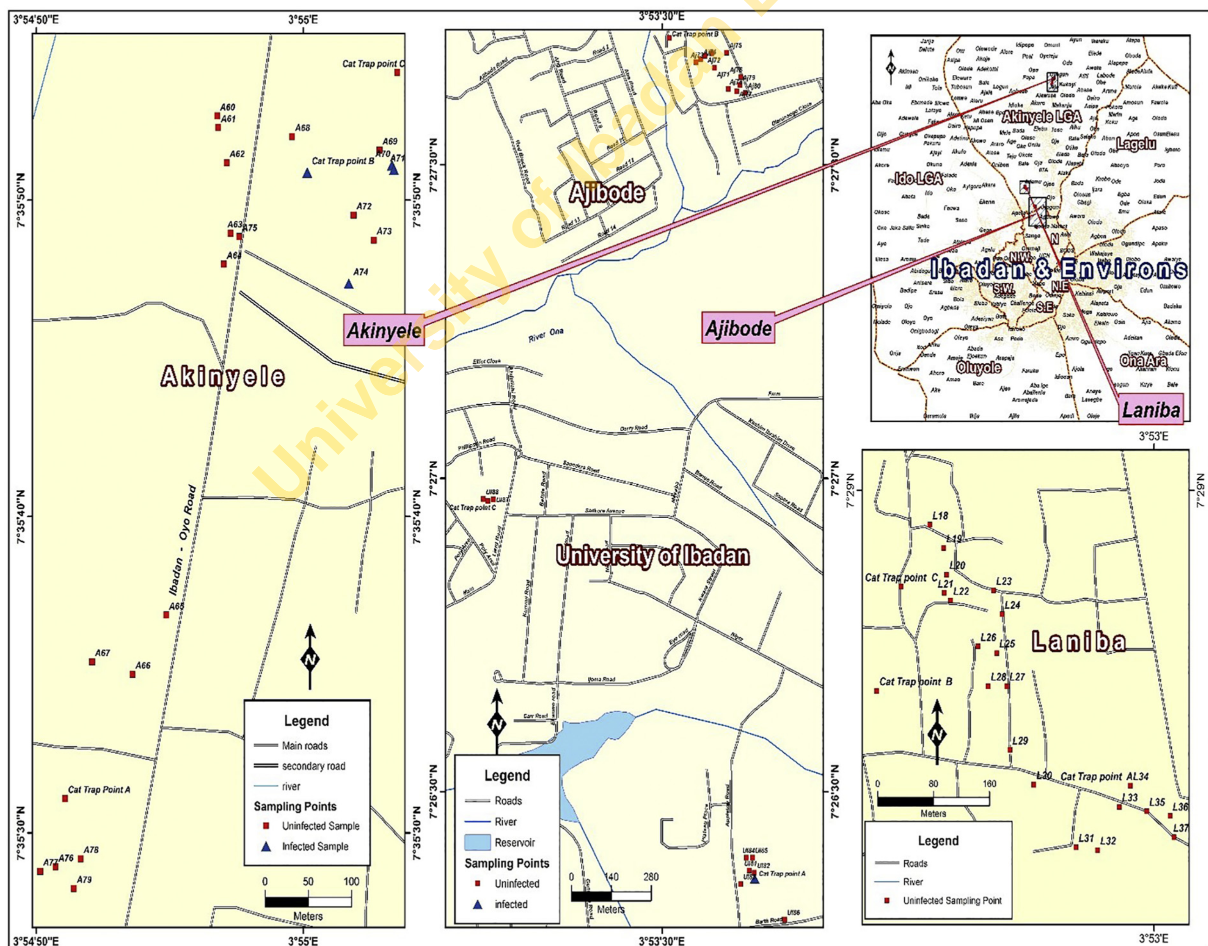

Fig. 1. Map showing parasite distribution in the study communities.

Although the population of cats in the communities was low, the few cats kept as companion animals, were also allowed to roam about freely. This was observed in the community where oocyst-contaminated soil was found, thus increasing the likelihood of human infection. This may enhance the transmission of *T. gondii* in the communities. Also, the presence of infected cats in certain areas within the communities where human activities are high suggests such places as potential high-risk regions. Studies have shown that stray cats are highly susceptible to *T. gondii* infection while feral cats are two times more likely to be infected than domestic cats (Kamani et al., 2010; Gyorke et al., 2011). Awoke et al. (2015) identified the presence of cats around homes as a risk factor for *T. gondii* infection; and the fact that the trapped cats in this study were shedding *T. gondii*-like oocysts during the time of capture is indicative of active transmission in the communities.

Since felids are the only known host to excrete *T. gondii* oocyst, detection of either IgG or IgM would suggest the infection status of cats (Jones and Dubey, 2010). The seroprevalence values in this study agree with those of Kamani et al. (2010) from North-eastern Nigeria but are in contrast with the lower seroprevalence reported by Alayande et al. (2012) in Northwestern Nigeria. It was also higher than the world average (30–40%) in cats as stated by Elmore et al. (2010) and Lilly and Wortham (2013). This indicates that the study communities may be highly contaminated (Jones and Dubey, 2010).

4.1. Study limitation

In this study, the low number of cats sampled, the limitation of microscopy (or morphology) in confidently identifying oocysts of *T. gondii* and the lack of specific/sensitive molecular identification methods were limitations which could potentially influence the interpretation of the results. Therefore, we referred to the encountered oocysts as *T. gondii*-like oocysts.

4.2. Conclusion

The spatial distribution of cats which tested positive for *T. gondii* and soil contaminated with *T. gondii*-like oocysts in areas of high human activities (residence and dumpsites) suggest that the risk of infection in this community is of sufficient concern. The level of soil contamination was higher in the areas where the number of feral cats was higher. The possibility of *T. gondii* transmission in the communities and the implications particularly for children who play closely with soil and immunocompromised individuals is of public health concern. Thus, the adoption of proactive measures including health education aimed at improving personal/environmental hygiene to interrupt transmission is recommended.

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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