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Survey on the contamination of *Toxoplasma gondii* oocysts in the soil of public parks of Wuhan, China

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ABSTRACT

Toxoplasma gondii of warm-blooded animals and humans is an important pathogenic agent throughout the world. Soil is increasingly recognized as an important source in the transmission of *Toxoplasma*. To attain the contamination status of *T. gondii* in the soil of public parks, a total of 252 soil samples were collected from September 2009 to August 2010 at different sites located in 6 public parks of Wuhan, Hubei, China and detected by PCR and loop-mediated isothermal amplification (LAMP). The detection limit of PCR/B1, PCR/529 and LAMP was determined to be 50, 5, and 5 tachyzoites in soil, respectively. Forty-one samples were found positive for *Toxoplasma* DNA by PCR on both genes, whereas LAMP products were generated in 58 samples ($\chi^2 = 3.6328$, $P = 0.0567$). All parks were found contaminated and no significant difference was found among the parks (PCR: $\chi^2 = 0.0072$, $P = 0.9325$; LAMP: $\chi^2 = 0.6101$, $P = 0.4347$). However, contamination was found with significantly different among the four seasons (PCR: $\chi^2 = 11.6066$, $P = 0.0007$; LAMP: $\chi^2 = 12.4636$, $P = 0.0004$), with a gradual decrease in the prevalence from spring to winter on both analyses. This is the first investigation on soil contamination of public parks in China by *T. gondii* oocysts. The results indicate that the soil of public parks contaminated with *T. gondii* oocysts may play a role in the epidemiology of toxoplasmosis and effective preventive measures should be considered. Moreover, the conventional PCR and LAMP used in the present study are applicable to detect *T. gondii* oocysts in soil samples.

1. Introduction

Toxoplasma gondii, an apicomplexan protozoal parasite capable of infecting all warm-blooded animals worldwide, including humans, is the causative agent of toxoplasmosis. This is one of the most prevalent parasitic infections in animals and humans (Dubey and Beattie, 1988). However, infections with *Toxoplasma* are generally asymptomatic except after congenital transmission, when associated with abortions or clinical diseases, and in immunocompromised individuals such as patients suffered organ transplantation and AIDS (Dubey, 2008; Dubey and Jones, 2008; Dumètre and Dardé, 2003). *T. gondii* infection in pregnant women can result in fetal diseases, with severe problems, including abortion, encephalitis, malformations, mental retardation, loss of vision and sub-clinical symptoms (Afonso et al., 2008; Dubey and Jones, 2008; Yang et al., 2009). *T. gondii* infection in AIDS and other immunocompromised patients can cause life-threatening disease (Dubey, 2008; Lass et al., 2009). Moreover, severe infections may lead to neonatal

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deaths and abortion in animals (Dubey and Jones, 2008; Dumètre and Dardé, 2003).

T. gondii infections seem to be strongly associated with soil contact, as the suspected source of outbreaks was the oocysts from soil. So far, soil-borne transmission toxoplasmosis outbreaks have been documented in USA and Brazil (Coutinho et al., 1982; Stagno et al., 1980). The oocysts were isolated from the soil during an outbreak of toxoplasmosis in a rural area of Brazil in spite of no direct evidence of association of outbreak with soil (Stagno et al., 1980). Dabritz and Conrad (2010) summarized the association of prevalence of *T. gondii* with soil in humans. Thus, soil is increasingly considered as an important source in the transmission of *Toxoplasma*.

So far, a few studies have been conducted worldwide to determine the status of environmental contamination of this parasite. Contamination was maximal at cat defecating sites. Afonso et al. (2008) found soil samples contaminated by *T. gondii* oocysts only at the defecation sites of the cats, where the level of contamination was high. Similarly, contaminated soil samples were only found in areas used by cats (Lass et al., 2009). dos Santos et al. (2010) found that *T. gondii* oocysts were widely distributed in the soil of elementary public schools from the northwest area of the state of São Paulo using mouse bioassays. However, the report regarding seasonal variations on soil contamination of public parks by *T. gondii* oocysts is still unavailable. Thus, the objective of present study was to determine the frequency of occurrence of *T. gondii* oocysts in the soil of public parks in Wuhan city, China with respect to health risks to animals and human beings, and to determine the possible existence of seasonal variations.

2. Materials and methods

2.1. T. gondii strain

T. gondii of RH strain was obtained from National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China. Tachyzoites of *T. gondii* (RH) strain were harvested from the ascites of BALB/c mice infected 5–6 days earlier by injecting 1 ml of 0.1 M phosphate buffered saline (PBS) pH 7.2 as described previously (Fang et al., 2009). *T. gondii* of the Prugniaud strain was provided by Xinxiang Medical College, Henan, China.

2.2. Collection of soil samples

Two hundred fifty-two soil samples were collected from September 2009 to August 2010 at different sites located in six public parks of Wuhan, Hubei, China, namely Zhongshan Park, Ziyang Park, Lotus Lake Park, Hongshan Park, Shouyi Square and Guqintai Culture Square (Table 2). Wuhan (29°58′and 31°22′ N latitude, 113°41′ and 115°05′ E longitude) lies in the subtropical zone with sufficient sunshine and abundant rainfall especially from May to July, and the mean relative humidity is high in all seasons of year. According to the climate of Wuhan, the whole year was divided into following four seasons: (i) spring (March to May) (ii) summer (June to August) (iii) autumn (September to November) and (iv) winter (December to February). Because of the influence of monsoon, summer is hot and rainy, while winter is cold and dry in Wuhan. In summer, the highest temperatures range from 37 to 40 °C, but the lowest temperatures are also higher than 29–30 °C and the extreme temperature of the ground may exceed to 50 °C at some places.

All parks are located in the urban area with high visitor flow rate. The soil samples were taken from areas where cats often appear. Soil was removed from the surface layer of the ground (top 5 cm) with stainless steel shovel, air-dried and sieved using a 20 mesh to remove stones and organic detritus. Finally, 0.5 g of the soil prepared in this way were taken for further analysis.

2.3. DNA extraction

Soil DNA extraction was performed using the commercial E.Z.N.A.™ Soil DNA Kit (OMEGA, USA) according to the manufacturer's instructions. One hundred microliters of DNA was eluted per sample, and DNA was stored at −20 °C until use for detection.

2.4. Conventional PCR

T. gondii B1 gene (AF179871) and 529 bp repetitive fragment (AF146527) were selected as the targets for PCR. The B1 gene consists of 35 copies, and it is highly conserved among strains of *T. gondii* (Burg et al., 1989). The 529 bp repetitive fragment consists of 200–300 copies in the genome of *T. gondii* (Homan et al., 2000). The B1 and 529 primer sequences are shown in Table 1. Both PCR were performed according to the conditions described by Burg et al. (1989) with the exceptions that the reaction mixture volume was 25 μl and 400 ng/μl of non-acetylated bovine serum albumin (Sigma) were added in the reaction mixture (Jiang et al., 2005). PCR products were analyzed by electrophoresis on a 1.0% agarose gel stained with ethidium bromide for visualization under a UV transilluminator.

2.5. LAMP

Amplification was performed according to the conditions described by Nie (2010). Six oligonucleotide primers were used for the LAMP assay targeting eight conserved regions within the sequence of MIC3 gene of *T. gondii* (Table 1). The LAMP products were visualized by 1.5% agarose gel electrophoresis. Visual inspection of LAMP amplicons in the reaction tube was performed by adding 1 μl of 1/10 dilution of SYBR Green I (Invitrogen, Australia) after the reaction, and the fluorescent signals of the solutions were observed under UV light.

Each sample was amplified in triplicate in order to determine the reproducibility of the PCR and LAMP. Moreover, to verify the effect of residual PCR inhibitors in all DNA samples, 2 μl of each DNA template mixed with 1 μl of genomic DNA from the *T. gondii* RH strain were co-amplified in PCR and LAMP.

2.6. Specificity of PCR and LAMP

DNA derived from *Schistosome* eggs, *Toxocara cati* eggs, *Streptococcus pneumoniae*, *Escherichia coli*, *Actinobacillus*