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Seroprevalence, disease awareness, and risk factors for *Toxocara canis* infection among primary schoolchildren in Makoko, an urban slum community in Nigeria

Pam V. Gyang[a,b,c], Olaoluwa P. Akinwale[c], Yueh-Lun Lee[d], Ting-Wu Chuang[a], Akwaowo B. Orok[e], Olusola Ajibaye[e], Chien-Wei Liao[a], Po-Ching Chen[a], Chia-Mei Chou[a], Ying-Chieh Huang[a], Ursula Barghouth[f], Chia-Kwung Fan[a,b,g,*]

[a] *Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[b] *Department of Molecular Parasitology and Tropical Diseases, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[c] *Public Health Division, Molecular Parasitology Research Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria*
[d] *Department of Microbiology and Immunology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[e] *Biochemistry and Nutrition Division, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria*
[f] *A.T. Still University School of Osteopathic Medicine in Arizona, USA*
[g] *Master Program in Global Health and Development, College of Public Health and Nutrition, Taipei Medical University, Taipei, Taiwan*

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ABSTRACT

In this study, we investigated the seroprevalence of *Toxocara canis* infection in southern Nigeria, which previously was unknown, in addition to evaluating disease awareness and potential risk factors for schoolchildren in an urban slum community. In total, 366 primary schoolchildren were investigated for the presence of anti-*Toxocara* IgG antibodies. Blood was collected and screened by a Western blot analysis based on the excretory–secretory antigens of larval *T. canis* (TcES), targeting low molecular weight bands of 24–35 kDa specific for *T. canis*. Children were considered seropositive if their serum reacted with TcES when diluted to a titer of 1:32. Questionnaires concerning possible risk factors were given to the schoolchildren to acquire data on this infection. The overall seroprevalence of *Toxocara* infection was 86.1% (315/366). The logistic regression analysis of risk factors showed that children's age (odds ratio (OR) = 2.88, 95% confidence interval (CI) = 1.08–7.66, $p = 0.03$), contact with dogs (OR = 0.51, 95% CI = 0.28–0.94, $p = 0.03$), the age of the dog (OR = 0.34, 95% CI = 0.18–0.68, $p = 0.002$), the feeding location of the dog (OR = 0.31, 95% CI = 0.12–0.79, $p = 0.01$), the consumption of raw vegetables (OR = 0.89, 95% CI = 0.54–1.48, $p = 0.004$), and the drinking of unboiled water (OR = 0.48, 95% CI = 0.26–0.90, $p = 0.02$) were risk factors associated with *Toxocara* infection. Although there was a high awareness of dogs being hosts of some parasites in this study, not much was known about *T. canis*. This is the first serological investigation of *T. canis* infection among primary schoolchildren in southern Nigeria. The high seroprevalence recorded is an indication of high transmission with the consequent risk of visceral or ocular larval migrans and neurologic toxocariasis in these children. Our findings suggest the need for prompt interventional measures, particularly health education on personal hygiene.

1. Introduction

Toxocariasis is a zoonotic parasitic infection with a wide geographical distribution, including developed and developing countries, and especially in communities with poor standards of hygiene and sanitation (Smith et al., 2009). An estimated 2 billion people are at risk of infection, and the geographical distribution is expanding as a result of human and animal movements, together with the effects of global warming (Jenkins et al., 2013). Toxocariasis is caused by dog roundworms, *Toxocara canis*, and cat roundworms, *T. cati*, to a lesser extent, both of which inhabit the lumen of definitive hosts (Overgaauw and van Knapen, 2013).

Worldwide, dogs are the most successful canids at adapting to human habitation and contribute to the physical, social,

* Corresponding author at: Department of Molecular Parasitology and Tropical Diseases, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. Tel.: +886 2 27395092; fax: +886 2 27395092.
E-mail address: tedfan@tmu.edu.tw (C.-K. Fan).

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and emotional well-being of their owners, especially children (Robertson et al., 2000). Global surveys have shown that the occurrence of *T. canis* ranges from 86% to 100% in puppies and 1% to 45% in adult dogs (Soriano et al., 2010; Fan et al., 2013). In Nigeria, prevalence in dogs was reported as 33.8%, 41.1%, and 56.6% in the north, south, and southwestern parts of the country, respectively (Dada et al., 1979; Arene, 1984; Sowemimo, 2009).

Humans are among the accidental hosts of this parasite, and infection is largely a result of ingestion of soil, water, or food contaminated with embryonated ova. Consumption of chicken and cow livers is another rare route (Choi et al., 2012). Although human infection with *T. canis* is typically asymptomatic, it can be associated with severe clinical syndromes due to organ injury by migrating larvae (Glickman et al., 1979). The predominant clinical syndromes are classified as visceral larva migrans (VLM), ocular larva migrans (OLM), and common, neurologic, and covert toxocariasis. All these depend on the organs affected and the specificity of the symptoms (Magnaval et al., 2001).

Children in their first decade of life appear to be most prone to this infection due to their geophagic behavior and mouthing of objects, which is further linked to a high risk of exposure at playgrounds contaminated with dog feces (Despommier, 2003). Toxocariasis in children has been associated with endophthalmitis, generalized lymphadenopathy, endomyocarditis, asthma, hepatosplenomegaly, and meningoencephalitis (Bächli et al., 2004; Nicoletti et al., 2007; Quattrocchi et al., 2012).

Since the parasites cannot mature to the adult stage in humans, the direct microscopic diagnosis of ova in stool is not possible. Using a biopsy for a diagnosis is also extremely difficult, and so serological methods are the diagnostic mainstay. A diagnosis relies on a *T. canis* larval excretory–secretory antigen (TcES)-based enzyme-linked immunosorbent assay (ELISA) (Fan et al., 2013; Macpherson, 2013). It was shown that at a threshold titer of 1:32, the sensitivity and specificity were 78% and 92%, respectively (Jones et al., 2008; Smith et al., 2009). However, in areas where polyparasitism is common (for example with *Ascaris lumbricoides*), antigenic cross-reactivity reduces the usefulness of such assays (Smith et al., 2009). Western blotting based on fractionated, native, and excretory–secretory (ES) antigens of *T. canis* larvae (TcES-WB) can yield superior specificity levels and exhibits reactivity to bands of low molecular weights (24–32 kDa) that were proven to be specific to *T. canis* infection (Smith et al., 2009). Polyparasitism was reported in Nigeria by several studies (Nock et al., 2003; Ugbomoiko et al., 2006; Oninla et al., 2010); hence in the present study, TcES-WB was used to detect *T. canis*-specific immunoglobulin G (IgG) and estimate the seroprevalence of *T. canis* infection in schoolchildren.

The only serological survey in Nigeria was conducted in 2000 in the north (Ajayi et al., 2000) using the TcES-ELISA. Clear ideas of the status and importance of toxocariasis within the country are still lacking. Hence this study, the first in the southern part, can provide much-needed current data on the seroprevalence of *T. canis*, especially among schoolchildren in the Makoko community, an urban slum with poor sanitation, unsafe drinking water, and no established de-worming programs.

2. Materials and methods

2.1. Ethical considerations

The guidelines laid down in the *Declaration of Helsinki* for procedures involving human subjects were strictly adhered to, and approval for this study was granted by the Institutional Review Board (IRB) of the Nigerian Institute of Medical Research (NIMR) (project no.: IRB/13/225).

Prior to the commencement of the study, permission was also obtained from the local education authority in the Local Government Area (the Mainland LGA). Approval was also obtained from the head teachers of each selected school and from parents/guardians of the children. Meetings were held to explain to teachers and pupils the objectives and protocol of the study, emphasizing that participation was voluntary, and withdrawal was allowed. Signed or thumb-printed consent was obtained from parents/guardians on behalf of their children before sample collection commenced.

2.2. Study area

This cross-sectional study was carried out in November 2013–March 2014, in Makoko, an urban slum located in the Mainland LGA in Lagos State, Nigeria. Makoko lies within latitude 6°28′ and 6°29′ and longitude 3°12′ and 3°13′. It has an estimated population of about 100,000 inhabitants (Simon et al., 2013). The settlement is located partly on land and partly on the Lagos lagoon. Ethnically, the community is dominated by the Ilajes and Eguns, and there are also Yorubas, Igbos and other ethnic groups (Oduwaye et al., 2010). The major occupation is fishing and trading, especially for those living on the lagoon.

Makoko suffers from serious environmental and infrastructural deficiencies, including inadequate access roads, schools, healthcare facilities, and housing (Simon et al., 2013). This study was conducted on the land portion of the community using the only three government-built primary schools (located in the same vicinity): Adekunle Anglican Church Primary School, Ayetoro African Church Primary School, and Makoko Anglican Church Primary School.

2.3. Study population and sampling strategy

The study population constituted of children attending the only three government-built, primary schools in the community. Medical laboratory scientists collected blood specimens after verifying that informed consent forms had been received from the pupils. Attached to the consent forms were structured questionnaires designed to capture information about the generalized symptoms of toxocariasis (cough, fever, and visual discomfort) and obtain basic demographic data regarding the age, gender, and parental educational level and occupation. Health workers used standard calibrated instruments to measure height and weight. The children, whose ages ranged 7–17 years with an overall mean age (standard deviation; SD) of 10.73 (2.11) years, were divided into three age groups, to maintain a sufficient sample size in the analysis: group 1 contained students who were <10 years old; group 2 children 10–12 years old; and group 3 children >12 years old. Parental occupation was also categorized into three groups: group 1 was unemployed; group 2 was unskilled workers; and group 3 was skilled workers. The study also examined other risk factors in the environment including contact with dogs, dog feeding practices, contact with soil, ingestion of unsafe water, and consumption of raw or undercooked meat. These were then used in the multivariate analysis.

2.4. Blood collection

About 3–5 ml of blood was drawn by venipuncture by healthcare workers/nurses. Each sample was allowed to clot in order to separate the serum, and then stored at −20 °C.

2.5. Toxocara *egg culture*

Adult *T. canis* worms were collected from the stool of dogs that have been treated with mebendazole. Eggs were harvested from the anterior third of the uteri of females, and were cultured as described by Bowman et al. (1987). The eggs were stirred in a 1% (w/v) sodium