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Field evaluation of a rapid diagnostic test to detect antibodies in human toxocariasis

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ABSTRACT

Human toxocariasis which is caused mainly by the larvae of *Toxocara canis* and *Toxocara cati*, is a worldwide zoonotic disease that can be a potentially serious human infection. The enzyme-linked immunosorbent assay (ELISA) using *T. canis* excretory–secretory (TES) antigens harvested from *T. canis* larvae is currently the serological test for confirming toxocariasis. An alternative to producing large amounts of *Toxocara* TES and improved diagnosis for toxocariasis is through the development of highly specific recombinant antigens such as the *T. canis* second stage larva excretory–secretory 30 kDa protein (recTES-30). The aim of this study was to evaluate the sensitivity and specificity of a rapid diagnostic kit (RDT, named as iToxocara kit) in comparison to recTES-30 ELISA in Serendah Orang Asli village in Selangor, Malaysia. A total of 133 subjects were included in the study. The overall prevalence rates by ELISA and RDT were 29.3% and 33.1%, respectively, with more positive cases detected in males than females. However, no association was found between toxocariasis and gender or age. The percentage sensitivity, specificity, positive predictive value and negative predictive value of RDT were 85.7%, 90.1%, 80% and 93.2%, respectively. The prevalence for toxocariasis in this population using both ELISA and RDT was 27.1% (36/133) and the K-concordance test suggested good agreement of the two tests with a Cohen's kappa of 0.722, $P < 0.01$. In addition, the followed-up Spearman rank correlation showed a moderately high correlation at $R = 0.704$ and $P < 0.01$. In conclusion, the RDT kit was faster and easier to use than an ELISA and is useful for the laboratory diagnosis of hospitalized cases of toxocariasis.

1. Introduction

Human toxocariasis which is caused mainly by the larvae of *Toxocara canis* and *Toxocara cati*, is a worldwide zoonotic disease that can be a potentially serious human infection. In Malaysia, studies have shown that the prevalence of human toxocariasis ranged from 19.6% to as high as 35.5% (Hakim et al., 1992, 1993). The disease normally presents as either visceral or ocular although some patients are asymptomatic. Common manifestations include persistent eosinophilia, fever, pneumonitis, visual field defects and neurological disturbances (Watthanakupanich, 2010). Cases of visceral larva migrans (VLM) are mostly asymptomatic or present with mild symptoms while cases of ocular larva migrans (OCM) may have chronic inflammation and permanent eye damage. Covert toxocariasis is most frequently found in children and the clinical symptoms range from fever, headache, abdominal pain to cervical lymphadenitis and hepatomegaly (Watthanakupanich, 2010).

As many of the symptoms of toxocariasis are nonspecific and mild, diagnosis of the disease can be quite difficult. Chronic eosinophilia, hepatomegaly, chronic pulmonary disease, or a history of exposure to puppies or contact with feces-contaminated soil are common indicators of infection. So in addition to taking patient history to determine exposure to risks, laboratory investigations are useful for the diagnosis and various combinations of tests are often used for confirmation. Microscopic or macroscopic examination of the parasite remains the gold standard for protozoan and helminthic parasites including *Toxocara* spp. *Toxocara* larva can be identified from affected tissue (Parsons et al., 1986) or under the retina depending on the site of infection (Singh et al., 2007).

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More recently, molecular tools such as polymerase chain reaction, DNA hybridization and DNA sequencing technology have been used to diagnose *Toxocara* infections (Rai et al., 1997; Wu et al., 1997; Van De et al., 2013). However, serological assays using immunological techniques are considered the most effective approach for laboratory diagnosis of *Toxocara* infection and most of the assays detect anti-*Toxocara* antibodies. The enzyme-linked immunosorbent assay (ELISA) using *T. canis* excretory–secretory (TES) antigens harvested from *T. canis* larvae is currently the serological test for confirming toxocariasis (Magnaval et al., 2001) but some cross reactions have been reported with other nematodes (Kennedy et al., 1987, 1989; Yamasaki et al., 2000). Other toxocariasis diagnostic methods that were developed based on TES to improve sensitivity and specificity included the indirect antibody competition ELISA (Nunes et al., 1999), dot-ELISA (Carmago et al., 1992), and antigen capture assays using monoclonal antibodies (Gillespie et al., 1993; Ngah et al., 1999). An alternative to producing large amounts of *Toxocara* TES and improved diagnosis for toxocariasis is through the development of highly specific recombinant antigens which share important antigenic and immunogenic structures with native TES. Recombinant antigens have been produced for *T. canis* second stage larva excretory–secretory 30 kDa protein (recTES-30) (Yamasaki et al., 1998, 2000) and 120 kDa protein (Fong et al., 2003) for diagnosis of human toxocariasis. The recTES-30 was reported to be sensitive and specific in ELISA and did not cross react with sera from individuals infected with *Ascaris* and hookworms (Yamasaki et al., 2000; Coêlho et al., 2005); however there was a low cross-reactivity with sera from individuals with paragonimiasis, ganthostomiasis and spirometriasis (Yamasaki et al., 2000). ELISA using recTES-30 has also been evaluated in two other formats namely dot-ELISA and immunoblot and all three formats were comparable in terms of sensitivity and specificity to recTES-30-ELISA (Lim et al., 1999). Both the dot-ELISA and immunoblot appeared to be more sensitive than recTES-30 ELISA and the dot-ELISA was technically quicker and easier to perform compared to ELISA.

In an effort to improve the convenience and time taken for diagnosis, recTES-30 was used in the development of an immunochromatographic device to detect anti-*Toxocara* antibodies (Yamasaki, 2012). The objective of this study was therefore to evaluate the sensitivity and specificity of this rapid diagnostic kit (RDT, named as iToxocara kit) in comparison to recTES-30-ELISA in an Orang Asli village in Selangor, Malaysia.

2. Materials and methods

2.1. Study area

The study site selected was Serendah Orang Asli village located in Hulu Selangor (3°21′50″N, 101°37′28″E) (Fig. 1), about 60 km away from Kuala Lumpur, Malaysia. The village has a population of about 250 people who are from the Temuan tribe.

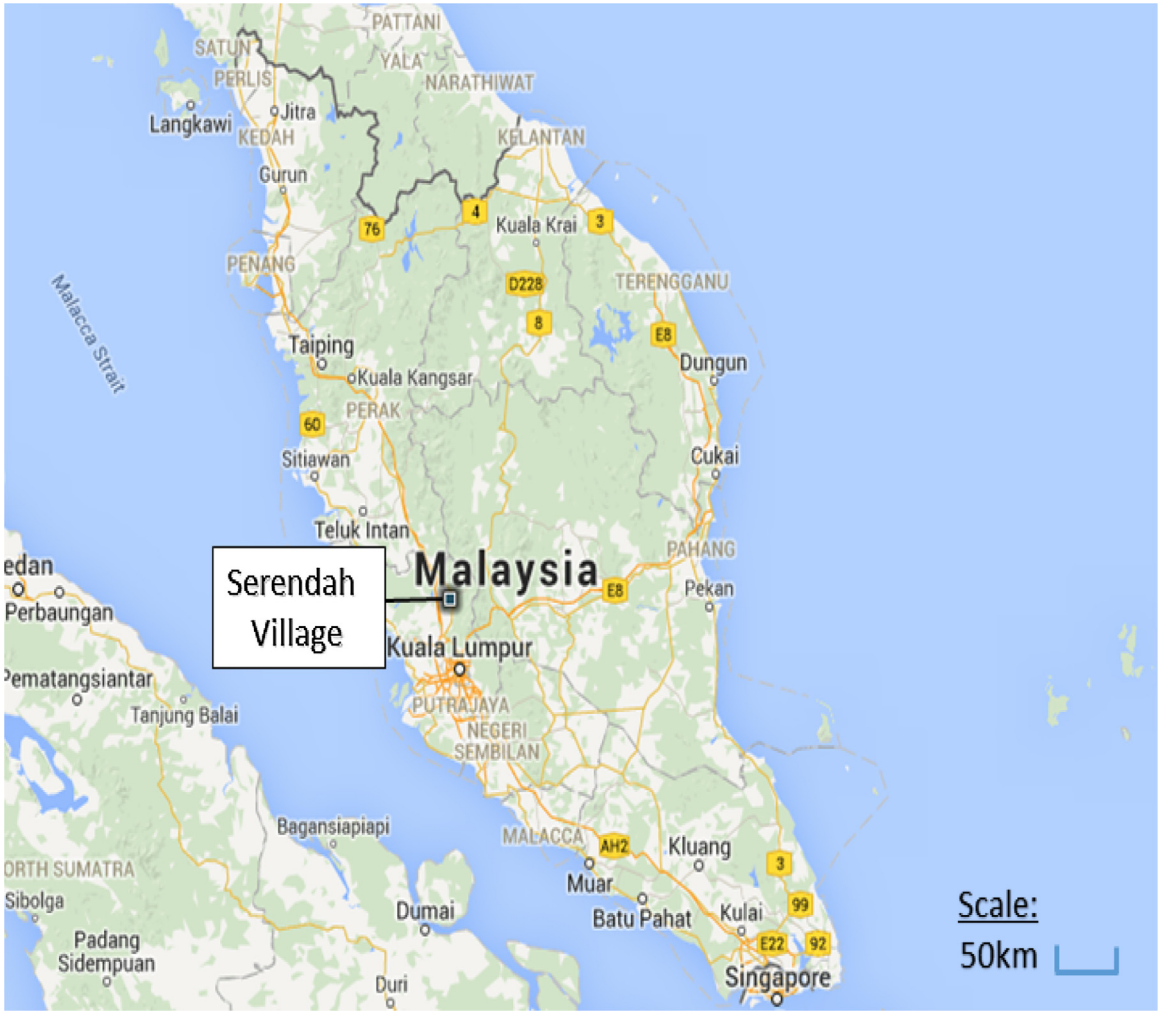

Fig. 1. Location map of Serendah Orang Asli Village, Selangor, Malaysia. (*Source*: http://www.click2map.com/).