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Short communication

Strongyloides stercoralis infection and re-infection in a cohort of children in Cambodia

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ABSTRACT

Information on *Strongyloides stercoralis* re-infection after ivermectin treatment is scarce in *S. stercoralis* endemic countries. In semi-rural Cambodia, we determined *S. stercoralis* infection and re-infection rates among schoolchildren, two years after ivermectin treatment (2 × 100 μg/kg PO, 24 h apart). The study was conducted among 484 children from four primary schools in semi-rural villages in Kandal province from 2009 to 2011, using Koga agar plate culture and the Baermann method on two stool samples per child. Complete data were available for 302 participants. We observed infections in 24.2% and 22.5% of the children at baseline and at follow-up, respectively. At baseline, 73 children were treated for *S. stercoralis* infection. At follow-up, one-third of those treated for *S. stercoralis* infection had been reinfected, while 19.6% of the 229 healthy children (at baseline) had been newly infected with *S. stercoralis*. Possession of shoes and defecation in toilet were negatively associated with *S. stercoralis* infection at follow-up. Infection and re-infection rates of *S. stercoralis* among schoolchildren are considerably high. However, 68.5% of infected children remained free of infection for at least two years. A large-scale cohort study is required to understand age-specific infection and re-infection dynamics in endemic countries.

1. Introduction

Strongyloides stercoralis, a soil-transmitted nematode, infects an estimated 30–100 million people worldwide [1]. *S. stercoralis* infection is endemic in the tropical and humid areas of Central and South America, Sub-Saharan Africa and South and Southeast Asia [2–4], and in temperate climates such as Japan, Australia, Spain, Italy, Romania and the United States [5,6]. Despite its negative impact on public health, *S. stercoralis* is still one of the most neglected infections among the so-called neglected tropical diseases [7].

The global prevalence of *S. stercoralis* infection is heterogeneously distributed, depending on the ecological and socioeconomic conditions in which the population live [8]. In many resource poor countries, *S. stercoralis* infection is generally underreported. One underlying reason for underreporting is that most available information on *S. stercoralis* originates from studies on other soil-transmitted helminths (STHs), such as *Ascaris lumbricoides*, hookworms and *Trichuris trichiura*, that use the Kato-Katz method, which only has a very low sensitivity for *S. stercoralis* infection [8,9]. Koga-agar plate (KAP) culture and the Baermann technique are considered to be the most appropriate diagnostic methods [10], however, they are hardly used, as *S. stercoralis* is rarely included in such studies. As a result, information on incidence rates of *S. stercoralis* infection is entirely missing [8].

Ivermectin is the medicine of choice for treating *S. stercoralis* infection [11]. Even though the efficacy of ivermectin treatment is high [10,12–14], no guideline exists for large-scale chemotherapy against strongyloidiasis [11]. A major reason for this is the lack of information about re-infection after successful treatment [8].

Using a sensitive diagnostic approach of multiple stool samples and multiple examination techniques, we assessed *S. stercoralis* infection and re-infection rates among schoolchildren in four primary schools in semi-rural villages in Kandal province, central-southern Cambodia. Specifically, we assessed *S. stercoralis* infection at baseline and two years after infected children had been treated with ivermectin.

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2. Materials and methods

2.1. Ethical considerations

The study was approved by the Ethics Committee of the Cantons of Basel-Stadt and Baselland, Switzerland (EKBB; number 21/09, dated 29 January 2009 and number 159/11, dated 19 May 2011) and by the National Ethics Committee for Health Research, Ministry of Health, Cambodia (NECHR; number 033, dated 20 March 2009 and number 30 dated 11 April 2011). Written informed consent was obtained from the children's parents, legal guardian or appropriate literate substitute for both the baseline (2009) and follow-up (2011) surveys. All relevant authorities (village chiefs, school teachers and headmasters) were informed of the study's purpose and procedures.

All children infected with *S. stercoralis* were treated with ivermectin under the direct supervision of a medical doctor [11]. All other diagnosed parasitic infections were treated according to the guidelines of the National Helminth Control Program of Cambodia [15].

2.2. Study setting

The study was carried out in four semi-rural villages (Ang, Roka, Koh Khel and Damrey Chhlang villages), located in the Saang District (11.22° N and 105.01° E longitude), Kandal province, 45 km south of Phnom Penh. These villages were selected because they were readily accessible by the project car, assuring rapid transfer of stool samples to the Parasitological Laboratory of the National Center for Parasitology, Entomology and Malaria Control (CNM) in Phnom Penh (up to 90 minutes travel time by car).

2.3. Study design

A school-based survey was conducted during the dry season between March and June 2009 (baseline) and again between May and June 2011 (follow-up). In the 2009 baseline survey, KAP culture and the Baermann method were performed on two stool samples per participant, to detect *S. stercoralis* infection. All *S. stercoralis* cases were followed-up three weeks after ivermectin treatment (2 × 100 μg/kg PO, 24 h apart) [16] to ensure that *S. stercoralis* larvae had been cleared. For the 2011 follow-up survey, all schoolchildren who had participated in the baseline survey were re-enrolled. They were examined using the same laboratory procedures as at baseline (two stool samples per child examined by KAP culture and the Baermann method).

2.4. Field and laboratory procedures

During home visits, after obtaining written informed consent from the children's parents or legal guardian, a pre-tested household questionnaire was administered to the head of household to obtain socioeconomic data, such as ownership of household assets. All schoolchildren present on the day of the school visit were interviewed using a pre-tested children's questionnaire, to obtain demographic data, personal risk-perception (knowledge about worm infection) and behavioural data (wearing shoes, personal hygiene practices). After the interview, each participant received a pre-labelled plastic container (ID code, name, sex, age and date) for stool sample collection and instructions on how to collect the faeces. The next morning, after collecting the filled stool container, another empty pre-labelled container was given to the child. Every participant was asked to provide two stool samples.

Within 2 h of receipt, the stool samples were sent to the laboratory at ambient temperature (25–30 °C). As soon as the specimen arrived in the laboratory, trained laboratory technicians from the Parasitological Laboratory of CNM examined the samples using KAP culture: a hazelnut-sized stool was placed on a nutrient agar plate and the closed Petri dish was incubated in a humid chamber at 28 °C for 48 h and evaluated for visible tracks created by larvae as they carried bacteria over the agar [17]. The Baermann technique was then performed by placing faecal specimens on a mesh screen in a funnel filled with warm water and connected to clamped-tubing. After 2 h any larvae present had crawled out of the stool suspension and migrated into the warm water, from where they were collected by centrifugation [18].

2.5. Follow-up study

From May to June 2011, two years after the baseline survey, the schoolchildren were individually re-assessed. The same field and laboratory procedures were performed as at baseline (i.e. written informed consent of the parents or legal guardian was obtained, two stool samples per child were collected and analysed by KAP culture and Baermann technique for detecting *S. stercoralis* larvae). In addition, questionnaires were used to obtain information about risk factors.

2.6. Data management and statistical analyses

Questionnaire and laboratory data collected from each individual for both surveys were entered twice and validated by EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses were performed with STATA version 12.1 (StataCorp.; College Station, TX, USA). Only participants with completed records in 2009 and 2011 (completed questionnaires and two stool samples examined with all diagnostic tests) were included in the final analyses.

Univariate logistic regression was used to associate infection status with the participants' demographic variables, hygienic practices, knowledge and recent medical history. *P*-values less than 0.05 indicated a significant association. Re-infection was defined as treated *S. stercoralis* cases in 2009 that were *S. stercoralis* positive in 2011.

3. Results

3.1. Study population and compliance

In total, 484 schoolchildren participated in the study in 2009, of whom 409 (84.5%) were present at the follow-up visit in 2011 (Fig. 1). The final analysis focused on 302 (62.6%) participants with complete data records (completed questionnaire and two stool samples analysed with all diagnostic methods) in 2011. Children were between 8 and 18 years old (median age 12 years). The ratio of males to females was close to 1.

The characteristics of participants obtained during the baseline survey (both compliant and non-compliant study participants) are given in Table 1. Participant characteristics were very similar between the two groups. However, non-compliant children were, in general, slightly older and had better personal hygiene practices than the children retained in the cohort.

3.2. Infection and re-infection

Fig. 2 shows the *S. stercoralis* cases diagnosed at baseline and at follow-up. Of the 302 children, 24.2% and 22.5% tested positively for *S. stercoralis* infection at baseline and at follow-up; respectively. Of the 73 children diagnosed and treated in 2009, 23 (31.5%) were re-infected in 2011. Of the 229 healthy children at baseline, 45 (19.6%) were infected with *S. stercoralis* at follow-up. Two of the 73 positive cases diagnosed at baseline were not *S. stercoralis* infection-free three weeks after ivermectin treatment.

The distribution of *S. stercoralis* infection and re-infection rate varied among the study villages (Fig. 3). Compared to the prevalence at baseline, the follow-up prevalence decreased in Roka and Ang villages, and increased in Damrey Chlong and Koh Khel villages. The re-infection rate of *S. stercoralis* in Damrey Chlong and Ang villages was 24.0% and 42.0%, respectively. However, the different prevalence and re-infection rates in the study villages were not statistically significant.