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The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village



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ABSTRACT

In Cambodia, intestinal parasitic infections are prevalent in humans and particularly in children. Yet, information on potentially zoonotic parasites in animal reservoir hosts is lacking. In May 2012, faecal samples from 218 humans, 94 dogs and 76 pigs were collected from 67 households in Dong village, Preah Vihear province, Cambodia. Faecal samples were examined microscopically using sodium nitrate and zinc sulphate flotation methods, the Baermann method, Koga Agar plate culture, formalin-ether concentration technique and Kato Katz technique. PCR was used to confirm hookworm, Ascaris spp., Giardia spp. and Blastocystis spp. Major gastrointestinal parasitic infections found in humans included hookworms (63.3%), Entamoeba spp. (27.1%) and Strongyloides stercoralis (24.3%). In dogs, hookworm (80.8%), Spirometra spp. (21.3%) and Strongyloides spp. (14.9%) were most commonly detected and in pigs Isospora suis (75.0%), Oesophagostomum spp. (73.7%) and *Entamoeba* spp. (31.6%) were found. Eleven parasite species were detected in dogs (eight helminths and three protozoa), seven of which have zoonotic potential, including hookworm, Strongyloides spp., Trichuris spp., Toxocara canis, Echinostoma spp., Giardia duodenalis and Entamoeba spp. Five of the parasite species detected in pigs also have zoonotic potential, including Ascaris spp., Trichuris spp., Capillaria spp., Balantidium coli and Entamoeba spp. Further molecular epidemiological studies will aid characterisation of parasite species and genotypes and allow further insight into the potential for zoonotic cross transmission of parasites in this community. © 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Intestinal parasitic infections (IPIs) are the most common infections among humans and domestic animals such as dogs, cats and pigs, particularly in the rural areas of Southeast Asia. Chronic infections with one or several of the most common soil-transmitted helminths (STHs), *Ascaris lumbricoides, Trichuris trichiura* and hookworms, might account for a global burden of 39 million disability-adjusted life years lost annually [1]. Another STH, *Strongyloides stercoralis*, is often neglected in helminth surveys [2,3], yet previous studies show high *S. stercoralis* infection rates in Cambodia [4]. School-aged children in the

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developing world are at highest risk of morbidity due to STHs and intestinal protozoan infections [5].

Many of the IPIs in animals, especially those with the larval stages of hookworms, *Gnathostoma* spp. and *Toxocara* spp., may result in zoonotic diseases such as eosinophilic enteritis [6], cutaneous larval migrans, and toxocariasis. In addition, dogs and pigs may also serve as definitive reservoir hosts for adult zoonotic parasites capable of forming patent infections in humans, including *Ancylostoma ceylanicum* (dogs) [7], *Ascaris* spp. (pigs) [8], *Trichuris* spp. (pigs) [9], *Fasciolopsis buski* (pigs) [10], *Echinostoma* spp.(dogs/pigs) [11], *Cryptosporidium* spp. (dogs) [12] and *Giardia duodenalis* (dogs) [13]. Current information on the prevalence of such parasites is vital for developing veterinary and public health strategies for their treatment and control [14].

Today, the national helminth control programme covers most schoolaged children in Cambodia. However, mass treatment only focuses on

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three major STHs (*Ascaris*/hookworm/*Trichuris*). Other nematodes like *S. stercoralis*, trematodes and protozoan infections are not addressed.

In rural Southeast Asia, little is known about the zoonotic potential of IPIs in humans and animals. Previous studies have shown a high prevalence of intestinal protozoan and helminthic parasites infecting Cambodian refugees and school children [4,15–20]. The role of domestic animals, such as dogs and pigs, as contributors to human IPI and as reservoir hosts for zoonotic parasites remains unexplored and/or the data are inaccessible. The current study was conducted to obtain information on the prevalence and diversity of IPIs among humans and domestic animals living in the same household in a rural Cambodian village.

2. Materials and Methods

2.1. Ethical considerations

The study protocol was approved by the Ethics Committee of the Canton of Basel, Switzerland and the National Ethics Committee for Health Research, Ministry of Health in Cambodia. All participants and relevant parties were informed of the purpose of the study. Written informed consent was obtained from all individuals prior to enrolment. All infections diagnosed in humans and animals were treated at the end of the study according to the Cambodian treatment guidelines.

2.2. Study design and area

The study was carried out in May 2012 in Dong village, Rovieng district, Preah Vihear province, Cambodia. Preah Vihear province is located in the north of Cambodia, bordering Thailand and Lao PDR (13°47'N 104°58'E). The climate is tropical, with warm and hot temperatures all year round and alternating dry and wet seasons. Households from Dong village were randomly selected from lists provided by the Ministry of Health. All household members (>2 years) and animals (dogs, pigs) were assessed for IPIs using a multiple diagnostic test approach on two stool samples for each human and one sample for each animal. Only animals owned by the household were included in the survey. Risk factors for infection of humans and animals were assessed based on information collected through questionnaire interviews and observations. The questionnaire mainly covered demographic information, self-reported symptoms, occupation, eating habits, personal hygiene practices and household assets. Information about the animals (i.e. age) was collected by questioning the respective owner.

2.3. Field procedures and sample collection

On the day of the first visit, informed consent was obtained from all household members and interviews were conducted with enrolled participants. Interviews with young children were conducted with the help of a parent or legal guardian. All enrolled participants received a prelabelled stool container. Participants were asked to fill the container with faeces passed the following morning. Upon collecting the first sample, a second stool container was given to participants for filling. The collected stool samples were transported within two hours following defecation to a laboratory in Rovieng Health Centre. Stool samples from each dog and pig present at the time of the visit and belonging to the household were obtained. For each animal, approximately five grams of faeces were collected from the rectum directly, placed into a sterile plastic faecal container and chilled immediately in a box containing ice. For each human, two stool samples given on consecutive days were analysed and for each animal, one sample was analysed.

2.4. Laboratory procedures

For each human stool sample, the following tests were performed: Kato Katz [21], Koga Agar culture [22], Baermann [23], formalin-ether concentration technique (FECT) [24] and sodium nitrate and zinc sulfate flotation [25] analysis. As they arrived in the laboratory, human samples were processed as follows:

First, duplicate Kato Katz smears were prepared. Stool was filtered using a nylon mesh and then placed on the standard Kato Katz template, leaving 41.7 mg of stool for examination on a microscopic slide. Examination was performed at 100x magnification [21] for hookworm, Taenia spp. and small and large trematode eggs (Opisthorchis/Clonorchis/ Dicrocoelium-like and Fasciola/Fasciolopsis/Echinostomes). Second, a Koga Agar test was prepared by placing a piece of stool (3-5 g) on a freshly produced Agar plate. The plates were then incubated for 48 hours at 28 °C. Larvae were washed from the plate into a tube, the liquid was centrifuged and the entire sediment was read at 40x magnification [22] for hookworm and *S. stercoralis* larvae. Third, a Baermann test was prepared. In brief, stool was placed in a wire-mesh situated in a funnel. A clamped tube was attached to the funnel while the funnel and tube were filled with tap water. Artificial light was used to stimulate larvae movement out of the stool and into the tube. After two hours, the tube-water was centrifuged. The entire sediment was read at 40x magnification [23] for S. stercoralis larvae. Fourth, stool samples were fixed in a 15 ml centrifuge tube with 10 ml of sodium-acetateformaldehyde (SAF) solution and subsequently analysed for protozoa (Entamoeba spp., G. duodenalis, Blastocystis, Endolimax nana, Isospora spp. and I. bütschlii) at the National Malaria Centre in Phnom Penh, Cambodia, using the FECT method [24]. Lastly, two grams of stool were fixed separately in 10% formaldehyde and subjected to sodium nitrate and zinc sulphate flotation [25] at the School of Veterinary Science, University of Queensland, Gatton campus, Australia.

For the animal samples, a sodium nitrate and zinc sulphate flotation analysis was performed in the same manner as for the human samples [25]. Additionally, all human and animal samples were fixed in 2.5% potassium dichromate for subsequent PCR screening for hookworm [26], Ascaris [27], Giardia [28–30] and Blastocystis [31].

2.5. Statistical analysis

The data were double-entered and validated in EpiData (www. epidata.dk). Statistical analysis was carried out using STATA version 12 (StataCorp LP; College Station, TX). We calculated the cumulative prevalence for all IPIs. The cumulative prevalence is the combined prevalence of the results from all applied diagnostic methods for a specific parasite. Potential risk factors were determined by matching the parasite infection status of humans and animals with corresponding questionnaire data, either at individual or household level. Possible risk factors were determined by regression analysis and were considered significant at p-levels smaller than 0.05.

3. Results

3.1. Study population

In this study, 67 households were enrolled, yielding 218 participants, of which 99 (45.4%) were male and 119 (54.6%) were female, with an average age of 30 years (range 2–84 years). Almost half (n = 107, 49.1%) of all participants worked as farmers in rice fields, while many others (n = 89, 40.8%) were pre-school or school-aged children. Altogether, 94 dogs were sampled; 39 (41.5%) were male and 55 (58.5%) were female, with an average age of 27 months. The dogs were further classified into puppies/ juveniles (<12 months old), adults (1–6 years old) and geriatrics (>6 years old) [25]. Seventy-six pigs were sampled; 35 (46.1%) were male and 41 (53.9%) were female, with an average age

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