Acta Tropica 141 (2015) 315–321

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Repeated stool sampling and use of multiple techniques enhance the sensitivity of helminth diagnosis: A cross-sectional survey in southern Lao People's Democratic Republic

Somphou Sayasone[a,b,c], Jürg Utzinger[b,c], Kongsap Akkhavong[a], Peter Odermatt[b,c,*]

[a] National Institute of Public Health, Ministry of Health, Vientiane, Lao Democratic People's Republic
[b] Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland
[c] University of Basel, P.O. Box, CH-4003 Basel, Switzerland

ARTICLE INFO

Article history:
Available online 16 September 2014

Keywords:
Diagnosis
Helminthiasis
Multiparasitism
Multiple stool sampling
Multiple diagnostic techniques
Lao People's Democratic Republic

ABSTRACT

Intestinal parasitic infections are common in Lao People's Democratic Republic (Lao PDR). We investigated the accuracy of the Kato-Katz (KK) technique in relation to varying stool sampling efforts, and determined the effect of the concurrent use of a quantitative formalin-ethyl acetate concentration technique (FECT) for helminth diagnosis and appraisal of concomitant infections. The study was carried out between March and May 2006 in Champasack province, southern Lao PDR. Overall, 485 individuals aged ≥6 months who provided three stool samples were included in the final analysis. All stool samples were subjected to the KK technique. Additionally, one stool sample per individual was processed by FECT. Diagnosis was done under a light microscope by experienced laboratory technicians. Analysis of three stool samples with KK plus a single FECT was considered as diagnostic 'gold' standard and resulted in prevalence estimates of hookworm, *Opisthorchis viverrini*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma mekongi* infection of 77.9%, 65.0%, 33.4%, 26.2% and 24.3%, respectively. As expected, a single KK and a single FECT missed a considerable number of infections. While our diagnostic 'gold' standard produced similar results than those obtained by a mathematical model for most helminth infections, the 'true' prevalence predicted by the model for *S. mekongi* (28.1%) was somewhat higher than after multiple KK plus a single FECT (24.3%). In the current setting, triplicate KK plus a single FECT diagnosed helminth infections with high sensitivity. Hence, such a diagnostic approach might be utilised for generating high-quality baseline data, assessing anthelminthic drug efficacy and rigorous monitoring of community interventions.

1. Introduction

Neglected tropical diseases remain of considerable public health importance, particularly in low-income and middle-income countries (Hotez et al., 2009; Murray et al., 2012). Indeed, hundreds of millions of people are affected by neglected tropical diseases, among which soil-transmitted helminthiasis is particularly widespread (Pullan et al., 2014; Utzinger et al., 2012). Of note, multiple species parasite infections often co-occur in the same eco-epidemiological setting, i.e. intestinal protozoa, soil-transmitted helminths, cestodes and trematodes (Phongluxa et al., 2013; Sayasone et al., 2009, 2011; Steinmann et al., 2010).

In Lao People's Democratic Republic (Lao PDR), multiparasitism is common (Sayasone et al., 2007, 2009). With regard to soil-transmitted helminths, *Ascaris lumbricoides* and *Trichuris trichiura* are highly prevalent in the mountainous and highland areas of the country, particularly in the north, while hookworm is highly prevalent across the country (Ayé Soukhathammavong et al., 2012; Laymanivong et al., 2014; Rim et al., 2003; Sayasone et al., 2011). The liver fluke *Opisthorchis viverrini* and five species of small intestinal trematodes (i.e. *Haplorchis taichui*, *Haplorchis yogokawai*, *Haplorchis pumilio*, *Phaneropsolus bonnei* and *Prosthodendrium molenkampi*) are endemic in the lowlands of the Mekong River basin (Chai et al., 2005, 2007; Eom et al., 2014; Sayasone et al., 2009). The blood fluke *Schistosoma mekongi* is endemic in the lower Mekong River, in the most southern part of the country (Laymanivong et al., 2014; Muth et al., 2010; Sayasone et al., 2011; Urbani et al., 2002).

Concurrent helminth infections pose serious challenges for the diagnosis in clinical settings and are of public health concern.

* Corresponding author at: Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland.
Tel.: +41 61 284 8214; fax: +41 61 284 8105.
E-mail address: peter.odermatt@unibas.ch (P. Odermatt).

http://dx.doi.org/10.1016/j.actatropica.2014.09.004
0001-706X/

Prevalence rates of parasitic infections are usually underestimated, and hence, a combination of different techniques on repeatedly collected stool samples is warranted to enhance the sensitivity of helminth diagnosis (Knopp et al., 2008; Marti and Koella, 1993). Importantly, the impact of concurrent infections on health is often overlooked. It is generally thought that light-intensity infection with a single parasite is associated with no or only little morbidity. However, there is growing evidence that infections from multiple parasite species, even at light intensity, may contribute to substantial morbidity to the host (Ezeamama et al., 2005; King and Bertino, 2008; Sayasone et al., 2012). For example, infection with soil-transmitted helminths result in a number of gastro-intestinal discomforts (e.g. abdominal pain, chronic diarrhoea and vomiting) and anaemia due to chronic blood lost in the intestinal tract due to hookworm infection (Brooker et al., 2008). In Lao PDR, *O. viverrini* and *S. mekongi* might lead to severe morbidity (Sayasone et al., 2012). Specifically, *O. viverrini* infection lead to severe hepatobiliar pathologies, and may develop to a fatal bile duct cancer (cholangiocarcinoma) (Sripa et al., 2012), while chronic infection with *S. mekongi* contribute to a formation of hepatomegaly, periportal fibrosis and portal hypertension (Biays et al., 1999; Keang et al., 2007). Additionally, in areas of co-infection, substantial excess morbidity has been observed, even among individuals with relatively low intensity infections (Sayasone et al., 2012).

For the diagnosis of intestinal parasites, widely used techniques are the Kato-Katz (KK) (Katz et al., 1972) and the formalin-ethyl acetate concentration technique (FECT) (Elkins et al., 1991). The KK technique is often employed in community-based epidemiological surveys, and requires mainly reusable test kits. It is recommended by the World Health Organization (WHO) as a suitable tool for helminth diagnosis, particularly *Schistosoma mansoni*, *Schistosoma japonicum*, *S. mekongi* and soil-transmitted helminths. However, the KK technique fails to detect *Strongyloides stercoralis*, arguably the most neglected of the soil-transmitted helminths (Olsen et al., 2009; Schär et al., 2013b), and intestinal protozoa. The KK technique, moreover, has a low sensitivity because it is processing only a small amount of faeces (41.7 mg). Light infections may be missed, consequently, misjudging infection status of patients and underestimating the 'true' community prevalence (Khieu et al., 2013; Kongs et al., 2001; Utzinger et al., 2001). The FECT allows for the concurrent diagnosis of helminths and intestinal protozoa (Utzinger et al., 2010). Analysis of multiple stool samples and the combination of different techniques increase the sensitivity of helminth diagnosis (Knopp et al., 2008; Steinmann et al., 2008).

The aim of this study was to investigate the effect of varying sampling efforts on the diagnostic accuracy of the KK technique and the combination of two methods (KK plus FECT) for detecting eggs of soil-transmitted helminths, *S. mekongi* and food-borne trematodes in rural communities in southern Lao PDR.

2. Materials and methods

2.1. Ethics statement

The study was reviewed by the institutional research commission of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland). Approval was granted by the ethics committee of Basel (EKBB, reference no. 255/06) and the national ethics committee of the Ministry of Health (MOH) in Vientiane (reference no. 027/NECHR). Permission for field work was obtained from the MOH and the provincial and district health officers. Village meetings were held and local authorities and villagers were given detailed explanations about the purpose, procedures, potential risks and benefit of the study. An information sheet in the local language was read to all household members and any questions were answered using lay terminology. Individual oral consent was obtained from all adult household members, while parents (or legal guardians) provided consent on behalf of their children. Consent was documented on a household registration sheet. A literate witness observing this procedure signed the consent form. Additionally, written informed consent was obtained from the heads of household. This consent procedure was approved by the aforementioned ethics committees.

At the end of the study, all individuals infected with *O. viverrini*, *S. mekongi*, soil-transmitted helminths and intestinal protozoa were treated according to national guidelines (MOH, 2004).

2.2. Study area and population surveyed

A cross-sectional survey was carried out between March and May 2006 in Champasack province, southern Lao PDR. Details of the study area and the population surveyed have been described elsewhere (Sayasone et al., 2011). In brief, the study was conducted in three eco-epidemiological settings, namely (i) the plains along the Mekong River basin where *O. viverrini* and hookworm co-exist; (ii) the small islands in the Mekong River in the southern part of the province where *S. mekongi*, *O. viverrini* and hookworm co-occur; and (iii) the mountainous and highland areas of the eastern part of province where hookworm and other soil-transmitted helminths are widespread. In each setting, three villages were selected in collaboration with district health authorities. At least 20 and up to 25 households were randomly selected in each village from readily available census data. All family members aged ≥6 months were invited to participate.

2.3. Study design

Eligible people were invited to submit three stool samples over five consecutive days. Unique identifiers were assigned to households and study participants. Sample containers were prepared for all members of study households. Participants' names and unique identifiers were marked on the containers that were distributed to the heads of household with detailed explanation on how to collect a fresh morning stool. After filled containers were collected, new empty containers were handed out until three stool samples were submitted by study participants. In each village, a house (usually a school or a temple) was designated as area of work for KK thick smear preparation, and subsequent microscopic examination.

Stool samples were processed on the spot by experienced laboratory technicians. A single KK thick smear was prepared from each stool sample, using standard plastic templates holding 41.7 mg of stool (Katz et al., 1972). Slides were allowed to clear for 30 min prior to examination under a microscope. The number of eggs was counted and recorded for each helminth species separately.

Additionally, exactly 300 mg of stool taken from one sample was fixed in a tube containing 10 ml of sodium acetate-acetic acid-formalin (SAF) (Marti and Escher, 1990). SAF-fixed samples were forwarded to the parasitological department of the Faculty of Medicine, National University of Lao PDR where the samples were subjected to FECT (Elkins et al., 1991). Presence of helminth species-specific infections and intensities were assessed with quality control of laboratory staff from Swiss TPH. A random sample of 10% of the samples were re-examined by a senior laboratory technician for quality control. If any differences were observed, the results were discussed until agreement was reached. During the laboratory work, senior technicians were available for any question.

2.4. Statistical analysis

Data were double-entered and cross-checked in EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses