Post-Travel Screening of Asymptomatic Long-Term Travelers to the Tropics for Intestinal Parasites Using Molecular Diagnostics

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Abstract. The incidence of asymptomatic travel-related parasitic infection is uncertain. Previous studies did not distinguish new incident infections, from past infections. Regardless of symptoms, we performed multiplex real-time polymerase chain reaction on pre- and post-travel stool samples of Dutch long-term travelers to the (sub)tropics. Serological screening for *Schistosoma* spp. was only performed in travelers to sub-Saharan Africa. In total, 679 travelers were included in the study. The follow-up rate was 82% (556 of 679). Participants' median travel duration was 12 weeks. There was one incident infection with *Strongyloides stercoralis*; there were none with *Entamoeba histolytica*, 4 with *Cryptosporidium* spp. (1%), and 22 with *Giardia lamblia* (4%). Nine of 146 travelers (6%) seroconverted for *Schistosoma* spp. Routine screening of stool samples for parasitic infection is not indicated for asymptomatic people, who travel to the (sub)tropics for up to 3 months. Screening for *Schistosoma* spp. should be offered to travelers with fresh-water contact in endemic regions.

INTRODUCTION

Asymptomatic infection with protozoa or helminths can cause morbidity long after the primary infection. Travelers to the tropics are at risk of such infections. Asymptomatic infection, in particular with parasites such as *Entamoeba histolytica*, *Strongyloides stercoralis*, or *Schistosoma* spp. can cause devastating morbidity later on in life. Early detection and eradication is beneficial. Some travel clinics offer post-travel screening, which includes screening for parasites. However, the prevalence of asymptomatic parasitic infections after travel remains uncertain. Two sizeable studies have been performed, in which people from highly industrialized countries were screened for parasitic infections, after having stayed in the tropics. Whitty and others¹ determined the use of post-travel screening in the United Kingdom. They studied two cohorts, totaling over 1,000 symptomatic and asymptomatic travelers and expatriates who stayed in the tropics or subtropics for at least 3 months. The majority had lived in the tropics for well over a year. A stool specimen was screened with stool concentrate microscopy. Gut helminths were detected in 3% of samples, as was *Giardia lamblia*. The study did not distinguish between *E. histolytica* and *Entamoeba dispar* cysts, which were detected in 9% of samples. Schistosomal serology was positive in 13% and was limited to those who had stayed in Africa. These results are not generalizable to most long-term non-expatriate travelers, whose travel duration is considerably shorter than 1 year. Furthermore, the sensitivity of traditional microscopic methods to detect parasitic infections is limited, even in experienced well-equipped laboratories. In addition, the available serological tests for *S. stercoralis* either have a limited sensitivity during the early stage of infection, or have a low specificity, and therefore are less useful for screening travelers.² A study by Ten Hove and others³ screened over 2,500 samples from symptomatic and asymptomatic people

who visited a large travel clinic in Belgium for a variety of reasons. The majority was born in Europe. The multiplex real-time polymerase chain reaction (PCR) analysis that they used was clearly more sensitive to detect infection with *E. histolytica* (prevalence 0.5%), *S. stercoralis* (0.8%), *G. lamblia* (6%), and *Cryptosporidium* spp. (1.3%) than the more traditional methods. This cross-sectional study could not distinguish recently contracted incident infections from infections that may have been contracted earlier, during past travel or during residency in the tropics.

We aimed to determine the use of routine post-travel screening for asymptomatic parasitic infections. The screening was restricted to parasites that may cause asymptomatic infection and late-onset morbidity. Therefore, we studied the incidence of infection with *E. histolytica*, *G. lamblia*, *Cryptosporidium* spp., *S. stercoralis*, and *Schistosoma* spp. in a cohort of Dutch long-term travelers who visited the tropics.

MATERIALS AND METHODS

Design and study population. This prospective cohort study was conducted in The Netherlands from July 2007 until November 2009. Healthy adults who visited the travel clinics at Leiden University Medical Center (LUMC) and Wageningen University and Research center (WUR) and who intended to travel to the (sub)tropics for more than 1 month were invited to take part. The majority of those who visit the travel clinic at LUMC are “general travelers.” However, relatively more hospital employees and (bio)medical students visit the travel clinic at LUMC compared with other out-of-hospital based travel clinics. The WUR focuses on the field of agriculture and the living environment and only university students and staff visit the travel clinic at WUR. All participants submitted stool samples and filled out web-based questionnaires before departure and 2 and 12 weeks after returning home. Pre- and post-travel serum samples were only obtained from those who visited sub-Saharan Africa. Stool specimens were sent by regular mail. Pre-travel samples were stored and analyzed later in case of a positive post-travel sample. Post-travel samples (i.e., the samples that were collected 2 weeks and 12 weeks after returning

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home) were processed directly upon arrival. Participants were notified of their result. In case of an infection, the participants' general practitioner was also notified. The study protocol was approved by the Medical Ethics Committee at LUMC and participants provided written informed consent.

Questionnaires. The first questionnaire (Q1) was sent before participants' departure and consisted of questions on past travel to the tropics, current illness, past and current bowel complaints, use of medication, and past infection or treatment of enteric parasites. The second questionnaire (Q2) was sent 2 weeks after participants had returned home and consisted of questions on travel characteristics and (treatment of) intestinal infection during travel. The third questionnaire (Q3) was sent 12 weeks after participants had returned home and dealt with persisting complaints and use of medication after travel. Travelers' diarrhea was defined as the passage of three or more unformed stools during a 24-hour period with or without additional symptoms.⁴

Laboratory methods. *Stool samples.* Participants were asked to fill an empty tube with stool and send it by regular mail to the diagnostic laboratory of LUMC. The DNA isolation and multiplex real-time PCR amplification were performed upon arrival of each post-travel sample. Pre-travel samples were stored at -20°C and analyzed later in case of a positive post-travel sample. The DNA isolation and multiplex real-time PCR were performed as described previously.^{3,5,6} For DNA isolation, 200 μL of feces suspension (~ 0.5 g/mL feces in

phosphate buffered saline [PBS] containing 2% polyvinylpyrrolidone [PVPP; Sigma, Steinheim, Germany]) was heated for 10 min at 100°C . After sodium-dodecyl-sulphate-proteinase K treatment (overnight at 55°C), DNA was isolated with the MagNA Pure LC 2.0 instrument using the MagNA Pure LC DNA isolation kit III (Roche, Almere, Nederland). In each sample, a fixed amount of phocin herpes virus 1 (PhHV-1) was added within the isolation lysis buffer, to serve as an internal control for the isolation procedure and to monitor inhibition of the multiplex real-time PCR assays. *Entamoeba histolytica*, *G. lamblia*, and *C. hominis/C. parvum* DNA amplification was performed in a multiplex real-time PCR including the PhHV internal control (HGC PCR).⁵ *Strongyloides stercoralis* DNA amplification was performed in a separate assay, also including PhHV-1 as an internal control.⁶ Amplification, detection and data analysis was performed on the CFX96 real-time detection system (BioRad, Veenendaal, The Netherlands).

Serum samples. Before travel and 12 weeks after travel a serum sample was collected from participants who had visited sub-Saharan Africa. Pre-travel samples were stored at -20°C and analyzed later in case of a positive post-travel sample. Antibodies to *Schistosoma mansoni*-derived somatic antigens (Adult Worm Antigen, AWA) were assessed by an indirect immunofluorescence assay for the detection of immunoglobulin G (IgM) antibodies, using paraffin sections of adult male *S. mansoni* with Rossmann fixative. The IgG antibodies to egg antigens (Soluble Egg Antigens, SEA) were assessed by

FIGURE 1. Flowchart of participants in the study of post-travel screening for intestinal parasites.

enzyme-linked immunosorbent assay.^{7,8} Stool samples of travelers who seroconverted were analyzed for *Schistosoma* spp. using PCR as has been described previously.⁹ No urine samples were collected.

Data editing. Travel destination was categorized according to the United Nations (UN) International Migrant Stock.¹⁰

Sample size. This was a descriptive study. We deemed an incidence rate of at least 1% for each separate parasitic infection to be a significant finding. Based on this assumption, we chose to screen 500 travelers.

RESULTS

Study population, travel characteristics, and travelers' diarrhea. Six hundred and seventy-nine travelers (84% LUMC, 16% WUR) provided informed consent of who 123 were excluded from the analyses because they submitted less than two stool samples (follow-up rate 556 of 679, 82%) (Figure 1); 200 participants visited sub-Saharan Africa of whom 54 did not submit a post-travel serum sample (follow-up rate 146 of 200,

TABLE 1
Demographic and travel-related characteristics of 556 Dutch travelers*

Characteristic	Leiden N = 467	Wageningen N = 89	All N = 556
Median age, years (IQR)	25 (23–32)	25 (23–26)	25 (23–30)
Gender, female, n (%)	323 (69)	52 (58)	375 (67)
Median travel duration, weeks (IQR)	13 (7–20)	17 (13–22)	12 (6–20)
Travel destination, n (%)†			
Latin America	111 (24)	23 (26)	134 (24)
Central America and Caribbean	38 (8)	6 (7)	44 (8)
South America	85 (18)	19 (21)	104 (19)
Africa	160 (34)	46 (52)	206 (37)
Northern Africa	4 (1)	1 (1)	5 (1)
Western Africa	39 (8)	17 (19)	56 (10)
Middle Africa	14 (3)	3 (3)	17 (3)
Eastern Africa	82 (18)	24 (27)	106 (19)
Southern Africa	42 (9)	4 (5)	46 (8)
Asia	184 (39)	18 (20)	202 (36)
Central and Western Asia	11 (2)	1 (1)	12 (2)
Eastern Asia	34 (7)	2 (2)	36 (7)
South-Eastern Asia	108 (23)	12 (14)	120 (22)
Southern Asia	67 (14)	6 (7)	73 (13)
Unknown	16 (3)	2 (2)	18 (3)
Main travel purpose, n (%)			
Study	170 (36)	75 (84)	307 (55)
Tourism	197 (42)	5 (6)	202 (36)
Volunteer work	58 (12)	4 (5)	62 (11)
Professional	15 (3)	2 (2)	17 (3)
Visit friends/relatives	5 (1)	1 (1)	6 (1)
Unknown	22 (5)	2 (2)	24 (4)
Type of travel, n (%)			
Backpacking	204 (44)	48 (54)	252 (45)
Self-arranged, not backpacking	184 (39)	37 (42)	221 (40)
Organized group travel	57 (12)	2 (2)	59 (11)
Type of accommodation, n (%)			
Guesthouse/small budget hotel	309 (66)	43 (48)	352 (63)
With locals in rural area	43 (9)	23 (26)	66 (12)
With locals in city	43 (9)	19 (21)	62 (11)
Large hotel	32 (7)	–	32 (6)
Camping/boat	17 (4)	3 (3)	20 (4)
Unknown	22 (5)	2 (2)	24 (4)

*IQR = interquartile range.

†Four participants traveled to more than one continent.

TABLE 2
Travelers' diarrhea, incidence proportions for 496 Dutch travelers

Characteristic	Leiden N = 416	Wageningen N = 80	All* N = 496
Travelers' diarrhea, n (%)	309/416 (74)	59/80 (74)	368/496 (74)
1 Episode	84/309 (27)	15/59 (25)	99/368 (27)
2 Episodes	82/309 (27)	20/59 (34)	102/368 (28)
More than 2 episodes	143/309 (46)	24/59 (41)	167/368 (45)
Accompanying symptoms, n (%)			
Abdominal cramps	178/309 (58)	26/59 (44)	204/368 (55)
Nausea	89/309 (29)	14/59 (24)	103/368 (28)
Vomiting	78/309 (25)	10/59 (17)	88/368 (24)
Fever	54/309 (17)	9/59 (15)	63/368 (17)
Blood in stool	10/309 (3)	3/59 (5)	13/368 (4)
Travelers' diarrhea by continent, n (%)			
Latin America	73/111 (66)	15/23 (65)	88/134 (66)
Africa	116/160 (73)	32/46 (70)	148/206 (72)
Asia	122/184 (66)	12/18 (67)	134/202 (66)

*Data is missing for 60 travelers who did not return the questionnaire after travel. Diarrhea was defined as the passage of three or more unformed stools during a 24-hour period.

73%). Participants' median age was 25 years (interquartile range [IQR] 23–30) and the median travel duration was 12 weeks (IQR 6–20 weeks). Among the participants from Leiden, South-Eastern Asia was the most popular travel destination. Among the participants from Wageningen, Western and Eastern Africa were the most popular destinations. The main travel purpose was study (55%) and the main mode of travel was qualified as backpacking (47%) (Table 1). The incidence proportion of travelers' diarrhea was very high (74%). The majority reported more than one episode, although it needs to be mentioned that a formal definition of what constituted an episode was not included in the questionnaire (Table 2).

Post-travel screening results: fecal parasites. The results of real-time PCR for all fecal parasites are summarized in Table 3. None of the 542 stool samples obtained 2 weeks after participants' return was positive for *E. histolytica*. Twelve weeks after travel only 1 of 437 samples (0.2%) was positive for *S. stercoralis* (Ct-value 27.5). This participant was asymptomatic and had traveled to Indonesia for 14 weeks of field work involving water management. No rhabditiform larvae were detected with microscopy of a direct smear of the same sample, possibly because the sample was no longer fresh. *Strongyloides* serology was positive. His pre-travel stool sample was negative, as was his sample taken 2 weeks after return (i.e., no specific amplification). He was treated with ivermectin. *Cryptosporidium* spp.-specific amplification was detected in four samples (1%). The pre-travel samples of these participants were negative for *Cryptosporidium*. With microscopy

TABLE 3
Results of routine post-travel screening of stool samples of long-term travelers to the tropics using multiplex real-time PCR and serological screening*

Characteristic	Leiden	Wageningen	All
<i>Entamoeba histolytica</i> , n (%)	0/456 (0)	0/86 (0)	0/542 (0)
<i>Giardia lamblia</i> , n (%)	19/456 (4)	10/86 (12)	29/542 (5)*
<i>Cryptosporidium</i> spp., n (%)	3/456 (1)	1/86 (1)	4/542 (1)
<i>Strongyloides stercoralis</i> , n (%)	0/369 (0)	1/78 (1)	1/437 (0.2)
<i>Schistosoma</i> spp.	7/113 (6)	2/33 (6)	9/146 (6)
Seroconversion, n (%)			

*Twenty-three of 542 (4%) were newly infected with *G. lamblia* after travel and 6 of 29 were already infected, but asymptomatic, before departure. Half of the travelers who were infected with *G. lamblia* after travel reported gastrointestinal complaints (i.e. abdominal discomfort and/or diarrhea), for which the majority did not consult a physician.

using carbol-fuchsin-staining *Cryptosporidium* oocysts were seen in 3 of 4 PCR-positive samples (75%). *Giardia lamblia*-specific amplification was detected in 29 individuals (5%). Six of these participants were also positive for *G. lamblia* before departure. These six participants did not have abdominal complaints before departure, nor did they develop complaints after returning home. With microscopy, *G. lamblia* cysts and/or trophozoites were seen in 23 of 26 (88%) PCR-positive samples. In the PCR, Ct-values ranged from 20.2 to 35.3 (median 26.7). As expected, negative microscopy was associated with higher Ct values (P value 0.002, Kruskal-Wallis test).

***Giardia lamblia* and *Cryptosporidium* spp., symptoms, risk factors, and treatment.** The four cases of cryptosporidium infection were detected in travelers returning from Western Africa (two cases) and Eastern Africa or Southern Asia (one case each) (Table 4). All four had experienced travelers' diarrhea and three had abdominal complaints in the first 2 weeks after having returned home. None were treated. Twelve weeks after travel *Cryptosporidium* spp. were no longer detected. The incidence proportion of travelers' diarrhea and travel duration was not higher in those who were newly infected with *G. lamblia*, compared with all other travelers. Half of all travelers infected with *G. lamblia* were asymptomatic at the time of diagnosis. Ct values for *G. lamblia* were not higher in symptomatic compared with asymptomatic participants. Gastrointestinal complaints (i.e., abdominal discomfort and/or diarrhea) were more common in those infected with *G. lamblia* (48%) than in their non-infected counterparts (31%) (relative risk [RR] 1.6, 95% confidence interval [CI] 1.0–2.4). Of note, two symptomatic participants who had contracted *G. lamblia* in Ghana did not respond to treatment with metronidazole.

***Giardia lamblia* and *Cryptosporidium* spp., household contacts.** We screened 27 household contacts of 11 *G. lamblia* infected travelers and eight household contacts of two *Cryptosporidium*-infected travelers. *Giardia lamblia* was detected in one household contact of a traveler who had contracted *G. lamblia* during travel, and in one household contact of a traveler whose pre- and post-travel sample was positive for *G. lamblia*. *Cryptosporidium* spp. was not detected.

Post-travel screening results: schistosomiasis. Twelve weeks after travel, nine of 146 travelers (6%) seroconverted for *Schistosoma* spp. All had been swimming in Lake Malawi or Lake Victoria. Seven were asymptomatic and two had

Katayama syndrome. Three had antibodies to both SEA and AWA. Six only had antibodies to AWA. The stool samples of these nine travelers were analyzed for *Schistosoma* spp. using PCR. Two, both with antibodies to AWA only, were positive. Treatment and follow-up was done by the travelers' general practitioners.

DISCUSSION

The incidence of asymptomatic infection with *E. histolytica* and *S. stercoralis* after travel was low. Only one infection with *S. stercoralis* was found in over 400 travelers and no infection with *E. histolytica* in over 500 travelers. Therefore, routine screening of asymptomatic travelers is not indicated for those who travel to the (sub)tropics for up to 3 months. The incidence of infection with *Schistosoma* spp. was higher. However, each case was associated with exposure to highly endemic lakes in Malawi and Tanzania. Therefore, screening for *Schistosoma* spp. can be limited to travelers with a history of exposure to fresh water exposure in endemic regions. In the study by Whitty and others,¹ freshwater exposure did not correlate with schistosomiasis. However, their result may be influenced by a considerable amount of recall bias, considering the fact that most participants had lived in the tropics for well over a year.

Infection with *G. lamblia* was seen in 5% and was associated with gastrointestinal complaints in 48%, meaning that half of the infected participants were asymptomatic. Participants, who already had *G. lamblia* in their stool before departure, did not have abdominal complaints. Infection is usually self-limiting and does not have long-term repercussions. Furthermore, the prevalence of asymptomatic *G. lamblia* infection in The Netherlands is comparable to the post-travel incidence in this study¹¹; therefore, routine screening of asymptomatic travelers for *G. lamblia* is not warranted.

This study has a number of strengths. First, the participants were recruited prospectively and samples were collected before and after travel. This allowed us to differentiate post-travel incident cases from pre-existent infections. Second, we used a well-validated multiplex real-time PCR to screen stool samples. This method is more sensitive than traditional methods to detect infection.^{3,12,13} Furthermore, it can differentiate pathogenic *E. histolytica* from apathogenic *E. dispar*. Finally, although 18% were lost to follow-up, there is no reason to assume that these participants had higher rates of symptomatic or asymptomatic infection. This study also has limitations. The results may not be representative of all travelers. First, the results pertain to travelers who seek health-related travel advice before travel and who traveled for ~3 months. Post-travel screening of specific groups of asymptomatic travelers, such as migrants, expatriates, or aid workers may yield higher infection rates. Second, half of the participants in this study were students. Although most students combined their stay abroad with travel for touristic purposes, this may limit the generalizability of this study.

To conclude, based on the low incidence of infection, routine screening for *E. histolytica*, *S. stercoralis*, *Cryptosporidium* spp., and *G. lamblia* is not indicated for asymptomatic people, who travel to the (sub)tropics for up to 3 months.

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TABLE 4

Incidence proportion of infection with *Giardia lamblia* and *Schistosoma* spp. according to travel destination

Characteristic	<i>Giardia lamblia</i> , n (%)	<i>Schistosoma</i> spp. Seroconversion, n (%)
Latin America*	7/133 (5)	—
Central America and Caribbean	2/44 (5)	—
South America	6/104 (6)	—
Africa	10/205 (5)	9/146 (6)
Northern Africa	0/5 (0)	—
Western Africa	7/56 (13)	0/37 (0)
Middle Africa	1/17 (6)	0/11 (0)
Eastern Africa	2/106 (2)	9/78 (12)
Southern Africa	0/46 (0)	0/27 (0)
Asia*	12/202 (6)	—
Central and Western Asia	1/12 (8)	—
Eastern Asia	2/36 (6)	—
South-Eastern Asia	3/120 (3)	—
Southern Asia	9/73 (12)	—

* A few participants traveled to more than one region or continent.

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