Molecular diagnostics of intestinal parasites in returning travellers

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Abstract A new diagnostic strategy was assessed for the routine diagnosis of intestinal parasites in returning travellers and immigrants. Over a period of 13 months, unprepared stool samples, patient characteristics and clinical data were collected from those attending a travel clinic. Stool samples were analysed on a daily basis by microscopic examination and antigen detection (i.e. care as usual), and compared with a weekly performed multiplex real-time polymerase chain reaction (PCR) analysis on Entamoeba histolytica, Giardia lamblia, Cryptosporidium and Strongyloides stercoralis. Microscopy and antigen assays of 2,591 stool samples showed E. histolytica, G. lamblia, Cryptosporidium and S. stercoralis in 0.3, 4.7, 0.5 and 0.1% of the cases, respectively. These detection rates were increased using real-time PCR to 0.5, 6.0, 1.3 and 0.8%, respectively. The prevalence of ten additional pathogenic parasite species identified with microscopy was, at most, 0.5%. A pre-selective decision tree based on travel history or gastro-intestinal complaints could not be made. With increased detection rates at a lower workload and the potential to extend with additional parasite targets combined with fully automated DNA isolation, molecular high-throughput screening could eventually replace microscopy to a large extent.

Introduction

Over the past decennia, outbound tourism showed a worldwide increase and it is expected that this trend will continue in the future [1]. As a consequence, it is likely that a growing number of international travellers will consult a doctor after their return. Gastro-intestinal disorders are one of the main reasons for returning travellers to seek medical advice [2, 3]. Moreover, several studies have shown that a large proportion of travellers and immigrants from tropical and subtropical countries harbour intestinal pathogens without clear gastro-intestinal problems [4–7]. Although this emphasises the need for a standard screening procedure for all travellers, the increasing numbers of samples will become a burden for routine diagnostic laboratories, especially during the peak periods of holidaying. Patients and diagnostic laboratories would, therefore, greatly benefit from the implementation of a sensitive high-throughput system for the screening of intestinal parasites.

Intestinal protozoan infections with Giardia lamblia and Cryptosporidium hominis/Cryptosporidium parvum are the main non-viral causes of diarrhoea in industrialised countries [8] and are even more frequently seen as the cause of gastro-intestinal complaints in returning travellers [2, 9, 10]. Although quite rare, the early diagnosis of Entamoeba histolytica is of vital importance because of the potential invasive character of this protozoan parasite. Intestinal helminth infections in travellers usually do not cause severe clinical complications. One important exception is Strongyloides stercoralis. Unlike other helminth infections, S. stercoralis is capable of maturing to the infective filariform stage in the intestinal lumen, causing auto-infection through larval penetration of the intestinal mucosa or the perianal skin. Even after decades, these chronic infections can develop into life-threatening hyper
infections in immune depressed patients, especially those receiving immunosuppressive therapy with corticosteroids [11]. Laboratory diagnosis of schistosomiasis, which is frequently diagnosed in travellers, mainly depends on serology rather than on the microscopic detection of ova in stool and urine in this particular population [12].

Traditionally, the laboratory diagnosis of intestinal protozoan and helminth infections relies on the detection of trophozoites, cysts, eggs and larvae by microscopic stool examination. Although microscopy is considered to be the gold standard, it is labour-intensive and its diagnostic performance critically depends on well-trained microscopists. To improve sensitivity, multiple specimens and concentration procedures, as well as a variety of staining methods, are needed to achieve ample sensitivity [13, 14]. Enzyme immunoassays [15, 16] and direct fluorescent-antibody assays [17] have been accepted as cost-effective alternative diagnostic methods for the detection of *G. lamblia* and Cryptosporidium in stools. The specific detection of *E. histolytica* cannot be achieved using microscopy alone, as cysts and (small) trophozoites of *E. histolytica* and non-pathogenic *E. dispar* are morphologically indistinguishable. Therefore, additional methods such as antigen detection or polymerase chain reaction (PCR) have to be employed [7]. The laboratory diagnosis of *S. stercoralis* is known to be problematic: the sensitivity and specificity of immunodiagnostic assays can vary considerably and the number of larvae in a stool sample can be very low. Multiple samples and concentration methods such as Baermann and copro-culture techniques are employed to increase the detection rates [18].

Although DNA-based methods for a variety of intestinal parasites have shown excellent sensitivity and specificity, until now, the introduction of these methods in daily laboratory practice has been limited. The introduction of real-time PCR combining several targets into one multiplex assay and the implementation of automated DNA-isolation methods offers the possibility of using DNA-based detection techniques in a high-throughput diagnostic approach. In a previous study, it was shown that in patients attending their general practitioner with gastrointestinal problems, only two parasitic pathogens were found in such a population, i.e., *G. lamblia* and *C. hominis/C. parvum* [19]. The sensitivity of the multiplex real-time PCR proved to be much higher as compared to microscopy in detecting these two parasitic infections. In returning travellers, a larger variety of parasitic infections can be expected. Presently, one of the constraints of multiplex real-time PCR is the restriction in the number of parasitic targets that can be detected simultaneously. Therefore, a careful assessment is needed for the choice of parasitic targets when molecular diagnostic techniques are implemented.

In this prospective study, the performance of real-time PCR for the detection of *E. histolytica*, *G. lamblia*, *C. hominis/C. parvum* and *S. stercoralis* DNA in faeces was compared with current diagnostic tools, which consist of microscopy and antigen detection in stool samples from patients attending a travel clinic. Patient characteristics and clinical data were recorded to define a practical diagnostic strategy for the implementation of molecular methods in the routine laboratory diagnosis of intestinal parasitic infections in returning travellers and immigrants.

### Materials and methods

**Sample collection**

Stool specimens were collected between April 2005 and May 2006 from outpatients attending the travel clinic of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. Age, gender, place of birth, travel history and gastrointestinal complaints were actively recorded by the attending physician and included in the database. Patients who were referred to the clinic specifically for HIV/AIDS-related issues were not included in this study. A successive sample from the same patient analysed within 30 days of the preceding sample was excluded from the study. Subsequently, each sample was considered to have been obtained from a new patient. The protocol was approved by the ethical committee of the ITM. Patients gave informed consent and the data were rendered anonymous according to the Belgian legislation.

Stool specimens were collected according to the routine procedure of the ITM: patients were asked to fill an empty tube with stool, preferably at the clinic, or otherwise to send it to the diagnostic laboratory of the ITM by regular mail. Aliquots of the samples were stored in the refrigerator until they were sent to the Leiden University Medical Centre (LUMC) for real-time PCR analysis on a weekly transport by regular mail. Molecular diagnostics were performed immediately after the arrival of the samples and the results were generated blinded to the result of the conventional stool examination at the ITM.

**Microscopy and copro-antigen detection**

Microscopic examination for the presence of ova and cysts was performed daily according to the standard routine procedures at the diagnostic laboratory of the ITM by the examination of unstained and iodine-stained direct smears with saline and unstained and iodine-stained wet mounts after formalin-ether concentration [20]. Additionally, carbol-fuchsine staining was performed on formalin-ether concentrates for the detection of coccidian parasites [21].
Both pathogenic and non-pathogenic parasites were recorded.

Copro-antigen enzyme-linked immunosorbent assays (ELISAs) for the detection of *Giardia, Cryptosporidium* and *E. histolytica/E. dispar* (ProSpecT, Remel, Lenexa, Kansas, USA) were performed on all specimens. The *E. histolytica*-specific copro-antigen tests (TechLab, Blacksburg, VA, USA) was performed if macroscopic examination revealed *E. histolytica/E. dispar* cysts and/or if the *E. histolytica/E. dispar* antigen ELISA showed a positive result. In the diagnostic procedure at the ITM before the start of this study, *E. histolytica/E. dispar*-positive samples were routinely tested with the *E. histolytica/E. dispar* (HD) PCR [22]. As in this study, *E. histolytica* PCR would be performed as part of the *E. histolytica/G. lamblia/Cryptosporidium* (HGC) PCR on all samples; HD PCR was performed only on the microscopy or antigen *E. histolytica/E. dispar*-positive samples retrospectively.

In clinically suspected strongyloidiasis cases, Baermann’s method was requested to detect *S. stercoralis* larvae [23, 24]. The detection of *Enterobius vermicularis* eggs was performed on demand using the scotch-tape method [25].

**DNA isolation**

For DNA isolation, 200 μl of faeces suspension (approximately 0.5 g/ml PBS containing 2% polyvinylpyrrolidone [PVPP; Sigma]) was heated for 10 min at 100°C. After sodium-dodecyl-sulphate-proteinase K treatment (overnight), DNA was isolated with QIAamp Tissue Kit spin columns (QIAGen, Hilden, Germany) [26]. In each sample, 10^3 plaque-forming units (PFU)/ml phocin herpes virus 1 (PhHV-1) was added within the isolation lysis buffer, to serve as an internal control for the isolation, amplification and detection of the multiplex real-time PCR assays [27].

**PCR amplification and detection**

*E. histolytica, G. lamblia* and *C. hominis/C. parvum* multiplex real-time PCR including PhHV-1 as an internal control (HGC PCR) was performed as described previously with some modifications [28]. *S. stercoralis* DNA amplification was performed in a separate assay, also including PhHV-1 as an internal control [29].

Amplification and detection reactions for HGC PCR were performed in a volume of 25 μl containing PCR buffer (Hotstar master mix, QIAGen, Venlo, The Netherlands), 5 mM MgCl2, 2.5 μg bovine serum albumin (BSA) (Roche Diagnostics, Almere, The Netherlands), 3.13 pmol of each *E. histolytica*-specific primer, 1.25 pmol VIC-labelled MGB probe for *E. histolytica* (Applied Biosystems, Warrington, UK), 3.13 pmol of each *G. lamblia* primer, 2.5 pmol *G. lamblia*-specific FAM-double-labelled probe (Biolegio, Mal-
an Access database (Microsoft, Redmond, WA, USA). Data
included patient’s demographic information, travel history
and clinical presentation, the laboratory results of the travel
clinic in Antwerpen and the real-time PCR results of the
laboratory for parasitology in Leiden. Travel destinations
were classified according to geographical regions: Central
America, South America, Asia, Northern Africa and sub-
Saharan Africa were considered as high-risk areas and
others (including Europe, United States of America and
Australia) as being at a low risk of contracting intestinal
infections. World travellers or sailors were also considered
to have a high risk of contracting intestinal infections.

Analysis was done with SPSS 14.0 (SPSS Inc., Chicago,
IL, USA). Continuous variables were described by the
range and median of all positive cases and were compared
between groups by the Mann-Whitney rank-sum test. For
this purpose, zero values in Ct-values were redefined as 50.
Statistical significance was considered at $P<0.05$. The Chi-
square distribution for the risk of acquiring pathogenic
intestinal parasites, recorded as high- or low-risk travel
destination, or for the presence of gastro-intestinal com-
plaints, was calculated as a proportion of parasite infections
detected by any of the used diagnostic techniques.

Results

Study group

Over a period of 13 months, 2,709 samples were collected
and analysed (Fig. 1). One hundred and eighteen samples
were excluded from the study population as the time
interval after the preceding sample of the same patient
was less than 30 days. The final number of participants
included in this study was 2,591. PhHV internal control was
amplified within the correct Ct range in all samples;
therefore, no samples were excluded due to inhibition.
The travellers’ ages varied from newborn to 85 years
(median 36) and 55.5% of the patient cohort was of the
male gender. Travel to 142 different countries or areas was
mentioned, most frequently to sub-Saharan Africa (50.9%).
The majority of travellers were born in Europe (73.7%) or
on the African continent (19.0%). Gastro-intestinal com-
plaints were mentioned by 897 (34.6%) patients and are
listed in more detail in Table 1, together with the other
patient’s characteristics.

Diagnosis of Entamoeba histolytica/Entamoeba dispar,
Giardia lamblia and Cryptosporidium spp.

The results of microscopy, copro-antigen and real-time
PCR for all faecal parasites are summarised in Table 2. E.
histolytica/E. dispar cysts and trophozoites were detected
by microscopy in 99 cases. Fifteen additional cases were
detected with E histolytica/E. dispar copro-antigen ELISA.
The presence of E. histolytica was confirmed with the
species-specific copro-antigen ELISA in seven cases. In
one of these seven samples, trophozoites with ingested red
blood cells were seen in the direct smear, representing the
only distinctive morphologic features of E. histolytica. E.
histolytica-specific amplification was detected using the
HGC PCR in 13 samples, with Ct-values between 20.7 and
38.7 (median 31.3). Retrospective analysis with HD PCR of
114 E. histolytica/E. dispar microscopy and/or copro-
antigen-positive samples confirmed 88 samples as E. dispar
and 14 samples as E. histolytica, whereas HD PCR
remained negative for both targets in 12 samples.

G. lamblia cysts and/or trophozoites were detected with
microscopy in 95 cases and 121 cases revealed a positive
result in the Giardia antigen ELISA. The Giardia antigen

![Fig. 1 Number of all collected stool samples (n=2,709) on a weekly basis between April 2005 and May 2006. Sharp increases of sample collections due to holidays are observed in August 2005 and at beginning of January 2006](image-url)
ELISA was positive in 28 microscopy-negative cases and did not confirm two microscopy-positive samples. G. lamblia-specific amplification was detected in 149 samples with Ct-values between 17.0 and 44.7 (median 29.8).

Cryptosporidium oocysts were detected in 12 carbol-fuchsin-stained samples. The copro-antigen tests confirmed all Cryptosporidium microscopy-positive samples and two additional samples were found positive in the Cryptosporidium antigen ELISA. C. hominis/C. parvum-specific amplification was detected in 31 samples with Ct-values between 24.4 and 39.5 (median 35.6).

Ct-values of samples positive with microscopy and/or copro-antigen tests were significantly lower as compared to samples with a negative result in the conventional methods (P < 0.001) for both G. lamblia and C. hominis/C. parvum (data not shown).

Discrepancies between real-time HGC PCR and conventional methods were observed in the following cases. E. histolytica real-time PCR was negative in one sample in which E. histolytica/E. dispar cysts were observed with microscopy and both copro-antigen ELISAs tested positive. Giardia real-time PCR remained negative in two cases in which cysts were seen in microscopy and in five cases in which only the copro-antigen assay tested positive. Cryptosporidium real-time PCR remained negative in two samples in which only the Cryptosporidium copro-antigen tested positive. The HGC multiplex real-time PCR detected a total of 49 additional cases which were not detected with microscopy and antigen tests.

Strongyloides stercoralis

Rhabditiform S. stercoralis larvae were detected with microscopy on concentrated smears in three samples. In one of these samples, the number of larvae was exceptionally high and the larvae were also detected in direct smear. Baermann’s method was performed on stool samples from 121 clinically suspected cases and was only found to be positive in the same sample that was found to be positive in the direct smear. S. stercoralis-specific amplification was detected in all three microscopy-positive samples and in 18 additional samples with Ct-values ranging from 24.5 to 39.5 (median of 33.3).

Other parasitic infections

Microscopy revealed 55 additional pathogenic parasites that were not targeted with real-time PCR. Mixed infections with two pathogenic parasite species were observed in 18 patients (Table 3). Non-pathogenic parasites as seen in direct smears and wet mounts after concentration are also summarised in Table 2.

Travel history, symptoms and intestinal parasites

Detected parasitic infections in relation to travel destination and gastro-intestinal complaints are summarised in Table 4.
The majority (69.2%) of the study participants travelled to areas where the exposure risk to intestinal parasites is considered to be high. Among those with high-risk travel destinations, infection rates were not significantly higher for any of the pathogenic parasites compared with those who travelled to low-risk areas. Gastro-intestinal complaints were correlated with *G. lamblia* and *C. hominis/C. parvum* detection (*P* < 0.001), but not with the detection of *E. histolytica*, *S. stercoralis* or other pathogenic parasitic infections.

**Table 2** Intestinal parasites in the study population (n=2,591) as detected with microscopy, copro-antigen test and real-time polymerase chain reaction (PCR)

| Pathogens                        | Total detected | Microscopy | Antigen | Real-time PCR |
|----------------------------------|----------------|------------|---------|---------------|
| *E. histolytica/E. dispar*       | 114            | 99         | 90<sup>a</sup> | -             |
| *E. histolytica*                 | 14             | 1<sup>b</sup> | 7<sup>d</sup> | 13            |
| *G. lamblia*                    | 156            | 95         | 121     | 149           |
| Cryptosporidium spp.            | 33             | 12         | 14      | 31            |
| *S. stercoralis*                | 21             | 3          | -       | 21            |
| *T. trichiura*                  | 14             | 14         | -       | -             |
| Hookworm                        | 10             | 10         | -       | -             |
| *A. lumbricoides*               | 8              | 8          | -       | -             |
| Trichostrongylus spp.           | 3              | 3          | -       | -             |
| *E. vermicularis*               | 1              | 1          | -       | -             |
| *S. mansoni*                    | 11             | 11         | -       | -             |
| *S. haematobium*                | 1              | 1          | -       | -             |
| *Taenia* spp.                   | 1              | 1          | -       | -             |
| *C. cayetanensis*               | 4              | 4          | -       | -             |
| *I. belli*                      | 2              | 2          | -       | -             |
| *Non-pathogens*                 |                |            |         |               |
| *E. coli*                       | 246            | 246        | -       | -             |
| *B. hominis*                    | 220            | 220        | -       | -             |
| *E. nana*                       | 139            | 139        | -       | -             |
| *E. hartmanii*                  | 59             | 59         | -       | -             |
| *I. butschlii*                  | 26             | 26         | -       | -             |
| *C. mesnilii*                   | 23             | 23         | -       | -             |
| *S. hominis*                    | 6              | 6          | -       | -             |

<sup>a</sup> Direct microscopy, after formalin-ether concentration, scotch tape test for *E. vermicularis*, Baermann test for *S. stercoralis*, and carbol-fuchsine staining for Cryptosporidium spp.

<sup>b</sup> Observed hematophagous trophozoites

<sup>c</sup> *E. histolytica / E. dispar* copro-antigen test (ProSpect, Remel, Lenexa, Kansas, USA)

<sup>d</sup> *E. histolytica* specific copro-antigen test (TechLab, Blackburg, Virginia, USA)

The diagnosis of intestinal parasitic infections in returned travellers traditionally relies on time-consuming analyses by experienced microscopists. Nowadays, the increasing number of travellers to a variety of exotic countries calls for new diagnostic approaches for the efficient processing of samples. In this study, multiplex real-time PCR was compared with the routine approach of microscopy and antigen-based methods, focussing on four target parasites, *E. histolytica*, *G. lamblia*, Cryptosporidium and *S. stercoralis*, respectively.

The results showed PCR to be more sensitive for the specific detection of *E. histolytica*, *G. lamblia*, *C. hominis/C. parvum* and *S. stercoralis*. Only a few unexplained discrepancies between the different detection methods were seen. These were mostly cases in which only the antigen test was positive. In one sample, *E. histolytica/E. dispar* cysts were seen with microscopy and both antigen ELISAs...
showed a positive result; however, *E. histolytica*-specific amplification was not successful in the initial HGC PCR. A positive result was obtained when the HGC real-time PCR was repeated, as well as in the HD real-time PCR. Internal and positive controls in the initial real-time PCR analysis were correct and the reason for the negative outcome of the first real-time PCR analysis remains unclear. This one case is discordant with the proven higher sensitivity of *E. histolytica* real-time PCR compared to stool antigen assays in a non-endemic setting [30, 31].

It is well known that the laboratory diagnosis of *S. stercoralis* requires multiple fresh stool samples using concentration techniques and/or copro-culture in order to improve detection rates [32]. In this study, the Baermann concentration technique was requested by the clinician in case of clinical suspicion, yet many Baermann tests were omitted because, for example, the sample was too old (had been sent by mail). Although the positive results of the *S. stercoralis* real-time PCR was confirmed by microscopy in three cases only, the other positive results were supported by serology (*n*=7), eosinophilia (*n*=5) and/or clinical presentation (*n*=4) in the majority of cases.

In this study population, ten additional pathogenic parasite species detected by microscopy were missed with only four targeted parasite species in the real-time PCR. However, the prevalence of each detected species was only 0.5% at most and the clinical significance of the majority of these parasitic infections is limited. Most of these infections were helminths, of which the eggs could be easily found with microscopy at low magnification of an unstained wet mount of the concentrated sample. Schistosomiasis is probably the most relevant and also probably the most underestimated infection in this study, as the laboratory diagnosis of *Schistosoma* infections in travellers relies mainly on serology [33]. The implementation of a *Schistosoma* real-time PCR [34] might be a worthwhile addition to a molecular diagnostic panel; however, its performance in a routine setting still needs further evaluation. Finally, two remaining protozoan infections, *Isospora belli* and *Cyclospora cayetanensis*, are important candidates as additional real-time PCR targets for patients returning from high-risk areas [35–37].

Designing an efficient diagnostic strategy requires a thorough exploration of possible predictors for parasite exposure in patient groups. A rationale for a specific diagnostic approach in a travel population is less evident compared with a patient group without extensive travel background [19]. In the travel population of this study, only a minority (34.6%) mentioned gastro-intestinal complaints. Stool examination was also performed as part of a routine screening procedure. The presence of gastro-intestinal complaints was a significant predictor only for the presence of *G. lamblia* and *C. hominis*. In the cases without complaints, however, the prevalence of *G. lamblia* was still higher than any of the other pathogenic parasites in the total population. Furthermore, travel destinations were of little predictive value for the presence of any of the parasitic infections. In conclusion, travel destinations or gastro-intestinal complaints did not provide a diagnostic key towards specific pathogenic parasites in this study. As already seen in other studies, an exception can be made for the diagnosis of schistosomiasis, as cases are mainly found in travellers returning from Africa [6, 33].

The overall low prevalence of intestinal parasitic infections found in this study emphasises the need for a rapid, sensitive and simple screening assay for the most important parasitic infections in all returned travellers, disregarding their travel history and the presence of gastro-intestinal complaints, which agrees with recommendations made in other studies [6, 38, 39]. The low
prevalence of additionally detected parasites raises doubts about the cost benefit of elaborate microscopic analyses of stool samples. An in-depth study on the cost per detected parasite or profit per diagnosis by different technical approaches will further elucidate the most beneficial strategy for a laboratory. For example, the staff utilisation for stool analysis with conventional techniques consists in this study of approximately one full-time equivalent (FTE) compared to 0.3 FTE for stool DNA isolation and real-time PCR analysis. Moreover, a fully automated DNA isolation process and extension of additional molecular targets on the already isolated DNA will have considerable impact on the cost-efficiency of the diagnostic procedures. Already, a growing number of routine diagnostic laboratories are implementing multiplex real-time PCR for the detection of intestinal microorganisms [19, 40, 41]. These standard PCR assays can be extended specifically for the travel population with one or more additional multiplex real-time PCR panels for an overlap of the most important intestinal parasitic infections.

Real-time PCR has proved to be a highly sensitive and specific technique for the detection of the majority of intestinal parasites found in returning travelers presenting at a travel clinic with and without gastro-intestinal complaints. The diagnostic approach for the detection of intestinal parasites in returning travelers in a routine diagnostic laboratory could be limited to real-time PCR for *E. histolytica*, *G. lamblia*, *Cryptosporidium* spp. and *S. stercoralis*. In addition, *Schistosoma* serology should be performed for travelers to Africa [6]. This approach could be complemented with additional multiplex PCR panels and/or a quick microscopic examination for the presence of helminth eggs. Fully automated procedures and combination with additional targets might replace microscopy in the future to a large extent.

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References

1. World Tourism Organization (2001) Global Forecast and Profiles of Market Segments. Tourism: 2020 Vision, Vol 7. World Tourism Organization, Madrid, Spain. ISBN 978-92-844-0466-7

2. Freedman DO, Weld LH, Kozarsky PE, Fisk T, Robins R, von Sonnenburg F, Keystone JS, Pandey P, Cetron MS; GeoSentinel Surveillance Network (2006) Spectrum of disease and relation to place of exposure among ill returned travelers. N Engl J Med 354:119–130. doi:10.1056/NEJMoa051331

3. Ansart S, Perez L, Vergely O, Danis M, Bricaire F, Caumes E (2005) Illnesses in travelers returning from the tropics: a prospective study of 622 patients. J Travel Med 12:312–318

4. Caruana SR, Kelly HA, Ngeow JY, Ryan NJ, Bennett CM, Chea L, Nuon S, Bak N, Skull SA, Biggs BA (2006) Undiagnosed and potentially lethal parasite infections among immigrants and refugees in Australia. J Travel Med 13:233–239. doi:10.1111/j.1708-8305.2006.00045.x

5. Saiman L, Aronson J, Zhou J, Gomez-Duarte C, Gabriel PS, Alonso M, Maloney S, Schulte J (2001) Prevalence of infectious diseases among internationally adopted children. Pediatrics 108:608–612. doi:10.1542/peds.108.4.608

6. Whitty CJ, Carroll B, Armstrong M, Dow C, Snashall D, Marshall T, Chiodini PL (2000) Utility of history, examination and laboratory tests in screening those returning to Europe from the tropics for parasitic infection. Trop Med Int Health 5:818–823. doi:10.1046/j.1365-3156.2000.00642.x

7. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J (2007) Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev 20:511–532. doi:10.1128/CMR.00004-07

8. de Wit MAS, Koopmans MPG, Kortbeek LM, Wannet WJB, Vinjé J, van Leusden F, Bartelds AIM, van Duynhoven YTHP (2001) Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. Am J Epidemiol 154:666–674. doi:10.1093/aje/154.7.666

9. Thielen NM, Guerrant RL (1998) Persistent diarrhea in the returned traveler. Infect Dis Clin N Am 12:489–501. doi:10.1016/S0891-5520(05)70015-5

10. Okhuysen PC (2001) Traveler’s diarrhea due to intestinal protozoa. Clin Infect Dis 33:110–114. doi:10.1086/320894

11. Concha R, Harrington W Jr, Rogers AI (2005) Intestinal strongyloidiasis: recognition, management, and determinants of outcome. J Clin Gastroenterol 39:203–211. doi:10.1097/01. mcg.0000152779.68900.33

12. Bottieau E, Clerinx J, Van den Enden E, Van Esbroeck M, Colebunders R, Van Gompel A, Van den Ende J (2007) Fever after a stay in the tropics: diagnostic predictors of the leading tropical conditions. Medicine 86:18–25. doi:10.1097/MD.0b013e3180305c48

13. Branda JA, Lin TY, Rosenberg ES, Halpern EF, Ferraro MJ (2006) A rational approach to the stool ova and parasite examination. Clin Infect Dis 42:972–978. doi:10.1086/500937

14. Morgan UM, Thompson RC (1998) Molecular detection of parasitic protozoa. Parasitology 117(Suppl):S73–S85

15. Weitzel T, Dittrich S, Möhl I, Adusu E, Jelinek T (2006) Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. Clin Microbiol Infect 12:656–659. doi:10.1111/j.1365-3156.2006.01457.x

16. Katanik MT, Schneider SK, Rosenblatt JE, Hall GS, Procop GW (2001) Evaluation of *ColorPAC* *Giardia*/*Cryptosporidium* rapid assay and ProSpecT *Giardia*/*Cryptosporidium* microplate assay for detection of *Giardia* and *Cryptosporidium* in fecal specimens. J Clin Microbiol 39:4523–4525. doi:10.1128/JCM.39.12.4523-4525.2001

17. Garcia LS, Shimizu RY (1997) Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. J Clin Microbiol 35:1526–1529

18. Steinhmann P, Zhou XN, Du ZW, Jiang Y, Wang LB, Wang XZ, Li LH, Marti H, Uetzminger J (2007) Occurrence of *Strongyloides stercoralis* in Yunnan Province, China, and comparison of diagnostic methods. PLoS Negl Trop Dis 1:e75. doi:10.1371/journal.pntd.0000075

19. ten Hove R, Schuurman T, Kooistra M, Möller L, van Lieshout L, Verweij JJ (2007) Detection of diarrhoea-causing protozoa in general practice patients in The Netherlands by multiplex real-
time PCR. Clin Microbiol Infect 13:1001–1007. doi:10.1111/j.1469-0691.2007.01788.x
20. Loughlin EH, Spitz SH (1949) Diagnosis of helminthiasis. JAMA 139:997–1000
21. Heine J (1982) A simple technic for the demonstration of cryptosporidia in feces. Zentralbl Veterinarmed B 29:324–327
22. Verweij JJ, Oostvogel F, Brienen EA, Nang-Beifubah A, Ziem J, Polderman AM (2003) Short communication: Prevalence of Entamoeba histolytica and Entamoeba dispar in northern Ghana. Trop Med Int Health 8:1153–1156. doi:10.1046/j.1360-2276.2003.01145.x
23. Jones CA, Abadie SH (1954) Studies in human strongyloidiasis. II. A comparison of the efficiency of diagnosis by examination of feces and duodenal fluid. Am J Clin Pathol 24:1154–1158
24. Pereira Lima J, Delgado PG (1961) Diagnosis of strongyloidiasis: importance of Baermann’s method. Am J Dig Dis 6:899–904. doi:10.1007/BF02231086
25. Graham CF (1941) A device for the diagnosis of Enterobius infection. Am J Trop Med Hyg 21:159–161
26. Verweij JJ, Blotkamp J, Brienen EAT, Aguirre A, Polderman AM (2000) Differentiation of Entamoeba histolytica and Entamoeba dispar cysts using polymerase chain reaction on DNA isolated from faeces with spin columns. Eur J Clin Microbiol Infect Dis 19:358–361. doi:10.1007/s100960050494
27. Niesters HG (2002) Clinical virology in real time. J Clin Virol 25:361. doi:10.1016/S1386-6532(02)00197-X
28. Verweij JJ, Blange RA, Templeton K, Schinkel J, Brienen EAT, van Rooyen MAA, van Lieshout L, Brienen EAT, Polderman AM (2003) Short communication: Prevalence of Entamoeba histolytica and Entamoeba dispar in northern Ghana. Trop Med Int Health 8:1153–1156. doi:10.1046/j.1360-2276.2003.01145.x
29. Verweij JJ, Canales M, Polman K, Ziem J, Brienen EA, Nang-Beifubah A, Ziem J, Polderman AM (2003) Short communication: Prevalence of Entamoeba histolytica and Entamoeba dispar in northern Ghana. Trop Med Int Health 8:1153–1156. doi:10.1046/j.1360-2276.2003.01145.x
30. Verweij JJ, Blotkamp J, Brienen EAT, Aguirre A, Polderman AM (2000) Differentiation of Entamoeba histolytica and Entamoeba dispar cysts using polymerase chain reaction on DNA isolated from faeces with spin columns. Eur J Clin Microbiol Infect Dis 19:358–361. doi:10.1007/s100960050494
31. Verweij JJ, Laeijendecker D, Brienen EA, Polderman AM (2003) Detection of Cyclospora cayetanensis in travellers returning from the tropics and subtropics using microscopy and real-time PCR. Int J Med Microbiol 293:199–202. doi:10.1016/j.ijmm.2003.08.003
32. Siddiqui AA, Berk SL (2001) Diagnosis of Strongyloides stercoralis infection. Clin Infect Dis 33:1040–1047. doi:10.1086/322707
33. Botteau E, Clerinx J, de Vega MR, Van den Enden E, Colebunders R, Van Esbroeck M, Vervoort T, Van Gompel A, Van den Ende J (2006) Imported Katayama fever: clinical and biological features at presentation and during treatment. J Infect 52:339–345. doi:10.1016/j.jinf.2005.07.022
34. ten Hove RJ, Verweij JJ, Vereecken K, Polman K, Dieye L, van Lieshout L (2008) Multiplex real-time PCR for the detection and quantification of Schistosoma mansoni and S. haematobium infection in stool samples collected in northern Senegal. Trans R Soc Trop Med Hyg 102:179–185. doi:10.1016/j.trstmh.2007.10.011
35. Ten Hove RJ, van Lieshout L, Brienen EA, Aranza Perez M, Verweij JJ (2008) Real-time polymerase chain reaction for detection of Isospora bellii in stool samples. Diagn Microbiol Infect Dis 61:280–283. doi:10.1016/j.diagmicrobio.2008.03.003
36. Varma M, Hester JD, Schaefer FW 3rd, Ware MW, Lindquist HD (2003) Detection of Cyclospora cayetanensis using a quantitative real-time PCR assay. J Microbiol Methods 53:27–36. doi:10.1016/S1050-3227(03)00252
37. Verweij JJ, Laeijendecker D, Brienen EA, van Lieshout L, Polderman AM (2003) Detection of Cyclospora cayetanensis in travellers returning from the tropics and subtropics using microscopy and real-time PCR. Int J Med Microbiol 293:199–202. doi:10.1016/j.ijmm.2003.08.003
38. Gushulak B, Funk M, Steffen R (2007) Global changes related to travelers’ health. J Travel Med 14:205–208. doi:10.1111/j.1708-3835.2007.00128.x
39. Bailey MS, Thomas R, Green AD, Bailey JW, Beeching NJ (2006) Helminth infections in British troops following an operation in Sierra Leone. Trans R Soc Trop Med Hyg 100:842–846. doi:10.1016/j.trmsh.2005.10.001
40. Schuurman T, de Boer RF, van Zanten E, van Slochteren KR, Scheper HR, Dijk-Alberts BG, Müller AV, Kooistra-Smid AM (2007) Feasibility of a molecular screening method for detection of Salmonella enterica and Campylobacter jejuni in a routine community-based clinical microbiology laboratory. J Clin Microbiol 45:3692–3700. doi:10.1128/JCM.00896-07
41. Mackay IM (2004) Real-time PCR in the microbiology laboratory. Clin Microbiol Infect 10:190–212. doi:10.1111/j.1198-743X.2004.00722.x