Review Article
Molecular Diagnostics for Soil-Transmitted Helminths

Elise M. O’Connell1* and Thomas B. Nutman1

1Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Abstract. Historically, the diagnosis of soil-transmitted helminths (STHs) (e.g., Strongyloides stercoralis, Trichuris trichiura, Ancylostoma duodenale, Necator americanus, and Ascaris lumbricoides) has relied on often-insensitive microscopy techniques. Over the past several years, there has been an effort to use molecular diagnostics, particularly quantitative polymerase chain reaction (qPCR), to detect intestinal pathogens. While some platforms have been approved by regulatory bodies (e.g., Food and Drug Administration) to detect intestinal bacteria, viruses, and protozoa, there are no approved tests currently available for STH. Although studies comparing qPCR to microscopy methods for STH are imperfect, due in large part to a lack of a sufficient gold standard, they do show a significant increase in sensitivity and specificity of qPCR compared with microscopic techniques. These studies, as well as the advantages and disadvantages of using qPCR for STH diagnosis, are discussed. Guidelines for those designing future studies utilizing qPCR are proposed for optimizing results, as is the proposition for using standardized molecular diagnostics routinely for STH in clinical laboratories and for field-based studies when possible.

INTRODUCTION

Soil-transmitted helminths (STHs) encompass a number of intestinal parasitic nematodes that are either acquired by larval burrowing through intact skin (Strongyloides stercoralis, the hookworms Ancylostoma duodenale and related spp., and Necator americanus) or by the fecal oral route (Ascaris lumbricoides and Trichuris trichiura). As a group, STH are on the World Health Organization’s (WHO’s) list of 17 neglected tropical diseases1 because of the significant morbidity they cause and their propensity to be poverty promoting. Although WHO does not include S. stercoralis on its formal list of STH, we include it here because it is highly prevalent and can be a significant cause of morbidity and mortality2 in low-, middle-, and high-income countries alike.

Infections with STHs are often clinically asymptomatic, but they can be associated with eosinophilia and/or prolonged gastrointestinal symptoms, which most often occur in returning travelers3–5 and immigrants.6–8 These infections are often underdiagnosed given the decreasing number of well-trained personnel with the competence in identifying eggs and/or larvae in traditional stool-based microscopic methods and the intermittent shedding of eggs and/or larvae by some of these parasites (e.g., Strongyloides).9,10

Over the past 20 years, there has been an increasing effort to use molecular diagnostics for STH in epidemiologic studies and for the diagnosis of individual patients in some high-income countries.11,12 With the recent Food and Drug Administration (FDA) approval of several molecular diagnostic tools for stool bacterial and a few protozoa pathogens,13,14 the use of twenty-first-century technology for the detection of intestinal pathogens has finally begun. However, there is no such FDA-approved molecular platform for diagnosing gastrointestinal helminth infections. In this review, we summarize the work that has been done in this growing field with specific attention paid to the strengths and limitations of using molecular diagnostics (particularly quantitative polymerase chain reaction [qPCR] platforms) in detecting STH.

AN INADEQUATE GOLD STANDARD

Part of the difficulty in determining the sensitivity and specificity of qPCR and other molecular-based diagnostics is the lack of a sufficient gold standard against which to compare these newer techniques, given the general insensitivity of stool-based microscopic methods commonly in use. For example, the Kato-Katz (KK) technique was initially developed to detect Schistosoma spp. eggs,15 but is currently the most commonly used technique in STH surveys.16 It is particularly problematic in accurately detecting hookworm infections as the stool must be prepared immediately,17 and the clarification step (i.e., glycerin) can make the eggs unrecognizable.18 Interestingly, it is partly due to the lack of sensitivity of KK in identifying the larvae of S. stercoralis that has informed the decision by the WHO to not include this pathogen as an STH.16

Some studies have attempted to deal with this (lack of sufficient gold standard) problem by considering true positives to be the sum of the positives found by microscopy and/or PCR.19–22 Others have used several different microscopic detection methods with or without the use of statistical modeling to project the true prevalence, sensitivity, and specificity for any given method.23 Regardless of the method used, with rare exception, studies comparing microscopy to molecular methods (primarily qPCR) to diagnose STH have found markedly increased sensitivities with molecular diagnostics (Table 1). Moreover, because many of the molecular diagnostic techniques are multiplexed24,42,43 or multiparameter,19,21,22 studies have also shown an increased ability to detect multiple concurrent infections using qPCR when compared with microscopy.19,21,43–45

APPLICATIONS OF QPCR AS AN STH DIAGNOSTIC

The WHO has set a worldwide goal to eliminate childhood morbidity caused by STH by 2020. Dictating which communities receive anthelmintic drugs, how often they receive them, and when community treatment is stopped is based on
population prevalence rates determined by a small community sampling. Therefore, the very sensitive nature of qPCR (even post-treatment where eggs are often no longer visible), the improved ability to detect multiple helminths in a given sample and the ability to reproducibly quantitate egg burden, would be a huge asset to control programs. 

Research studies are already utilizing qPCR for mass screening of stool samples for STH in malaria and in vaccine studies (currently underway) as stool can be stored relatively easily (see about preservatives in the section Technical Considerations in Using qPCR as an STH diagnostic) and sent offsite for qPCR not only for STH but also for protozoa and other gastrointestinal pathogens. 

One of the most common criticisms of qPCR is the high cost compared with traditional methods. In one price comparison, a multiplex platform was estimated to cost about the same in consumables as the cost of microscopy, and consumption cost for multi-parallel singleplex qPCR of one group was estimated to be almost half the cost of microscopy on a per test basis. Thus, depending on the technique used, cost may not be a prohibitive factor.

**TECHNICAL CONSIDERATIONS IN USING qPCR AS AN STH DIAGNOSTIC**

Despite the sensitive, rapid, and quantitative nature of qPCR for the diagnosis of STH, there are important methodological considerations in test design and results interpretation. Stool contains bile acids and other substances that inhibit the PCR product amplification. Early on in the use of molecular diagnostics for fecal pathogens, there were significant sensitivity problems when DNA extraction techniques that were not specifically designed to remove these inhibitors were used. PCR inhibitors are largely removed without problem using 1) more recent in-house developed protocols, 2) tissue kits with additional inhibitor removal steps, and 3) stool and soil-engineered kits for DNA extraction. 

A more clinically validated study will also amplify an internal control for each specimen to ensure efficient PCR inhibitor removal and to eliminate the possibility of false negatives in the molecular diagnostic results. 

Sensitivity can also be decreased by formalin fixation of the stool prior to DNA extraction. While specific stool preservation methods have not been directly compared in helminth detection through qPCR, in studies assessing the impact of different preservative measures on protozoal DNA amplification, it has been shown that samples stored in potassium dichromate can be stored at room temperature for prolonged periods before qPCR without any significant loss in sensitivity. Unlike bacteria, which have cell walls that are easy to lyse, some physical stress is required to optimize the release of the nucleic acids from helminth eggs or larvae (and even some protozoa). While freeze-thaw cycles, heating, and/or sonication do appear to offer an advantage over standard lysis buffer methods, the use of a tissue homogenization (“bead beating”) step with beads resistant to degradation (i.e., not glass) likely offers the most thorough disruption of parasite ova, thereby increasing molecular-based assay sensitivities significantly. The amount of stool extracted, the type of physical disruption method used, as well as the relative efficiency of commercial kits in extracting DNA likely explain much of the variability in sensitivity in published studies (see Table 1). 

When interpreting molecular diagnostic results, it is also important to know the DNA sequence being amplified (see Table 2) and the limitations inherent in testing for a widely conserved sequence compared with sequence(s) that are species specific. In terms of qPCR platforms, singleplex (often using a multi-parallel approach) offers slightly more sensitivity compared with multiplexed assays (in which reagents in a given tube/well may be limiting). However, it may be possible to optimize a multiplex format to be equally sensitive to a singleplex approach. Multiplexed assays do, however, require more sophisticated and expensive equipment and labeled probes that may be less universally available (and more costly). 

**QPCR IN THE DETECTION OF EACH OF THE MAJOR STH**

*Acanthocephalus brasiliensis.* At an estimated prevalence rate of 819 million infections worldwide, *A. brasiliensis* is by far...
the most common STH.66 Several large studies have shown a clear relationship between stool DNA concentration as determined by qPCR and egg counts.19,21,43 Interestingly, there is also a good correlation between DNA quantification in the stool (presumably reflecting egg DNA) and the number of expelled adult worms after albendazole treatment.21 The evaluation of the existing data on *A. lumbricoides* across multiple studies (Table 1) has been aided enormously by the fact that all of the studies have targeted the internal transcribed spacer 1 region (see Table 2). In addition, of all the STH, KK identifies *A. lumbricoides* the most easily. More reliable reproducibility likely explains the relatively small gap (still in favor of the molecular diagnostics) in sensitivities between KK and qPCR for *Ascaris* (Table 1).

**Hookworms.** Necator americanus and *A. duodenale* are classically the two species of hookworm considered to be the most prevalent and clinically relevant worldwide. However, in certain regions of the world, Ancylostoma ceylanicum and *Oesophagostomum bifurcum* are very prevalent intestinal parasites, both of which are indistinguishable from *N. americanus* and *A. duodenale* using standard microscopic diagnostic methods. Thus, in areas where molecular testing has yet to characterize the exact species of infecting hookworm, the presumed predominant hookworm species may not always be present. This inability to accurately distinguish among the hookworms species morphologically has led to some confusion in that highly species-specific primer/probe combinations have led to some “false negatives” when comparing stool microscopy to qPCR.17,61 Indeed, egg-spiking experiments have determined that qPCR can detect a single hookworm egg in 200 mg of stool36 suggesting qPCR is extraordinarily sensitive, but the eggs seen by microscopy may not be the species that the primer/probe set was designed to detect.

Under optimal microscopic conditions, stool egg counts, stool larval counts, and clinical outcomes have been highly correlated with qPCR cycle times.21,23,24,43 For example, a study on hookworm infection in Malawian children found a significant interrelationship between the burden of hookworms as determined by qPCR, particularly *A. duodenale*, and the severity of iron deficiency and anemia.67 **Trichuris trichiura.** Trichuris infections in some populations are associated with iron deficiency anemia.68–70 The ova of *Trichuris* spp. are notorious for being more difficult to break open in the DNA extraction process than any other STH. Indeed, it is clear that a tissue homogenization step is imperative to achieve egg disruption. This parasite is the least well studied in the context of molecularly based diagno-

---

**Table 2**

| Organism | Sensitivities reported by qPCR | Sensitivities by microscopy |
|----------|--------------------------------|-----------------------------|
| Ascaris lumbricoides | 85.7%, 198% | 71.4%, 172% |
| Hookworms | 96.9%, 178% | 31.3%, 172% |
| Strongyloides stercoralis | 76.0%, 183% | 16.7%, 172% |
| Trichuris trichiura | 100%, 152% | 88%, 152% |

qPCR = quantitative polymerase chain reaction; STH = soil-transmitted helminth.

unless otherwise stated, gold standard in determining true positives was the sum of positives by qPCR and microscopy method used. study specific microscopy methods (if described) are listed below.

† single stool sample prepared with a combination of Kato-Katz, wet preparation, and formal-ether concentration methods.

§ single stool sample subjected to Baermann funnel concentration and Koga agar plate culture.

‡ sensitivity excludes those positive by Koga agar and negative by Baermann funnel. see text for details.

§§ no internal controls used to rule out PCR inhibition in this study.

¶¶ mathematical modeling to determine gold standard taking into account results of single stool subjected to FLOTAC, Kato-Katz, Baermann, and qPCR.

†† gold standard a combination of nested PCR, Koga agar plate culture, and formalin ether concentration.

††† single stool sample prepared by Kato-Katz, with duplicate slides assessed by two technicians.

¶¶¶ gold standard a combination of formalin-ethyl acetate concentration, agar plate culture, and Harada Mori technique.

---

**Proposed Guidelines in Using qPCR to Diagnose STH in Research Studies**

Given the variability in sensitivity and specificity of qPCR found in previous studies, we propose some guidelines for use
Search Tool74 searches. Once identified, the primers/probes sequences should be used for a particular target. Alternatively, of false-negative results due to PCR inhibitors, samples should and PCR surveys, one should ideally know the endemic hookworm reactive organisms to determine specificity. In performing stool DNA from the species of interest and from potentially cross-derived from these new targets can be tested using genomic actual species of infecting parasitic helminth found in the stool.

CONCLUSION

The highly sensitive, rapid, and scalable nature of qPCR makes its utilization in diagnosing STH extremely appealing over insensitive and labor-intensive traditional microscopic methods. We have suggested here its superiority over microscopy methods (particularly KK) in sensitivity while still providing a quantitative measure of infection intensity. There are, however, important technical lessons that have been learned that provide a framework to maximize the utility of this potentially valuable molecular approach, which we have highlighted here. Until now molecular detection of STH has been restricted to the research setting. Given the neglected nature of STH and the large cost of the regulatory processes, moving forward in developing a FDA- or European Union-approved platform would require significant political will and/or philanthropic efforts. Nevertheless, given that surveillance and monitoring for many other pathogens are being done using standardized (but not commercialized) molecular techniques, we would argue that use of qPCR in detecting STH would likely provide a more accurate and cost-effective approach to the WHO STH elimination strategy and should be considered seriously.

Received April 5, 2016. Accepted for publication June 29, 2016.

Published online August 1, 2016.

Financial support: This work was supported by the Division of Intramural Research (DIR) of the National Institute of Allergy and Infectious Diseases.

Authors’ addresses: Elise M. O’Connell and Thomas B. Nutman Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, E-mails: oconnell@niaid.nih.gov and tnutman@niaid.nih.gov.

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

REFERENCES

1. WHO, 2015. Investing to Overcome the Global Impact of Neglected Tropical Diseases: Third WHO Report on Neglected Tropical Diseases 2015. Holmes P, ed. Geneva, Switzerland: World Health Organization, 161–167.

2. Keiser PB, Nutman TB, 2004. Strongyloides stercoralis in the immunocompromised population. Clin Microbiol Rev 17: 208–217.

3. Gill GV, Welch E, Bailey JW, Bell DR, Breechini NJ, 2004. Chronic Strongyloides stercoralis infection in former British Far East prisoners of war. QJM 97: 789–795.

4. Schulte C, Krebs B, Jelincic T, Nothdurft HD, von Sonnenburg F, Loscher T, 2002. Diagnostic significance of blood eosinophilia in returning travelers. Clin Infect Dis 34: 407–411.

5. Naidu P, Yanow SK, Kowalewska-Grochowska KT, 2013. Eosinophilia: a poor predictor of Strongyloides stercoralis infection in refugees. Can J Infect Dis Med Microbiol 24: 93–96.

6. Calderaro A, Montecchini S, Rossi S, Gorrini C, DeConto F, Medici MC, Chezzi C, Arcangelo MC, 2014. Intestinal parasitoses in a tertiary-care hospital located in a non-endemic setting during 2006–2010. BMC Infect Dis 14: 254.

7. Becker SL, Sieto B, Silue KD, Adjonass L, Kone S, Hatz C, Kern WV, N’Goran EK, Utzinger J, 2011. Diagnosis, clinical features, and self-reported morbidity of Strongyloides stercoralis and hookworm infection in a co-endemic setting. PLoS Negl Trop Dis 5: e1292.

8. O’Brien DP, Leder K, Matchett E, Brown GV, Torresi J, 2006. Illness in returned travelers and immigrants/refugees: the 6-year experience of two Australian infectious diseases units. J Travel Med 13: 145–152.

9. Schar F, Hattendorf J, Khiev V, Muth S, Char MC, Marti HP, Odermatt P, 2014. Strongyloides stercoralis larvae excretion patterns before and after treatment. Parasitology 141: 892–897.

10. Ramanathan R, Burbelo P, Groot S, Iadarola M, Neva F, Nutman T, 2008. A luciferase immunoprecipitation systems assay enhances the sensitivity and specificity of diagnosis of Strongyloides stercoralis infection. J Infect Dis 198: 444–451.

11. Soonawala D, van Lieshout L, den Boer MA, Claas EC, Verweij JJ, Godkewitsch A, Ratering M, Visser LG, 2014. Post-travel screening of asymptomatic long-term travelers to the tropics for intestinal parasites using molecular diagnostics. Am J Trop Med Hyg 90: 835–839.

12. ten Hove RJ, van Esbroeck M, Vervoort T, van den Ende J, van Lieshout L, Verweij JJ, 2009. Molecular diagnostics of intestinal parasites in returning travellers. Eur J Clin Microbiol Infect Dis 28: 1045–1053.

13. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, Rogatcheva M, Kanack KJ, Bourzac KM, 2015. Multi-center evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol 53: 915–925.

14. Reddington K, Tuite N, Minogue E, Barry T, 2014. A current overview of commercially available nucleic acid diagnostics approaches to detect and identify human gastroenteritis pathogens. Biomed Quantit Anal 1: 3–7.

15. Sleigh S, Hoff R, Mott K, Barreto M, de Paiva TM, Pedrosa J, Odermatt P, 2014. Eosinophilia: a poor predictor of Schistosoma mansoni eggs in faeces. Trans R Soc Trop Med Hyg 76: 403–405.

16. WHO, 2015. Assessing the Epidemiology of Soil-Transmitted Helminths During a Transmission Assessment Survey in the Global Programme for the Elimination of Lymphatic Filariasis. Geneva, Switzerland: WHO Press.

17. van Mens SP, Aryeetey Y, Yazdanbakhsh M, van Lieshout L, Boakye D, Verweij JJ, 2013. Comparison of real-time PCR and Kato smear microscopy for the detection of hookworm infections in three consecutive faecal samples from schoolchildren in Ghana. Trans R Soc Trop Med Hyg 107: 269–271.

18. Odongo-Aginya EI, Kabaterine N, Ludwig S, Wabinga H, Fenwick A, Montresor A, 2007. Substitution of malachite green with nigrosin-eosin yellow stain in the Kato-Katz method: microscopic appearance of the helminth eggs. Afr J Health Sci 7: 33–36.

19. Mejia R, Vicuna Y, Broncano N, Sandoval C, Vaca M, Chico M, Cooper PJ, Nutman TB, 2013. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities.
to resource-limited at-risk populations. Am J Trop Med Hyg 88: 1041–1047.

20. Arndt MB, John-Stewart G, Richardson BA, Singa B, van Lieshout L, Verweij JJ, Sangare LR, Mbogo LW, Nauliikha JM, Walton JL. 2013. Impact of helminth diagnostic test performance on the estimation of risk factors and outcomes in HIV-positive adults. PLoS One 8: e18915.

21. Easton AV, Oliveira RG, O’Connell EM, Keph A, Mwandawiro CS, Njenga SM, Kihara JH, Mwatele C, Odiere MR, Brooker SJ, Webster JP, Anderson RM, Nutman TB. 2016. Multi-parallel qPCR provides increased sensitivity and diagnostic breadth for gastrointestinal parasites of humans: field-based inferences on the impact of mass deworming. Parasit Vectors 9: 38.

22. Pilotte N, Papaiakovou M, Grant JR, Bierwert LA, Llewellyn S, McCarthy JS, Williams SA. 2016. Improved PCR-based detection of soil transmitted helminth infections using next generation sequencing approach to assay design. PLoS Negl Trop Dis 10: e0004578.

23. Knopp S, Salim N, Schindler T, Karagiannis Voules DA, Rothen J, Lweno O, Mohammed AS, Singo R, Benninghoff M, Nsojo AA, Genton B, Daubenberger C. 2014. Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann, and PCR methods for the detection of light-intensity hookworm and Strongyloides stercoralis infections in Tanzania. Am J Trop Med Hyg 90: 535–545.

24. Verweij JJ, Brienen EA, Ziem J, Yelifari L, Polderman AM, Verweij JJ, Canales M, Pollman K, Ziem J, Brienen EA, Verweij JJ. 2008. PCR-based coprodiagnostic tools reveal dogs as a major reservoir of hookworm infection. Vet Parasitol 155: 411–415.

25. Llewellyn S, Inpankaew T, Nery SV, Gray DJ, Verweij JJ, Clements AC, Gomes SJ, Traub R, McCarthy JS, 2016. Application of a multiplex quantitative PCR to assess prevalence and intensity of intestinal parasite infections in a controlled clinical trial. PLoS Negl Trop Dis 10: e0004380.

26. Basuni M, Muhi J, Othman N, Verweij JJ, Ahmad M, Miswan N, Rahumatullah A, Aziz FA, Zainudin NS, Noordin R. 2011. A pentaplex real-time PCR assay for detection of four species of soil-transmitted helminths, Ascaris lumbricoides, Trichuris trichiura, Necator americanus, and hookworm in human stool samples. Am J Trop Med Hyg 84: 574–580.

27. Sharrifdini M, Mirhendi H, Ashrati K, Hosseini M, Mohebali M, Khodadadi H, Kia EB. 2015. Comparison of nested polymerase chain reaction and real-time polymerase chain reaction with parasitological methods for detection of Strongyloides stercoralis in human fecal samples. Am J Trop Med Hyg 93: 1285–1291.

28. Moghaddassani H, Mirhendi H, Hosseini M, Rokni MB, Mowlavi GH, Kia EB. 2011. Molecular diagnosis of Strongyloides stercoralis infection by PCR detection of specific DNA in human stool samples. Iran J Parasitol 6: 23–30.

29. Kato-Katz, FLOTAC, Baermann, and PCR methods for the detection of light-intensity hookworm and Strongyloides stercoralis infections in Tanzania. Am J Trop Med Hyg 90: 535–545.

30. Traub RJ, Inpankaew T, Sutthikornchai C, Sukthana Y, Thompson CS, Njenga SM, Kihara JH, Mwatele C, Odiere MR, Brooker SJ, Webster JP, Anderson RM, Nutman TB. 2016. Multi-parallel qPCR provides increased sensitivity and diagnostic breadth for gastrointestinal parasites of humans: field-based inferences on the impact of mass deworming. Parasit Vectors 9: 38.

31. Llewellyn S, Inpankaew T, Nery SV, Gray DJ, Verweij JJ, Clements AC, Gomes SJ, Traub R, McCarthy JS, 2016. Application of a multiplex quantitative PCR to assess prevalence and intensity of intestinal parasite infections in a controlled clinical trial. PLoS Negl Trop Dis 10: e0004380.

32. Basuni M, Muhi J, Othman N, Verweij JJ, Ahmad M, Miswan N, Rahumatullah A, Aziz FA, Zainudin NS, Noordin R. 2011. A pentaplex real-time PCR assay for detection of four species of soil-transmitted helminths, Ascaris lumbricoides, Trichuris trichiura, Necator americanus, and hookworm in human stool samples. Am J Trop Med Hyg 84: 574–580.

33. Sharrifdini M, Mirhendi H, Ashrati K, Hosseini M, Mohebali M, Khodadadi H, Kia EB. 2015. Comparison of nested polymerase chain reaction and real-time polymerase chain reaction with parasitological methods for detection of Strongyloides stercoralis in human fecal samples. Am J Trop Med Hyg 93: 1285–1291.

34. Llewellyn S, Inpankaew T, Sutthikornchai C, Sukthana Y, Thompson RC. 2008. PCR-based coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by Ankylostoma caninum in temple communities in Bangkok. Vet Parasitol 155: 67–73.

35. Loreille O, Roumat E, Verneau O, Bouchet F, Hanni C. 2001. Ancient DNA from Ascaris: extraction amplification and sequences from eggs collected in coprolites. Int J Parasitol 31: 1101–1106.

36. Wang JX, Pan CS, Cui LW. 2012. Application of a real-time PCR method for detecting and monitoring hookworm Necator americanus infections in southern China. Asian Pac J Trop Biomed 2: 925–929.

37. Verweij JJ, Pit DS, van Lieshout L, Baeta SM, Dery GD, Gasser RB, Polderman AM. 2001. Determining the prevalence of Oesophagostomum bifurcum and Necator americanus infections using specific PCR amplification of DNA from faecal samples. Trop Med Int Health 6: 726–731.

38. Romstad A, Gasser RB, Monti JR, Polderman AM, Nansen P, Pit DS, Chilton NB. 1997. Differentiation of Oesophagostomum bifurcum from Necator americanus by PCR using genetic markers in spacer ribosomal DNA. Mol Cell Probes 11: 169–176.

39. Romstad A, Gasser RB, Nansen P, Polderman AM, Monti JR, Chilton NB. 1997. Characterization of Oesophagostomum bifurcum and Necator americanus by PCR-RFLP of rDNA. J Parasitol 83: 963–966.

40. Ramachandran S, Gam AA, Neva FA. 1997. Molecular differences between several species of Strongyloides and comparison of selected isolates of S. stercoralis using a polymerase chain reaction-linked restriction fragment length polymorphism approach. Am J Trop Med Hyg 56: 61–65.

41. Nissen S, Al-Jubary A, Hansen TV, Olsen A, Christensen H, Thamsborg SM, Nejsum P. 2012. Genetic analysis of Trichuris suis and Trichuris trichiura recovered from humans and pigs in a sympatric setting in Uganda. Vet Parasitol 188: 68–77.

42. Phumphisut O, Younnuan T, Sanguankiat S, Maipanich W, Pubampen S, Komalamisra C, Adisakwattana P. 2014. Triplex polymerase chain reaction assay for detection of major soil-transmitted helminths, Ascaris lumbricoides, Trichuris trichiura, Necator americanus, in fecal samples. Southeast Asian J Trop Med Public Health 45: 267–275.

43. gwankiaw K, Nery SV, Gray DJ, Verweij JJ, Clements AC, Gomes SJ, Traub R, McCarthy JS, 2016. Application of a multiplex quantitative PCR to assess prevalence and intensity of intestinal parasite infections in a controlled clinical trial. PLoS Negl Trop Dis 10: e0004380.

44. Pinsoneul WA, Ouis IS, Li DJ, Ljueng S, Wadstrom T. 2005. Characterization of the PCR inhibitory effect of bile to optimize real-time PCR detection of Helicobacter species. FEMS Immunol Med Microbiol 44: 177–182.

45. Sato M, Sanguankiat S, Younnuan T, Pongvongsa T, Keomoungkhoun M, Phimmayoi I, Boupa B, Moji K, Waikagul J. 2010. Coprodiagnostic tool for detection of selected intestinal helminths and protozoa in human feces using real-time PCR. Trop Biomed 29: 43–44.

46. Basuni M, Muhi J, Othman N, Verweij JJ, Ahmad M, Miswan N, Rahumatullah A, Aziz FA, Zainudin NS, Noordin R. 2011. A pentaplex real-time polymerase chain reaction assay for detection of four species of soil-transmitted helminths. Am J Trop Med Hyg 84: 338–343.

47. Yooseph S, Kirkness EF, Tran TM, Harkins DM, Jones MB, Lasala MB, Tekiel VS, Gonzalez Cappa SM, 2013. An improved rapid method for the detection of Strongyloides stercoralis in coprolites. Vet Parasitol 188: 338–346.

48. Al-Soud WA, Ouis IS, Li DJ, Ljueng S, Wadstrom T. 2005. Characterization of the PCR inhibitory effect of bile to optimize real-time PCR detection of Helicobacter species. FEMS Immunol Med Microbiol 44: 177–182.
52. Liu J, Gratz J, Amour C, Kibigi G, Becker S, Janaki L, Verweij JJ, Taniuchi M, Sobuz SU, Haque R, Haveckert DM, Houpt ER, 2013. A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. Clin Microbiol 51: 472–480.
53. Taniuchi M, Verweij JJ, Noor Z, Sobuz SU, Licsbou L, Petri WA Jr, Haque R, Houpt ER, 2011. High-throughput multiplex PCR and probe-based detection with Lumixen beads for seven intestinal parasites. Am J Trop Med Hyg 84: 332–337.
54. Harmon AF, Williams ZB, Holler LD, Hildreth MB, 2007. Comparison of three different preservatives for morphological and real-time PCR analyses of Haemonchus contortus eggs. Vet Parasitol 145: 361–365.
55. Williams RB, Theo P, Marshall RN, Marshall JA, 2010. Coccidial oocysts as type-specimens: long-term storage in aqueous potassium dichromate solution preserves DNA. Syst Parasitol 76: 69–76.
56. Wilke H, Robertson LJ, 2009. Preservation of Giardia cysts in stool samples for subsequent PCR analysis. J Microbiol Methods 78: 292–296.
57. Kuk S, Yazar S, Cetinkaya U, 2012. Improved methods for isolating DNA from Ostertagia ostertagi eggs in cattle feces. Vet Parasitol 185: 297–302.
58. Schar F, Odermatt P, Khieu V, Panning M, Wain J, Taniuchi M, Hatz CF, 2013. Evaluation of real-time PCR for Strongyloides stercoralis and hookworm as diagnostic tool in asymptomatic schoolchildren in Cambodia. Acta Trop 126:89–92.
59. Becker SL, Piraisoody N, Klamme S, Marti H, Silue KD, Panning M, Nickel B, Kern WV, Herrmann M, Hatz CF, N’Goran EK, Utzinger J, von Muller L, 2015. Real-time PCR for detection of Strongyloides stercoralis in human stool samples from Cote d’Ivoire: diagnostic accuracy, inter-laboratory comparison and patterns of hookworm co-infection. Acta Trop 150: 210–217.
60. Cimino RO, Jeun R, Juarez M, Cajal PS, Vargas P, Echazu A, Bryan PE, Nasser J, Krolewicski A, Mejia R, 2015. Identification of human intestinal parasites affecting an asymptomatic peri-urban Argentinian population using combination real-time polymerase chain reaction. Parasit Vectors 8: 380.
61. Gordon CA, McManus DP, Acosta LP, Olveda RM, Williams GM, Ross AG, Gray DJ, Gobert GN, 2015. Multiplex real-time PCR monitoring of intestinal helminths in humans reveals widespread polyparasitism in Northern Samar, the Philippines. Int J Parasitol 45: 477–483.
62. Hu W, Wu S, Yu X, Abdullahi AY, Song M, Tan L, Wang Z, Jiang B, Li G, 2015. A multiplex PCR for simultaneous detection of three zoonotic parasites, Ancylostoma ceylanicum, A. caninum, and Giardia lamblia, assemblage A. BioMed Res Int 2015: 406168.
63. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ, 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors 7: 37.
64. Jonker FA, Calis JC, Phiri K, Brienen EA, Khoofi H, Brabin BJ, Verweij JJ, van Hensbroek MB, van Lieshout L, 2012. Real-time PCR demonstrates Ancylostoma duodenale is a key factor in the etiology of severe anaemia and iron deficiency in Malawian pre-school children. PLoS Negl Trop Dis 6: e1555.
65. Genta M, Yewhalaw D, Talessy K, Getaecht Y, Zeynudin A, 2012. Anaemia and associated risk factors among pregnant women in Gilgel Gibe dam area, southwest Ethiopia. Parasit Vectors 5: 296.
66. Gyorkos TW, Gilbert NL, Larocque R, Casapia M, Montresor A, 2012. Re-visiting Trichuris trichiura intensity thresholds based on anaemia during pregnancy. PLoS Negl Trop Dis 6: e1783.
67. Ngui R, Lim YA, Chong Kin L, Sek Chuen C, Jaffar S, 2012. Association between anaemia, iron deficiency anaemia, neglected parasitic infections and socioeconomic factors in rural children of west Malaysia. PLoS Negl Trop Dis 6: e1550.
68. Demeler J, Kruger N, Kruecken J, von der Heyden VC, Ramunke S, Kutler U, Mitsch S, Lopez Cepeda M, Knox M, Vercruyse J, Geldhof P, Harder A, von Samson-Himmelstjerna G, 2013. Phylogenetic characterization of β-tubulins and development of pyrosequencing assays for benzimidazole resistance in cattle nematodes. PLoS One 8: e70212.
69. Areekul P, Putaporntip C, Pattanawong U, Sethicharoenchai P, Jongwutisues S, 2010. Trichuris vulpis and T. trichiura infections among schoolchildren of a rural community in northwestern Thailand: the possible role of dogs in disease transmission. Asian Biomed 4: 69–76.
70. Ahmad AF, Hadip F, Ngui R, Lim YA, Mahmud R, 2013. Serological and molecular detection of Strongyloides stercoralis infection among an Orang Asli community in Malaysia. Parasitol Res 112: 2811–2816.
71. Autschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. J Mol Biol 215: 403–410.