Published in final edited form as:
Trans R Soc Trop Med Hyg. 2016 November 1; 110(11): 657–663. doi:10.1093/trstmh/trw078.

Molecular speciation of soil-transmitted helminths egg isolates collected during six drug efficacy trials in endemic countries

Santosh George[a,b], **Peter Geldhof**[a], **Marco Albonico**[c], **Shaali M. Ame**[d], **Jeffrey M. Bethony**[e], **Dirk Engels**[f], **Zeleke Mekonnen**[g], **Antonio Montresor**[f], **Hem Sopheak**[h], **Louis-Albert Tchuem-Tchuenté**[i], **Nguyen Thu Huong**[j], **Gagandeep Kang**[b], **Jozef Vercruysse**[a], and **Bruno Levecke**[a,*]

[a]Department of Virology, Parasitology and Immunology, Ghent University, Faculty of Veterinary Medicine, Merelbeke, Belgium [b]Department of Gastrointestinal Sciences, Christian Medical College, Vellore, India [c]Fondazione Ivo de Carneri, Milan, Italy [d]Public Health Laboratory, Ivo de Carneri, Chake-chake, Pemba, Pemba Island, Zanzibar, Tanzania [e]Microbiology, Immunology, and Tropical Medicine, George Washington University Medical Center, Washington, United States of America [f]Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland [g]Department of Medical Laboratory Sciences and Pathology, Jimma University, Jimma, Ethiopia [h]Clinical laboratory, Institut Pasteur in Cambodia, Phnom Penh, Cambodia [i]Centre for Schistosomiasis and Parasitology, Faculty of Sciences, University of Yaoundé I, PO Box 812 Yaoundé, Cameroon [j]Department of Parasitology, National Institute of Malariology, Parasitology and Entomology, Ha Noi, Vietnam

Abstract

Background—The diagnosis of soil-transmitted helminths (STHs; *Ascaris*, *Trichuris* and hookworms) is traditionally based on the demonstration of eggs in stool using microscopic techniques. While molecular techniques are more appropriate to speciate STH species they are

*Corresponding author: Tel: +32 9 264 74 04; bruno.levecke@ugent.be (BL), Faculty of Veterinary Medicine, Laboratory of Parasitology, Salisburylaan 133, B-9820 Merelbeke, Belgium.

Authors' contribution
SG, PG, GK, and BL designed the molecular speciation; MA, DE, AM, JV and BL designed the collection of isolates; SMA, JMB, ZM, HS, L-A T-T, NTH, JV and BL collected the isolates; SG, PG, GK and BL analyzed and interpreted the data; SG and BL wrote the manuscript; PG and GK reviewed the manuscript. All authors have read and approved the final manuscript. BL is the guarantor of the paper.

Competing interests
None declared.

Ethical approval
The overall protocol of the mebendazole trial was approved by the Ethic committee of the Faculty of Medicine, Ghent University (reference no. 2011/374),which was followed by a local ethical approval at each trial site. For Brazil, ethical approval was obtained from the Institutional Review Board (IRB) from Centro de Pesquisas René Rachou (reference no. 21/2008). For Cambodia, from the National Ethic Committee for Health Research (reference no. 185). For Cameroon, from the National Ethics Committee (reference no. 147/CNE/DNM/11). For Ethiopia, from IRB of Jimma University (reference no. RPGE/09/2011). For United Republic of Tanzania, from the Zanzibar Health Research Council (reference no. 20/ZAMREC/0003/JUNE/2012), and for Vietnam, by the Ethical Committee of National Institute of Malariology, Parasitology and Entomology and the Ministry of Health (reference no. 752/QD-VSR). The parents of all subjects included in the studies signed an informed consent form. In Brazil and Ethiopia an informed consent form was obtained from children aged 10 or 11 years and above. In Cambodia and Ethiopia, a verbal assent was obtained from all children and these procedures were approved by their respective IRB. This trial was registered under the ClinicalTrials.gov, identifier no. NCT01379326

Europe PMC Funders Author Manuscripts

seldom applied. In this study we speciated STH isolates using molecular techniques to gain insights into the distribution of both human and animal STH species in the human host.

Methods—We speciated 207 STH isolates from stool collected during six drug efficacy trials conducted in Brazil, Cambodia, Cameroon, Ethiopia, Tanzania and Vietnam applying a PCR/RFLP-based approach.

Results—DNA of *Ascaris* was detected in 71 (34.2%) samples, of which all were identified as the human roundworm *A. lumbricoides.* In 87 (42.0%) samples, DNA of *Trichuris* spp. was found and further speciation demonstrated the presence of the human *T. trichiura* (100%) and the canine *T. vulpis* (3.3%). Hookworms were identified in 87 (42.0%) samples, with *N. americanus* (57.4%) being the predominant species followed by *A. duodenale* (31.5%).

Conclusions—Our study indicates that STH infections in humans are predominantly caused by human STH species. They also suggest that zoonotic transmission occurs on a local scale.

Keywords

Ascaris lumbricoides; *Trichiura trichiura*; *Trichuris vulpis*; *Necator americanus*; *Ancylostoma duodenale*; zoonosis

Introduction

The soil-transmitted helminths (STHs) are a group of parasitic worms that infect both humans and animals through contact with worm eggs or larvae present in the soil (referring to their common name). The primary species that infect humans are *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Necator americanus* and *Ancylostoma duodenale* (hookworms).1 In 2010, it was estimated that ~819 million people were infected with *A. lumbricoides*, ~465 million with *T. trichiura,* and ~439 million with hookworms, resulting in a global disease burden of ~5.2 million disability adjusted life years (DALYs; 19.9% of the DALYs attributable to Neglected Tropical Disease).2 Periodic treatment of at-risk populations with one of the two benzimidazole drugs (albendazole and mebendazole) has been advocated as a cheap and effective means of reducing the worm burden and its related morbidity.3 Where possible, it is also recommended to use improved water, sanitation and hygiene (WASH) to minimize the rates of re-infection4, with the ultimate goal to eliminate soil-transmitted helminthiasis as a public health problem by 2020.5

Although it is commonly accepted that STH infections in humans are caused only by one of the four human STH species, studies indicate that a variety of animal STHs can develop to egg-laying adult worms in humans.6–8 Important animal STH species that are known to cause patent infections in humans, either experimentally or naturally, are *Ascaris suum* and *Trichuris suis* from pigs9, and *Ancylostoma ceylanicum* from dogs and cats10. In addition, there are a few animal STH species for which human patent infections are only recently confirmed (*Trichuris vulpis* of dogs)8, 11 or suggested (*Ancylostoma caninum*)12.

The studies designed to speciate STHs derived from humans so far indicate that the role of animals as a zoonotic reservoir should not be underestimated, as animal STH species are attributable for a considerable proportion of the STH infections in humans. In addition, they

highlight important geographical variation in the distribution of animal STH species in humans. *Ascaris* infections in developed countries, where human STH species are non-endemic, are almost exclusively caused by pig-to-human transmission (e.g., USA13; Denmark14; UK15; Japan16), whereas zoonotic transmission has only been occasionally reported in countries where STHs pose an important burden on public health, such as Uganda17 (<1% of the human derived *Ascaris* worms were of pig origin), Zanzibar18 (2%) and China19 (14%). Zoonotic *Trichuris* infections have been reported in Uganda20 (10% of the human derived *Trichuris* worms were of pig origin) and Thailand8 (11% of the speciated *Trichuris* egg isolates from human stool contained *T. vulpis*). *A. ceylanicum* infections are reported in variety of Asian countries, including Cambodia21 (52% of the speciated hookworm egg isolates derived from human stool contained *A. ceylanicum*), Malaysia22 (23%), Laos23 (17%), Thailand24 (6%) and India7, 25 (5%). Recently, *A. caninum* was also detected in 17% of the egg isolates derived from human stool in India, suggesting that, in contrast with current knowledge 26–27, *A. caninum* may be able to develop to egg-laying adult in humans.12 Note that the animal hookworms were at least the second most prevalent hookworm species in each of the aforementioned studies involving hookworms. Albeit based on a small number of hookworm egg isolates, *A. ceylanicum* infections were rather homogenously distributed across Malaysian villages (~ 20%)22, whereas it was only found in two of the 50 samples identified positive for hookworm from a tribal area in India (5%).7 A local scattered distribution was also observed for *A. caninum*, the canine hookworm being identified in 7 out of the 10 tribal villages in India (frequency within villages ranging from less than 5% up to 100%).12

These studies indeed contribute to a growing body of literature on the role of animals as a reservoir for STH infections in humans, and although a ‘One Health’ approach has been proposed for *A. ceylanicum*10, it remains unclear whether there truly is a need for additional measures to reduce the animal-to-human transmission. Probably the most important reason for the lack of evidence is the means to diagnose STH in large-scale epidemiological surveys. Traditionally, the diagnosis of STHs is based on the demonstration of eggs in stool. 28 Although majority of the current microscopic techniques are cheap and ease-of-use in the field, they do not allow unraveling the importance of animal STH species in humans. This is because, it is impossible to differentiate these animal STH species from their human counterparts on the morphology of the eggs: eggs of both human and animal roundworm (45 – 75 x 35 – 50 μm) and hookworms (55 – 79 x 35 – 45 μm) are identical.29–30 Although the eggs of the canine whipworm *T. vulpis* (70 – 90 x 32 – 41 μm) are traditionally larger than the human whipworm *T. trichiura* (50 – 58 x 22 – 27 μm), speciation of the eggs based on the size remains unreliable. There is an overlap in the length of the eggs of both species that could mislead diagnosis based on the egg dimension only. Yoshikawa and colleagues (1989) reported the presence of both small (57 x 26 μm) and large (78 x 30 μm) eggs in the uteri of adult female *T. trichiura* worms.31 Moreover, this misdiagnosis may worsen when *T. trichiura* eggs are recovered shortly after treatment, as the egg morphology may change due to the administration of benzimidazoles, increased size being one of the morphological changes.32 As a consequence of this, reports drawing conclusions on zoonotic *T. vulpis* infections based on the size of eggs should be interpret with caution.11, 32 With the recent advances in molecular technologies a variety of techniques (e.g. PCR, PCR-RFLP and

Europe PMC Funders Author Manuscripts

qPCR7–8, 33–34) have been developed to molecularly speciate the different STH species, but they are so far rarely applied. The present study aimed to assess the distribution of both animal and human STH across different geographical settings where STHs are endemic.

Material and Methods

Selection of isolates

The STH isolates used for the present study were collected as part of a multicentric drug efficacy study designed to assess the efficacy of a single-oral dose of 500 mg mebendazole against STH infections in children. This study was conducted in six STH-endemic countries across Africa (Cameroon, Ethiopia and Tanzania), Asia (Cambodia and Vietnam) and Latin-America (Brazil). The details of this drug efficacy study have been described elsewhere.35 Each of the different study sites preserved approximately 100 stool samples of subjects excreting eggs of any STH species at baseline (1 gram of stool in 10 ml of 70% ethanol). The detection of eggs of stool was based on the McMaster egg counting method.36 These samples were subsequently sent to the Laboratory of Parasitology, Ghent University, Belgium for the molecular differentiation of the isolates.

Per study site a random set of 40 samples were selected for further molecular speciation, except for Tanzania and Vietnam. Given the high frequency of mixed STH infections, representing *Ascaris*, *Trichuris* and hookworm infections, we only selected 20 samples from Tanzania. For Vietnam, samples were lost during shipment, and as a consequence of this a molecular speciation could only be performed on 27 samples.

Extraction of DNA from eggs in stool

Genomic DNA was extracted from STH eggs using the QIAamp DNA stool mini kit. To this end, 200 µl of the stool suspension (1g in 10 ml of 70% ethanol) was used to extract DNA according to the manufacturer's recommendations. However, prior to DNA extraction the suspension was subjected to 3 freeze-thaw cycles (liquid nitrogen for 2 min and subsequently transferring them to 95°C for 5 min).

Molecular speciation

We applied one general semi-nested PCRs separately for each of the three STH genera (*Ascaris*, *Trichuris* and hookworms). The primers for each of these PCRs targeted the ITS-1, 2 and 5.8s region and were designed using EditSeq™ and MegAlign™ (Lasergene®, DNASTAR, Inc). All the reactions were performed in a volume of 25 µl containing 2.5 µl DNA, 0.5 µl of each primer (10 mM), 0.5 µl dNTP (10 µM), 1 µl $MgCl_2$ (25 µM), 5 µl GoTaq Flexi buffer, 14.875 µl PCR-grade water and 0.125 µl GoTaq Flexi DNA polymerase. Both a negative (water) and positive (control DNA) control was included in each run. The following conditions were used: 2 min at 95°C (initial denaturation), 34 cycles of 30 s at 95°C (denaturation), 30 s at 55 °C (annealing), 30 s at 72°C (extension), followed by a single step of 10 min at 72°C (final extension). The amplified product was detected using 1.5% agarose gel electrophoresis using ethidium bromide. Further speciation was based on restriction fragment length polymorphism (RFLP) for *Ascaris* and hookworm, and species-specific PCRs for *Trichuris*.

Ascaris

The semi-nested *Ascaris* PCR was performed using the first round forward primer AsITF-Ext (5'-CCGGGCAAAAGTCGTAACAA-3') and the second round forward primer AsITF-Int(5'-TCCGAACGTGCACATAAGTAC-3') along with the common reverse primer As ITSR - (5'-CATATACATCATTATTGTCACGC-3'). These primers were designed using the sequence of *A. lumbricoides* (GenBank accession nos. AB571298, AB571297, AB571301) and *A. suum* (GenBank accession nos. AB571302, AB576592). The PCR resulted in a product size of 850 bp. Differentiation between *A. lumbricoides* and *A. suum* was performed as described by Zhu and colleagues.37 In short, the second round PCR product was digested using restriction enzyme HaeIII at 37°C for 13 hours. HaeIII digests PCR products of *A. lumbricoides* into two (515 bp and 334 bp) and of *A. suum* into three (515 bp, 228 bp and 106 bp). The digested product was detected using 2% agarose gel electrophoresis using ethidium bromide.

Trichuris

The semi-nested PCR was performed using the common forward primer UGTF (5'-TGACAACGGTTAACGGAGAAT-3'), the first-round reverse primer UGTR-Ext (5′-TCAAGTCGCCAAGGACACTC-3′) and the second-round reverse primer UGTR-Int (5′-CGACTCCTGCTTAGGACGAC-3′). These primers were designed using the sequence of *T. trichiura* (GenBank accessionnos. GQ301554, GQ301555, GQ352554), *T. suis* (GenBank accessionnos. AM993010, AM993012, AM993014, AM993016) and *T. vulpis* (GenBank accessionno. AM234616). The first-round PCR resulted in a product of 399 bp, while the second-round PCR resulted in a product of 327 bp. Unlike *Ascaris* and hookworm, differentiation of *T. trichiura* and *T. suis* from *T. vulpis* was done using species-specific primers that bind to the interspecies conserved regions of the SSUrRNA region of *Trichuris* genome as described by Areekul and colleagues.8 This PCR differentiates *T. trichuria*/*T. suis* from *T. vulpis* giving a product size of 207 bp and 212 bp respectively. The amplified product was detected using 1.5% agarose gel electrophoresis using ethidium bromide. A subset of the *Trichuris* were sequenced and compared with reference sequences using MegAlign (Lasergene®, DNASTAR, Inc).

Hookworm

A semi-nested hookworm PCR was performed as previously described by George and colleagues.7 The first round of this PCR results in an amplicon of 597 bp and 449 bp for *N. americanus* and *Ancylostoma* spp, respectively. While the second PCR product results in anamplicon of 552 bp for *N. americanus* and 404-408 bp for *Ancylostoma* spp. Further characterization of *Ancylostoma* spp. was done using RFLP. To this end, the second-round PCR products were digested using the restriction enzymes MvaI and Psp1406I at 37°C for 13 hours. MvaI digests PCR products of *A. ceylanicum* into two (340 bp and 64 bp), but does not digest *A. duodenale* and *A. caninum*. Psp1406I digests *A. duodenale* PCR products into two (255 bp and 149 bp), but does not digest *A. ceylanicum* and *A. caninum.* The lysed product was detected using 2% agarose gel electrophoresis using ethidium bromide.

Results

In the present study a total of 207 STH isolates from an equal number of subjects were examined for the presence of both human and animal STH species. Of them, 165 (79.7%) were found positive for at least one of the three general semi-nested PCRs. DNA of *Ascaris* was detected in 71 (34.2%) samples, of which all were identified to be the human *A. lumbricoides.* In 87 (42.0%) samples, DNA of *Trichuris* spp. was found and further speciation indicated the presence of *T. trichiura* in all the samples. In 7 samples from Cameroon, DNA of the canine *T. vulpis* was also detected. Hookworm DNA was detected in 104 samples (50.2%). Majority of hookworm isolates were identified as *N. americanus* (n = 73; 35.2%) followed by *A. duodenale* (n = 40; 31.5%). No animal hookworm species were found. Mixed *N. americanus* and *A. duodenale* were observed in 9 samples (0.4%). The distribution of the different STH species is provided in Table 1.

Discussion

It is traditionally accepted that STH infections in humans are caused by the human STH species only (*A. lumbricoides*, *T. trichiura*, *N. americanus* and *A. duodenale*). However, recent epidemiological studies applying molecular techniques indicate that the role of animals as a zoonotic reservoir for STH infections in humans should not be underestimated. 8–10, 12 In the present study we molecularly speciated STH isolates collected from children during a drug efficacy study in six STH-endemic countries across Africa, Asia and Latin America, with the aim to gain insights into the distribution of both human and animal STH species.

Our results highlighted that the STH infections were almost exclusively caused by human STH species. Only in Cameroon DNA of the canine *T. vulpis* was detected in 7 out 23 subjects infected with *Trichuris* (Table 1). Our findings are in line with molecular studies conducted in northwestern Thailand where they found 6 out of 80 subjects excreting eggs *T. vulpis*8, and they contribute to the evidence that *T. vulpis* may cause patent infections in humans. The burden of disease caused by this animal STH species in humans however is unclear. This is because majority of the human clinical cases were only suspected for *T. vulpis* infections (*T. trichiura* infections could always be ruled out).10 In the present study, no animal round- and hookworms were identified. The absence of zoonotic *Ascaris* transmission is confirms literature, indicating that pig-to-human transmission is mainly found in countries where human STH are not endemic (cfr. Introduction). Moreover, in some countries pigs are already rare or absent due to cultural habits (e.g., Ethiopia and Tanzania (Pemba)), and hence zoonotic transmission is already unexpected, though pig-to-human transmission cannot be entirely excluded.18 This is in contrast for animal hookworm species, were evidence of zoonotic transmission was absent, particularly for *A. ceylanicum* in Asian countries. This animal hookworm has been previously detected in humans from Asian countries also included in the present study, such as Cambodia (52% of the hookworm egg isolates derived from human stool)21, highlighting once more the geographical variation in the transmission of zoonotic hookworm species.7, 12, 22, 38 At this stage it is difficult to explain this geographical variation. The most apparent factors might be differences in (i) the prevalence of animal STHs in their natural hosts, (ii) the population size of dogs or cats and

(iii) the way these animals and human populations interact with each other. The latter is probably the most important, as animals are often abundant and infected in the involved STH-endemic countries. For example, in Vietnam *A. ceylanicum* are highly prevalent in dogs (half of the dogs are infected with hookworms, of which more than 60% identified as *A. ceylanicum*).34

The present study has three major limitations. First, this study was embedded into a multi-centric clinical trials designed to assess the efficacy of mebendazole against STH infections in children. As a consequence of this, our STH egg isolates do not represent a random sample from the total population of STHs. Second, we deployed general genus primers into our PCR protocols for *Ascaris*, *Trichuris* and hookworms. An important disadvantage of this approach is that it will amplify DNA of the most abundant species, and hence we might have missed potential mixed infections with human and animal STH species (e.g. *A. ceylanicum*). Third, we are not able to draw conclusions on the absence of *T. suis* infections, as the applied primer set did not allow to differentiate the swine from the human whipworm. However, sequence results of a selection of *T. trichiura/T. suis* products only revealed *T. trichiura*. Finally, we were not able to quantify the STH species infections within a sample; rather we confirmed the presence or absence of different STH species. This quantification of STHs would be particularly interesting to assess the relative contribution of *T. vulpis* and *T. trichiura* in the isolates from Cameroon. In conclusion, our study indicates that STH infections in humans are predominantly caused by human STH species, and suggest that zoonotic transmission mainly occurs on a more local scale. As a consequence of this, it will be important to further the speciation of human derived STHs to identify hot spots of zoonotic transmission, and to subsequently develop and implement local control strategies to reduce animal-to-human transmission.

Acknowledgements

For Brazil, we would like to acknowledge Renata C. Diniz for her invaluable help with the communication and organization with the volunteers in the rural area and the technical support provided by the staff in Americaninhas, MG. For Cambodia, we would like to acknowledge the contributions of various members: Dr Muth Sinuon (National Center for Parasitology, Entomology and Malaria, CNM), Dr Philippe Guyant (Partners for Development), staff of the Medical Microbiology Laboratory (Institut Pasteur in Cambodia), National Center for Parasitology, Entomology and Malaria, and Partners for Development who helped to facilitate the study. For Ethiopia, we would like to acknowledge Mio Ayana, Dereje Jirata, Dereje Atomisa, Tesfaye Demie, Nuredin Abduselam, Mitiku Bajaro, Mestawet Getachew, and Teshome Bekana for their technical assistance both in the laboratory and in the field. In addition, we would like to thank study subjects, schoolteachers, and directors of the schools. For the United Republic of Tanzania, we would like to thank staff participating in the study, teachers, and students and, lastly, parents of the students for giving consent for their children to participate. For Vietnam, we would like to thank Dr. Tran Cong Dai (WHO Country Office) who greatly facilitated the communications with National Institute of Malariology, Parasitology and Entomology, and provided technical support. In addition, we would like to acknowledge the contributions of various members and staff of Parasitology Department, National Institute of Malariology, Parasitology and Entomology, and the Centre of Control Malaria in Cao Bang and Dien Bien provinces in collection and analysis of samples. Finally, we would like to thank Dr. Deepthi Kattula from Christian Medical College, Vellore, India for her help in analyzing the data, her work has been truly invaluable.

Funding

This work was supported by a Special Research Fund of Ghent University (https://www.ugent.be/nl/onderzoek/financiering/bof). The multi-centric drug efficacy trial was financially supported by World Health Organization (grant no APW200432311, http://apps.who.int/whocc/Detail.aspx?cc_ref=BEL-42&cc_code=bel).SG is supported by the Special Research Fund of Ghent University and the Division of Gastrointestinal Sciences, Christian Medical College, Vellore, India (www.cmcwtrl.in). BL is a postdoctoral fellow of FWO (Ref No. 05–05 1.2.853.13; www.fwo.be).

Europe PMC Funders Author Manuscripts

References

1. Bethony J, Brooker S, Albonico M, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet. 2006; 367(9521):1521–32. S0140-6736(06)68653-4 [pii]. DOI: 10.1016/S0140-6736(06)68653-4 [PubMed: 16679166]
2. Pullan RL, Smith JL, Jasrasaria R, et al. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors. 2014; 7:37. 1756-3305-7-37 [pii]. doi: 10.1186/1756-3305-7-37 [PubMed: 24447578]
3. WHOs. , editor. WHO. Helminth control in school age children: a guide for managers of control programmes. 2011.
4. Strunz EC, Addiss DG, Stocks ME, et al. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. PLoS Med. 2014; 11(3):e1001620. PMEDICINE-D-13-02702 [pii]. doi: 10.1371/journal.pmed.1001620 [PubMed: 24667810]
5. WHO. Soil-transmitted helminthiases: eliminating soil-transmitted helminthiases sa a public health probelm in children. Progress report 2001 - 2010 and strategic plan 2011 - 2020. World health Organization. 2012
6. Traub RJ, Inpankaew T, Sutthikornchai C, et al. PCR-based coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by *Ancylostoma ceylanicum* in temple communities in Bangkok. Vet Parasitol. 2008; 155(1–2):67–73. S0304-4017(08)00235-5 [pii]. DOI: 10.1016/j.vetpar.2008.05.001 [PubMed: 18556131]
7. George S, Kaliappan SP, Kattula D, et al. Identification of *Ancylostoma ceylanicum* in children from a tribal community in Tamil Nadu, India using a semi-nested PCR-RFLP tool. Trans R Soc Trop Med Hyg. 2015; 109(4):283–5. trv001 [pii]. DOI: 10.1093/trstmh/trv001 [PubMed: 25618132]
8. Areekul P, P C, Pattanawong U, Sitthicharoenchai P, Jongwutiwes S. *Trichuris vulpis* and *T. trichiura* infections among schoolchildren of a rural community in northwestern Thailand: the possible role of dogs in disease transmission. Asian Biomedicine. 2010; 4(1):49–60.
9. Nejsum P, Betson M, Bendall RP, et al. Assessing the zoonotic potential of *Ascaris suum* and *Trichuris suis*: looking to the future from an analysis of the past. J Helminthol. 2012; 86(2):148–55. S0022149X12000193 [pii]. DOI: 10.1017/S0022149X12000193 [PubMed: 22423595]
10. Traub RJ. *Ancylostoma ceylanicum*, a re-emerging but neglected parasitic zoonosis. Int J Parasitol. 2013; 43(12–13):1009–15. S0020-7519(13)00203-8 [pii]. DOI: 10.1016/j.ijpara.2013.07.006 [PubMed: 23968813]
11. Traversa D. Are we paying too much attention to cardio-pulmonary nematodes and neglecting old-fashioned worms like *Trichuris vulpis*? Parasit Vectors. 2011; 4:32. 1756-3305-4-32 [pii]. doi: 10.1186/1756-3305-4-32 [PubMed: 21385441]
12. George S, Levecke B, Kattula D, et al. Molecular Identification of Hookworm Isolates in Humans, Dogs and Soil in a Tribal Area in Tamil Nadu, India. PLoS Negl Trop Dis. 2016; 10(8):e0004891. [PubMed: 27486798]
13. Anderson TJ. *Ascaris* infections in humans from North America: molecular evidence for cross-infection. Parasitology. 1995; 110(Pt 2):215–9. [PubMed: 7885739]
14. Nejsum P, Parker ED Jr, Frydenberg J, et al. Ascariasis is a zoonosis in denmark. J Clin Microbiol. 2005; 43(3):1142–8. 43/3/1142 [pii]. DOI: 10.1128/JCM.43.3.1142-1148.2005 [PubMed: 15750075]
15. Bendall RP, Barlow M, Betson M, et al. Zoonotic ascariasis, United Kingdom. Emerg Infect Dis. 2011; 17(10):1964–6. DOI: 10.3201/eid1710.101826 [PubMed: 22000387]
16. Arizono N, Yoshimura Y, Tohzaka N, et al. Ascariasis in Japan: is pig-derived *Ascaris* infecting humans? Jpn J Infect Dis. 2010; 63(6):447–8. [PubMed: 21099099]
17. Betson M, Nejsum P, Bendall RP, et al. Molecular epidemiology of ascariasis: a global perspective on the transmission dynamics of *Ascaris* in people and pigs. J Infect Dis. 2014; 210(6):932–41. jiu193 [pii]. DOI: 10.1093/infdis/jiu193 [PubMed: 24688073]
18. Sparks AM, Betson M, Oviedo G, et al. Characterization of *Ascaris* from ecuador and zanzibar. J Helminthol. 2015; 89(4):512–5. S0022149X14000431 [pii]. DOI: 10.1017/S0022149X14000431 [PubMed: 26017334]

Europe PMC Funders Author Manuscripts

19. Zhou C, Li M, Yuan K, et al. Pig *Ascaris*: an important source of human ascariasis in China. Infect Genet Evol. 2012; 12(6):1172–7. S1567-1348(12)00149-9 [pii]. DOI: 10.1016/j.meegid.2012.04.016 [PubMed: 22561394]
20. Nissen S, Al-Jubury A, Hansen TV, et al. Genetic analysis of *Trichuris suis* and *Trichuris trichiura* recovered from humans and pigs in a sympatric setting in Uganda. Vet Parasitol. 2012; 188(1–2): 68–77. S0304-4017(12)00116-1 [pii]. DOI: 10.1016/j.vetpar.2012.03.004 [PubMed: 22494938]
21. Inpankaew T, Schar F, Dalsgaard A, et al. High prevalence of *Ancylostoma ceylanicum* hookworm infections in humans, Cambodia, 2012. Emerg Infect Dis. 2014; 20(6):976–82. DOI: 10.3201/eid2006.131770 [PubMed: 24865815]
22. Ngui R, Lim YA, Traub R, et al. Epidemiological and genetic data supporting the transmission of *Ancylostoma ceylanicum* among human and domestic animals. PLoS Negl Trop Dis. 2012; 6(2):e1522. PNTD-D-11-00674 [pii]. doi: 10.1371/journal.pntd.0001522 [PubMed: 22347515]
23. Conlan JV, Khamlome B, Vongxay K, et al. Soil-transmitted helminthiasis in Laos: a community-wide cross-sectional study of humans and dogs in a mass drug administration environment. Am J Trop Med Hyg. 2012; 86(4):624–34. 86/4/624 [pii]. DOI: 10.4269/ajtmh.2012.11-0413 [PubMed: 22492147]
24. Jiraanankul V, Aphijirawat W, Mungthin M, et al. Incidence and risk factors of hookworm infection in a rural community of central Thailand. Am J Trop Med Hyg. 2011; 84(4):594–8. 84/4/594 [pii]. DOI: 10.4269/ajtmh.2011.10-0189 [PubMed: 21460016]
25. Kaliappan SP, George S, Francis MR, et al. Prevalence and clustering of soil-transmitted helminth infections in a tribal area in southern India. Trop Med Int Health. 2013; 18(12):1452–62. DOI: 10.1111/tmi.12205 [PubMed: 24237860]
26. Croese J, Loukas A, Opdebeeck J, et al. Human enteric infection with canine hookworms. Ann Intern Med. 1994; 120(5):369–74. [PubMed: 8304653]
27. Landmann JK, Prociv P. Experimental human infection with the dog hookworm, *Ancylostoma caninum*. Med J Aust. 2003; 178(2):69–71. doi: lan10157_fm [pii]. [PubMed: 12526725]
28. McCarthy JS, Lustigman S, Yang GJ, et al. A research agenda for helminth diseases of humans: diagnostics for control and elimination programmes. PLoS Negl Trop Dis. 2012; 6(4):e1601. PNTD-D-11-01002 [pii]. doi: 10.1371/journal.pntd.0001601 [PubMed: 22545166]
29. Leles D, Gardner SL, Reinhard K, et al. Are *Ascaris lumbricoides* and *Ascaris suum* a single species? Parasit Vectors. 2012; 5:42. 1756-3305-5-42 [pii]. doi: 10.1186/1756-3305-5-42 [PubMed: 22348306]
30. Brooker S, Bethony J, Hotez PJ. Human hookworm infection in the 21st century. Adv Parasitol. 2004; 58:197–288. S0065308X04580041 [pii]. DOI: 10.1016/S0065-308X(04)58004-1 [PubMed: 15603764]
31. Yoshikawa H, Yamada M, Matsumoto Y, et al. Variations in egg size of *Trichuris trichiura*. Parasitol Res. 1989; 75(8):649–54. [PubMed: 2771930]
32. Steinmann P, Rinaldi L, Cringoli G, et al. Morphological diversity of *Trichuris* spp. eggs observed during an anthelminthic drug trial in Yunnan, China, and relative performance of parasitologic diagnostic tools. Acta Trop. 2015; 141(Pt B):184–9. S0001-706X(14)00279-4 [pii]. DOI: 10.1016/j.actatropica.2014.08.018 [PubMed: 25174679]
33. Traub RJ, Robertson ID, Irwin P, et al. Application of a species-specific PCR-RFLP to identify *Ancylostoma* eggs directly from canine faeces. Vet Parasitol. 2004; 123(3–4):245–55. S0304401704002651 [pii]. DOI: 10.1016/j.vetpar.2004.05.026 [PubMed: 15325050]
34. Ng-Nguyen D, Hii SF, Nguyen VA, et al. Re-evaluation of the species of hookworms infecting dogs in Central Vietnam. Parasit Vectors. 2015; 8:401. 10.1186/s13071-015-1015-y [pii]. doi: 10.1186/s13071-015-1015-y [PubMed: 26216353]
35. Levecke B, Montresor A, Albonico M, et al. Assessment of anthelmintic efficacy of mebendazole in school children in six countries where soil-transmitted helminths are endemic. PLoS Negl Trop Dis. 2014; 8(10):e3204. PNTD-D-13-01560 [pii]. doi: 10.1371/journal.pntd.0003204 [PubMed: 25299391]
36. Ministry of Agriculture FaF. Manual of veterinary parasitological laboratory techniques. London: Her Majesty's Stationery Office (HMSO); 1986.

37. Zhu X, Chilton NB, Jacobs DE, et al. Characterisation of *Ascaris* from human and pig hosts by nuclear ribosomal DNA sequences. Int J Parasitol. 1999; 29(3):469–78. doi: S0020751998002264 [pii]. [PubMed: 10333331]

38. Ngui R, Ching LS, Kai TT, et al. Molecular identification of human hookworm infections in economically disadvantaged communities in Peninsular Malaysia. Am J Trop Med Hyg. 2012; 86(5):837–42. 86/5/837 [pii]. DOI: 10.4269/ajtmh.2012.11-0446 [PubMed: 22556084]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Distribution of *Ascaris*, *Trichuris* and hookworm spp. in six different endemic countries

Country	N	*Ascaris*		*Trichuris*		Hookworm	
		A. lumbricoides	*A. suum*	*T. trichiura*	*T. vulpis*	*N. americanus*	*A. duodenale*
Brazil	40	20	0	19	0	17	3
Cambodia	40	0	0	0	0	21	10
Cameroon	40	17	0	23	7	3	14
Ethiopia	40	15	0	15	0	10	3
Tanzania	20	14	0	20	0	8	10
Vietnam	27	5	0	10	0	14	0
Total	207	71	0	87	7	73	40