Medical Sciences

Strongyloides stercoralis in the non endemic setting and beyond: an epidemiological, clinical, diagnostic and treatment challenge

PhD thesis submitted for the degree of doctor in de medische wetenschappen at the University of Antwerp to be defended by Dora Buonfrate

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Introduction

Strongyloidiasis is the infection caused by nematode worms of the genus *Strongyloides*. In humans, strongyloidiasis is caused mainly by *Strongyloides stercoralis*, rarely by *S. fuelleborni fuelleborni* and *S. fuelleborni kelleyi* {Nutman, 2017; Streit, 2008; Bradbury, 2021}. Strongyloidiasis is listed by the WHO as a Neglected Tropical Disease (NTD), along with infections by the other soil-transmitted helminths (STH), namely hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* {Buonfrate, 2020}. However, it has been only recently added to the WHO list, several years later compared to the other STH.

S. stercoralis does not infect only humans, but also dogs, cats, and non-human primates. The role of animals in human infection is not yet clear, and there are concerns about the potential zoonotic transmission of *Strongyloides stercoralis* from dogs to humans {Bradbury, 2021}.

The life cycle of the parasite is complex (Figure 1), comprising a free-living and a parasitic cycle {Streit, 2008 #1}.

The infected host releases rhabditiform larvae (L1, Fig. 2) in stool and, in case defecation occurs in suitable external environment (in soil, mostly in hot moist climate), L1 can start the free-living cycle.

Once in the soil, L1 molt either directly into infective filariform larvae (iL3, Fig. 3) or, through different stages, into adult worms.

The latter are female and male adults, who mate producing a new generation of iL3. The iL3 present in the soil can penetrate the skin of human and non-human hosts leading to the parasitic cycle. In humans, the iL3 migrate through the blood vessels to the lungs, ascend the tracheobronchial tree and are swallowed into the intestinal tract. During their migration across the human body, the larvae molt into different stages, and eventually mature into adult females that settle in the small intestine. There, they reproduce by parthenogenesis and lay eggs.

The newborn rhabditoid larvae (L1) hatch out of the eggs when still in the bowel, and are released with feces.

Figure 1. *S. stercoralis* life cycle. From: https://www.cdc.gov/parasites/strongyloides/biology.html

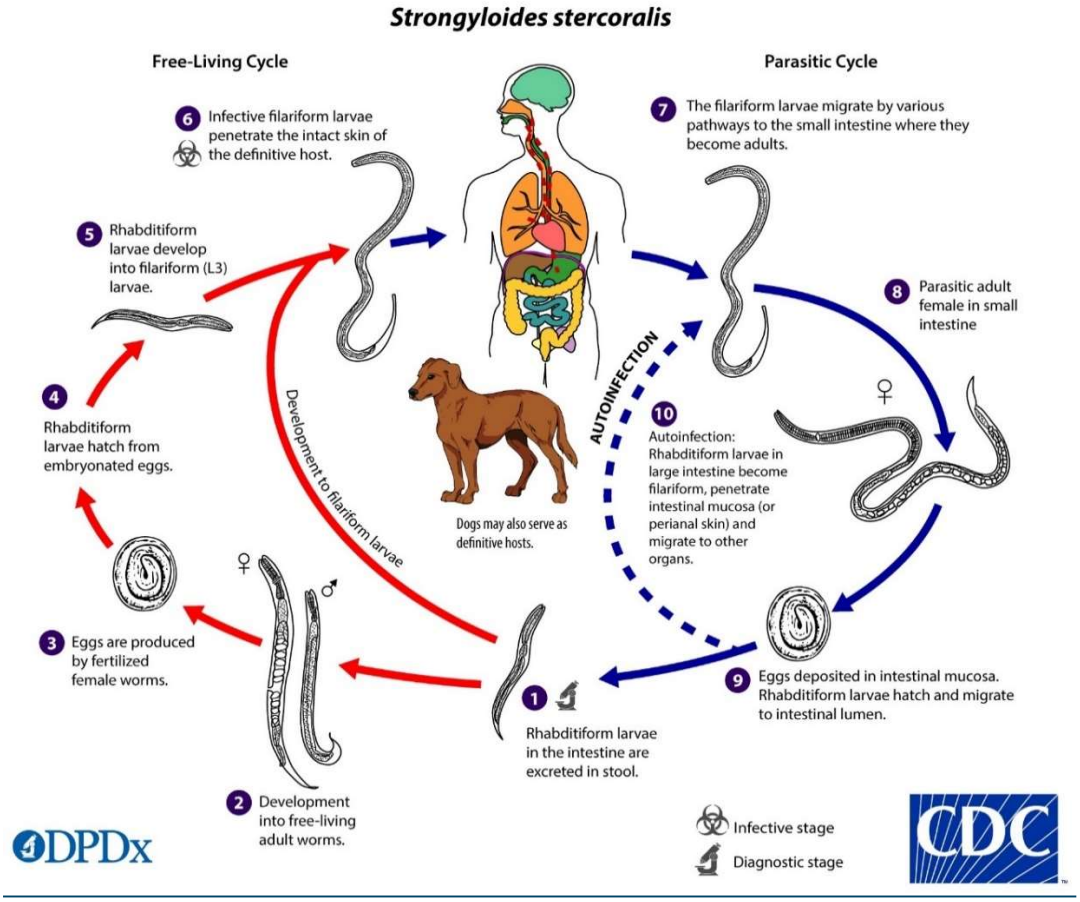


Figure 2. Rhabditiform larva from stool in agar plate culture; x40 magnification

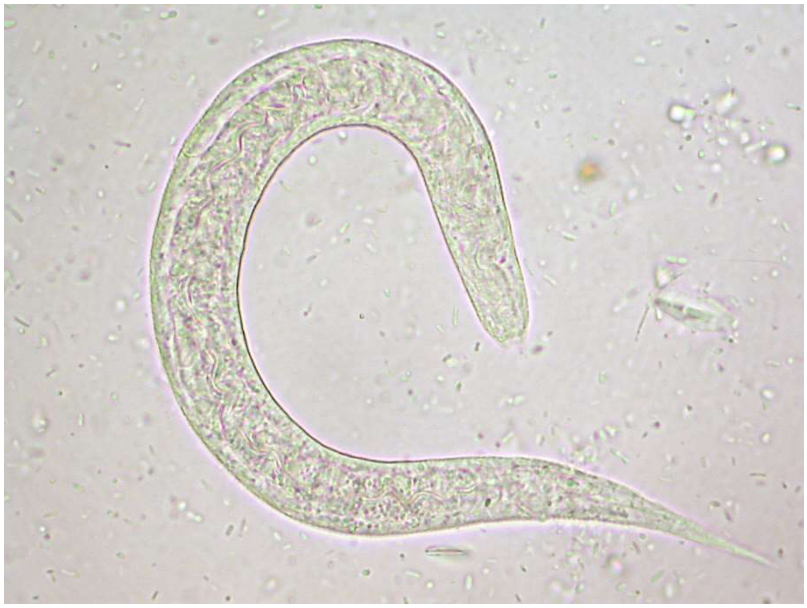
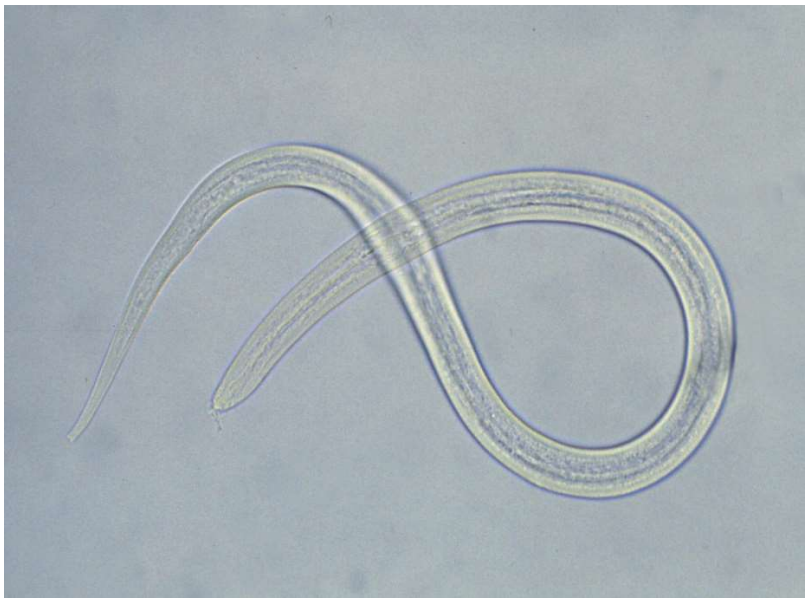


Figure 3. Filariform larva from stool in agar plate culture; x40 magnification



Some L1 molt into iL3 before leaving the body, and can re-infect the host by penetration of the rectum mucosa or the perianal skin. This "auto-infective cycle" leads to chronic infection also in individuals who are not re-exposed to an external source of infection, so strongyloidiasis persists life-long in individuals who do not receive proper antiparasitic treatment {Streit, 2008; Nutman, 2017}. For this reason, *S. stercoralis* infection can be diagnosed in people who left an endemic area even decades before. Therefore, in some parts of Europe strongyloidiasis can be observed not only in migrants from endemic areas, but also in elderly people without history of travels abroad, who acquired the infection in their youth, when sanitary conditions and the sewage system in the country were inappropriate {Buonfrate, 2016}.

Chronic infection can be either asymptomatic or characterized by symptoms which are mostly non-specific, as they may also be present in other conditions (e.g. abdominal pain, diarrhea, urticaria). Symptoms can have different grades of severity and can be either intermittent or chronic {Olsen, 2009}. Differences in studies' methods of diagnosis, settings and populations have limited an exhaustive characterization of the morbidity caused by chronic strongyloidiasis. Evidence from observational studies carried out in endemic areas are often limited by coinfections with other parasites, so it can be difficult to discriminate between symptoms caused by a single infection. In non-endemic areas, most observational studies did not specifically focus on morbidity {Buonfrate 2021}. As a result, there is still some uncertainty on the frequencies of some clinical manifestations, and the association of some (possibly relevant) symptoms with chronic strongyloidiasis has not been caught. When immunocompromise occurs (either iatrogenic etiology or due to immunosuppressant conditions), the autoinfective cycle accelerates, leading to an increased larval load (called "hyperinfection"). This can cause mechanical obstruction, thus worsening of symptoms (e.g. paralytic ileus, asthma attack). Moreover, the larvae (and even other worm stages) can disseminate throughout the body, carrying intestinal bacteria and thus potentially leading to gram negative sepsis and meningitis. The larval spread to organs which are outside the normal route of migration is also known as "dissemination". However, it should be noted that it is often difficult to discriminate between hyperinfection/dissemination, that both indicate the severe progression of the infection {Buonfrate, 2013}. Hyperinfection/disseminated strongyloidiasis is a life-threatening syndrome {Nutman, 2017}, with an estimated case fatality rate of about 80% {Buonfrate, 2013}. In fact, the scarce attention to the definition of morbidity in chronic infection might be due to the fact that dissemination has been long perceived as the clinically-relevant condition caused by strongyloidiasis, compared to less worrisome symptoms like pruritus and abdominal pain. Detecting the infection in the chronic indolent phase is however mandatory to treat the infection before a possible progression to its severe form. Corticosteroids administration, TNF-alpha inhibitors, HTLV-1 infection and malignancies are among factors that showed a clear association with the development of hyperinfection {Buonfrate, 2013}. Although some uncertainty persists (such as the length of treatment or the dosage of immunosuppressant therapies leading to severe strongyloidiasis), it is recommended that candidates to immunosuppression should be screened and, when positive, eventually treated for strongyloidiasis before they might progress to hyperinfection {Nutman, 2017}. For HTLV-1 patients, treatment is unfortunately even more challenging, due to a reduced response to the first-line option (single dose of ivermectin); moreover, HTLV-1 seems to stimulate a progression of strongyloidiasis, and *vice versa* {Ye, 2022}.

Unfortunately, a diagnostic gold standard lacks, resulting in misdiagnosis {Buonfrate, 2015}. The microscopical examination of stool has low sensitivity, and this not only impacts on individual diagnosis, but also on prevalence estimates {Buonfrate, 2020}. Other diagnostic tests are available, and the choice depends on the setting (endemic versus non-endemic area) and purpose (screening versus individual diagnosis). Baermann method (a filtration method that can concentrate the worms present in stool) and agar plate culture have better sensitivity than microscopy, but they are seldom done in routine practice. Real-time PCR is also available; though its accuracy seems similar to that of Baermann method and agar plate culture, it has some advantages over those techinques, such as the fact that it can be done on stool samples preserved in alcohol, easing transport to reference laboratories (the method is still not widely available). Many serological assays, both commercial and in-house, are also available. Despite some differences between different assays, currently serology is considered the available most sensitive technique. There are some concerns about its

specificity, and recombinant-antigen based assays have been developed to overcome this issue, with discrepant results. All these techniques have advantages and disadvantages that should be considered, and the choice of a test depends on purpose, setting and local expertise. No test fits all. A high index of suspicion is hence needed to reduce misdiagnosis. Unfortunately, there is poor awareness of strongyloidiasis among health care workers, and this can cause inaqdequate clinical management, even with prescription of steroids to treat symptoms and signs (e.g. wheezing, eosinophilila) attributed to other clinical conditions {De l'Étoile-Morel, 2022; Boulware, 2007}.

As a consequence of the diagnostic issues, for long time the global burden was very difficult to be estimated, due to unreliable prevalence figures based on poorly-accurate diagnostic tests. In endemic areas, where most cases are present, surveys generally rely on coprological methods, ending up in an underestimation of prevalence. Serology has been seldom used in the field, while it is more widely used for screening of cohorts of immigrants in non-endemic areas; this might also affect figures, with an overestimation of the prevalence. Overall, the diagnostic issue affected also the definition of morbidity/mortality, hampering estimation of disability-adjusted life years (DALYs). Further, the lack of a diagnostic gold standard limits the comparison of the test accuracy between different studies, that might use different reference standards. In most diagnostic studies stool microscopy is still considered the reference test, and only recently researchers have been considering more sophisticated statistical methods (such as Bayesian latent class analysis) for estimating the test accuracy {Buonfrate, 2022}. Finally, the poor sensitivity of stool microscopy can also affect the results of randomized controlled trials, with an overestimation of the treatment efficacy. Further research in the diagnostic area is hence of paramount importance, not only for an easier diagnosis, but also in consideration of its influence on different aspects of the disease.

Grey areas lingered in the therapeutic area as well, for many years. Albendazole was considered a first line treatment option, mostly based on expert opinion and sometimes due to the fact that it was the only anthelminthic drug available in many settings. Finally, a Cochrane systematic review published in 2016 demonstrated that its efficacy was much lower than ivermectin {Henriquez-Camacho, 2016}, however leaving an open question about the doses to be administered (single or multiple?). In this thesis, I address many of the knowledge gaps of strongyloidiasis in the non-endemic setting, specifically: 1) The “actual global burden” of the disease, which is currently based on an educated guess; 2) the true chronic morbidity, beyond the most severe cases; 3) the best diagnostic strategy, in absence of a gold standard test; 4) the optimal treatment of non-complicated cases, in light of the findings of Henriquez-Camacho et al.

In order to address those aspects, the thesis will have the following specific objectives:

- To estimate the prevalence of strongyloidiasis at global level and in different populations in a non-endemic area (Northern Italy)
- To review the clinical presentation and laboratory profile of infected individuals
- To assess the accuracy of diagnostic tests for the diagnosis and post-treatment follow up
- To assess the efficacy of different doses of ivermectin for the treatment of chronic strongyloidiasis

Each chapter of this thesis addresses one main objective, evolving from epidemiology to treatment. Specifically:

- The first chapter brings an up-to-date review of the main epidemiological, clinical, diagnostic and treatment issues in the non-endemic setting, as a general introduction to the many challenges

related to this condition worldwide. A narrative review serves as introduction: **Buonfrate D et al. Imported Strongyloidiasis: Epidemiology, Presentations, and Treatment. Curr Infect Dis Rep. 2012**

- The second chapter focuses on epidemiology. It includes: 1) a study reporting new estimates of the global prevalence of strongyloidiasis based on a mathematical model; the study has been published in Pathogens: **Buonfrate D et al. The global prevalence of *Strongyloides stercoralis* infection. Pathogens. 2020**. 2) Another study included in this chapter is a case-control study assessing the epidemiological characteristics of the infection in Italy, both in migrants and in Italians. The study was published in Eurosurveillance: **Buonfrate D et al. Epidemiology of *Strongyloides stercoralis* in northern Italy: results of a multicentre case–control study, February 2013 to July 2014.** Euro Surveill. **2016**

- The third chapter addresses the clinical and laboratory profile of chronic strongyloidiasis. It includes a systematic review focusing on clinical and laboratory characteristics of patients with strongyloidiasis at presentation and their evolution after treatment. **Buonfrate D et al. Clinical and laboratory features of *Strongyloides stercoralis* infection at diagnosis and after treatment: a systematic review and meta-analysis. Clin Microbiol Infect 2021**

- The fourth chapter relates to the critical issue of diagnosis. It includes: 1) a scoping review of available diagnostic tools, describing their accuracy and potential role. **Buonfrate D et al. The diagnosis of human and companion animal *Strongyloides stercoralis* infection: Challenges and solutions. A scoping review. Adv Parasitol. 2022;118:1-84. doi: 10.1016/bs.apar.2022.07.001.** 2) a systematic review with meta-analysis on the accuracy of polymerase chain reaction (PCR) for the diagnosis of strongyloidiasis: , **Buonfrate D et al. Accuracy of molecular biology techniques for the diagnosis of *Strongyloides stercoralis* infection - A systematic review and meta-analysis.** PLoS Negl Trop Dis. **2018 Feb 9;12(2):e0006229. doi: 10.1371/journal.pntd.0006229.** 3) a diagnostic study evaluating the use of serology for the follow up of infected patients.: Buonfrate D et al. **Accuracy of five serologic tests for the post-treatment follow up of *Strongyloides stercoralis* infection.** PLoS Negl Trop Dis. **2015 Feb 10;9(2):e0003491. doi: 10.1371/journal.pntd.0003491.**

- The fifth chapter deals with therapy, with the results of a randomized controlled trial conducted in European travel clinics that compared different doses of ivermectin for the treatment of chronic strongyloidiasis: **Buonfrate D et al. Strong Treat 1 to 4 – Randomized phase 3 Clinical Trial of Multiple versus Single Dose Ivermectin for *Strongyloides stercoralis* infection. Lancet Infect Dis. 2019 Nov;19(11):1181-1190. doi: 10.1016/S1473-3099(19)30289-0. Epub 2019 Sep 23**

The information brought on by the different chapters will finally be integrated and commented into a general discussion.

References

1. Nutman, T.B. Human infection with Strongyloides stercoralis and other related Strongyloides species. *Parasitology* **2017**, *144*, 263-273, doi:10.1017/s0031182016000834.
2. Streit, A. Reproduction in Strongyloides (Nematoda): a life between sex and parthenogenesis. *Parasitology* **2008**, *135*, 285-294, doi:10.1017/s003118200700399x.
3. Bradbury, R.S.; Pafčo, B.; Nosková, E.; Hasegawa, H. Strongyloides genotyping: a review of methods and application in public health and population genetics. *International journal for parasitology* **2021**, *51*, 1153-1166, doi:10.1016/j.ijpara.2021.10.001.
4. Buonfrate, D.; Bisanzio, D.; Giorli, G.; Odermatt, P.; Fürst, T.; Greenaway, C.; French, M.; Reithinger, R.; Gobbi, F.; Montresor, A., et al. The Global Prevalence of Strongyloides stercoralis Infection. *Pathogens (Basel, Switzerland)* **2020**, *9*, doi:10.3390/pathogens9060468.
5. Buonfrate, D.; Baldissera, M.; Abrescia, F.; Bassetti, M.; Caramaschi, G.; Giobbia, M.; Mascarello, M.; Rodari, P.; Scattolo, N.; Napoletano, G., et al. Epidemiology of Strongyloides stercoralis in northern Italy: results of a multicentre case-control study, February 2013 to July 2014. *Euro surveillance : bulletin Europeen sur les*

maladies transmissibles = European communicable disease bulletin **2016**, *21*, doi:10.2807/1560-7917.es.2016.21.31.30310.

6. Olsen, A.; van Lieshout, L.; Marti, H.; Polderman, T.; Polman, K.; Steinmann, P.; Stothard, R.; Thybo, S.; Verweij, J.J.; Magnussen, P. Strongyloidiasis--the most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2009**, *103*, 967-972, doi:10.1016/j.trstmh.2009.02.013.
7. Buonfrate D, Fittipaldo A, Vlieghe E, Bottieau E. Clinical and laboratory features of Strongyloides stercoralis infection at diagnosis and after treatment: a systematic review and meta-analysis. Clin Microbiol Infect. 2021 Nov;27(11):1621-1628. doi: 10.1016/j.cmi.2021.07.016.
8. Buonfrate, D.; Requena-Mendez, A.; Angheben, A.; Muñoz, J.; Gobbi, F.; Van Den Ende, J.; Bisoffi, Z. Severe strongyloidiasis: a systematic review of case reports. *BMC infectious diseases* **2013**, *13*, 78, doi:10.1186/1471-2334-13-78.
9. Ye L, Taylor GP, Rosadas C. Human T-Cell Lymphotropic Virus Type 1 and *Strongyloides stercoralis* Co-infection: A Systematic Review and Meta-Analysis. Front Med (Lausanne). 2022 Feb 14;9:832430. doi: 10.3389/fmed.2022.832430. PMID: 35237633; PMCID: PMC8882768.
10. Buonfrate, D.; Formenti, F.; Perandin, F.; Bisoffi, Z. Novel approaches to the diagnosis of Strongyloides stercoralis infection. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2015**, *21*, 543-552, doi:10.1016/j.cmi.2015.04.001.
11. Buonfrate D, Tamarozzi F, Paradies P, Watts MR, Bradbury RS, Bisoffi Z. The diagnosis of human and companion animal Strongyloides stercoralis infection: Challenges and solutions. A scoping review. Adv Parasitol. 2022;118:1-84. doi: 10.1016/bs.apar.2022.07.001.
12. Henriquez-Camacho, C.; Gotuzzo, E.; Echevarria, J.; White, A.C., Jr.; Terashima, A.; Samalvides, F.; Pérez-Molina, J.A.; Plana, M.N. Ivermectin versus albendazole or thiabendazole for Strongyloides stercoralis infection. *The Cochrane database of systematic reviews* **2016**, *2016*, CD007745, doi:10.1002/14651858.CD007745.pub3.

Summary

Strongyloides stercoralis is a soil-transmitted helminth which can cause a fatal syndrome in immunosuppressed individuals. The parasitic infection is mostly present in disadvantaged areas of the world, characterized by poor sanitation and lack of adequate sewage disposal, causing the contamination of the soil with human stool. In such settings, *Strongyloides* larvae are dispersed in the environment through human faeces and moult in the soil into an infective stage. The infective larvae can penetrate human skin and start the parasitic cycle. Peculiar to *S. stercoralis*, an auto-infective cycle in humans, which leads to a chronic, long-life (if not treated) infection. Due to auto-infection, we can diagnose strongyloidiasis in people who left an endemic area even decades ago. In some individuals, the infection may not cause evident signs or symptoms, but there is still the risk of developing the life-threatening form of infection later on in life, in case of emergence of an immunosuppressant condition or treatment. Other people do present unrelenting or fluctuating signs and symptoms mostly affecting the skin, the intestine and the respiratory tract, and might seek clinical care for these. Unfortunately, the infection is often misdiagnosed because health care providers are seldom aware of this condition (which is precisely included in the WHO list of Neglected Tropical Diseases), and proper diagnostic tests might not be prescribed. Microscopic examination of stool samples, which is traditionally used to detect intestinal parasites, has low sensitivity for this infection, thus it might be falsely negative. In fact, there is no diagnostic gold standard. This issue has hampered for long time both individual management of the infection and estimates of global prevalence (causing part of the neglect of strongyloidiasis). Also, the dose of ivermectin to be administered for chronic infection was undefined for long time.

Main objective of this doctoral work was to address the main critical aspects of strongyloidiasis in the non-endemic setting, covering a wide spectrum of areas, from epidemiology to clinical presentation, diagnosis, treatment and follow up. Specific objectives were:

- To estimate the prevalence of strongyloidiasis at global level and in different populations in a non-endemic area (Northern Italy)
- To review the clinical presentation and laboratory profile of infected individuals
- To assess the accuracy of diagnostic tests for the diagnosis and post-treatment follow up
- To assess the efficacy of different doses of ivermectin for the treatment of chronic strongyloidiasis

The objectives are addressed here with a collection of published papers, ranging from narrative and systematic reviews, case-control studies, original diagnostic studies and a randomized controlled trial.

The first chapter is a narrative review, describing the main characteristics of strongyloidiasis in the non-endemic setting. It reports the state-of-the-art and the grey areas on this topic before the following studies were carried out, and thus serves as an introduction to the whole work. In the second chapter, I deal with epidemiological aspects. Global prevalence of strongyloidiasis was estimated using a mathematical model, and resulted in 613.9 (95% CI: 313.1–910.1) million people infected worldwide, figures much larger than previous estimated (about 30-100 million people). This partly explains the neglect of this infection, whose prevalence was dramatically underestimated for years. The chapter also reports the results of a screening survey carried out in Northern Italy. Among Italians born before 1960, 8% (97/1,137) of those with

eosinophilia were positive for strongyloidiasis, compared to 1% (13/1,178) of those with normal eosinophil count (adjusted odds ratio (aOR) 8.2; 95% confidence interval (CI): 4.5–14.8). Among immigrants, the infection was found in 17% (36/214) of individuals with eosinophilia and in 2% (3/172) of those with normal eosinophil count (aOR 9.6; 95% CI: 2.9–32.4). These results demonstrate that eosinophilia is an important diagnostic clue, both for migrants and for Italians. The third chapter deals with clinical presentation and laboratory profile of chronic infection, and is composed by a systematic review with meta-analysis, whose main results are: a) About 50% people with chronic infection complain symptoms, mostly abdominal pain in 51.9% (95%CI 50.2-53.6) individuals, diarrhea in 43.6% (95%CI 41.7-45.6), itching in 36.3% (95%CI 34.5-37.9), skin rash/urticaria in 30.4% (95%CI 28.8-32.0), respiratory symptoms in 29.6% (95%CI 27.7-31.4), and nausea/vomiting in 8.1% (95%CI 6.4-9.9). b) About 77% infected people might have eosinophilia at presentation. c) both symptoms and eosinophilia tend to clear after treatment. This demonstrates that strongyloidiasis deserves attention also in absence of immunocompromise, as it can cause relevant disturbances that can be solved with treatment.

The collection of papers composing the fourth chapter address the diagnostic area. In a narrative review I comment the advantages and disadvantages of the available diagnostic tests for strongyloidiasis, pointing out that the use of highly sensitive diagnostic tests (that is the case of serological assays) is of primary importance. In a systematic review with meta-analysis I demonstrate that the sensitivity of real-time PCR for *S. stercoralis* is unsatisfactory (64.4, 95% CI 46.2±77.7); hence, once again, serology is preferred as screening tool. Finally, in an original diagnostic study, I demonstrate that serology can be used for post-treatment monitoring, although time to seroconversion can take up to 12 months. However, if a quantitative result is available, the decrease of antibody titre can be used in a shorter period of time to evaluate response to treatment.
Finally, the last chapter is composed by a multicenter randomized controlled trial (“Strong Treat” study) comparing a single dose versus multiple doses of 200 μg/kg of ivermectin for the treatment of chronic uncomplicated strongyloidiasis. The study showed that a single dose is as effective as multiple doses and better tolerated.

Dutch Summary

Strongyloides stercoralis is een worm die via de bodem en de omgeving wordt overgedragen, en die bij ernstig immuungedeprimeerde personen een zeldzaam maar fataal ziektebeeld kan veroorzaken.
Deze parasitaire infectie komt wereldwijd vooral voor in armere regio's, gekenmerkt door slechte of afwezige sanitaire voorzieningen, waardoor de bodem wordt verontreinigd met menselijke ontlasting. In dergelijke omstandigheden worden *Strongyloides*-larven via menselijke ontlasting in het milieu verspreid en vervellen ze in de bodem tot een infectieus stadium. De besmettelijke larven kunnen de menselijke huid binnendringen en een parasitaire cyclus starten.
Eigen aan *S. stercoralis* is de mogelijkheid van een auto-infectiecyclus bij de mens, die kan leiden tot een chronische, langdurige (indien niet behandeld) infectie. Omwille van zo'n auto-infectie kan strongyloïdiasis worden vastgesteld bij mensen die een endemisch gebied zelfs tientallen jaren geleden hebben verlaten. Bij sommige mensen veroorzaakt de infectie geen duidelijke tekens of symptomen, maar bestaat nog steeds het risico dat de levensbedreigende vorm van infectie later in het leven ontstaat, in geval van een immunosuppressieve aandoening of behandeling. Andere mensen vertonen niet aflatende of wisselende klinische tekens en symptomen die vooral de huid, de darmen en de luchtwegen aantasten, en kunnen daarvoor klinische zorg opzoeken. Helaas wordt de infectie vaak verkeerd gediagnosticeerd, omdat zorgverleners zelden goed op de hoogte zijn van deze aandoening (die zeer recent werd opgenomen in de WHO-lijst van verwaarloosde tropische ziekten); de juiste diagnostische tests worden ook niet altijd voorgeschreven. Microscopisch onderzoek van stoelgangsmonsters, traditioneel gebruikt om darmparasieten op te sporen, heeft een lage gevoeligheid voor deze infectie, leidend tot een vals negatief resultaat. In feite bestaat er geen gouden diagnostische standaard. Dit probleem heeft lange tijd zowel de individuele behandeling van de infectie als schattingen van de wereldwijde prevalentie belemmerd (waardoor strongyloïdiasis deels werd verwaarloosd). Ook de toe te dienen dosis ivermectine voor een chronische infectie is lange tijd niet exact vastgesteld.

Het hoofddoel van dit doctoraatswerk was om de meest kritische aspecten van strongyloïdiasis in de niet-endemische setting te behandelen, waarbij een breed spectrum van gebieden werd bestreken, van epidemiologie tot klinische presentatie, diagnose, behandeling en follow-up. Specifieke doelstellingen waren:
- Het inschatten van de prevalentie van strongyloïdiasis op mondiaal niveau en in verschillende populaties in een niet-endemisch gebied (Noord-Italië).
- Beoordelen van de klinische presentatie en het laboratoriumprofiel van geïnfecteerde personen.
- Beoordelen van de nauwkeurigheid van diagnostische tests voor de diagnose en follow-up na behandeling.
- Beoordelen van de doeltreffendheid van verschillende doses ivermectine voor de behandeling van chronische strongyloïdose.
De doelstellingen worden hier behandeld aan de hand van een verzameling gepubliceerde artikelen, variërend van narratieve en systematische reviews, case-control studies, originele diagnostische studies en een gerandomiseerde gecontroleerde trial.

Het eerste hoofdstuk is een narratieve review, waarin de belangrijkste kenmerken van strongyloïdiasis in een niet-endemische setting worden beschreven. Het vermeldt de stand van zaken en de zones van onzekerheid over dit onderwerp voordat de volgende studies werden uitgevoerd, en dient dus als inleiding op het hele werk.

In het tweede hoofdstuk behandel ik de epidemiologische aspecten. De wereldwijde prevalentie van strongyloïdiasis werd geschat met behulp van een wiskundig model, en resulteerde in een inschatting van 613,9 (95% CI: 313,1-910,1) miljoen mensen die wereldwijd besmet zijn, cijfers die veel hoger liggen dan eerder geschat (ongeveer 30-100 miljoen mensen). Dit verklaart ten dele de verwaarlozing van deze infectie, waarvan de prevalentie immers jarenlang dramatisch werd onderschat. Het hoofdstuk meldt ook de resultaten van een screeningsonderzoek in Noord-Italië. Onder Italianen geboren vóór 1960 was 8% (97/1.137) van degenen die zich presenteerden met eosinofilie positief voor strongyloïdiasis, vergeleken met 1% (13/1.178) van degenen met een normaal aantal eosinofielen (adjusted odds ratio (aOR) 8,2; 95% betrouwbaarheidsinterval (CI): 4,5-14,8). Bij immigranten werd de infectie aangetroffen bij 17% (36/214) van de personen met eosinofilie en bij 2% (3/172) van degenen met een normaal eosinofielgetal (aOR 9,6; 95% CI: 2,9-32,4). Deze resultaten tonen aan dat eosinofilie een belangrijke diagnostische aanwijzing is, zowel voor migranten als voor Italianen.

Het derde hoofdstuk behandelt de klinische presentatie en het laboratoriumprofiel van chronische infectie en bestaat uit een systematische review met meta-analyse, waarvan de belangrijkste resultaten zijn: a) ongeveer 50% mensen met chronische infectie hebben symptomen, voornamelijk buikpijn in 51,9% (95%CI 50,2-53,6) personen, diarree in 43,6% (95%CI 41. 7-45,6), jeuk bij 36,3% (95%CI 34,5-37,9), huiduitslag/urticaria bij 30,4% (95%CI 28,8-32,0), ademhalingssymptomen bij 29,6% (95%CI 27,7-31,4), en misselijkheid/braken bij 8,1% (95%CI 6,4-9,9). b) ongeveer 77% van de besmette personen heeft mogelijk eosinofilie bij presentatie. c) zowel de symptomen als de eosinofilie verdwijnen meestal na behandeling. Dit toont aan dat strongyloïdiasis ook in afwezigheid van immuundeficiëntie aandacht verdient, aangezien het relevante symptomen kan veroorzaken die met behandeling kunnen worden verholpen.

De verzameling artikelen in het vierde hoofdstuk gaat over de diagnostiek. In een verhalend overzicht geef ik commentaar op de voor- en nadelen van de beschikbare diagnostische tests voor strongyloïdose, waarbij ik erop wijs dat het gebruik van zeer gevoelige diagnostische tests (dat wil zeggen serologische tests) van primair belang is. In een systematische review met meta-analyse toon ik aan dat de gevoeligheid van real-time PCR voor *S. stercoralis* onvoldoende is (64,4, 95% CI 46,2±77,7); vandaar dat alsnog de voorkeur wordt gegeven aan serologie als screeningsinstrument. Tenslotte toon ik in een origineel diagnostisch onderzoek aan dat serologie kan worden gebruikt voor controle na behandeling, hoewel de tijd tot seroconversie tot 12 maanden kan duren. Indien echter een kwantitatief resultaat beschikbaar is, kan de afname van de antilichaamtiter in kortere tijd worden gebruikt om de respons op de behandeling te evalueren.
Het laatste hoofdstuk tenslotte is samengesteld uit een multicenter gerandomiseerde gecontroleerde studie ("Strong Treat"-studie) waarin een enkelvoudige dosis versus meervoudige doses van 200 µg/kg ivermectine worden vergeleken voor de behandeling van chronische ongecompliceerde strongyloïdiasis. De studie toonde aan dat een enkelvoudige dosis even doeltreffend is als meervoudige doses en beter wordt verdragen.

Chapter 1 Imported strongyloidiasis: epidemiology, presentations, and treatment

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Abstract

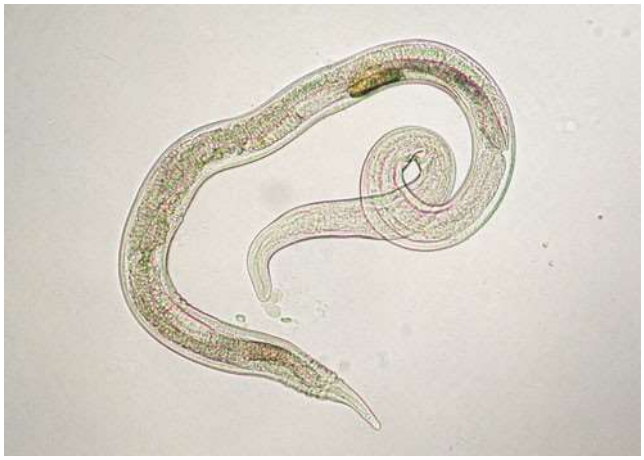
Strongyloidiasis is extremely more frequent in immigrants than in travellers. Clinical presentations do not differ significantly between the two groups, and the most frequent picture is a chronic infection characterized by in- termittent, mild, non-specific symptoms. Acute presentationis rare but it has been reported in travellers. Screening of asymptomatic subjects is not generally recommended, while a presumptive treatment with ivermectin might be justified for all travellers and immigrant patients presenting unexplained eosinophilia and/or compatible symptoms, even in case of negative test results. In fact, delayed diagnosis and treatment has life-threatening consequences in patients withconditions predisposing to development of hyperinfection and dissemination.

Introduction

Strongyloides stercoralis (*S. stercoralis,* see Fig. 1) is anematode widely distributed in tropical and subtropical areas, but small foci of low endemicity are also present in temperate climate—countries, like some parts of Southern Europe and USA (Appalachia). Estimates of prevalence indicate that 30 to 100 million people are infected world- wide, but the number is presumably higher because the diagnostic methods traditionally used (stool examination,culture) lack sensitivity [1]. Despite the high burden of the infection, only recently did the WHO add strongyloidiasis tothe list of neglected parasitic diseases [1, 2].

Transmitted through direct penetration of human skin putin contact with contaminated soil or sand, this peculiarparasite is characterized by a unique capability to replicateindefinitely inside the host ("auto-infective cycle"), withoutfurther exposures to an infected site [3]. Due to this characteristic, the infection can be diagnosed in persons that havebeen living in non-endemic countries for many years, butacquired the infection decades earlier in endemic countries.Thus, following the flows of migration and travel, the parasite can be found in patients presenting to health serv-ices in Western countries, where the diagnosis might be difficult due to the lack of familiarity of physicians with tropical diseases. This was clearly outlined in a study con- ducted in the USA, where 363 resident physicians were challenged with case presentations of strongyloidiasis and other helminth infections [4]. The results showed limited recognitionof *Strongyloides* infection and poor knowledge of helminths ingeneral. In particular, 23% of the US residents participating atthe study decided to prescribe empiric corticosteroid therapy in case of eosinophilia, without conducting previous investigations in order to exclude the presence of possible parasites, aprocedure that can cause life-threatening consequences in case of strongyloidiasis [5]. The authors conclude that all physicians should at least be aware of the risksof this infection and consider the potential exposure to*Strongyloides* before prescribing immunosuppressive therapies. The need for a better approach to the diagnosis and management of strongyloidiasis in non-endemic countries isalso stressed by Nuesch et al. [6], who state that this is probably the imported helminth infection with the highest impact on health.

Fig. 1 *Strongyloides stercoralis* adult female



Methods

- Search 1: To collect papers on epidemiology of importedcases we searched MEDLINE using the following search strategy: (strongyloid* AND (Humans[Mesh] AND "last 10 years"[PDat])) AND ((travel*) OR (imported) OR (mi-grant*) OR (immigrant*)). The search was conducted onthe 20th October 2011. Only papers written in English,French, and Spanish were reviewed. Papers published within the last 5 years were preferentially analyzed, thoughwe extended the analysis to older papers in case of paucityof more recent literature.

– Search 2: To collect papers on hyperinfection syndromes/ disseminated cases, we searched MEDLINE using the following search strategy: *disease* (strongyl*, anguillulose) AND *severity of cases* (disseminat*, hyperinfect*, severe, death, fatal, mortality) OR *disease* (strongyl*, anguillu- lose) AND *associated conditions* (tumor*, cancer, haema-tolog*, lymphom*, leukem*, leukaem*, neoplas*,malignan*, HTLV*, HIV, AIDS, hypogammaglobuline- mia, rheumat*, "biological agents", diabet*, transplant*, COPD, steroid*, glucocorticoid*, Immunosuppression [MeSH], Immunocompromised Host [MeSH]) and limiting the search to papers published since 2006, related tohumans and to the above mentioned languages. Date ofsearch in Pubmed: 20th May 2011.

Incidence of Travel-Related Strongyloidiasis

In literature published since 2005, we found only one paperdescribing a study specifically designed to investigate the risk of acquiring strongyloidiasis in travellers [7]. In this prospective study, subjects attending a pre-travel visit at thetravel clinic of the Public Health Service of Amsterdam were tested for *Strongyloides* (using an in–house ELISA) before departure and 2 and 6 weeks after return from an endemic country. Among 1178, previously negative peopletested, 3 subjects (0.25%) showed seroconversion (incidence rate 6.5 per 1000 persons-month), all returning from Asia. Interestingly, serology performed before the journey was positive in 29 out of 1207 travellers (2.4%); the authorsobserved that an increasing number of travels to endemic areas leads to a cumulative risk of infection (the same was true for other parasitic infections such as schistosomiasis, filariasis, toxocariasis). Since the incidence rates for all the parasites studied were low, the authors conclude that there is no indication to perform routine screening of returning travellers. The same opinion is shared by Bottieau et al.[8], who remark that screening of asymptomatic, returning travellers would only be justified in case of a stay longer than 3 months in a tropical area. They also suggest that physicians shouldn't rely on eosinophil count and stoolexamination for screening, as the sensitivity of both is too low; even serology has limited value in detecting recent *S. stercoralis* infections, although some researchers have found serology to be more sensitive in migrants than in travellers [6, 9]; however, the authors did not specify the time elapsed from exposure to serology testing in travellers in their study, and possibly a delayed testing might lead to ahigher proportion of positive results.

The approach should be different in subjects with aconsistent travel history presenting to physicians with symptoms and/or eosinophilia. In this case, the possibility of helminthic infections should be considered: experts of the British Infection Society propose a schematic approach based on symptoms/region visited, although it is suggested to performconcentrated stool microscopy and *Strongyloides* serology inall cases, regardless the country visited [10].

Prevalence of Strongyloidiasis in Immigrants and Refugees

Since 2006, we found three papers reporting the results of cross-sectional surveys [11–13] and one of prospective survey [14], aimed at investigating the prevalence of parasite infections in immigrants and/or refugees from low-middle income countries to countries of low or no endemicity for *S.stercoralis*. Data are summarized in Table 1.

Table 1: Prevalence of strongyloidiasis in immigrants and/or refugees

Paper	Country	Population	Diagnostic method	Positive/tested subjects. N (%)
Gualdieri 2011	Italy	Immigrants	Microscopy	2/514 (0.4)
Hochberg 2011	USA	Immigrants HIV+	Microscopy and serology	Microscopy:0/128; serology:33/128(26)
Posey 2007	USA	Refugees	Serology	214/462 Sudaneses (46);23/100 Somali Bantu (23)
Caruana 2006	Australia	Immigrants and refugees	Microscopy and serology	Microscopy:10/321; serology:84/354

Clearly, prevalence data varied according with the diagnostic methods used: serology was found to be much more sensitive than stool microscopy, even in case of people with HIV infection [11],while specificity might be hampered by cross-reactivity withother parasites [13, 15]. We believe that the true prevalence is likely to be between the estimates based on direct methods andthose based on serology.

As for the countries of origin of the immigrants, Caruanaet al. [13] analyzed only subjects from East Africa and fromCambodia, finding a higher prevalence in the latter group: apositive serology was detected in 82/230 (36%) of Cambodians versus 2/124 (2%) of East Africans. In the paper by Gualdieri et al. [14] subjects tested came from a larger number of countries, but the nationality of the two subjectsdiagnosed by microscopy (the only method used in this study) is not specified. Hochberg et al. [11] in the US founda high proportion of positive serology (33/128 or 26%) amongHIV patients coming from the following countries: Mexico(12 patients), Honduras (4), Ethiopia (3), El Salvador (2), Zambia (2) and one each in Argentina, Congo, Cuba, Grenada,Guatemala, India, Kenya, Niger, Tanzania and Vietnam. How-ever, some areas had insufficient representation for a reliablecomparison of the country-related risk. In another study con-ducted in two Italian hospitals from 2000 to 2009 [16], 15 (11%) of 138 HIV–positive immigrants were infected by *S.stercoralis*. Diagnosis was made with serology (an in—houseindirect immunofluorescence antibody test—IFAT) in 11 patients, while 2 had positive stool microscopy and 2 positivestool culture. In this study, all but one positive patient were Africans (14 positive of 107 tested). The remaining positivesubject came from Central/South America. None of the 4 patients from Asia/Oceania tested positive.

Other studies retrospectively analyzed wider aspects of the health profile of asylum seekers or immigrants. The servicePRAIDA [17] "Programme régional d'accueil et d'intégrationdes demandeurs d' asile", the program for reception and integration of asylum seekers of Montréal, Canada, collecteddata about the refugees screened from 2000 to 2004; amongthe 231 patients tested for strongyloidiasis (with EIA), 40 were positive (17.3%). Logistic regression analysis found noassociation between the continent of origin and the risk of infection. The authors remark that the prevalence found washigher than previous studies based on direct methods; they also argue that false positive results due to cross-reactivity (i.e.with filariae) did not seem to significantly affect their result,given that most subjects with a positive result for strongyloidiasis tested negative for filariasis. Both serology and stoolmicroscopy were performed in African immigrants attendingoutpatient clinics at the Royal Melbourne Hospital since 2003 to 2006, in a retrospective audit [18]. Of 145 stool samples examined, only 2 (1.4%) were positive for *Strongyloides* larvae by microscopy, while 32 of 179 tested (17.9%) had positive serology. Both patients with positive stool also had apositive serology.

Since serology has been used to test immigrants, a high prevalence of strongyloidiasis has been observed [11, 12, 17,18]. Although the need for immigrant screening should not be overstated, the potential severity of the disease warrants a proper diagnosis and possibly a presumptive treatment in some subgroups of migrants at high risk of developing severe infection [19]. For instance, transplant candidates should be extensively screened for infectious/parasitic diseases that could reactivate after transplant, and in case of immigrant patients it is mandatory to extend the screening to infectionsthat are endemic in their countries of origin. Fitzpatrick et al.[20] describe the results of the extended screening programproposed for Hispanic kidney transplant candidates. For strongyloidiasis, of 75 patients tested with serology, 5 (6.7%) were positive: they were all asymptomatic, and only one had eosinophilia; these findings confirm the need for screening all patients at high risk of developing the life-threatening stages of strongyloidiasis,

irrespectively of the presence of clinical symptoms/signs or eosinophilia.

Clinical Presentation

In most cases, strongyloidiasis is a chronic asymptomatic infection, frequently associated with eosinophilia as theunique finding that could raise suspicion. When symptoms occur, they are usually mild and non-specific, and involve the gastrointestinal tract (abdominal pain, diarrhea), therespiratory tract (cough, dyspnea) and the skin (pruritus, rash) [10, 21]. Another, rarer manifestation is an itchy, serpiginous rash that develops as the result of subcutaneouslarval migration: *larva currens*. It is mostly observed aroundthe trunk, upper legs and buttocks and it moves quickly (around 5–15 cm/h), lasting for some hours to a few days [10, 22].

Acute strongyloidiasis is rarely reported, but it should beconsidered in tourists returning from highly endemic countries with compatible symptoms and signs: within a few days from exposure, a Loeffler's syndrome usuallyoccurs, along with skin signs (urticaria, itch). Experimentalhuman infections demonstrated that a local reaction at the site of entry may appear and may last up to several weeks [5]. A recent paper [23] described a case of acute strongyloidiasis in a couple of tourists returning to Italy fromSoutheast Asia where in addition to the classical Loeffler's syndrome, a transient splenomegaly and increased serum AST and ALT were recorded.

Severe, potentially fatal clinical syndromes occur especially in immunosuppressed patients, who can experience hyperinfection (HI) or dissemination (DS). As it has been outlined above, corticosteroid therapy is the most frequent trigger of both [24]. Other conditions commonly associatedto HI/DS are malignancies (particularly lymphomas), organtransplantation, HTLV-1 infection. Malnutrition, alcohol abuse and diabetes have also been associated with the severeforms of strongyloidiasis [5, 24]. Hyperinfection is usually defined as an increased larval load, causing severe symptoms [25]; the larvae in this case remain confined to the respiratory and gastrointestinal tracts. Sometimes during HI, gram–negative sepsis and meningitis develop because *Strongyloides* larvae can carry enteric bacteria through the bowel mucosa into the host's circulation [15]. On the other hand, in DS larvae are found virtually in any organs [5]. Themortality associated to these two syndromes is significant: for HI it is estimated to be around 15%, while it is exceedingly high (87%) in case of dissemination [24]. Unfortunately,just like in case of chronic infection, symptoms in HI/DS are nonspecific; moreover, eosinophilia is often absent, thereforediagnosis is often delayed. However, in contrast with the low sensitivity of direct methods in case of chronic strongyloidiasis, in hyperinfection and dissemination the larval output is so accelerated and increased that diagnosis is easily done withdirect examination of the clinical samples (stool, sputum, andother) [25].

The results of the MEDLINE search 2 defined above, permitted to identify 290 papers. We reviewed the full text of the papers and selected the cases of hyperinfection syndromes/ disseminated strongyloidiasis diagnosed in low-endemicity countries in patients with previous stays in endemic countries.We identified 37 papers accounting for 41 case reports. None of the cases were related to travel, while 36 cases were diag nosed in immigrants. A total of 15 of the 36 patients (41.7%) were Hispanic (originating from Spanish–speaking countriesin Central America, South America or the Caribbeans) [26–39]. An interesting finding concerns 5 cases occurring intransplant recipients who did not have a history of possible exposure to *S. stercoralis*, but who received the transplantedorgan from Hispanic donors [40–45]. One of the patients developed intercurrent cytomegalovirus sepsis and died [45]. Strongyloidiasis was retrospectively confirmed in 4 of thosedonors [40, 41, 43–45], and subsequent investigations wereconducted on all the patients who had received organs from the same infected donors. This procedure led to the diagnosis of strongyloidiasis in a kidney recipient, who had already developed hyperinfection but was successfully treated [41].

Studies Comparing Strongyloidiasis in Travellers versus Immigrants

In a retrospective study of 33 imported cases in Spain, González et al. [21] reviewed clinical, epidemiological and biochemical characteristics of immigrants (23 patients) and travellers (10) diagnosed with strongyloidiasis at the Hospital Clinic (University of Barcelona) in a 3–year time. A high proportion of

the travellers had visited Sub-Saharan Africa (40%), while the immigrants mostly came from South America (69.6%). About half of the patients had a chronic, asymptomatic infection (the diagnostic work-up was usuallycarried out to investigate eosinophilia in these cases). No significant differences in clinical presentations were found between immigrants and travellers. The levels of eosinophilcount were also compared between the two groups, and >therange of variation was similar (with eosinophilia frequently lacking in severe disease). An analogous retrospective analysis was conducted on 31 patients (12 travellers and 19 immigrants) diagnosed with strongyloidiasis in two referral centres in Switzerland from 1998 to 2002 [15]. In contrast to the Spanish study, only 16% of the patients were asymptomatic, while the others underwent the diagnostic work up because of nonspecific symptoms: immigrants had less frequently abdominal symptoms (47% vs 75% in travellers) but more frequently respiratory symptoms (25% vs 8%). Neither study found statistically significant differences in clinical presentations between immigrants and travellers.

Treatment

Currently, ivermectin is the best therapeutic option for strongyloidiasis. The most recent trials comparing albendazole and ivermectin confirm the superiority of the latter in termsof efficacy: in particular, Nontasut et al. [46] treated 33 patients with albendazole 400 mg for 5 days and 78 patientswith ivermectin 0.2 mg/Kg single dose, finding cure rates of78.8% vs 98.7%, respectively. Supputtamongkol et al. [47]compared single and double doses (given 2 weeks apart) ofivermectin with high dose albendazole (800 mg daily for7 days); the parasitological cure rates were 96.8% and93.1% in the single dose and in the double doses regimens of ivermectin, respectively, and 63.3% in the albendazole group. It was not possible to demonstrate a difference in efficacy between the two ivermectin groups. However, some experts empirically recommend repeated doses, arguing thatthe "classical" single dose [48, 49] is often insufficient to eradicate the infection, especially in immunocompromised subjects [25].

In another study [50] ivermectin was compared to thiabendazole, finding no difference in efficacy but better tolerability with ivermectin. This is the only study conducted sofar in which the treatment efficacy was evaluated not only with direct methods but also with serology (an in-house IFAT), and was found to be much lower than in any previous trial (below 70% for both drugs). Given the low sensitivity of diagnostic methods, the authors suggest that the treatmentefficacy could have been overestimated in previous trials, asnegative stool cultures/examinations after treatment are tooinsensitive to prove eradication of the infection. Other groups evaluated serology in monitoring the response to therapy, though not in randomized controlled trials; in particular, Biggs et al. [51] showed its usefulness in immigrantsand refugees.

On the other hand, particularly in case of refugees who could be difficult to follow up, some guidelines consider presumptive treatment before their resettlement to be more cost-effective than screening [12].

Further research is needed to determine the optimal doseschedule of ivermectin (unquestionably the best available drug) to cure strongyloidiasis. Future trials should take intoconsideration the accuracy of the currently available diagnostictools for trial inclusion and cure monitoring.

Conclusions

The increase in travel and migration facilitate the spread ofpathogens and diseases all around the world. Strongyloidiasis, widely distributed in large parts of Asia, Africa and South America, has become an emerging global infection that has migrated from developing regions to industrialized areas [24]. Clinicians should be aware of this infection as well as the conditions exposing patients to the risk of hyper-infection and dissemination.

Travellers are at relatively low risk of acquiring strongyloidiasis, therefore routine screening in the absence of symptoms is probably of no value. On the other hand, it is important to investigate eosinophilia in subjects that have visited endemic countries. It is less easy to define common guidelines for the management of asymptomatic immigrantsand in particular refugees: in the latter group a presumptive treatment could also be worthwhile if a high prevalence of strongyloidiasis has been demonstrated previously. Doubtlessly, clinicians must be even more careful with patients needing chemotherapy,

corticosteroids or presenting any condition exposing to the risk of hyperinfection or dissemination, and a presumptive treatment with ivermectin would be justified for all traveller and immigrant patients presenting unexplained eosinophilia and/or compatible symptoms,even in the setting of negative test results.

Important knowledge gaps still remain regarding theoptimal management of this peculiar parasitic infection. In particular, more research is needed to find the optimal tool,or combination of tools, for individual diagnosis, prevalence studies and monitoring of treatment efficacy.

References

1. Olsen A, van Lieshout L, Marti H, et al. Strongyloidiasis—the most neglected of the neglected tropical diseases? Trans R Soc Trop Med Hyg. 2009;103(10):967–72.
2. World Health Organization: Strongyloidiasis fact sheet. Available at: http://www.who.int/neglected_diseases/diseases/strongyloidiasis/en/.Accessed December 2011.
3. Prendki V, Fenaux P, Durand R, et al. Strongyloidiasis in man75 years after initial exposure. Emerg Infect Dis. 2011;17(5):931–2.
4. Boulware DR, Stauffer WM, Hendel-Paterson BR, et al. Maltreat-ment of Strongyloides infection: case series and worldwide physicians-in-training survey. Am J Med 2007;120(6):545 e541–548.
5. Keiser PB, Nutman TB. Strongyloides stercoralis in the Immuno-compromised Population. Clin Microbiol Rev. 2004;17(1):208–17.
6. Nuesch R, Zimmerli L, Stockli R, et al. Imported strongyloidosis: a longitudinal analysis of 31 cases. J Travel Med. 2005;12(2):80–4.
7. Baaten GG, Sonder GJ, van Gool T, et al. Travel-related schis- tosomiasis, strongyloidiasis, filariasis, and toxocariasis: the risk ofinfection and the diagnostic relevance of blood eosinophilia. BMCInfect Dis. 2011;11:84.
8. Bottieau E, Van Gompel A. Screening the asymptomatic traveler returning from the tropics. Rev Med Suisse. 2009;5(202):1016–21.
9. Sudarshi S, Stumpfle R, Armstrong M, et al. Clinical presentationand diagnostic sensitivity of laboratory tests for Strongyloides stercoralis in travellers compared with immigrants in a non- endemic country. Trop Med Int Health: TM & IH. 2003;8 (8):728–32.
10. Checkley AM, Chiodini PL, Dockrell DH, et al. Eosinophilia in returning travellers and migrants from the tropics: UK recommen-dations for investigation and initial management. J Infect. 2010;60(1):1–20.
11. Hochberg NS, Moro RN, Sheth AN, et al. High prevalence of persistent parasitic infections in foreign-born, HIV-infected personsin the United States. PLoS Negl Trop Dis. 2011;5(4):e1034.
12. Posey DL, Blackburn BG, Weinberg M, et al. High preva- lence and presumptive treatment of schistosomiasis and stron- gyloidiasis among African refugees. Clin Infect Dis: an official publication of the Infectious Diseases Society of America. 2007;45(10):1310–5.
13. Caruana SR, Kelly HA, Ngeow JY, et al. Undiagnosed and poten-tially lethal parasite infections among immigrants and refugees inAustralia. J Travel Med. 2006;13(4):233–9.
14. Gualdieri L, Rinaldi L, Petrullo L, et al. Intestinal parasites in immigrants in the city of Naples (southern Italy). Acta Trop. 2011;117(3):196–201.
15. Newberry AM, Williams DN, Stauffer WM, et al. Strongyloides hyperinfection presenting as acute respiratory failure and gram- negative sepsis. Chest. 2005;128(5):3681–4.
16. Mascarello M, Gobbi F, Angheben A, et al. Prevalence of Strong-yloides stercoralis infection among HIV-positive immigrants attend-ing two Italian hospitals, from 2000 to 2009. Ann Trop Med Parasitol2011;In Press.
17. Ouimet MJ, Munoz M, Narasiah L, et al. Current pathologies among asylum seekers in Montreal: prevalence and associated riskfactors. Canadian Journal of Public Health Revue canadienne de sante publique. 2008;99(6):499–504.
18. Gibney KB, Mihrshahi S, Torresi J, et al. The profile of health problems in African immigrants attending an infectious disease unit in Melbourne, Australia. Am J Trop Med Hyg. 2009;80(5):805–11.
19. Keystone JS. Can one afford not to screen for parasites in high-risk immigrant populations? Clin Infect Dis: an official publication of the Infectious Diseases Society of America. 2007;45(10):1316–8.
20. Fitzpatrick MA, Caicedo JC, Stosor V, et al. Expanded infectious diseases screening program for Hispanic transplant candi- dates. Clin Infect Dis: an official journal of the Transplantation Society. 2010;12(4):336–41.
21. Gonzalez A, Gallo M, Valls ME, et al. Clinical and epidemiolog- ical features of 33 imported Strongyloides stercoralis infections. Trans R Soc Trop Med Hyg. 2010;104(9):613–6.
22. Gonzalez Martinez F, Mellado Pena MJ, Angulo Gonzalez de Lara R, et al. Larva Currens as a differential diagnosis of skin lesions inimmigrant children. Anales de pediatria (Barcelona, Spain : 2003)2010;73(2):102–104.
23. Angheben A, Mistretta M, Gobbo M, et al. Acute strongyloidiasis inItalian tourists returning from Southeast Asia. J

Travel Med. 2011;18(2):138–40.
24. Marcos LA, Terashima A, Dupont HL, et al. Strongyloides hyper-infection syndrome: an emerging global infectious disease. Trans R Soc Trop Med Hyg. 2008;102(4):314–8.
25. Feely NM, Waghorn DJ, Dexter T, et al. Strongyloides stercoralis hyperinfection: difficulties in diagnosis and treatment. Anaesthesia.2010;65(3):298–301.
26. Basile A, Simzar S, Bentow J, et al. Disseminated Strongyloides stercoralis: hyperinfection during medical immunosuppression. J Am Acad Dermatol. 2010;63(5):896–902.
27. Fusco DN, Downs JA, Satlin MJ, et al. Non-oral treatment with ivermectin for disseminated strongyloidiasis. Am J Trop Med Hyg. 2010;83(4):879–83.
28. Grijsen M, van den Berk G, Hoekstra E, et al. Intestinal strongyloidiasis as a presenting symptom of HTLV-1-associated adult T-cell leukemia/lymphoma. Endoscopy. 2009;41 Suppl 2: E271–272.
29. Roman-Velez JM, Martinez-Camacho RN, Alayon-Laguer D, et al.An unusual presentation of alveolar hemorrhage. Prim Care Respir J:journal of the General Practice Airways Group. 2009;18(4):337–9.
30. Rojo-Marcos G, Cuadros-Gonzalez J, Gonzalez-Juarez MJ, et al. Strongyloides stercoralis hyperinfection syndrome in a Colombianpatient receiving immunosuppressive treatment. Enferm Infecc Microbiol Clin. 2009;27(7):432–4.
31. Agarwal VK, Khurana HS, Le HX, et al. 30-year-old HIV-positive female with diffuse alveolar hemorrhage. J Intensive Care Med. 2009;24(3):200–4.
32. Balagopal A, Mills L, Shah A, et al. Detection and treatment of Strongyloides hyperinfection syndrome following lung transplan-tation. Transpl Infect Dis. 2009;11(2):149–54.
33. Khasawneh F, Sreedhar R, Chundi V. Strongyloides hyperinfection: an unusual cause of respiratory failure. Ann Intern Med. 2009;150(8):570–1.
34. N'Dri K, Kouame KE, Sahari R, et al. Strongyloides stercoralis hyperinfection syndrome with acute meningoencephalitis associat-ed to HTLV-1 and HTLV-2 virus co-infection. Med Mal Infect. 2008;38(3):162–4.
35. Janssen R, Vlaminckx BJ, Seldenrijk CA, et al. Strongyloides stercoralis hyperinfection mimicking accelerated form of idiopathic pulmo-nary fibrosis. Lancet Infect Dis. 2008;8(7):456.
36. Miller MA, Church LW, Salgado CD. Strongyloides hyperinfection: a treatment dilemma. Am J Med Sci. 2008;336(4):358–61.
37. Rotolo U, Scarlata F, Giordano S, et al. Nephrotic syndrome and Gram-negative sepsis in a patient with strongyloidiasis: a case report. Le infezioni in medicina: rivista periodica di eziologia, epidemiologia, diagnostica, clinica e terapia delle patologie infettive. 2007;15(1):59–62.
38. Foreman EB, Abraham PJ, Garland JL. Not your typical strongyloidesinfection: a literature review and case study. South Med J. 2006;99(8):847–52.
39. Gupta S, Jain A, Fanning TV, et al. An unusual cause of alveolar hemorrhage post hematopoietic stem cell transplantation: a case report. BMC Cancer. 2006;6:87.
40. Brugemann J, Kampinga GA, Riezebos-Brilman A, et al. Two donor-related infections in a heart transplant recipient: one common, the other a tropical surprise. J Heart Lung Transplant. 2010;29(12):1433–7.
41. Hamilton KW, Abt PL, Rosenbach MA, et al. Donor-derived Strongyloides stercoralis infections in renal transplant recipients. Transplantation. 2011;91(9):1019–24.
42. Patel G, Arvelakis A, Sauter BV, et al. Strongyloides hyperinfection syndrome after intestinal transplantation. Transpl Infect Dis. 2008;10(2):137–41.
43. Rodriguez-Hernandez MJ, Ruiz-Perez-Pipaon M, Canas E, et al. Strongyloides stercoralis hyperinfection transmitted by liver allo- graft in a transplant recipient. Am J Transplant: official journal of the American Society of Transplantation and the American Societyof Transplant Surgeons. 2009;9(11):2637–40.
44. van Hattem S, Schuttelaar ML. Disseminated strongyloidiasis causedby heart donor-to-host transmission presenting with purpura. Clin Exp Dermatol. 2010;35(4):e149–150.
45. Weiser JA, Scully BE, Bulman WA, et al. Periumbilical parasitic thumbprint purpura: strongyloides hyperinfection syndrome acquired from a cadaveric renal transplant. Transpl Infect Dis 2011;13(1):58–62.
46. Nontasut P, Muennoo C, Surapol S-N, et al. Prevalence of strongyloides in Northern Thailand and treatment with ivermectin and albendazole. Southeast Asian J Trop Med Public Health. 2005;36(2):442–4.
47. Suputtamongkol Y, Premasathian N, Bhumimuang K, et al. Effica- cy and safety of single and double doses of ivermectin versus 7-dayhigh dose albendazole for chronic strongyloidiasis. PLoS Negl TropDis. 2011;5(5):e1044.
48. Caumes E, Keystone JS. Acute strongyloidiasis: a rarity. Chronic strongyloidiasis: a time bomb! J Travel Med. 2011;18(2):71–2.
49. Gilbert DN, Moellering RC, Eliopoulos GM, et al. The Sanford guide to antimicrobial therapy. Forty-First Edition 2011;137.
50. Bisoffi Z, Buonfrate D, Angheben A, et al. Randomized clinicaltrial on ivermectin versus thiabendazole for the treatment of strongyloidiasis. PLoS Negl Trop Dis. 2011;5(7):e1254.

51. Biggs BA, Caruana S, Mihrshahi S, et al. Management of chronicstrongyloidiasis in immigrants and refugees: is serologic testing useful? Am J Trop Med Hyg. 2009;80(5):788–91.

Chapter 2 Worldwide epidemiology of strongyloidiasis in high (the global South) and low endemic areas (Italy as an example)

2.1 The global prevalence of *Strongyloides stercoralis* infection

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Abstract

Strongyloidiasis is a common neglected tropical disease in tropical and sub-tropical climatic zones. At the worldwide level, there is high uncertainty about the strongyloidiasis burden. This uncertainty represents an important knowledge gap since it affects the planning of interventions to reduce the burden of strongyloidiasis in endemic countries. This study aimed to estimate the global strongyloidiasis prevalence. A literature review was performed to obtain prevalence data from endemic countries at a worldwide level from 1990 to 2016. For each study, the true population prevalence was calculated by accounting for the specificity and the sensitivity of testing and age of tested individuals. Prediction of strongyloidiasis prevalence for each country was performed using a spatiotemporal statistical modeling approach. The country prevalence obtained from the model was used to estimate the number of infected people per country. We estimate the global prevalence of strongyloidiasis in *2017* to be 8.1% (95% CI: 4.2–12.4%), corresponding to 613.9 (95% CI: 313.1–910.1) million people infected. The South-East Asia, African, and Western Pacific Regions accounted for 76.1% of the global infections. Our results could be used to identify those countries in which strongyloidiasis prevalence is highest and where mass drug administration (MDA) should be deployed for its prevention and control.

Introduction

Strongyloidiasis is the infection caused by the soil-transmitted helminth (STH) *Strongyloides stercoralis*. Its global prevalence was previously estimated at 30–100 million infected people [1], but these estimates were subsequently questioned in light of diagnostic issues that characterized the few studies available at that time [2]. Moreover, neither the source nor the methods which formed the basis for these estimates were reported in the paper. Indeed, the diagnostic methods commonly used in the field for other STHs, such as Kato–Katz and direct smear examination, have a very low sensitivity for *S. stercoralis* [3]. The Baermann method and Koga agar plate culture (APC) have a higher sensitivity than stool microcopy, but still miss a large proportion of infections [3]; polymerase chain reaction (PCR) is highly specific, but not more sensitive than the Baermann method and APC [4]. Serology is the most sensitive method, although false positive results are possible, due to cross-reactions and long-term persistence of antibodies [3]. Overall, none of the available diagnostic tests can be considered the gold standard for the diagnosis of strongyloidiasis. Recently, a paper estimated the prevalence of strongyloidiasis as a ratio to hookworm, in order to partly overcome the diagnostic issue [5].

The morbidity caused by *S. stercoralis* is not well defined compared to other STHs. A systematic review evaluated the clinical burden caused by strongyloidiasis and reported that urticaria (reported by 33% of infected individuals in the included studies), abdominal pain (62%), and diarrhea (50%) might be frequently affecting people with strongyloidiasis [6]. Although the results of the review were limited by the paucity of studies focusing on this topic, the clinical relevance of *S. stercoralis* infection cannot be disregarded, because in immunosuppressed individuals it can lead to a syndrome (hyperinfection/dissemination) that is invariably fatal if not promptly and properly cured and is often fatal despite treatment [7]. Ivermectin (IVM) is the drug of choice for the treatment of *S. stercoralis* infection [7], and it has been recently included in the WHO list of essential medicines for this purpose [8]. Unfortunately, IVM is often not easily available outside specific donation programs aimed at the elimination of lymphatic filariasis (LF) and onchocerciasis.

Currently, no specific strategies for the control of *S. stercoralis* infection have been implemented in endemic areas. This is mostly due to the knowledge gap regarding the global prevalence of the disease and the difficult access to quality-assured and affordable IVM.

In this work, we aimed to estimate the prevalence of strongyloidiasis at a global and country level, using a spatiotemporal statistical modeling approach.

Results

Review of the Literature

The flow of the literature review is described in Figure 1. The review of the literature identified 146 articles (Supplementary File 1) with data on the prevalence of strongyloidiasis from 43 countries (Figure 2). Brazil and Thailand were the countries with the highest number of studies. Twenty studies used either the Baermann method or stool culture (including agar plate, Harada Mori or any other cultural method) as diagnostic methods; a combination of diagnostic tests including Baermann method and/or stool culture was used in 22 studies. PCR was used in 7 studies, and a further 2 studies used it in combination with other tests. Serology was used in 16 studies, and a further 3 studies used serology in combination with other tests. The remaining studies used less sensitive diagnostic methods (mostly Kato–Katz and single/multiple direct smears). The prevalence reported was adjusted considering the diagnostic test used, as described above in the Methods section.

Figure 1. Flow chart describing the review process.

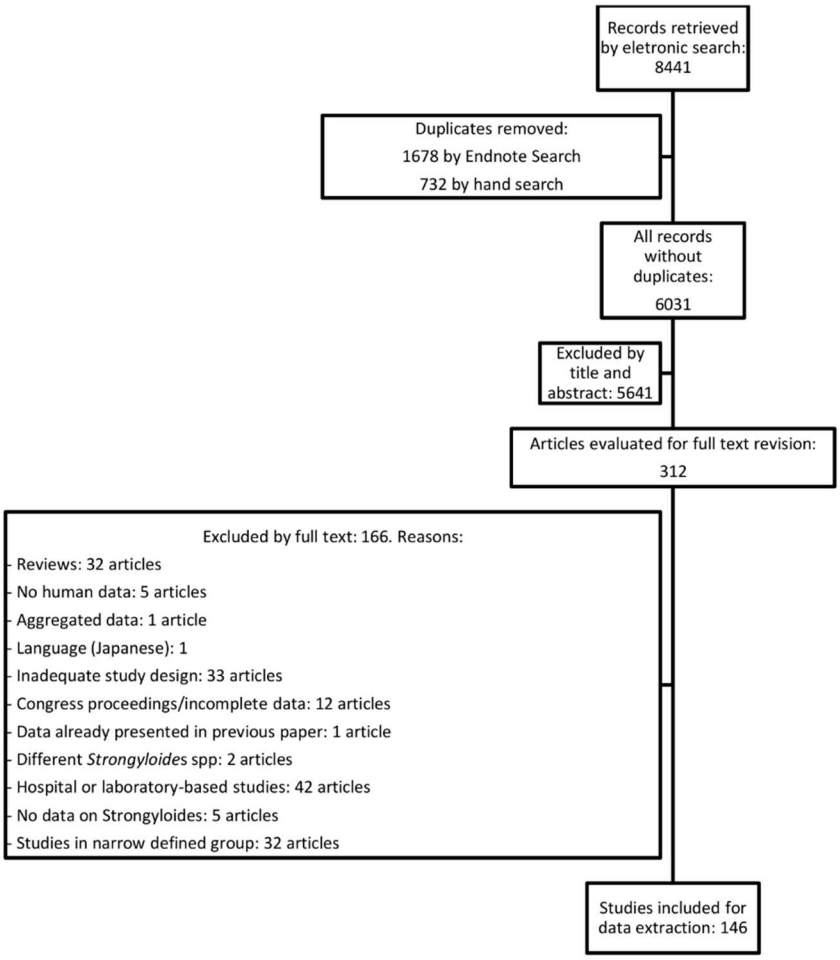
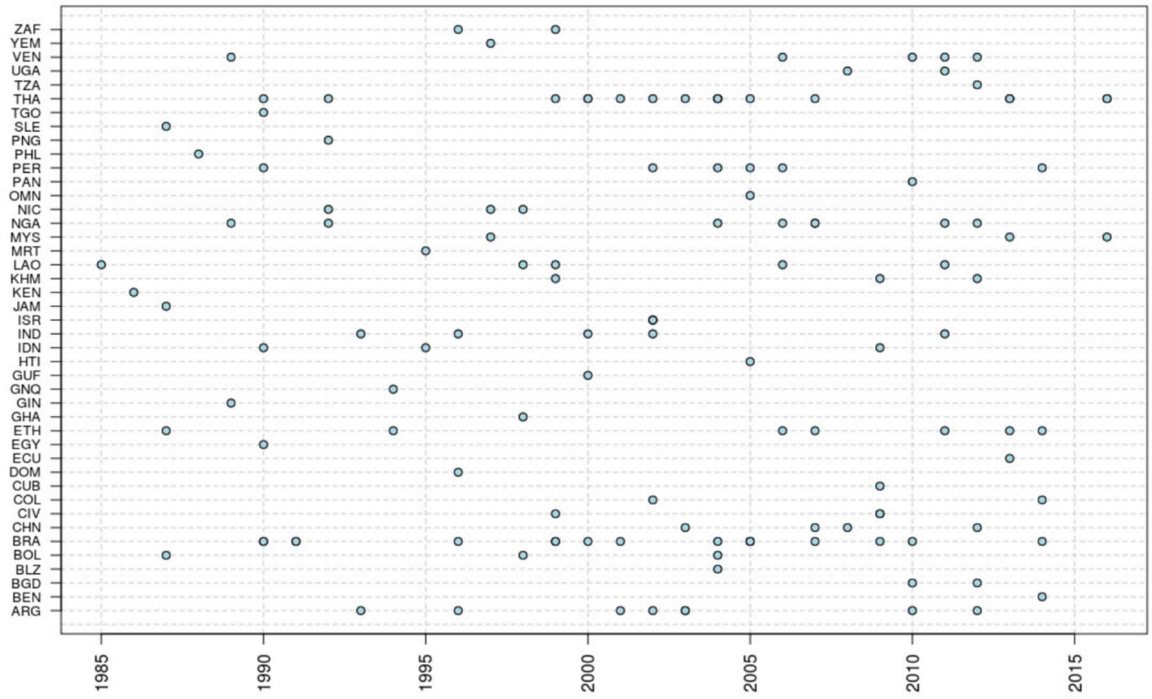


Figure 2. Year of survey reported in the research publications included in the literature review by country. In the Y-axis, country codes are reported according to ISO 3 (legend available in Supplementary Table 2).



Global and Regional (WHO) Prevalence According to the Model

The best model describing strongyloidiasis prevalence included GDP, percentage of rural population, territory roughness, sanitation, annual mean temperature, and annual precipitation:

$STG\text{-}PR = GDP + RURAL + RUG + SANIT + TEMP + RAIN + REGION_{RND}$

This model was used to estimate strongyloidiasis prevalence for each country at the worldwide level with a 95% confidence interval (95% CI).

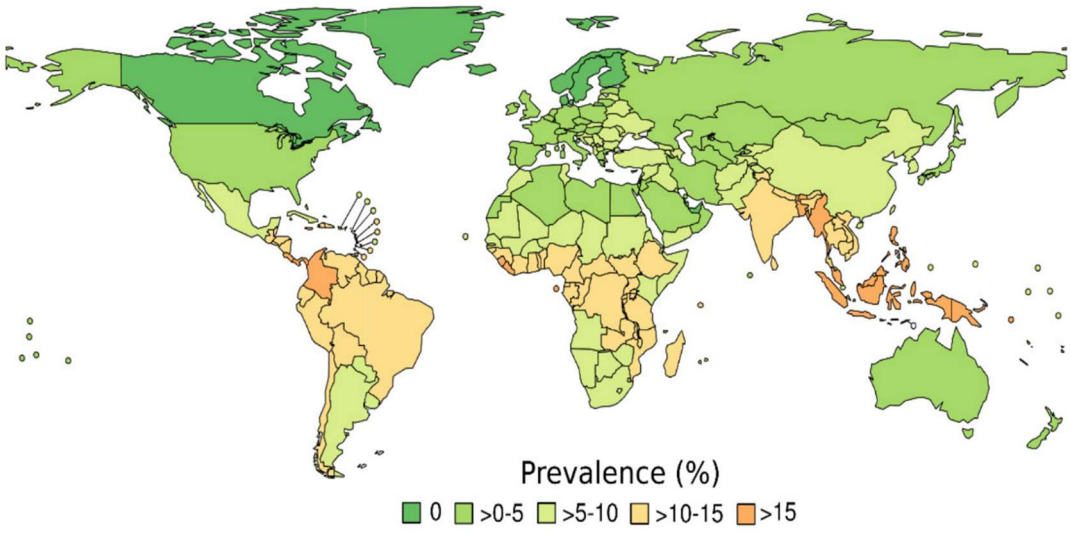
Strongyloidiasis prevalence and number of infected people are reported at the world and regional levels in Table 1 and Figure 3.

Table 1. Global and regional S. stercoralis prevalence (STG-PR), the number of infected individuals.

WHO REGION	STG-PR (95% CI)	POPULATION INFECTED (95% CI) [MILLION]
AFR	10.3% (5.3–15.3)	108.1 (55.1–160.9)
AMR	6.9% (3.5–10.2)	69.8 (35.5–103.9)
EMR	5.8% (2.9–8.6)	39.4 (20.1–58.8)
EUR	2.8% (1.4–4.1)	26.1 (13.3–38.8)
SEAR	12.1% (6.1–17.9)	237.3 (129.9–353.3)
WPR	7.13% (3.6–10.6)	133.2 (68.1–198.4)
World	8.1% (4.2–12.4%)	613.9 (313.1–910.1)

AFR: African Region; AMR: American Region; EMR: Eastern Mediterranean Region; EUR: European Region; SEAR; South-East Asia Region; WPR: Western Pacific Region.

Figure 3. Estimated strongyloidiasis prevalence (STG-PR) for 2017, as predicted by the best statistical model.



In 2017, the estimated strongyloidiasis prevalence was 8.1% (95% CI: 4.2%–12.4%), which corresponds to 613.9 (95% CI: 313.1–910.1) million people infected with Strongyloides. Referring to the WHO regions, the highest number of infected people live in the South-East Asia Region (SEAR) estimated at 237.3 (95% CI: 129.9–353.3) million, followed by Western Pacific Region (WPR) with 133.2 (95% CI: 68.1–198.4) million, and African Region (AFR) with 108.1 (95% CI: 55.1–160.9) million; combined this represents 76.1% of the total infected population worldwide (Table 1).

At the regional level, high *S. stercoralis* prevalence was estimated for countries in AFR (10.3%; 95% CI: 5.3–15.3%), for the Americas Region (AMR) (6.9%; 95% CI: 3.5–10.2%), and SEAR (12.1%; 95% C.I.: 6.1–17.9%). In the AMR, countries in Central America and the northern part of South America had the highest prevalence (Figure 3), particularly Panama (15.7%; 95% CI: 8–23.4%), Costa Rica (15.7%; 95% CI: 8–23.4%), and Colombia (18.4%; 95% CI: 9.4–27.4%). S. stercoralis prevalence in AFR was estimated to be high in sub-Saharan countries, with prevalence in West Africa higher compared with the rest of the AFR countries and Sierra Leone (17%; 95% CI: 8.7–25.3%), Liberia (16%; 95% CI: 8.4–24.6%), and Sao Tome and Principe (20.7%; 95% CI: 10.6–30.8%) having particularly high estimated prevalence. All countries in the SEAR had high levels of strongyloidiasis prevalence, with the highest prevalence estimated for Myanmar (19.2%; 95% CI: 9.8–28.6%). Low prevalence of infection (<0.1%) was estimated for high-income countries in temperate zones, with countries at the northern latitudes having the lowest prevalence.

Discussion

Different to the previous review on the global prevalence [9], here we provide new estimates of *S. stercoralis* prevalence at a global level. Our results suggest that, similar to other STHs, a large number of people are infected by *S. stercoralis* worldwide and they are mostly distributed in sub-Saharan Africa, Latin America and East Asia [10]. The global prevalence resulting from our modeling is ten times higher than previous estimates, ranging between 30 to 100 million people [1]. Although these estimates are regularly cited by articles on *S. stercoralis*, we were unable to find any evidence base for these estimates as well as for previous (assessing the prevalence at 3 to 30 million cases [1,11]) (under)estimation. This may be the main reason why *S. stercoralis* infection has lagged behind other STHs in being addressed in countries' STH control programming. Recently, a systematic review and meta-analysis [12] on schistosomiasis and *S. stercoralis* prevalence in migrants from endemic countries to non-/low-endemic countries (defined as the United States, Canada, Australia, New Zealand, Western Europe, and Israel) reported similar prevalence figures to ours for the migrants' main geographic areas of origin.

S. stercoralis infection affects an important proportion of the world population and this calls for action. Not surprisingly, almost all cases of the severe, usually fatal form of the disease are reported, precisely, in non-endemic countries, with very few cases reported from the highly-endemic geographic areas [13]. This means that most of the deaths caused by this parasite are simply undetected. Moreover, besides the disseminated disease that is caused by immune suppression in chronically infected patients, the clinical burden of the chronic, uncomplicated *S. stercoralis* infection is still poorly known [6,14], reflecting the paucity of studies and the general lack of funding for research on this parasite.

The number of studies reporting data on the prevalence of *S. stercoralis* infection is still scarce and sparse in time and space. This paucity of data with very few country-level surveys is the main limitation in our study. Furthermore, many studies included in our review still relied on diagnostic tests with a low sensitivity which would have resulted in an underestimation of the prevalence. Adjustment for test accuracy and a robust model that could fill the gap in data in many areas were necessary. Because we did not have enough data to have time series of testing results from the same location for a long period, we were not able to account in our model for the effect of MDA based on IVM targeting other parasites.

A consensus should be reached on priority research areas that could support operational *S. stercoralis* prevention and control programming. If no action is taken a preventable disease will keep on taking thousands if not hundreds of thousands of lives. Indeed, because *S. stercoralis* causes a long-lasting infection, the proportion of infections in adults is higher than in children. While IVM is effective against *S. stercoralis* infection, a mass drug administration program only for *S. stercoralis* would be difficult to implement due to cost constraints. Ad hoc cost–benefit analyses might help to identify the best strategies to tackle *S. stercoralis* operationally, either on its own or in combination/integrated with other worm infections.

At least in a preliminary phase, prior to operational activities being implemented based on prevalence data from studies conducted one or two decades ago, it would be important to conduct surveys to estimate the prevalence of *S. stercoralis* in specific (e.g., high burden) areas and confirm modeling outputs presented here. There is an urgent need for guidelines indicating the optimal diagnostic methods for such surveys to allow for homogeneous and reliable estimates of prevalence.

Materials and Methods

Prevalence data was modeled based on data retrieved from a literature review and a number of sources that provide data on key predictors of *S. stercoralis* prevalence. The review of the literature was performed in May 2017 in three databases (PubMed, WHOLIS, ISI Web of Science), using the MeSH terms "Strongyloides" and "Strongyloidiasis", with no date of publication or language restrictions. The methods for the literature search are described in Supplementary File 2.

Country population data were obtained from the World Bank website [15], including total population, percentage of the population living in rural areas, and the fraction of the population by age, up to age 14 and older than 14 years. Additional data on the gross domestic product (GDP) per capita and percentage of GDP spent on health (for all sectors and the public sector) were collected from the World Bank website. Strongyloidiasis prevalence is also linked with the level of sanitation in a country [16]. To include this information in our analyses, we accessed the percentage of the population with access to a proper latrine from the UNICEF website [17].

Environmental factors also affect strongyloidiasis prevalence [18]. To account for environmental characteristics of each country, we included in our analyses the terrain ruggedness index [19] and land use characteristics (i.e., the percentage of the total country area that is desert, agricultural, and forest). These data were collected from the FAO website and Nunn and Puga [20]. *S. stercoralis* larvae living in soil have a high chance of surviving in humid and warm weather [21]. To account for country climate suitability for strongyloidiasis prevalence in our analyses, we included country data on annual average temperature and total annual rainfall from the World Bank website.

All tabular data were imported into a database based on SQLite [22]. Geographical data were processed using QGIS [23], and statistical analyses performed using the R language [24] through the RStudio interface [25]. All the analyses were performed using free and open-source software installed on a Linux Mint 18 platform.

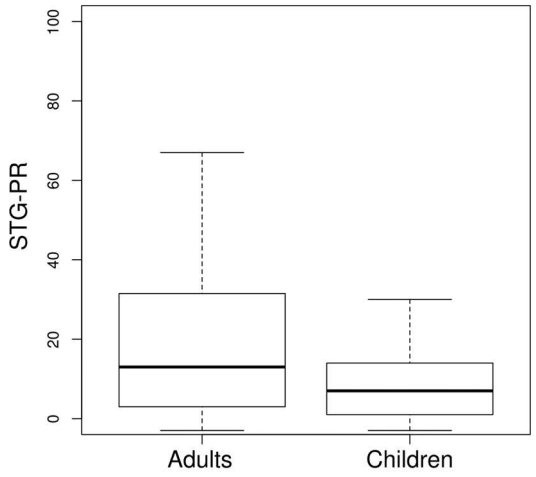
Estimation of Country Prevalence

To estimate the final country prevalence of strongyloidiasis, we had to adjust the data reported in each survey to the accuracy of the diagnostic test used and the age of tested individuals. The reported strongyloidiasis prevalence extracted through the literature review was adjusted using the specificity and sensitivity of each diagnostic test, reported by studies comparing the different diagnostic tests [25] and systematic reviews [3,4,26]. In particular, the following ranges of sensitivity were considered: direct stool examination/Kato–Katz: 5–21% [26]; formol ethyl-acetate concentration technique (FECT): 9–48% [4, 26]; Baermann method and APC: 45–89% [26]; PCR: 62–72%[4]; IFAT: 81–98% [25]; ELISA crude antigen: 73–100 [25]; NIE-ELISA: 71–84% [25]; LIPS: 84–97% [25]. We performed the accuracy adjustment using the methods described in the scientific literature [27]. This correction used the direct relation between prevalence, sensitivity and specificity (details in Supplementary File 3).

For each country, a final weighted mean of the adjusted prevalence by the sample size of each study was computed. After adjusting by test accuracy, we needed to calculate the all-age strongyloidiasis prevalence at the country level. Many studies did not sample all age groups but focused on particular ones. In order to estimate strongyloidiasis prevalence for the entire population of countries without an all-age prevalence, we separately estimated prevalence for children and adults. We considered two age groups: children < 15 years of age and individuals ≥ 15 years of age. We compared the infection prevalence of these two age groups (Figure 4) to compute the groups' prevalence ratio. The ratio was obtained by calculating the mean of the ratio of the prevalence of the two age groups using those studies in which both age groups were sampled. The ratio was used as an adjusting factor to obtain the unknown strongyloidiasis prevalence of one of the age groups based on the known prevalence of the other age group. The prevalence of the two age

groups was used to calculate the strongyloidiasis prevalence of the entire country's population. The calculation took into consideration the proportion of the population belonging to the two age groups.

Figure 4. Boxplot of strongyloidiasis prevalence (STG-PR) in adults (≥15 years of age) and children. The prevalence showed in the graph was adjusted by test accuracy.



Statistical Methods

A model approach was implemented to calculate the strongyloidiasis prevalence at the worldwide level for the year 2017. A generalized linear mixed model (GLMM) was used to investigate the relationship of strongyloidiasis prevalence with economic and environmental factors [28]. The model was built using variables that could have an effect on strongyloidiasis prevalence levels in a country:

$$\text{STG-PR} = \text{GDP} + \text{GDPHealth} + \text{EDU} + \text{RURAL} + \text{CROP} + \text{FOREST} + \text{RUG} + \text{SANIT} + \text{TEMP} + \text{RAIN} + \text{REGION}_{\text{RND}}$$

where GDP is per capita gross domestic product, GDPHealth is the percentage of GDP allocated to health expenditure, EDU is the percentage of the population who attended primary education, RURAL is the percentage of the population living in a rural setting, CROP is the percentage of the country's land allocated for agriculture, FOREST is the percentage of the country's land covered by forest, RUG is the ruggedness index of the country, SANIT is the percentage of the population with access to a proper latrine, TEMP is the mean annual temperature, RAIN is the total annual rainfall, and $\text{REGION}_{\text{RND}}$ is the region as a random effect.

To determine the most important variable associated with strongyloidiasis prevalence, a model selection approach was applied to identify which variable had the highest ability to predict strongyloidiasis prevalence. A set of possible models were created, starting from the full model formula and varying the included variables (Table S2). Model selection was performed using the Akaike Information Criteria (AIC) of each model. The model with the lowest AIC was identified as the best one and was used to predict strongyloidiasis prevalence for the year 2017 [29].

Conclusions

Global prevalence of *S. stercoralis* is probably higher than previously thought. Ad hoc surveys should be carried out in areas where prevalence is estimated as high, prior to designing operational programs to control *S. stercoralis*.

References

1. Bethony, J.; Brooker, S.; Albonico, M.; Geiger, S.M.; Loukas, A.; Diemert, D.J.; Hotez, P.J. Soil-transmitted helminth infections: Ascariasis, trichuriasis, and hookworm. *Lancet* **2006**, *367*, 1521–1532.
2. Bisoffi, Z.; Buonfrate, D.; Montresor, A.; Requena-Méndez, A.; Muñoz, J.; Krolewiecki, A.J.; Gotuzzo, E.; Mena, M.A.; Chiodini, P.; Anselmi, M.; et al. *Strongyloides stercoralis*: A plea for action. *PLoS Negl. Trop. Dis.* **2013**, *7*, e2214.
3. Buonfrate, D.; Formenti, F.; Perandin, F.; Bisoffi, Z. Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. *Clin. Microbiol. Infect.* **2015**, *21*, 543–552.
4. Buonfrate, D.; Requena-Méndez, A.; Angheben, A.; Cinquini, M.; Cruciani, M.; Fittipaldo, A.V.; Giorli, G.; Gobbi, F.; Piubelli, C.; Bisoffi, Z. Accuracy of molecular biology techniques for the diagnosis of *Strongyloides stercoralis* infection—A systematic review and meta-analysis. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006229.
5. Fleitas, P.; Travacio, M.; Martí-Soler, H.; Socías, M.E.; Lopez, W.R.; Krolewiecki, A.J. The *Strongyloides stercoralis*-hookworms association as a path to the estimation of the global burden of strongyloidiasis: A systematic review. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0008184.
6. Tamarozzi, F.; Martello, E.; Giorli, G.; Fittipaldo, A.V.; Staffolani, S.; Montresor, A.; Bisoffi, Z.; Buonfrate, D. Morbidity associated with *Strongyloides stercoralis* infection: A systematic review and meta-analysis. *Am. J. Trop. Med. Hyg.* **2019**, *100*, 1305–1311.
7. Nutman, T.B. Human infection with *Strongyloides stercoralis* and other related *Strongyloides* species. *Parasitology* **2017**, *144*, 263–273.
8. World Health Organization. WHO Model List of Essential Medicines. 2019. Available online: https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf?ua=1 (accessed on 12 June 2020).
9. Schär, F.; Trostdorf, U.; Giardina, F.; Khieu, V.; Muth, S.; Marti, H.; Vounatsou, P.; Odermatt, P. *Strongyloides stercoralis*: Global Distribution and Risk Factors. *PLoS Negl. Trop. Dis.* **2013**, *7*, e2288.
10. World Health Organization. Soil-transmitted helminth infections. Available online: https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections (accessed on 27 May 2020).
11. Genta, R.M. Global prevalence of strongyloidiasis: Critical review with epidemiologic insights into the prevention of disseminated disease. *Rev. Infect. Dis.* **1989**, *11*, 755–767.
12. Asundi, A.; Beliavsky, A.; Liu, X.J.; Akaberi, A.; Schwarzer, G.; Bisoffi, Z.; Requena-Méndez, A.; Shrier, I.; Greenaway, C. Prevalence of strongyloidiasis and schistosomiasis among migrants: A systematic review and meta-analysis. *Lancet Glob. Health* **2019**, *7*, e236–e248.
13. Buonfrate, D.; Requena-Méndez, A.; Angheben, A.; Muñoz, J.; Gobbi, F.; Ende, J.V.D.; Bisoffi, Z. Severe strongyloidiasis: a systematic review of case reports. *BMC Infect. Dis.* **2013**, *13*, 78.
14. Forrer, A.; Khieu, V.; Schaer, F.; Hattendorf, J.; Marti, H.; Neumayr, A.; Char, M.C.; Hatz, C.; Muth, S.; Odermatt, P. *Strongyloides stercoralis* is associated with significant morbidity in rural Cambodia, including stunting in children. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005685.
15. Brasitus, T.A. Parasites ana malabsorption. *Am. J. Med.* **1979**, *67*, 1058–1065.
16. Khieu, V.; Schaer, F.; Marti, H.; Sayasone, S.; Duong, S.; Muth, S.; Odermatt, P. Diagnosis, treatment and risk factors of *Strongyloides stercoralis* in schoolchildren in Cambodia. *PLoS Negl. Trop. Dis.* **2013**, *7*, e2035.
17. UNICEF. UNICEF Data. Available online: https://data.unicef.org/ (accessed on 11 July 2019).
18. Khieu, V.; Schaer, F.; Forrer, A.; Hattendorf, J.; Marti, H.; Duong, S.; Vounatsou, P.; Muth, S.; Odermatt, P. High prevalence and spatial distribution of *Strongyloides stercoralis* in rural Cambodia. *PLoS Negl. Trop. Dis.* **2014**, *8*, e2854.
19. Riley, S.J.; DeGloria, S.D.; Elliot, R. Index that quantifies topographic heterogeneity. *Intermt. J. Sci.* **1999**, *5*, 23–27.
20. Nunn, N.; Puga, D. Ruggedness: The blessing of bad geography in Africa. *Rev. Econ. Stat.* **2012**, *94*, 20–36.
21. Schär, F.; Giardina, F.; Khieu, V.; Muth, S.; Vounatsou, P.; Marti, H.; Odermatt, P. Occurrence of and risk factors for *Strongyloides stercoralis* infection in South-East Asia. *Acta Trop.* **2016**, *159*, 227–238.
22. Consortium, S. SQLite. Available online: https://www.sqlite.org/consortium.html (accessed on 11 July 2019).
23. Caraballo, L.; Coronado, S. Parasite allergens. *Mol. Immunol.* **2018**, *100*, 113–119.
24. R Team, R.D.C. The R Foundation. Available online: https://www.r-project.org/foundation/ (accessed on 11 July 2019).
25. Bisoffi, Z.; Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Gobbo, M.; Bonafini, S.; Angheben, A.; et al. Diagnostic accuracy of five serologic tests for *Strongyloides stercoralis* infection. *PLoS Negl. Trop. Dis.* **2014**, *8*, e2640.
26. Campo, L.P.; Gutiérrez, L.A.; Cardona, J.A. Diagnosis of *Strongyloides stercoralis* infection: meta-analysis on evaluation of conventional parasitological methods (1980–2013). *Rev. Esp. Salud Publica* **2014**, *88*, 581–600.
27. Blaker, H. Confidence curves and improved exact confidence intervals for discrete distribution. *Can. J. Stat.* **2000**, *29*, 681.

Faraway, J. Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models; Chapman & Hall/CRC: Boca Raton, Florida, USA 2005.

29. Burnham, K.; Anderson, D. Model Selection and multi-model inference A practical information-theoretic approach; Springer: Fort Collins, Colorado, USA 2013.

2.2 Epidemiology of *Strongyloides stercoralis* infection in northern Italy: results of a multicentre case–control study, February 2013 to July 2014

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Abstract

Strongyloides stercoralis is a soil-transmitted helminth widely diffused in tropical and subtropical regions of the world. Autochthonous cases have been also diagnosed sporadically in areas of temperate climate. We aimed at defining the epidemiology of strongyloidiasis in immigrants and Italians living in three northern Italian Regions. Screening for *S. stercoralis* infection was done with serology, confirmation tests were a second serological method or stool agar culture. A case–control approach was adopted and patients with a peripheral eosinophil count ≥ 500/mcL were classified as cases. Of 2,701 individuals enrolled here 1,351 were cases and 1,350 controls; 86% were Italians, 48% women. Italians testing positive were in 8% (97/1,137) cases and 1% (13/1,178) controls (adjusted odds ratio (aOR) 8.2; 95% confidence interval (CI): 4.5–14.8), while positive immigrants were in 17% (36/214) cases and in 2% (3/172) controls (aOR 9.6; 95% CI: 2.9–32.4). Factors associated with a higher risk of infection for all study participants were eosinophilia ($p < 0.001$) and immigration ($p = 0.001$). Overall, strongyloidiasis was nine-times more frequent in individuals with eosinophilia than in those with normal eosinophil count.

Introduction

Strongyloides stercoralis is a soil-transmitted helminth affecting millions of people worldwide [1,2]. Its trans- mission occurs in areas where poor hygienic conditions and humid, warm climate permit the free-living cycle of the parasite. The larvae present in the soil can penetrate human skin, therefore barefoot walking and agricultural activities pose people at risk of acquiring the infection. *S. stercoralis* produces larvae that can reinfect the host by a so-called auto-infective cycle, a peculiarity shared only by *Capillaria* spp [3], so that an infected person remains infected life-long, if not properly treated [4]. This is the reason why strongyloidiasis can be diagnosed in people who have left endemic countries already several years before.

The few studies conducted in the United States (US) and in Europe to evaluate the prevalence of strongyloidiasis in immigrants and refugees from endemic countries, either through population or hospital-based studies, probably underestimated the real burden of the infection as long as microscopic stool examination was the only test used for screening [5]. In fact, the methods commonly employed for stool micros- copy such as formalin-ether concentration, have a low sensitivity. Preferred faecal-based methods for the detection of *S. stercoralis* are Baermann funnel concentration and agar plate culture (APC), but the method that has so far demonstrated the highest sensitivity is serology [6]. Studies conducted in the field, classically underestimate the burden of strongyloidiasis if there is no special focus on this infection i.e. through using an appropriate diagnostic method. This is why the 'old' estimates of prevalence from the late 1980s and 1990s [7,8] were recently questioned [1,2].

The transmission of strongyloidiasis occurs especially in tropical and subtropical areas. However, in some temperate countries, autochthonous transmission occurred in the past [9,10], or might be still ongoing [11,12]. Therefore, cases of *S. stercoralis* infection can be diagnosed in people who have never moved from the Mediterranean coast.

Strongyloidiasis can be fatal in immunocompromised patients so prompt diagnosis and effective treatment are crucial for all those infected, in order to prevent later complications, such as disseminated strongyloidiasis [4]. Chronic infection is characterised by mild, unspecific symptoms such as pruritus, abdominal pain or discomfort, respiratory impairment which are not easily attributable to *S. stercoralis* and there is no full agreement among experts on considering eosinophilia as a predictor of the infection [13]. However, in non- endemic countries a high eosinophil count might be a sufficient index of suspicion in travellers or in patients over 65 years with history of barefoot walking in a formerly endemic area [14,15].

The treatment of choice for strongyloidiasis is ivermectin that has demonstrated a higher efficacy than albendazole [16]. Although the drug is included in the World Health Organization (WHO) list of essential medicines [17], it is not accessible for the vast majority of infected people in the world [18,19]. In fact, this essential drug is still donated to endemic countries, but with the strict limitation of use for *Wuchereria bancrofti* and *Onchocerca volvulus* control programmes [20]. In Italy, ivermectin has never been registered for human use.

In a previous pilot study, we screened 132 Italian individuals born in 1940 or earlier, with eosinophilia and no significant travel history, presenting to the clinical laboratories of two health districts. The serology test, an in-house immunofluorescence antibody test (IFAT), was positive in 28% of cases, suggesting that strongyloidiasis can be a relevant cause of eosinophilia in this group of individuals [9].

In the present study, we extended the previous screening in order to estimate the prevalence of strongyloidiasis in six provinces of three Italian Regions. The population analysed included both adult immigrants and Italians born before 1952, with or with- out eosinophilia.

Study design and setting

We conducted a multicentre case–control study.

Participants were enrolled between 2 February 2013 and 27 July 2014. The enrolling sites were the outpa- tient blood sampling sectors of seven hospitals located in three Italian Regions: Veneto (Negrar, San Bonifacio, and Treviso sites), Lombardia (Brescia, Mantova sites), and Friuli Venezia Giulia

(Trieste, Udine sites) (Figure 1). The Centre for Tropical Diseases of Negrar (CTD) and the Health Prevention Department, Verona, were the coordinating centres.

Figure 1. Map of northern Italy showing where participating sites are situated, study of *Strongyloides stercoralis* epidemiology, northern Italy, February 2013–July 2014

On 1 January 2013, according to the Italian National Institute of Statistics [21], the total resident population in the six provinces of the three Regions involved in the study was 4,215,423 people (3,742,724 Italian and 472,699 foreign residents). With regard to Italian residents, 1,074,367 (28.7%) were > 60 years (born before 1952), which was the age cut off for inclusion of Italians in the present study. As for immigrants, 351,347 (74.3%) were > 17 years old, which was the age criterion for their inclusion in the present study.

Participants

Investigators proposed the screening to individuals meeting the inclusion criteria and consecutively presenting as outpatients to perform a full blood count to one of the collaborating laboratories, during 20 randomly-selected weeks. For the study purpose, we adopted the following definitions:

- Cases: individuals with peripheral eosinophil count ≥ 500/mcL;
- Controls: individuals with eosinophil count < 500/ mcL;
- Italians: individuals born and resident in Italy;
- Immigrants: individuals who were born in an endemic area and resided there for at least the first two years of life, and without Italian citizenship.

Each selected week, every centre had to recruit 10 cases and 10 controls. Inclusion criteria were: Italians born before 1952 (as in CTD experience with hundreds of patients the infection was extremely rare in younger Italian individuals with no travel history), immigrants aged ≥ 18 years. Each participant gave informed writ- ten consent. Individuals included in the study received a copy of the result of the

test(s) performed and, in case of positive or uncertain result, a treatment with ivermectin (200 μg/kg, stat dose) was offered. A case report form (CRF) with essential clinical data was filled for those with positive test results.

Laboratory methods

Screening for *S. stercoralis* infection was performed with a commercial ELISA test, IVD Research, CA, USA ELISA (IVD ELISA) during 2013, then Bordier ELISA until the end of the study period, due to unavailability of the former test; positive samples were tested with an in-house IFAT [22]. Discordant samples were analysed with Bordier ELISA during 2013, until when IVD ELISA was used as the screening test. Subsequently, due to unavailability of the latter test, Bordier ELISA was used as the screening test, and a third testing for discord- ant samples was no longer possible. The three tests have been described in detail elsewhere [23]. Patients testing positive in the screening tests were invited to supply a faecal sample for Koga agar plate culture for *S. stercoralis* [24] and/or for copro-parasitological test (formalin-ether concentration). For the study purpose, patients were defined as 'positive' in case of two concordant positive serologic tests and/or a positive screening test AND a positive APC / copro-parasitological test. Individuals with only one positive screening test and negative stool test were classified as 'uncertain' in case a third serologic test was unavailable.

Study size

There is scarce data on the prevalence of strongyloidiasis in Italy. Previous, smaller studies, found a prevalence between 10 and 15% in Italians with eosinophilia aged >60 and >68 years, respectively, and around 4% in controls of the same age group with normal eosinophil count [9,14]. On the basis of these surveys, the study size was calculated considering an odds ratio (OR) for suspected/confirmed strongyloidiasis in cases vs controls of 3, a case/control ratio of 1:1, a prevalence of strongyloidiasis in the control group of 3%, a confidence level at 95%, a study power of 80% and a design effect of 1.5. Eventually, a total of 950 Italians was to be tested, 475 cases and controls, respectively. Therefore, we initially established to enroll at least 500 individuals per group (1,000 Italian individuals in total).

The literature demonstrates a high variability in the prevalence of strongyloidiasis in immigrants, depending on their country of origin and on the screening method used [5]. Studies based on serology demonstrated a prevalence between 10 and 36%, irrespective of the eosinophil count. To calculate the study size we assumed an OR (for strongyloidiasis in cases vs controls) of 3 and a study power 80%. Based on these data, the minimum number of immigrants to be tested was 185 for each group, resulting in a total number of 370. Therefore, we attempted to enroll 200 individuals per group, 400 immigrants in total.

Overall, the minimal sample size required was of 1,400 individuals. As the sample size calculation was based on very weak estimates, particularly for Italian individuals for whom no formal, previous prevalence study was available, the proposed target sample was twice as large i.e. 2,800 individuals, 200 cases and 200 controls to be recruited by each study site.

Statistical methods

Data at each centre were entered in a pre-structured

Excel file and analysed using Stata 10 software (Stata Corp., Texas, US). For quantitative variables, data distribution was checked for normality by Shapiro-Francia test.

Since data were not normally distributed, they were analysed using the non-parametric Mann–Whitney test and the variations among groups were calculated as medians with interquartile ranges (IQR). Associations among categorical variables were analysed by Pearson's chi-squared test or Fisher's exact test as appropriate, and presented as observed frequencies and proportions. Trend analysis

was performed by chi-squared test for linear trend. The OR of finding the outcome of interest (i.e. *S. stercoralis* infection) in relationship to the eosinophil count (defining cases and controls) and to other variables of interest (sex, age, recruitment site, geographical area of origin) were calculated by logistic regression. For all tests, the level chosen to indicate statistical significance was $p < 0.05$ (two-tailed).

Ethical issues

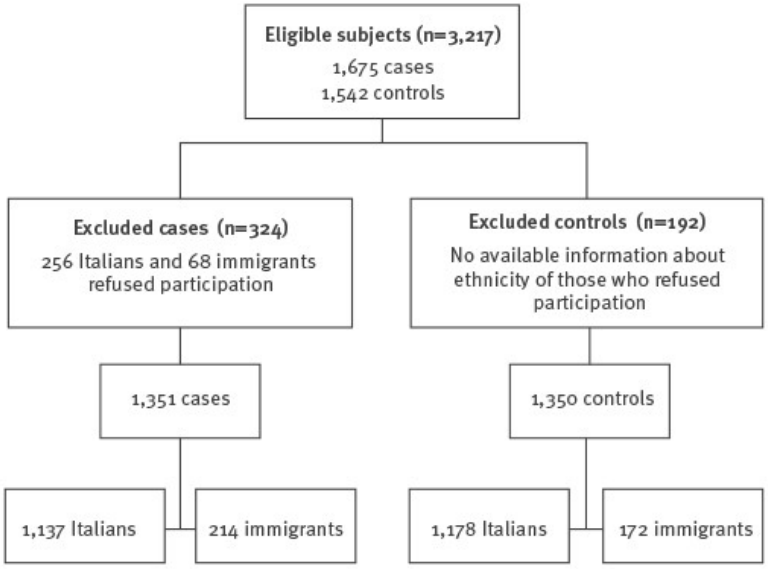
The Ethics Committee of the coordinating centres (Comitato Etico della Provincia di Verona) approved the study protocol on 17 January 2012. The study protocol was then submitted to the Ethics Committees of each of the study sites, and formally approved. All study participants received an information sheet and a letter for their general practitioner, explaining aim and methods of the study; signed informed consent form was required.

Results

Participants

A total of 3,217 individuals fulfilled the inclusion criteria; 516 were not included in the study because they were unable to give informed consent or refused to participate. The total number of individuals included in the study and analysed was 2,701 (Figure 2).

Figure 2. Flowchart for inclusion of participants



The study population comprised 1,392 men (52%) and 1,309 (48%) women. Each participating centre recruited ca 400 individuals.

Among 2,315 Italians, the proportion of women was 41% (n=464) for the 1,137 cases and 53% (n=625) for the 1,178 total controls. Median age was 73 years (range: 61–99; IQR: 67–78) and 72 years (range: 61–94; IQR: 67–77) for cases and controls, respectively. Median value of eosinophil count was 630/mcL (range: 500–24,890; IQR: 550–790) and 150/mcL (range: 0–490; IQR: 100–220) for cases and controls, respectively.

Among 386 immigrants, women represented 48% (n=103) of the 214 cases, and the proportion was higher for the 172 controls, 68% (n=117). Median age was 38 years (range: 18–87; IQR: 30–48) and 40 years (range: 18–83; IQR: 29–53) for cases and controls, respectively. Median value of eosinophil count was 655/mcL (range: 500–2,380; IQR: 570–830) and 145/mcL (range: 0–460; IQR: 70–240) for the cases and the controls, respectively.

Immigrants originated from Europe, especially eastern Europe and the Balkans (26%, n=101), Asia (22%,

n=83), Sub-Saharan Africa (21%, n=82), North Africa and Middle East (18%, n=68), and Latin America (13%, n=52).

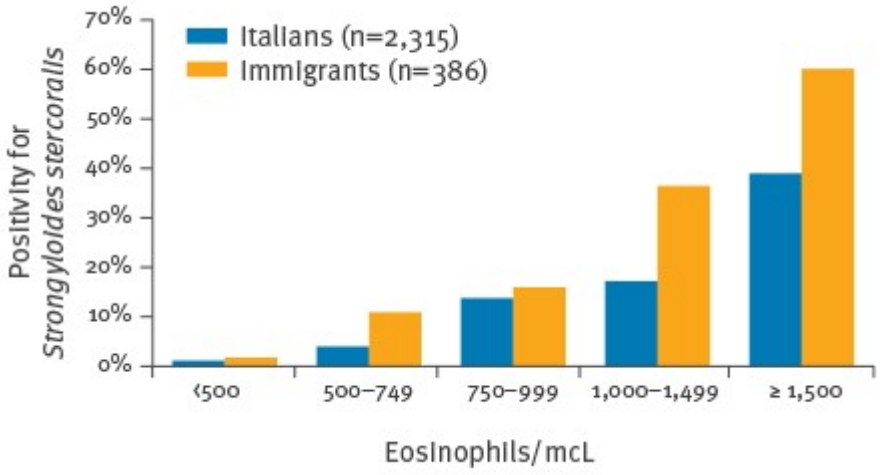
Prevalence in cases and controls

Overall, of 2,701 participating individuals, 149 (5%) were classified as positive (110 Italians and 39 immigrants) and 32 (1%) as uncertain (29 Italians and 3 immigrants).

Among Italians with eosinophilia (cases) 8% (97/1,137) were positive vs 1% (13/1,178) without eosinophilia. Considering a total population of the same age group of 1,074,367 in the six provinces, and an average of 4% of subjects of the same age with eosinophilia (data not shown), we obtain a rough estimate of 4,000 Italians over 60 years of age with *S. stercoralis* infection.

Among immigrants, positive cases were 17% (36/214) vs 2% positive controls (3/172), respectively. The proportion of positives was significantly higher among cases, both for Italians ($p < 0.001$) and immigrants ($p < 0.001$). Moreover, the higher the eosinophil count, the higher was the proportion of infected individuals, in both groups. Among Italians, the proportion of positive individuals ranged from 4% (31/780) for those with eosinophil counts between 500 and 749/mcL, to 39% (21/54) for those with eosinophil counts ≥ 1,500/mcL ($p < 0.001$) (Figure 3).

Figure 3. Percentage of positivity for *Strongyloides stercoralis* in relation to eosinophil count in Italians and in immigrants



Among immigrants, this proportion ranged from 11% (15/138) for those with eosinophils between 500 and 749/mcL, to 60% (6/10) for those with eosinophils ≥ 1,500/mcL ($p < 0.001$), albeit numbers were small in the latter group. Moreover, among the Italian cases, the proportion of positive individuals showed an upward trend with increasing age ($p < 0.001$) and varied depending on the study site ($p = 0.01$), with a peak in individuals born before 1936 (46/380; 12%) and in those recruited in the sites located in agricultural regions of the Po valley (e.g. San Bonifacio site: 19/146; 13%).

Immigrant cases had the following distribution, according to the region of origin of the patients: Latin America (6/31), Sub-Saharan Africa (14/48), Asia (11/55), Europe (2/44), and North Africa (3/36).

Some of the individuals who were positive in the screening test refused to provide a stool sample, therefore the results of stool tests were available only for 70% (104/149) of patients with positive serology of which 28% (n=29/104) had a positive stool result. Ninety-nine of 149 positive patients (66%), plus seven individuals with uncertain result, received ivermectin treatment, offered free of charge to all eligible patients. Information about possible risk factors for complicated strongyloidiasis was available for 83% (124/149) positive individuals: 16% (20/124) presented a current or past condition considered to constitute a risk for the development of severe strongyloidiasis. In the latter group, most (17/20) were treated, while two individuals refused and one died of metastatic breast cancer soon after being tested.

Analysis on the subgroup of 54 of 149 positive individuals who answered the questionnaire showed that the majority had signs and symptoms compatible with strongyloidiasis (Table 1) and had been exposed to a risk factor for infection (farm work 32/54; walking barefoot in earlier years 37/54). Only two of 43 responding Italians reported a stay longer than one month in endemic countries, where they might have had contact with contaminated soil. The remaining Italians did not present a relevant travel history, so we assume that the infection was probably acquired in Italy.

Table 1. Signs and symptoms compatible with strongyloidiasis in individuals testing positive who answered a questionnaire

Signs and symptoms	**Number of Italians (%) n = 43**	**Number of immigrants (%) n = 11**	**Total number (%) n = 54**
Pruritus	23 (53.5)	4 (36.4)	27 (50)
Skin rash	13 (30.2)	2 (18.2)	15 (27.8)
Respiratory symptoms	16 (37.2)	3 (27.3)	19 (35.2)
Abdominal pain	9 (20.9)	1 (9.1)	10 (18.5)
Diarrhoea	1 (2.3)	2 (18.2)	3 (5.6)

By logistic regression, eosinophilia ($p < 0.001$) and immigration ($p = 0.001$) were independent risk factors for infection for all participants. After adjusting for birth cohort, sex and site of recruitment for Italians, or age, sex and geographical area of origin for immigrants, presence of eosinophilia ≥ 500/mcL was significantly associated with infection both in Italians (adjusted OR: 8.18; 95% CI: 4.53–14.76; $p < 0.001$) and in immigrants (aOR: 9.62; 95% CI: 2.85–32.41; $p < 0.001$). Among Italians, year of birth and site of recruitment maintained a significant association with infection also at the multivariate analysis (Table 2); the same occurred among immigrants with regard to area of origin (Table 3).

Table 2. Logistic regression analysis of factors associated with Italians testing positive for *Strongyloides stercoralis*

Factors	**OR**	**95% CI**	**P value**
Eosinophil count ≥ 500/mcL	8.18	4.53–14.76	< 0.001
Sex (male vs female)	0.93	0.63–1.39	0.730
Year of birth			
1947–1951	1.00	Reference	NA.

1937–1946	2.56	1.24–5.28	0.011
1936 or before	3.95	1.90–8.20	< 0.001
Recruitment site			
Trieste	1.00	Reference	NA
Udine	1.34	0.54–3.32	0.52
Negrar	1.67	0.69–4.00	0.25
Mantova	2.33	1.00–5.41	0.050
Brescia	2.47	1.07–5.70	0.033
Treviso	2.97	1.33–6.64	0.008
San Bonifacio	3.43	1.54–7.68	0.003

CI: confidence interval; NA: not applicable; OR: odds ratio.

Table 3. Logistic regression analysis of factors associated with testing positive for *Strongyloides stercoralis* among immigrant individuals

Factors	**OR**	**95% CI**	**P value**
Eosinophil count ≥ 500/mcL	9.62	2.85–32.41	< 0.001
Sex (male vs female)	1.70	0.82–3.54	0.16
Age (+ 1 year)	1.00	0.97–1.03	0.83
Geographical area of origin			
Europe	1.00	Reference	NA
North Africa/Middle East	1.77	0.28–11.27	0.55
Asia	5.01	1.02–24.59	0.047
Latin America	6.33	1.20–33.40	0.030
Sub-Saharan Africa	9.54	2.01–45.19	0.004

Discussion

The high number of screened individuals, especially Italians, in our study, permitted to obtain a valuable estimate of the prevalence of strongyloidiasis in the studied regions in the north of Italy: 8 and 17%, respectively, in Italians and immigrants with eosinophilia, 1 and 2% in those with a normal eosinophil count, irrespective of signs/symptoms of the infection. This finding is relevant for autochthonous Italians, for whom prevalence data were previously limited and patchy, and this study demonstrated a considerable proportion of infected individuals. In addition, 2% of Italian controls without eosinophilia with positive/uncertain test result is worth of note. The findings indicate that the infection is not an extinguished problem among elderly Italians living in the study areas.

The geographical pattern of infection prevalence is consistent with a higher transmission in agricultural areas of Po valley during the first decades of the past century, with a downward trend over time likely due to improvement of hygiene and sanitary conditions. Parts of the country, in the centre and in the south, presented in the past characteristics that make a location suitable for the free-living cycle of *S. stercoralis*. It is thus probable that a similar epidemiological picture might be prevalent in a large part, if not in the whole, of Italy. This could also be true for other countries in the Mediterranean basin, where sporadic autochthonous strongyloidiasis cases have been diagnosed [10,12].

Among immigrants, the proportion of positive individuals was high among cases with eosinophil counts ≥ 500/mcL, particularly if individuals originated from Sub-Saharan Africa, Asia, and Latin America.

Prevalence data are fundamental to implement screening and prevention programmes. We believe our results support the establishment of risk categories for screening individuals at risk of developing strongyloidiasis, such as elderly Italians (and, probably, Europeans from other Mediterranean countries) and immigrants with eosinophilia. In the latter group, it might be even cost-effective to treat all patients without testing [4]**.** This should, however, be demonstrated by a well-designed study, also considering that a pre-treatment diagnostic evaluation (obligatorily including serology) is crucial to monitor cure at follow-up [25]**.**

One in three of the infected individuals refused the treatment that was offered free of charge after a thorough explanation of the risk associated with untreated, chronic infection. Even general practitioners were not always keen to collaborate. Our experience suggests that strongyloidiasis is not always perceived as a relevant health problem, not only by the general population, but also by the medical community. To overcome this problem, it would be advisable to create national guidelines for the screening and management of eosinophilia that should consider strongyloidiasis among the differential diagnoses. Moreover, considering that strongyloidiasis can be fatal in immunocompromised individuals, *S. stercoralis* should be included in guidelines/protocols for screening of candidate patients for immune-suppressant therapies, such as the oncological and rheumatological ones.

Limitations

We faced some difficulties in finding eligible immigrants for inclusion in the study, therefore the number of immigrants enrolled was slightly lower than planned. This was the reason, in addition to that provided in the Methods part, to recruit a higher number of Italians than the initially calculated sample size, as we did not deem it appropriate, to stop the recruitment in this group and continue only with immigrants. We did not include in the analysis the countries of origin as numbers for such analysis were too small and instead we analysed the continents/macro-areas. We still believe the results are useful, considering the paucity of similar data in the literature. Although the included individuals were not randomly extracted from the general population, the enrolment of out-patients, coming to the hospital laboratory to perform a very simple and common test (full blood count), results in a sample that can be comparable to the general population in that age range, in particular for the larger Italian group. The controls were unmatched, but consecutively recruited on a randomly selected day on a 1:1 basis, within the same main group (Italian or immigrant) and age range.

Finally, the accuracy of serology is high, but false-positive and false-negative results can occur [23]. The use of a second, confirmatory serological test in addition to the faecal-based tests, when available, was aimed to increase the specificity of the results. Sensitivity can be lower in immunocompromised individuals [26], however, we believe that this may have had a minimal influence on the overall results, given the high number of individuals screened [19]. The screening test had to be changed, however Bordier ELISA and IVD demonstrated similar accuracy in our previous study [23]. Therefore, we believe that the number of patients positive at screening might not have been substantially different with IVD ELISA. On the other hand, this change entailed the lack of a third serology test, therefore patients with discordant results had to be classified as uncertain. PCR was not available at our Centre before 2014, hence we could not use this method, that showed good accuracy compared with APC and Baermann technique [27,28]. PCR is less cumbersome than the traditional faecal-based methods and the samples can be stored (either frozen or with ethanol), therefore it could have been a useful tool considering the high number of individuals screened.

Conclusions

The improvement of hygienic conditions and sanitation, and the availability of deworming drugs are likely to successfully control most helminth infections in endemic areas in the forthcoming years. However, the lack of mass drug administration programmes specifically targeting *S. stercoralis* (using ivermectin) might lead to long-term persistence of this infection in some individuals. It is also important to note that, due to the peculiarity of the auto-infective cycle of *S. stercoralis*, this parasite may remain once the other helminth infections have disappeared. This has been observed in Italy. Physicians should be aware of the categories of patients that would require screening for *S. stercoralis* infection.

References

1. Schar, F.; Trostdorf, U.; Giardina, F.; Khieu, V.; Muth, S.; Marti, H.; Vounatsou, P.; Odermatt, P. Strongyloides stercoralis: Global Distribution and Risk Factors. *PLoS neglected tropical diseases* **2013**, *7*, e2288, doi:10.1371/journal.pntd.0002288.1.
2. Bisoffi, Z.; Buonfrate, D.; Montresor, A.; Requena-Mendez, A.; Munoz, J.; Krolewiecki, A.J.; Gotuzzo, E.; Mena, M.A.; Chiodini, P.L.; Anselmi, M., et al. Strongyloides stercoralis: a plea for action. *PLoS neglected tropical diseases* **2013**, *7*, e2214, doi:10.1371/journal.pntd.0002214.
3. Dronda, F.; Chaves, F.; Sanz, A.; Lopez-Velez, R. Human intestinal capillariasis in an area of nonendemicity: case report and review. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **1993**, *17*, 909-912.
4. Mejia, R.; Nutman, T.B. Screening, prevention, and treatment for hyperinfection syndrome and disseminated infections caused by Strongyloides stercoralis. *Current opinion in infectious diseases* **2012**, *25*, 458-463, doi:10.1097/QCO.0b013e3283551dbd.
5. Buonfrate, D.; Angheben, A.; Gobbi, F.; Munoz, J.; Requena-Mendez, A.; Gotuzzo, E.; Mena, M.A.; Bisoffi, Z. Imported strongyloidiasis: epidemiology, presentations, and treatment. *Current infectious disease reports* **2012**, *14*, 256-262, doi:10.1007/s11908-012-0248-6.
6. Requena-Mendez, A.; Buonfrate, D.; Bisoffi, Z.; Munoz, J. Advances in the diagnosis of human strongyloidiasis. *Current Tropical Medicine Reports* **2014**, *1*, 207-215.
7. Genta, R.M. Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. *Reviews of infectious diseases* **1989**, *11*, 755-767.
8. Jorgensen, T.; Montresor, A.; Savioli, L. Effectively controlling strongyloidiasis. *Parasitology today (Personal ed.)* **1996**, *12*, 164.
9. Abrescia, F.F.; Falda, A.; Caramaschi, G.; Scalzini, A.; Gobbi, F.; Angheben, A.; Gobbo, M.; Schiavon, R.; Rovere, P.; Bisoffi, Z. Reemergence of strongyloidiasis, northern Italy. *Emerging infectious diseases* **2009**, *15*, 1531-1533, doi:10.3201/eid1509.090191.
10. Martinez-Perez, A.; Lopez-Velez, R. Is strongyloidiasis endemic in Spain? *PLoS neglected tropical diseases* **2015**, *9*, e0003482, doi:10.1371/journal.pntd.0003482.
11. Russell, E.S.; Gray, E.B.; Marshall, R.E.; Davis, S.; Beaudoin, A.; Handali, S.; McAuliffe, I.; Davis, C.; Woodhall, D. Prevalence of Strongyloides stercoralis antibodies among a rural Appalachian population--Kentucky, 2013. *The American journal of tropical medicine and hygiene* **2014**, *91*, 1000-1001, doi:10.4269/ajtmh.14-0310.
12. Duvignaud, A.; Pistone, T.; Malvy, D. Strongyloidiasis in a young French woman raises concern about possible ongoing autochthonous transmission in Spain. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* **2015**, *42*, 43-44, doi:10.1016/j.ijid.2015.11.015.
13. Requena-Mendez, A.; Chiodini, P.; Bisoffi, Z.; Buonfrate, D.; Gotuzzo, E.; Munoz, J. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS neglected tropical diseases* **2013**, *7*, e2002, doi:10.1371/journal.pntd.0002002.
14. Pirisi, M.; Salvador, E.; Bisoffi, Z.; Gobbo, M.; Smirne, C.; Gigli, C.; Minisini, R.; Fortina, G.; Bellomo, G.; Bartoli, E. Unsuspected strongyloidiasis in hospitalised elderly patients with and without eosinophilia. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2006**, *12*, 787-792, doi:10.1111/j.1469-0691.2006.01500.x.
15. Checkley, A.M.; Chiodini, P.L.; Dockrell, D.H.; Bates, I.; Thwaites, G.E.; Booth, H.L.; Brown, M.; Wright, S.G.; Grant, A.D.; Mabey, D.C., et al. Eosinophilia in returning travellers and migrants from the tropics: UK recommendations for investigation and initial management. *The Journal of infection* **2010**, *60*, 1-20, doi:10.1016/j.jinf.2009.11.003.

16. Suputtamongkol, Y.; Premasathian, N.; Bhumimuang, K.; Waywa, D.; Nilganuwong, S.; Karuphong, E.; Anekthananon, T.; Wanachiwanawin, D.; Silpasakorn, S. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. *PLoS neglected tropical diseases* **2011**, *5*, e1044, doi:10.1371/journal.pntd.0001044.
17. WHO. WHO Model List of Essential Medicines. *Available at: http://www.who.int/medicines/publications/essentialmedicines/en/* **2015**.
18. Krolewiecki, A.J.; Lammie, P.; Jacobson, J.; Gabrielli, A.F.; Levecke, B.; Socias, E.; Arias, L.M.; Sosa, N.; Abraham, D.; Cimino, R., et al. A public health response against Strongyloides stercoralis: time to look at soil-transmitted helminthiasis in full. *PLoS neglected tropical diseases* **2013**, *7*, e2165, doi:10.1371/journal.pntd.0002165.
19. Anselmi, M.; Buonfrate, D.; Guevara Espinoza, A.; Prandi, R.; Marquez, M.; Gobbo, M.; Montresor, A.; Albonico, M.; Racines Orbe, M.; Martin Moreira, J., et al. Mass Administration of Ivermectin for the Elimination of Onchocerciasis Significantly Reduced and Maintained Low the Prevalence of Strongyloides stercoralis in Esmeraldas, Ecuador. *PLoS neglected tropical diseases* **2015**, *9*, e0004150, doi:10.1371/journal.pntd.0004150.
20. WHO. Preventive chemotherapy in human helminthiasis. Coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers. *Geneva. World Health Organization* **2006**.
21. www.istat.it. **Accessed January 12th 2016**.
22. Boscolo, M.; Gobbo, M.; Mantovani, W.; Degani, M.; Anselmi, M.; Monteiro, G.B.; Marocco, S.; Angheben, A.; Mistretta, M.; Santacatterina, M., et al. Evaluation of an indirect immunofluorescence assay for strongyloidiasis as a tool for diagnosis and follow-up. *Clinical and vaccine immunology : CVI* **2007**, *14*, 129-133, doi:10.1128/cvi.00278-06.
23. Bisoffi, Z.; Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Gobbo, M.; Bonafini, S.; Angheben, A., et al. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. *PLoS neglected tropical diseases* **2014**, *8*, e2640, doi:10.1371/journal.pntd.0002640.
24. Koga, K.; Kasuya, S.; Khamboonruang, C.; Sukhavat, K.; Ieda, M.; Takatsuka, N.; Kita, K.; Ohtomo, H. A modified agar plate method for detection of Strongyloides stercoralis. *The American journal of tropical medicine and hygiene* **1991**, *45*, 518-521.
25. Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Degani, M.; Tais, S.; Angheben, A.; Requena-Mendez, A., et al. Accuracy of five serologic tests for the follow up of Strongyloides stercoralis infection. *PLoS neglected tropical diseases* **2015**, *9*, e0003491, doi:10.1371/journal.pntd.0003491.
26. Mascarello, M.; Gobbi, F.; Angheben, A.; Gobbo, M.; Gaiera, G.; Pegoraro, M.; Lanzafame, M.; Buonfrate, D.; Concia, E.; Bisoffi, Z. Prevalence of Strongyloides stercoralis infection among HIV-positive immigrants attending two Italian hospitals, from 2000 to 2009. *Annals of tropical medicine and parasitology* **2011**, *105*, 617-623, doi:10.1179/2047773211y.0000000006.
27. Becker, S.L.; Piraisoody, N.; Kramme, S.; Marti, H.; Silue, K.D.; Panning, M.; Nickel, B.; Kern, W.V.; Herrmann, M.; Hatz, C.F., et al. 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 tropica* **2015**, *150*, 210-217, doi:10.1016/j.actatropica.2015.07.019.
28. Saugar, J.M.; Merino, F.J.; Martin-Rabadan, P.; Fernandez-Soto, P.; Ortega, S.; Garate, T.; Rodriguez, E. Application of real-time PCR for the detection of Strongyloides spp. in clinical samples in a reference center in Spain. *Acta tropica* **2015**, *142*, 20-25, doi:10.1016/j.actatropica.2014.10.020.

Chapter 3 Clinical and laboratory features of *Strongyloides stercoralis* infection at diagnosis and after treatment: a systematic review and meta-analysis

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Abstract

Background. The clinical and laboratory characterization of *Strongyloides stercoralis* infection at diagnosis and after treatment is still poorly defined.
Objectives. Primary objective was to describe pattern and frequency of clinical and laboratory characteristics associated to *S. stercoralis* infection. Secondary objectives: 1) Comparison of characteristics reported in endemic *versus* non-endemic areas; 2) Evaluation of the resolution of identified characteristics after treatment.
Methods. We searched PubMed, EMBASE, LILACS and CENTRAL up to May 2021.
Eligible studies were randomized controlled trial (RCT) for the treatment of *S. stercoralis* infection and prospective observational studies reporting data on symptoms caused by strongyloidiasis. in individuals diagnosed with a highly specific test. Quality assessment was performed to assess the risk of bias. Demographic and clinical data were summarized using descriptive statistics. Meta-analysis was done by pooling the proportion of participants with symptoms with random effects model.
Results. Twenty studies were included: nine RCTs and 13 observational studies. Overall, symptoms were reported in 50.4% cases (95% CI 47.6 - 53.1), and were more often reported in non-endemic (58.6%, 95% CI 55.0-62.2) than in endemic (35.7%, 95% CI 31.4-39.9) areas. The removal of a paper of lower quality did not impact on figures. Frequency of symptoms tended to reduce after treatment. Three studies reported the proportion of participants with eosinophilia before and after treatment: 76.9% of participants (95% CI 73.4-80.4) had eosinophilia at diagnosis, reducing to 27.4% (95% CI 24.0-30.7) after treatment.
Conclusions. About half of infected people complain at least of one symptom and almost 70% have eosinophilia. The frequency of symptoms and eosinophilia decreased after treatment, though the association with cure is not clearly defined. Providing relief from symptoms and eosinophilia is another reason, in addition to prevention of disseminated disease, for promoting screening and treatment of individuals with strongyloidiasis.

Introduction

Strongyloides stercoralis infection is a neglected tropical disease infecting hundreds of millions of people in disadvantaged areas of the world {Buonfrate, 2020; Krolewiecki, 2013}. Transmitted through the contact with soil contaminated by infective larvae, the infection perpetuates indefinitely in humans, due to a peculiar auto-infective cycle. The infection is known to cause potentially a fatal syndrome in immunosuppressed individuals. However, there are still some grey areas in the definition of the clinical presentation and burden associated to chronic infection {Nutman, 2017}. A systematic literature review of prospective studies {Tamarozzi, 2019} found an association between strongyloidiasis and urticaria, abdominal pain and diarrhea in affected populations, but highlighted also the need for more studies carried out with adequate design and diagnostic methods. Indeed, the use of inappropriate diagnostic tests often represents a major issue for a correct classification of cases {Nutman, 2017}. In the field, the diagnostic tests used for surveys on soil-transmitted helminths (STH) are usually stool-based microscopic methods with a very low sensitivity for *S. stercoralis* (such as Kato-Katz, microscopic examination of formol-ether concentrated feces), and this results in an underestimation of cases {Buonfrate, 2015}. More sensitive diagnostic tests, such as Baermann method and Koga agar plate culture are more logistically-demanding and seldom used. PCR is not yet widely available, in particular in endemic areas, because of cost constraints and the need for highly- equipped laboratories. Its specificity is virtually 100%, with good sensitivity. Finally, serology has high sensitivity but cross-reactions with other nematodes should be considered {Buonfrate, 2015}.

In this study, we aimed to revise the clinical and diagnostic characteristics of people with strongyloidiasis. To partly overcome a possible misclassification of infected/uninfected patients, we evaluated symptoms in well-characterized populations, that is patients included in randomized clinical trials (RCT) and in prospective observational case series, hence excluding retrospective datasets.

The primary objective was to estimate the pattern and frequency of symptoms and frequency of eosinophilia associated to *S. stercoralis* infection as reported at diagnosis. Secondary objectives were to compare these features in endemic *versus* non-endemic areas, and to assess the resolution of symptoms and eosinophilia after treatment.

Methods

A systematic review of the literature was performed, including all papers with the following study designs: (1) RCT for the treatment of *S. stercoralis* infection; (2) prospective observational studies reporting information on treatment and including individuals diagnosed with strongyloidiasis using highly specific tests (microscopic examination of feces, concentration methods such as Baermann, Koga agar plate stool culture and/or polymerase chain reaction [PCR]). We included studies independently of the age groups and immunological status of the target population and of the medication received. Hence, we excluded retrospective observational studies, those where participants were included based on serology only and/or when data on the type of diagnostic method could not be extracted for each participant.

Search strategy

Two different search strategies were carried out for RCT and observational studies. For both strategies, the search was done in Pubmed, EMBASE, LILACS and the Cochrane Central Register of Controlled Trials (full search strategy reported in the Supplementary File). Search was conducted in November 2020 and updated

in May 2021. For RCTs, search was limited to papers published from 2009 to 29th May 2021, and for observational studies the search was not restricted to any date. No language restriction applied. We also reviewed the list of references from studies identified by the electronic search in order to find other potential eligible studies.

Study selection and data extraction

The articles retrieved were reviewed for eligibility independently by two authors (DB and AF). Studies were considered eligible if they had a prospective design and reported symptoms caused by strongyloidiasis in a cohort of individuals. Disagreement was resolved by discussion between the two authors. The information was collected in a previously - piloted Excel database by a reviewer and checked by a second reviewer. Variables for which data were sought were: study design and setting, frequency and pattern of any symptom possibly related to strongyloidiasis (as per the Investigators' evaluation) and eosinophil cell count at diagnosis/baseline (before drug administration), as well as frequency/pattern of symptoms and eosinophil count during each selected follow-up visits, treatment administered. Additional information to be collected included age group (adults/children) and presence of immunosuppressed individuals.

Quality assessment

Two reviewers (DB and AF) independently assessed the risk of bias of the included studies, and resolved the disagreements by discussion. Assessment of observational studies was done with the New Castle – Ottawa scale for cohort studies {Wells}. Quality was assessed at study level, and was expressed as low (in case a single or 2 stars were assigned to the study), high (3 or 4 stars), or very high (5-6 stars).
Assessment of the RCT was done with using the criteria outlined in the Cochrane 'Risk of bias' tool {Higgins, 2011}; we report here only the assessment for the RCT not included previously in a Cochrane review {Henriquez-Camacho, 2016 #26}. Based on the results of the quality assessment, we planned to repeat the analyses removing papers of low quality.
To minimize the risk of publication bias, we conducted a comprehensive search across numerous databases, with no language restrictions, and included RCTs along with observational studies.

Statistical analysis

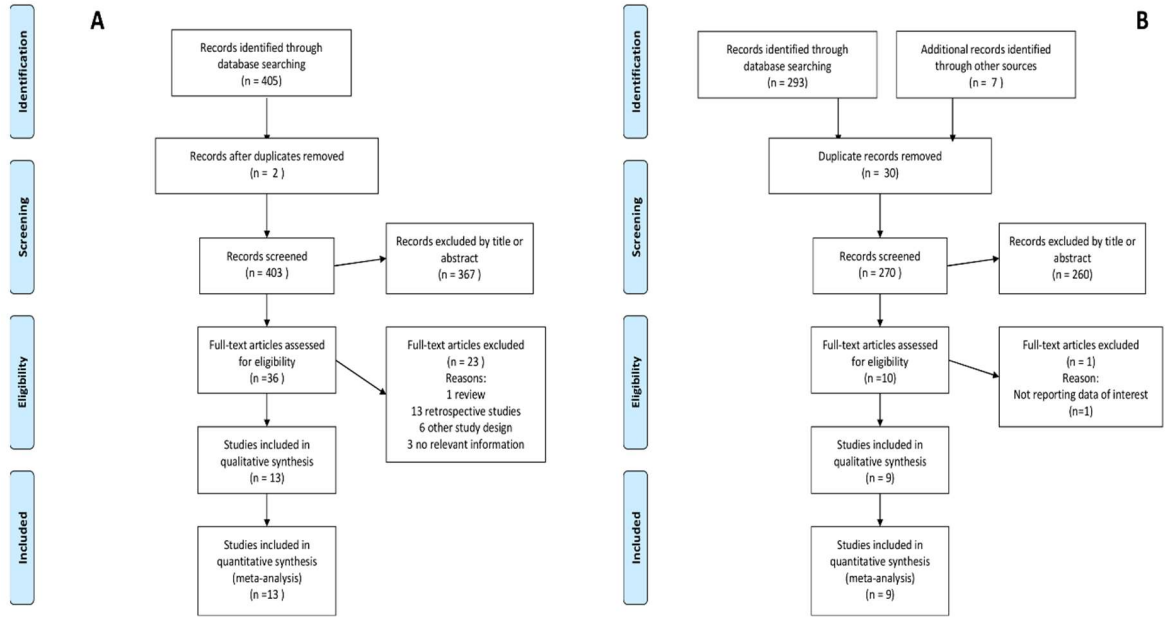
To characterize the populations described in the studies included in this review, demographic and clinical data were summarized using descriptive statistics.
The total number of participants with symptoms and number of participants for each symptom were analyzed using meta-analysis techniques, specifically by pooling the proportion of participants with symptoms using random effects model to account for variability among studies. Analysis was performed stratifying data by setting (endemic or non-endemic) as planned previously, and an exploratory analysis by study design (RCTs or observational studies) was added to explore sources of heterogeneity. I^2 statistic was used to quantify the degree of variability between studies, and heterogeneity was regarded as low if I^2 was < 50%, moderate if was ≥ 50% <74%, high if ≥ 75%. Pooled proportions were calculated and forest plots elaborated using STATA software version 14.0 and the metan package. The forest plots were used to explore heterogeneity between studies, and to display the effect estimates and confidence intervals of each study and the summary estimate.

Results

Study selection and characteristics

Figure 1 reports the study flow for the selection of observational studies (A) and RCTs (B).

Figure 1. PRISMA flow charts for observational studies (A) and randomized controlled trials (B)



The literature search identified nine RCT{Gann, 1994; Marti, 1996; Suputtamongkol, 2011; Barda, 2017; Buonfrate, 2019; Hofmann, 2021; Datry, 1994; Suputtamongkol, 2008; Bisoffi, 2011}, six of which included in a Cochrane systematic review{Henriquez-Camacho, 2016}, the remaining three were published afterwards {Buonfrate, 2019; Barda, 2017; Hofmann, 2021}, and 13 observational studies{Berk, 1987;Oliver, 1989;Shikiya, 1992; Cremades Romero, 1997 ;Sanchez, 2001; Rodríguez Calabuig, 2001;Zaha, 2002;Marcos, 2005;Herrera, 2006; Forrer, 2016;Chacon, 2010;Khieu, 2014}. Hence, overall there were 22 studies included in this review. Table 1 summarizes the setting, population and design of the included studies.

Table 1. Main characteristics of included studies

Paper	Design	Country	N partic	Pop	Diagnosis	Treatment
Gann 1994	rct	United States	53	Adults and children	FECT, Kato-katz, Baermann	IVM 200 mcg/Kg single dose vs IVM 200 mcg/Kg for 2 days vs TBZ (50 mg/Kg/day) twice/day for 3 days
Datry 1994°	rct	France	53	Adults and children	FECT, Kato-katz, Baermann	IVM 150-200 mcg/Kg single dose vs ALB 200
Marti 1996	rct	Tanzania	417	Adults and children	Baermann	IVM 200 mcg/Kg single dose vs ALB 400 mg/day 3 days
Supputtamongkol 2008§	rct	Thailand	42	Adults	FECT	IVM 200 mcg/Kg single dose vs ALB 800 mg/day for 7 days
Supputtamongkol 2011§	rct	Thailand	90	Adults	direct smear, FECT, modified Koga APC	IVM 200 mcg/Kg single dose vs IVM 2 days vs ALB twice/day for 7 days
Bisoffi 2011	rct	Italy	223	Adults and children	serology, FECT, APC	IVM 200 mcg/Kg single dose vs TBZ 50 mg/Kg/day for 2 days
Barda 2017	rct	Laos	127	Adults and children	Baermann	IVM 200 mcg/Kg single dose vs MOX
Buonfrate 2019	rct	Italy, Spain, United Kingdom	309	Adults and children	serology, FECT, APC, PCR	IVM 200 mcg/Kg single dose vs IVM 200 mcg/Kg for 4 days
Hofmann 2021	rct	Laos	209	Adults	Baermann	MOX (ascending doses)* vs placebo
Berk 1987	obs	USA	23	Adults	direct smear	TBZ 50 mg/Kg/day for 2 days
Oliver 1989	obs	Australia	18	Adults	direct smear, FECT, Harada-mori culture	MBZ OR TBZ
Shikiya 1992	obs	Japan	23	Adults	APC	IVM 200 mcg/Kg, 2 doses given 2 weeks apart
Cremades 1997	obs	Spain	37	Adults	direct smear, APC	TBZ 25 mg/Kg /day for 3 days
Sanchez 2001	obs	Spain	152	Adults	direct smear, APC	TBZ 5 days
Rodriguez calabuig 2001	obs	Spain	47	Adults	direct smear, APC	TBZ 3 days OR IVM 200 mcg/Kg single dose
Zaha 2002	obs	Japan	50	Adults	APC	IVM 200 mcg/Kg for 2 days
Marcos 2005	obs	Peru	33	Adults and children	Baermann, SSTT	TBZ 50 mg/Kg/day for 3 days
Herrera 2006	obs	Peru	50	Adults and children	Baermann, SSTT	TBZ
Chacon 2010	obs	Venezuela	44	Adults and children	direct smear, FECT, Baermann	IVM200 mcg/Kg for 2 days
Becker 2011	obs	Cote d'Ivoire	37	Adults and children	Baermann, Koga APC, Kato -katz	IVM 200 mcg/Kg single dose
Khieu 2014	obs	Cambodia	601	Children	Baermann, Koga APC, Kato -katz	IVM 200 mcg/Kg single dose
Forrer 2016	obs	Cambodia	853	Adults and children	Baermann, Koga APC	IVM 200 mcg/Kg single dose

N Partic: number of participatns; P: population; rct: randomized clinical trial ; obs: observational study ; FECT: formol-ether concentration ; APC: agar plate culture ; SSTT: sedimentation in tube ; IVM: ivermectin; ALB: albendazole; TBZ: thiabendazole, MOX: moxidectin. ° : included only for the evaluation of eosinophilia, no information about symptoms reported; §: included also an unknown number of immunosuppressed patients. *: treatment groups: 2 mg, 4 mg, 6 mg, 8 mg, 10 mg, 12 mg moxidectin.

One RCT was included for the evaluation of eosinophilia only, as it did not report information about symptoms. Eleven studies (50%) were conducted in endemic settings. Overall, in the RCTs 1,523 participants were included, with a range of 42 to 417 per trial. In the observational studies, a total of 1,968 participants were included, with a range of 18 to 853. Diagnosis was based on parasitological tests only (either Baermann, microscopic examination of feces, stool culture) in all studies (n=11) carried out in endemic areas and in 8/10 studies in the non-endemic setting. A parasitological test and/or a PCR method were used along with serology in the two remaining RCTs {Buonfrate, 2019; Mascarello, 2011}.

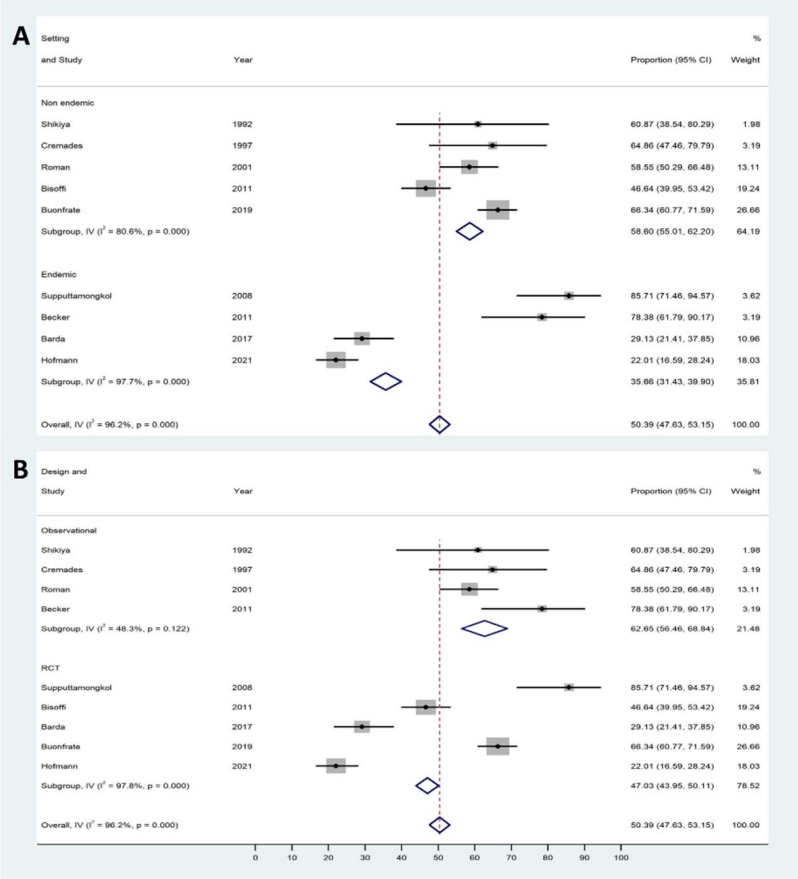
Risk of bias

The results of the quality assessment are reported in the Supplementary File. Overall, the quality of the papers was deemed high/very high, in particular the ascertainment of *S. stercoralis* infection was accurately described. Only one study {Shikiya, 1992} reported scarce information for all domains of the New Castle – Ottawa scale, and its quality was deemed low. All included papers were deemed at a low risk of selective reporting.

Evaluation of symptoms

Nine studies {Suputtamongkol, 2008; Buonfrate, 2019; Barda, 2017; Bisoffi, 2011; Sanchez, 2001; Becker, 2011; Cremades Romero, 1997; Shikiya, 1992; Hofmann, 2021} reported the number of patients with and without symptoms. Among them, four studies were conducted in endemic areas {Barda, 2017; Suputtamongkol, 2008; Becker, 2011; Hofmann, 2021} and five in a non-endemic setting {Buonfrate, 2019; Bisoffi, 2011; Sanchez, 2001; Cremades Romero, 1997; Shikiya, 1992}. Overall, according to the pooled proportions, symptoms were reported in 50.4% cases (95% CI 47.6 - 53.1) (Figure 2), with a significant difference in the proportion of individuals reporting symptoms between settings (Figure 2A): 58.6% (95% CI 55.0-62.2) in non-endemic versus 35.7% (95% CI 31.4-39.9) in endemic areas. For both settings, the heterogeneity was high, as indicated by I^2> 75. When considering the study design (Figure 2B), we found higher frequency of symptoms in observational studies (62.6%, 95% CI 56.5-68.8), which showed low heterogeneity (I^2 48.3%), compared to RCTs (47.0%, 95% CI 43.9-50.1), which presented high heterogeneity. Results were comparable when the paper of low quality was removed from the analysis (not shown). The symptoms most frequently reported were abdominal pain in 51.9% (95% CI 50.2-53.6) individuals, diarrhea in 40.2% (95% CI 38.4-41.9), itching in 33.8% (95% CI 32.2-35.4), skin rash/urticaria in 29.7% (95% CI 27.6-30.7), respiratory symptoms in 29.6% (95% CI 27.7-31.4), and nausea/vomiting in 8.1% (95% CI 6.4-9.9). Also in this case, the removal of the article by Shykyia et al did not impact on the figures (not shown).

Figure 2. Proportion of people reporting symptomatic infection. Figure 2A is a forest plot showing the frequency of symptomatic infection reported in each study. The diamonds synthetize overall frequency (at the bottom of the figure) and for each setting (non-endemic/ endemic)**.** Figure 2B is a forest plot showing the frequency of symptomatic infection reported in each study. The diamonds synthetize overall frequency among studies (at the bottom of the figure) and for study design (observational studies and randomized controlled trials).

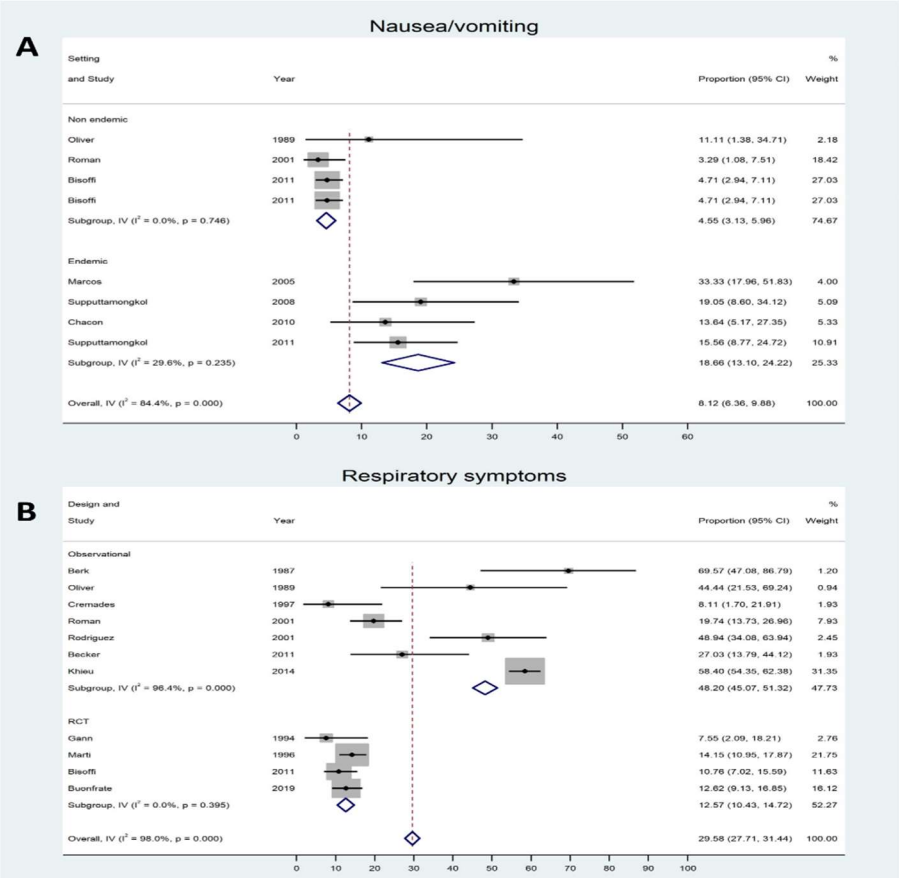


Abdominal pain, diarrhea, skin rash/urticaria, respiratory symptoms, and nausea/vomiting were more often reported in endemic than in non-endemic areas. Frequency of itching did not significantly change between settings. The analyses were affected by high heterogeneity for all symptoms but nausea/vomiting, for which we found low heterogeneity within settings (Figure 3A): frequency was 18.7% (95% CI 13.1-24.2) with I^2=29.6 in endemic areas, versus 4.5 (95% CI 3.1-5.9), I^2=0, in non-endemic areas. When we consider the study design, we observe that abdominal pain, diarrhea, skin rash/urticaria, respiratory symptoms and itching were more frequently reported in observational studies than in RCTs. Nausea/vomiting was reported in a similar frequency between RCTs and observational studies. Also grouping for study design resulted in high heterogeneity when considering each specific symptom; low heterogeneity (I^2=0) was found only for respiratory symptoms in RCTs (Figure 3 B), which were reported by 12.6% (95% CI 10.4-14.7) individuals.

Post-treatment evaluation was available only for three RCTs conducted in non-endemic areas {Buonfrate, 2019; Zaha, 2002; Gann, 1994}, and two conducted in endemic areas {Marti, 1996; Hofmann, 2021}. The latter two included a study comparing a single dose of 200 µg/kg ivermectin with albendazole 400 mg/day for 3 days, and a phase 2a trial comparing ascending doses of moxidectin with placebo {Hofmann, 2021}. All the other studies included a single dose ivermectin, either as single treatment {Zaha, 2002}, or in comparison to multiple doses {Buonfrate, 2019; Gann, 1994}. One of the latter studies comprised also an arm of thiabendazole treatment {Gann, 1994}. The time-points for follow up ranged from 24 days and 12 months, and frequency of all symptoms was reduced compared to baseline (Figure 4). Hofmann et al {Hofmann, 2021} reported also a follow-up after treatment of 3 and 24 hours after treatment, but these time points were not considered here (they were deemed important to collect data about adverse events due to treatment). Symptoms that completely/almost completely cleared at follow up were diarrhea and difficulty in maintaining weight. About a two-fold reduction was observed from baseline to follow up in the number of individuals reporting abdominal pain and skin rash, and a more modest reduction was observed in the frequency of respiratory symptoms and itching. The frequency is reported in absolute numbers, as

for most studies it was difficult to retrieve the number of people for whom the information was available, hence the values were not reported as percentages.
Only one study explored a possible association between symptom resolution and response to treatment, finding a higher proportion of symptom resolution in the group of participants who achieved clearance from the infection.
A reduction in the proportion of patients complaining symptoms was observed also in the single study from endemic country which assessed it at a follow up visit 24 days after treatment {Marti, 1996}.

Figure 3. Frequency of selected symptoms in each study, comparison between settings/study design and overall proportion. Figure 3A is a forest plot showing the frequency of nausea/vomiting reported in each study. The diamonds synthetize the overall frequency (at the bottom), and for each setting (non-endemic/endemic). Figure 3B is a forest plot showing the frequency of respiratory symptoms reported in each study. The diamonds synthetize the overall frequency (at the bottom), and for study design (observational studies and randomized controlled trials).



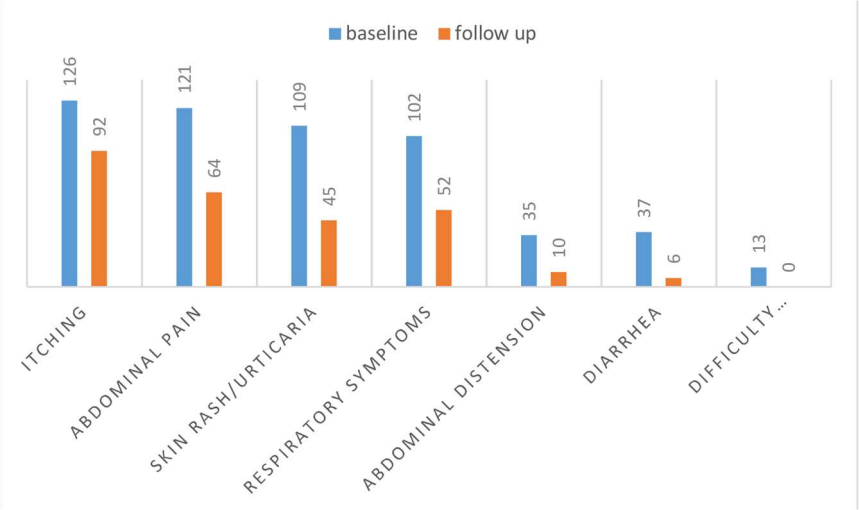
Eosinophil count

The definition of eosinophilia was different among studies, based either on eosinophil count > 400 cells/µL, >440 cells/µL, > 500 cells/µL, or >5% of the total white blood cell count. Six studies reported the mean values of eosinophils/µL, which were comprised between 533.9 (±375.3 standard deviation) and 2,157. Median values were reported only by two studies: 914 and 1,350 eosinophils/µL.

The proportion of participants with eosinophilia (according to the definition reported in each paper) was reported in 10 studies. Among them, there were only two studies (one RCT {Suputtamongkol, 2011} and one observational {Chacon, 2010}) carried out in endemic areas; one of them did not report the threshold used to define eosinophilia. Overall, of the 1047 individuals tested, 726 had eosinophilia (pooled frequency 69.3%, 95% CI 66.4-72.1).

Three studies reported the proportion of participants with eosinophilia before and after treatment {Gann, 1994; Buonfrate, 2019; Mascarello, 2011}. In these studies, 76.9% of participants (95% CI 73.4-80.4) had eosinophilia at diagnosis, reducing to 27.4% (95% CI 24.0-30.7) from 3 to 12 months after treatment. One trial [6] reported a significant decrease in the eosinophil count from baseline to 17 days after treatment, when median values resulted within the normal range. This was more accentuated in participants who had final cure as assessed at 12 months post-treatment.

Figure 4. Patients reporting each symptom: comparison between baseline and follow up. Figures refer to absolute number of patients with each symptom at baseline and at follow up.



Discussion

In this systematic review strictly focusing on strongyloidiasis cases with appropriate diagnostic ascertainment, we found that about a half of infected people reported at least one symptom probably related to this diagnosis, mainly abdominal pain, diarrhea, itching, skin rash/urticaria and respiratory symptoms. The frequency of symptomatic illness was higher in non-endemic settings, although differences were sometimes broad between studies {Barda, 2017; Suputtamongkol, 2008}. More reliable data (based on low heterogeneity within studies) on the proportion of symptomatic people were retrieved from observational studies, which found about 63% of symptomatic individuals. At diagnosis, eosinophilia was

reported in most individuals, although this information was available for a limited number of studies. After treatment, the frequency of both symptoms and eosinophilia substantially decreased, although persisted in a sizeable subset of patients.

In a large retrospective study in the non-endemic setting, conducted by the +REDIVI Spanish Collaborative Network {Salvador, 2019}, symptoms were reported in only 17.9% of the strongyloidiasis cases (mostly immigrants, 66.9%). In this cohort of 1,245 patients however, only 21.9% individuals had a parasite-based diagnosis, while 61% cases were defined as probable (positive serology and eosinophilia) and 17.1% possible (positive serology, no eosinophilia). The high risk of misclassification due to false positive results likely resulted in a dilution of *Strongyloides*-associated symptoms. On the other hand, asymptomatic infection might be common and emerges only when systematic screening is performed. In other words, the low frequency of symptomatic illness in studies conducted non-endemic areas is likely due to the fact that the diagnostic screening is based on epidemiological risk, rather than on symptoms like in tropical countries. The bias towards symptomatic people would entail that only the tip of the iceberg is reported, rather than the entire spectrum of disease. However, in endemic setting this could be balanced by the presence of co-infections with similar clinical presentation, which could cause an overestimation of symptoms frequency due to *S. stercoralis* (for instance abdominal pain, diarrhea). Moreover, in endemic areas the continuous exposure to re-infection could lead to higher *Strongyloides* larval load, resulting in more frequent and more severe symptoms. This aspect is, however, difficult to be assessed, as infection intensity is not usually quantified for *S. stercoralis*. Finally, in all settings, the proportion of symptoms could be underestimated since some of them may be intermittent (thus, not being present at study enrollment) while others are seldomly reported in association with strongyloidiasis. Considering the symptom pattern of strongyloidiasis, a descriptive prospective study conducted in Spain {Martinez-Pérez, 2020} among 158 participants found lower frequency of abdominal pain (21%) and urticaria/itching (16%), but this was likely related with the exclusive use of serology as diagnostic method, as commented above. Another smaller retrospective Spanish study {González, 2010} in which strongyloidiasis was diagnosed by stool microscopy, found in contrast that half of the participants (15/33) had symptoms, with gastrointestinal complaints, respiratory presentation and pruritus in 93.7%, 12.5% and 6.3% of them, respectively). Of note, in this retrospective study also, symptoms ceased after treatment in 75% of the patients (time from treatment to follow up is not reported).

In the +REDIVI Network study {Salvador, 2019}, about 82% of patients had eosinophilia, but the authors acknowledged that an overestimation was possible due to co-infections, which were not reported in the paper. Few retrospective studies assessed the evolution of eosinophil count after treatment. Eosinophilia normalized in about 50% of the patients reported in the study here above {González, 2010}. In the subgroup of papers reporting the information about eosinophil count, the reduction of eosinophilia was significant (from 76.9% to 27.4%) but not complete at the time of assessment. It is unclear whether persistence of eosinophilia strictly reflects treatment failure. In the only study that investigated association between cure and decrease in eosinophil count [6], a reduction of eosinophil count was observed both in participants who were cured and in those who were not, but the decrease was more pronounced in the former group. In this trial, non-normalization of eosinophil count could be due to the rather strict criteria to define cure, but also to a partial response to treatment, since ivermectin is not 100% effective (incomplete larval clearance). Similarly, it can be expected that clinical and laboratory response to albendazole would be partial as well, since this drug has a lower efficacy than ivermectin for the treatment of strongyloidiasis [7]. Correlation between disappearance of symptoms, normalization of eosinophilia, complete larval clearance and the potential to cause severe disease later in life should be thoroughly studied in future treatment trials.

Limitations of this review include the fact that only a few studies reported the overall proportion of participants with and without symptoms, highlighting the need for further research in this aspect. Also, the estimation of the reduction of symptoms and eosinophilia frequencies was weakened by the difficulty to

consistently extract precise absolute numbers. Selective reporting might have affected the results of our synthesis. Among additional issues that could not be addressed is the possible influence of immunological status and presence of co-infections on clinical presentation and eosinophilia, as most papers did not include immunosuppressed individuals (and those that have, did not detail symptoms/eosinophil count in relation to immunological status and /or co-infections). The considerable statistical heterogeneity found in most analyses limits general interpretation. Among the strengths of this work, it must be mentioned that the inclusion of studies where diagnosis was only made by parasite-based methods substantially reduced the uncertainty generated by the exclusive use of serology. Moreover, although some retrospective datasets are very large [31], prospective studies offer higher quality and more reliable data.

In conclusion, the morbidity caused by strongyloidiasis in immunocompetent populations has long been considered a marginal aspect of the infection, while the emphasis has almost always been put on the disseminated disease (hyperinfection syndrome) in immunocompromised individuals {Tamarozzi, 2019; Nutman, 2017}. While strongyloidiasis is often asymptomatic, it is also associated with invalidating symptoms in about half of infected people and eosinophilia is present almost 70% of them. Both symptoms and eosinophilia improve after treatment, even if the clinical response was sometimes partial and the association with cure could not be fully ascertained. In any case, relief from symptoms and eosinophilia is another reason, in addition to prevention of disseminated disease, for promoting screening and treatment of individuals at risk in non-endemic areas and to intensify control initiatives in endemic countries.

References

1. Buonfrate D, Bisanzio D, Giorli G, Odermatt P, Fürst T, Greenaway C, French M, Reithinger R, Gobbi F, Montresor A, Bisoffi Z. The Global Prevalence of *Strongyloides stercoralis* Infection. Pathogens. 2020 Jun 13;9(6):468. doi: 10.3390/pathogens9060468. PMID: 32545787; PMCID: PMC7349647.
2. Krolewiecki AJ, Lammie P, Jacobson J, Gabrielli AF, Levecke B, Socias E, Arias LM, Sosa N, Abraham D, Cimino R, Echazú A, Crudo F, Vercruysse J, Albonico M. A public health response against Strongyloides stercoralis: time to look at soil-transmitted helminthiasis in full. PLoS Negl Trop Dis. 2013 May 9;7(5):e2165. doi: 10.1371/journal.pntd.0002165. PMID: 23675541; PMCID: PMC3649958.
3. Nutman TB. Human infection with Strongyloides stercoralis and other related Strongyloides species. Parasitology. 2017 Mar;144(3):263-273. doi: 10.1017/S0031182016000834. Epub 2016 May 16. PMID: 27181117; PMCID: PMC5563389.
4. Tamarozzi F, Martello E, Giorli G, Fittipaldo A, Staffolani S, Montresor A, Bisoffi Z, Buonfrate D. Morbidity Associated with Chronic *Strongyloides stercoralis* Infection: A Systematic Review and Meta-Analysis. Am J Trop Med Hyg. 2019 Jun;100(6):1305-1311. doi: 10.4269/ajtmh.18-0895. PMID: 30963990; PMCID: PMC6553888.
5. Buonfrate D, Formenti F, Perandin F, Bisoffi Z. Novel approaches to the diagnosis of Strongyloides stercoralis infection. Clin Microbiol Infect. 2015 Jun;21(6):543-52. doi: 10.1016/j.cmi.2015.04.001. Epub 2015 Apr 14. PMID: 25887711.
6. GA Wells, B Shea, D O'Connell, J Peterson, V Welch, M Losos, P Tugwell. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at : https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
7. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011 Oct 18;343:d5928. doi: 10.1136/bmj.d5928. PMID: 22008217; PMCID: PMC3196245.
8. Henriquez-Camacho C, Gotuzzo E, Echevarria J, White AC Jr, Terashima A, Samalvides F, Pérez-Molina JA, Plana MN. Ivermectin versus albendazole or thiabendazole for Strongyloides stercoralis infection. Cochrane Database Syst Rev. 2016 Jan 18;2016(1):CD007745. doi: 10.1002/14651858.CD007745.pub3. PMID: 26778150; PMCID: PMC4916931.
9. Gann PH, Neva FA, Gam AA. A randomized trial of single- and two-dose ivermectin versus thiabendazole for treatment of strongyloidiasis. J Infect Dis. 1994 May;169(5):1076-9. doi: 10.1093/infdis/169.5.1076. PMID: 8169394.

10. Marti H, Haji HJ, Savioli L, Chwaya HM, Mgeni AF, Ameir JS, Hatz C. A comparative trial of a single-dose ivermectin versus three days of albendazole for treatment of Strongyloides stercoralis and other soil-transmitted helminth infections in children. Am J Trop Med Hyg. 1996 Nov;55(5):477-81. doi: 10.4269/ajtmh.1996.55.477. PMID: 8940976.
11. Suputtamongkol Y, Premasathian N, Bhumimuang K, Waywa D, Nilganuwong S, Karuphong E, Anekthananon T, Wanachiwanawin D, Silpasakorn S. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. PLoS Negl Trop Dis. 2011 May 10;5(5):e1044. doi: 10.1371/journal.pntd.0001044. PMID: 21572981; PMCID: PMC3091835.
12. Barda B, Sayasone S, Phongluxa K, Xayavong S, Keoduangsy K, Odermatt P, Puchkov M, Huwyler J, Hattendorf J, Keiser J. Efficacy of Moxidectin Versus Ivermectin Against Strongyloides stercoralis Infections: A Randomized, Controlled Noninferiority Trial. Clin Infect Dis. 2017 Jul 15;65(2):276-281. doi: 10.1093/cid/cix278. PMID: 28369530.
13. Buonfrate D, Salas-Coronas J, Muñoz J, Maruri BT, Rodari P, Castelli F, Zammarchi L, Bianchi L, Gobbi F, Cabezas-Fernández T, Requena-Mendez A, Godbole G, Silva R, Romero M, Chiodini PL, Bisoffi Z. Multiple-dose versus single-dose ivermectin for Strongyloides stercoralis infection (Strong Treat 1 to 4): a multicentre, open-label, phase 3, randomised controlled superiority trial. Lancet Infect Dis. 2019 Nov;19(11):1181-1190. doi: 10.1016/S1473-3099(19)30289-0. Epub 2019 Sep 23. PMID: 31558376.
14. Hofmann D, Sayasone S, Sengngam K, Chongvilay B, Hattendorf J, Keiser J. Efficacy and safety of ascending doses of moxidectin against Strongyloides stercoralis infections in adults: a randomised, parallel-group, single-blinded, placebo-controlled, dose-ranging, phase 2a trial. Lancet Infect Dis. 2021 Aug;21(8):1151-1160. doi: 10.1016/S1473-3099(20)30691-5. Epub 2021 Mar 30. PMID: 33798487.
15. Datry A, Hilmarsdottir I, Mayorga-Sagastume R, Lyagoubi M, Gaxotte P, Biligui S, Chodakewitz J, Neu D, Danis M, Gentilini M. Treatment of Strongyloides stercoralis infection with ivermectin compared with albendazole: results of an open study of 60 cases. Trans R Soc Trop Med Hyg. 1994 May-Jun;88(3):344-5. doi: 10.1016/0035-9203(94)90110-4. PMID: 7974685.
16. Suputtamongkol Y, Kungpanichkul N, Silpasakorn S, Beeching NJ. Efficacy and safety of a single-dose veterinary preparation of ivermectin versus 7-day high-dose albendazole for chronic strongyloidiasis. Int J Antimicrob Agents. 2008 Jan;31(1):46-9. doi: 10.1016/j.ijantimicag.2007.08.014. Epub 2007 Nov 26. PMID: 18023151.
17. Bisoffi Z, Buonfrate D, Angheben A, Boscolo M, Anselmi M, Marocco S, Monteiro G, Gobbo M, Bisoffi G, Gobbi F. Randomized clinical trial on ivermectin versus thiabendazole for the treatment of strongyloidiasis. PLoS Negl Trop Dis. 2011 Jul;5(7):e1254. doi: 10.1371/journal.pntd.0001254. Epub 2011 Jul 26. PMID: 21814588; PMCID: PMC3144183.
18. Berk SL, Verghese A, Alvarez S, Hall K, Smith B. Clinical and epidemiologic features of strongyloidiasis. A prospective study in rural Tennessee. Arch Intern Med. 1987 Jul;147(7):1257-61. PMID: 3606282.
19. Oliver NW, Rowbottom DJ, Sexton P, Goldsmid JM, Byard R, Tooth M, Thomson KS. Chronic strongyloidiasis in Tasmanian veterans--clinical diagnosis by the use of a screening index. Aust N Z J Med. 1989 Oct;19(5):458-62. doi: 10.1111/j.1445-5994.1989.tb00306.x. PMID: 2590096.
20. Shikiya K, Kinjo N, Uehara T, Uechi H, Ohshiro J, Arakaki T, Kinjo F, Saito A, Iju M, Kobari K. Efficacy of ivermectin against Strongyloides stercoralis in humans. Intern Med. 1992 Mar;31(3):310-2. doi: 10.2169/internalmedicine.31.310. PMID: 1611180.
21. Cremades Romero MJ, Igual Adell R, Ricart Olmos C, Estellés Piera F, Pastor-Guzmán A, Menéndez Villanueva R. Infección por Strongyloides stercoralis en la comarca de La Safor (Comunidad Valenciana) [Infection by Strongyloides stercoralis in the county of Safor, Spain]. Med Clin (Barc). 1997 Jul 5;109(6):212-5. Spanish. PMID: 9289550.
22. Sánchez PR, Guzman AP, Guillen SM, Adell RI, Estruch AM, Gonzalo IN, Olmos CR. Endemic strongyloidiasis on the Spanish Mediterranean coast. QJM. 2001 Jul;94(7):357-63. doi: 10.1093/qjmed/94.7.357. PMID: 11435631.
23. Rodríguez Calabuig D, Igual Adell R, Oltra Alcaraz C, Sánchez Sánchez P, Bustamante Balen M, Parra Godoy F, Nagore Enguidanos E. Actividad laboral agrícola y estrongiloidiasis. Estudio caso-control [Agricultural occupation and strongyloidiasis. A case-control study]. Rev Clin Esp. 2001 Feb;201(2):81-4. Spanish. doi: 10.1016/s0014-2565(01)70755-2. PMID: 11345610.
24. Zaha O, Hirata T, Kinjo F, Saito A, Fukuhara H. Efficacy of ivermectin for chronic strongyloidiasis: two single doses given 2 weeks apart. J Infect Chemother. 2002 Mar;8(1):94-8. doi: 10.1007/s101560200013. PMID: 11957127.
25. Marcos L, Terashima A, Samalvides F, Alvarez H, Lindo F, Tello R, Canales M, Demarini J, Gotuzzo E. Tiabendazol para el control de la infección por Strongyloides stercoralis en una zona hiperendémica en el

Perú [Thiabendazole for the control of Strongyloides stercoralis infection in a hyperendemic area in Peru]. Rev Gastroenterol Peru. 2005 Oct-Dec;25(4):341-8. Spanish. PMID: 16333389.
26. Herrera J, Marcos L, Terashima A, Alvarez H, Samalvides F, Gotuzzo E. Factores asociados a la infección por strongyloides stercoralis en individuos de una zona endémica en el Perú [Factors associated with strongyloides stercoralis infection in an endemic area in Peru]. Rev Gastroenterol Peru. 2006 Oct-Dec;26(4):357-62. Spanish. PMID: 17211485.
27. Forrer A, Khieu V, Schindler C, Schär F, Marti H, Char MC, Muth S, Odermatt P. Ivermectin Treatment and Sanitation Effectively Reduce Strongyloides stercoralis Infection Risk in Rural Communities in Cambodia. PLoS Negl Trop Dis. 2016 Aug 22;10(8):e0004909. doi: 10.1371/journal.pntd.0004909. PMID: 27548286; PMCID: PMC4993485.
28. Chacón, Nathalie; Durán, Carmen; Rossomando, María J. Factores predictivos de infección para estrongiloidosis en pacientes procedentes de áreas urbanas / Predictors of infection in strongyloidiasis patients from urban areas. Bol. venez. infectol *;* 21(1): 41-47, ene.-jun. 2010. Tab
29. Khieu V, Schär F, Marti H, Bless PJ, Char MC, Muth S, Odermatt P. Prevalence and risk factors of Strongyloides stercoralis in Takeo Province, Cambodia. Parasit Vectors. 2014 May 12;7:221. doi: 10.1186/1756-3305-7-221. PMID: 24886763; PMCID: PMC4029906.
30. Becker SL, Sieto B, Silué KD, Adjossan L, Koné S, Hatz C, Kern WV, N'Goran EK, Utzinger J. Diagnosis, clinical features, and self-reported morbidity of Strongyloides stercoralis and hookworm infection in a Co-endemic setting. PLoS Negl Trop Dis. 2011 Aug;5(8):e1292. doi: 10.1371/journal.pntd.0001292. Epub 2011 Aug 23. PMID: 21886853; PMCID: PMC3160297.
31. Salvador F, Treviño B, Chamorro-Tojeiro S, Sánchez-Montalvá A, Herrero-Martínez JM, Rodríguez-Guardado A, Serre-Delcor N, Torrús D, Goikoetxea J, Zubero Z, Velasco M, Sulleiro E, Molina I, López-Vélez R, Pérez-Molina JA; on behalf the +REDIVI Collaborative Network. Imported strongyloidiasis: Data from 1245 cases registered in the +REDIVI Spanish Collaborative Network (2009-2017). PLoS Negl Trop Dis. 2019 May 16;13(5):e0007399. doi: 10.1371/journal.pntd.0007399. PMID: 31095570; PMCID: PMC6541302.
32. Martinez-Pérez A, Soriano-Pérez MJ, Salvador F, Gomez-Junyent J, Villar-Garcia J, Santin M, Muñoz C, González-Cordón A, Salas-Coronas J, Sulleiro E, Somoza D, Treviño B, Pecorelli R, Llaberia-Marcual J, Lozano-Serrano AB, Quinto L, Muñoz J, Requena-Méndez A, Strong-Semtsi Working Group OBOT. Clinical Features Associated with Strongyloidiasis in Migrants and the Potential Impact of Immunosuppression: A Case Control Study. Pathogens. 2020 Jun 23;9(6):507. doi: 10.3390/pathogens9060507. PMID: 32585975; PMCID: PMC7350355.
33. González A, Gallo M, Valls ME, Muñoz J, Puyol L, Pinazo MJ, Mas J, Gascon J. Clinical and epidemiological features of 33 imported Strongyloides stercoralis infections. Trans R Soc Trop Med Hyg. 2010 Sep;104(9):613-6. doi: 10.1016/j.trstmh.2010.06.001. Epub 2010 Jul 16. PMID: 20637483.

Chapter 4 Diagnostic tools for screening, diagnosis and follow up in the non-endemic setting

4.1 The diagnosis of human and companion animal *Strongyloides stercoralis* infection: Challenges and solutions. A scoping review

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Abstract

Strongyloidiasis is the infection caused by soil-transmitted nematodes of *Strongyloides* species, infecting humans and some animals. *Strongyloides stercoralis* is the species with most clinical and epidemiological relevance in humans and dogs, due to its high prevalence and its capacity of inducing a life-threatening hyperinfection. Diagnosis of strongyloidiasis is challenging, due to the absence of a single reference standard test with high sensitivity and specificity, which also hampers the estimation of the accuracy of diagnostic tests. In this work, we review the deployment and performance of the parasitological, immunological, molecular tests for the diagnosis of strongyloidiasis in humans and in dogs. Further, we comment the available evidence from genotyping studies that have addressed the zoonotic potential of *S. stercoralis.* Finally, we discuss the use of different diagnostic methods in relation to the purpose (i.e screening, individual diagnosis, inclusion in a clinical trial) and the setting (endemic/non-endemic areas) and report the accuracy figures reported by systematic reviews on either parasitological, serological or molecular techniques published in literature.

Introduction

Strongyloidiasis is a parasitic infection caused by nematodes of the genus *Strongyloides*. An estimated 600 million people worldwide are infected by the species *S. stercoralis*, while rare human infections by *S. fuelleborni fuelleborni* have been reported in Africa and Asia {Buonfrate, 2020; Bradbury, 2021}. *S. fuelleborni kellyi* appears to be restricted to the island of New Guinea {Bradbury, 2021 #6}. Some *S. stercoralis* strains can also infect non-human primates, cats and dogs, posing concerns about a possible zoonotic transmission {Gonzalez Akimori, 2021; Bradbury, 2021}.

S. stercoralis is a soil-transmitted helminth: infection occurs by skin penetration of infective third-stage filariform larvae (iL3) living in the soil in areas with poor sanitation. The iL3 classically migrate through the blood vessels to the lungs, ascend the tracheobronchial tree and are swallowed into the intestinal tract, but they may also migrate directly through other tissues to the gastrointestinal tract {Page, 2018}. During the migration route, they mature into different stages, and eventually settle in the wall of the small intestine as adult females {Streit, 2008; Nutman, 2017}. The parasitic female reproduces by parthenogenesis, laying eggs that hatch into rhabditiform larvae (L1). In humans, these larvae are either excreted with faeces or transform into filariform larvae, which can penetrate the intestinal wall or the perianal skin, leading to reinfection. This is called “auto-infective cycle”, and causes the indefinite persistence of the infection, if left untreated {Nutman, 2017}. When defecation occurs in the external environment, the L1 can either precociously develop into iL3 (direct or homogonic cycle) or into non- infective larvae, which go through different stages and eventually mature into free-living adult worms of both sexes (indirect or heterogonic cycle). The free-living adult worms reproduce sexually and their offspring moult twice to mature into iL3 {Streit, 2008}. This second generation of the free-living cycle do not transform into adults and must find a host to complete the life cycle {Page, 2018}.

As a result of the auto-infective cycle of *S. stercoralis* in humans, strongyloidiasis is a chronic disease. A large proportion of infected individuals present with no symptoms, while others present with intermittent, non-specific symptoms mostly affecting the skin and/or the gastrointestinal and/or the respiratory tracts {Buonfrate, 2021}. Eosinophilia may be intermittent or absent {Naidu, 2013}, and this sign is frequent also in other parasitic infections, thus not specific {O'Connell, 2016}. Immunosuppressed individuals can present with an accelerated autoinfective cycle, which leads to an increased larval load. This condition, named hyperinfection, usually manifests with worsening of symptoms and sometimes with sepsis by enteric bacteria, which can enter the bloodstream through intestinal ulceration {Nutman, 2017}. In the most severe hyperinfection, there is a catastrophic dissemination of large numbers of larvae throughout body, with a high fatality rate {Buonfrate, 2013}.

For chronic uncomplicated infection, first line treatment is ivermectin 200 µg/kg in a single dose, while albendazole demonstrates lower efficacy {Buonfrate, 2019; Henriquez-Camacho, 2016}. Recent evidence suggests that moxidectin may become an alternative to the first line treatment {Hofmann, 2021}. The treatment of infection in immunosuppressed individuals and hyperinfection relies on expert’s opinion, and repeated doses of ivermectin are recommended up to 2 weeks from clearance from infection at microscopy examination of stool and/or previously-positive biological material {Nutman, 2017}. In literature, hyperinfection cases treated with subcutaneous administration of ivermectin have been reported, some of them showing a good response to the treatment {Barrett, 2016}.

S. f. fuelleborni is primarily a parasite of primates in Africa and Asia, but human infection is also rarely reported {Nutman, 2017}. The disease resembles infection with *S. stercoralis*, though auto-infection seems unlikely as for this species as eggs rather than larvae are passed {Pampiglione, 1972; Potters, 2020}. High intensity infections with *S. f. kellyi* due to external autoinfection rather than true autoinfection, has been associated with “swollen belly syndrome”, a severe protein-losing enteropathy in infants in New Guinea. In

older ages, the prevalence and morbidity of this infection progressively reduces {Bradbury, 2021}. There is scant evidence available about the best diagnostic and treatment approach for these subspecies.

The diagnosis of *S. stercoralis* infection is challenging, due to the intermittent shedding of larvae in the faeces and consequent low sensitivity of parasitological diagnosis. In addition, there is the absence of a single reference test with high sensitivity and specificity {Requena-Méndez, 2013; Basáñez, 2004}. The lack of a single reference standard hampers a correct evaluation of novel diagnostic tests, the accuracy of which is often assessed against a panel of microscopy-positive samples, leading to overestimation of the sensitivity and underestimation of the specificity of the novel test {Buonfrate, 2013}. Alternative statistical methods have been recommended for the estimation of the accuracy of diagnostic tests in the absence of a single reference test {Rutjes, 2007}, but these are still seldom applied. The application of different reference standards for the evaluation of accuracy sometimes hampers the comparison between diagnostic studies, thus this limitation should be taken into consideration also for meta-analyses.

Further, the choice of a diagnostic test should be based on the specific context and situation. Indeed, a diagnostic tool which is suitable for individual diagnosis in a non-endemic, high-income country, might not be so for use in screening surveys in endemic areas. The World Health Organization (WHO) has recently included the control of strongyloidiasis among the 2030 targets {Montresor, 2020}, and the identification of a test that could be deployed in the field is compelling.

In this work, we review the tests for the diagnosis of strongyloidiasis in human and veterinary medicine, reporting assays used in routine practice for screening and diagnosis as well as experimental assays under evaluation/development.

Methods for literature search

Search strategy

We searched literature published from 1990 to November 6th 2021 and available in the following databases: MEDLINE, Google Scholar, Embase, Cochrane Central Register of Controlled Trials, LILACS. The complete list of key words is reported in Table 1. Each author evaluated the record list generated by the search strategy and retrieved the papers to be included in the section he/she was in charge of. Further papers were retrieved from the reference list of included papers. DB and FT also searched the records to retrieve systematic reviews on diagnostic methods for strongyloidiasis.

Results

The search permitted to identify 3663 records, which reduced to 3446 after duplicate removal. Eventually, 257 full-text papers were reviewed and quoted in this work. Three systematic reviews were identified and included here.

Coproparasitological methods

The peculiar life cycle of *S. stercoralis* means that larvae, not eggs, are eliminated with faeces. Furthermore, in chronic infections, the elimination of such larvae is scant and intermittent {Nutman, 2017}. These two features are at the basis of the techniques used for the coproparasitological diagnosis of strongyloidiasis, and of their performances. Also, these features are responsible for the current absence of a reference standard for the diagnosis of infection to be used in clinical practice and research studies, since no single

technique (coproparasitological, but also molecular and serological), at any given time, can achieve 100% sensitivity and specificity {Buonfrate, 2013}.

Coproparasitological techniques classically used for the retrieval of helminth eggs and protozoan cysts, such as direct smear-based approaches (e.g. direct smear [DS], Kato-Katz [KK]), and gravity-based concentration techniques such as formalin-ether-based concentration techniques (FECT) and flotation techniques, have consistently lower sensitivity than methods developed for the specific retrieval of *S. stercoralis* larvae in faeces {Campo Polanco, 2014}. These in turn basically exploit the capacity of the parasite larvae to migrate actively, variably combined with their capacity to enter the free-living life cycle, and are based on sedimentation (e.g. Baermann technique and its modifications), and culture (e.g. Agar Plate Culture [APC] and its modification). These techniques, although more sensitive, have several drawbacks that can impact on sensitivity in routine diagnostic use, including the need of fresh (ideally processed within a few hours from passing), unpreserved and unrefrigerated faeces. Concerning the latter, Ines et al {Inês, 2011} compared the viability of *S. stercoralis* larvae using Baermann and APC when using fresh stool and stool stored at 4°C for 24h, 48h and 72h, and found that viability (and in parallel the sensitivity of the techniques) halved every 24h of refrigeration. Furthermore, these techniques require longer times to obtain a result (up to 72h or more for APC) and they carry the possible risk of infection for the operator manipulating samples possibly containing iL3. Finally, for all coproparasitological techniques requiring microscopic examination, knowledge that larvae of other parasites may be observed, and skills in differentiating them, are absolutely needed.

In general, it can be difficult to compare the accuracy of the different methods because of the huge variability in terms of setting, study design, reference test/combination of tests used, and actual specific protocol applied for each technique, including quantity of faeces processed and number of slides examined by microscopy at the end of the technique. Studies that made a dedicated comparison of these techniques are listed in Table 2. Below we provide an overview of the techniques described in the literature and used in several settings for the diagnosis of strongyloidiasis, and their performances.

Direct smear and Kato-Katz techniques

The microscopic examination of faecal smears obtained by mixing a small quantity of stool with saline or iodine (direct smear technique [DS]) is probably the most widely used coproparasitological technique, especially in non-specialized centres, because of its simplicity and rapidity, although at the cost of very low sensitivity in the presence of light infections. This is understandable if one considers that only 2-5 mg of faeces are examined. For the diagnosis of strongyloidiasis, sensitivity is in the range of 0-18% {Campo Polanco, 2014; Koga, 1991; de Kaminsky, 1993; Jongwutiwes, 1999; Lau Chong, 2005; Machicado, 2012; Meurs, 2017; Chankongsin, 2020}. Figures around 30-50% sensitivity were also reported {Getaneh, 2010; Hailegebriel, 2017; Hernández-Chavarría, 2001}, but seem implausible.

The Kato-Katz (KK) technique is a semi-concentration method widely used for the quantitative assessment of eggs in faeces, especially in the context of epidemiological studies and control programs for the other soil-transmitted helminths {Cools, 2019}. It was developed for the diagnosis of schistosomiasis in 1954 by Kato and Miura {Kato, 1954} and later adapted for field use by Katz and colleagues {Katz, 1972}. The technique is not appropriate for the diagnosis of strongyloidiasis since it consistently fails to diagnose the infection {Carvalho, 2012; Lopez, 2016; Machicado, 2012; Schär, 2014}.

Flotation techniques

Flotation techniques are based on the ability of parasite eggs and cysts to float on the surface of a solution (e.g. zinc sulfate) with higher density and adhere onto a glass slide placed on the solution meniscus surface

{Willis, 1921; Faust, 1938}. Flotation techniques have been assessed for the retrieval of *S. stercoralis* larvae, generally with poor results (sensitivity 4-8%) {Carvalho, 2012; Schär, 2014}. Furthermore, the hyperosmotic flotation solution rapidly distorts the larvae, making specific identification difficult. FLOTAC, and its modification Mini-FLOTAC, are flotation-based techniques for the quantitative diagnosis of faecal parasite elements (e.g. ova and cysts) using a "closed" device {Cringoli, 2010; Cringoli, 2013}. When evaluated for the diagnosis of strongyloidiasis by Glinz and colleagues {Glinz, 2010}, their performance was poor, with only 2 positive samples detected of the 38 diagnosed by APC.

Sedimentation techniques

Protocols of faecal concentration based on the sedimentation of filtered, homogenized faeces have been applied since the early 1900s and have taken different names in the published literature {Lutz, 1919 ; Hoffman, 1934; Lumbreras, 1962; Faust, 1939}. The Spontaneous in Tube Sedimentation Technique (STST), originally described by Tello and colleagues in 1988 {Tello, 2012}, consists in the homogenization of faeces (about 10g) with saline, followed by a spontaneous sedimentation step of the strained faeces in a 50 ml conical tube filled with saline or water. After at least 45 min, the sediment is examined by microscopy. The sedimentation can also be achieved by centrifugation {Inês, 2016}. The Lumbreras rapid sedimentation test {Lumbreras, 1962}, also widely used especially in Latin America, allows the homogenized and filtered faeces to settle for 45 min in a 200 ml conical vase filled with water. After discarding 2/3 of the supernatant, the vase is filled again and the sedimentation allowed for further 45 min. The sediment is then examined by microscopy, or the process repeated a few times before microscopy examination, until the supernatant is clear, depending on the protocol {Maco Flores, 2002}. When applied for the diagnosis of strongyloidiasis, these sedimentation techniques were reported having sensitivity in the range of 27%-75% {Blatt, 2003; Carvalho, 2012; Inês, 2011; Lau Chong, 2005; Lopez, 2016; Machicado, 2012; Hailu, 2021}.

Formalin-Ether Concentration Technique (FECT) and its modifications

FECT is a centrifugation-sedimentation method based on the different specific gravity of water and parasites (cysts, ova and larvae). Specifically, the parasites present in the formalin-fixed sample are heavier than the formalin-ether solution and settle in the sediment of the tube containing the mixture. In short, after centrifugation of homogenized faeces strained through gauze, four layers are formed, from the bottom: sediment, formalin, plug of lighter debris, and ether. Subsequent decanting of the supernatant and examination of the sediment by microscopy complete the process. The starting quantity of stool reported in the literature ranges from as much as an "apricot-size" to as little as 500mg.

The prototype of the FECT method was originally described by Telemann in 1908 {Telemann, 1908} and modified by Ritchie in 1948 {Ritchie, 1948}, after whom the technique is commonly named. Several modifications of the "Ritchie" technique have been described since, with slight changes in the quantity of faeces used and in the procedure steps {Allen, 1970; Ridley, 1956}. To overcome the disadvantages of the use of diethyl ether, which is flammable, explosive if exposed to light, and highly volatile with dispersion of anaesthetic vapour, several other chemicals have been evaluated as a substitute, first of all ethyl-acetate {Young, 1979}. However, this chemical also is hazardous and several drawbacks in the processing of the sample and reading of the slide have been described {Erdman, 1981}. Many further modifications of the FECT technique by use of different chemicals, less dangerous and/or more readily available, have been published, including gasoline {Ahmadi, 2009}, detergent {Anecimo, 2012; Kightlinger, 1990}, Tween {Ahmadi, 2007}, d-Limonene (Hemo-De®) {Neimeister, 1987}. Commercial devices for faecal examination using the FECT principle were also applied {Amor, 2016; Carvalho, 2012; Meurs, 2017; Perry, 1990}. Generally speaking, all studies applying this vast array of modifications obtained similar results in respect to the (poorly sensitive) retrieval of *S. stercoralis* larvae. Reported sensitivity ranges from 6-60% {Aramendia Aa, 2020; Arakaki, 1990; Blatt, 2003; Carvalho, 2012; Intapan, 2005; Jongwutiwes, 1999; Kobayashi, 1996;

Anamnart, 2013; Getaneh, 2010; Hailegebriel, 2017; Schär, 2014}. In a systematic review and meta-analysis, Campo-Polanco and colleagues {Campo Polanco, 2014} estimated the global sensitivity of the FECT in 48% (95%CI 42-54%). Noteworthy, the reference standard of the reviewed studies were faecal-based techniques, only.

Anamnart et al {Anamnart, 2010} evaluated the factors affecting the recovery rate of *S. stercoralis* by using FECT (2-4 g of known *S. stercoralis*-positive faeces), finding that the use of fresh faeces with only short time exposure to formalin (<2h), use of a wire mesh instead of gauze, and a longer centrifugation time improved the recovery rate of larvae, however sensitivity raised only from 14% to 28% {Anamnart, 2010}. By examining the other layers formed upon centrifugation, they found that a large proportion of larvae were actually trapped in the debris plug, in the gauze, and in the formalin layer {Anamnart, 2010}. Furthermore, it has been argued that dead larvae are more difficult to discern in the sediment than moving larvae in a clear background as obtained by Baermann or culture techniques {Siddiqui, 2001}. In a study that compared the microscopic detection of *Strongyloides* larvae in three stool specimens using direct smear, formalin ethyl acetate concentration and the solvent free Parasep SF faecal concentrator (Apacor, Workingham, England), the Parasep SF concentrator was the least sensitive method {Moody, 2013}.

Baermann funnel technique and its modifications

This technique was originally described in 1917 by the author naming it, who used it for the detection of hookworm larvae in soil, taking advantage of the thermotropism and hydrotrophism of larvae stimulating larvae to migrate spontaneously {Baermann, 1917}, and later applied also to retrieve *S. stercoralis* larvae from faces {Moraers, 1948; Coutinho, 1951}. The Baermann technique uses a specific apparatus consisting in a funnel fit with a short piece of tubing to the stem, closed at the other end by a clamp; the apparatus is supported by a stand or rack. Faeces (generally about 10 g) are placed in gauze and suspended in lukewarm water (30-45°C) filling the apparatus, so that the sample only lightly touches the water. The active movement of larvae out of the faecal mass towards the water is allowed for a variable time at room temperature (generally between 25-30°C), during which the larvae sediment at the lower extremity of the tube; sometimes a light is placed underneath the apparatus to further stimulate larval migration. Then, the last volume of water is transferred in a tube by loosening the clamp, centrifuged, and the sediment observed under the microscope. Several modifications have been applied to the original technique, for example the use of a sedimentation flask instead of a funnel, with faeces partially {Lumbreras, 1959; Lumbreras, 1963; Rugai, 1954 } or completely {de Kaminsky, 1993} introduced in the lukewarm water; the use of laboratory tubes {Gelaye, 2021; Hernández Chavarria, 2001}, or commercial kits {Willcox, 1989}, or other materials more readily available in resource-poor settings, such as recycled plastic bottles {Graeff-Teixeira, 1997 }, with overall comparable results. Figure 1 shows a Baermann apparatus.

The technique has been also applied following APC culture {Inês, 2011} or coupled with a pre-incubation step with activated charcoal {Amor, 2016; Aramendia Aa, 2020; Gelaye, 2021}. Gelaye and colleagues {Gelaye, 2021 }, comparing three variations of the Baermann technique, foud that the pre-incubation step of 3g of stool with activated charcoal for 18-24h at room temperature, followed by 2.5h filtration through gauze, gave better results (sensitivity 87% when using results of all the three techniques combined as reference standard) than the same technique without pre-incubation (sensitivity 22.1%), and than the use of 10g of stool pre-incubated with charcoal but allowed to filter through tissue paper for 1h (sensitivity 26.7%). The authors {Gelaye, 2021} ascribed the better performance to the incubation procedure, which allowed the parasite to enter the free-living cycle, and the application of gauze instead of tissue paper in the filtration step, as well as the use of a smaller amount of faeces, both allowing larvae to move more freely towards the water.

Much variation in this technique is also described regarding the amount of faeces used, ranging from as much as >30 g {Amor, 2016; Aramendia Aa, 2020; Salazar, 1995} to as little as 2 g {Gelaye, 2021}, and in the filtration time, from 1h to overnight {Gelaye, 2021; de Kaminsky, 1993; Graeff-Teixeira, 1997; Hailegebriel, 2017}.

In published papers, the reported sensitivity of the Baermann technique ranges from 20-100% {Knopp, 2008; Hernández-Chavarría, 2001}, most commonly however falling in the range of 40-80% {Aramendia Aa, 2020; Becker, 2015; Blatt, 2003; de Kaminsky, 1993; Carvalho, 2012; Hailegebriel, 2017; Inês, 2011; Khieu, 2013; Krolewiecki, 2010; Lau Chong, 2005; Machicado, 2012; Meurs, 2017; Chankongsin, 2020; Schär, 2014}. In a systematic review and meta-analysis, Campo-Polanco and colleagues {Campo Polanco, 2014} estimated the global sensitivity of the Baermann technique at 72% (95%CI 67-76%).

Agar Plate Culture (APC) and its modifications

The use of agar plate to culture *S. stercoralis* was first described by Arakaki and colleagues in 1988, based on the observation that occasionally bacterial colonies grew in the form of furrows in coprocultures using nutrient agar plates for bacteriology {Arakaki, 1988}. The APC technique takes advantage of the capacity of the parasite to develop its free-living cycle, and the method was standardized by Koga and colleagues {Koga, 1991}. Briefly, about 2-3g of stool are placed at the centre of a nutrient 1.5% agar sterile medium (containing 0.5% meat extract, 1% peptone and 0.5% NaCl) in a Petri dish and incubated at room temperature (26-33°C). Cultures are maintained generally for 48h, although prolongation until 7 days may increase the detection rate {Jongwutiwes, 1999}. Tracks resulting from bacterial growth along the path of larval movement may not be always macroscopically evident, or even visible after examination of the plate by microscopy; therefore, careful washing of the plate surface using 10% formalin or water and observation of the sediment by microscopy are required to complete the reading {Koga, 1991}.

In their original study, Arakaki and colleagues {Arakaki, 1988 found that using a 37°C incubation of finger-head size stool for 48h, *S. stercoralis* could be detected in 4.5-5.7% samples from patients accessing their clinic, compared to 0% by DS, 0.3% by Harada-Mori test tube culture, and 0.8% by FECT. Based on results from cultures with serial dilution of faeces containing a known number of larvae, Koga and colleagues {Koga, 1992} estimated that at least 3 g of stool should be used. Optimal culture conditions were further described by Kaewrat and colleagues {Kaewrat, 2020} and confirmed by Senthong and colleagues {Sengthong, 2020}, using a yeast extract-based agar with 0.5% NaCl, pH 6.0 and incubation temperature of 29-30°C. However, also non-nutrient agar using 1% plain food agar has been tested and reported similar performance to nutrient agar {Sukhavat, 1994}. A recent study in Thailand found that use of Oxoid™ nutrient agar adjusted to pH 6.0 was superior to the Koga agar formulation for the recovery of larvae {Kaewrat, 2020}.

To prevent larvae from crawling out of the plate, with consequent alteration of results and risk of infection for the operator, Arakaki et al {Arakaki, 1988} suggested the use of a double-walled Petri dish with 25% glycerine half-filling the outer space between the plates {Arakaki, 1990}. This has been replaced by simply sealing the Petri dish using sealing films {Koga, 1991}.

A modification of the APC technique was described by Khanna and colleagues {Khanna, 2015}, which consisted in the cutting of a 1cm wide canal around a square area of 2x2 cm in an Agar plate, filled with saline or water. About 2g of stool are placed on the central square area and incubated for 3 days; larvae and adults could then be retrieved from the liquid in the canal using a pipette introduced through a hole in the plate lid, without the need to open the plate and wash the surface of the agar. While the authors declared that this technique “had better yield of parasites” compared to the conventional APC method {Khanna, 2015}, no actual figures were provided.

APC is generally considered the most sensitive coproparasitological technique for the diagnosis of *S. stercoralis* infection, with reported sensitivity in the range of 60-98% {Arakaki, 1990; Blatt, 2003 ; Hailegebriel, 2017; Hailu, 2021; Hernández-Chavarría, 2001; Inês, 2011; Intapan, 2005; Jongwutiwes, 1999; Khieu, 2013; Kobayashi, 1996; Koga, 1991; Lau Chong, 2005; Machicado, 2012; Chankongsin, 2020; Schär, 2014} although lower figures (20-50%) are also reported {Anamnart, 2013; Becker, 2015; Buonfrate, 2017 ; de Kaminsky, 1993; Knopp, 2008; Krolewiecki, 2010}. In a systematic review and meta-analysis, Campo-Polanco and colleagues {Campo Polanco, 2014} estimated the global sensitivity of the APC technique in 89% (95%CI 86-92%).

Filter paper-based culture techniques

The Harada-Mori technique was described in 1955 for the diagnosis of hookworm by the authors after whom it is named {Harada, 1955}. Around 1g of fresh faeces are smeared on a 2-3 cm wide filter paper strip. One of the clear extremities of the strip is dipped into water contained in a tube and incubated at 20-30 °C for 3-10 days. The water is then centrifuged and the sediment examined under the microscope for the presence of larvae.

Another filter paper-based culture technique consists in mixing equal parts of fresh stool and activated charcoal or vermiculite in the centre of a Petri dish layered with moisten filter paper, incubated for several days while maintaining the moisture; larvae are retrieved from the plate by further addition of water at the end of the incubation period, and observed under a microscope {Hailegebriel, 2017; Meurs, 2017; Polderman, 2010}.

These methods have generally lower sensitivities (in the range of 19%-57%) compared to the APC and Baermann techniques {Blatt, 2003; Hailegebriel, 2017; Jongwutiwes, 1999; Kobayashi, 1996; Koga, 1991; Krolewiecki, 2010; Machicado, 2012; Meurs, 2017}**.**

Other reported techniques based on larval migration

A number of other techniques taking advantage of larval migration have been described. Dancescu {Dancescu, 1968} described a method consisting in mixing equal parts of charcoal and faeces (6-9 g each) in a cone-shape inside a small box of transparent plastic, with the top of the cone touching the inner surface of the transparent lid of the box. After incubation, larvae can be observed in the condensation drops through microscopic observation of the inner surface of the lid. Lau-Chong and colleagues {Lau Chong, 2005 #1751} applied this technique in a field study in Peru, finding a sensitivity of 92.3% when the reference standard was positivity in any of the applied DS, STST, Baermann in flask, and APC.

The water-emergence technique consists in producing a central depression in a fresh stool specimen, filled with lukewarm water, and incubated at 37°C for 1h to allow larvae to migrate into the dip. These can be then recovered and examined by microscopy. In their study in the hospital setting in Ethiopia, Gataneh and colleagues {Getaneh, 2010} found that this technique had a sensitivity of 85.1% when the reference standard was positivity in any of the applied DS, FECT and water-emergence tests.

Strategies to improve detection rates by coproparasitological techniques

Different coproparasitological techniques can be applied on the same sample to achieve better sensitivity since it is a common finding that there is imperfect or even limited overlap between samples found positive with one or with another result from different methods in an examined population {Anamnart, 2010; Aramendia, 2020; Becker, 2015; de Kaminsky, 1993; Inês, 2011; Intapan, 2005; Kobayashi, 1996}. The same

applies when molecular techniques (PCR on stool) are performed in parallel to coproparasitological techniques such as APC or Baermann {Kristanti, 2018; Amor, 2016; Aramendia Aa, 2020; Becker, 2015; Buonfrate, 2017; Krolewiecki, 2010; Meurs, 2017; Schär, 2013; Chankongsin, 2020}.

To deal with the problem of intermittent elimination of larvae and of the low number of larvae generally eliminated in chronic infections, the diagnostic sensitivity could also be increased by increasing the amount of faeces examined, and examining multiple samples produced in different days from the same individual {Dacal, 2018; Dreyer, 1996; Khieu, 2013; Knopp, 2008; Lopez, 2016; Nielsen, 1987; Repetto, 2016; Sato, 1995; Steinmann, 2007; Uparanukraw, 1999}. However, infection confirmation proves generally difficult. Sato and colleagues {Sato, 1995} applied multiple coproparasitological techniques (DS, FECT, and APC) to re-confirm *S. stercoralis* infection 5 and 18 months after initial diagnosis and found that maximum rate of reconfirmation was achieved by APC. However, this reconfirmation rate was unsatisfactory, with only 60% of infections re-detected by APC when performed once, and 85% when re-performed on three specimens collected on different days. Re-detection rates by other techniques ranged from 16% (DS) - 24% (FECT) when performed on one stool specimen, to 45% (FECT) – 51% (DS) when performed on three specimens {Sato, 1995}. Uparanukraw and colleagues {Uparanukraw, 1999} found that 9.8% of individuals initially deemed negative based on a single APC-negative result were actually infected when up to 6 consecutive specimens over a month were cultured; furthermore, up to 8 consecutive examinations by APC over two months were necessary to re-confirm all 56 *S. stercoralis*-positive individuals initially diagnosed as infected in their cohort. Dreyer et al {Dreyer, 1996} applied the Baermann-Moraes technique weekly over 2 months using 25 g of faeces and examining the whole sediment of 40 ml sedimentation fluid (about 100 slides/sample) in 108 patients with proven *S. stercoralis* infection, finding that the maximum sensitivity (n=72; 66.7%) for the scheduled timeframe was achieved at the 4th weekly examination. The remaining 36 patients had a positive Baermann only between week 9 and 21, always with weekly examinations. Furthermore, elimination was not constant week to week in individual patients, although those with detectable larvae over consecutive weeks tended to eliminate larvae more constantly over time than the others. Single specimen examination had a sensitivity ranging from 19.4 to 36.1% {Dreyer, 1996}. In a study conducted in Tanzania, it was estimated that up to 12 samples collected in different days would have to be analysed by APC and up to 20 by Baermann if ≤1% false negative results were allowed {Knopp, 2008}. Similarly, when using the mathematical model developed by Marti and Koella {Marti, 1993} to estimate the "true" prevalence of helminth infection based on examination of multiple stool samples, Steinmann and colleagues in China {Steinmann, 2007} found that sensitivity of single APC or single Baermann were 63% and 48%, respectively, while increased to 95% and 86%, respectively, when 3 stool samples collected on consecutive days were examined; using APC with 3 samples, the estimated *S. stercoralis* prevalence in the investigated province (11.7%) reached the "true" prevalence calculated by the mathematical model (12.3% ± 5.1%).

Identification of *S. stercoralis* in diagnostic preparations and differential diagnosis with other parasites

Already during the first studies using the APC {Arakaki, 1990}, the emergence of larvae of different species was observed, especially after 48h incubation, flagging the importance of differentiating the retrieved parasites {Arakaki, 1990; Jongwutiwes, 1999; Inês, 2011; Koga, 1991}. The same applies in case of delayed processing from the time of defecation.

The shape of tracks on agar and the movement pattern of the larvae could be of some use in their identification, but these parameters cannot be completely relied upon, and careful morphological identification is always required {Jongwutiwes, 1999; Inês, 2011}. Furthermore, tracks are not always visible in positive samples; therefore, it is important to carry out microscopic examination of the surface and of

the plate surface washing sediment also of plates with no visible furrows {de Kaminsky, 1993}. Depending on the time elapsing from stool production to microscopic examination of the processed sample, several stages of *S. stercoralis* can be observed, which should be differentiated from larvae of other parasites hatching from eggs upon culture, depending on areas of co-endemicity {Grove, 1996; Blotkamp, 1993; Bradbury, 2019; Bradbury, 2021}.

L1 (Figure 2) are 180-240 µm long, with a shallow buccal capsule and a muscular oesophagus which occupies the anterior one-third of the body, divided into an anterior cylindrical corpus and a posterior rounded bulb. L1 can be observed within 1-2 days in freshly passed stool or after culture, produced by free-living adult worms (Figures 3 and 4) developed in cultured fecal samples. *S. stercoralis* L1 can be differentiated from hookworm, *Trichostrongylus* spp., *Oesophagostomum* spp., *Ternidens deminutus* and *Rhabditis* spp. L1 mainly on the basis of the short buccal capsule and prominent rhomboid genital primordium placed about half-way down the length of the larva (Fig. 5). Also, *S. stercoralis* L1 larvae, as well as those of *T. deminutus*, have an evident genital primordium, contrary to the other species; however, rhabditiform larvae of the latter have a longer tail, with larvae measuring 300-360 µm.

Through an L2 stage, characterized by a progressive lengthening of the body and of the oesophagus, iL3 develop after further 1-2 days of culture (Fig 6). *S. stercoralis* filariform larvae are 490-630 µm long and slender, with a typical filariform oesophagus, and are characterized by a notched tail and a 1:1 esophagus to intestine ratio (Fig 6). Figure 7 shows the morphology of filariform larvae of different helminths.

Hatched larvae of *S. f. fuelleborni* and of S*. f. kellyi* are indistinguishable from those of *S. stercoralis*; these species can be differentiated in their cultured free-living adult stages, although morphological differences are subtle {Speare, 1989}.

Unfortunately, little attention is given today to the maintenance of “classic” parasitology skills, including morphology, in the curriculum of health-care professional figures who may be involved in various aspects of diagnostic parasitology. This often leads to incorrect species identification, adding to the problems in accurate mapping of infection and evaluation of diagnostic techniques and treatment interventions inherent in the biology of the parasite.

Immunodiagnosis

Serodiagnosis with enzyme-linked immunosorbent assays (ELISAs)

The use of tests based on antibody detection has diffused widely in non-endemic countries for the screening of strongyloidiasis. This is mostly due to the availability of commercial enzyme-linked immunosorbent assays (ELISAs), which are easy to perform and less time-consuming compared to parasitological methods. In addition, several in-house assays have been described in the literature. Overall, the accuracy reported is good, though differences in the reference standard and panel of samples used to estimate sensitivity and specificity limit the evaluation of the performance of individual tests, and the comparison between different assays. The ELISAs also differentiate into assays based on crude antigens retrieved from *Strongyloides* larvae and those using recombinant antigens, as specified in the following paragraphs. Table 3 reports the sensitivity and specificity values of different ELISAs, as estimated in selected diagnostic studies. We included in the table only studies comparing at least 2 different assays and reporting confidence intervals.

Crude antigen-based ELISAs

ELISAs commercially available at the time of writing detect IgG-class antibodies to crude antigens (CrAg) extracted from different species of *Strongyloides,* including *S. stercoralis*, *S. ratti*, and *S. papillosus* {Bon, 2010; Buonfrate, 2021; van Doorn, 2007}. The use of antigens from species not infecting humans is due to the risk of infectivity with *S. stercoralis* for the laboratory staff and to an easier access to antigen sources, due to the possibility of maintaining the life cycle of the parasites in the laboratory {Huaman, 2003; Nutman, 2017; Sykes, 2011}.

Overall, the high sensitivity, ranging from 83 to 95% {Fradejas, 2018; Bon, 2010; van Doorn, 2007; Bisoffi, 2014; Buonfrate, 2021; Ruantip, 2019} is the main strength of the CrAg ELISAs available on the market. Specificity, ranging from 42.1 to 97.4% {Fradejas, 2018; Ruantip, 2019; Bon, 2010; Bisoffi, 2014; Buonfrate, 2021; van Doorn, 2007} is influenced by possible cross-reactions with other helminths, such as filarial nematodes and *Schistosoma* spp {van Doorn, 2007; Bisoffi, 2014}. Although the result provided in routine practice is qualitative, increasing levels of the normalised optical density (nOD), which is the signal to cut-off ratio, were associated to higher specificity in diagnostic studies {Bisoffi, 2014}.

A commercial CrAg ELISA demonstrated excellent agreement of results obtained from serum compared to those obtained from dried blood spots collected on filter paper {Formenti, 2016; Tilli, 2021}. This would be useful for fieldwork, as transport and storage of samples from remote areas would be easier.

In literature, different in-house CrAg ELISAs have also been evaluated, with sensitivity and specificity ranges similar to those observed for the commercial assays {Lindo, 1993; Loutfy, 2002; Machado, 2003; Sithithaworn, 2003; van Doorn, 2007; Ruantip, 2019; Eamudomkarn, 2015}.

Seroreversion usually occurs after treatment, although it requires several months {Loutfy, 2002; Page, 2006; Buonfrate, 2015; Biggs, 2009}. Thus, false positive cases due to previous infections are presumably limited to recently treated cases. The seroreversion allows using these tests to monitor the response to treatment and, in case of quantitative result, a decrease of the nOD can be gradually observed over time {Buonfrate, 2015}.

Drawbacks of the CrAg ELISAs are the lower specificity compared to parasitological methods, variable reproducibility between different batches of the assays, due to different antigen lots, and the difficulties related to the production of filariform larvae for antigen extraction, which is time-consuming and hampers the production of large quantities of the assay {Pak, 2014}. Moreover, sensitivity might be lower in immunosuppressed individuals, with a reduced antibody response {Abdul-Fattah, 1995; Ahmed, 2019} and in very early infection {Ming, 2019}.

Recombinant antigen-based ELISAs

In order to overcome these issues, ELISAs based on recombinant antigens have been implemented, most of them currently available for research-use-only (RUO). The recombinant antigens most frequently used are a 31-kDa antigen derived from the L3 cDNA library, named NIE {Ravi, 2002}, and the *S. stercoralis* immunoreactive antigen, SsIR {Ramanathan, 2008}. The sensitivity and specificity of ELISAs based on NIE range between 70.8-97% and 57.9%-95% {Ramanathan, 2008; Ruantip, 2019; Fradejas, 2018; Anderson, 2014; Bisoffi, 2014}, respectively. Similar to what observed for CrAg ELISAs, a RUO NIE ELISA proved useful for post-treatment monitoring, both at individual and at community level {Buonfrate, 2015; Vargas, 2017; Ramanathan, 2008}. Further, the same test demonstrated excellent agreement between results obtained on serum with those obtained from dried blood spots {Mounsey, 2014}. More recently, ELISAs using a combination of NIE and SsIR have been implemented, to further improve the accuracy of the tests. A novel NIE/SsIR assay detecting IgG demonstrated good performance in a retrospective diagnostic study {Tamarozzi, 2021}, with 78% sensitivity (95% CI: 72–83%) and 98% specificity (95% CI: 96–100%). In the

same study, a NIE/SsIR ELISA detecting IgG4 showed lower accuracy, with sensitivity and specificity of 70% (64–76) and 97% (95–100), respectively.

Other Experimental ELISAs

Some experimental ELISAs detecting different immunoglobulin isotypes and subclasses (such as IgG1, IgG4, IgE and IgA) {Atkins, 1997; Arifin, 2013; Norsyahida, 2013; Ahmad, 2020; Rodrigues, 2007; Ahmad, 2020 have been evaluated, with the aim of improving the specificity of the assay and/or the capacity of diagnosing acute infection. In particular, IgG4 is the subclass most studied, deemed promising for the high specificity for helminth intestinal infections {Arifin, 2019; Norsyahida, 2013}. However, the results are still inconsistent, and the reduced sensitivity of these assays compared to IgG-based ELISAs, in particular during chronic infection (such as IgG1 and IgE which are probably downregulated during chronic infection) {Arifin, 2013; Atkins, 1997; Ramanathan, 2008; Ahmad, 2020; Kubofcik, 2016} has so far limited the production.

In the last decade, the Phage Display technology has been deployed to identify peptides mimicking *S. stercoralis* antigens (mimotopes), which can be used for the implementation of phage ELISAs {Levenhagen, 2021}. Phage-based tests would have the advantages of high reproducibility, low cost and potential massive production {Levenhagen, 2021; Feliciano, 2014; Miguel, 2020}. Moreover, the Phage Display technology has been used to select functional probes that can detect immune complexes circulating in *S. stercoralis* infected individuals. Tests based on these probes would have the advantage of identifying active infection {Levenhagen, 2021; Miguel, 2020}.

Also, the use of biological materials other than blood/serum has been evaluated, in order to make sample collection more accessible and accepted by individuals. An in-house ELISA to detect IgG in urine had fair agreement (k= 0.615) with the results of the same assay using serum {Ruantip, 2019}. Other authors found either IgG, IgA or IgG immune complexes in saliva, using in-house ELISAs {Bosqui, 2015; Bosqui, 2017}. These techniques still need further evaluation of their accuracy.

Other assays based on antibody detection

Among the different assays that have been evaluated for the detection of *S. stercoralis* antibodies, the luciferase immunoprecipitation system (LIPS) is probably the one that demonstrated the best performance so far, in particular in terms of specificity, compared to CrAg and recombinant antigen ELISAs {Ramanathan, 2008; Bisoffi, 2014; Krolewiecki, 2010}. The test is based on the fusion of a specific antigen with the enzyme reported *Renilla* luciferase (Ruc). A mixture of Ruc and patient serum is transferred to a plate containing A/G beads which capture IgG molecules. The antibody bound Ruc-antigen is then visualized by luminometer {Ramanathan, 2008}. LIPS is a technology which allows obtaining a quantification of antigen-specific antibodies in a relatively short time. Compared to ELISAs, the background noise is extremely reduced, leading to a high signal {Ramanathan, 2008}. LIPS assays based on either NIE or the combination of NIE and SsIR were evaluated for the detection of IgG and IgG4 to *S. stercoralis*. Overall, sensitivity and specificity ranged from 87 to 100% and from 91 to 100%, with the lowest sensitivity values estimated in the IgG4 format {Ramanathan, 2008; Krolewiecki, 2010; Bisoffi, 2014}. Despite the excellent performance, this assay is not widely available, and the ELISA format is still considered a more practical solution for routine use.

Other in-house techniques that have been described in the literature include immunofluorescence tests (IFAT) and immunoblot techniques {Lindo, 1993; Boscolo, 2007; Bisoffi, 2014; Conway, 1993; Machado, 2008; Silva, 2003; Andreetta Corral, 2019}. Immunoblots have also been used to identify other potential biomarkers {Sudré, 2007; Varatharajalu, 2011; Corral, 2015; Andreetta Corral, 2019}. The assays differ widely between each other for the target proteins. Overall, the sensitivity and specificity of the assays

range between 82%-97% and 96-100% {Lindo, 1993; Silva, 2003}, respectively. A biplex Western blot based on the recombinant antigens NIE and SsRI detecting IgG4 was implemented recently, showing high sensitivity and specificity in an experimental study {de Souza, 2021}. IFAT assays have been implemented using antigens from either *S. stercoralis* or different species; overall, they demonstrated good sensitivity and specificity, ranging from 87%- 95% and 87%-100%, respectively {Bisoffi, 2014; Buonfrate, 2017; Costa-Cruz, 1997; Koosha, 2004; Silva, 2003; Gottardi, 2015}. The test proved also useful for post-treatment monitoring {Buonfrate, 2015; Boscolo, 2007}. Although accuracy proved similar or slightly better than ELISA assays, they have a more laborious execution and interpretation, so they are less deployed in routine practice.

Other assays were implemented with the aim of developing rapid, easy-to-use tests, which could also be deployed in remote endemic areas. These are the gelatin particle agglutination tests (GPAT) and the immunochromatographic tests (ICT). Experimental GPAT were implemented through sensitization of gelatin particles with either *S. stercoralis* or other species {Ahmed, 2019; Huaman, 2003; Sithithaworn, 2005}. They demonstrated good accuracy, with increased sensitivity compared to ELISAs, in particular for diagnosis in immunocompromised individuals {Ahmed, 2019; Huaman, 2003}. However, the number of published studies is still limited for a thorough evaluation of this technique.

ICT, or lateral-flow assays, are easy-to-use and rapid point-of-care tests. Some prototypes based on *S. stercoralis* CrAg showed good sensitivity (ranging from 91% to 93.3%) and specificity (from 83.7%-97.7%) {van Doorn, 2007; Sadaow, 2020}. To overcome the same limitations listed for the CrAg - ELISAs, ICT based on recombinant antigens were also implemented. A dipstick test detecting IgG4 and based on the combination of NIE and Ss1a produced promising results {Yunus, 2019} and was afterwards implemented in a cassette format, but with the inclusion of a single recombinant antigen (NIE) {Noordin, 2021}. Another group of researchers evaluated two SsIR - ICT, either detecting IgG or IgG4 {Sadaow, 2020}. Overall, the ICT based on recombinant antigens showed good performance, with 82-91% and 84-100% sensitivity and specificity, respectively {Sadaow, 2020; Noordin, 2021; Yunus, 2019}. Due to these promising preliminary results, and appeal of use, these recombinant antigen-based ICT deserve further evaluation.

Tests based on antigen detection

Coproantigen tests

The coproantigen tests detect antigen biomarkers present in stool. This method is usually deemed useful to diagnose infections in the pre-patent period {Little, 2019 #3482}, and to differentiate current from previous infections, due to the rapid negativization after treatment. A coproantigen can also be deployed in a point-of-care rapid test format. As for the diagnosis of strongyloidiasis, a few coproantigen ELISAs have been evaluated mostly in experimental studies in rats {Chaves, 2015; Nageswaran, 1994; Sykes, 2011}. A proof of principle study {Sykes, 2011} in humans is also available. The assays were implemented using either anti-*S. venezuelensis* or *S. ratti* IgG. Overall, they proved successful in detecting the target antigens, but further development and studies in humans are needed.

Skin test reaction

Some experimental studies describing diagnosis through skin test reaction have been published. They were based on intradermal injection of extracts retrieved from disrupted larvae from either homologous or heterologous *Strongyloides* species, causing immediate hypersensitivity {Pellegrino, 1961; Porto, 2001; Sato, 1986}. Reduced reactions were observed in individuals with HTLV-1 co-infection {Neva, 2001; Porto, 2001}. No further implementation of such tests has been reported in the last two decades.

Molecular diagnosis

The use of nucleic acid amplification tests (NAATs) such as PCR and Loop-Mediated Isothermal Amplification (LAMP) tests for the diagnosis of strongyloidiasis offers several advantages over traditional coprological and serological approaches, in research studies and for clinical diagnostics. Samples may be easily preserved and tested weeks, months, or years after collection. Preserved specimens may be easily transported long distances at ambient temperature, even in adverse tropical environments, and batch tested in the destination laboratory. Preservation of specimens for DNA analysis also eliminates the risk of laboratory acquired infection inherent in working with human faeces, serum and cultured *Strongyloides* larvae {Bradbury, 2020}. However, it must be recognised NAATs are not 100% sensitive or specific for the diagnosis of *S. stercoralis* and there is an absence of clinical validation data for most available NAATs. Furthermore, pre-analytical variables such as method of preservation and method of DNA extraction, and analytical variables, such as the presence of PCR inhibitors in the sample, the quality of PCR reagents, and the efficiency of the PCR cycler employed, can markedly affect the performance of these tests. Although these assays may detect free *S. stercoralis* DNA in faeces in the absence of visible larvae, false negatives also do occur {Verweij, 2009}.

PCR assays for *Strongyloides stercoralis*

Several conventional, nested, and real-time PCRs targeting variously portions of the *S. stercoralis 5.8S rRNA*, *18S rRNA*, *28S rRNA*, ITS-1, ITS-2, or DNA repeats in the *S. stercoralis* genome, have been published {Kramme, 2011; Holt, 2017; Verweij, 2009; Pilotte, 2016; Repetto, 2013; Sitta, 2014; Lodh, 2016; Ghasemikhah, 2017; Sharifdini, 2015; Robertson, 2017; Nilforoushan, 2007}. Few of these are validated against a suitable reference standard. On review, the nested PCR described by Sharafidini et al. {Sharifdini, 2015} appears to have the greatest diagnostic sensitivity, but more validation is required. The ease of amplicon contamination leading to false positives when using a nested approach means very comprehensive PCR product contamination precautions must be taken if routine diagnostic use of this assay is intended. Furthermore, in modern diagnostic laboratories, there is a general preference for real-time PCR assays due to their improved sensitivity compared to conventional PCR and capacity to provide at least partial quantification of larval load {Llewellyn, 2016}. A recent systematic review of the literature revealed that behind a high specificity, the sensitivity of PCR was unsatisfactory {Buonfrate, 2018} suggesting that PCR could not be considered superior to other parasitological technique such as Baermann test {Buonfrate, 2018; Chankongsin, 2020}.

A *Strongyloides* real-time PCR (qPCR) assay developed by Verweij et al. {Verweij, 2009}, with or without modifications, remains the most comprehensively validated and widely employed NAAT assay in both diagnostic and research laboratories. This assay targets a 101 bp portion of the 18S ribosomal small subunit gene. It should be noted that the target is not specific to *S. stercoralis* and other species of *Strongyloides* will be amplified by this assay, including *S. f. fuelleborni*, *Strongyloides venezuelensis, S. ratti* and other *Strongyloides* spp. {Saugar, 2015; Barratt, 2019}. Only the Verweij PCR has been used in the testing of dogs. This has been performed in Italy, Australia, Kiribati, and elsewhere {Buonfrate, 2017; Beknazarova, 2019; Beknazarova, 2020; Zendejas-Heredia, 2021} but no diagnostic validation studies have been performed using the assay on faecal samples from this host. It has been demonstrated that the assay will amplify both lineages of *S. stercoralis* (lineage A; reported from humans, dogs, cats, and non-human primates, and lineage B; apparently restricted to dogs) {Beknazarova, 2019}. The assay has not yet been used for the diagnosis of strongyloidiasis in cats or non-human primates. It is not known if it will amplify other canine and feline *Strongyloides* spp., such as *Strongyloides tumefaciens* and *Strongyloides felis*, though it does amplify cryptic *Strongyloides* spp. of Australian dogs {Beknazarova, 2019}.

Holt, et al. {Holt, 2017} modified the Verweij PCR by designing an alternative forward primer to improve specificity in their laboratory in the Northern Territory of Australia. This modified version had a 471 bp

product. Though the adaptation compared favourably with APC, it was not thoroughly validated {Holt, 2017}. Holt's modified forward primer has been employed both in an individual PCR assay, and as part of a five-target multiplex assay targeting three intestinal protozoa, *S. stercoralis* and equine herpes virus (EHV) control {Llewellyn, 2016}. This assay was determined to be semi-quantitative when tested against dilutions of a plasmid control. When tested against 467 samples from East Timor and 213 from Cambodia, only one PCR positive (from East Timor) was detected. This was despite four of the Cambodian samples having *Strongyloides* larvae detected by microscopy {Llewellyn, 2016}. Given the known high prevalence of *S. stercoralis* in Cambodia and likely moderate to high prevalence in East Timor, this result is surprising and a comprehensive validation of this PCR is indicated prior to further use. Barda et al. {Barda, 2018} also modified the forward primer of the Verweij et al. {Verweij, 2009} PCR and conducted validation studies of this modification using Baermann sedimentation on two separate stools as a reference standard. The assay was also clinically validated using two DNA extraction techniques. The method of DNA extraction greatly affected the sensitivity and specificity of the PCR assay (Table 4).

Another *S. stercoralis* q PCR assay was published by Pilotte et al. {Pilotte, 2016}. This assay adopted a new approach to primer design, by scanning the genome for repetitive elements which could be used as PCR targets. This approach is designed to yield a more sensitive assay due to high copy number repeats being chosen. The assay was validated against a panel of 79 stool samples collected in East Timor and already tested using Holt's primers {Llewellyn, 2016}, only one of which was positive for *S. stercoralis*. The assay correlated well against this panel, but a far more robust clinical validation using a high sensitivity reference standard is required. The assay has been used thus far in a small number of parasite prevalence studies {Benjamin-Chung J, 2021; Bradbury, 2020; Bradbury, 2021}.

Chankongsin et al {Chankongsin, 2020} recently described an *S. stercoralis* TaqMan (Thermo Fisher Scientific) qPCR based on the primers on primers originally developed for using a fluorescence energy transfer (FRET) qPCR described by Kramme et al {Kramme, 2011}. This was clinically validated in the Lao PDR and found to have a moderate sensitivity (66.8%) when compared to a combination of only APC and Baermann sedimentation as a reference standard.

Commercial multiplex qPCR panels are increasingly being used in diagnostic laboratories. Only one such panel, the The Allplex™ GI-Helminth (I) Assay, includes primers for *S. stercoralis*. These primers and their target are commercial-in-confidence. The Assay demonstrated an 80.0% sensitivity and 97.3% specificity when tested against a panel of faeces positive for various parasites by microscopy {Autier, 2018}. This sensitivity was increased to 86% with the addition of a bead-beating step prior to DNA extraction. It should be noted that various techniques had been used individually or in combination to assemble the reference panel. A prospective clinical validation performed in an endemic community and using a high sensitivity reference test would be of benefit to better determine the assay performance characteristics.

Recently, a promising *S. stercoralis* duplex droplet PCR assay has been described. Validation studies of this assay compared to microscopic techniques (FECT and APC) are promising, with a 98% sensitivity and 90% specificity reported. The limit of detection was a single larva and no cross-reactions with other parasites were reported {Iamrod, 2021}.

Loop-mediated isothermal amplification (LAMP) assays for *Strongyloides* Diagnosis

LAMP assays have advantages over PCR in that they require less complex equipment for performance. By using technology based on polymerases with strand-displacement activity that can amplify nucleic acid under isothermal conditions, equipment is simplified without the need for the for the cycling reactions of PCR {Watts, 2014}. There is also the potential to use lyophilized reagents that are stable at room temperature {Watts, 2019}. Like PCR, stool diagnosis with LAMP also requires highly specific primers due to the presence

of DNA from a large number of other sources, including foodstuffs and other organisms {Watts, 2014; Verweij, 2009}. Two LAMP assays have been developed for *Strongyloides* diagnosis {Watts, 2014; Fernández-Soto, 2016}.

The primers developed by Watts et al. {Watts, 2014} target a 184 bp region on the 28S ribosomal subunit gene and are comprised of forward and backward outer primers (F3, B3), forward and backward inner primers (FIP, BIP) and a loop primer (LB) to reduce the reaction time. The assay also employed Syto-82 (Life Technologies), to allow visualisation of positive results without opening the reaction tube to reduce the risk of laboratory contamination with amplified product. The analytical specificity of the primer set was testing using human DNA and a range of DNA extracted from gut bacteria, fungi and parasites and testing 30 *Strongyloides*-negative stool specimens from a low prevalence area {Watts, 2014}. The analytical sensitivity / limit of detection (LoD) was determined using serial dilutions of a plasmid with target DNA with detection to <10 copies (Watts et al., 2014). Serial dilutions were also made of DNA extracted from negative stool spiked with single *S. ratti* larvae with a LAMP assay limit of detection of 10^{-2} dilution, compared to 10^{-3} for a PCR assay based on the method developed by Verweij et al. {Watts, 2014; Sultana, 2013; Verweij, 2009; Watts, 2019}.

The Watts et al. {Watts, 2014} LAMP assay was also compared to a PCR, based on the method by Verweij et al. {Verweij, 2009}, in a survey of DNA extracts from three different locations: Dhaka, Sydney and northern Queensland. Based on the primer design the assay will also detect *Strongyloides* spp. other than *S. stercoralis*, including *S. ratti* {Watts, 2014}. While concordance with negative results (negative percentage agreement) was 100% with the LAMP and PCR assays, the concordance of positive results (positive percentage agreement) ranged from 91.6% to 71% depending on the source of the DNA extracts {Watts, 2019}. Using the Verweij et al. {Verweij, 2009} PCR as a reference, the total positive percentage agreement was 77.4% (95% C.I. 63.8% to 87.7%) and the total negative percentage agreement was 100% (95% C.I. 98.9% to 100%). Some of this variation may have been due to DNA degradation in some of the samples and a clinical study that compared freshly extracted stool samples would be valuable. Modifying reagents such as the DNA polymerase may also improve sensitivity. While this assay shows promise, a clinical validation against a high sensitivity phenotypic reference standard such as APC, Baermann sedimentation, or both is still indicated.

The LAMP assay developed by Fernández-Soto et al. {Fernández-Soto, 2016} targets a 329 bp region on the 18S ribosomal subunit gene and is comprised of forward and backward outer primers (F3, B3), forward and backward inner primers (FIP, BIP) {Fernández-Soto, 2016}. Positive results were confirmed using the addition of SYBR Green (Invitrogen) at the completion of the reaction {Fernández-Soto, 2016}. On analytical specificity testing, there was no amplification of DNA from a range of gut parasites (Fernández-Soto et al., 2016). The LoD of the LAMP assay was determined using serial dilutions of *S. venezuelensis* larval DNA and was found to be 0.01 ng {Fernández-Soto, 2016}. This was also the limit of detection for a PCR assay based on the F3 and B3 LAMP primers.

The Fernández-Soto et al. {Fernández-Soto, 2016} LAMP assay was effective in detecting *Strongyloides* DNA in urine and stool using a rat model (*S. venezuelensis*). Out of a series of 11 human stool samples, 7 were positive using microscopy-based diagnostics and the LAMP assay {Fernández-Soto, 2016}. Subsequently, urine from 24 individuals diagnosed with strongyloidiasis on the basis of microscopy-based methods or serology were tested with the LAMP assay and the PCR assay based on the F3 and B3 primers {Fernández-Soto, 2020}. Twelve of the 24 urine samples were positive using the LAMP assay and all were negative on the PCR assay {Fernández-Soto, 2020}. A cause of the discrepancy may be that the PCR reaction was inhibited by protein in the urine and the LAMP assay was less affected {Fernández-Soto, 2020}. Further development of the LAMP assay would include additional analytical specificity testing using DNA extracted from gut bacteria and fungi {Fernández-Soto, 2016} and clinical validation studies.

NAAT Testing on Non-Stool Specimens (urine, respiratory samples, serum, cerebral spinal fluid)

A number of preliminary studies have investigated the diagnosis of strongyloidiasis using urine, respiratory specimens and serum. In terms of urine specimens, Fernández-Soto et al. {Fernández-Soto, 2020} used a LAMP assay to detect *S. stercoralis* DNA in urine from 12 of 24 patients diagnosed with strongyloidiasis either by FECT, APC or serology (also see the *Loop-mediated isothermal amplification (LAMP) assays for* Strongyloides *Diagnosis* section of this review). Serology positive patients had the poorest correlation {Fernández-Soto, 2020}. This study also used a touchdown PCR based on the LAMP primers and applied this to positive patient's urine samples, while the assay amplified *S. venezuelensis* DNA, it did not yield a product in any urine samples tested {Fernández-Soto, 2020}.

Use of the Verweij et al. {Verweij, 2009} qPCR on urine and faeces had better correlation, with urine yielding slightly less positives than faeces {Formenti, 2019}. Lodh et al. {Lodh, 2016} developed a conventional PCR targeting a DNA-repeats and with a 125 bp product. This was applied to testing faeces and urine of people from an endemic area of Argentina. The analysis of urine yielded almost double the number of positive results when compared to faecal analysis. As the authors acknowledge, more validation is required before the meaning of these results can be accurately interpreted.

To demonstrate proof-of-concept, microscopy-positive broncho-alveolar lavage (BAL) fluid was positive for *Strongyloides* on a PCR based on the Verweij et al. {Verweij, 2009} method and the Watts et al. {Watts, 2014} LAMP assay, after DNA was extracted using the High Pure DNA template preparation kit (Roche) {Watts, 2019}. Some serum samples from broncho-alveolar lavage and stool microscopy-positive persons were also positive using these assays, following serum DNA extraction using the using the NucliSENS easyMag system (bioMerieux), however, further validation for these specimen types, including analytical and clinical sensitivity testing is required.

Gorgani-Firouzjaee et al {Gorgani-Firouzjaee, 2018} also used their conventional adaptation of the Verweij et al. {Verweij, 2009} qPCR to screen the serum of immunosuppressed patients and compare this to microscopy and APC of their faecal samples. A six-fold higher number of positives were detected in the serum samples, but only five were sequenced (it is not stated if these five were from any of the six positives by coprological methods) and a *cox1* conventional genotyping target PCR yielded no amplification in the same DNA extracts {Gorgani-Firouzjaee, 2011}.

The Verweij PCR has been employed to detect and diagnose *S. stercoralis* infection in individual patient sputum {Beknazarova, 2019}. The Saugar SYBR Green modification of the Verweij PCR was used to simultaneously detected *S. stercoralis* DNA in BAL fluid, cerebrospinal fluid (CSF), serum, and tissue biopsies from the duodenum, stomach, oesophagus and bone marrow of an HIV positive patient with disseminated strongyloidiasis and multi-organ failure, leading to appropriate treatment and recovery {Le Pogam, 2020}.

It is important not to interpret positive results by NAAT in the absence of a positive reference test result as indicative of higher sensitivity of NAAT assay when used in urine, fluid, sputum, or serum matrices unless comprehensive validations are conducted to support this assumption. Validation studies should include an analytical specificity testing including faecal, urethral and vaginal flora which may be found contaminating urine in small quantities. The difficulties in interpreting comparison studies of PCR assays on urine, serum and faeces highlight the need for proper validation of assays prior to use in research or diagnostics.

Pre-analytical variables in NAAT testing

As important as choice of NAAT assay for diagnostic use is the choice of preservation and DNA extraction method. NAATs may be performed on any sample preserved in a way that maintains DNA integrity. It should be noted that formalin cross-links DNA and is thus not an appropriate preservative for use in NAAT testing. DNA extraction from stool requires an efficient nucleic acid extraction process due to reaction inhibitors, for example humic and tannic acids and nucleases, and the intermittent larval output in chronic infection {Watts, 2019; Dreyer, 1996}. The same pre-analytical variables which affect PCR also affect LAMP testing.

Faeces and other tissue and fluids for *S. stercoralis* PCR testing have previously been extracted from fresh faeces or faeces variously preserved by storage by freezing at -20°C {Campo-Polanco, 2018 }, -80°C {Bradbury, 2021 #6}, 100% ethanol {Robertson, 2017}, 96% ethanol {Meurs, 2017}, 70% ethanol {Becker, 2015}, Zymo DNA/RNA Shield (Zymo Research) {Zendejas-Heredia, 2021}, 5% KCr_2 {Llewellyn, 2016} and dimethyl sulfoxide, disodium EDTA with saturated NaCl (DESS) {Beknazarova, 2017}. Beknazarova et al. {Beknazarova, 2017} compared DESS at a ratio of 1:1 and 1:3 with neat dog faeces spiked with larvae of *S. ratti* and stored at room temperature. DNA was extracted using the PowerSoil kit (Qiagen) then subjected to the Verweij qPCR. PCR products were detected for all samples with a slightly lower Ct for unpreserved faeces at day 0 (average Ct 16 vs Ct 18 for DESS preserved samples, possibly a due to the dilution effect of the preservative). After 56 days, but those stored in DESS had an on average 5 points lower Ct in the PCR. No other validation studies have been performed on the efficacy of these various preservation methods and it cannot be assumed that preservation validation studies for other STH {Ayana, 2019}, which are passed as eggs, will have corresponding efficacy for the preservation of *Strongyloides* larvae. Similarly, no other studies have been performed regarding the effect of time in storage on NAAT assay results for *Strongyloides*. Commercial fixatives such as Total-Fix (Medical Chemical Corporation) and EcoFix (Cardinal Health) are now commonly used in U.S. laboratories performing multiplex PCR assays, but these also have not been validated in comparison trials.

The choice of DNA extraction method will also impact the results of NAAT testing. A major consideration when performing NAATs on faeces is the presence of PCR inhibitors in the sample matrix. Kits designed for soil or faecal extraction generally are superior for removal of such inhibitors than tissue or blood extraction kits, though the latter may be more effective when dealing with samples of sputum, fluid, or tissue for *Strongyloides* NAAT testing. One comparison study has been performed on DNA extraction kits for *Strongyloides* testing using the Verweij PCR {Sultana, 2013}. This study employed human faeces spiked with between 1 and 10 larvae of *S. ratti*. It compared Qiagen PowerSoil kit (now superseded by the Qiagen PowerFecal Pro kit), the Ultra Clean Fecal DNA kit (MoBio Laboratories,) with prior bead beating, a modification of the QiaAmp Tissue kit (Qiagen,) incorporating polyvinylpolypyrrolidone pre-treatment, and the NucliSens EasyMag (bioMerieux) automated DNA extraction platform with prior bead beating. This study found that the MoBio PowerSoil kit led to the greatest analytical sensitivity based on a model of *S. ratti* spiked into stool {Sultana, 2013}. Barda et al. {Barda, 2018} compared DNA extraction in the QiaAmp kit with and without pre-treatment in preoteinase K using their modification of the Verweij qPCR and found much improved sensitivity using proteinase K pre-treatment {Barda, 2018}. Fernández-Soto et al. {Fernández-Soto, 2016 #986} used a Bioparapred-Midi column (Leti Diagnostics) to concentrate the stool pellet prior to DNA extraction. Several other DNA extraction protocols have been employed in various studies (Table 4), but without comparison to other methods.

Analytical variables

The assay described by Verweij et al. {Verweij, 2009}, sometimes with minor variations, remains the most thoroughly validated assay, with ten clinical validation studies employing sensitive reference standards having been published (Table 4). Many of these papers report a wide range of sensitivity and specificity results, which may be attributable to variations pre-analytical and analytical processes more than variables in the populations studied. When used as a qPCR with the primers and probe designed by Verweij et al. {Verweij, 2009 #3236} and validated against an optimal reference standard (Baermann sedimentation or

APC), this assay was has demonstrated sensitivities ranging between 34.5% and 100% and a specificity of between 77.1% and 98.9% when compared to phenotypic tests (Table 4). The wide variation in assay performance may be attributed to not just the sampled cohort and pre-analytical variables, but also to a surprising array of modifications to the cycling conditions and Ct value cut-offs employed. A modification based on the primers designed by Verweij et al. {Verweij, 2009} and using real-time SYBR Green (Invitrogen) detection {Saugar, 2015. The Verweij et al. {Verweij, 2009} primers have also been used in a conventional PCR form, though without validation {Gorgani-Firouzjaee, 2018}. Although the primers employed are the same, such changes in analytical processes may impact and sensitivity and specificity. Reagent storage and quality and the PCR cycler used, and the frequency of maintenance, may also influence on assay performance. These observations apply to other examples where NAAT tests have been used by different groups.

Given the variability in sample preservation, DNA extraction, primers, PCR cycling conditions and reference standards employed, it is difficult to determine the true sensitivity and specificity of many NAAT tests for *S. stercoralis*. Even the most widely validated assay, the Verweij et al. {Verweij, 2009} real time PCR, can yield vastly different values depending on these variables. This makes comparison of data from different trials and population surveys difficult. Standardisation of NAAT testing protocols for *S. stercoralis* would greatly benefit the WHO 2030 goals for the for the implementation of control strategies for this parasite {Montresor, 2020} by allowing comparability of survey data. A thorough validation of all assays, including pre-analytical variables, is necessary to determine what the optimal molecular testing approach for the detection of *S. stercoralis* infection is. This will be necessary if the results of such assays are used to guide interventions.

Quantification of larval Output by PCR

Unlike PCR for bacterial or viral infections, where millions or billions of copies of the pathogen genome may be extracted in a single specimen, there is often a very low number of individual parasitic elements in faeces {Dreyer, 1996}. When performing Baermann sedimentation or APC, large amounts (often >1 g) of stool are used. However, most commercial DNA extraction kits recommend using only 250 mg of stool. As larval numbers in faeces are often low and larvae are not equally distributed throughout the specimen, this presents a risk of sampling error.

Some authors have suggested qPCR as a semi-quantitative approach to determining larval load {Llewellyn, 2016}. A low Ct on qPCR may suggest high numbers of excreted larvae and possibly indicate hyperinfection. However, outside of this scenario and unlike egg excretion for other STH, larval excretion in strongyloidiasis is intermittent {Page, 2018; Dreyer, 1996} and therefore unreliable as a measure of parasite load. Moreover, the autoinfective cycle of *S. stercoralis* means that the intensity of infection can vary with the host's immune status {Page, 2018}. Another important consideration is that the more developed L3 and free-living adult stages will yield higher quantities of DNA. The effect of worm development on quantification results in PCRs for other STH has been discussed in depth elsewhere {Papaiakovou, 2019}. Stool which has not been preserved promptly after passage is not ideal for such quantification approaches {Papaiakovou, 2019}.

Quality Control and Quality Assurance

Quality control is imperative to any NAAT testing. For example, reagents need to be in-date, staff need to be appropriately trained, and equipment maintained and monitored. Non-template and inhibition controls must be performed, particularly given the large number of inhibitors present in stool. Samples not yielding an amplicon for the inhibition control should be excluded from analysis in research studies, or another sample collected and testing repeated in the context of diagnostic use.

Where therapy will be administered as part of a research study, treatment program or routine clinical care, it is essential from an ethical standpoint that the diagnostic tests used are appropriately validated and that

there an ongoing process of test quality assurance and laboratory accreditation. The Dutch Foundation for Quality Assessment in Medical Laboratories (SKML) offers a The Helminth External Molecular Quality Assessment Scheme (HEMQAS) {SKML} which includes *S. stercoralis*. The Royal College of Pathologists of Australasia Quality Assurance Program (RCPA QAP) is also currently investigating the feasibility of a QAP for *Strongyloides* nucleic acid tests (Watts, personal communication, 2022).

Where laboratories cannot access an external quality assurance scheme (EQAS), periodic blinded testing within a laboratory and blinded interlaboratory specimen exchange are alternative approaches to quality assurance.

Validation

The most pressing issue for the diagnosis of, and population screening for, strongyloidiasis is the need for appropriate analytical and clinical validation of tests prior to their use. This is particularly important if test results will form the basis for treatment. Examples of guidelines for the laboratory (analytical) validation of tests are published by the FDA and the CLSI {Garrett, 2008} (US Food and Drug Administration, 2017; CLSI, 2008). The STARD Guidelines provide a framework for the clinical evaluation of diagnostic tests {Cohen, 2016}.

Any validation approach should include the complete sample preservation, transport, and DNA extraction approach. Many publications of prevalence studies have employed either unvalidated assays or the Verweij PCR but using alternative pre-analytical processes which may alter the reported sensitivity and specificity of that test (Table 4). Cases of amplification in samples not found to contain *S. stercoralis* by other methods should not be attributed to improved sensitivity of the PCR used without reference to the comprehensive analytical specificity testing of the assay. Analytical validation *in silico* using sequences from DNA sequence databases is insufficient. Examples of *Strongyloides* PCR assays amplifying non-target eukaryotic elements in faeces, including *Blastocystis* spp., other helminths, yeasts, and even human genes exist {Sitta, 2014; Robertson, 2017}. Many reported assays have had some form of *in vitro* analytical validation performed, but the number and breadth of organisms tested for analytical specificity and the choice of template for analytical specificity vary greatly (table 5).

The absence of a single high sensitivity and reference standard for the detection of *S. stercoralis* infection complicates validation studies. A frustrating approach used in many validation reports is the application of a composite reference standard which includes the test being validated for statistical analysis of sensitivity and specificity. This approach will always yield 100% specificity for the test being validated as there can be no false positives. The approach is based on the assumption that all positives from a NAAT assay are true positives - an assumption for which there is not valid scientific basis. The most accurate measurements of clinical sensitivity and specificity would be in those studies that used more than one sensitive coprological larval recovery method for the detection of *Strongyloides* in stool. Specifically, we recommend a combination of Baermann sedimentation and APC, in the hands of experienced parasite morphologists who can easily differentiate *Strongyloides* larvae from those of other helminths.

At a minimum, eight important validation procedures that should be adhered to by all developing new diagnostic PCR assays. These validation conditions are;

a) The novel PCR products should be sequenced to confirm that that the true target is being amplified.
b) The optimal cycling conditions should be determined by testing in a temperature gradient against a positive clinical sample of known DNA concentration.
c) The limit of detection (analytical sensitivity) of the assay should be determined using the nucleic acid extraction method that will be used in testing
e) The novel PCR should always be validated for analytical specificity against a panel of organisms likely

to be present in the tested sample matrix (e.g. urine, faeces, soil, food).
d) The novel PCR should be validated against a panel of clinical samples containing the target pathogen.
f) A panel of known negative clinical samples of the same sample type should be analysed to confirm specificity and the absence of false positives.
g) A prospective, blinded, clinical validation study of the assay should be performed in an endemic population, using a highly reliable reference assay or composite reference standard as a comparator.
h) Unusual or unexpected positive amplification by the novel PCR during these validations should always be regarded as a potential 'false positive' unless the positive result is supported by sequence analysis

Only when the above requirements have been met can a diagnostic NAAT assay for *S. stercoralis* (or any other target organism) be considered valid. Thus far, the only assay which meets most of these requirements is the Verweij et al {Verweij, 2009} qPCR, or versions with minor modifications. Although other published PCRs may have similar, or even superior, performance characteristics, these have not been fully validated and thus all results generated using these assays should be interpreted with caution.

Statistical Analysis of Validation Data

The calculation of disease prevalence in a population may be greatly affected by the choice of test used. To correct for these deficiencies, Bayesian statistical modelling has been applied to estimate disease prevalence {Black, 2002; Dendukuri, 2001; Dendukuri, 2010; Stamey, 2005; Joseph, 1995}. These approaches recognise the limitations of morphological (reference standard) identification of larvae for *Strongyloides* diagnosis, that increasing the range of tests in a study (e.g. microscopic methods and PCR) will give a closer approximation to the true prevalence and that test results are not independent {Dendukuri, 2010}. Software has been developed to facilitate the calculation of study sample size based on the number of tests and such technology could be applied to epidemiological research {Dendukuri, 2010}.

Diagnosing *S. stercoralis* infection in domestic animals

S. stercoralis affects not only humans and non-human primates but also dogs, wild canids and cats {Monteiro, 2016; Nyambura Njuguna, 2017; Wulcan, 2019; Ko, 2020}.

Among *Strongyloides* species infecting companion animals and large livestock, *S. stercoralis* is the only species considered potentially zoonotic {Thamsborg, 2017}. The only other species having a zoonotic potential is *S. f. fuelleborni,* which infects wild non-human primates. Attempts to find an animal reservoir for *S. f. kellyi* in New Guinea have thus far been unfruitful {Bradbury, 2021}.

In dogs, *S. stercoralis* occurs worldwide with prevalence from 0 to 50%, with most studies indicating <5% {Thamsborg, 2017}. Infection in dogs, as in humans, is considered common/endemic in tropical and subtropical regions, although epidemiological data from low income countries are limited {Gonçalves, 2007; Schär, 2014; Jaleta, 2017}. In Europe, prevalence data and data from case reports and case series have been recently reviewed by Ottino and colleagues {Ottino, 2020}. Sporadic canine infections have been described in numerous countries, with some hot spots described in southern Italy {Paradies, 2017}, Slovakia {Štrkolcová, 2017} and Australia {Beknazarova, 2017}.

In dogs, *S. stercoralis* infection represents both a veterinary clinical issue and a public health challenge, because in some dogs the infection can induce severe symptoms and can be fatal, and the potential role of dogs (and cats?) in the transmission to humans is a matter of concern. Thus, the role of diagnosis is crucial both in clinical practice and in epidemiological surveys.

Diagnosis of strongyloidiasis in dogs, in clinical practice

In dogs *S. stercoralis* infection can cause mild to severe symptoms, mostly affecting the gastroenteric tract {Dillard, 2007; Paradies, 2017} but pulmonary and dermatological signs have also been described {Cervone, 2016; Dashchenko, 2020}. Associated changes in haematological and biochemical indices (anaemia, leucocytosis, eosinophilia, increased acute phases proteins) could help to include the infection in the list of differential diagnosis in dogs with compatible signs, but they are not specific and not constant. The absence of eosinophilia does not exclude strongyloidiasis {Paradies, 2017}.

A correct diagnosis in clinical practice is crucial also considering the infection does not respond to commonly used deworming drugs for dogs (such as tetrahydropyridines); ivermectin per os is recommended {Paradies, 2019; Yang, 2013}. No evidence is available in the literature about the widely sponsored use of a deworming spot-on formulation containing moxidectin for prevention and treatment of parasitic infections to treat strongyloidiasis. It is possible that the use of such formulation may hamper the diagnosis without being effective in controlling the infection due to a possible reduction in the parasite load.

The Baermann test remains the most used method for the diagnosis of *S. stercoralis* infection in veterinary practice. In most of the case reports and case series reported in literature {Basso, 2019; Cervone, 2016; Cvetkovikj, 2018; Dillard, 2007; Paradies, 2017; Eydal, 2016} definitive diagnosis was reached using Baermann sedimentation. A direct faecal smear may be useful to diagnose the infection in severely affected dogs {Paradies, 2017; Cvetkovikj, 2018}.

The possibility of false negative results using Baermann test in clinical practice has been documented, and the need of other tests to support the diagnosis already suggested {Paradies, 2017}. The larval output is irregular and may be low {Siddiqui, 2001}. Even dogs with severe clinical signs may yield a negative result in a single test {Paradies, 2017}. Furthermore, Baermann sedimentation is not routinely employed in clinical practice and larvae could be incorrectly identified as *Angiostrongylus* species {Hall, 2020}. Thus, the possibility that even cases of patent strongyloidiasis remain undiagnosed in clinical practice is not negligible due to the lack of rapid and efficacious diagnostic methods.

Behind their specific limitations, serological tests and PCR (see below) are not commercially available for veterinary use, thus they are not routinely used in clinics. Unfortunately, rapid antigenic tests, as recently implemented for human infection {Balachandra, 2021} are not available and/or validated in dogs. These snap tests could be very useful in clinical practice and their validation in dogs is desirable.

Post-mortem diagnosis in affected animals could be reached by means of intestinal scarification and microscopic identification of all *S. stercoralis* stages. The histopathology on intestinal biopsies documents the presence of an inflammatory disease (i.e. lymphoplasmacellular enteritis) and could reveal the presence of larvae, ova and adult females mostly in the duodenum gland crypt. It has to be known to practitioners that histology from in vivo endoscopic biopsy in dogs with gastrointestinal signs could not reveal the infection in few cases (Paradies, not published data), thus a negative biopsy does not exclude the diagnosis and other tests need to be used in the diagnostic plan in these dogs. The possibility that biopsy could not reveal the infection in few cases was reported also in humans {Mittal, 2009; Montes, 2010}.

In cats *S. stercoralis* infection was recently associated to colonic epithelial grossly, multifocal nodules evidenced at post-mortem examination in which the parasites were localized {Wulcan, 2019}. Morphologically similar nodules were previously associated to *S. tumefaciences* but it is possible to hypothesise that a misdiagnosis of species could have occurred in the past based on morphology alone.

Diagnostic tests used for epidemiological surveys

Epidemiological surveys aimed at estimating the prevalence of parasitic infections (including strongyloidiasis) in dogs have been carried out with different coprological methods, including the Baermann concentration method {Paradies, 2017; Iatta, 2019; Sauda, 2018}, flotation {Zanzani, 2014; Mircean, 2012; Riggio, 2013}, FLOTAC (Wright et al., 2016), FECT {Eydal, 2016} {Papazahariadou, 2007} and APC {Štrkolcová, 2017}. Regardless, it is known that *S. stercoralis* remains frequently undetected in parasitological survey for various reasons, such as the intermittent larval shedding and low parasite burden. Moreover, the deployment of tests with low sensitivity for this parasite (such as flotation) further contributes to an underestimation of its prevalence. Experiments in human samples have shown that both Baermann and APC are more sensitive when multiple samples from consecutive days are examined {Albonico, 2016}.

Differences between diagnosis in humans and in dogs

One of the most important differences in the diagnostic approach to *S. stercoralis* infection in humans and dogs is that in humans the risk of missed diagnosis is not acceptable, because the infection is long-lasting due to autoinfection and potentially fatal at any time during life (due to hyperinfection). Thus, in humans, the use of the most sensitive diagnostic techniques to exclude the infection is mandatory. Serology, which has shown the highest sensitivity, has been suggested for screening at a population and individual level in this context {Albonico, 2016; Requena-Méndez, 2017; Buonfrate, 2018}, while PCR could be a valid option as a confirmatory test in case of positive serology or for the screening of immunosuppressed patients for whom the sensitivity of serology decreases {Buonfrate, 2018}. Conversely, in dogs the infection can be self- limiting, and autoinfection/hyperinfection is not common {Thamsborg, 2017}, thus the use of a highly-sensitivity but low specificity test for screening could be questionable.

As in humans, serology increases sensitivity. In studies where more than one diagnostic method has been used for the diagnosis of *S. stercoralis* infection, the prevalence of the infection recorded by serology using IFAT or ELISA was higher than that estimated by Baermann {Júnior, 2006; Gonçalves, 2007}. In the prevalence study by Strkolcova et al. {Štrkolcová, 2017} the seroprevalence was 55% compared to a prevalence of 10% at faecal detection. An in-house IFAT, routinely used for *S. stercoralis* diagnosis in humans and showing good performance in dogs {Buonfrate, 2017} resulted the most sensitive test in a study comparing the accuracy of serological, molecular and coprological tests {Iatta, 2019}. This IFAT resulted more sensitive of the commercial ELISA based on somatic antigens from *S. ratti* larvae for the diagnosis of canine strongyloidiasis {Buonfrate, 2017; Iatta, 2019; Paradies, 2017}. The specificity of IFAT increases with titres >1:320 in dogs {Iatta, 2019}. It is not known if *Strongyloides* serology in dogs seroreverts to negative within 18 months following treatment, as has been observed in human infections {Kearns, 2017}. Therefore, the clinical significance of positive serology in dogs remains unknown. This aspect must be considered in dogs where the infection is frequently self-limiting. Furthermore, no data are available on the persistence of antibodies after natural elimination of the infection but it has been demonstrated that serology can remain positive for three months after treatment {Paradies, 201}. Other intrinsic limitations for performing IFAT in dogs are the difficulty in setting up the test and the lack of validation in dogs.

Molecular methods had never been applied in dogs until 2017. The Verweij qPCR {Verweij, 2009} was first evaluated in dogs by Buonfrate et al. {Buonfrate, 2017 #4} and demonstrated the good performance for the diagnosis of *S. stercoralis* infection in dogs. A recent survey of dogs in remote Aboriginal communities in northern Australia using the same qPCR found a high prevalence {Beknazarova, 2019}. In dogs {Iatta, 2019} qPCR showed 100% specificity and a sensitivity higher than Baermann sedimentation, diagnosing several positive samples that were not revealed by Baermann probably because qPCR also detected unviable larvae

that could be missed at microscopy. The authors suggested that coupling qPCR with Baermann could be an optimal approach to detect patent infections and reservoir dogs, although missed diagnoses could occur.

Studies suggest that positive PCR results in carnivore faecal samples decline significantly after three days from collection at room temperature {Santini, 2007}. Long term DNA preservation by cryopreservation or the use of chemical preservatives at room temperature {Zhou, 2019; Beknazarova, 2017} has been successfully employed, further discussion of which may be found in the *Pre-analytical variables in NAAT testing* section of this review.

Is *Strongyloides stercoralis* infection a zoonosis? From epidemiological evidence to haplotypes

To what extent strongyloidiasis is a zoonotic disease has been the subject of controversy in the literature for several decades, and what the potential is for dogs may serve as a source for human *S. stercoralis* infection, or vice versa, still remains a challenge.

Until 2017 the question of the identity of *S. stercoralis* from dogs to humans had not been thoroughly explored. Nine historical studies attempted to infect dogs with human strains from various parts of the world had been made, but most infections were refractory or self-cured within weeks {Galliard, 1951; Fuelleborn, 1927; Sandground, 1928; Sandosham, 1952}. A human isolate from Vietnam did cause lasting infection in a dog {Galliard, 1951} and another from the Burma/Thailand region caused infection lasting 15 months in an immunosuppressed dog {Grove, 1982}. The single published attempt to infect a human with a dog strain in the USA failed {Augustine, 1939}.

There remains only one apparently confirmed transmission of *S. stercoralis* from dogs to a human documented in the scientific literature {Georgi, 1974}. In this case, the infected person demonstrated marked clinical symptoms (vomiting, abdominal pain and diarrhoea) and mounted an unusually high eosinophil response (13.6 $x10^9$ cells/L), suggesting poor adaptation of the parasite to the human host. An unexplained case of autochthonous *S. stercoralis* infection in a girl from the United Kingdom raised the question of potential transmission from dog faeces while walking barefoot in parks {Sprott, 1987}. Studies in the Anami Islands of Japan found no relationship between *Strongyloides* infection in dogs and their owners, despite a relatively high prevalence in each (10% and 2.8%, respectively) {Takano, 2009}. Although zoonotic transmission from dogs is speculated and may be a phenomenon dependent upon the geographical origin of strains {Barratt, 2020}, it remains unclear why more infections in household contacts of infected dogs are not reported.

Attempts to distinguish *S. stercoralis* isolates from dogs and humans by means of molecular analysis and genotyping gave contradictory results against {Ramachandran, 1997; Hasegawa, 2009; Hasegawa, 2010} or in support of {Schär, 2014} the zoonotic potential.

In 2017, two studies collecting samples from different geographical areas of Asia demonstrated that two distinct *cox1* clades of *S. stercoralis* exist, one found in both humans and dogs and the other restricted to dogs {Jaleta, 2017; Nagayasu, 2017}. This demonstrated that dogs carry *S. stercoralis* genotypically indistinguishable from humans and non-human primates (NHPs) at the targets sequenced in addition to a dog specific population. The human/dog clade was associated with a single haplotype of HVR-IV (*cox1* lineage A), the other clade (dog only) had a unique HVR-IV haplotype (*cox1* lineage B). In dogs, the HVR IV haplotype may apparently be used to distinguish the two populations of *S. stercoralis* {Jaleta, 2017; Aupalee, 2020}.

Beknazarova et al. {Beknazarova, 2019} who found lineage A in both dogs and humans and lineage B affecting some dogs only in remote Australian communities. A limitation of this study, which incorporated deep sequencing of genotyping amplicons directly from faeces was that no control for coprophagia of human

faeces by dogs was included. This is common activity for dogs in areas with poor sanitation and at least some of the genotypes identified may have been passage of *Strongyloides* DNA or larvae following consumption of human or animal faeces {Beknazarova, 2019}. Interestingly, in further sequencing studies the parasites isolated from dogs from Thailand {Sanpool, 2019}, Europe {Basso, 2019; Barratt, 2019}, USA {Barratt, 2019} and Japan {Montresor, 2020 #3} belong to the human/dog infective type (lineage A). A novel HVR-IV haplotype (haplotype E) has been identified in humans in China {Zhou, 2019} and from an Australian dog {Beknazarova, 2019}.

It is now accepted that dogs can carry strains of *S. stercoralis* genetically very similar to those affecting humans, in addition to a dog-specific population {Jaleta, 2017; Nagayasu, 2017; Beknazarova, 2019}. Although these studies provided evidence for zoonotic transmission, they cannot demonstrate that a direct transmission from dogs to humans occurs. A Barrat and Sapp {Barratt, 2020} analysis recently suggested that some canine infections could represent incidental infections with strains originating from humans resulting in transient infection. The role of the cat as reservoir of infection is unexplored. Only one *S. stercorali*s isolate from a cat has been sequenced at *cox1* and was within the human/dog/NHP infecting lineage A {Wulcan, 2019}.

It should be noted that the short length and limited discrimination of currently used genotyping targets disallows true phylogenetic comparison and longer more discriminatory targets must be identified before the question of zoonotic transmission can be finally settled. The true role of domestic animals, specifically dogs (and cats) in the transmission and maintenance of *S. stercoralis* infections of humans remains unclear {Beknazarova, 2019; Barratt, 2020; Bradbury, 2021}.

It has been demonstrated that *S. stercoralis* free- living cycle has only one generation and do not continue indefinitely persist in the environment as incorrectly perceived in the past, thus there is not an ongoing source of infection in the soil {Page, 2018}. Although the most important reservoir of infection still remains people, the control of environmental reservoirs throughout sanitation and dog treatment may be required as part of the control strategies to break the life cycle of the parasite {Beknazarova, 2017; Page, 2018; Thamsborg, 2017}. The WHO recently included the control of strongyloidiasis among the 2030 targets {Montresor, 2020}. To reach this aim, the development of long read sequencing for *S. stercoralis* to definitively determine if dogs, or cats, act as reservoirs of human infection is of paramount importance {Bradbury, 2021}.

Systematic reviews with meta-analysis on diagnostic tests for human strongyloidiasis

Through the electronic search described in the methods, we identified 3 systematic reviews with meta-analysis evaluating diagnostic methods for strongyloidiasis: one comparing coproparasitological techniques {Campo Polanco, 2014}, one on serology {Kalantari, 2020}, and one on molecular methods {Buonfrate, 2018}.

Main characteristics and findings of the reviews are summarized in Table 7.

Table 7. Characteristics and findings of systematic reviews with meta-analysis of diagnostic tests for strongyloidiasis

From the results of these reviews, APC would appear to be the method with the highest sensitivity, but again the wide differences in the procedures applied for the same assay (e.g number of plates cultured in APC, different DNA extraction methods for PCR), in the characteristics of the diagnostic assays themselves

(e.g. different antigens used for serology assays), and in the reference tests for the estimation of accuracy hamper the comparison of methods and obtaining a definitive result.

The choice of a diagnostic test based on purpose and setting

Finally, the choice of diagnostic technique(s) to be implemented and the intensity of the diagnostic effort must be guided by the final aim of the investigation and the trade-off balance between easiness/implementability of the diagnostic procedure, consequences of inaccurate diagnosis and cost-effectiveness of treatment.

- In a context of initial epidemiological mapping of infection distribution, likely followed by preventive chemotherapy with ivermectin, extreme accuracy of the diagnosis at individual level is not required, since the estimates of prevalence would be used to evaluate a possible intervention with ivermectin at population level, and not an individual administration based on diagnosis on a single person {Buonfrate, 2021}. In this scenario, serology assays based on widely available techniques such as ELISA, preferencing sensitivity and ease of use, might be currently the best option, also considering the possible collection of blood spots on filter paper {Formenti, 2016; Mounsey, 2014}. However, accurate evaluation of cross-reactivity of assays is needed. In the same epidemiology investigation setting, but in the context of monitoring and evaluation of a control program, especially when infection prevalence falls, false positive results become a more important problem, and increased diagnostic effort towards specificity should be implemented (e.g. with the use of specific coproparasitological techniques directed at the retrieval of larvae, molecular techniques, or the two combined). In order to compare baseline and follow up prevalence, it has been suggested that both serology and a faecal-based test would be used for baseline assessment, so that any of the two methods can be used in different phases of monitoring (depending on prevalence thresholds) 3570}.
- Especially outside the clinical context, the acceptability by the target population should also be considered for the choice of a test. Moreover, feasibility should be evaluated in terms of costs, technical skills (laboratory staff who can differentiate larvae for instance), logistics (such as transport of samples to referral laboratories), facilities and equipment (maintenance of cold chain, presence of equipment for molecular biology, ect). Outside endemic areas, screening of populations with high epidemiological risk (such as migrants from endemic countries) should be based on a sensitive diagnostic tool (i.e. serology) {Requena-Méndez, 2017}. A stool test is not mandatory; however, it will provide an indication of larval burden and in heavy infections may be useful to monitor treatment efficacy. Considering the possible harm of the infection against the high tolerability profile of ivermectin, treatment of all seropositive cases would be recommended {Requena-Méndez, 2017}.
- For individual diagnosis in people with suspected infection (including those at risk of developing hyperinfection/disseminated infection, such as candidates to immunosuppressant treatments) a combination of serology with a sensitive faecal test (which could be parasitological or molecular, depending on the expertise/equipment of the laboratory) would be suggested, in order to further increase sensitivity and to monitor treatment response {Requena-Méndez, 2017}.
- In immunocompromised people, serology might be negative {Abdul-Fattah, 1995; Ahmed, 2019}. However, the combination of serology with a sensitive faecal test would be still recommended.
- Finally, in the context of a clinical trial of drugs efficacy, particular care must be taken to use a sensitive faecal test, which should be better repeated over time in the follow-up phase, to safely rule out the persistence of the infection {Sato, 1995; Dreyer, 1996}. In non-endemic areas, clinical trials relying on serologic follow-up have also been conducted {Buonfrate, 2018; Mascarello, 2011}. In this case, time-point(s) for follow-up should be at least 5-6 months after treatment, in order to observe either a seroconversion or a decline in titre {Buonfrate, 2015; Loutfy, 2002; Page, 2006; Biggs, 2009;

Ramanathan, 2008}. Because of the high risk of re-infection during a long-term follow up, serology would not be adequate for clinical trials in endemic area.

A summary of these points is presented in Table 6.

Conclusions

With this review we have attempted to outline, as accurately and completely as possible, the state of the art and future perspectives for the diagnosis of *S. stercoralis*, a parasite with unique characteristics. While traditional faecal methods, which use microscopic identification have a high specificity with trained operators, the reduced sensitivity of these methods, due to variable larval output and pre-analytical limitations, has been the main cause of the underestimation of the prevalence of this helminth and consequently of its importance for public health. Routine clinical diagnosis is affected by the limitations of diagnostic tests and it is important to consider the diagnosis when there are epidemiological risk factors. In addition, research studies can be impacted by diagnostic limitations as the infection may be present, even in apparently uninfected control groups.

However, significant progress has been made and is ongoing, with many different and new diagnostic techniques available and others being implemented. The challenge faced now is to determine the most appropriate diagnostics or combination of diagnostics for different purposes, the most immediate being the assessment of the burden of infection and disease in endemic countries and monitoring of progress towards the ambitious goals set by the WHO for 2030.

References

1. Abdul-Fattah, M.M.; Nasr, M.E.; Yousef, S.M.; Ibraheem, M.I.; Abdul-Wahhab, S.E.; Soliman, H.M. Efficacy of ELISA in diagnosis of strongyloidiasis among the immune-compromised patients. *Journal of the Egyptian Society of Parasitology* **1995**, *25*, 491-498.
2. Ahmad, H.; Balachandra, D.; Arifin, N.; Nolan, T.J.; Lok, J.B.; Hayat Khan, A.; Yunus, M.H.; Noordin, R. Diagnostic Potential of an IgE-ELISA in Detecting Strongyloidiasis. *The American journal of tropical medicine and hygiene* **2020**, *103*, 2288-2293, doi:10.4269/ajtmh.20-0265.
3. Ahmadi, N.A.; Damraj, F.-a. A field evaluation of formalin-gasoline technique in the concentration of stool for detection of intestinal parasites. *Parasitology research* **2009**, *104*, 553-557, doi:10.1007/s00436-008-1229-4.
4. Ahmadi, N.A.; Pakdad, K. Tween as a Substitute for Diethyl Ether in the Formalin-Ether Sedimentation Technique. *Iran J Publ Health* **2007**, *36*, 91-95.
5. Ahmed, S.A.; El-Moselhy, A.; El-Moammaly, A.; El-Shewy, K. Strongyloides stercoralis in Patients on Corticosteroids Therapy Using Enzyme-Linked Immunosorbent Assay and Gelatin Particles Indirect Agglutination Tests: A Diagnostic Approach. *Acta parasitologica* **2019**, *64*, 394-405, doi:10.2478/s11686-019-00060-w.
6. Albonico, M.; Becker, S.L.; Odermatt, P.; Angheben, A.; Anselmi, M.; Amor, A.; Barda, B.; Buonfrate, D.; Cooper, P.; Gétaz, L., et al. StrongNet: An International Network to Improve Diagnostics and Access to Treatment for Strongyloidiasis Control. *PLoS neglected tropical diseases* **2016**, *10*, e0004898-e0004898, doi:10.1371/journal.pntd.0004898.
7. Allen, A.V.; Ridley, D.S. Further observations on the formol-ether concentration technique for faecal parasites. *J Clin Pathol* **1970**, *23*, 545-546, doi:10.1136/jcp.23.6.545.
8. Amor, A.; Rodriguez, E.; Saugar, J.M.; Arroyo, A.; López-Quintana, B.; Abera, B.; Yimer, M.; Yizengaw, E.; Zewdie, D.; Ayehubizu, Z., et al. High prevalence of Strongyloides stercoralis in school-aged children in a rural highland of north-western Ethiopia: the role of intensive diagnostic work-up. *Parasites & vectors* **2016**, *9*, 617-617, doi:10.1186/s13071-016-1912-8.
9. Anamnart, W.; Intapan, P.M.; Maleewong, W. Modified formalin-ether concentration technique for diagnosis of human strongyloidiasis. *The Korean journal of parasitology* **2013**, *51*, 743-745, doi:10.3347/kjp.2013.51.6.743.

10. Anamnart, W.; Pattanawongsa, A.; Intapan, P.M.; Maleewong, W. Factors affecting recovery of Strongyloides stercoralis larvae: an approach to a newly modified formalin-ether concentration technique for diagnosis of strongyloidiasis. *Journal of clinical microbiology* **2010**, *48*, 97-100, doi:10.1128/JCM.01613-09.
11. Anderson, N.W.; Klein, D.M.; Dornink, S.M.; Jespersen, D.J.; Kubofcik, J.; Nutman, T.B.; Merrigan, S.D.; Couturier, M.R.; Theel, E.S. Comparison of three immunoassays for detection of antibodies to Strongyloides stercoralis. *Clinical and vaccine immunology : CVI* **2014**, *21*, 732-736, doi:10.1128/CVI.00041-14.
12. Andreetta Corral, M.; de Paula, F.M.; Meisel, D.; Abdala, E.; Figueiredo Costa, S.; Camera Pierrotti, L.; Yamashiro, J.; do Nascimento Gonçalves, E.M.; Castilho, V.L.P.; Chieffi, P.P., et al. IgG reactivity with 40-35 kDa soluble and membrane antigen of Strongyloides venezuelensis in immunocompromised patients. *Acta Trop* **2019**, *190*, 357-360, doi:10.1016/j.actatropica.2018.12.020.
13. Anecimo, R.S.; Tonani, K.A.; Fregonesi, B.M.; Mariano, A.P.; Ferrassino, M.D.; Trevilato, T.M.; Rodrigues, R.B.; Segura-Munoz, S.I. Adaptation of Ritchie's Method for Parasites Diagnosing with Minimization of Chemical Products. *Interdiscip Perspect Infect Dis* **2012**, *2012*, 409757, doi:10.1155/2012/409757.
14. Arakaki, T.; Hasegawa, H.; Asato, R.; Ikeshiro, T.; Kinjo, F.; Saito, A.; Iwanaga, M. A new method to detect Strongylides stercoralis from human stool. *Japan J Trop Med Hyg* **1988**, *16*, 11-17.
15. Arakaki, T.; Iwanaga, M.; Kinjo, F.; Saito, A.; Asato, R.; Ikeshiro, T. Efficacy of agar-plate culture in detection of Strongyloides stercoralis infection. *The Journal of parasitology* **1990**, *76*, 425-428.
16. Aramendia Aa, A.M.Z.D.D.E.S.J.M.H.Z.H.T.Y.M.P.M.V.R.E.; Benito, A. Epidemiology of intestinal helminthiases in a rural community of Ethiopia: is it time to expand control programs to include strongyloides stercoralis and the entire community? *PLoS neglected tropical diseases* **2020**, *14*, 1-14.
17. Arifin, N.; Hanafiah, K.M.; Ahmad, H.; Noordin, R. Serodiagnosis and early detection of Strongyloides stercoralis infection. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* **2019**, *52*, 371-378, doi:10.1016/j.jmii.2018.10.001.
18. Arifin, N.; Riazi, M.; Mahmoud, S.S.; Yunus, M.H.; Low, H.C.; Mohamed, Z.; Noordin, R. Improvement of serodiagnosis of human strongyloidiasis. *Tropical Medicine and International Health* **2013**, *18*, 61-61, doi:10.1111/tmi.12162 LK - http://BT8EP3TD6D.search.serialssolutions.com?sid=EMBASE&issn=13602276&id=doi:10.1111%2Ftmi.12162&atitle=Improvement+of+serodiagnosis+of+human+strongyloidiasis&stitle=Trop.+Med.+Int.+Health&title=Tropical+Medicine+and+International+Health&volume=18&issue=&spage=61&epage=&aulast=Arifin&aufirst=N.&auinit=N.&aufull=Arifin+N.&coden=&isbn=&pages=61-&date=2013&auinit1=N&auinitm=.
19. Atkins, N.S.; Lindo, J.F.; Lee, M.G.; Conway, D.J.; Bailey, J.W.; Robinson, R.D.; Bundy, D.A. Humoral responses in human strongyloidiasis: correlations with infection chronicity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1997**, *91*, 609-613, doi:10.1016/s0035-9203(97)90049-3.
20. Augustine, D.; Davey, D. Observations on a natural infection with Strongyloides In the dog. *Journal of Parasitology Research* **1939**, *25*, 117-119.
21. Aupalee, K.; Wijit, A.; Singphai, K.; Rödelsperger, C.; Zhou, S.; Saeung, A.; Streit, A. Genomic studies on Strongyloides stercoralis in northern and western Thailand. *Parasites & vectors* **2020**, *13*, 250-250, doi:10.1186/s13071-020-04115-0.
22. Autier, B.; Belaz, S.; Razakandrainibe, R.; Gangneux, J.P.; Robert-Gangneux, F. Comparison of three commercial multiplex PCR assays for the diagnosis of intestinal protozoa. *Parasite* **2018**, *25*, 48, doi:10.1051/parasite/2018049.
23. Ayana, M.; Cools, P.; Mekonnen, Z.; Biruksew, A.; Dana, D.; Rashwan, N.; Prichard, R.; Vlaminck, J.; Verweij, J.J.; Levecke, B. Comparison of four DNA extraction and three preservation protocols for the molecular detection and quantification of soil-transmitted helminths in stool. *PLoS Negl Trop Dis* **2019**, *13*, e0007778, doi:10.1371/journal.pntd.0007778.
24. Baermann, G. *A simple method for the detection of Ankylostomum (nematde) larvae in soil tests*; Javasche Boekhandel & Drukkerij: 1917.
25. Balachandra, D.; Ahmad, H.; Arifin, N.; Noordin, R. Direct detection of Strongyloides infection via molecular and antigen detection methods. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* **2021**, *40*, 27-37, doi:10.1007/s10096-020-03949-x.
26. Barda, B.; Wampfler, R.; Sayasone, S.; Phongluxa, K.; Xayavong, S.; Keoduangsy, K.; Schindler, C.; Keiser, J. Evaluation of Two DNA Extraction Methods for Detection of Strongyloides stercoralis Infection. *Journal of clinical microbiology* **2018**, *56*, doi:10.1128/JCM.01941-17.
27. Barda B, C.J.; Keiser, J. Assessment of the safety and efficacy of oral Moxidectin, Synriam, Synriam-Praziquantel combination versus Praziquantel in schoolchildren infected with Schistosoma haematobium and Schistosoma mansoni. *Tropical medicine and international health. 20*.

28. Barratt, J.L.N.; Lane, M.; Talundzic, E.; Richins, T.; Robertson, G.; Formenti, F.; Pritt, B.; Verocai, G.; Nascimento de Souza, J.; Mato Soares, N., et al. A global genotyping survey of Strongyloides stercoralis and Strongyloides fuelleborni using deep amplicon sequencing. *PLoS neglected tropical diseases* **2019**, *13*, e0007609-e0007609, doi:10.1371/journal.pntd.0007609.
29. Barratt, J.L.N.; Sapp, S.G.H. Machine learning-based analyses support the existence of species complexes for Strongyloides fuelleborni and Strongyloides stercoralis. *Parasitology* **2020**, *147*, 1184-1195, doi:10.1017/s0031182020000979.
30. Barrett, J.; Broderick, C.; Soulsby, H.; Wade, P.; Newsholme, W. Subcutaneous ivermectin use in the treatment of severe Strongyloides stercoralis infection: two case reports and a discussion of the literature. *The Journal of antimicrobial chemotherapy* **2016**, *71*, 220-225, doi:10.1093/jac/dkv315.
31. Basáñez, M.-G.; Marshall, C.; Carabin, H.; Gyorkos, T.; Joseph, L. Bayesian statistics for parasitologists. *Trends in parasitology* **2004**, *20*, 85-91, doi:10.1016/j.pt.2003.11.008.
32. Basso, W.; Grandt, L.-M.; Magnenat, A.-L.; Gottstein, B.; Campos, M. Strongyloides stercoralis infection in imported and local dogs in Switzerland: from clinics to molecular genetics. *Parasitology research* **2019**, *118*, 255-266, doi:10.1007/s00436-018-6173-3.
33. Becker, S.L.; Piraisoody, N.; Kramme, S.; Marti, H.; Silué, K.D.; Panning, M.; Nickel, B.; Kern, W.V.; Herrmann, M.; Hatz, C.F., et al. Real-time PCR for detection of Strongyloides stercoralis in human stool samples from Côte d'Ivoire: diagnostic accuracy, inter-laboratory comparison and patterns of hookworm co-infection. *Acta tropica* **2015**, *150*, 210-217, doi:10.1016/j.actatropica.2015.07.019.
34. Beknazarova, M.; Barratt, J.L.N.; Bradbury, R.S.; Lane, M.; Whiley, H.; Ross, K. Detection of classic and cryptic Strongyloides genotypes by deep amplicon sequencing: A preliminary survey of dog and human specimens collected from remote Australian communities. *PLoS neglected tropical diseases* **2019**, *13*, e0007241-e0007241, doi:10.1371/journal.pntd.0007241.
35. Beknazarova, M.; Millsteed, S.; Robertson, G.; Whiley, H.; Ross, K. Validation of DESS as a DNA Preservation Method for the Detection of Strongyloides spp. in Canine Feces. *International journal of environmental research and public health* **2017**, *14*, doi:10.3390/ijerph14060624.
36. Beknazarova, M.; Whiley, H.; Traub, R.; Ross, K. Opportunistic Mapping of Strongyloides stercoralis and Hookworm in Dogs in Remote Australian Communities. *Pathogens (Basel, Switzerland)* **2020**, *9*, doi:10.3390/pathogens9050398.
37. Benjamin-Chung J, C.Y.S.M.A.E.A.P.A.J.L.A.S.L.S.J.R.M.P.S.M.H.; Arnold, B.F. Household finished flooring and soil-transmitted helminth and Giardia infections among children in rural Bangladesh and Kenya: a prospective cohort study. *The lancet global health* **2021**, *9*, e301-e308.
38. Biggs, B.-A.; Caruana, S.; Mihrshahi, S.; Jolley, D.; Leydon, J.; Chea, L.; Nuon, S. Management of chronic strongyloidiasis in immigrants and refugees: is serologic testing useful? *The American journal of tropical medicine and hygiene* **2009**, *80*, 788-791.
39. Bisoffi, Z.; Buonfrate, D.; Angheben, A.; Boscolo, M.; Anselmi, M.; Marocco, S.; Monteiro, G.; Gobbo, M.; Bisoffi, G.; Gobbi, F. Randomized clinical trial on ivermectin versus thiabendazole for the treatment of strongyloidiasis. *PLoS neglected tropical diseases* **2011**, *5*, e1254-e1254, doi:10.1371/journal.pntd.0001254.
40. Bisoffi, Z.; Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Gobbo, M.; Bonafini, S.; Angheben, A., et al. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. *PLoS neglected tropical diseases* **2014**, *8*, e2640-e2640, doi:10.1371/journal.pntd.0002640.
41. Black, M.A.; Craig, B.A. Estimating disease prevalence in the absence of a gold standard. *Statistics in medicine* **2002**, *21*, 2653-2669, doi:10.1002/sim.1178.
42. Blatt, J.M.; Cantos, G.A. Evaluation of techniques for the diagnosis of Strongyloides stercoralis in human immunodeficiency virus (HIV) positive and HIV negative individuals in the city of Itajaí, Brazil. *Braz. j. infect. dis* **2003**, *7*, 402-408.
43. Blotkamp, J.; Krepel, H.P.; Kumar, V.; Baeta, S.; Van't Noordende, J.M.; Polderman, A.M. Observations on the morphology of adults and larval stages of Oesophagostomum sp. isolated from man in northern Togo and Ghana. *J Helminthol* **1993**, *67*, 49-61, doi:10.1017/s0022149x00012840.
44. Bon, B.; Houze, S.; Talabani, H.; Magne, D.; Belkadi, G.; Develoux, M.; Senghor, Y.; Chandenier, J.; Ancelle, T.; Hennequin, C. Evaluation of a rapid enzyme-linked immunosorbent assay for diagnosis of strongyloidiasis. *Journal of clinical microbiology* **2010**, *48*, 1716-1719, doi:10.1128/JCM.02364-09.
45. Boscolo, M.; Gobbo, M.; Mantovani, W.; Degani, M.; Anselmi, M.; Monteiro, G.B.; Marocco, S.; Angheben, A.; Mistretta, M.; Santacatterina, M., et al. Evaluation of an indirect immunofluorescence assay for strongyloidiasis as a tool for diagnosis and follow-up. *Clinical and vaccine immunology : CVI* **2007**, *14*, 129-133, doi:10.1128/CVI.00278-06.

46. Bosqui, L.R.; Gonçalves, A.L.R.; Gonçalves-Pires, M.d.R.F.; Custodio, L.A.; de Menezes, M.C.N.D.; Murad, V.A.; de Paula, F.M.; Pavanelli, W.R.; Conchon-Costa, I.; Costa-Cruz, J.M., et al. Detection of parasite-specific IgG and IgA in paired serum and saliva samples for diagnosis of human strongyloidiasis in northern Paraná state, Brazil. *Acta tropica* **2015**, *150*, 190-195, doi:10.1016/j.actatropica.2015.07.026.
47. Bosqui, L.R.; Gonzaga, H.T.; Gonçalves-Pires, M.d.R.d.F.; de Paula, F.M.; Almeida, R.S.; Pavanelli, W.R.; Conchon-Costa, I.; Costa-Cruz, J.M.; da Costa, I.N. Avidity as a criterion for diagnosis of human strongyloidiasis increases specificity of IgG ELISA. *Diagnostic microbiology and infectious disease* **2017**, *89*, 262-264, doi:10.1016/j.diagmicrobio.2017.08.008.
48. Bradbury, R. Strongyloides fuelleborni kellyi in New Guinea: neglected, ignored and unexplored. *Microbiology Australia* **2021**, *42*, 169-172.
49. Bradbury, R.; Herwaldt, B.L. Section VIII-C: Parasitic Agents. In *Biosafety in Microbiological and Biomedical Laboratories*, Potts, P.J.M.J., Ed. Washington DC, 2020; pp. 223-238.
50. Bradbury, R.S. Ternidens deminutus Revisited: A Review of Human Infections with the False Hookworm. *Trop Med Infect Dis* **2019**, *4*, doi:10.3390/tropicalmed4030106.
51. Bradbury, R.S.; Arguello, I.; Lane, M.; Cooley, G.; Handali, S.; Dimitrova, S.D.; Nascimento, F.S.; Jameson, S.; Hellmann, K.; Tharp, M., et al. Parasitic Infection Surveillance in Mississippi Delta Children. *The American journal of tropical medicine and hygiene* **2020**, *103*, 1150-1153, doi:10.4269/ajtmh.20-0026.
52. Bradbury, R.S.; Lane, M.; Arguello, I.; Handali, S.; Cooley, G.; Pilotte, N.; Williams, J.M.; Jameson, S.; Montgomery, S.P.; Hellmann, K., et al. Parasitic Disease Surveillance, Mississippi, USA. *Emerg Infect Dis* **2021**, *27*, 2201-2204, doi:10.3201/eid2708.204318.
53. Bradbury, R.S.; Pafčo, B.; Nosková, E.; Hasegawa, H. Strongyloides genotyping: a review of methods and application in public health and population genetics. *Int J Parasitol* **2021**, *51*, 1153-1166, doi:10.1016/j.ijpara.2021.10.001.
54. Brasil, R.; Badaró, R.; Andrade, J.A.F.d.; Andrade, T.M.d.; Carvalho, E.M. Diagnóstico sorológico da estrongiloidíase humana, através do método imunoenzimático TT - Immunoenzymatic serological diagnosis of human strongyloidiasis. *Rev. Soc. Bras. Med. Trop* **1988**, *21*, 139-143.
55. Buonfrate, D.; Bisanzio, D.; Giorli, G.; Odermatt, P.; Fürst, T.; Greenaway, C.; French, M.; Reithinger, R.; Gobbi, F.; Montresor, A., et al. The Global Prevalence of Strongyloides stercoralis Infection. *Pathogens* **2020**, *9*, doi:10.3390/pathogens9060468.
56. Buonfrate, D.; Fittipaldo, A.; Vlieghe, E.; Bottieau, E. Clinical and laboratory features of Strongyloides stercoralis infection at diagnosis and after treatment: a systematic review and meta-analysis. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2021**, 10.1016/j.cmi.2021.07.016, doi:10.1016/j.cmi.2021.07.016.
57. Buonfrate, D.; Gobbi, F.; Angheben, A.; Bisoffi, Z. Strongyloides stercoralis: the need for accurate information. *Lancet* **2018**, *391*, 2322-2323, doi:10.1016/s0140-6736(18)30864-x.
58. Buonfrate, D.; Marrone, R.; Silva, R.; Mirisola, C.; Ragusa, A.; Mistretta, M.; Perandin, F.; Bisoffi, Z. Prevalence of Strongyloidiasis in a Cohort of Migrants in Italy and Accuracy of a Novel ELISA Assay for S. stercoralis Infection, a Cross-Sectional Study. *Microorganisms* **2021**, *9*, doi:10.3390/microorganisms9020401.
59. Buonfrate, D.; Paradies, P.; Iarussi, F.; Formenti, F.; Perandin, F.; Otranto, D.; Bisoffi, Z. Serological and molecular tests for the diagnosis of Strongyloides stercoralis infection in dogs. *Parasitology research* **2017**, *116*, 2027-2029, doi:10.1007/s00436-017-5468-0.
60. Buonfrate, D.; Perandin, F.; Formenti, F.; Bisoffi, Z. A retrospective study comparing agar plate culture, indirect immunofluorescence and real-time PCR for the diagnosis of Strongyloides stercoralis infection. *Parasitology* **2017**, *144*, 812-816, doi:10.1017/S0031182016002559.
61. Buonfrate, D.; Requena-Mendez, A.; Angheben, A.; Cinquini, M.; Cruciani, M.; Fittipaldo, A.; Giorli, G.; Gobbi, F.; Piubelli, C.; Bisoffi, Z. Accuracy of molecular biology techniques for the diagnosis of Strongyloides stercoralis infection-A systematic review and meta-analysis. *PLoS neglected tropical diseases* **2018**, *12*, e0006229-e0006229, doi:10.1371/journal.pntd.0006229.
62. Buonfrate, D.; Requena-Mendez, A.; Angheben, A.; Muñoz, J.; Gobbi, F.; Van Den Ende, J.; Bisoffi, Z. Severe strongyloidiasis: a systematic review of case reports. *BMC infectious diseases* **2013**, *13*, 78-78, doi:10.1186/1471-2334-13-78.
63. Buonfrate, D.; Salas-Coronas, J.; Muñoz, J.; Maruri, B.T.; Rodari, P.; Castelli, F.; Zammarchi, L.; Bianchi, L.; Gobbi, F.; Cabezas-Fernández, T., et al. Multiple-dose versus single-dose ivermectin for Strongyloides stercoralis infection (Strong Treat 1 to 4): a multicentre, open-label, phase 3, randomised controlled superiority trial. *The Lancet. Infectious diseases* **2019**, *19*, 1181-1190, doi:10.1016/S1473-3099(19)30289-0.
64. Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Degani, M.; Tais, S.; Angheben, A.; Requena-Mendez, A., et al. Accuracy of five serologic tests for the follow up of Strongyloides

stercoralis infection. *PLoS neglected tropical diseases* **2015**, *9*, e0003491-e0003491, doi:10.1371/journal.pntd.0003491.
65. Buonfrate, D.; Vazquez-Villegas, J.; Munoz, J.; Chiodini, P.L.; Trevino-Maruri, B.; Zanotti, P.; Zammarchi, L.; Bianchi, L.; Gobbi, F.; Soriano-Perez, M.J., et al. Strong treat 1 to 4: A randomized, open-label clinical trial on multiple versus single dose of ivermectin for the treatment of strongyloidiasis. *American Journal of Tropical Medicine and Hygiene* **2018**, *99*, 399-399.
66. Buonfrate, D.; Zammarchi, L.; Bisoffi, Z.; Montresor, A.; Boccalini, S. Control programs for strongyloidiasis in areas of high endemicity: an economic analysis of different approaches. *Infect Dis Poverty* **2021**, *10*, 76, doi:10.1186/s40249-021-00858-9.
67. Campo Polanco, L.; Gutiérrez, L.A.; Cardona Arias, J. [Diagnosis of Strongyloides Stercoralis infection: meta-analysis on evaluation of conventional parasitological methods (1980-2013)]. *Revista espanola de salud publica* **2014**, *88*, 581-600, doi:10.4321/S1135-57272014000500004.
68. Campo-Polanco, L.F.; Sarmiento, J.M.H.; Mesa, M.A.; Franco, C.J.V.; López, L.; Botero, L.E.; Builes, L.A.G. Strongyloidiasis in humans: diagnostic efficacy of four conventional methods and real-time polymerase chain reaction. *Revista da Sociedade Brasileira de Medicina Tropical* **2018**, *51*, 493-502, doi:10.1590/0037-8682-0055-2018.
69. Carvalho, G.L.X.d.; Moreira, L.E.; Pena, J.L.; Marinho, C.C.; Bahia, M.T.; Machado-Coelho, G.L.L. A comparative study of the TF-Test®, Kato-Katz, Hoffman-Pons-Janer, Willis and Baermann-Moraes coprologic methods for the detection of human parasitosis. *Memorias do Instituto Oswaldo Cruz* **2012**, *107*, 80-84, doi:10.1590/s0074-02762012000100011.
70. Cervone, M.; Giannelli, A.; Otranto, D.; Perrucci, S. Strongyloides stercoralis hyperinfection in an immunosuppressed dog from France. *Revue Veterinaire Clinique* **2016**, *51*, 55-59.
71. Chankongsin, S.; Wampfler, R.; Ruf, M.T.; Odermatt, P.; Marti, H.; Nickel, B.; Keoluangkhot, V.; Neumayr, A. Strongyloides stercoralis prevalence and diagnostics in Vientiane, Lao People's Democratic Republic. *Infect Dis Poverty* **2020**, *9*, 133, doi:10.1186/s40249-020-00750-y.
72. Chaves, L.A.; Gonçalves, A.L.R.; Paula, F.M.; Silva, N.M.; Silva, C.V.; Costa-Cruz, J.M.; Freitas, M.A.R. Comparison of parasitological, immunological and molecular methods for evaluation of fecal samples of immunosuppressed rats experimentally infected with Strongyloides venezuelensis. *Parasitology* **2015**, *142*, 1715-1721, doi:10.1017/S0031182015001298.
73. Cohen, J.F.; Korevaar, D.A.; Altman, D.G.; Bruns, D.E.; Gatsonis, C.A.; Hooft, L.; Irwig, L.; Levine, D.; Reitsma, J.B.; de Vet, H.C., et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *BMJ open* **2016**, *6*, e012799, doi:10.1136/bmjopen-2016-012799.
74. Conway, D.J.; Bailey, J.W.; Lindo, J.F.; Robinson, R.D.; Bundy, D.A.; Bianco, A.E. Serum IgG reactivity with 41-, 31-, and 28-kDa larval proteins of Strongyloides stercoralis in individuals with strongyloidiasis. *The Journal of infectious diseases* **1993**, *168*, 784-787, doi:10.1093/infdis/168.3.784.
75. Cools, P.; Vlaminck, J.; Albonico, M.; Ame, S.; Ayana, M.; José Antonio, B.P.; Cringoli, G.; Dana, D.; Keiser, J.; Maurelli, M.P., et al. Diagnostic performance of a single and duplicate Kato-Katz, Mini-FLOTAC, FECPAKG2 and qPCR for the detection and quantification of soil-transmitted helminths in three endemic countries. *PLoS Negl Trop Dis* **2019**, *13*, e0007446, doi:10.1371/journal.pntd.0007446.
76. Corral, M.A.; Paula, F.M.; Gottardi, M.; Meisel, D.M.C.L.; Chieffi, P.P.; Gryschek, R.C.B. Membrane fractions from Strongyloides venezuelensis in the immunodiagnosis of human strongyloidiasis TT - Frações de membrana de Strongyloides venezuelensis para o imunodiagnóstico da estrongiloidíase humana. *Rev. Inst. Med. Trop. Säo Paulo* **2015**, *57*, 77-80.
77. Costa-Cruz, J.M.; Bullamah, C.B.; Gonçalves-Pires, M.d.R.; Campos, D.M.; Vieira, M.A. Cryo-microtome sections of coproculture larvae of Strongyloides stercoralis and Strongyloides ratti as antigen sources for the immunodiagnosis of human strongyloidiasis. *Revista do Instituto de Medicina Tropical de Sao Paulo* **1997**, *39*, 313-317, doi:10.1590/s0036-46651997000600001.
78. Coutinho, J.; Campos, R.; Amato Neto, V. Nota sobre o diagnostico e prevalencia de estrongiloidose em Sao Paulo. *Rev Clin de Sao paulo* **1951**, *27*, 11-20.
79. Cringoli, G.; Rinaldi, L.; Albonico, M.; Bergquist, R.; Utzinger, J. Geospatial (s)tools: integration of advanced epidemiological sampling and novel diagnostics. *Geospatial health* **2013**, *7*, 399-404, doi:10.4081/gh.2013.97.
80. Cringoli, G.; Rinaldi, L.; Maurelli, M.P.; Utzinger, J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nat Protoc* **2010**, *5*, 503-515, doi:10.1038/nprot.2009.235.
81. Cvetkovikj, A.; Rashikj, L.; Celeska, I.; Petrov, E. First Case of Strongyloides Stercoralis Infection in a Dog in the Republic of Macedonia. *Macedonian Veterinary Review* **2018**, *41*, 95-98.

82. Dacal, E.; Saugar, J.M.; Soler, T.; Azcárate, J.M.; Jiménez, M.S.; Merino, F.J.; Rodríguez, E. Parasitological versus molecular diagnosis of strongyloidiasis in serial stool samples: how many? *Journal of helminthology* **2018**, *92*, 12-16, doi:10.1017/S0022149X17000050.
83. Dancescu, P. Investigations on the intensity of the infection in a strongyloidiasis focus. The coal culture method. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1968**, *62*, 490-495, doi:10.1016/0035-9203(68)90131-4.
84. Dashchenko, S.; Soroka, N.; Semenko, O. DISTRIBUTION OF STRONGYLOIDES STERCORALIS AMONG DOGS OF DIFFERENT HOUSING GROUPS IN KYIV AND KYIV REGION, CLINICAL MANIFESTATIONS AND DIAGNOSTIC METHODS. *EUREKA: Health Sciences* **2020**, *5*, 99-107.
85. de Kaminsky, R.G. Evaluation of three methods for laboratory diagnosis of Strongyloides stercoralis infection. *The Journal of parasitology* **1993**, *79*, 277-280.
86. de Souza, J.N.; Langford, I.I.; Wang, Y.; Soares, N.M.; Handali, S. Development of rSs-NIE-1 and rSs-IR Recombinant Antigen-Based Immunoblot for Detection of Antibody to Strongyloides stercoralis. *The American journal of tropical medicine and hygiene* **2021**, *104*, 2038-2041, doi:10.4269/ajtmh.20-1539.
87. Dendukuri, N.; Bélisle, P.; Joseph, L. Bayesian sample size for diagnostic test studies in the absence of a gold standard: Comparing identifiable with non-identifiable models. *Statistics in medicine* **2010**, *29*, 2688-2697, doi:10.1002/sim.4037.
88. Dendukuri, N.; Joseph, L. Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics* **2001**, *57*, 158-167, doi:10.1111/j.0006-341x.2001.00158.x.
89. Dillard, K.J.; Saari, S.A.; Anttila, M. Strongyloides stercoralis infection in a Finnish kennel. *Acta veterinaria Scandinavica* **2007**, *49*, 37-37, doi:10.1186/1751-0147-49-37.
90. Dreyer, G.; Fernandes-Silva, E.; Alves, S.; Rocha, A.; Albuquerque, R.; Addiss, D. Patterns of detection of Strongyloides stercoralis in stool specimens: implications for diagnosis and clinical trials. *Journal of clinical microbiology* **1996**, *34*, 2569-2571, doi:10.1128/jcm.34.10.2569-2571.1996.
91. Eamudomkarn, C.; Sithithaworn, P.; Sithithaworn, J.; Kaewkes, S.; Sripa, B.; Itoh, M. Comparative evaluation of Strongyloides ratti and S. stercoralis larval antigen for diagnosis of strongyloidiasis in an endemic area of opisthorchiasis. *Parasitology research* **2015**, *114*, 2543-2551, doi:10.1007/s00436-015-4458-3.
92. Erdman, D.D. Clinical comparison of ethyl acetate and diethyl ether in the formalin-ether sedimentation technique. *J Clin Microbiol* **1981**, *14*, 483-485, doi:10.1128/jcm.14.5.483-485.1981.
93. Eydal, M.; Skirnisson, K. Strongyloides stercoralis found in imported dogs, household dogs and kennel dogs in Iceland. *Icelandic Agricultural Sciences* **2016**, *29*, 39-51.
94. Faust, E.; D'Antonio, J.; Odon, V.; Miller, M.; Perez, C.; Sawitz, W.; Thomen, F.; Tobie, J.; Walker, H. A Critical Study of Clinical Laboratory Technics for the Diagnosis of Protozoan Cysts and Helminth Eggs in Feces. *American Journal of Tropical Medicine and Hygiene* **1938**, *18*, 169-183.
95. Faust, E.C.; Sawitz, W.; Tobie, J.; Odon, V.; Peres, C.; Linicome, D. Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces. *J Parasitol* **1939**, *25*, 241-262.
96. FDA. US Food and Drug Administration. Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests - Guidance for Industry and FDA Staff. Availabe online: (accessed on 20 December 2021).
97. Feliciano, N.D.; Ribeiro, V.d.S.; Santos, F.d.A.A.; Fujimura, P.T.; Gonzaga, H.T.; Goulart, L.R.; Costa-Cruz, J.M. Bacteriophage-fused peptides for serodiagnosis of human strongyloidiasis. *PLoS neglected tropical diseases* **2014**, *8*, e2792-e2792, doi:10.1371/journal.pntd.0002792.
98. Fernández-Soto, P.; Celis-Giraldo, C.T.; Collar-Fernández, C.; Gorgojo, Ó.; Camargo, M.; Muñoz, J.; Salas-Coronas, J.; Patarroyo, M.A.; Muro, A. Strong-LAMP Assay Based on a Strongyloides spp.-Derived Partial Sequence in the 18S rRNA as Potential Biomarker for Strongyloidiasis Diagnosis in Human Urine Samples. *Disease markers* **2020**, *2020*, 5265198-5265198, doi:10.1155/2020/5265198.
99. Fernández-Soto, P.; Sánchez-Hernández, A.; Gandasegui, J.; Bajo Santos, C.; López-Abán, J.; Saugar, J.M.; Rodríguez, E.; Vicente, B.; Muro, A. Strong-LAMP: A LAMP Assay for Strongyloides spp. Detection in Stool and Urine Samples. Towards the Diagnosis of Human Strongyloidiasis Starting from a Rodent Model. *PLoS neglected tropical diseases* **2016**, *10*, e0004836-e0004836, doi:10.1371/journal.pntd.0004836.
100. Formenti, F.; Buonfrate, D.; Prandi, R.; Marquez, M.; Caicedo, C.; Rizzi, E.; Guevara, A.G.; Vicuña, Y.; Huerlo, F.R.; Perandin, F., et al. Comparison of S. stercoralis Serology Performed on Dried Blood Spots and on Conventional Serum Samples. *Frontiers in microbiology* **2016**, *7*, 1778-1778, doi:10.3389/fmicb.2016.01778.
101. Formenti, F.; La Marca, G.; Perandin, F.; Pajola, B.; Romano, M.; Santucci, B.; Silva, R.; Giorli, G.; Bisoffi, Z.; Buonfrate, D. A diagnostic study comparing conventional and real-time PCR for Strongyloides stercoralis on urine and on faecal samples. *Acta tropica* **2019**, *190*, 284-287, doi:10.1016/j.actatropica.2018.12.001.

102. Fradejas, I.; Herrero-Martínez, J.M.; Lizasoaín, M.; Rodríguez de Las Parras, E.; Pérez-Ayala, A. Comparative study of two commercial tests for Strongyloides stercoralis serologic diagnosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2018**, *112*, 561-567, doi:10.1093/trstmh/try101.
103. Fuelleborn, F. Über das Verhalten der Larven von Strongyloides Stercoralis, Hakenwürmern und Ascaris lumbricoides im Körper des Wirtes. *Arch. Schiffs.-u. Tropenhyg* **1927**, *31*, 1-56.
104. Galliard, H. [Experimental infestation with Strongyloides stercoralis in Tonkin; part 2]. *Ann Parasitol Hum Comp* **1951**, *26*, 67-84.
105. Galliard, H. [Study on the experimental infestation with Strongyloides stercoralis in Tonkin. XII. Action of physical and chemical factors on the endogenous and exogenous development]. *Ann Parasitol Hum Comp* **1951**, *26*, 201-227.
106. Garrett, P.; Lasky, F.; Meier, K. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline. In *Clinical and Laboratory Standards Institute*, 2nd ed.; Wayne, PA, 2008.
107. Gelaye, W.; Williams, N.A.; Kepha, S.; Junior, A.M.; Fleitas, P.E.; Marti-Soler, H.; Damtie, D.; Menkir, S.; Krolewiecki, A.J.; van Lieshout, L., et al. Performance evaluation of Baermann techniques: The quest for developing a microscopy reference standard for the diagnosis of Strongyloides stercoralis. *PLoS neglected tropical diseases* **2021**, *15*, e0009076-e0009076, doi:10.1371/journal.pntd.0009076.
108. Georgi, J.R.; Sprinkle, C.L. A case of human strongyloidosis apparently contracted from asymptomatic colony dogs. *The American journal of tropical medicine and hygiene* **1974**, *23*, 899-901, doi:10.4269/ajtmh.1974.23.899.
109. Getaneh, A.; Medhin, G.; Shimelis, T. Cryptosporidium and Strongyloides stercoralis infections among people with and without HIV infection and efficiency of diagnostic methods for Strongyloides in Yirgalem Hospital, southern Ethiopia. *BMC research notes* **2010**, *3*, 90-90, doi:10.1186/1756-0500-3-90.
110. Ghasemikhah, R.; Tabatabaiefar, M.A.; Shariatzadeh, S.A.; Shahbazi, A.; Hazratian, T. A PCR-Based Molecular Detection of Strongyloides stercoralisin Human Stool Samples from Tabriz City, Iran. *Scientia pharmaceutica* **2017**, *85*, doi:10.3390/scipharm85020017.
111. Glinz, D.; Silué, K.D.; Knopp, S.; Lohourignon, L.K.; Yao, K.P.; Steinmann, P.; Rinaldi, L.; Cringoli, G.; N'Goran, E.K.; Utzinger, J. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for Schistosoma mansoni and soil-transmitted helminths. *PLoS neglected tropical diseases* **2010**, *4*, e754-e754, doi:10.1371/journal.pntd.0000754.
112. Gonçalves, A.L.R.; Machado, G.A.; Gonçalves-Pires, M.R.F.; Ferreira-Júnior, A.; Silva, D.A.O.; Costa-Cruz, J.M. Evaluation of strongyloidiasis in kennel dogs and keepers by parasitological and serological assays. *Veterinary parasitology* **2007**, *147*, 132-139, doi:10.1016/j.vetpar.2007.03.016.
113. Gonzalez Akimori, D.; Dalessandro, E.J.; Nolan, T.J.; Stieha, C.R.; Lok, J.B.; Stoltzfus, J.D.C. Transcriptional profiles in Strongyloides stercoralis males reveal deviations from the Caenorhabditis sex determination model. *Sci Rep* **2021**, *11*, 8254, doi:10.1038/s41598-021-87478-3.
114. Gorgani-Firouzjaee, T.; Kalantari, N.; Javanian, M.; Ghaffari, S. Strongyloides stercoralis: detection of parasite-derived DNA in serum samples obtained from immunosuppressed patients. *Parasitology research* **2018**, *117*, 2927-2932, doi:10.1007/s00436-018-5985-5.
115. Gottardi, M.; Paula, F.M.; Corral, M.A.; Meisel, D.M.C.; Costa, S.F.; Abdala, E.; Pierrotti, L.C.; Yamashiro, J.; Chieffi, P.P.; Gryschek, R.C.B. Immunofluorescence assay for diagnosis of strongyloidiasis in immunocompromised patients. *Infectious diseases (London, England)* **2015**, *47*, 550-554, doi:10.3109/23744235.2015.1028096.
116. Graeff-Teixeira, C.; Medeiros, E.; Zanini, G.M.; Brasil, C.A.; Cardozo, B.L.; Dalpiaz, M.G.; Bisol, L.W. Inexpensive alternative material for the isolation of larvae with the Baermann method. *Mem Inst Oswaldo Cruz* **1997**, *92*, 399-400, doi:10.1590/s0074-02761997000300017.
117. Grove, D.I. Human strongyloidiasis. *Adv Parasitol* **1996**, *38*, 251-309, doi:10.1016/s0065-308x(08)60036-6.
118. Grove, D.I.; Northern, C. Infection and immunity in dogs infected with a human strain of Strongyloides stercoralis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1982**, *76*, 833-838, doi:10.1016/0035-9203(82)90120-1.
119. Hailegebriel, T.; Petros, B.; Endeshaw, T. Evaluation of Parasitological Methods for the Detection of Strongyloides Stercoralis among Individuals in Selected Health Institutions In Addis Ababa, Ethiopia. *Ethiop J Health Sci* **2017**, *27*, 515-522, doi:10.4314/ejhs.v27i5.10.
120. Hailu, T.; Nibret, E.; Amor, A.; Munshea, A. Strongyloides Stercoralis Infection in Ethiopia: Systematic Review and Meta-analysis on Prevalence and Diagnostic Methods. *Helminthologia* **2021**, *58*, 17-27, doi:10.2478/helm-2021-0010.

121. Hailu, T.; Nibret, E.; Amor, A.; Munshea, A.; Anegagrie, M. Agar Plate Culture: An Alternative Sensitive Routine Laboratory Detection Method for Strongyloides stercoralis and Hookworm Parasites. *Iranian journal of parasitology* **2021**, *16*, 72-80, doi:10.18502/ijpa.v16i1.5514.
122. Hall, E.; Lyriaki, M.; Whitworth, F.; Black, V. Severe strongyloidosis in dogs. *Vet Rec* **2020**, *186*, 354-355, doi:10.1136/vr.m1077.
123. Harada, Y.; Mori, O. A new method for culturing hookworm. *Yonago Acta Med* **1955**, *1*, 177-179.
124. Hasegawa, H.; Hayashida, S.; Ikeda, Y.; Sato, H. Hyper-variable regions in 18S rDNA of Strongyloides spp. as markers for species-specific diagnosis. *Parasitology research* **2009**, *104*, 869-874, doi:10.1007/s00436-008-1269-9.
125. Hasegawa, H.; Sato, H.; Fujita, S.; Nguema, P.P.; Nobusue, K.; Miyagi, K.; Kooriyama, T.; Takenoshita, Y.; Noda, S.; Sato, A., et al. Molecular identification of the causative agent of human strongyloidiasis acquired in Tanzania: dispersal and diversity of Strongyloides spp. and their hosts. *Parasitol Int* **2010**, *59*, 407-413, doi:10.1016/j.parint.2010.05.007.
126. Henriquez-Camacho, C.; Gotuzzo, E.; Echevarria, J.; White, A.C., Jr.; Terashima, A.; Samalvides, F.; Pérez-Molina, J.A.; Plana, M.N. Ivermectin versus albendazole or thiabendazole for Strongyloides stercoralis infection. *Cochrane Database Syst Rev* **2016**, *2016*, CD007745, doi:10.1002/14651858.CD007745.pub3.
127. Hernández Chavarria, F. Strongyloides stercoralis: un parásito subestimado TT - Strongyloides stercoralis: an underestimated parasite. *Parasitol. día* **2001**, *25*, 40-49.
128. Hernández-Chavarría, F.; Avendaño, L. A simple modification of the Baermann method for diagnosis of strongyloidiasis. *Memorias do Instituto Oswaldo Cruz* **2001**, *96*, 805-807, doi:10.1590/s0074-02762001000600011.
129. Hoffman, W.; Pons, J.; Janer, J. Sedimentation concentration method in Schistosomiasis mansoni. *Puerto Rico J Publ Health* **1934**, *9*, 283-298.
130. Hofmann, D.; Sayasone, S.; Sengngam, K.; Chongvilay, B.; Hattendorf, J.; Keiser, J. Efficacy and safety of ascending doses of moxidectin against Strongyloides stercoralis infections in adults: a randomised, parallel-group, single-blinded, placebo-controlled, dose-ranging, phase 2a trial. *The Lancet. Infectious diseases* **2021**, *21*, 1151-1160, doi:10.1016/S1473-3099(20)30691-5.
131. Holt, D.C.; Shield, J.; Harris, T.M.; Mounsey, K.E.; Aland, K.; McCarthy, J.S.; Currie, B.J.; Kearns, T.M. Soil-Transmitted Helminths in Children in a Remote Aboriginal Community in the Northern Territory: Hookworm is Rare but Strongyloides stercoralis and Trichuris trichiura Persist. *Tropical medicine and infectious disease* **2017**, *2*, doi:10.3390/tropicalmed2040051.
132. Huaman, M.C.; Sato, Y.; Aguilar, J.L.; Terashima, A.; Guerra, H.; Gotuzzo, E.; Kanbara, H. Gelatin particle indirect agglutination and enzyme-linked immunosorbent assay for diagnosis of strongyloidiasis using Strongyloides venezuelensis antigen. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2003**, *97*, 535-538, doi:10.1016/s0035-9203(03)80017-2.
133. Iamrod, K.; Chaidee, A.; Rucksaken, R.; Kopolrat, K.Y.; Worasith, C.; Wongphutorn, P.; Intuyod, K.; Pinlaor, S.; Sithithaworn, J.; Sithithaworn, P., et al. Development and Efficacy of Droplet Digital PCR for Detection of Strongyloides stercoralis in Stool. *The American journal of tropical medicine and hygiene* **2021**, 10.4269/ajtmh.21-0729, doi:10.4269/ajtmh.21-0729.
134. Iatta, R.; Buonfrate, D.; Paradies, P.; Cavalera, M.A.; Capogna, A.; Iarussi, F.; Šlapeta, J.; Giorli, G.; Trerotoli, P.; Bisoffi, Z., et al. Occurrence, diagnosis and follow-up of canine strongyloidiosis in naturally infected shelter dogs. *Parasitology* **2019**, *146*, 246-252, doi:10.1017/S0031182018001312.
135. Inês, E.D.J.; Pacheco, F.T.F.; Pinto, M.C.; Mendes, P.S.d.A.; Da Costa-Ribeiro, H., Jr.; Soares, N.M.; Teixeira, M.C.A. [Concordance between the zinc sulphate flotation and centrifugal sedimentation methods for the diagnosis of intestinal parasites]. *Biomedica : revista del Instituto Nacional de Salud* **2016**, *36*, 519-524, doi:10.7705/biomedica.v36i4.2799.
136. Inês, E.d.J.; Souza, J.N.; Santos, R.C.; Souza, E.S.; Santos, F.L.; Silva, M.L.S.; Silva, M.P.; Teixeira, M.C.A.; Soares, N.M. Efficacy of parasitological methods for the diagnosis of Strongyloides stercoralis and hookworm in faecal specimens. *Acta tropica* **2011**, *120*, 206-210, doi:10.1016/j.actatropica.2011.08.010.
137. Intapan, P.M.; Maleewong, W.; Wongsaroj, T.; Singthong, S.; Morakote, N. Comparison of the quantitative formalin ethyl acetate concentration technique and agar plate culture for diagnosis of human strongyloidiasis. *Journal of clinical microbiology* **2005**, *43*, 1932-1933, doi:10.1128/JCM.43.4.1932-1933.2005.
138. Jaleta, T.G.; Zhou, S.; Bemm, F.M.; Schär, F.; Khieu, V.; Muth, S.; Odermatt, P.; Lok, J.B.; Streit, A. Different but overlapping populations of Strongyloides stercoralis in dogs and humans-Dogs as a possible source for zoonotic strongyloidiasis. *PLoS Negl Trop Dis* **2017**, *11*, e0005752, doi:10.1371/journal.pntd.0005752.
139. Jongwutiwes, S.; Charoenkorn, M.; Sitthichareonchai, P.; Akaraborvorn, P.; Putaporntip, C. Increased sensitivity of routine laboratory detection of Strongyloides stercoralis and hookworm by agar-plate culture.

Transactions of the Royal Society of Tropical Medicine and Hygiene **1999**, *93*, 398-400, doi:10.1016/s0035-9203(99)90132-3.
140. Joseph, L.; Gyorkos, T.W.; Coupal, L. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. *American journal of epidemiology* **1995**, *141*, 263-272, doi:10.1093/oxfordjournals.aje.a117428.
141. Júnior, A.F.; Gonçalves-Pires, M.R.F.; Silva, D.A.O.; Gonçalves, A.L.R.; Costa-Cruz, J.M. Parasitological and serological diagnosis of Strongyloides stercoralis in domesticated dogs from southeastern Brazil. *Veterinary parasitology* **2006**, *136*, 137-145, doi:10.1016/j.vetpar.2005.10.022.
142. Kaewrat, W.; Sengthong, C.; Yingklang, M.; Intuyod, K.; Haonon, O.; Onsurathum, S.; Dangtakot, R.; Saisud, P.; Sangka, A.; Anutrakulchai, S., et al. Improved agar plate culture conditions for diagnosis of Strongyloides stercoralis. *Acta tropica* **2020**, *203*, 105291-105291, doi:10.1016/j.actatropica.2019.105291.
143. Kalantari, N.; Chehrazi, M.; Ghaffari, S.; Gorgani-Firouzjaee, T. Serological assays for the diagnosis of Strongyloides stercoralis infection: a systematic review and meta-analysis of diagnostic test accuracy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2020**, *114*, 459-469, doi:10.1093/trstmh/trz135.
144. Kato, K.; Miura, M. Comparative examinations. *Japanese Journal of Parasitology* **1954**, *3*, 35.
145. Katz, N.; Chaves, A.; Pellegrino, J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo* **1972**, *14*, 397-400.
146. Kearns, T.M.; Currie, B.J.; Cheng, A.C.; McCarthy, J.; Carapetis, J.R.; Holt, D.C.; Page, W.; Shield, J.; Gundjirryirr, R.; Mulholland, E., et al. Strongyloides seroprevalence before and after an ivermectin mass drug administration in a remote Australian Aboriginal community. *PLoS neglected tropical diseases* **2017**, *11*, e0005607-e0005607, doi:10.1371/journal.pntd.0005607.
147. Khanna, V.; Tilak, K.; Prakash, P.Y.; Mukhopadhyay, C. Modified agar plate culture method for culture of Strongyloides stercoralis. *Tropical parasitology* **2015**, *5*, 136-138, doi:10.4103/2229-5070.162535.
148. Khieu, V.; Schär, F.; Marti, H.; Sayasone, S.; Duong, S.; Muth, S.; Odermatt, P. Diagnosis, treatment and risk factors of Strongyloides stercoralis in schoolchildren in Cambodia. *PLoS neglected tropical diseases* **2013**, *7*, e2035-e2035, doi:10.1371/journal.pntd.0002035.
149. Kightlinger, L.; Kightlinger, M.B. Examination of faecal specimens by the formalin-detergent technique. *Trans R Soc Trop Med Hyg* **1990**, *84*, 417-418, doi:10.1016/0035-9203(90)90341-b.
150. Knopp, S.; Mgeni, A.F.; Khamis, I.S.; Steinmann, P.; Stothard, J.R.; Rollinson, D.; Marti, H.; Utzinger, J. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS neglected tropical diseases* **2008**, *2*, e331-e331, doi:10.1371/journal.pntd.0000331.
151. Ko, P.P.; Suzuki, K.; Canales-Ramos, M.; Aung, M.; Htike, W.W.; Yoshida, A.; Montes, M.; Morishita, K.; Gotuzzo, E.; Maruyama, H., et al. Phylogenetic relationships of Strongyloides species in carnivore hosts. *Parasitol Int* **2020**, *78*, 102151, doi:10.1016/j.parint.2020.102151.
152. Kobayashi, J.; Hasegawa, H.; Soares, E.C.; Toma, H.; Dacal, A.R.; Brito, M.C.; Yamanaka, A.; Foli, A.A.; Sato, Y. Studies on prevalence of Strongyloides infection in Holambra and Maceió, Brazil, by the agar plate faecal culture method. *Revista do Instituto de Medicina Tropical de Sao Paulo* **1996**, *38*, 279-284, doi:10.1590/s0036-46651996000400007.
153. Koga, K.; Kasuya, S.; Khamboonruang, C.; Sukhavat, K.; Ieda, M.; Takatsuka, N.; Kita, K.; Ohtomo, H. A modified agar plate method for detection of Strongyloides stercoralis. *The American journal of tropical medicine and hygiene* **1991**, *45*, 518-521, doi:10.4269/ajtmh.1991.45.518.
154. Koga, K.; Kasuya, S.; Ohtomo, H. How effective is the agar plate method for Strongyloides stercoralis? *The Journal of parasitology* **1992**, *78*, 155-156.
155. Koosha, S.; Fesharaki, M.; Rokni, M.B. Comparison of enzyme-linked immunosorbent assay and indirect immunofluorescence assay in the diagnosis of human strongyloidiasis. *Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology* **2004**, *23*, 214-216.
156. Kramme, S.; Nissen, N.; Soblik, H.; Erttmann, K.; Tannich, E.; Fleischer, B.; Panning, M.; Brattig, N. Novel real-time PCR for the universal detection of Strongyloides species. *Journal of medical microbiology* **2011**, *60*, 454-458, doi:10.1099/jmm.0.025338-0.
157. Kristanti, H.; Meyanti, F.; Wijayanti, M.A.; Mahendradhata, Y.; Polman, K.; Chappuis, F.; Utzinger, J.; Becker, S.L.; Murhandarwati, E.E.H. Diagnostic comparison of Baermann funnel, Koga agar plate culture and polymerase chain reaction for detection of human Strongyloides stercoralis infection in Maluku, Indonesia. *Parasitology research* **2018**, *117*, 3229-3235, doi:10.1007/s00436-018-6021-5.
158. Krolewiecki, A.J.; Ramanathan, R.; Fink, V.; McAuliffe, I.; Cajal, S.P.; Won, K.; Juarez, M.; Di Paolo, A.; Tapia, L.; Acosta, N., et al. Improved diagnosis of Strongyloides stercoralis using recombinant antigen-based serologies

in a community-wide study in northern Argentina. *Clinical and vaccine immunology : CVI* **2010**, *17*, 1624-1630, doi:10.1128/CVI.00259-10.
159. Kubofcik, J.; Nutman, T.B. Next generation immunoassays provide one-step species-specificity for the diagnosis of filarial infections and strongyloides stercoralis in travelers and immigrants. *American Journal of Tropical Medicine and Hygiene* **2016**, *95*, 600-600, doi:10.4269/ajtmh.abstract2016 LK - http://BT8EP3TD6D.search.serialssolutions.com?sid=EMBASE&issn=00029637&id=doi:10.4269%2Fajtmh.abstract2016&atitle=Next+generation+immunoassays+provide+one-step+species-specificity+for+the+diagnosis+of+filarial+infections+and+strongyloides+stercoralis+in+travelers+and+immigrants&stitle=Am.+J.+Trop.+Med.+Hyg.&title=American+Journal+of+Tropical+Medicine+and+Hygiene&volume=95&issue=5&spage=600&epage=&aulast=Kubofcik&aufirst=Joseph&auinit=J.&aufull=Kubofcik+J.&coden=&isbn=&pages=600-&date=2016&auinit1=J&auinitm=.
160. Lau Chong, C.; Samalvides Cuba, F.; Terashima Iwashita, A. Evaluación de técnicas parasitológicas en el diagnóstico de estrongiloidiasis por Strongyloides stercoralis TT - Efficacy of five methods for detection of Strongyloides stercoralis in human stool specimens. *Rev. méd. hered* **2005**, *16*, 11-18.
161. Le Pogam, A.; Lopinto, J.; Pecriaux, A.; Fartoukh, M.; Guitard, J.; Voiriot, G. Strongyloides stercoralis disseminated infection in an HIV-infected adult. *PLoS Negl Trop Dis* **2020**, *14*, e0008766, doi:10.1371/journal.pntd.0008766.
162. Levenhagen, M.A.; Fujimura, P.T.; Santos, F.d.A.A.; Vecchi, L.; Alves-Balvedi, R.P.; Madurro, A.G.B.; Madurro, J.M.; Costa-Cruz, J.M.; Goulart, L.R. Innovative approaches to improve serodiagnosis of Strongyloides stercoralis infection. *The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases* **2021**, *25*, 101545-101545, doi:10.1016/j.bjid.2021.101545.
163. Lindo, J.F.; Conway, D.J.; Atkins, N.S.; Bianco, A.E.; Robinson, R.D.; Bundy, D.A.P. Prospective evaluation of an ELISA and Western Blot for the diagnosis of endemic strongyloides stercoralis infection - abstract. *West Indian med. j* **1993**, *42*, 50-50.
164. Little, S.E.; Barrett, A.W.; Beall, M.J.; Bowman, D.D.; Dangoudoubiyam, S.; Elsemore, D.A.; Liotta, J.; Lucio-Forster, A.; McCrann, D.J.; Snowden, K.F., et al. Coproantigen Detection Augments Diagnosis of Common Nematode Infections in Dogs. *Topics in companion animal medicine* **2019**, *35*, 42-46, doi:10.1053/j.tcam.2019.04.001.
165. Llewellyn, S.; Inpankaew, T.; Nery, S.V.; Gray, D.J.; Verweij, J.J.; Clements, A.C.A.; Gomes, S.J.; Traub, R.; McCarthy, J.S. Application of a Multiplex Quantitative PCR to Assess Prevalence and Intensity Of Intestinal Parasite Infections in a Controlled Clinical Trial. *PLoS neglected tropical diseases* **2016**, *10*, e0004380-e0004380, doi:10.1371/journal.pntd.0004380.
166. Lodh, N.; Caro, R.; Sofer, S.; Scott, A.; Krolewiecki, A.; Shiff, C. Diagnosis of Strongyloides stercoralis: Detection of parasite-derived DNA in urine. *Acta tropica* **2016**, *163*, 9-13, doi:10.1016/j.actatropica.2016.07.014.
167. Lopez, M.; Morales, M.L.; Konana, M.; Hoyer, P.; Pineda-Reyes, R.; White, A.C., Jr.; Garcia, H.H.; Lescano, A.G.; Gotuzzo, E.; Cabada, M.M. Kato-Katz and Lumbreras rapid sedimentation test to evaluate helminth prevalence in the setting of a school-based deworming program. *Pathogens and global health* **2016**, *110*, 130-134, doi:10.1080/20477724.2016.1187361.
168. Loutfy, M.R.; Wilson, M.; Keystone, J.S.; Kain, K.C. Serology and eosinophil count in the diagnosis and management of strongyloidiasis in a non-endemic area. *The American journal of tropical medicine and hygiene* **2002**, *66*, 749-752, doi:10.4269/ajtmh.2002.66.749.
169. Lumbreras, H. Aplicación de la Técnica de Baermann modificada en copa para el diagnóstico y control terapéutico. *Rev Med Per* **1959**, *30*, 21.
170. Lumbreras, H. Strongyloidosis: I. Evaluacion de la "tecnica de Baermann modificada en copa" en el estudiod e la strongyloidosis *Revista Medica Peruana* **1963**, *22*.
171. Lumbreras, H.; Cantella, R.; Burga, R. Acerca de un procedimiento de sedimentacion rapida para investigar huevos de Fasciola hepatica en las heces, su evaluacion y uso en el campo. *Rev Med Peru* **1962**, *31*, 167–174
172. Lutz, A. Schistosomum mansoni e a Schistosomatose segundo observações, feitas no Brazil. *Mem Inst Oswaldo Cruz* **1919**, *11*, 121-155.
173. Machado, E.R.; Faccioli, L.H.; Costa-Cruz, J.M.; Lourenço, E.V.; Roque-Barreira, M.C.; Gonçalves-Pires, M.d.R.d.F.; Ueta, M.T. Strongyloides venezuelensis: the antigenic identity of eight strains for the immunodiagnosis of human strongyloidiasis. *Experimental parasitology* **2008**, *119*, 7-14, doi:10.1016/j.exppara.2007.12.008.
174. Machado, E.R.; Ueta, M.T.; de Fátima Gonçalves-Pires, M.d.R.; Alves de Oliveira, J.B.; Faccioli, L.H.; Costa-Cruz, J.M. Strongyloides venezuelensis alkaline extract for the diagnosis of human strongyloidiasis by enzyme-linked immunosorbent assay. *Memorias do Instituto Oswaldo Cruz* **2003**, *98*, 849-851, doi:10.1590/s0074-02762003000600024.

175. Machicado, J.D.; Marcos, L.A.; Tello, R.; Canales, M.; Terashima, A.; Gotuzzo, E. Diagnosis of soil-transmitted helminthiasis in an Amazonic community of Peru using multiple diagnostic techniques. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2012**, *106*, 333-339, doi:10.1016/j.trstmh.2012.03.004.
176. Maco Flores, V.; Marcos Raymundo, L.; Terashima, A.; Samalvides Cuba, F.; Miranda Sanchez, E. Fas2-ELISA y la técnica de sedimentación rápida modificada por lumbreras en el diagnostico de la infeccion por Fasciola hepatica. *Rev Med Hered* **2002**, *13*, 49-57.
177. Marti, H.; Koella, J.C. Multiple stool examinations for ova and parasites and rate of false-negative results. *J Clin Microbiol* **1993**, *31*, 3044-3045, doi:10.1128/jcm.31.11.3044-3045.1993.
178. Meurs, L.; Polderman, A.M.; Vinkeles Melchers, N.V.S.; Brienen, E.A.T.; Verweij, J.J.; Groosjohan, B.; Mendes, F.; Mechendura, M.; Hepp, D.H.; Langenberg, M.C.C., et al. Diagnosing Polyparasitism in a High-Prevalence Setting in Beira, Mozambique: Detection of Intestinal Parasites in Fecal Samples by Microscopy and Real-Time PCR. *PLoS neglected tropical diseases* **2017**, *11*, e0005310-e0005310, doi:10.1371/journal.pntd.0005310.
179. Miguel, C.B.; Levenhagen, M.A.; Costa-Cruz, J.M.; Goulart, L.R.; Alves, P.T.; Ueira-Vieira, C.; Brito, P.K.M.O.; Gomes, A.O.; Lazo-Chica, J.E.; Oliveira, C.J.F., et al. scFv against HSP60 of Strongyloides sp. and Its Application in the Evaluation of Parasite Frequency in the Elderly. *Disease markers* **2020**, *2020*, 4086929-4086929, doi:10.1155/2020/4086929.
180. Ming, D.K.; Armstrong, M.; Lowe, P.; Chiodini, P.L.; Doherty, J.F.; Whitty, C.J.M.; McGregor, A.C. Clinical and Diagnostic Features of 413 Patients Treated for Imported Strongyloidiasis at the Hospital for Tropical Diseases, London. *The American journal of tropical medicine and hygiene* **2019**, *101*, 428-431, doi:10.4269/ajtmh.19-0087.
181. Mircean, V.; Györke, A.; Cozma, V. Prevalence and risk factors of Giardia duodenalis in dogs from Romania. *Veterinary parasitology* **2012**, *184*, 325-329, doi:10.1016/j.vetpar.2011.08.022.
182. Mittal, S.; Sagi, S.V.; Hawari, R. Strongyloidiasis: endoscopic diagnosis. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **2009**, *7*, e8-e8, doi:10.1016/j.cgh.2008.08.045.
183. Monteiro, M.F.M.; Ramos, R.A.N.; Calado, A.M.C.; Lima, V.F.S.; Ramos, I.C.d.N.; Tenório, R.F.L.; Faustino, M.A.d.G.; Alves, L.C. Gastrointestinal parasites of cats in Brazil: frequency and zoonotic risk. *Revista brasileira de parasitologia veterinaria = Brazilian journal of veterinary parasitology : Orgao Oficial do Colegio Brasileiro de Parasitologia Veterinaria* **2016**, *25*, 254-257, doi:10.1590/S1984-29612016019.
184. Montes, M.; Sawhney, C.; Barros, N. Strongyloides stercoralis: there but not seen. *Current opinion in infectious diseases* **2010**, *23*, 500-504, doi:10.1097/QCO.0b013e32833df718.
185. Montresor, A.; Mupfasoni, D.; Mikhailov, A.; Mwinzi, P.; Lucianez, A.; Jamsheed, M.; Gasimov, E.; Warusavithana, S.; Yajima, A.; Bisoffi, Z., et al. The global progress of soil-transmitted helminthiases control in 2020 and World Health Organization targets for 2030. *PLoS Negl Trop Dis* **2020**, *14*, e0008505, doi:10.1371/journal.pntd.0008505.
186. Moody, A.; Rao, S.; Subirats, M. *Evaluation of Parasep SF, Ether and Ethyl Acetate Free Faecal Concentrators*; Apacaor: Berkshire, UK, 2013.
187. Moraers, G. Contribucao para o studo do Strongyloides stercoralis e da strongiloidosis no Brasil. *Revista do Servicio Especial de Saude Publica* **1948**, *1*, 507-624.
188. Mounsey, K.; Kearns, T.; Rampton, M.; Llewellyn, S.; King, M.; Holt, D.; Currie, B.J.; Andrews, R.; Nutman, T.; McCarthy, J. Use of dried blood spots to define antibody response to the Strongyloides stercoralis recombinant antigen NIE. *Acta tropica* **2014**, *138*, 78-82, doi:10.1016/j.actatropica.2014.07.007.
189. Nagayasu, E.; Aung, M.; Hortiwakul, T.; Hino, A.; Tanaka, T.; Higashiarakawa, M.; Olia, A.; Taniguchi, T.; Win, S.M.T.; Ohashi, I., et al. A possible origin population of pathogenic intestinal nematodes, Strongyloides stercoralis, unveiled by molecular phylogeny. *Sci Rep* **2017**, *7*, 4844, doi:10.1038/s41598-017-05049-x.
190. Nageswaran, C.; Craig, P.S.; Devaney, E. Coproantigen detection in rats experimentally infected with Strongyloides ratti. *Parasitology* **1994**, *108 (Pt 3)*, 335-342, doi:10.1017/s0031182000076174.
191. Naidu, P.; Yanow, S.K.; Kowalewska-Grochowska, K.T. Eosinophilia: A poor predictor of Strongyloides infection in refugees. *The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale* **2013**, *24*, 93-96, doi:10.1155/2013/290814.
192. Neimeister, R.; Logan, A.L.; Gerber, B.; Egleton, J.H.; Kleger, B. Hemo-De as substitute for ethyl acetate in formalin-ethyl acetate concentration technique. *J Clin Microbiol* **1987**, *25*, 425-426, doi:10.1128/jcm.25.2.425-426.1987.
193. Neva, F.A.; Gam, A.A.; Maxwell, C.; Pelletier, L.L. Skin test antigens for immediate hypersensitivity prepared from infective larvae of Strongyloides stercoralis. *The American journal of tropical medicine and hygiene* **2001**, *65*, 567-572, doi:10.4269/ajtmh.2001.65.567.

194. Nielsen, P.B.; Mojon, M. Improved diagnosis of strongyloides stercoralis by seven consecutive stool specimens. *Zentralblatt fur Bakteriologie, Mikrobiologie, und Hygiene. Series A, Medical microbiology, infectious diseases, virology, parasitology* **1987**, *263*, 616-618, doi:10.1016/s0176-6724(87)80207-9.
195. Nilforoushan, M.R.; Mirhendi, H.; Rezaian, M.; Meamar, A.R.; Kia, E.B. A DNA-based identification of Strongyloides stercoralis isolates from Iran. *Iran J Publ Health* **2007**, *36*, 16-20.
196. Noordin, R.; Anuar, N.S.; Juri, N.M.; Wongphutorn, P.; Ruantip, S.; Kopolrat, K.Y.; Worasith, C.; Sithithaworn, J.; Sithithaworn, P. Evaluation of a Rapid IgG4 Lateral Flow Dipstick Test to Detect Strongyloides stercoralis Infection in Northeast Thailand. *The American journal of tropical medicine and hygiene* **2021**, *105*, 688-691, doi:10.4269/ajtmh.21-0317.
197. Norsyahida, A.; Riazi, M.; Sadjjadi, S.M.; Muhammad Hafiznur, Y.; Low, H.C.; Zeehaida, M.; Noordin, R. Laboratory detection of strongyloidiasis: IgG-, IgG4 - and IgE-ELISAs and cross-reactivity with lymphatic filariasis. *Parasite immunology* **2013**, *35*, 174-179, doi:10.1111/pim.12029.
198. Nutman, T.B. Human infection with Strongyloides stercoralis and other related Strongyloides species. *Parasitology* **2017**, *144*, 263-273, doi:10.1017/S0031182016000834.
199. Nyambura Njuguna, A.; Kagira, J.M.; Muturi Karanja, S.; Ngotho, M.; Mutharia, L.; Wangari Maina, N. Prevalence of Toxoplasma gondii and Other Gastrointestinal Parasites in Domestic Cats from Households in Thika Region, Kenya. *BioMed research international* **2017**, *2017*, 7615810-7615810, doi:10.1155/2017/7615810.
200. O'Connell, E.M.; Nutman, T.B. Molecular Diagnostics for Soil-Transmitted Helminths. *The American journal of tropical medicine and hygiene* **2016**, *95*, 508-513, doi:10.4269/ajtmh.16-0266.
201. Ottino, L.; Buonfrate, D.; Paradies, P.; Bisoffi, Z.; Antonelli, A.; Rossolini, G.M.; Gabrielli, S.; Bartoloni, A.; Zammarchi, L. Autochthonous Human and Canine Strongyloides stercoralis Infection in Europe: Report of a Human Case in An Italian Teen and Systematic Review of the Literature. *Pathogens (Basel, Switzerland)* **2020**, *9*, doi:10.3390/pathogens9060439.
202. Page, W.; Judd, J.A.; Bradbury, R.S. The Unique Life Cycle of Strongyloides stercoralis and Implications for Public Health Action. *Trop Med Infect Dis* **2018**, *3*, doi:10.3390/tropicalmed3020053.
203. Page, W.A.; Dempsey, K.; McCarthy, J.S. Utility of serological follow-up of chronic strongyloidiasis after anthelminthic chemotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2006**, *100*, 1056-1062, doi:10.1016/j.trstmh.2005.12.006.
204. Pak, B.J.; Vasquez-Camargo, F.; Kalinichenko, E.; Chiodini, P.L.; Nutman, T.B.; Tanowitz, H.B.; McAuliffe, I.; Wilkins, P.; Smith, P.T.; Ward, B.J., et al. Development of a rapid serological assay for the diagnosis of strongyloidiasis using a novel diffraction-based biosensor technology. *PLoS neglected tropical diseases* **2014**, *8*, e3002-e3002, doi:10.1371/journal.pntd.0003002.
205. Pampiglione, S.; Ricciardi, M.L. Experimental infestation with human strain Strongyloides fulleborni in man. *Lancet* **1972**, *1*, 663-665, doi:10.1016/s0140-6736(72)90464-3.
206. Papaiakovou, M.; Gasser, R.B.; Littlewood, D.T.J. Quantitative PCR-Based Diagnosis of Soil-Transmitted Helminth Infections: Faecal or Fickle? *Trends Parasitol* **2019**, *35*, 491-500, doi:10.1016/j.pt.2019.04.006.
207. Papazahariadou, M.; Founta, A.; Papadopoulos, E.; Chliounakis, S.; Antoniadou-Sotiriadou, K.; Theodorides, Y. Gastrointestinal parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece. *Vet Parasitol* **2007**, *148*, 170-173, doi:10.1016/j.vetpar.2007.05.013.
208. Paradies, P.; Buonfrate, D.; Iatta, R.; Iarussi, F.; Giorli, G.; Palmieri, V.O.; Sasanelli, M.; Perandin, F.; Šlapeta, J.; Capogna, A., et al. Efficacy of ivermectin to control Strongyloides stercoralis infection in sheltered dogs. *Acta tropica* **2019**, *190*, 204-209, doi:10.1016/j.actatropica.2018.11.014.
209. Paradies, P.; Iarussi, F.; Sasanelli, M.; Capogna, A.; Lia, R.P.; Zucca, D.; Greco, B.; Cantacessi, C.; Otranto, D. Occurrence of strongyloidiasis in privately owned and sheltered dogs: clinical presentation and treatment outcome. *Parasites & vectors* **2017**, *10*, 345-345, doi:10.1186/s13071-017-2275-5.
210. Pellegrino, J.; Chaia, G.; Memoria, J.M. [Observations on the intradermal reaction to Strongyloides ratti antigen in strongyloidosis patients]. *Revista do Instituto de Medicina Tropical de Sao Paulo* **1961**, *3*, 181-185.
211. Perry, J.L.; Matthews, J.S.; Miller, G.R. Parasite detection efficiencies of five stool concentration systems. *J Clin Microbiol* **1990**, *28*, 1094-1097, doi:10.1128/jcm.28.6.1094-1097.1990.
212. Pilotte, N.; Papaiakovou, M.; Grant, J.R.; Bierwert, L.A.; Llewellyn, S.; McCarthy, J.S.; Williams, S.A. Improved PCR-Based Detection of Soil Transmitted Helminth Infections Using a Next-Generation Sequencing Approach to Assay Design. *PLoS neglected tropical diseases* **2016**, *10*, e0004578-e0004578, doi:10.1371/journal.pntd.0004578.
213. Polderman, A.M.; Eberhard, M.; Baeta, S.; Gasser, R.B.; Van Lieshout, L.; Magnussen, P.; Olsen, A.; Spannbrucker, N.; Ziem, J.; Horton, J. The rise and fall of human oesophagostomiasis. *Advances in parasitology* **2010**, *71*, 93-155.

214. Porto, A.F.; Oliveira Filho, J.; Neva, F.A.; Orge, G.; Alcântara, L.; Gam, A.; Carvalho, E.M. Influence of human T-cell lymphocytotropic virus type 1 infection on serologic and skin tests for strongyloidiasis. *The American journal of tropical medicine and hygiene* **2001**, *65*, 610-613, doi:10.4269/ajtmh.2001.65.610.
215. Potters, I.; Micalessi, I.; Van Esbroeck, M.; Gils, S.; Theunissen, C. A rare case of imported Strongyloides fuelleborni infection in a Belgian student. *Clinical Infection in Practice* **2020**, *7-8*, doi:10.1016/j.clinpr.2020.100031 LK - http://BT8EP3TD6D.search.serialssolutions.com?sid=EMBASE&issn=25901702&id=doi:10.1016%2Fj.clinpr.2020.100031&atitle=A+rare+case+of+imported+Strongyloides+fuelleborni+infection+in+a+Belgian+student&stitle=Clin.+Infect.+Pract.&title=Clinical+Infection+in+Practice&volume=7-8&issue=&spage=&epage=&aulast=Potters&aufirst=Idzi&auinit=I.&aufull=Potters+I.&coden=&isbn=&pages=-&date=2020&auinit1=I&auinitm=.
216. Ramachandran, S.; Gam, A.A.; Neva, F.A. 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. *The American journal of tropical medicine and hygiene* **1997**, *56*, 61-65, doi:10.4269/ajtmh.1997.56.61.
217. Ramanathan, R.; Burbelo, P.D.; Groot, S.; Iadarola, M.J.; Neva, F.A.; Nutman, T.B. A luciferase immunoprecipitation systems assay enhances the sensitivity and specificity of diagnosis of Strongyloides stercoralis infection. *The Journal of infectious diseases* **2008**, *198*, 444-451, doi:10.1086/589718.
218. Ravi, V.; Ramachandran, S.; Thompson, R.W.; Andersen, J.F.; Neva, F.A. Characterization of a recombinant immunodiagnostic antigen (NIE) from Strongyloides stercoralis L3-stage larvae. *Molecular and biochemical parasitology* **2002**, *125*, 73-81, doi:10.1016/s0166-6851(02)00214-1.
219. Repetto, S.A.; Alba Soto, C.D.; Cazorla, S.I.; Tayeldin, M.L.; Cuello, S.; Lasala, M.B.; Tekiel, V.S.; González Cappa, S.M. An improved DNA isolation technique for PCR detection of Strongyloides stercoralis in stool samples. *Acta tropica* **2013**, *126*, 110-114, doi:10.1016/j.actatropica.2013.02.003.
220. Repetto, S.A.; Ruybal, P.; Solana, M.E.; López, C.; Berini, C.A.; Alba Soto, C.D.; Cappa, S.M.G. Comparison between PCR and larvae visualization methods for diagnosis of Strongyloides stercoralis out of endemic area: A proposed algorithm. *Acta tropica* **2016**, *157*, 169-177, doi:10.1016/j.actatropica.2016.02.004.
221. Requena-Méndez, A.; Buonfrate, D.; Gomez-Junyent, J.; Zammarchi, L.; Bisoffi, Z.; Muñoz, J. Evidence-Based Guidelines for Screening and Management of Strongyloidiasis in Non-Endemic Countries. *Am J Trop Med Hyg* **2017**, *97*, 645-652, doi:10.4269/ajtmh.16-0923.
222. Requena-Méndez, A.; Chiodini, P.; Bisoffi, Z.; Buonfrate, D.; Gotuzzo, E.; Muñoz, J. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS neglected tropical diseases* **2013**, *7*, e2002-e2002, doi:10.1371/journal.pntd.0002002.
223. Ridley, D.S.; Hawgood, B.C. The value of formol-ether concentration of faecal cysts and ova. *J Clin Pathol* **1956**, *9*, 74-76, doi:10.1136/jcp.9.1.74.
224. Riggio, F.; Mannella, R.; Ariti, G.; Perrucci, S. Intestinal and lung parasites in owned dogs and cats from central Italy. *Vet Parasitol* **2013**, *193*, 78-84, doi:10.1016/j.vetpar.2012.11.026.
225. Ritchie, L.S. An ether sedimentation technique for routine stool examinations. *Bulletin of the U.S. Army Medical Department. United States. Army. Medical Department* **1948**, *8*, 326.
226. Robertson, G.J.; Koehler, A.V.; Gasser, R.B.; Watts, M.; Norton, R.; Bradbury, R.S. Application of PCR-Based Tools to Explore Strongyloides Infection in People in Parts of Northern Australia. *Tropical medicine and infectious disease* **2017**, *2*, doi:10.3390/tropicalmed2040062.
227. Rodrigues, R.M.; de Oliveira, M.C.; Sopelete, M.C.; Silva, D.A.O.; Campos, D.M.B.; Taketomi, E.A.; Costa-Cruz, J.M. IgG1, IgG4, and IgE antibody responses in human strongyloidiasis by ELISA using Strongyloides ratti saline extract as heterologous antigen. *Parasitology research* **2007**, *101*, 1209-1214, doi:10.1007/s00436-007-0602-z.
228. Ruantip, S.; Eamudomkarn, C.; Techasen, A.; Wangboon, C.; Sithithaworn, J.; Bethony, J.M.; Itoh, M.; Sithithaworn, P. Accuracy of Urine and Serum Assays for the Diagnosis of Strongyloidiasis by Three Enzyme-Linked Immunosorbent Assay Protocols. *The American journal of tropical medicine and hygiene* **2019**, *100*, 127-129, doi:10.4269/ajtmh.18-0569.
229. Rugai, E.; Mattos, T.; Brisola, A.P. Nova técnica para isolar larvas de mematóides das fezes - Modificação do Método de Baermann. *Revista do Instituto Adolfo Luz* **1954**, *14*, 5-8.
230. Rutjes, A.W.; Reitsma, J.B.; Coomarasamy, A.; Khan, K.S.; Bossuyt, P.M. Evaluation of diagnostic tests when there is no gold standard. A review of methods. *Health technology assessment (Winchester, England)* **2007**, *11*, iii, ix-51, doi:10.3310/hta11500.
231. Sadaow, L.; Sanpool, O.; Rodpai, R.; Boonroumkaew, P.; Maleewong, W.; Intapan, P.M. Development of immunochromatographic device as a point-of-care tool for serodiagnosis of human strongyloidiasis cases.

European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology **2020**, *39*, 465-470, doi:10.1007/s10096-019-03745-2.
232. Salazar, S.A.; Gutierrez, C.; Berk, S.L. Value of the agar plate method for the diagnosis of intestinal strongyloidiasis. *Diagnostic microbiology and infectious disease* **1995**, *23*, 141-145, doi:10.1016/0732-8893(95)00247-2.
233. Sandground, J. Some studies on susceptibility, resistance, and acquired immunity to infection with Strongyloides Stercoralis (Nematoda) in dogs and cats. *American Journal of Hygiene* **1928**, *8*, 507-538.
234. Sandosham, A. An investigation into the association of creeping eruption with Strongyloides infection contracted in the Far East. *Journal of Helminthology* **1952**, *26*, 1-24.
235. Sanpool, O.; Intapan, P.M.; Rodpai, R.; Laoraksawong, P.; Sadaow, L.; Tourtip, S.; Piratae, S.; Maleewong, W.; Thanchomnang, T. Dogs are reservoir hosts for possible transmission of human strongyloidiasis in Thailand: molecular identification and genetic diversity of causative parasite species. *J Helminthol* **2019**, *94*, e110, doi:10.1017/s0022149x1900107x.
236. Santini, A.; Lucchini, V.; Fabbri, E. Ageing and environmental factors affect PCR success in wolf (canis lupus) excremental DNA sample. *Molecular Ecology Notes* **2007**, *7*, 955-961.
237. Sato, Y.; Kobayashi, J.; Toma, H.; Shiroma, Y. Efficacy of stool examination for detection of Strongyloides infection. *The American journal of tropical medicine and hygiene* **1995**, *53*, 248-250, doi:10.4269/ajtmh.1995.53.248.
238. Sato, Y.; Otsuru, M.; Takara, M.; Shiroma, Y. Intradermal reactions in strongyloidiasis. *Int J Parasitol* **1986**, *16*, 87-91, doi:10.1016/0020-7519(86)90070-6.
239. Sauda, F.; Malandrucco, L.; Macrì, G.; Scarpulla, M.; De Liberato, C.; Terracciano, G.; Fichi, G.; Berrilli, F.; Perrucci, S. Leishmania infantum, Dirofilaria spp. and other endoparasite infections in kennel dogs in central Italy. *Parasite* **2018**, *25*, 2, doi:10.1051/parasite/2018001.
240. Saugar, J.M.; Merino, F.J.; Martín-Rabadán, P.; Fernández-Soto, P.; Ortega, S.; Gárate, T.; Rodríguez, E. Application of real-time PCR for the detection of Strongyloides spp. in clinical samples in a reference center in Spain. *Acta tropica* **2015**, *142*, 20-25, doi:10.1016/j.actatropica.2014.10.020.
241. Schär, F.; Inpankaew, T.; Traub, R.J.; Khieu, V.; Dalsgaard, A.; Chimnoi, W.; Chhoun, C.; Sok, D.; Marti, H.; Muth, S., et al. The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. *Parasitology international* **2014**, *63*, 597-603, doi:10.1016/j.parint.2014.03.007.
242. Schär, F.; Odermatt, P.; Khieu, V.; Panning, M.; Duong, S.; Muth, S.; Marti, H.; Kramme, S. Evaluation of real-time PCR for Strongyloides stercoralis and hookworm as diagnostic tool in asymptomatic schoolchildren in Cambodia. *Acta tropica* **2013**, *126*, 89-92, doi:10.1016/j.actatropica.2012.12.012.
243. Schnyder, M.; Reichler, I.M.; Eichenberger, R.M.; Hofer-Inteeworn, N.; Kümmerle-Fraune, C.; Grimm, F. Strongyloides stercoralis in Swiss dogs - a retrospective study suggests an increasing occurrence of this potentially zoonotic parasite as a consequence of dog imports. *Schweizer Archiv fur Tierheilkunde* **2022**, *164*, 89-104, doi:10.17236/sat00340.
244. Sengthong, C.; Yingklang, M.; Intuyod, K.; Hongsrichan, N.; Pinlaor, S. An optimized agar plate culture improves diagnostic efficiency for Strongyloides stercoralis infection in an endemic community. *Parasitology research* **2020**, *119*, 1409-1413, doi:10.1007/s00436-020-06626-2.
245. Sharifdini, M.; Mirhendi, H.; Ashrafi, K.; Hosseini, M.; Mohebali, M.; Khodadadi, H.; Kia, E.B. Comparison of Nested Polymerase Chain Reaction and Real-Time Polymerase Chain Reaction with Parasitological Methods for Detection of Strongyloides stercoralis in Human Fecal Samples. *The American journal of tropical medicine and hygiene* **2015**, *93*, 1285-1291, doi:10.4269/ajtmh.15-0309.
246. Siddiqui, A.A.; Berk, S.L. Diagnosis of Strongyloides stercoralis infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2001**, *33*, 1040-1047, doi:10.1086/322707.
247. Silva, L.P.; Barcelos, I.S.; Passos-Lima, A.B.; Espindola, F.S.; Campos, D.M.; Costa-Cruz, J.M. Western blotting using Strongyloides ratti antigen for the detection of IgG antibodies as confirmatory test in human strongyloidiasis. *Mem Inst Oswaldo Cruz* **2003**, *98*, 687-691, doi:10.1590/s0074-02762003000500017.
248. Sithithaworn, J.; Sithithaworn, P.; Janrungsopa, T.; Suvatanadecha, K.; Ando, K.; Haswell-Elkins, M.R. Comparative assessment of the gelatin particle agglutination test and an enzyme-linked immunosorbent assay for diagnosis of strongyloidiasis. *Journal of clinical microbiology* **2005**, *43*, 3278-3282, doi:10.1128/JCM.43.7.3278-3282.2005.
249. Sithithaworn, P.; Srisawangwong, T.; Tesana, S.; Daenseekaew, W.; Sithithaworn, J.; Fujimaki, Y.; Ando, K. Epidemiology of Strongyloides stercoralis in north-east Thailand: application of the agar plate culture technique compared with the enzyme-linked immunosorbent assay. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2003**, *97*, 398-402, doi:10.1016/s0035-9203(03)90069-1.

250. Sitta, R.B.; Malta, F.M.; Pinho, J.R.; Chieffi, P.P.; Gryschek, R.C.B.; Paula, F.M. Conventional PCR for molecular diagnosis of human strongyloidiasis. *Parasitology* **2014**, *141*, 716-721, doi:10.1017/S0031182013002035.
251. SKML. Parasitology section. Availabe online: (accessed on 14 December 2022).
252. Speare, R. Identification of species of Strongyloides. In *Strongyloidiasis: A major roundworm infection of man*, D.I., G., Ed. Basingstoke: Taylor and Francis: 1989; pp. 11-84.
253. Sprott, V.; Selby, C.D.; Ispahani, P.; Toghill, P.J. Indigenous strongyloidiasis in Nottingham. *British medical journal (Clinical research ed.)* **1987**, *294*, 741-742, doi:10.1136/bmj.294.6574.741-a.
254. Stamey, J.D.; Seaman, J.W.; Young, D.M. Bayesian sample-size determination for inference on two binomial populations with no gold standard classifier. *Statistics in medicine* **2005**, *24*, 2963-2976, doi:10.1002/sim.2167.
255. Steinmann, P.; Zhou, X.-N.; Du, Z.-W.; Jiang, J.-Y.; Wang, L.-B.; Wang, X.-Z.; Li, L.-H.; Marti, H.; Utzinger, J. Occurrence of Strongyloides stercoralis in Yunnan Province, China, and comparison of diagnostic methods. *PLoS neglected tropical diseases* **2007**, *1*, e75-e75, doi:10.1371/journal.pntd.0000075.
256. Streit, A. Reproduction in Strongyloides (Nematoda): a life between sex and parthenogenesis. *Parasitology* **2008**, *135*, 285-294, doi:10.1017/s003118200700399x.
257. Štrkolcová, G.; Goldová, M.; Bocková, E.; Mojžišová, J. The roundworm Strongyloides stercoralis in children, dogs, and soil inside and outside a segregated settlement in Eastern Slovakia: frequent but hardly detectable parasite. *Parasitology research* **2017**, *116*, 891-900, doi:10.1007/s00436-016-5362-1.
258. Sudré, A.P.; Siqueira, R.C.; Barreto, M.G.M.; Peralta, R.H.S.; Macedo, H.W.; Peralta, J.M. Identification of a 26-kDa protein fraction as an important antigen for application in the immunodiagnosis of strongyloidiasis. *Parasitology research* **2007**, *101*, 1117-1123, doi:10.1007/s00436-007-0596-6.
259. Sukhavat, K.; Morakote, N.; Chaiwong, P.; Piangjai, S. Comparative efficacy of four methods for the detection of Strongyloides stercoralis in human stool specimens. *Annals of tropical medicine and parasitology* **1994**, *88*, 95-96, doi:10.1080/00034983.1994.11812843.
260. Sultana, Y.; Jeoffreys, N.; Watts, M.R.; Gilbert, G.L.; Lee, R. Real-time polymerase chain reaction for detection of Strongyloides stercoralis in stool. *The American journal of tropical medicine and hygiene* **2013**, *88*, 1048-1051, doi:10.4269/ajtmh.12-0437.
261. Sykes, A.M.; McCarthy, J.S. A coproantigen diagnostic test for Strongyloides infection. *PLoS neglected tropical diseases* **2011**, *5*, e955-e955, doi:10.1371/journal.pntd.0000955.
262. Takano, Y.; Minkami, K.; Kodama, S. Cross infection of Strongyloides between humans and dogs in the Amami Islands, Japan. *Tropical Medicine & Health* **2009**, *37*, 149.
263. Tamarozzi, F.; Longoni, S.S.; Mazzi, C.; Pettene, S.; Montresor, A.; Mahanty, S.; Bisoffi, Z.; Buonfrate, D. Diagnostic accuracy of a novel enzyme-linked immunoassay for the detection of IgG and IgG4 against Strongyloides stercoralis based on the recombinant antigens NIE/SsIR. *Parasites & vectors* **2021**, *14*, 412-412, doi:10.1186/s13071-021-04916-x.
264. Telemann, W. Eine Methode zur Erleich Terung der Auffindung von Parasiten-eiern In den Faeces. *Dtsche. Med. Wochenschr* **1908**, *34*, 1510-1511.
265. Tello, R.; Terashima, A.; Marcos, L.A.; Machicado, J.; Canales, M.; Gotuzzo, E. Highly effective and inexpensive parasitological technique for diagnosis of intestinal parasites in developing countries: spontaneous sedimentation technique in tube. *Int J Infect Dis* **2012**, *16*, e414-416, doi:10.1016/j.ijid.2011.12.017.
266. Thamsborg, S.M.; Ketzis, J.; Horii, Y.; Matthews, J.B. Strongyloides spp. infections of veterinary importance. *Parasitology* **2017**, *144*, 274-284, doi:10.1017/s0031182016001116.
267. Tilli, M.; Botta, A.; Mantella, A.; Nuti, B.; Bartoloni, A.; Boccalini, S.; Zammarchi, L. Community-based seroprevalence survey of schistosomiasis and strongyloidiasis by means of Dried Blood Spot testing on Sub-Saharan migrants resettled in Italy. *The new microbiologica* **2021**, *44*, 62-65.
268. Uparanukraw, P.; Phongsri, S.; Morakote, N. Fluctuations of larval excretion in Strongyloides stercoralis infection. *The American journal of tropical medicine and hygiene* **1999**, *60*, 967-973, doi:10.4269/ajtmh.1999.60.967.
269. van Doorn, H.R.; Koelewijn, R.; Hofwegen, H.; Gilis, H.; Wetsteyn, J.C.F.M.; Wismans, P.J.; Sarfati, C.; Vervoort, T.; van Gool, T. Use of enzyme-linked immunosorbent assay and dipstick assay for detection of Strongyloides stercoralis infection in humans. *Journal of clinical microbiology* **2007**, *45*, 438-442, doi:10.1128/JCM.01735-06.
270. Varatharajalu, R.; Parandaman, V.; Ndao, M.; Andersen, J.F.; Neva, F.A. Strongyloides stercoralis excretory/secretory protein strongylastacin specifically recognized by IgE antibodies in infected human sera. *Microbiology and immunology* **2011**, *55*, 115-122, doi:10.1111/j.1348-0421.2010.00289.x.

271. Vargas, P.; Krolewiecki, A.J.; Echazú, A.; Juarez, M.; Cajal, P.; Gil, J.F.; Caro, N.; Nasser, J.; Lammie, P.; Cimino, R.O. Serologic Monitoring of Public Health Interventions against Strongyloides stercoralis. *The American journal of tropical medicine and hygiene* **2017**, *97*, 166-172, doi:10.4269/ajtmh.16-0857.
272. Verweij, J.J.; Canales, M.; Polman, K.; Ziem, J.; Brienen, E.A.T.; Polderman, A.M.; van Lieshout, L. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2009**, *103*, 342-346, doi:10.1016/j.trstmh.2008.12.001.
273. Watts, M.R.; James, G.; Sultana, Y.; Ginn, A.N.; Outhred, A.C.; Kong, F.; Verweij, J.J.; Iredell, J.R.; Chen, S.C.A.; Lee, R. A loop-mediated isothermal amplification (LAMP) assay for Strongyloides stercoralis in stool that uses a visual detection method with SYTO-82 fluorescent dye. *The American journal of tropical medicine and hygiene* **2014**, *90*, 306-311, doi:10.4269/ajtmh.13-0583.
274. Watts, M.R.; Kim, R.; Ahuja, V.; Robertson, G.J.; Sultana, Y.; Wehrhahn, M.C.; Bradbury, R.S.; Gilbert, G.L.; Lee, R. Comparison of Loop-Mediated Isothermal Amplification and Real-Time PCR Assays for Detection of Strongyloides Larvae in Different Specimen Matrices. *Journal of clinical microbiology* **2019**, *57*, doi:10.1128/JCM.01173-18.
275. WHO. World Health Organization. Diagnostic methods for the control of strongyloidiasis. Availabe online: (accessed on
276. Willcox, H.P.; Coura, J.R. [A new design of the Baermann, Moraes, Coutinho's technique for the isolation of nematode larva]. *Memorias do Instituto Oswaldo Cruz* **1989**, *84*, 563-565, doi:10.1590/s0074-02761989000400015.
277. Willis, H. A simple levitation method for the detection of hook¬worm ova. *Med J Aust* **1921**, *2*, 375-376.
278. Wulcan, J.M.; Dennis, M.M.; Ketzis, J.K.; Bevelock, T.J.; Verocai, G.G. Strongyloides spp. in cats: a review of the literature and the first report of zoonotic Strongyloides stercoralis in colonic epithelial nodular hyperplasia in cats. *Parasit Vectors* **2019**, *12*, 349, doi:10.1186/s13071-019-3592-7.
279. Yang, M.; Gebeheyu, E.; Jung, S.; Kwon, O. TREATMENT OF NATURALLY ACQUIRED STRONGYLOIDES STERCORALIS INFECTION IN A DOG WITH IVERMECTIN *The Journal of Animal & Plant Sciences* **2013**, *23*, 337-339.
280. Young, K.H.; Bullock, S.L.; Melvin, D.M.; Spruill, C.L. Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. *J Clin Microbiol* **1979**, *10*, 852-853, doi:10.1128/jcm.10.6.852-853.1979.
281. Yunus, M.H.; Arifin, N.; Balachandra, D.; Anuar, N.S.; Noordin, R. Lateral Flow Dipstick Test for Serodiagnosis of Strongyloidiasis. *The American journal of tropical medicine and hygiene* **2019**, *101*, 432-435, doi:10.4269/ajtmh.19-0053.
282. Zanzani, S.A.; Di Cerbo, A.R.; Gazzonis, A.L.; Genchi, M.; Rinaldi, L.; Musella, V.; Cringoli, G.; Manfredi, M.T. Canine fecal contamination in a metropolitan area (Milan, north-western Italy): prevalence of intestinal parasites and evaluation of health risks. *TheScientificWorldJournal* **2014**, *2014*, 132361-132361, doi:10.1155/2014/132361.
283. Zendejas-Heredia, P.A.; Crawley, A.; Byrnes, H.; Traub, R.J.; Colella, V. Zoonotic Soil-Transmitted Helminths in Free-Roaming Dogs, Kiribati. *Emerg Infect Dis* **2021**, *27*, 2163-2165, doi:10.3201/eid2708.204900.
284. Zhou, S.; Harbecke, D.; Streit, A. From the feces to the genome: a guideline for the isolation and preservation of Strongyloides stercoralis in the field for genetic and genomic analysis of individual worms. *Parasites & vectors* **2019**, *12*, 496-496, doi:10.1186/s13071-019-3748-5.

Table 1. Data summary from a selection studies comparing the diagnostic accuracy of different microscopic/coproculture assays for the detection of *Strongyloides stercoralis* from a single stool sample.

Location	**Honduras**	**Thailand**	**Thailand**	**Japan**	**Thailand**	**Brazil**	**Brazil**	**China**	**Ethiopia**	**Mozambique**	**Iran**
Reference	de Kaminsky, 1993	Jongwutiwes et al. 1999	Koga et al. 1991	Sato et al. 1995	Sato et al. 1995	Sato et al. 1995	Blatt & Cantos 2003	Steinmann et al. 2007	Hailegebriel et al. 2017	Meurs et al. 2017	Mohammadi-Meskin et al. 2018
Total specimens	427	1085	137	713	205	432	424	179	351	323	163
Positive by any method	70	191	31	109	98	49	23	21	43	100	27
(% positive)	**(16%)**	**(18%)**	**(23%)**	**(15%)**	**(48%)**	**(11%)**	**(5%)**	**(12%)**	**(12%)**	**(31%)**	**(17%)**
DS Positive	9	20	7	28	ND	ND	ND	ND	21	26	16
(%total positives)	**(13%)**	**(10%)**	**(23%)**	**(26%)**					**(49%)**	**(26%)**	**(59%)**
FEC Positive	ND	104	ND	44	54	13	16	ND	23	ND	8
(%total positives)		**(54%)**		**(40%)**	**(55%)**	**(26%)**	**(70%)**		**(53%)**		**(5%)**
Baermann	33	ND	ND	ND	ND	ND	16	9*	31	37	27
(%total positives)	**(47%)**						**(70%)**	**(43%)**	**(72%)**	**(37%)**	**(100%)**
H-M Positive	ND	93	18	30	ND	14	8	ND	19	70	26
(%total positives)		**(49%)**	**(58%)**	**(27%)**		**(29%)**	**(35%)**		**(44%)**	**(70%)**	**(96%)**
APC Positive	28§	186	30	105	95	46	23	13**	ND	83	22
(%total positives)	**(40%)**	**(97%)**	**(97%)**	**(96%)**	**(97%)**	**(94%)**	**(100%)**	**(62%)**		**(83%)**	**(81%)**
Charcoal culture	ND	ND	ND	ND	ND	ND	ND	ND	16	ND	ND
(%total positives)									**(37%)**		
Spontaneous sedimentation	ND	ND	ND	ND	ND	ND	14	ND	ND	ND	ND
(%total positives)							**(61%)**				

ND = not done, APC: Agar plate culture, DS: Direct smear microscopy, FEC: Formalin-ether concentration, H-M: Harada-Mori culture
* 18 (85.7% of total positives) detected when three stools tested
** 21 (100% of total positives) detected when three stools tested
§ 48 hour - incubation only

Table 3. Selection of studies reporting the diagnostic accuracy of different serology assays

		Crude Antigen ELISAs			Recombinant antigen ELISAs				Other assays	
Study	**Reference standard**	**ELISA SciMedx Denville, NJ, US**	**ELISA Microwell IVD Research, Inc., Carlsbad, CA, US**	**ELISA Bordier Affinity Products SA, Crissier, Switzerland**	**NIE ELISA NovaTec Immunodiagnostica, Dietzenbach, Germany**	**NIE ELISA InBios Int, Inc, Seattle, WA, US**	**NIE ELISA by the National Institute of Health, Bethesda, Maryland, US**	**NIE-SsIR ELISA InBios Int, Inc, Seattle, WA, US***	**NIE LIPS by the National Institute of Health, Bethesda, Maryland, US**	**IFAT (in-house technique from IRCCS Sacro Cuore Don Calabria, Negrar, Verona, Italy)**
Bisoffi et al, 2014	Parasitological tests (including FECT, Baermann and APC)		Sens 91.2% (95%CI 86.0-96.4%) Spec 91.9% (95%CI 88.8-95.1%)	Sens 89.5% (95%CI 83.8-95.1) Spec 88.8% (95%CI 85.1-92.4%)			Sens 75.4% (95%CI 67.5-83.3%) Spec 89.5% (95%CI 85.9-93.0%)		Sens 85.1% (95%CI 78.5-91.6%) Spec 95.4% (95%CI 93.0-97.9%)	Sens 93.9% (95%CI 89.4-98.3%) Spec 82.5% (95%CI 78.0-86.9%)
	Composite reference standard (including the above-mentioned tests and serology)		Sens 92.3% (95%CI 87.8-96.9%) Spec 97.4% (95%CI 95.5-99.3%)	Sens 90.8% (95%CI 85.8-95.7%) Spec 94.0% (95%CI 91.2-96.9%)			Sens 70.8% (95%CI 62.9-78.6%) Spec 91.1% (95%CI 87.7-94.5%)		Sens 83.8% (95%CI 77.5-90.2%) Spec 99.6% (95%CI 98.9-100.0%)	Sens 94.6% (95%CI 90.7-98.5%) Spec 87.4% (95%CI 83.4-91.3%)
Fradejas et al, 2018	Parasitological tests (including	Sens 94.7% (95%CI 75.4-99%)			Sens 78.9% (95%CI 56.7-91.5%)					

	FECT, APC) and PCR)	Spec 72.3% (95%CI 58.2-83.1%)			Spec 85.1% (95%CI 72.3-92.6%)					
	Composite reference standard (including the above-mentioned tests, serology and epidemiological risk factors)	Sens 89.2% (95%CI 80.7-94.2%) Spec 89.3% (95%CI 83.1-93.4%)			Sens 72.3% (95%CI 81.8-80.8%) Spec 85.1% (95%CI 72.3-92.6%)					
Ruantip et al, 2019	Parasitological methods (including APC and FECT)	Sens 91% (95%CI 75.8-97.8) Spec 42.1% (95%CI 21.1-66.0%)				Sens 82.9% (95%CI 65.7-92.8%) Spec 57.9% (95%CI 40.0-78.9%)				
Tamarozzi et al, 2021	Parasitological tests (including FECT, APC) and PCR)							Sens of IgG 93% (95%CI 88-97%) Spec of IgG 91% (95%CI 88-95%)		

							Sens of IgG4 81% (95%CI 74-87) Spec of IgG4 94% (95%CI 91-97%)		
Composite reference standard (including the above-mentioned tests and serology)							Sens of IgG 76% (95%CI 71-82%) Spec of IgG 98% (95%CI 96-100%) Sens of IgG4 75% (95%CI 69-81) Spec of IgG4 91% (95%CI 88-95%)		

Table 4. Analytical and pre-analytical variables and calculated diagnostic performance values* for all *Strongyloides* PCR assay validation studies where a high sensitivity coproculture technique was available for use as a reference standard

PCR assay	n tested (n +ve by reference method)	Source	Preservation method	Preservation time	DNA Extraction method	PCR Target (amplicon size)	Cycling conditions	Inhibition control	Reference Method	Sensitivity (95% C.I.) (using phenotypic reference standards only)	Specificity (95% C.I.) (using phenotypic reference standards only)	Sensitivity (95% C.I.) (using a composite reference standard§)	Reference
Real-time PCR (Verweij)	212 (54)	Ghana	Frozen or in ethanol*	ns	QIAamp Tissue Kit (Qiagen) with pre-heating in 2% polyvinylpolypyrolidone	*18S rRNA* (101 bp)	95 °C for 15 min, 50 cycles of 95 °C for 15 s, 60 °C for 60 s	Phocine herpesvirus	Baermann & 2x Modified charcoal coproculture	61.1% (46.9% to 74.1%)	92.4% (87.1% to 96.0%)	72.0% (60.4% to 81.8%)	Verweij et al. 2009
Real-time PCR (Verweij)	160 (41)	Bangladesh	-20 C freezing	1 year	MoBio Powersoil kit	*18S rRNA* (101 bp)	95 °C for 15 min, 50 cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 30 s	Commerical synthetic DNA internal control	3x Harada-Mori	34.5% (20.1% to 50.6%)	99.2% (95.4% to 100%)	33.3% (19.6% to 49.6%)	Sultana, et al. 2013
Real-time PCR (Verweij)	501	Cambodia	-20 C freezing	ns	QIAmp DNA Stool Kit	*18S rRNA* (101 bp)	95 °C for 10 min, 40 cycles of 95 °C for	Spiking duplicate samples with *S. stercoralis 18s rRNA*	APC & Baermann	61% (49.6% to 71.6%)	92.7% (87.8% to 96.0%)	66.3% (55.9% to 75.7%)	Schär, et al. 2013

							15 s, 60 °C for 30 s	plasmid control					
Real-time PCR (Verweij)	466 (85)	Iran	70% ethanol at RT	ns	phenol–chloroform–isoamyl alcohol extraction with prior rounds of freeze-thawing	*18S rRNA* (101 bp)	95 °C for 10 min, 40 cycles of 95 °C for 15 s, 60 °C for 60 s	None	APC & FEC	84.7% (75.3% to 91.6%)	95.8% (93.3% to 97.6%)	75.2% (66.4% to 82.7%)	Sharifdini et al. 2015
Real-time PCR (Verweij)	256 (28)	Côte d'Ivoire	70% ethanol	ns	QIAmp DNA Stool Kit	*18S rRNA* (101 bp)	95 °C for 15 min, 40 cycles of 95 °C for 15 s, 60 °C for 30 s	ns	APC & Baermann	50% (30.7% to 69.3%)	92.5% (88.3% to 95.6%)	54.39% (40.7% to 67.6%)	Becker et al. 2015
Real-time PCR (Verweij)	256 (28)	Côte d'Ivoire	70% ethanol	ns	QIAmp DNA Stool Kit	*18S rRNA* (101 bp)	95 °C for 15 min, 50 cycles of 95 °C for 15 s, 60 °C for 60 s	ns	APC & Baermann	70% (45.7% to 88.1%)	91.2% (86.8% to 94.6%)	59.7% (45.8% to 72.4%)	Becker et al. 2015
Real-time PCR (Verweij)	237 (35)	Argentina, Paraguay, Bolivia, Peru	-20 C freezing	ns	Overnight in GTES buffer, followed by mechanical disruption and QIAamp DNA Stool Mini Kit extraction	*18S rRNA* (101 bp)	ns	Stools spiked with *Trypanosoma cruzi* culture and *T. cruzi* PCR performed on DNA extract	APC & FEC	100% (90.0% to 100%)	82.18% (76.2% to 87.2%)	100% (90.0% to 100%)	Repetto et al. 2016

Real-time PCR (Verweij)	223 (20)	Italy (incl. immigrants & travelers)	Fresh faeces	ns	Roche Magnapure LC.2 with overnight pre-freezing in 2% polyvinylpolypyrolidone, followed by one round of freze-thaw	*18S rRNA* (101 bp)	95 °C for 15 min, 50 cycles of 95 °C for 15 s, 60 °C for 60 s	Phocinceherpesvirus	APC	72.0% (50.6% to 87.9%)	98.9% (96.4% to 99.9%)	56.8% (41.0% to 71.7%)	Buonfrate et al. 2017b
Real-time PCR (Verweij)	323 (100)	Mozambique	96% ethanol	ns	QIAamp Tissue Kit (Qiagen) with pre-heating in 2% polyvinylpolypyrolidone followed by proteinase K pre-treatment	*18S rRNA* (101 bp)	95 °C for 15 min, 50 cycles of 95 °C for 15 s, 60 °C for 60 s	Phocinceherpesvirus	APC, Baermann, FEC, Direct smear microscopy	88.8% (80.8% to 94.3%)	77.1% (70.1% to 82.6%)	92.3% (86.7% to 96.1%)	Meurs et al. 2017
Real-time PCR (Verweij)	143 (4)	Colombia	-20 C freezing	ns	ns	*18S rRNA* (101 bp)	ns	TaqMan® Exogenous Internal Positive control (Applied Biosystems)	APC, Harada-Mori, Direct smear microscopy & FEC	75% (20.1% to 100%)	79.1% (72.0% to 86.2%)	97.0% (84.2 to 99.9%)	Campo-Polanco, et al. 2018
Real-time PCR (Verweij) - Sybr Green modification	231 (12)	Spain (incl. immigrants & travelers)	Fresh faeces	ns	QiaAmp DNA stool Mini kit with pre-concentration in Bioparapred-Midi columns	*18S rRNA* (101 bp)	95 °C for 15 min, 50 cycles of 95 °C for 10 s, 60 °C for 30 s, 72 °C for 30 s	Spiking duplicate samples with *S. venezuelensis* L3 DNA	3x Harada-Mori	93.8% (69.8% to 99.8%)	86.5% (81.2% to 90.8%)		Saugar et al. 2015
Real-time PCR (Verweij) -	396 (48)	Ethiopia	Fresh faeces at 4° C	ns	QiaAmp DNA stool Mini kit with pre-concentration in	*18S rRNA* (101 bp)	95 °C for 15 min, 50 cycles of 95 °C for 15 s, 60 °C	Spiking duplicate samples with *S.*	Baermann	43.1% (29.4 to 57.7%)	91% (87.5%	97.1% (85.1%	Amor Aramendia, et al. 2016

Sybr Green modification					Bioparapred-Midi columns		for 10 s, 72 °C for 30 s	*venezuelensis* L3 DNA			to 57.8%)	to 99.9%)	
Real-time PCR (Verweij) in multiplex	193 (46)	Tanzania	-20 C freezing	Between 0 days & 1 year	QIAamp Tissue Kit (Qiagen) with pre-heating in 2% polyvinylpolypyrolidone	*18S rRNA* (101 bp)	95 °C for 15 min, 50 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s	ns	Baermann	17.39% (7.8% to 31.4%)	93.9% (88.7% to 97.2%)	30.9% (19.1% to 44.8%)	Knopp, et al. 2014
Barda modification of Verweij real-time PCR	95 (69)	Lao PDR	ethanol**	ns	QIAamp Tissue Kit (Qiagen)	*18S rRNA* (184 bp)	50 °C for 2 min, 95 °C for 10 min, 45 cycles of 95 °C for 15 s, 58 °C for 60 s	plasmid	2x Baermann	27.5%	76.9%	31.3% (21.3% to 42.6%)	Barda et al. 2018
Barda modification of Verweij real-time PCR	95 (69)	Lao PDR	ethanol**	ns	QIAamp Tissue Kit (Qiagen) with proteinase K pre-treatment	*18S rRNA* (184 bp)	50 °C for 2 min, 95 °C for 10 min, 45 cycles of 95 °C for 15 s, 58 °C for 60 s	ns	2x Baermann	76.8%	65.4%	77.5% (66.8% to 86.1%)	Barda et al. 2018
Real-time PCR (Chankongsin)	104 (27)	Lao PDR	90% ethanol at RT	ns	QIAamp Tissue Kit (Qiagen) with proteinase K pre-treatment	*28S rRNA* (92 bp)	50 °C for 2 min, 95 °C for 10 min, 45 cycles of 95 °C for 15 s, 58 °C for 60 s	ns	APC, Baermann & Direct smear microscopy	66.8% (46.0% to 83.5%)	89.6% (80.6% to 95.4%)	74.3% (56.7% to 87.5%)	Chankongsin et al. 2020

Nested PCR (Sharifdini)	466 (85)	Iran	70% ethanol at RT	ns	phenol–chloroform–isoamyl alcohol extraction with prior rounds of freeze-thawing	*cox1* (Outer: 509 bp, Inner: 261 bp)	Outer: 95 °C for 6 min, 35 cycles of 95 °C for 45 s, 55 °C for 60 s, 72 °C for 60 s, final extension 72 °C for 6 min Inner: 95 °C for 2 min, 25 cycles of 95 °C for 15 s, 60 °C for 30 s, 70 °C for 30 s, final extension 72 °C for 6 min	ns	APC, FEC	100% (95.8% to 100%)	91.6% (88.4% to 94.2%)	100% (96.9% to 100%)	Sharifdini et al. 2015
Nested PCR (Moghaddassani) using novel outer primers and Nilforoushan (2007) inner primers	46 (16)	Iran	70% ethanol	ns	Modification of QIAamp® DNA stool MiniKit with prior 70% ethanol/ether concentration	ITS 1 to *5.8S rRNA* to ITS 2 region (Outer: 750 bp, Inner: 680 bp)	Outer: 95 °C for 7 min, 30 cycles of 94 °C for 45 s, 55 °C for 90 s, 72 °C for 90 s, final extension 72 °C for 5 min Inner: 94 °C for 3 min, 30 cycles of 94 °C for 45 s, 60 °C for 45 s, 72 °C for 60	ns	APC	75% (47.6% to 92.7%)	100% (88.4% to 100%)	57.1% (34.0% to 78.2%)	Moghaddassani et al. 2011

							s, final extension 72 °C for 5 min						
Conventional PCR (Moghaddassani)	46 (16)	Iran	70% ethanol	ns	Modification of QIAamp® DNA stool MiniKit with prior 70% ethanol/ether concentration	ITS 1 to *5.8S rRNA* region (114 bp)	95 °C for 5 min, 30 cycles of 94 °C for 30 s, 58 °C for 45 s, 72 °C for 45 s, final extension 72 °C for 5 min	ns	APC	76.2% (52.8% to 91.8%)	100% (86.3% to 100%)	76.2% (52.8% to 91.8%)	Moghaddassani et al. 2011

ns: not stated

*****Diagnostic sensitivities were calculated in this work from data provided in the reference using the reported results of a single or a combination high sensitivity coproculture technique as a reference standard
**percentage not stated

§ A composite of the results of all methods combined, including the assay being validated. In some cases, this includes additional phenotypic reference methods to those stated in the reference method column

GTES Buffer: glycine, Tris/Cl, EDTA, SDS buffer

APC: Agar plate culture; Baermann: Baermann sedimentation; FEC: Formalin-ethyl acetate sedimentation; *18S rRNA*: 18S ribsomal RNA small subunit gene; *cox1*: Cytochrome oxidase 1 gene; ITS 1: Internal Trascribed Spacer 1 gene; ITS 2: Internal Trascribed Spacer 2 gene

Table 5. Analytical validation data for all *Strongyloides* PCR and LAMP assay validation studies

Assay	Analytical sensitivity (limit of detection)	Analytical specificity testing	Reference
Verweij real-time PCR	10^{-2} *S. stercoralis* DNA extract (life stage and number used not stated)	PARASITES: *Ancylostoma caninum, Ancylostoma duodenale, Angiostrongylus cantonensis, Ascaris lumbricoides, Cryptosporidium hominis, Cryptosporidium parvum Cyclospora cayetanensis, Dientamoeba fragilis, Enterocytozoon bieneusi, Encephalitozoon intestinalis, Entamoeba dispar, Entamoeba histolytica, Faciola hepatica, Giardia duodenalis, Necator americanus, Oesophagostomum bifurcum, Schistosoma mansoni, Taenia saginata, Trichostrongylus vitrinus, Trichuris trichiura* BACTERIA: *Bacillus cereus, Bacteroides fragilis, Campylobacter jejuni, Campylobacter upsaliensis, Clostridioides difficile, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli* O:157, *Mycobacterium tuberculosis, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella* Enteritidis, *Salmonella* Typhimurium, *Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Yersinia enterocolitica,* FUNGI: *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis,*	Verweij et al. 2009, Suktana et al. 2013
Verweij real-time PCR	ns	PARASITES: *A. duodenale, N. americanus, Ascaris suum, Dirofilaria immitis, Echinococcus granulosus, Loa loa, S. Mansoni, Toxocara canis, Trichinella spiralis, T. trichiura, Wuchereria bancrofti* BACTERIA: Nil FUNGI: Nil	Schär et al. 2015
Verweij real-time PCR	0.1 pg of DNA of *S. venezuelensis* L3 larva DNA	PARASITES: *Anisakis simplex, C. parvum, E. dispar, E. histolytica, G. duodenalis,* hookworm (species not stated), *L. loa, T. saginata, T. spiralis, T. trichura, S. mansoni, W.a bancrofti* BACTERIA: Nil FUNGI: Nil	Saugar, et al. 2015
Verweij real-time PCR	10^{-4} dilution of DNA of one *S. stercoralis* L3 larva	PARASITES: *A. lumbricoides, Blastocystis* sp., *Cryptosporidium* sp., *Cystoisospora belli, Dicrocoelium denriticum, Entamoeba coli, E. histolytica, Enterobius vermicularis, E. bieneusi, F. hepatica, G. duodenalis, Hymenolepis nana, Rhabditis axei, T. saginata, Trichostrongylus* sp., BACTERIA: Nil FUNGI: *Candida albicans*	Sharifdini et al. 2015

Modification of Verweij real-time PCR	ns	PARASITES: *A. lumbricoides, Blastocystis* sp.*, Cryptosporidium* spp., *Cyclospora* spp., *E. coli, E. dispar, Entamoeba hartmanni, E. histolytica, Entamoeba moshkovskii, Endolimax nana, G. duodenalis, Iodamoeba bütschlii, S. mansoni* BACTERIA: Nil FUNGI: Nil	Barda et al. 2018
Pilotte real-time PCR	10 fg/µl of *S. stercoralis* genomic DNA (initial volume input and life stage not stated)	PARASITES: *A. Lumbricoides, Ancylostoma duodenale, N. americanus, T. trichiura* BACTERIA: *E. coli* FUNGI: Nil	Pilotte et al. 2016
Chankongsin Real-time PCR	S. stercoralis DNA plasmid control and extracted DNA from S. stercoralis. Results not reported.	PARASITES: *Blastocystis* sp.*, Chilomastix mesnili, Cryptosporidium* sp.*, C. cayetanensis, C. belli, D. fragilis, Encephalitozoon hellen, E. coli, E. dispar, E. hartmanni, E. histolytica, E. moshkovskii, G. duodenalis, H. nana, I. bütschlii, S. mansoni, Taeni*a spp., *Trichostrongylus* spp. BACTERIA: Nil FUNGI: Nil	
Verweij primers as a conventional PCR*	One *S. stercoralis* L3 larva x10^{-2}/g stool when DNA extracted by in house method* One *S. stercoralis* L3 larva x10^{-1}/g stool when DNA extracted by QIAamp DNA Stool Mini Kit	PARASITES: *A. lumbricoides, Blastocystis* sp., *Cryptosporidium* sp., *C. belli, Taenia* sp., *G. duodenalis,* hookworm (species not stated)*, H. nana, T. trichiura* BACTERIA: *Salmonella enterica, S. aureus, P. aeruginosa* FUNGI: *C. albicans* VIRUSES: Rotavirus	Repetto et al. 2013
Repetto conventional PCR*	One *S. stercoralis* L3 larva x10^{-2}/g stool when DNA extracted by in house method* One *S. stercoralis* L3 larva/g stool when DNA extracted by QIAamp DNA Stool Mini Kit	PARASITES: *A. lumbricoides, Blastocystis* sp., *C. belli, Cryptosporidium* sp., *G. duodenalis,* hookworm (species not stated)*, H. nana, Taenia* sp., *T. trichiura* BACTERIA: *S. enterica, S. aureus, P. aeruginosa* FUNGI: *C. albicans* VIRUSES: Rotavirus	Repetto et al. 2013

Sharifdini Nested PCR	10^{-3} dilution of DNA of one *S. stercoralis* filariform larva	PARASITES: *A. lumbricoides, Blastocystis* sp., *Cryptosporidium* sp.*, C. Belli, D. denriticum, E. coli, E. histolytica, E. vermicularis, E. bieneusi, F. hepatica, G. duodenalis, H. nana, R. axei, T. saginata, Trichostrongylus* sp. BACTERIA: Nil FUNGI: *C. albicans*	Sharifdini et al. 2015
Moghaddassani conventional PCR	ns	PARASITES: *E. coli, E. histolytica, G. duodenalis, H. Nana, Trichostrongylus colubriformis* BACTERIA: *E. coli, Citrobacter* sp. FUNGI: *C. albicans*	Moghaddassani et al. 2011
Moghaddassani Nested PCR	ns	PARASITES: *H. nana, E. coli, E. histolytica, G. duodenalis, T. colubriformis,* BACTERIA: *E. coli, Citrobacter* sp. FUNGI: *C. albicans* VIRUSES: Nil	Moghaddassani et al. 2011
Holt modification of Verweij real-time PCR	ns	PARASITES: Nil BACTERIA: Nil FUNGI: Nil VIRUSES: Nil	Holt et al. 2017
Holt modification of Verweij real-time PCR in multiplex	At elast 10^{-7} dilution of a plasmid DNA control (concentration not specified)	PARASITES: *Ascaris* spp., *Cryptosporidium* spp., *E. dispar*, *E. histolytica*, *G. duodenalis*, hookworm (species not stated)*, T. trichiura*, BACTERIA: Nil FUNGI: Nil	Lewellyn et al. 2016
Ghasemikhah conventional PCR	ns	PARASITES: *G. duodenalis, H. nana, T. colubriformis .* BACTERIA: Nil FUNGI: Nil	

Watts LAMP		PARASITES: *A. caninum, A. cantonensis, A. lumbricoides, Blastocystic* sp.*, C. parvum, D. fragilis, E. vermicularis, F. hepatica, G. duodenalis, N. americanus, S. japonicum, T. saginata, T. trichiura.* BACTERIA: *B. cereus, B. fragilis, C. jejuni, Citrobacter freundii, C. difficile, E. faecalis, E. coli, M. tuberculosis, P. mirabilis, S.* Typhimurium, *S. sonnei, S. aureus, Vibrio cholerae, Vibrio parahaemolyticus, Y. enterocolitica.* FUNGI: *A. flavus, A. fumigatus, C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, Cryptococcus neoformans, Penicillium chrysogenum.*	Watts et al. 2014
Fernández-Soto LAMP		PARASITES: *A. simplex, Brugia pahangi, C. parvum, Calicophoron daubneyi, D. dendriticum, Echinostoma caproni, E. granulosus, E. histolytica, F. hepatica, G. duodenalis,* hookworm (species not stated)*, Hymenolepis diminuta, L. loa, P. malariae, P. ovale, P. vivax, Schistosoma bovis, S. haematobium, S. intercalatum, S. japonicum, S. mansoni, T. saginata, Taenia taeniformis, T. spiralis,* BACTERIA: Nil FUNGI: Nil	Fernández-Soto et al. 2016

*DNA isolated by incubation in glycine, Tris/Cl, EDTA, SDS (GTES) buffer, followed by three rounds of freeze-thawing, then incubation in nematode lysis buffer, followed by phenol/chloroform/isoamyl alcohol extraction

Table 6. Diagnostic test recommendation based on setting and purpose

Population-based Testing		Serological Tests	Stool Conventional / Molecular Tests*	Notes
Endemic Areas	**Epidemiological Mapping**	+	(+)	Ease of use and acceptability of serology Sub-population for stool-based testing for quality assurance and baseline data
	Evaluation of Control Program efficacy	(+)	(+)	Test a sub-population include stool testing (delayed sero-reversion / re-infection)
Non-endemic Areas	**General screening of populations with risk factors (eg refugee health clinics)**	+	(+)	Ease of use of serology Consider stool testing seropositive to determine disease burden / follow treatment efficacy
Individual Testing**				
Risk factor based	**Immunocompromise (pre-treatment or existing)**	+	+	Serology sensitivity decreased

				Maximise diagnostic potential where possible
	Symptomatic / Clinical Indication	+	+	Use available tests
Clinical Trial	**Drug Therapy**	(+)	+	Stool testing for clearance preferred Sero-reversion in non-endemic areas

*conventional and / or molecular stool test based on availability / feasibility; **endemic or non-endemic areas

Table 7. Main characteristics and findings of systematic reviews estimating the accuracy of different diagnostic tests

Study	Reference test(s) used in the included papers	Diagnostic methods evaluated	Sensitivity (95%CI); specificity (95%CI)	Notes
Campo Polanco et al., 2014	Combination of the results of all parasitological tests applied on the panel of stool	1. Stool microscopy without concentration	Se 21% (16-26); Sp 100%*	
		2. Formol-ether concentration stool microscopy	Se 48% (42-54); Sp100%*	
		3. Baermann method	Se 72% (67-76); Sp100%*	
		4. Agar plate culture	Se 89% (86-92); Sp100%*	
Kalantari et al., 2020	Faecal-based techniques, including culture, Baermann, DS, FECT, Kato-Katz	Serology (IFAT and ELISA)	Se 71.7% (56-83.4); Sp 90% (81-95)	Different sensitivity depending on the *Strongyloides* species used as antigenic preparation: from 81% (60-92) with *S. venezuelenzis* to 49% (23-74) with *S. ratti*

Buonfrate et al., 2018	Coproparasitological methods only	PCR (conventional, nested and real-time)	Se 71.8% (52.3-85.5); Sp 93.5% (90.3-95.6)	For real-time PCR: 56.5% (39.2-72.4) and 95.4% (91.7-97.5) against combination of methods and 64.4% (46.2-77.7) and 64.4% (46.2-77.7) against parasitological methods only
	A combination of coproparasitological and serological methods	PCR (conventional, nested and real-time)	Se 61.8% (42-78.4); Sp 95.3% (92-97.2)	

4.2 Accuracy of molecular biology techniques for the diagnosis of *Strongyloides stercoralis* infection - A systematic review and meta-analysis

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Abstract

Background. *Strongyloides stercoralis* infection is a neglected tropical disease which can lead to severe symptoms and even death in immunosuppressed people. Unfortunately, its diagnosis is hampered by the lack of a gold standard, as the sensitivity of traditional parasitological tests (including microscopic examination of stool samples and coproculture) is low. Hence, alter- native diagnostic methods, such as molecular biology techniques (mostly polymerase chain reaction, PCR) have been implemented. However, there are discrepancies in the reported accuracy of PCR. Methodology. A systematic review with meta-analysis was conducted in order to evaluate the accuracy of PCR for the diagnosis of *S*. *stercoralis* infection. The protocol was registered with PROSPERO International Prospective Register of Systematic Reviews (record: CRD42016054298). Four- teen studies, 12 of which evaluating real-time PCR, were included in the analysis. The specific- ity of the techniques resulted high (ranging from 93 to 95%, according to the reference test(s) used). When all molecular techniques were compared to parasitological methods, the sensitiv- ity of PCR was assessed at 71.8% (95% CI 52.2–85.5), that decreased to 61.8% (95% CI 42.0–78.4) when serology was added among the reference tests. Similarly, sensitivity of real- time PCR resulted 64.4% (95% CI 46.2–77.7) when compared to parasitological methods only, 56.5% (95% CI 39.2–72.4) including serology. Conclusions. PCR might not be suitable for screening purpose, whereas it might have a role as a confirmatory test.

INTRODUCTION

Strongyloides stercoralis is a soil-transmitted helminth (STH) affecting around 370 million people worldwide, particularly in remote rural areas {Buonfrate, 2013}. Chronic strongyloidiasis is characterized by non-specific, mostly mild symptoms involving the gastrointestinal tract (abdominal pain, diarrhea), the respiratory system (symptoms resembling asthma, chronic obstructive pulmonary disease), the skin (pruritus, rash) {Greaves, 2013}. However, in immunosuppressed individuals the infection can become severe, with complications due to a heavier load of parasites, including intestinal obstruction, paralytic ileus, respiratory failure, death {Greaves, 2013}. Hence it is recommended to diagnose and treat strongyloidiasis when still in the chronic, indolent phase. First-line treatment is with ivermectin, which demonstrated a good safety profile and is highly effective for chronic infection {Henriquez-Camacho, 2016 #20}. On the other hand, treatment of the severe syndrome is more complicated as failures tend to occur with the standard regimens {Mejia, 2012}. A gold standard for the diagnosis of strongyloidiasis is still lacking {Buonfrate, 2013 #29}. Microscopic examination of stools has insufficient sensitivity, and enrichment techniques (Ritchie's method, for instance) and examination of multiple samples can only partially improve the performance of the method{Campo Polanco, 2014}. The Baermann method has a sensitivity about four times higher than formol-ether concentration technique (FECT); however, it is a cumbersome method and the sensitivity remains not adequate, either {Requena-Mendez, 2013; Campo Polanco, 2014}. Sensitivity of agar plate culture (APC – and in particular, the technique described by Koga) is comparable to the one demonstrated by Baermann.{Buonfrate, 2013 #29} There are different serological tests, some of which are commercially available. Globally, serology demonstrated high sensitivity (ranging from 70 to 95%, depending on the test, according to a diagnostic study on multiple serological tests){Bisoffi, 2014}, but there are concerns about its specificity, because of possible cross-reactions with other parasites and long-term persistence of antibodies after an effective treatment. A recombinant antigen (NIE) has been used in order to increase specificity of serological methods such as ELISA and luciferase immunoprecipitation system (LIPS) {Ramanathan, 2008; Krolewiecki, 2010}. The latter test (NIE-LIPS) in particular demonstrated a high specificity and an equivalent sensitivity compared with the other serological tests, but the technique at the moment is not commercially available, and has been used so far only for study purpose {Bisoffi, 2014}.

Molecular methods have been implemented in this context, with the aim to achieve the highest sensitivity, preserving a high specificity. However, different studies report either better {Verweij, 2009} or worse {Knopp, 2014 #6} accuracy of molecular methods compared to other fecal-based methods. Some variables (such as setting in which the research was conducted, population, type of molecular technique, comparator) might influence the global evaluation of their accuracy. In conclusion, the accuracy of molecular biology techniques for the diagnosis of *S. stercoralis* infection should be better defined, and so should their role in different settings.

Aim of this work was to review the accuracy of molecular biology techniques for the diagnosis of *S. stercoralis* infection.

METHODS

The protocol was registered with PROSPERO International Prospective Register of Systematic Reviews (record: CRD42016054298) on December 29th, 2016.

Search strategy and selection criteria

A systematic literature search was carried on January 20th, 2017. The following databases were searched for relevant studies:

- Cochrane Central Register of Controlled Trials (CENTRAL 2017, Issue 1);
- MEDLINE (PubMed) (1966 to 20 January 2017);
- EMBASE (Embase.com) (1974 to 20 January 2017);

- Latin American and Caribbean Health Science Information Database (LILACS) (Bireme) (1982 to 20 January 2017);
- ClinicalTrials.gov
- World Health Organization (WHO) International Clinical Trials Registry Platform

All relevant studies were reviewed, regardless of language or publication status (published, unpublished, in press, and ongoing). The reference lists of all included studies for other potentially relevant research and authors' personal collections (grey literature) were also reviewed.

Selection of studies

Inclusion criteria:

- Cohort studies
- All studies evaluating a molecular biology technique (either conventional polymerase chain reaction (PCR), nested PCR, real-time PCR (qPCR), or loop-mediated isothermal amplification (LAMP) in comparison to serology and/or fecal-based methods "specific" for the diagnosis of *S. stercoralis* infection (Baermann method, agar plate culture, Harada-Mori culture, combination of fecal methods).
- Studies that pooled multiple intestinal parasites into one outcome measure (for example, multiplex PCR including other soil-transmitted helminthes) were included when it was possible to disaggregate the data.
- Studies conducted in endemic as well as in non-endemic areas.
- Studies conducted on either immunocompetent or immunosuppressed patients.

Exclusion criteria:

- Case-control studies
- Non-human studies
- Duplicate publications

Two authors, DB and ARM, reviewed the titles and abstracts yielded by the search, and identified all studies that potentially met the inclusion criteria. DB contacted some authors requesting additional information on published data and/or other potentially relevant unpublished data. After obtainment of the full text articles of the records selected as potentially relevant, DB and ARM independently assessed whether or not each study met the inclusion criteria using an eligibility form in Excel. When DB and ARM did not reach a consensus, a third reviewer (AA) made the final inclusion decision.

Data collection process

DB and ARM independently performed the data extraction, that included sensitivity and specificity values, and other covariates, namely: reference test (divided in four categories: serology, culture, Baermann, combination of parasitological exams), setting (endemic/non-endemic area), population (children, adults, all ages, not specified), immunological status (immunocompetent, immunosuppressed, not specified). For study purpose, infected and not infected were all subjects resulting positive and negative, respectively, to the reference standard test(s). The sensitivity of the index test(s) was calculated as the proportion of true positives (positive at the index test over all infected), and the specificity as the proportion of true negatives (negative at the index test over all not-infected). Studies evaluating more than one molecular method or using more than one reference standard test were split into sub-studies. Any disagreements regarding the data extraction was solved by discussion between the two authors. When necessary, a third review author (AA) facilitated the discussion until consensus was reached.

Risk of bias (quality) assessment

DB and ARM independently assessed the methodological quality of each included study using the QUADAS-2 tool. Hence, four key domains were evaluated in terms of risk of bias: patient selection, index test, reference standard, flow and timing. When necessary, a third review author, AA, facilitated discussion until consensus was reached. All assessments were summarized in 'Risk of bias' tables.

Statistical analysis

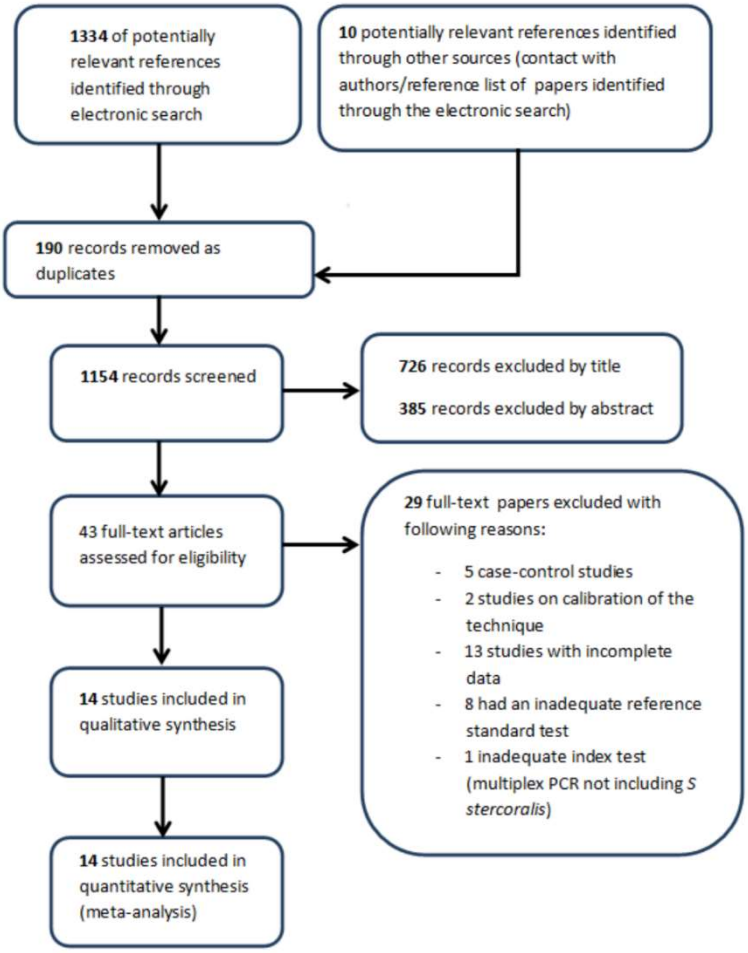
The values of sensitivity and specificity were automatically computed in RevMan 2014 (Version 5·3{http://community.cochrane.org/tools/review-production-tools/revman-5}). Individual study results

were graphically expressed by plotting the estimates of sensitivity and specificity and their 95% confidence intervals (CIs) through both forest plots and receiver operating characteristics (ROC) space. Heterogeneity was firstly evaluated by inspecting forest plots to detect overlapping 95% CIs, then by using a bivariate random-effects model {Reitsma, 2005} to obtain estimates of the between-study variation in sensitivity and specificity and the correlation between the two. The same bivariate model was used to assess the operating point sensitivity and specificity of the diagnostic tests under scrutiny, together with likelihood ratios and summary diagnostic odds ratio (DOR), taking both heterogeneity and threshold effect into account. Also, for each study, we estimated the true prevalence using the apparent prevalence, test sensitivity and specificity, as described by Rogan and Gladen {Rogan, 1978}. Finally, we used the hierarchical summary ROC (HSROC) model {Rutter, 2001} to obtain an adjusted ROC curve that summarized the results of all studies. All analyses were performed using all articles first, then they were repeated considering only those with parasitological methods (defined as the use of either stool culture, Baermann, or a combination of the two) as the reference test. This was considered the primary analysis. In order to have a more precise estimate of the influence of the real-time PCR, we also conducted a secondary analysis repeating the primary only on studies that used real-time PCR as the index test. All analyses were performed using Stata IC 13.0.

RESULTS

The electronic search identified 1334 records from the following databases: MEDLINE (448 records retrieved), Embase (516 records), CENTRAL (Cochrane library, 4 records), Lilacs (362 records); search on trial registries permitted to identify 4 further studies. The study flow is summarized in figure 1.

Figure 1. Study flow chart



Eventually, 14 studies were included both in quantitative and qualitative analyses. However, some studies evaluated either more than a single molecular method on the same pool of patients (in comparison to the same reference test) or a single molecular method on different subsets of patients (according to the results of different reference tests). In particular: two studies evaluated more than one molecular method (de Paula et al{Paula, 2015} tested both conventional and real-time PCR, Sharifdini et al {Sharifdini, 2015} tested both nested and real-time PCR), one study {Becker, 2015} evaluated the same real-time PCR method performed in two different laboratories, and one study {Buonfrate, 2017} evaluated the same index test (real-time PCR) both on patients positive to serology and on patients positive to APC. To handle and examine all these cases, we considered any experiment reported in a published paper as a separate study (Table 1).

Table 1. Main characteristics of the study sets included in the analysis

Study	Index test(s) and PCR target	Reference test(s)*	Setting	Population
Ahmad 2013{Ahmad, 2013 #1}	Nested PCR ITS1 region of the rRNA genes	Serology	Endemic country	All ages
Amor 2016{Amor, 2016 #2}	Real-time PCR 18S rRNA gene	Baermann	Endemic country	Children
Becker 2015_a {Becker, 2015 #4}	Real-time PCR 18S rRNA gene	Combination of parasitological methods	Endemic country	All ages
Becker 2015_b{Becker, 2015 #4}	Real-time PCR 18S rRNA gene	Combination of parasitological methods	Endemic country	All ages
Buonfrate 2017_a{Buonfrate, 2017 #28}	Real-time PCR 18S rRNA gene	APC	Non endemic country	Adults
Buonfrate 2017_b{Buonfrate, 2017 #28}	Real-time PCR 18S rRNA gene	Serology		
De Paula 2015_a {Paula, 2015 #5}	Conventional 18S rRNA gene	APC	Endemic country	Not specified
De Paula 2015_b{Paula, 2015 #5}	Real-time PCR 18S rRNA gene	APC	Endemic country	Not specified
Knopp 2014{Knopp, 2014 #6}	Real-time PCR 18S rRNA gene	Baermann	Endemic country	All ages
Lohd 2016{Lodh, 2016 #7}	Conventional PCR Specific interspersed repetitive sequence	Combination of parasitological methods	Endemic country	All ages

Meurs 2017{Meurs, 2017 #9}	Real-time PCR 18S rRNA gene	Combination of parasitological methods	Endemic country	All ages
Shar 2013{Schar, 2013 #11}	Real-time PCR 18S rRNA gene	Combination of parasitological methods	Endemic country	Children
Sharifdini 2015_a{Sharifdini, 2015 #12}	Nested 18S rRNA gene	Combination of parasitological methods	Endemic country	Not specified
Sharifdini 2015_b{Sharifdini, 2015 #12}	Real-time PCR 18S rRNA gene	Combination of parasitological methods	Endemic country	Not specif
Sultana 2013{Sultana, 2013 #13}	Real-time PCR 18S rRNA gene	APC	Endemic country	Not specified
Ten Hove 2009{ten Hove, #36}	Real-time PCR 18S rRNA gene	Baermann	Non endemic country	Adults
Verweij 2009{Verweij, 2009 #14}	Real-time PCR 18S rRNA gene	Combination of parasitological methods	Endemic country	All ages
Zueter 2014{Zueter, 2014 #15}	Real-time PCR 18S rRNA gene	Serology	Endemic country	Adults

ITS1= Internal transcribed spacer 1
rRNA= ribosomal RNA

Therefore, 4 out of the 14 included studies generated more than one set of sensitivity and specificity estimates. Globally, the included studies comprised a total of 3060 participants (from 54 {Ahmad, 2013} to 466 {Sharifdini, 2015} individuals tested). Of note, 12 of 14 studies evaluated a real-time PCR technique, and all of them used the method described by Verweij *et al* {Verweij, 2009}, which employs primers targeting the *S. stercoralis* 18S ribosomal RNA gene.. A different target DNA was used in a couple of studies {Ahmad, 2013; Lodh, 2016} only. Four studies evaluated either conventional {Lodh, 2016; Paula, 2015} or nested {Ahmad, 2013; Sharifdini, 2015} PCR. In addition, information on immunological status of the individuals tested was collected: only one study was conducted in immunocompromised patients{Zueter, 2014}. Three studies compared PCR with serology. In a couple of cases the serology was a commercial ELISA

test based on somatic antigens from *Strongyloides* L3 larvae {Ahmad, 2013; Zueter, 2014}, while the other study used an in-house IFAT based on intact *S. stercoralis* filariform larvae {Buonfrate, 2017}.
The samples were mostly kept frozen or preserved in ethanol until DNA extraction. In a few studies, the samples were kept at room temperature or refrigerated, and processed within a short time. Only one study did not report the method for preserving the stool sample before the DNA extraction {Zueter, 2014}. Another study protocol entailed the use of filter papers {Lodh, 2016}. DNA extraction was performed with a commercial kit in almost all cases (S3 Table). Only Sharifdini *et al* {Sharifdini, 2015} used an in-house method described previously. The DNA extraction method was not reported in one case {Zueter, 2014}. Most studies reported the use of controls for PCR inhibition (9 studies out of 14, S1 Table), and seven studies entailed the controls for DNA extraction. Neither PCR inhibition nor DNA extraction controls were reported by four studies. The validation of the PCR methods included the determination of a limit-of-detection (LOD) in four studies {Paula, 2015; Lodh, 2016; Sharifdini, 2015; Sultana, 2013} only. Shar *et al {Schar, 2013}* reported the determination of LOD in the methods, but the value was not specified in the results.
Figures 2 and 3 show the results of the qualitative evaluation, in terms of rating for each included study and overall methodological quality, respectively.

Figure 2. Risk of bias and applicability concerns summary

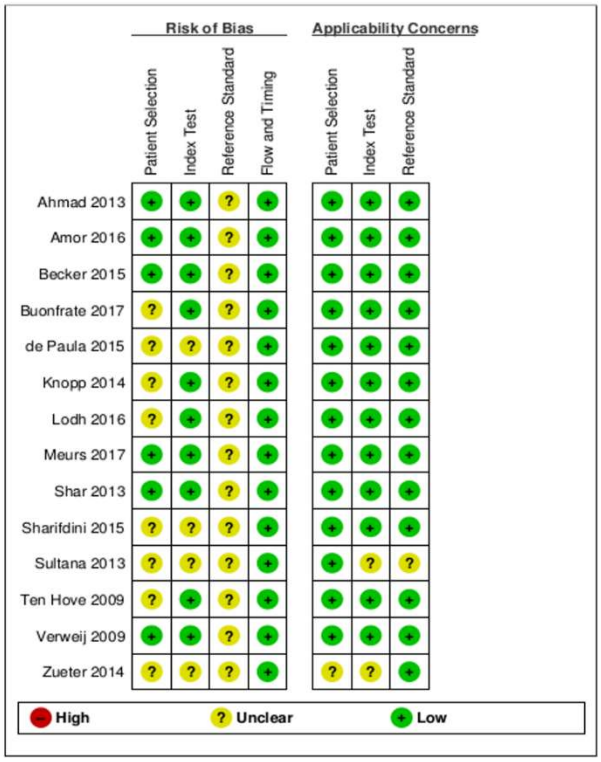
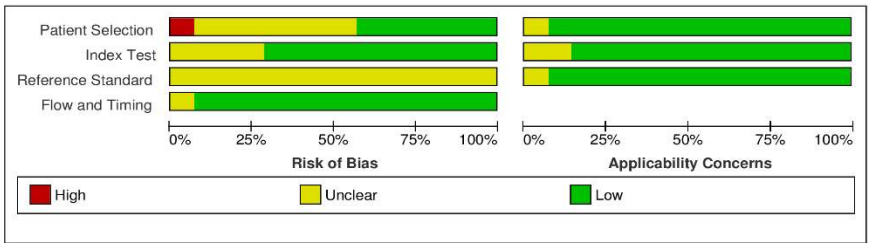
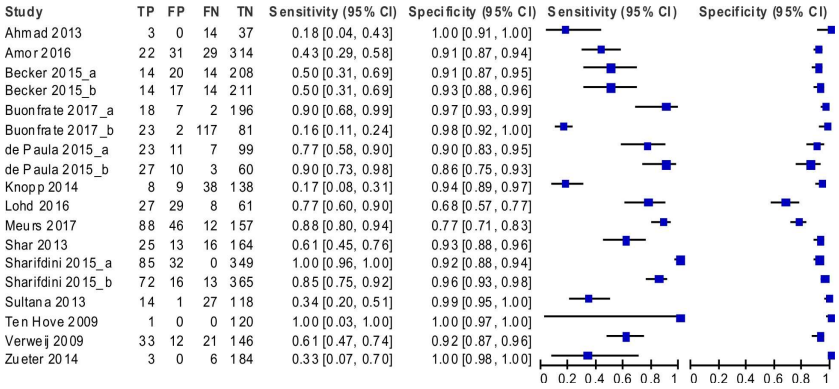


Figure 3. Risk of bias and applicability concerns graph



As reported in the introduction, the evaluation of diagnostic tests for *S. stercoralis* is hampered by the lack of a gold standard. Therefore, the risk of bias associated to the reference test (possible incorrect classification) was assessed as unclear for all studies. Only two studies{Knopp, 2014; Buonfrate, 2017} applied any of the methods suggested for reporting diagnostic accuracy in absence of a gold standard {Reitsma, 2005}. In particular, Buonfrate et al {Buonfrate, 2017} used a composite reference standard (CRS), while Knopp et al {Knopp, 2014} applied a Bayesan latent class analysis (BLCA). Data from these studies were extracted, similarly to the other studies, in relation to the comparison of PCR to the other tests (without considering CRS or BLCM), in order to obtain a more homogenous evaluation of the index test. However, the results of CRS and BLCM were then compared to the global results of included studies. In the domain of the patient selection, the risk of bias was assessed as unclear for 7 studies. For 6 out of 7 studies, the reason was that the papers did not clearly report some relevant details about the patient sampling: whether the sampling was random or consecutive, or inappropriate exclusions were avoided. For one paper, the unclear risk was mainly due to the retrospective design of the study {Buonfrate, 2017; Buonfrate, 2017}. Finally, one paper clearly reported that patient sampling was not random, hence the risk of bias was assessed as high. {Knopp, 2014} However, applicability concerns were assessed as low for all studies except one that evaluated the PCR accuracy in a cohort of cancer patients {Zueter, 2014; Zueter, 2014}. Figure 4 shows the accuracy reported in each study.

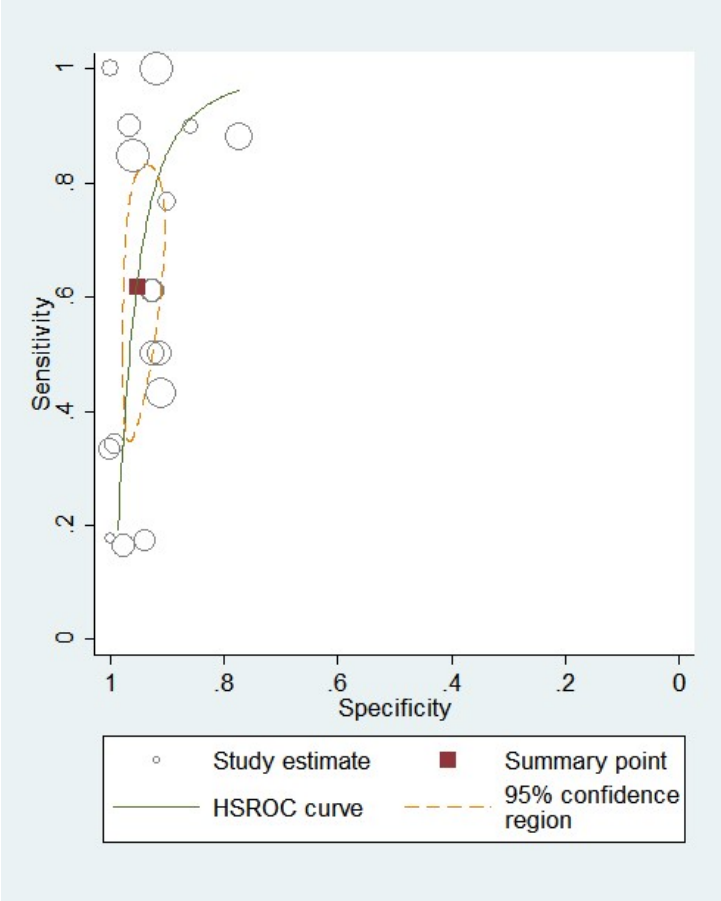
Figure 4. Forest plot showing the accuracy of the molecular techniques according to the single study sets.



The forest plot showed discrepancies in the results of the studies, particularly regarding sensitivity. As we included studies comparing PCR with different reference tests, this heterogeneity was partially expected. Nonetheless, we assessed the between-study variation in sensitivity and the degree of correlation between sensitivity and specificity by using the bivariate random effects approach introduced by Reitsma *et al* {Reitsma, 2005}. The variance of the logit of the sensitivity resulted 2.50 (95% CI: 1.12 to 5.49) and the correlation between logit of sensitivity and logit of specificity resulted -0.51 (95% CI: -0.82 to 0.02). Thus,

we fitted a bivariate model to take into account heterogeneity as much as possible and to obtain pooled accuracy estimates of PCR versus all other techniques (Table 2). Globally, the accuracy of all PCR techniques resulted in a sensitivity of 61.8% (95% CI: 42.0 to 78.4) and a specificity of 95.2% (95% CI: 92.0 to 97.2). A visual summary of these findings, comprehensive of a confidence area of the estimates and a summary ROC curve obtained through hierarchical random effects approach (HSROC){Rutjes, 2007} is displayed in Figure 5.

Figure 5. Accuracy of all PCR techniques (comparison with both fecal and serological tests): ROC curve



When studies comparing PCR with serology-positive patients were excluded from analysis, the sensitivity resulted 71.8% (95% CI: 52.2 to 85.5), and specificity 93.4% (95% CI: 90.3 to 95.6). Real-time PCR techniques were then analyzed separately (Table 2), showing sensitivity and specificity values of 56.5% (95% CI 39-72) and 95.4% (95% CI 92-97), respectively.

Table 2. Summary Estimates of Diagnostic Accuracy of PCR techniques for the diagnosis of *Strongyloides stercoralis* infection

Reference Test	All PCRs [a]		Real Time PCR	
Serology or parasitological methods [b]	**Estimate (95% CI)**	**S.E.**	**Estimate (95% CI)**	**S.E.**
Sensitivity	61.85% (42.0-78.4)	9.70	56.50% (39.2-72.4)	8.79
Specificity	95.27% (92.0-97.2)	1.28	95.38% (91.7-97.5)	1.40
DOR	32.7 (15.3-70.0)	12.6	26.8 (13.2-54.8)	9.77
LR+	13.1 (8.0-21.3)	3.2	12.2 (7.1-21.0)	3.38
LR-	0.40 (0.24-0.65)	0.09	0.45 (0.31-0.67)	0.08
1/LR-	2.5 (1.53-4.06)	0.62	2.2 (1.49-3.22)	0.43
Parasitological methods [b] only				
Sensitivity	71.76% (52.23-85.52)	8.72	64.42% (46.2-77.7)	8.31
Specificity	93.46% (90.35-95.61)	1.32	93.93% (90.3-96.3)	1.49
DOR	36.3 (15.4-85.4)	15.8	26.8 (12.7-56.6)	10.21
LR+	10.9 (7.2-16.6)	2.31	10.4 (6.4-16.8)	2.55
LR-	0.30 (0.16-0.55)	0.09	0.4 (0.25-0.60)	0.09
1/LR-	3.3 (1.49-3.22)	1.01	2.6 (1.66-3.98)	0.57

PCR, polymerase chain reaction; S.E., standard error; DOR, diagnostic odds ratio; LR, likelihood ratio;
S.E. Estimates for sensitivity and specificity are here reported in %.
[a] Studies included conventional PCR, nested PCR, real-time PCR
[b] Either Baermann method, agar plate culture, Harada-Mori culture, or a combination of fecal methods

Excluding serology from the analysis, the sensitivity and specificity values resulted 63.4% (95% CI 46-78) and 93.9% % (95% CI 90-96), respectively. The summary ROC curve is displayed in Figure 6.
Figure 6. Accuracy of real-time PCR (comparison with fecal tests): ROC curve.
Of note, the only study using a CRS (including serology) to assess the accuracy of real-time PCR demonstrated a sensitivity of 56.8%{Buonfrate, 2017}, which is almost the same value found with the meta-analysis.
On the other hand, the only study using a Bayesian approach {Knopp, 2014} demonstrated an extremely low sensitivity (11.6%) of real-time PCR.

DISCUSSION

Conventionally, PCR for *S. stercoralis* is considered 100% specific on the basis of the intrinsic characteristics of the technique. Although not confirming this value, the meta-analysis demonstrated a high specificity of PCR for the diagnosis of *S. stercoralis* infection, ranging from 93 to 95% according to the reference test. Moreover, it must be considered that the different reference standards used in the studies (implying that a sample PCR positive, but negative to all other fecal tests, is classified as a false positive) have most probably caused some underestimation of the specificity. On the other hand, the sensitivity resulted unsatisfactory, regardless of the reference test used: from 56% sensitivity when real-time PCR was compared to any other methods (including serology), to 71% when the results of any PCR techniques (either conventional, nested or real-time) were compared to fecal methods only. One possible explanation for this low sensitivity, particularly when compared with serological tests, is the irregular larval output observed in chronic strongyloidiasis. Therefore, PCR techniques might face the same problem as the conventional

parasitological techniques. As a matter of fact, PCR has not proven to be diagnostically superior to other parasitological techniques such as the Baermann method or APC, particularly in low-density infections where the larval output is low and irregular {Buonfrate, 2013 #29}. Moreover, one cause of the low sensitivity of PCR might be the small quantity of fecal sample analyzed {Requena-Mendez, 2014}, particularly relevant when the larvae are scarcely shed in feces.

Unfortunately, only a few included studies assessed the LOD of their techniques, that could permit a more accurate evaluation of the sensitivity of the PCR in relation to different levels of larval shedding. This information would be useful also to compare different techniques used in different studies, and should be better reported.

On the other hand, the sample preservation methods were reported by all but one authors of the included studies: they were all adequate, and presumably did not affect the results of the PCR. Also, DNA extraction was almost always conducted with commercial kits based on silica-membrane-DNA purification. All the automated methods used were highly reliable and the studies resulted homogeneous in relation to this aspect. Only one study reported an in-house method for DNA isolation that implies an organic solvent extraction and alcohol precipitation.

One reason for the low sensitivity might be represented by the presence of PCR inhibitors, commonly found in fecal samples. In fact, some authors did not report the use of controls for PCR inhibition. Knopp *et al*, who found the lowest sensitivity value of real-time PCR (when not considering the studies comparing PCR with serology) declared that the absence of controls for PCR inhibition was one of the limitations of their study. Therefore, we cannot exclude that PCR inhibition occurred and affected the results of some studies. However, most included papers reported the use of controls for PCR inhibition, and sensitivity resulted variable and seldom achieved 90%. In any case, these controls are of primary importance to confirm the correct execution of the PCR, and are therefore recommended both in research studies and in routine practice.

Analogously, the use of controls for DNA extraction was not reported by all authors, and it cannot be ruled out that a low efficiency in DNA extraction affected the results of PCR. Also in this case, the use of such controls is recommended both in routine and in research activities.

The interpretation of the results from a clinical point of view is resumed in the summary of findings table (table 3).

Table 3. Summary of findings table

Interpretative criteria to define: Index vs. Reference Test	Effect (95% CI)	Number of studies	Mean Prevalence (95% CI)	What do these results mean?
All PCR[a] vs. Serology or parasitological methods[b]	Sensitivity: 61.8% (42.0-78.4) Specificity: 95.3% (92.0-97.2)	17	21.1% (13.8 to 28.4)	Assuming (based on the mean prevalence) 21 out of 100 patients with SSI, eight would be missed by a single PCR test (38% of 21). Of the 79 patients without SSI, four (5%) would have a false positive result of the
Al PCR vs. parasitological methods only	Sensitivity: 71.8% (52.2-85.5) Specificity: 93.5% (90.3-95.6)	14	18.5% (13.4 to 23.6)	Assuming 18 out of 100 patients with SSI, five would be missed by a single PCR test. Of the 82 patients without SSI, five would have a false positive result of the PCR test.
Real-time PCR vs. Serology or parasitological methods	Sensitivity: 56.5% (39.2-72.4) Specificity: 95.4% (91.7-97.5)	14	20.5% (11.6 to 29.4)	Assuming 20 out of 100 patients with SSI, nine would be missed by a single PCR test. Of the other 80, four will have a false positive result of the PCR test.
Real-time PCR vs. parasitological methods only	Sensitivity: 64.4% (46.2-77.7) Specificity: 93.9% (90.3-96.3)	12	20.3% (9.9 to 30.8)	Assuming 20 out of 100 patients with SSI, seven would be missed by a single PCR test. Of the other 80, five would have a false positive result of the PCR test.

PCR, polymerase chain reaction; SSI, *S. stercoralis* infection. Estimates for sensitivity and specificity are here reported in %.

[a] Studies included conventional PCR, nested PCR, real-time PCR

[b] Either Baermann method, agar plate culture, Harada-Mori culture, or a combination of fecal methods

Indeed, PCR is not adequate for universal screening of strongyloidiasis, as it would entail an excessive risk of missing diagnoses of a potentially fatal infection. It could rather be a valid option as a confirmatory test in case of positive serology. Moreover, it could be used as an alternative to other fecal-based tests for the screening of immunosuppressed patients, for whom the sensitivity of serology decreases {Luvira, 2016}. However, also in this latter group it should be used in addition to serology, in order to increase case-detection in these patients particularly at risk of developing severe infection.

Unfortunately, as it results from the qualitative evaluation of the included studies, we suggest that the lack of a gold standard for the diagnosis may hamper the results of diagnostic studies. This problem is frequently encountered in parasitology. The comparison of PCR with the fecal methods which proved to be sufficiently sensitive for the diagnosis of strongyloidiasis (namely, Baermann and APC) could be seen as the best option to validate the accuracy of PCR, as they all rely on larval shedding, indicating the presence of active infection. However, the sensitivity of Baermann and APC is still inadequately low to safely rule out the infection, when resulting negative. For this reason, using them as reference tests tends to result in an overestimation of the sensitivity of PCR. Serology detects the antibodies against larval antigens, hence it does not rely on the presence of larvae in stool, that is often inconstant. Despite the possibility of false positive results (as reported in the introduction), we decided to add the comparison with serology to highlight that the sensitivity of PCR is presumably lower than that found when compared with the other fecal methods.

Although methods to assess the test accuracy in the absence of a gold standard have been proposed {Reitsma, 2005}, they are seldom applied, as it resulted from our review, too (only a couple of studies proposed an alternative model for the classification of the results). Indeed, our investigation highlighted that, in absence of a validated reference standard, different studies considered different reference tests for the evaluation of the accuracy of PCR, leading to difficulties in the direct comparison of the results.

Another limitation of our study is that it was not possible to analyze the influence of setting and age on the accuracy, because of the relatively low number of studies included in the meta-analysis. Due to the distinct pools of patients (defined through the different reference tests) of the PCR experiments included in the analysis, a certain degree of heterogeneity was inevitably expected. Indeed, the measure of correlation between sensitivity and specificity provided evidence of a heterogeneity that should not be ignored. This heterogeneity may be also largely caused by variations between tests in terms of country setting, population age or by a threshold effect. Nonetheless, the utilization of statistical techniques that take this heterogeneity into account for the estimation of summary measures, such as the bivariate model by Reitsma *et al* {Reitsma, 2005}, allowed for exhaustive and robust estimates as shown in Table 2. As the number of studies included did not allow for a proper analysis of all possible sub-cases of index-reference tests, these estimates shall be considered as pooled accuracy measures of the PCR techniques versus all other techniques.

Conclusions

In summary, the results of this review suggest that, although the PCR technique is highly specific, it should not yet be recommended for universal screening, nor as a stand-alone method for the individual diagnosis of *S. stercoralis* infection. However, PCR has a role as a confirmatory test. Additional studies investigating the accuracy of this and other diagnostic tests for this infection, using appropriate methods to cope with the absence of a gold standard, are needed to improve the screening and management of this neglected infection.

References

1. Ahmad, A.F.; Hadip, F.; Ngui, R.; Lim, Y.A.; Mahmud, R. Serological and molecular detection of Strongyloides stercoralis infection among an Orang Asli community in Malaysia. *Parasitology research* **2013**, *112*, 2811-2816, doi:10.1007/s00436-013-3450-z.
2. Amor, A.; Rodriguez, E.; Saugar, J.M.; Arroyo, A.; Lopez-Quintana, B.; Abera, B.; Yimer, M.; Yizengaw, E.; Zewdie, D.; Ayehubizu, Z., et al. High prevalence of Strongyloides stercoralis in school-aged children in a rural

highland of north-western Ethiopia: the role of intensive diagnostic work-up. *Parasites & vectors* **2016**, *9*, 617, doi:10.1186/s13071-016-1912-8.
3. Basuni, M.; Muhi, J.; Othman, N.; Verweij, J.J.; Ahmad, M.; Miswan, N.; Rahumatullah, A.; Aziz, F.A.; Zainudin, N.S.; Noordin, R. A pentaplex real-time polymerase chain reaction assay for detection of four species of soil-transmitted helminths. *The American journal of tropical medicine and hygiene* **2011**, *84*, 338-343, doi:10.4269/ajtmh.2011.10-0499.
4. Becker, S.L.; Piraisoody, N.; Kramme, S.; Marti, H.; Silue, K.D.; Panning, M.; Nickel, B.; Kern, W.V.; Herrmann, M.; Hatz, C.F., et al. 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 tropica* **2015**, *150*, 210-217, doi:10.1016/j.actatropica.2015.07.019.
5. Bisoffi, Z.; Buonfrate, D.; Montresor, A.; Requena-Mendez, A.; Munoz, J.; Krolewiecki, A.J.; Gotuzzo, E.; Mena, M.A.; Chiodini, P.L.; Anselmi, M., et al. Strongyloides stercoralis: a plea for action. *PLoS neglected tropical diseases* **2013**, *7*, e2214, doi:10.1371/journal.pntd.0002214.
6. Bisoffi, Z.; Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Gobbo, M.; Bonafini, S.; Angheben, A., et al. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. *PLoS neglected tropical diseases* **2014**, *8*, e2640, doi:10.1371/journal.pntd.0002640.
7. Buonfrate, D.; Formenti, F.; Perandin, F.; Bisoffi, Z. Novel approaches to the diagnosis of Strongyloides stercoralis infection. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2015**, *21*, 543-552, doi:10.1016/j.cmi.2015.04.001.
8. Buonfrate, D.; Perandin, F.; Formenti, F.; Bisoffi, Z. A retrospective study comparing agar plate culture, indirect immunofluorescence and real-time PCR for the diagnosis of Strongyloides stercoralis infection. *Parasitology* **2017**, 10.1017/s0031182016002559, 1-5, doi:10.1017/s0031182016002559.
9. Campo Polanco, L.; Gutierrez, L.A.; Cardona Arias, J. [Diagnosis of Strongyloides Stercoralis infection: meta-analysis on evaluation of conventional parasitological methods (1980-2013)]. *Revista espanola de salud publica* **2014**, *88*, 581-600, doi:10.4321/s1135-57272014000500004.
10. Greaves, D.; Coggle, S.; Pollard, C.; Aliyu, S.H.; Moore, E.M. Strongyloides stercoralis infection. *BMJ (Clinical research ed.)* **2013**, *347*, f4610, doi:10.1136/bmj.f4610.
11. Henriquez-Camacho, C.; Gotuzzo, E.; Echevarria, J.; White, A.C., Jr.; Terashima, A.; Samalvides, F.; Perez-Molina, J.A.; Plana, M.N. Ivermectin versus albendazole or thiabendazole for Strongyloides stercoralis infection. *The Cochrane database of systematic reviews* **2016**, 10.1002/14651858.CD007745.pub3, CD007745, doi:10.1002/14651858.CD007745.pub3.
12. http://community.cochrane.org/tools/review-production-tools/revman-5. Availabe online: (accessed on
13. Knopp, S.; Salim, N.; Schindler, T.; Karagiannis Voules, D.A.; Rothen, J.; Lweno, O.; Mohammed, A.S.; Singo, R.; Benninghoff, M.; Nsojo, A.A., et al. Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann, and PCR methods for the detection of light-intensity hookworm and Strongyloides stercoralis infections in Tanzania. *The American journal of tropical medicine and hygiene* **2014**, *90*, 535-545, doi:10.4269/ajtmh.13-0268.
14. Krolewiecki, A.J.; Ramanathan, R.; Fink, V.; McAuliffe, I.; Cajal, S.P.; Won, K.; Juarez, M.; Di Paolo, A.; Tapia, L.; Acosta, N., et al. Improved diagnosis of Strongyloides stercoralis using recombinant antigen-based serologies in a community-wide study in northern Argentina. *Clinical and vaccine immunology : CVI* **2010**, *17*, 1624-1630, doi:10.1128/cvi.00259-10.
15. Lodh, N.; Caro, R.; Sofer, S.; Scott, A.; Krolewiecki, A.; Shiff, C. Diagnosis of Strongyloides stercoralis: Detection of parasite-derived DNA in urine. *Acta tropica* **2016**, *163*, 9-13, doi:10.1016/j.actatropica.2016.07.014.
16. Luvira, V.; Trakulhun, K.; Mungthin, M.; Naaglor, T.; Chantawat, N.; Pakdee, W.; Phiboonbanakit, D.; Dekumyoy, P. Comparative Diagnosis of Strongyloidiasis in Immunocompromised Patients. *The American journal of tropical medicine and hygiene* **2016**, *95*, 401-404, doi:10.4269/ajtmh.16-0068.
17. Mejia, R.; Nutman, T.B. Screening, prevention, and treatment for hyperinfection syndrome and disseminated infections caused by Strongyloides stercoralis. *Current opinion in infectious diseases* **2012**, *25*, 458-463, doi:10.1097/QCO.0b013e3283551dbd.
18. Mejia, R.; Vicuna, Y.; Broncano, N.; Sandoval, C.; Vaca, M.; Chico, M.; Cooper, P.J.; Nutman, T.B. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. *The American journal of tropical medicine and hygiene* **2013**, *88*, 1041-1047, doi:10.4269/ajtmh.12-0726.
19. Meurs, L.; Polderman, A.M.; Vinkeles Melchers, N.V.; Brienen, E.A.; Verweij, J.J.; Groosjohan, B.; Mendes, F.; Mechendura, M.; Hepp, D.H.; Langenberg, M.C., et al. Diagnosing Polyparasitism in a High-Prevalence Setting in Beira, Mozambique: Detection of Intestinal Parasites in Fecal Samples by Microscopy and Real-Time PCR. *PLoS neglected tropical diseases* **2017**, *11*, e0005310, doi:10.1371/journal.pntd.0005310.
20. Paula, F.M.; Malta Fde, M.; Marques, P.D.; Sitta, R.B.; Pinho, J.R.; Gryschek, R.C.; Chieffi, P.P. Molecular diagnosis of strongyloidiasis in tropical areas: a comparison of conventional and real-time polymerase chain

reaction with parasitological methods. *Memorias do Instituto Oswaldo Cruz* **2015**, *110*, 272-274, doi:10.1590/0074-02760140371.
21. Ramanathan, R.; Burbelo, P.D.; Groot, S.; Iadarola, M.J.; Neva, F.A.; Nutman, T.B. A luciferase immunoprecipitation systems assay enhances the sensitivity and specificity of diagnosis of Strongyloides stercoralis infection. *The Journal of infectious diseases* **2008**, *198*, 444-451, doi:10.1086/589718.
22. Reitsma, J.B.; Glas, A.S.; Rutjes, A.W.; Scholten, R.J.; Bossuyt, P.M.; Zwinderman, A.H. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *Journal of clinical epidemiology* **2005**, *58*, 982-990, doi:10.1016/j.jclinepi.2005.02.022.
23. Repetto, S.A.; Alba Soto Cd Fau - Cazorla, S.I.; Cazorla Si Fau - Tayeldin, M.L.; Tayeldin Ml Fau - Cuello, S.; Cuello S Fau - Lasala, M.B.; Lasala Mb Fau - Tekiel, V.S.; Tekiel Vs Fau - Gonzalez Cappa, S.M.; Gonzalez Cappa, S.M. An improved DNA isolation technique for PCR detection of Strongyloides stercoralis in stool samples.
24. Requena-Mendez, A.; Buonfrate, D.; Bisoffi, Z.; Munoz, J. Advances in the Diagnosis of Human Strongyloidiasis. *Curr Trop Med Rep* **2014**, *1*, 207-215.
25. Requena-Mendez, A.; Chiodini, P.; Bisoffi, Z.; Buonfrate, D.; Gotuzzo, E.; Munoz, J. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS neglected tropical diseases* **2013**, *7*, e2002, doi:10.1371/journal.pntd.0002002.
26. Rogan, W.J.; Gladen, B. Estimating prevalence from the results of a screening test. *American journal of epidemiology* **1978**, *107*, 71-76.
27. Rutjes, A.W.; Reitsma, J.B.; Coomarasamy, A.; Khan, K.S.; Bossuyt, P.M. Evaluation of diagnostic tests when there is no gold standard. A review of methods. *Health technology assessment (Winchester, England)* **2007**, *11*, iii, ix-51.
28. Rutter, C.M.; Gatsonis, C.A. A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations. *Statistics in medicine* **2001**, *20*, 2865-2884.
29. Saugar, J.M.; Merino, F.J.; Martin-Rabadan, P.; Fernandez-Soto, P.; Ortega, S.; Garate, T.; Rodriguez, E. Application of real-time PCR for the detection of Strongyloides spp. in clinical samples in a reference center in Spain. *Acta tropica* **2015**, *142*, 20-25, doi:10.1016/j.actatropica.2014.10.020.
30. Schar, F.; Odermatt, P.; Khieu, V.; Panning, M.; Duong, S.; Muth, S.; Marti, H.; Kramme, S. Evaluation of real-time PCR for Strongyloides stercoralis and hookworm as diagnostic tool in asymptomatic schoolchildren in Cambodia. *Acta tropica* **2013**, *126*, 89-92, doi:10.1016/j.actatropica.2012.12.012.
31. Sharifdini, M.; Mirhendi, H.; Ashrafi, K.; Hosseini, M.; Mohebali, M.; Khodadadi, H.; Kia, E.B. Comparison of Nested Polymerase Chain Reaction and Real-Time Polymerase Chain Reaction with Parasitological Methods for Detection of Strongyloides stercoralis in Human Fecal Samples. *The American journal of tropical medicine and hygiene* **2015**, *93*, 1285-1291, doi:10.4269/ajtmh.15-0309.
32. Sultana, Y.; Jeoffreys, N.; Watts, M.R.; Gilbert, G.L.; Lee, R. Real-time polymerase chain reaction for detection of Strongyloides stercoralis in stool. *The American journal of tropical medicine and hygiene* **2013**, *88*, 1048-1051, doi:10.4269/ajtmh.12-0437.
33. Sun, W.; Wang, K.; Gao, W.; Su, X.; Qian, Q.; Lu, X.; Song, Y.; Guo, Y.; Shi, Y. Evaluation of PCR on bronchoalveolar lavage fluid for diagnosis of invasive aspergillosis: a bivariate metaanalysis and systematic review. *PloS one* **2011**, *6*, e28467, doi:10.1371/journal.pone.0028467.
34. ten Hove, R.J.; van Esbroeck M Fau - Vervoort, T.; Vervoort T Fau - van den Ende, J.; van den Ende J Fau - van Lieshout, L.; van Lieshout L Fau - Verweij, J.J.; Verweij, J.J. Molecular diagnostics of intestinal parasites in returning travellers. D - NLM: PMC2758195 EDAT- 2009/05/06 09:00 MHDA- 2009/12/16 06:00 CRDT- 2009/05/06 09:00 PHST- 2008/11/25 00:00 [received] PHST- 2009/03/31 00:00 [accepted] PHST- 2009/05/06 09:00 [entrez] PHST- 2009/05/06 09:00 [pubmed] PHST- 2009/12/16 06:00 [medline] AID - 10.1007/s10096-009-0745-1 [doi] PST - ppublish, doi:D - NLM: PMC2758195 EDAT- 2009/05/06 09:00 MHDA- 2009/12/16 06:00 CRDT- 2009/05/06 09:00 PHST- 2008/11/25 00:00 [received] PHST- 2009/03/31 00:00 [accepted] PHST- 2009/05/06 09:00 [entrez] PHST- 2009/05/06 09:00 [pubmed] PHST- 2009/12/16 06:00 [medline] AID - 10.1007/s10096-009-0745-1 [doi] PST - ppublish.
35. Verweij, J.J.; Canales, M.; Polman, K.; Ziem, J.; Brienen, E.A.; Polderman, A.M.; van Lieshout, L. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2009**, *103*, 342-346, doi:10.1016/j.trstmh.2008.12.001.
36. Whiting, P.F.; Rutjes Aw Fau - Westwood, M.E.; Westwood Me Fau - Mallett, S.; Mallett S Fau - Deeks, J.J.; Deeks Jj Fau - Reitsma, J.B.; Reitsma Jb Fau - Leeflang, M.M.G.; Leeflang Mm Fau - Sterne, J.A.C.; Sterne Ja Fau - Bossuyt, P.M.M.; Bossuyt, P.M. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies.
37. Zueter, A.M.; Mohamed, Z.; Abdullah, A.D.; Mohamad, N.; Arifin, N.; Othman, N.; Noordin, R. Detection of Strongyloides stercoralis infection among cancer patients in a major hospital in Kelantan, Malaysia. *Singapore medical journal* **2014**, *55*, 367-371.

4.3 Accuracy of five serologic tests for the post-treatment follow up of *Strongyloides stercoralis* infection

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Abstract

Background. Traditional faecal-based methods have poor sensitivity for the detection of *S. stercoralis*, therefore are inadequate for post-treatment evaluation of infected patients who should be carefully monitored to exclude the persistence of the infection. In a previous study we demonstrated high accuracy of five serology tests for the screening and diagnosis of strongyloidiasis. Aim of this study is to evaluate the performance of the same five tests for the follow up of patients infected with *S. stercoralis.* Methods. Retrospective study on anonymized, cryo-preserved samples available at the Centre for Tropical Diseases (Negrar, Verona, Italy). Samples were collected before and from 3 to 12 months after treatment. The samples were tested with two commercially-available ELISA tests (IVD, Bordier), two techniques based on a recombinant antigen (NIE-ELISA and NIE-LIPS) and one in-house IFAT. The results of each test were evaluated both in relation to the results of fecal examination and to those of a composite reference standard (classifying as positive a sample with positive stools and/or at least three positive serology tests). The associations between the independent variables age and time and the dependent variable value of serological test (for all five tests), were analyzed by linear mixed-effects regression model. Results. A high proportion of samples demonstrated for each test a seroreversion or a relevant decline (optical density/relative light units halved or decrease of at least two titers for IFAT) at follow up, results confirmed by the linear mixed effects model that showed a trend to seroreversion over time for all tests. In particular, IVD-ELISA (almost 90% samples demonstrated relevant decline) and IFAT (almost 87%) had the best performance. Considering only samples with a complete negativization, NIE-ELISA showed the best performance (72.5% seroreversion). Conclusions. Serology is useful for the follow up of patients infected with *S. stercoralis* and determining test of cure.

Introduction

Strongyloides stercoralis infection is widely distributed in tropical, subtropical countries and even in areas of temperate climate {Buonfrate, 2013 }. Strongyloidiasis probably affects at least 370 million people worldwide {Buonfrate, 2013 } and represents a threat for immunosuppressed people, who tend to develop the fatal complications of the infection {Greaves, 2013; Buonfrate, 2013}. Therefore, it is mandatory to diagnose the infection during the chronic phase, which is often indolent and can be more easily treated {Greaves, 2013}.

The diagnosis of *S. stercoralis* infection is characterized by poor sensitivity of fecal-based methods {Buonfrate, 2013 #29}. Therefore, other diagnostic tools have been developed and demonstrated better sensitivity {Requena-Mendez, 2013; Bisoffi, 2014}. Polymerase chain reaction (PCR) is still based on in-house techniques {Hasegawa, 2009; Schar, 2013; Verweij, 2009}, performed only in reference centers, and is not necessarily more sensitive than fecal culture {Knopp, 2014 }. Serology is more sensitive, though not 100% specific {Buonfrate, 2013 }. Some serology kits are commercially available {van Doorn, 2007; Bon, 2010}. A high sensitivity is also necessary when evaluating the response to the treatment, as treatment failures leave the patient exposed to the risk of developing a potentially fatal, disseminated strongyloidiasis at any time in his/her life {Buonfrate, 2013 }. Negative fecal-based methods cannot safely exclude persistence of infection {Dreyer, 1996; Buonfrate, 2013}, therefore the follow up of infected patients should also rely on more sensitive techniques as markers of cure. Although some authors have observed a decline of optical density (OD)/titers of serology tests over time, a wider comparative evaluation has not been carried out so far, and a clear definition of cure has not yet been established {Boscolo, 2007; Karunajeewa, 2006; Kobayashi, 1994; Lindo, 1996; Loutfy, 2002; Page, 2006; Salvador, 2014; Biggs, 2009}. We recently published the results of a study comparing the accuracy of five serologic tests for the diagnosis of *S. stercoralis* infection {Bisoffi, 2014}: two commercial ELISA tests (Bordier ELISA, IVD-ELISA), two tests based on the recombinant antigen NIE (ELISA and luciferase immunoprecipitation system, LIPS) and one in-house indirect immunofluorescence antibody test (IFAT). The study demonstrated a good performance of the tests, and in particular NIE-LIPS demonstrated the best accuracy for the diagnosis of *S. stercoralis.* The same tests were also evaluated on sera collected pre and post treatment in the present study.

Thus, the aim of this study was to compare the performance of the five tests for the follow up of patients after treatment in order to identify if antibody decline could be used a surrogate marker for cure, in addition to stool negativization.

Methods

Study population and data collection. This was a retrospective study on archived, anonymized sera available at the Centre for Tropical Diseases (CTD). Samples were classified according to a composite reference standard (a procedure suggested for evaluation of diagnostic tests when there is no gold standard) {Reitsma, 2009; Rutjes, 2007} as a) positive: positive fecal tests and/or at least 3/5 positive serologic tests; b) negative: negative fecal tests and less than 3 positive results out of the 5 serologic tests.

The inclusion criteria were: samples resulting positive before treatment, according to the composite reference standard), and available follow up serum sample/s, from 3 to 12 months after treatment. Treatment administered was ivermectin (stat dose of 200 µg/kg), with the exception of 6 cases treated with thiabendazole (two daily doses of 25 mg/kg for two days) in the earlier period. The exclusion criterion was travel history to endemic areas between treatment and follow up. The results of stool examination/agar culture were registered and entered in the study database.

Test methods. Parasitological tests used were: at least 3 stool samples examined with microscopy (formol-ether concentration) and Koga agar plate culture {Ines Ede, 2011; Siddiqui, 2001}. These methods were performed at the CTD. The serology tests evaluated were: the CTD in-house immunofluorescence technique (IFAT) {Boscolo, 2007}, two commercial ELISA tests (Bordier ELISA {van Doorn, 2007} and IVD ELISA {Bon, 2010}) and two techniques based on the recombinant antigen NIE (NIE-ELISA {Krolewiecki, 2010} and NIE-LIPS {Ramanathan, 2008}. IFAT and the two commercial ELISA tests were executed by senior staff of the CTD Negrar (Verona), Italy, while NIE-LIPS and NIE-ELISA were up to senior staff of the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH), Bethesda, US and of the Instituto de Investigaciones en Enfermedades Tropicales of the University of Salta/CONICET, Oran, Argentina. Lab staff were blinded to the patients' data and to the results of the other tests.

Definitions of response to therapy. Cure was operationally defined by negative composite reference standard (see above) at follow up or at least by: negative stool examination/coproculture and decrease of at least half of initial eosinophil count.

For the evaluation of each test, we assessed, over the denominator of patients cured according to the operational definition reported above: a) the proportion of initially positive tests that were negative at follow up; b) the proportion of those showing a decrease of at least half of initial OD/relative light units (RLU) values (for ELISA tests and LIPS, respectively) or decrease of at least two titers (for IFAT). This was taken as an empirical measure of response to therapy.

Sampling. The STARD flow chart (Figure 1) describes the selection of the samples tested. Among the 130 subjects responding to our definition of positive, 8 were excluded because follow up samples were not available. Of the remaining 122, 6 had a positive fecal result at follow up. Of the 116 testing negative at follow up, 98 met the criterion of cure as defined above, of which: 57 were negative according to the composite reference standard, and 41 showed a decrease of at least half of initial eosinophil count. Two subjects were excluded because their follow up sample was collected less than 3 months after the baseline sample. Eventually, 96 subjects were included in the analysis.

Statistical methods. Primarily, the performance of each test was calculated as the proportion of samples demonstrating seroreversion or a quantitative decrease (as indicated above) over all positive samples (for the same test) at baseline. Uncertainty was quantified using 95% confidence intervals.

To reduce the limitations due to the different time intervals between treatment and observation (from 3 to 12 months), we used the following methods. The associations between the independent variables age and time and the dependent variable value of serological test (for all five tests), were analyzed by linear mixed-effects regression model. Linear mixed model is a generalization of traditional linear regression, which adjusts for the correlation between repeated measurements within each subject and finds the best linear fit to the data across all individuals {Finucane, 2007; Symanski, 2001}. More specifically, a unique identification number for each subject and time was treated as a random effect in the model and age was treated as fixed effect. Time was entered as random effect because measurements of the value of serological tests over time were not taken at regular time points. Interaction term between age and time was evaluated to include in the regression model by using Likelihood Ratio Test. Introduction of an interaction term is necessary where the effect of one variable (time) is affected by the presence or value of another variable (age). Unstructured covariance matrix was selected since this is the structure that appears to fit the data the best, based upon the Akaike's information criterion (AIC).

Analyses were done by using SAS (version 9.1; SAS Institute, Inc, Cary, NC). We considered differences to be statistically significant when the p-value was <0.05.

Ethical issues. Although this was a retrospective study on anonymously coded, cryo-preserved samples, the study protocol was nevertheless submitted to the Ethics Committee of the Coordinating Site (Comitato Etico Provinciale di Verona) for approval. The latter acknowledged the study protocol and formally authorized the study (protocol n. 13286/09.11.01 of 24th April, 2012).

Results

The sample selection and the laboratory analyses were performed during the second semester of 2012. The median age of the population considered was 42 years (IQ range 22.5 – 67). Table 1 shows the time (in months) elapsed from baseline to follow up.

Table 1. Number of patients who had the follow up sample in each two-month period of time. For each time frame, it is also showed the number of patients who had positive versus negative stool microscopy and culture at baseline.

Months from baseline to follow up visit	Positive at fecal-based methods	Negative at fecal-based methods	Total
3 - 4	29	5	34
5 - 6	25	0	25
7 - 8	17	2	19
9 - 10	4	0	4
11 - 12	12	2	14
Total	87	9	96

Every patient had a baseline evaluation both with serology and with parasitological methods. Only 9/96 (0.9%) patients had negative stools at baseline; according to the composite reference standard, these patients were included in the analysis because they had at least 3 out of 5 positive serologic results. All but these 9 patients, had also parasitological evaluation at the time of collection of the follow up serum sample. All had negative stool microscopy and culture at follow up (data not reported in Table 1), as this was the first required criterion for the definition of cure.

Table 2 shows, for each test, the percentage of serum samples showing response according to the pre-defined criteria.

Table2. Number of samples which demonstrated cure at follow up, for each test. Total baseline samples positive at composite reference standard: 96. Negativization concerns for each test the samples that were positive at baseline and negative at follow-up; response also includes samples that, albeit not yet negative at follow-up, showed a decrease in OD, RLU or titer, respectively, as explained in the text.

Test	Positives at baseline	Response	%	Negativization	%
IFAT	91	79	86.8	36	39.6
NIE-LIPS	82	65	79.3	35	42.7
NIE-ELISA	69	56	81.2	50	72.5
IVD	88	79	89.8	48	54.5
Bordier	86	71	82.6	47	54.7

For each serologic test, we considered for this analysis only the samples that were positive at baseline. For instance, among the 96 samples resulting positive at baseline according to the composite reference standard, 91 had a positive IFAT result (see column “Positives at baseline”). The column “Negativization” comprises the samples which were positive at baseline and negative at follow-up, while the column “Response” includes the latter, plus the samples that, albeit remaining positive, showed a decrease of at least half of initial OD/relative light units (RLU) values (for ELISA tests and LIPS, respectively) or two titers (for IFAT). IVD-ELISA (almost 90% samples demonstrated response) and IFAT (almost 87%) had the best performance. When considering only samples with a complete negativization, NIE-ELISA showed the best performance (72.5% of seroreversion).

Figures from 2 to 6 show the results of the mixed effects model for all five serological tests. They represent the prediction of the trends of the values of serology, from the baseline evaluation (0 on the x-axis) to the moment in which the result became negative (0 on the y-axis). Thus, significantly negative trends over time were detected for all tests. Moreover, the intersection of the interpolation line with the x-axis predicts the average time (days) required to obtain the negativization of the serology test. Therefore, NIE-ELISA and IVD-ELISA showed the most rapid predicted negativization (about 1 year from baseline evaluation).

Figure 1. Results of the mixed effects model for IFAT

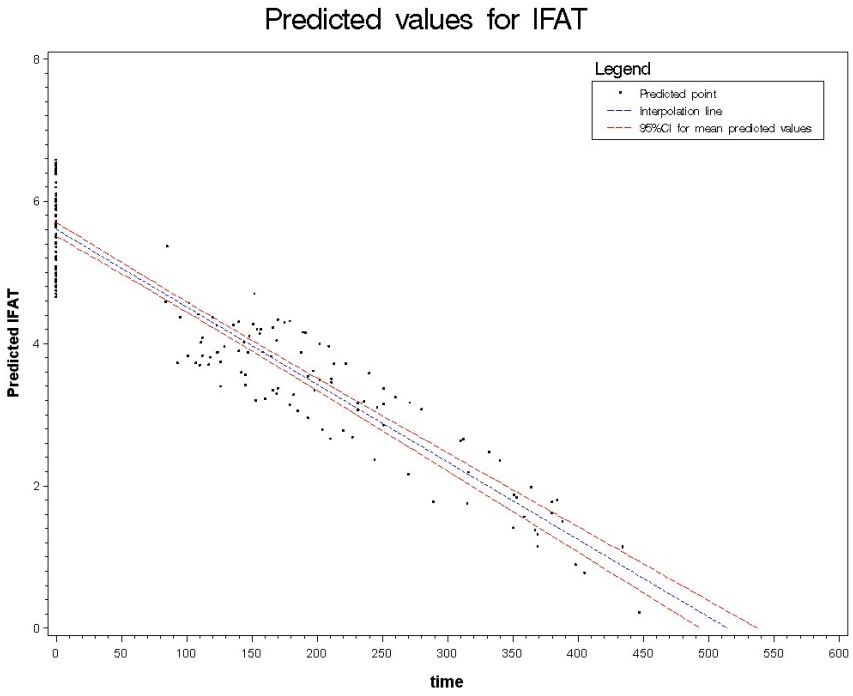


Figure 2. Results of the mixed effects model for IVD-ELISA

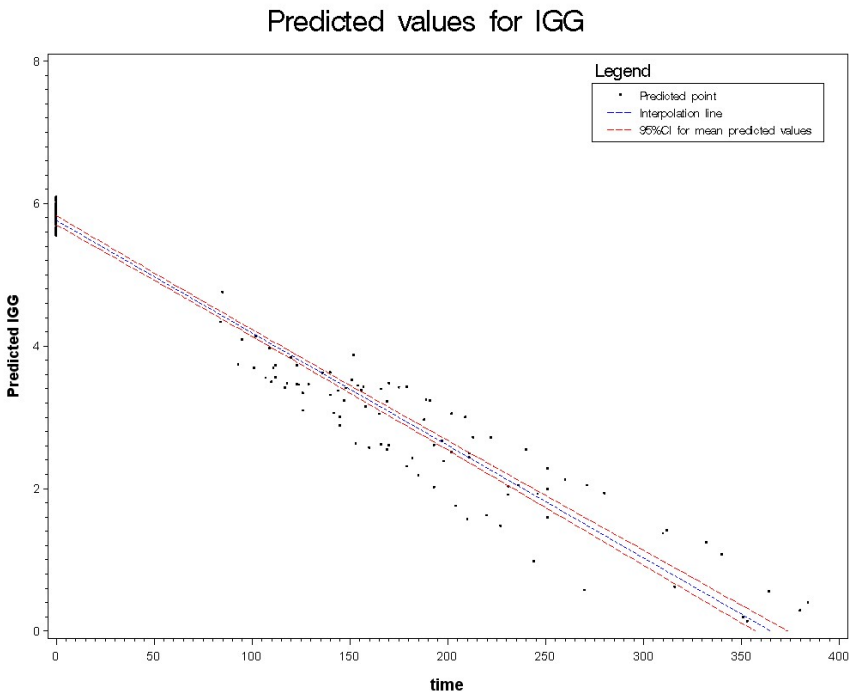


Figure 3. Results of the mixed effects model for Bordier-ELISA

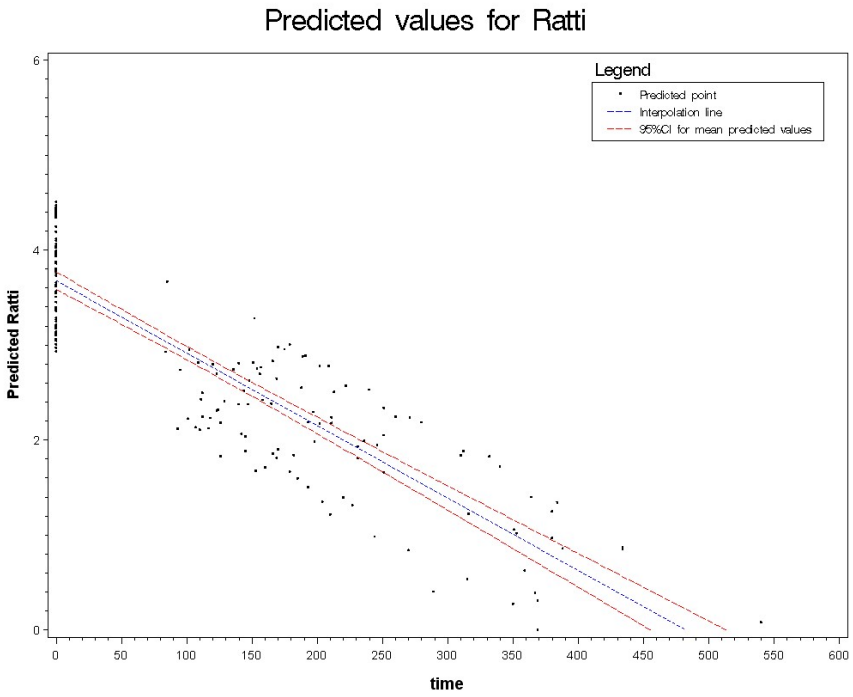


Figure 4. Results of the mixed effects model for NIE-LIPS

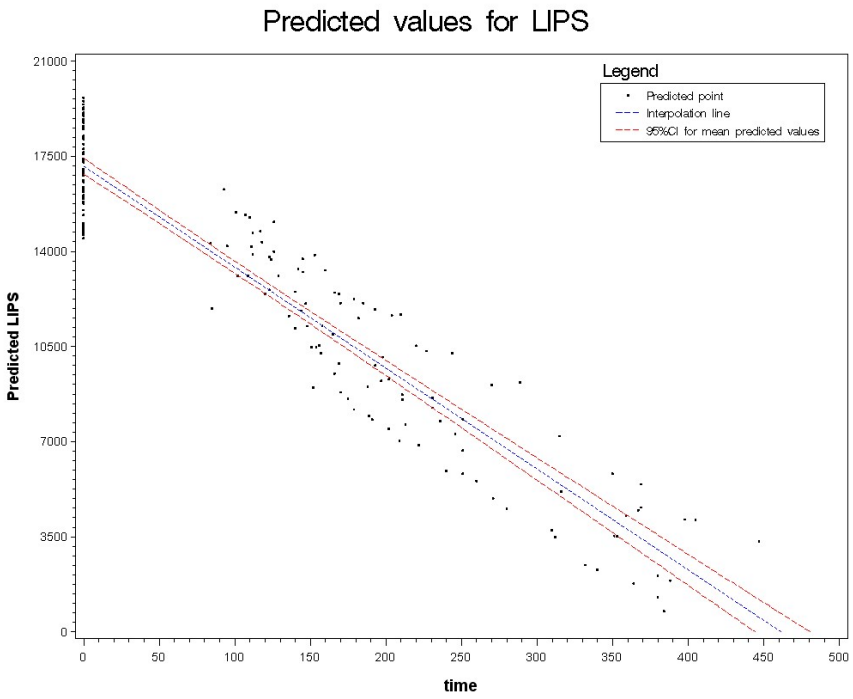
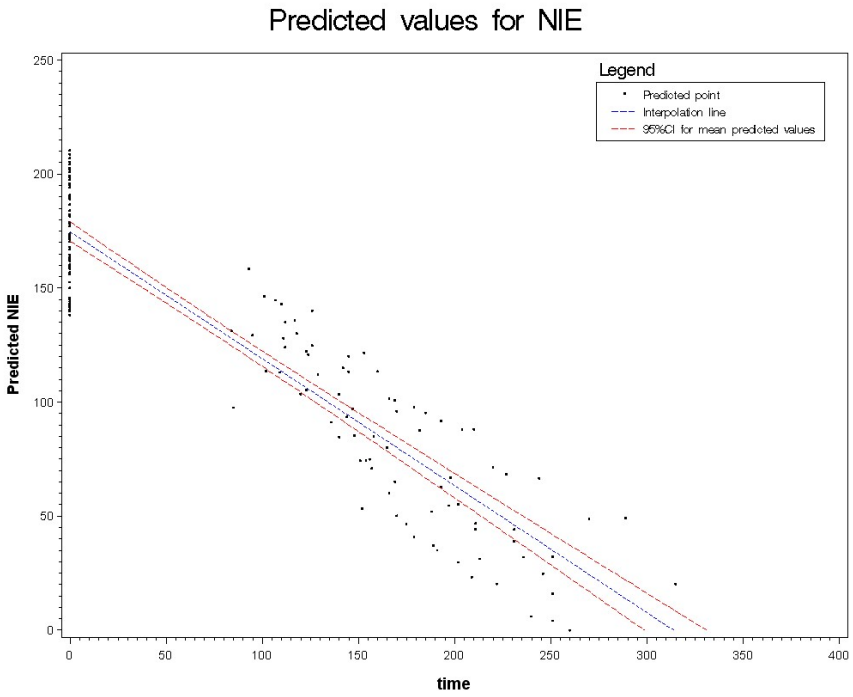


Figure 5. Results of the mixed effects model for NIE-ELISA



Interaction terms between age and time were not statistically significant, meaning that effect of time was not affected by age in the outcome variable.

Discussion

The results of this study indicate that serology tests for the diagnosis of strongyloidiasis tend to serorevert after effective treatment. All the tests evaluated demonstrated to be useful for monitoring, and the choice of a specific test is mainly influenced by diagnostic accuracy, costs and availability. It is worth of note that the setting in which this study was performed excluded the possibility of re-infection, that is always possible in endemic areas. Therefore, the following recommendations are primarily applicable to non-endemic areas. We suggest that serology, when affordable, should be routinely introduced in the diagnosis of strongyloidiasis, by virtue of its higher sensitivity, when compared with fecal methods. Serologic tests are the only available method to assess cure for patients with (false) negative fecal test results before treatment. Moreover, serology should be also performed in cases found positive in stool, in order to obtain a baseline result to be subsequently monitored at follow up. Negativization of fecal tests alone is not a sufficiently reliable marker of cure, due, again, to their sub-optimal sensitivity. It should also be considered that, while the excretion of larvae in stools stops within a few days after an effective treatment {Schar, 2014}, it takes several months to demonstrate negativization of serology. Therefore, patients should be monitored at 6 and 12 months after treatment, to be able to demonstrate decrease and/or negativization of the serologic results, and thus be safely considered cured. In areas where re-exposure can be excluded, a serological value failing to decrease should be cautiously interpreted as a treatment failure. In this case, the time-interval for evaluation after therapy is crucial, as our model shows that, especially for low values of OD/titer, it can be necessary to extend the follow up to more than 12 months. False positive results of serology might also be considered for those patients who do not show a response after one year, especially when the initial serology values were under a determined cutoff, as was showed by our previous study {Bisoffi, 2014}. The possible cross-reactivity with other parasitic infections was also investigated in the same study and appeared to be of limited importance. A combined diagnostic strategy (serology plus a suitable fecal method such as Baermann technique or Koga agar plate culture) is required at baseline evaluation, considering that a positive fecal result means 100% certainty of infection {Buonfrate, 2013}.

Study Limitations. Based on the operational case definition of cure, we obtained the denominator of "cured" patients on which we assessed the decline in titer of the different serologic tests. In the absence of a gold standard for cure, we cannot rule out that some patients might have been misclassified, i.e. considered cured when they were not, also considering that the eosinophil count can fluctuate. It is therefore possible that in some cases the lack of serologic response to cure could be due to misclassification. Moreover, the follow up samples were available at different time intervals after treatment, because of the retrospective design of the study. A three-month time could be a period of time too short to observe a decrease in the values of serology, therefore it cannot be excluded that a longer and more homogeneous period of observation would have demonstrated better performance of the tests in terms of percentage of seroreversion (as seen in table 2). However, the application of the mixed effects model permitted to have a prediction of the decrease over time, making it possible to demonstrate a tendency to seroreversion for all tests. Another limitation is related to the different treatment used (ivermectin or thiabendazole). Although the two drugs demonstrated a comparable efficacy {Mascarello,

2011 #30} we cannot exclude a difference in the rapidity of the response to treatment. However, the patients treated with thiabendazole were just a few (6 subjects), thus not allowing a separate analysis.

Conclusion and further research needs

Our results demonstrate that each of the serology tests considered can be used for monitoring patients who received a treatment for *S. stercoralis* infection. Serology, in combination with fecal-based methods, should be used as the preferred tool for the follow up. Validation of PCR techniques for the follow up might be a useful support for situations of uncertainty (such as patients with serology values that do not seem to decrease over time). Further investigations are necessary to extend these considerations to endemic areas, where re-infection might be an issue.

References

1. Biggs, B.A.; Caruana, S.; Mihrshahi, S.; Jolley, D.; Leydon, J.; Chea, L.; Nuon, S. Management of chronic strongyloidiasis in immigrants and refugees: is serologic testing useful? *The American journal of tropical medicine and hygiene* **2009**, *80*, 788-791.
2. Bisoffi, Z.; Buonfrate, D.; Angheben, A.; Boscolo, M.; Anselmi, M.; Marocco, S.; Monteiro, G.; Gobbo, M.; Bisoffi, G.; Gobbi, F. Randomized clinical trial on ivermectin versus thiabendazole for the treatment of strongyloidiasis. *PLoS neglected tropical diseases* **2011**, *5*, e1254, doi:10.1371/journal.pntd.0001254.
3. Bisoffi, Z.; Buonfrate, D.; Montresor, A.; Requena-Mendez, A.; Munoz, J.; Krolewiecki, A.J.; Gotuzzo, E.; Mena, M.A.; Chiodini, P.L.; Anselmi, M., et al. Strongyloides stercoralis: a plea for action. *PLoS neglected tropical diseases* **2013**, *7*, e2214, doi:10.1371/journal.pntd.0002214.
4. Bisoffi, Z.; Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Gobbo, M.; Bonafini, S.; Angheben, A., et al. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. *PLoS neglected tropical diseases* **2014**, *8*, e2640, doi:10.1371/journal.pntd.0002640.
5. Boscolo, M.; Gobbo, M.; Mantovani, W.; Degani, M.; Anselmi, M.; Monteiro, G.B.; Marocco, S.; Angheben, A.; Mistretta, M.; Santacatterina, M., et al. Evaluation of an indirect immunofluorescence assay for strongyloidiasis as a tool for diagnosis and follow-up. *Clinical and vaccine immunology : CVI* **2007**, *14*, 129-133, doi:10.1128/cvi.00278-06.
6. Buonfrate, D.; Requena-Mendez, A.; Angheben, A.; Munoz, J.; Gobbi, F.; Van Den Ende, J.; Bisoffi, Z. Severe strongyloidiasis: a systematic review of case reports. *BMC infectious diseases* **2013**, *13*, 78, doi:10.1186/1471-2334-13-78.
7. Finucane, M.M.; Samet, J.H.; Horton, N.J. Translational methods in biostatistics: linear mixed effect regression models of alcohol consumption and HIV disease progression over time. *Epidemiologic perspectives & innovations : EP+I* **2007**, *4*, 8, doi:10.1186/1742-5573-4-8.
8. Greaves, D.; Coggle, S.; Pollard, C.; Aliyu, S.H.; Moore, E.M. Strongyloides stercoralis infection. *BMJ (Clinical research ed.)* **2013**, *347*, f4610, doi:10.1136/bmj.f4610.
9. Hasegawa, H.; Hayashida, S.; Ikeda, Y.; Sato, H. Hyper-variable regions in 18S rDNA of Strongyloides spp. as markers for species-specific diagnosis. *Parasitology research* **2009**, *104*, 869-874, doi:10.1007/s00436-008-1269-9.
10. Karunajeewa, H.; Kelly, H.; Leslie, D.; Leydon, J.; Saykao, P.; Biggs, B.A. Parasite-specific IgG response and peripheral blood eosinophil count following albendazole treatment for presumed chronic strongyloidiasis. *Journal of travel medicine* **2006**, *13*, 84-91, doi:10.1111/j.1708-8305.2006.00004.x.
11. Knopp, S.; Salim, N.; Schindler, T.; Karagiannis Voules, D.A.; Rothen, J.; Lweno, O.; Mohammed, A.S.; Singo, R.; Benninghoff, M.; Nsojo, A.A., et al. Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann, and PCR methods for the detection of light-intensity hookworm and Strongyloides stercoralis infections in Tanzania. *The American journal of tropical medicine and hygiene* **2014**, *90*, 535-545, doi:10.4269/ajtmh.13-0268.
12. Kobayashi, J.; Sato, Y.; Toma, H.; Takara, M.; Shiroma, Y. Application of enzyme immunoassay for postchemotherapy evaluation of human strongyloidiasis. *Diagnostic microbiology and infectious disease* **1994**, *18*, 19-23.

13. Krolewiecki, A.J.; Ramanathan, R.; Fink, V.; McAuliffe, I.; Cajal, S.P.; Won, K.; Juarez, M.; Di Paolo, A.; Tapia, L.; Acosta, N., et al. Improved diagnosis of Strongyloides stercoralis using recombinant antigen-based serologies in a community-wide study in northern Argentina. *Clinical and vaccine immunology : CVI* **2010**, *17*, 1624-1630, doi:10.1128/cvi.00259-10.
14. Lindo, J.F.; Atkins, N.S.; Lee, M.G.; Robinson, R.D.; Bundy, D.A. Parasite-specific serum IgG following successful treatment of endemic strongyloidiasis using ivermectin. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1996**, *90*, 702-703.
15. Loutfy, M.R.; Wilson, M.; Keystone, J.S.; Kain, K.C. Serology and eosinophil count in the diagnosis and management of strongyloidiasis in a non-endemic area. *The American journal of tropical medicine and hygiene* **2002**, *66*, 749-752.
16. Page, W.A.; Dempsey, K.; McCarthy, J.S. Utility of serological follow-up of chronic strongyloidiasis after anthelminthic chemotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2006**, *100*, 1056-1062, doi:10.1016/j.trstmh.2005.12.006.
17. Requena-Mendez, A.; Chiodini, P.; Bisoffi, Z.; Buonfrate, D.; Gotuzzo, E.; Munoz, J. The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS neglected tropical diseases* **2013**, *7*, e2002, doi:10.1371/journal.pntd.0002002.
18. Salvador, F.; Sulleiro, E.; Sanchez-Montalva, A.; Saugar, J.M.; Rodriguez, E.; Pahissa, A.; Molina, I. Usefulness of Strongyloides stercoralis Serology in the Management of Patients with Eosinophilia. *The American journal of tropical medicine and hygiene* **2014**, 10.4269/ajtmh.13-0678, doi:10.4269/ajtmh.13-0678.
19. Schar, F.; Hattendorf, J.; Khieu, V.; Muth, S.; Char, M.C.; Marti, H.P.; Odermatt, P. Strongyloides stercoralis larvae excretion patterns before and after treatment. *Parasitology* **2014**, *141*, 892-897, doi:10.1017/s0031182013002345.
20. Schar, F.; Odermatt, P.; Khieu, V.; Panning, M.; Duong, S.; Muth, S.; Marti, H.; Kramme, S. Evaluation of real-time PCR for Strongyloides stercoralis and hookworm as diagnostic tool in asymptomatic schoolchildren in Cambodia. *Acta tropica* **2013**, *126*, 89-92, doi:10.1016/j.actatropica.2012.12.012.
21. Segarra-Newnham, M. Manifestations, diagnosis, and treatment of Strongyloides stercoralis infection. *The Annals of pharmacotherapy* **2007**, *41*, 1992-2001, doi:10.1345/aph.1K302.
22. Symanski, E.; Chan, W.; Chang, C.C. Mixed-effects models for the evaluation of long-term trends in exposure levels with an example from the nickel industry. *The Annals of occupational hygiene* **2001**, *45*, 71-81.
23. Verweij, J.J.; Canales, M.; Polman, K.; Ziem, J.; Brienen, E.A.; Polderman, A.M.; van Lieshout, L. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2009**, *103*, 342-346, doi:10.1016/j.trstmh.2008.12.001.

Chapter 5 Strong Treat 1 to 4 – Randomized phase 3 Clinical Trial of Multiple versus Single Dose Ivermectin for *Strongyloides stercoralis* infection

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Abstract

Background. *Strongyloides stercoralis* infection is a neglected condition that places immune compromised people at risk of hyperinfection and death. Ivermectin is the drug of choice, but there is no definitive evidence on the optimal dose. This trial aimed to assess whether multiple doses of ivermectin were superior to a single dose for the treatment of strongyloidiasis. Methods. A multicentre, open-label, randomized phase 3 controlled trial. Participants were enrolled in three countries (Italy, Spain and the United Kingdom). Patients diagnosed with *S. stercoralis* infection were eligible. Consented patients were randomly assigned (1:1) to receive either one dose of ivermectin 200 mcg/kg or 4 doses (given on days 1, 2, 15 and 16). Primary end point was the proportion of participants with clearance of *S. stercoralis* infection at 12 months. Findings. Three hundred and nine participants recruited between March 2013 and May 2017 were randomly assigned to one dose (n=155) or 4 doses (n=154) of ivermectin. The response to treatment at 12 months was 102/118 (86.44%,95% CI 79.11 to 91.48) for the single dose arm and 96/113 (84.96%,95% CI 77.22 to 90.39) for the 4 doses arm, with no significant difference (p-value 0.7471). All adverse events were of mild intensity and were more frequent in the multiple doses arm. The trial was terminated early for futility. Interpretation. Multiple doses of ivermectin did not demonstrate higher efficacy and had worse tolerability than single dose. A single dose should therefore be preferred for the treatment of uncomplicated strongyloidiasis.

Introduction

Strongyloides stercoralis is a soil-transmitted helminth (STH) with a wide distribution, primarily in tropical and subtropical regions {Nutman, 2017}. Previous estimates of prevalence (30-100 million cases) have been questioned recently, and the real prevalence is probably much higher {Bisoffi, 2013; Krolewiecki, 2013}. In contrast to other STH, *S. stercoralis* larvae, generated inside the bowel by parthenogenetic females, can reinfect the host ("autoinfection cycle"), leading to chronic infection. Acute infection is rarely reported in travellers, and the index of suspicion is usually low as clinical manifestations (mostly fever, cough, urticaria) are also commonly observed in other infections. Hence, misdiagnosis is possible. Most chronically-infected individuals are asymptomatic or present with non-specific symptoms affecting mostly the gastrointestinal tract, lungs and skin {Nutman, 2017}. However, strongyloidiasis can turn into a disseminated, life-threatening disease in cases of immunosuppression due to underlying conditions and/or medical treatment {Nutman, 2017; Keiser, 2004}. There is no internationally agreed gold standard for laboratory diagnosis. Stool microscopy has very low sensitivity, due to the irregular and often low larval output in chronic, non disseminated infection {Siddiqui, 2001}. The Baermann technique and Koga agar plate culture (APC) are more sensitive, but still miss a proportion of infections {Siddiqui, 2001}. Hence, it has been argued that these methods are not totally reliable for monitoring treatment efficacy {Dreyer, 1996}. Nucleic acid amplification tests demonstrated similar sensitivity to Baermann and APC {Buonfrate, 2018}. Conversely, serology demonstrated high sensitivity {Buonfrate, 2015}. Although cross-reactivity with other helminth infections is possible, serological specificity is close to 100% above defined cut-off values {Bisoffi, 2014}. Serology is also suitable for post-treatment monitoring, and criteria to define response to treatment with this method have been assessed by diagnostic studies {Buonfrate, 2015; Biggs, 2009; Kobayashi, 1994}. A Cochrane meta-analysis supports ivermectin as the drug of choice, as it has better tolerability than thiabendazole, which showed similar efficacy, and superior efficacy to albendazole, which had comparable, good tolerability, for the treatment of strongyloidiasis {Henriquez-Camacho, 2016}. Regimens using multiple doses of ivermectin have been tested {Shikiya, 1992; Gann, 1994; Zaha, 2002; Suputtamongkol, 2011}. In particular, a second dose given two weeks later has been proposed, based on the duration of the autoinfection cycle {Zaha, 2002; Suputtamongkol, 2011}. Alternatively, 200 μg/kg on 2 consecutive days is recommended by some experts {Organization, 2008; Nutman, 2017}, although recent evidence seemed to contrast this suggestion {Suputtamongkol, 2011}. Overall, there is no conclusive evidence to support any of the multiple dose regimens {Henriquez-Camacho, 2016 }. There is even more uncertainty regarding the management of disseminated disease, which is often fatal despite treatment with ivermectin {Barrett 2013}.

This trial aimed to assess whether multiple doses ivermectin were superior to a single dose for the treatment of non-disseminated strongyloidiasis.

Methods

Study design and participants

This was a multicentre, open-label, randomized controlled phase 3 superiority trial. Recruiting sites were in Italy (IRCCS Sacro Cuore Don Calabria Hospital, Negrar, Verona; ASST Spedali Civili General Hospital, Brescia; Azienda Ospedaliero Universitaria Careggi, Florence; Anna Meyer Children's University Hospital, Florence), Spain (Hospital de Poniente, El Ejido, Almería; Barcelona Institute for Global Health, ISGlobal-CRESIB; Unitat de Medicina Tropical Vall d'Hebron-Drassanes, Barcelona), and the United Kingdom (University College London Hospitals NHS; Cambridge University Hospital NHS). The protocol was approved by the local competent Ethics Committees for all study sites and registered with ClinicalTrials.gov (ID:

NCT01570504). All individuals diagnosed with *S. stercoralis* infection (with any test in use at the sites; case definition for inclusion in the trial is reported in the inclusion criteria) were assessed for eligibility. All participants provided written informed consent before trial entry. The inclusion criteria were: male and female individuals older than 5 years and weighing > 15 kg; current residence in non-endemic area; either positive faecal tests for *S. stercoralis* and positive serology (at any titre) OR a positive serological test at high titre (as defined below in this paragraph), irrespective of the results of faecal tests. Exclusion criteria were: pregnancy or lactation; disease of the central nervous system; disseminated strongyloidiasis; known immunosuppression; treatment with ivermectin in the previous year; lack of consent. Reasons for exclusion of participants living in endemic areas and/or presenting immunosuppressant conditions were to exclude, respectively, possible re-infection and dissemination, that in both cases could influence the response to treatment, and entail a different approach in the latter case. For screening and evaluation of eligibility, any diagnostic test for *S. stercoralis* infection in use in each site (serology, parasitological examination, agar/charcoal stool culture, PCR) was considered valid. None of the sites used Baermann method. For the follow up, the same serological assay used for diagnosis had to be repeated at the 6 and 12 months visit; participants with positive faecal tests at baseline had to be tested also with either PCR or charcoal/agar stool culture.The serological assays used were: an in-house immunofluorescence test (IFAT){Boscolo, 2007} and two commercially-available ELISA tests (*Strongyloides ratti* ELISA by Bordier Affinity Products; IVD *Strongyloides* Serum Antibody Detection Microwell ELISA by IVD Research Inc). For routine use, positive results for IFAT are assessed as ≥1/20. The manufacturers of the two commercial tests report the following indications for the interpretation of the results: positive samples defined by absorbance greater than 0.2 OD units (IVD), and positive results assessed when the absorbance of the analyzed sample is higher than the absorbance of the weak positive control (provided in the kit, for Bordier ELISA). For study purposes, the results of the ELISA tests were reported as normalized optical density (signal to cut-off ratio). The cut-off values to define “high titre” were assessed as ≥160 for IFAT, ≥ 2 for IVD ELISA, ≥ 2.5 for Bordier ELISA {Bisoffi, 2014}. Each study site used (both for inclusion and follow up) the serological assay available for routine practice. PCR (according to the method developed by Verweij et al) {Verweij, 2009} was introduced in 2016 with an amendment to the study protocol, and was used (as an alternative to APC){Buonfrate, 2017 #4} for inclusion and follow up of the participants almost exclusively (except a few cases) at the IRCCS Sacro Cuore Don Calabria Hospital, Negrar, Verona.

Randomization and masking

Participants were randomly assigned to receive either ivermectin (Stromectol 3mg tablets, Merck Sharp & Dohme) 200 µg/kg (maximum dose 21 mg) as a single dose (arm A), or ivermectin 200 µg/kg on days 1, 2, 15 and 16 (arm B), with an allocation ratio 1:1. Randomization was performed centrally using a computer-generated, blinded allocation sequence. Randomization with permuted blocks was stratified by participating study sites and the assignment was displayed in the eCRF. Participants and clinicians were not masked to the intervention, while laboratory staff and the study statistician were. The trial conduct was overseen by the study steering committee.

Procedures

Participants took ivermectin orally on an empty stomach with water and fasted for two hours after drug intake. Drug administration on day 1 was performed under direct observation. Participants in the multiple-dose arm received the remaining tablets for (home) self-administration. All participants received a phone call on days 2 and 16 to collect information about possible adverse events and to remind participants in arm B to take the further doses of ivermectin. On day 17, full blood count (FBC) and alanine aminotransferase (ALT)

were checked, and the participant was invited to report any symptom which had occurred since the last time he/she was asked (either phone calls or day 1, in case the participant could not be contacted by phone). Further, adverse events reported during any unscheduled visit were registered in the electronic case report form (eCRF). Clinical and complete laboratory assessments, including FBC, *Strongyloides* serology, *Strongyloides* stool culture and/or PCR, were repeated at 6 (T1) and 12 months (T2) after treatment. Volume of blood withdrawn was 8 mL on day 17, while 13 mL were taken on both follow up visits.

Outcomes

The primary outcome was the proportion of participants with clearance of *S. stercoralis* infection at T2, defined by negative APC or PCR AND negative serology, or serology with a decrease in titre (defined as a decrease of two titres in IFAT; two-fold reduction of normalized OD in case of ELISA {Buonfrate, 2015}). Secondary outcomes included partial response to treatment at T2 (defined by negative stool tests and positive serology with decrease in titre, remaining over the cutoff defining cure), all-cause mortality during the 12 months of follow-up, adverse events (AE), proportion of participants with symptoms cleared or improved at T2; variation in ALT at day 17, and variation of eosinophil count at T2 compared with baseline.

Grading of AE used for study purpose was: 0=none; 1=mild (any symptoms possibly related to ivermectin, not necessitating medication); 2=moderate (any symptom resolved with medication, not requiring hospitalization); 3=severe (necessity of hospitalization); 4=near fatal (necessity of intensive care); 4=fatal. Relative tolerability of the two regimens was assessed on the basis of a possible association of the AE with ivermectin. This was based on the evaluation of the Investigator and on AE reported in literature (according to which we expected high tolerability, with mostly mild symptoms).

Statistical analysis

The sample size was calculated based on an expected 15% increase in efficacy with the multiple dose over the single dose regimen, which was assumed to have a 70% efficacy based on a previous trial {Mascarello, 2011 #30}. The study was set with a 90% statistical power, 5% alpha level, and a 2-sided conservative alternative hypothesis. This required a sample size of 161 participants in each study arm. Allowing for a possible loss to follow-up of 15% of participants and the possible inclusion of an additional 4% false positive cases (despite the high specificity of “high” titres of serology, it is still possible that some cases were misclassified as positive), the target sample size was 400 participants; 200 per study arm.

Statistical analysis was performed in the full analysis set (FAS); the primary endpoint analysis was also performed in the per protocol set (PPS). In FAS, participants were classified according to the treatment arm assigned by randomization. The PPS excluded from the FAS participants who deviated from the assigned treatment regimen. The treatment response at 6 months and the sustained response from 6 to 12 months were compared to check for possible biases in the results obtained from the FAS, due to losses to follow-up.

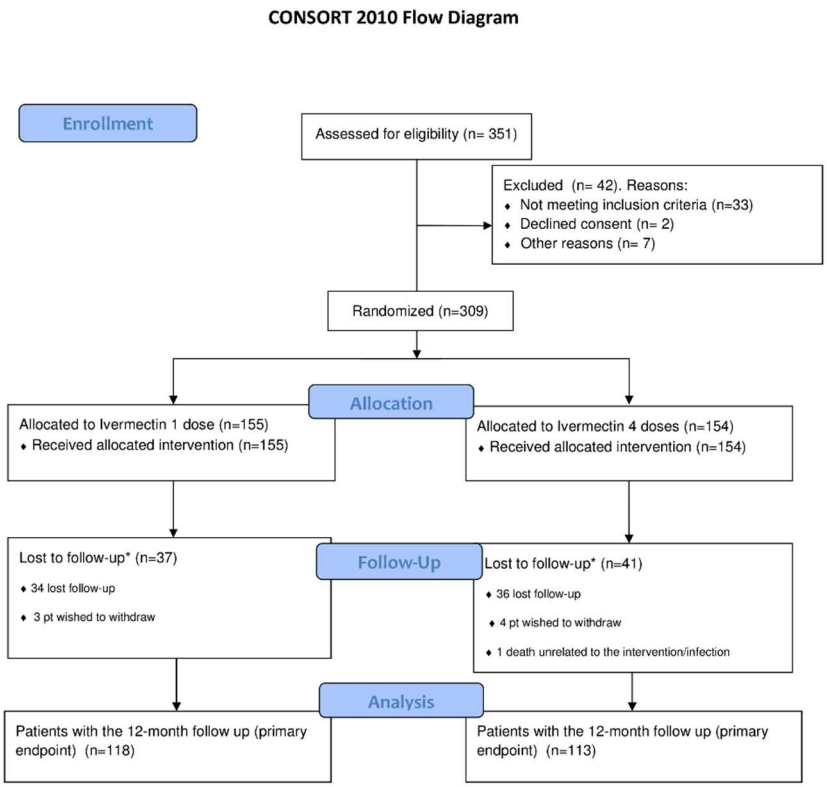
Demographic and clinical data were summarized using descriptive statistics. The significance level of statistical tests was fixed at 5%. Two samples unpaired medians were compared using Mann-Whitney (MW) or Kolmogorov-Smirnov test (KS) as appropriate, and the Dwass-Steel-Critchlow-Fligner (DSCF) method{Dwass, 1960; Steel, 1960; Critchlow, 1991} for multiple comparisons. Paired medians were compared using Wilcoxon Signed Rank test (WSR) and p-values (p) adjusted for multiple comparisons. The proportion of responders and 95% confidence intervals (CI) were summarized in a 2x2 contingency table. Chi-

square test (X^2), or Fisher test (F) if appropriate, was performed to compare treatment differences. Treatment differences within enrolling countries were also assessed and pooled differences calculated to estimate the contribution of each country to the overall estimation of treatment response. Clinical and demographical predictor variables were included in the full multivariate logistic regression, to model the probability of a participant responding to the treatment. Candidate models were compared using Akaike's information criterion, clinical and statistical relevance of candidate variables, and classification tables. Parameters were estimated using Firth's penalization {Heinze, 2002; Firth, 1993}. The study protocol included indications for an interim analysis in case of slow participant accrual. The analysis was performed using the sequential design approach with the O' Brien-Fleming spending functions {O'Brien, 1979} to evaluate whether based on trial interim results the null or alternative hypotheses fell within the rejection or acceptance regions. Data analysis was performed using Stata/SE14.

Results

The first participant was randomized in March 2013 and the last one in May 2017. The study was completed in May 2018. Of the 351 patients evaluated for eligibility, 309 were randomized. The participants' flow and reasons for exclusion are summarized in Figure 1.

Figure 1. Participants' flow



Details of the number of participants missing each time-point evaluation are reported in Figure S1. Globally, the number of missing participants was similar between the two arms at each time point.

Recruitment was stopped by the study steering committee before reaching the planned sample size, based on an interim analysis that showed that the probability of finding a significant difference favouring the 4 doses, in case the study reached the planned 400 participants, was well below 1%. The complete interim analysis, including the probability calculation, is reported in the Supplementary File 2. The baseline characteristics of the participants in the two arms did not differ significantly in terms of demographics, clinical presentation and lab values, as reported in Table 1.

Tables

Table 1. Participants' baseline symptoms and characteristics*

	Single dose **(n=155)**	**Multiple doses** **(n=154)**
Age, years	42 (34-60)	44 (36-65)
Female	63 (40.7%)	59(38.3%)
Weight, kg	71 (62-80)	71 (64-80)
Eosinophils/mcL	800 (500-1,250)	770 (450-1,200)
WBC/mcL	7,160 (5,900-8,620)	6,930 (5,950-8,370)
ELISA (nOD)	4.5(3-7.6)	4.1(3-6.6)
IFAT ≥160	46/54 (85%)	51/58 (88%)

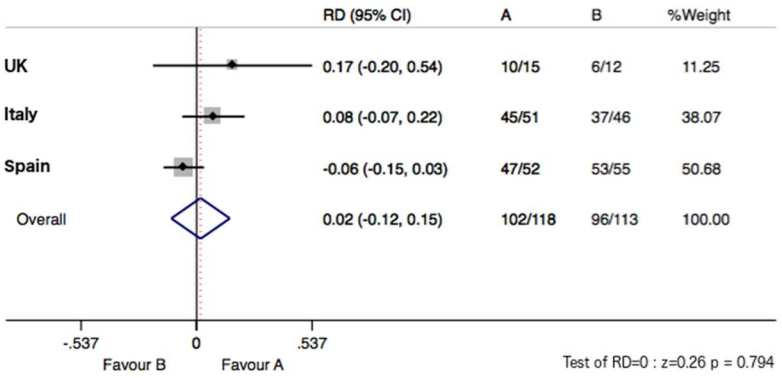
Country of enrolment		
Italy	66/155 (42.58%)	64/154(41.56%)
Spain	72/155(46.45%)	73/154(47.40%)
UK	17/155(10.97%)	17/154(10.97%)
Continent where the infection was presumably acquired[§]		
Europe	28 (19.1%)	36(25%)
Asia	13(8.8%)	5(3.5%)
Latin America	59(40.1%)	52(36.1%)
Africa	47(32%)	51(35.4%)
Pruritus	56 (18.1%)	49 (15.9%)
Skin rash	31 (10%)	23 (7.4%)
Abdominal pain	41 (13.3%)	39 (12.6%)
Respiratory symptoms	18 (5.8%)	21 (6.8%)

*data are in median(IQR) or frequencies(%); [§]18 missing data

Countries of presumed acquisition of the infection are shown in Supplementary File. One hundred and forty-six participants were enrolled on the basis of positive faecal tests, as follows: microscopy (67 participants), stool culture (111), PCR (17), a combination of faecal tests (96). At 6-months, 259 participants were followed up and 231 completed the 12-month follow up (FAS). All participants received their assigned treatment, but one in the single dose arm took a further 4 doses before the 6-month follow-up visit (excluded from the PPS). In the FAS, the 12 month cure rate was 86.44% (95% CI 79.11 to 91.48, 102 participants cured of 118) for arm A and 84.96% (95% CI 77.22 to 90.39, 96/113) for arm B, with no significant difference: risk difference 1.48% (95%CI -7.55 to 10.52), p-value 0.75. In the PPS, the 12 month cure rate was 87.18% (95% CI 79.92 to 92.07, 102/117) for arm A and 84.96% (95% CI 77.22 to 90.39, 96/113) for arm B, with no statistical difference in

efficacy between the two regimes: risk difference of 2.22% (95%CI -6.73 to 11.12), p-value 0.62. Similarly, an exploratory analysis of the efficacy at 6 months found a response of 107/128 (83.59%,95% CI 76.22 to 89.01) for arm A and 108/131 (82.44%,95% CI 75.03 to 88.01) for arm B, with no statistical difference in efficacy between the two treatment regimens (proportions difference of 1.12%, 95%CI -8 – 10.29, p-value 0.81). The sustained response from 6 (data available for 215 participants) to 12 months was 96.02% (169/176, with 81/84 in arm A and 88/92 in arm B), and the relative risk associated with non-sustained treatment response calculated as 0.065 (95% CI 0.03 to 0.14). We further investigated the difference in efficacy of the regimens by exploring the results within and between countries of enrolment. Figure 2 shows the weight of each country to the overall difference of 2% between group A and B. From the heterogeneity statistics, the variation among country results can be attributed to random variation.

Figure 2: Risk difference between regimens in primary outcome, overall and by country of enrolment

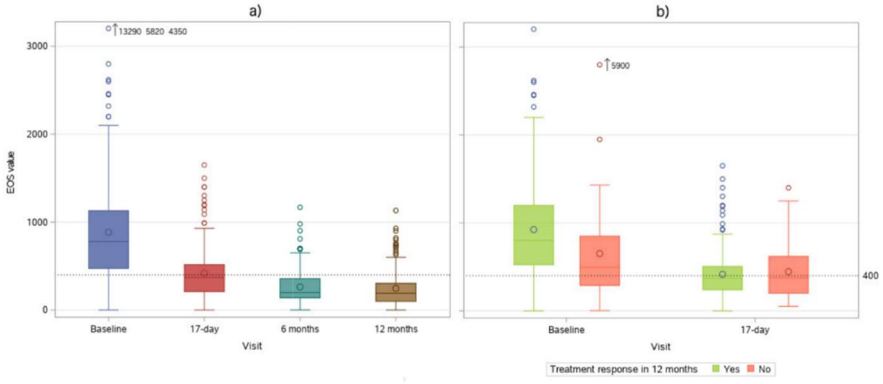


Partial response to treatment (among cases classified as failures according to the primary outcome) at T2 in arm A was 11/16 (68.75%, 95%CI 44.40 – 85.84) and 13/17 in arm B (76.47%, 95%CI 52.74 – 90.44), with proportions difference of 7.72% (95%CI -22.65 – 38.09). At T2, clearance or improvement of the symptoms reported at baseline was observed in 83.08% of participants in group A and in 82.09% of participants in group B. In both study arms, the proportion of participants reporting clearance or improvement of symptoms was higher in the subgroup of participants who achieved cure at T2. The efficacy analysis was also performed stratifying the study population into two main subgroups, according to the diagnostic criterion of enrolment: participants with positive faecal tests (subgroup F+) and participants with negative faecal tests but high serology (subgroup F-). The treatment efficacy (assessed with the same criteria used for the primary end-point) was 34/36 (94.44%,95% CI 81.86 to 98.46) for arm A and 43/49 (87.76%,95% CI 75.76 to 94.27) for arm B in the subgroup F+, and 57/72 (79.17%,95% CI 68.43 to 86.95) in arm A and 45/56 (80.36%,95% CI 68.16 to 88.66) in arm B in subgroup F-. In subgroup F+, when efficacy was assessed only on the basis of the results of faecal tests, the cure rate was 36/36 (100%,95% CI 90.36 to 100) in arm A and 48/49 (97.96, 95% CI 89.31 to 99.64) in arm B. In all sub-analyses, there was no clinical difference between the two regimens.

Two hundred and eighty-nine participants attended the 17-day visit, and all had white blood cell count within the normal range of values. Only 11 participants had ALT values > 55 U/L (median 71 U/L, IQR 57-81), but none of these cases was considered clinically relevant. The median eosinophil count showed a statistically significant decrease (WSR adjusted p <0.001) from baseline (789; IQR 485-1,204) to 17 day visit (371; IQR 220-540), as shown in figure 3a. Median eosinophil values were also significantly different (WSR adjusted p ≤ 0.001) from the 17 day visit to 6 months (200; IQR 140-358) follow-up, but not from 6 to 12 months (196;

IQR 100-310) follow-up (WSR adjusted p= 0.06). Moreover, median eosinophil values were significantly different by cure status at baseline (KS p≤ 0.001) but not at day 17 visit (KS p= 0.36), as shown in Figure 3b: the decrease in the median eosinophil values was accentuated in participants who achieved cure at T2.

Figure 3a) Median eosinophil values at the different time points in all recruited participants. 3b) Median eosinophil values from baseline to 17 days visit by participant's cure status at T2.



A multivariate logistic regression model was fitted to explore underlying differences between responders and non-responders to treatment at T2 (Table 2).

Table 2. Logistic regressions exploring underlying differences between responders and non-responders to treatment at T2 (n=231)

Variable	Variable profile[†]		Univariate assessment		Multivariate Model	
			Odds ratio (95% CI)	**p-value**	**Odds ratio (95% CI)**	**p-value**
Age	1 unit change		0.98 (0.96-0.99)	0.015	0.97 (0.95-0.99)	0.010
ELISA baseline (54 missing data)	1 unit change		1.38 (1.09-1.75)	0.007	-	-
	n (% R)					
Sex	Male	Female				
	143 (84)	88 (89)	1.46 (.67-3.20)	0.35	-	-
	Yes	No				
Has visited endemic country?	90 (81)	141 (89)	1.81 (0.87-3.78)	0.11	-	-
Skin rash	42 (76)	189 (88)	2.29 (1.002-5.23)	0.049	2.97 (1.20-7.33)	0.02
Abdominal pain	62 (90)	169 (84)	0.60 (0.24-1.49)	0.27	-	-
Pruritus	82 (79)	149 (89)	2.16 (1.03-4.52)	0.04	-	-
Respiratory symptoms	28 (75)	203 (87)	2.34 (0.91-5.98)	0.08	-	-

Eosinophil count baseline	<= 400	> 400				
	50 (74)	181 (89)	2.84 (1.30-6.18)	0.009	4.12 (1.74-9.74)	0.001
IFAT baseline	>=160	>160				
(15 missing data)	70 (84)	10 (90)	1.22 (0.18-8.31)	0.84	-	-
Likely region of *S. stercoralis* infection‡:						
(8 missing data)						
Europe						
	51 (86)		-	-	-	-
Asia						
	15(60)		0.25 (0.07-.88)	0.03		
America						
	80(89)		1.27 (0.44-3.55)	0.65		
Africa						
	77(91)		1.58 (0.53-4.75)	0.41		

†reference values are on the left column ‡4 from Central and Caribbean America, 4 from South Africa

A better response to treatment was associated with younger age (OR 0.97, p-value 0.01, 95% CI 0.95 to 0.99); eosinophils >400/mcL at baseline (OR 4.51, p-value <0.001, 95% CI 1.87 to 10.87); absence of skin rash (OR 2.5, p-value 0.04, 95% CI 1.02 to 5.90).

The adverse events (AE) reported were all of mild intensity, and more frequent in group B (Table 3).

Table 3. Complete description of mild adverse events

Adverse event	**Timepoint of reported AE**		**Group A**			**Group B**			**Ratio B/A**	**Grade**
			Number of participants	Day of occurrence of AE*		**Number of participants**	Day of occurrence of AE*			
Abdominal pain	Routine visit		2	10(6-13)		2	16(16-16)		1	1
Pruritus	Routine visit		2	16(16-16)		1	16(16-16)		0.5	1
Vomiting	Phone call		1	16(16-16)		1	14(8-15)		1	1
Drowsiness	Phone call and Routine visit		9	2(1-2)		16	12(1-15)		1.8	1
Fatigue	Phone call and Routine visit		6	3(1-16)		6	15(9-27)		1	1/2*
Headache	Phone call and Routine visit		12	1(1-4)		14	7(1-15)		1.2	1/2*
Hypotension	Phone call and Routine visit		2	3(2-4)		2	3(2-9)		1	1
Nausea	Phone call and Routine visit		7	2(1-9)		12	15(5-15)		1.7	1
Total			41			54			1.3	

*day on which the participant reported the symptoms, that did not necessarily occurr on the day of the report

One participant enrolled in arm B died 34 days after the last dose of ivermectin. The participant, aged 86, had underlying chronic conditions that were closely associated with the cause of death, that was judged unrelated to ivermectin treatment.

Discussion

This RCT showed that a 4-dose ivermectin regimen offers no advantage in terms of efficacy over single dose treatment and is less well tolerated. These findings are consistent with the results of two previous smaller trials, that compared one versus two doses ivermectin, given either on two consecutive days {Gann, 1994 #17} or two weeks apart {Suputtamongkol, 2011}. In both studies, efficacy was assessed with faecal tests, and was close to 100%, as was with our study when the same criterion of cure was applied. In light of these results and on a previous trial that found higher efficacy of multiple doses (on days 1,2,15, and 16) compared to a single dose in a small cohort of patients with HIV infection {Torres}, we chose to test a single versus a 4-dose regimen, in order to provide conclusive evidence as to whether dosage is an issue in the treatment of uncomplicated strongyloidiasis.

Diagnosing strongyloidiasis and measuring treatment efficacy is challenging. Negative faecal tests cannot reliably rule out *Strongyloides* infection, so the efficacy of an intervention tends to be overestimated when assessed by these methods only {Dreyer, 1996}. To our knowledge, only one previous trial of treatment for strongyloidiasis used serology to assess the efficacy of the intervention {Mascarello, 2011}. Compared to that trial, in this study the possible inclusion of false positive cases was limited by the introduction of serological cut-off values for inclusion of participants who had negative stools. Nevertheless, it is still possible that some participants were erroneously classified as infected, and this may have partially contributed to underestimation of treatment efficacy.

Although serology tends to decrease slowly over time in cured patients {Buonfrate, 2015}, the primary outcome showed similar responses at T1 and T2, hence a 6 - month follow- up might be sufficient. The eosinophil count showed a rapid decrease, that was significantly accentuated in cured participants. However, a decrease, albeit smaller, was also observed in participants who did not achieve cure, and might suggest a partial response to treatment. Thus, it is not possible to predict cure on the basis of a reduction in eosinophil count shortly after treatment.

In this study, a single dose was better tolerated than multiple doses, another factor to favour the single dose. Overall, including the 4-dose arm, AE were few and of mild intensity, confirming the excellent tolerability of ivermectin. A raised eosinophil count and younger age were associated with a better outcome: both parameters might indicate that a robust immune system is required for a good response to treatment. The strengths of our study include the use of sensitive diagnostic methods to assess cure and the long follow up period compared to previous trials. Furthermore, the study was performed in a non-endemic area, excluding the possibility of re-infection as a confounder. Despite this, the results are also relevant for endemic countries. The ability to use a single dose of a well tolerated, safe drug argues for provision of easier access to treatment where the infection is concentrated. Single dose treatment is more convenient for patients, with an option for directly observed administration. Moreover, community treatment of the other soil-transmitted helminths (namely, hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*) is currently based on a single dose of albendazole {World Health Organization, 2017}. A co-administration with ivermectin would enhance the effectiveness of community control programs by targeting *S. stercoralis*, too. The main limitation is that the sample size was smaller than originally planned. While this did not affect the results of the primary outcome (as the probability analysis showed), it has

possibly limited the interpretation of some sub-analyses. Another limitation is that we cannot assure adherence to the dose schedule for participants in the 4-dose arm, although telephone contact may have increased compliance. It must also be stressed that in real clinical practice no directly observed treatment (DOT) would be feasible. Finally, we could not assess the HTLV-1 status of the participants (this infection has been associated to a reduced response to treatment {Nutman, 2017 }), but we believe that the randomization permitted to balance the possible presence of individuals with HTLV-1 between the study arms. Moreover, while the overall efficacy of the intervention might have been partly reduced in case of inclusion of participants with HTLV-1 infection, the influence on the primary outcome is presumably irrelevant, as the prevalence of HTLV-1 infection is very low {Verdonck, 2007}. In conclusion, single dose ivermectin should be the preferred regimen for the treatment of strongyloidiasis in the immunocompetent patient.

References

1. Barrett, J.; Broderick, C.; Soulsby, H.; Wade, P.; Newsholme, W. Subcutaneous ivermectin use in the treatment of severe Strongyloides stercoralis infection: two case reports and a discussion of the literature.
2. Caumes E Fau - Keystone, J.S.; Keystone, J.S. Acute strongyloidiasis: a rarity. Chronic strongyloidiasis: a time bomb!
3. Choksi, T.T.; Madison, G.; Dar, T.; Asif, M.; Fleming, K.; Clarke, L.; Danilewitz, M.; Hennawy, R. Multiorgan Dysfunction Syndrome from Strongyloides stercoralis Hyperinfection in a Patient with Human T-Cell Lymphotropic Virus-1 Coinfection After Initiation of Ivermectin Treatment.
4. Torres, J.R.; Isturiz R Fau - Murillo, J.; Murillo J Fau - Guzman, M.; Guzman M Fau - Contreras, R.; Contreras, R. Efficacy of ivermectin in the treatment of strongyloidiasis complicating AIDS.
5. Dwass, M. Some k-Sample Rank-Order Tests. In *Contributions to Probability and Statistics*, Press, S.U., Ed. Stanford, CA, 1960.
6. Steel, R. A Rank Sum Test for Comparing All Pairs of Treatments. *Technometrics* **1960**, *2*, 197-207.
7. O'Brien, P.; Fleming, T. A Multiple Testing Procedure for Clinical Trials. *Biometrics* **1979**, *35*, 549–556.
8. Critchlow, D.; Fligner, M. On Distribution-Free Multiple Comparisons in the One-Way Analysis of Variance. *Communications in Statistics—Theory and Methods* **1991**, *20*, 127-139.
9. Shikiya, K.; Kinjo, N.; Uehara, T.; Uechi, H.; Ohshiro, J.; Arakaki, T.; Kinjo, F.; Saito, A.; Iju, M.; Kobari, K. Efficacy of ivermectin against Strongyloides stercoralis in humans. *Internal medicine (Tokyo, Japan)* **1992**, *31*, 310-312.
10. Firth, D. Bias Reduction of Maximum Likelihood Estimates. *Biometrika* **1993**, *80*, 27-38.
11. Gann, P.H.; Neva, F.A.; Gam, A.A. A randomized trial of single- and two-dose ivermectin versus thiabendazole for treatment of strongyloidiasis. *The Journal of infectious diseases* **1994**, *169*, 1076-1079.
12. Kobayashi, J.; Sato, Y.; Toma, H.; Takara, M.; Shiroma, Y. Application of enzyme immunoassay for postchemotherapy evaluation of human strongyloidiasis. *Diagnostic microbiology and infectious disease* **1994**, *18*, 19-23.
13. Dreyer, G.; Fernandes-Silva, E.; Alves, S.; Rocha, A.; Albuquerque, R.; Addiss, D. Patterns of detection of Strongyloides stercoralis in stool specimens: implications for diagnosis and clinical trials. *Journal of clinical microbiology* **1996**, *34*, 2569-2571.
14. Siddiqui, A.A.; Berk, S.L. Diagnosis of Strongyloides stercoralis infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2001**, *33*, 1040-1047, doi:10.1086/322707.
15. Heinze, G.; Schemper, M. A Solution to the Problem of Separation in Logistic Regression. *Statistics in Medicine* **2002**, *21*, 2409-2419.
16. Zaha, O.; Hirata, T.; Kinjo, F.; Saito, A.; Fukuhara, H. Efficacy of ivermectin for chronic strongyloidiasis: two single doses given 2 weeks apart. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy* **2002**, *8*, 94-98, doi:10.1007/s101560200013.
17. Keiser, P.B.; Nutman, T.B. Strongyloides stercoralis in the Immunocompromised Population. *Clinical microbiology reviews* **2004**, *17*, 208-217.
18. Boscolo, M.; Gobbo, M.; Mantovani, W.; Degani, M.; Anselmi, M.; Monteiro, G.B.; Marocco, S.; Angheben, A.; Mistretta, M.; Santacatterina, M., et al. Evaluation of an indirect immunofluorescence assay for

strongyloidiasis as a tool for diagnosis and follow-up. *Clinical and vaccine immunology : CVI* **2007**, *14*, 129-133, doi:10.1128/cvi.00278-06.
19. van Doorn, H.R.; Koelewijn, R.; Hofwegen, H.; Gilis, H.; Wetsteyn, J.C.; Wismans, P.J.; Sarfati, C.; Vervoort, T.; van Gool, T. Use of enzyme-linked immunosorbent assay and dipstick assay for detection of Strongyloides stercoralis infection in humans. *Journal of clinical microbiology* **2007**, *45*, 438-442, doi:10.1128/jcm.01735-06.
20. Verdonck, K.; Gonzalez, E.; Van Dooren, S.; Vandamme, A.M.; Vanham, G.; Gotuzzo, E. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *The Lancet. Infectious diseases* **2007**, *7*, 266-281, doi:10.1016/s1473-3099(07)70081-6.
21. Organization, W.H. *WHO Model Formulary*; Marc C. Stuart, Maria Kouimtzi, Hill., S.R., Eds.; Switzerland, 2008.
22. Biggs, B.A.; Caruana, S.; Mihrshahi, S.; Jolley, D.; Leydon, J.; Chea, L.; Nuon, S. Management of chronic strongyloidiasis in immigrants and refugees: is serologic testing useful? *The American journal of tropical medicine and hygiene* **2009**, *80*, 788-791.
23. Verweij, J.J.; Canales, M.; Polman, K.; Ziem, J.; Brienen, E.A.; Polderman, A.M.; van Lieshout, L. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2009**, *103*, 342-346, doi:10.1016/j.trstmh.2008.12.001.
24. Bon, B.; Houze, S.; Talabani, H.; Magne, D.; Belkadi, G.; Develoux, M.; Senghor, Y.; Chandenier, J.; Ancelle, T.; Hennequin, C. Evaluation of a rapid enzyme-linked immunosorbent assay for diagnosis of strongyloidiasis. *Journal of clinical microbiology* **2010**, *48*, 1716-1719, doi:10.1128/jcm.02364-09.
25. Bisoffi, Z.; Buonfrate, D.; Angheben, A.; Boscolo, M.; Anselmi, M.; Marocco, S.; Monteiro, G.; Gobbo, M.; Bisoffi, G.; Gobbi, F. Randomized clinical trial on ivermectin versus thiabendazole for the treatment of strongyloidiasis. *PLoS neglected tropical diseases* **2011**, *5*, e1254, doi:10.1371/journal.pntd.0001254.
26. Suputtamongkol, Y.; Premasathian, N.; Bhumimuang, K.; Waywa, D.; Nilganuwong, S.; Karuphong, E.; Anekthananon, T.; Wanachiwanawin, D.; Silpasakorn, S. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. *PLoS neglected tropical diseases* **2011**, *5*, e1044, doi:10.1371/journal.pntd.0001044.
27. Bisoffi, Z.; Buonfrate, D.; Montresor, A.; Requena-Mendez, A.; Munoz, J.; Krolewiecki, A.J.; Gotuzzo, E.; Mena, M.A.; Chiodini, P.L.; Anselmi, M., et al. Strongyloides stercoralis: a plea for action. *PLoS neglected tropical diseases* **2013**, *7*, e2214, doi:10.1371/journal.pntd.0002214.
28. Krolewiecki, A.J.; Lammie, P.; Jacobson, J.; Gabrielli, A.F.; Levecke, B.; Socias, E.; Arias, L.M.; Sosa, N.; Abraham, D.; Cimino, R., et al. A public health response against Strongyloides stercoralis: time to look at soil-transmitted helminthiasis in full. *PLoS neglected tropical diseases* **2013**, *7*, e2165, doi:10.1371/journal.pntd.0002165.
29. Bisoffi, Z.; Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Gobbo, M.; Bonafini, S.; Angheben, A., et al. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. *PLoS neglected tropical diseases* **2014**, *8*, e2640, doi:10.1371/journal.pntd.0002640.
30. Buonfrate, D.; Formenti, F.; Perandin, F.; Bisoffi, Z. Novel approaches to the diagnosis of Strongyloides stercoralis infection. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2015**, *21*, 543-552, doi:10.1016/j.cmi.2015.04.001.
31. Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Degani, M.; Tais, S.; Angheben, A.; Requena-Mendez, A., et al. Accuracy of five serologic tests for the follow up of Strongyloides stercoralis infection. *PLoS neglected tropical diseases* **2015**, *9*, e0003491, doi:10.1371/journal.pntd.0003491.
32. Buonfrate, D.; Baldissera, M.; Abrescia, F.; Bassetti, M.; Caramaschi, G.; Giobbia, M.; Mascarello, M.; Rodari, P.; Scattolo, N.; Napoletano, G., et al. Epidemiology of Strongyloides stercoralis in northern Italy: results of a multicentre case-control study, February 2013 to July 2014. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* **2016**, *21*, doi:10.2807/1560-7917.es.2016.21.31.30310.
33. Henriquez-Camacho, C.; Gotuzzo, E.; Echevarria, J.; White, A.C., Jr.; Terashima, A.; Samalvides, F.; Perez-Molina, J.A.; Plana, M.N. Ivermectin versus albendazole or thiabendazole for Strongyloides stercoralis infection. *The Cochrane database of systematic reviews* **2016**, 10.1002/14651858.CD007745.pub3, CD007745, doi:10.1002/14651858.CD007745.pub3.
34. Buonfrate, D.; Perandin, F.; Formenti, F.; Bisoffi, Z. A retrospective study comparing agar plate culture, indirect immunofluorescence and real-time PCR for the diagnosis of Strongyloides stercoralis infection. *Parasitology* **2017**, *144*, 812-816, doi:10.1017/s0031182016002559.

35. Nutman, T.B. Human infection with Strongyloides stercoralis and other related Strongyloides species. *Parasitology* **2017**, *144*, 263-273, doi:10.1017/s0031182016000834.
36. Organization, W.H. *Guideline: Preventive Chemotherapy to Control Soil-Transmitted Helminth Infections in At-Risk Population Groups*; World Health Organization 2017.: Geneva, 2017.
37. Buonfrate, D.; Requena-Mendez, A.; Angheben, A.; Cinquini, M.; Cruciani, M.; Fittipaldo, A.; Giorli, G.; Gobbi, F.; Piubelli, C.; Bisoffi, Z. Accuracy of molecular biology techniques for the diagnosis of Strongyloides stercoralis infection-A systematic review and meta-analysis. *PLoS neglected tropical diseases* **2018**, *12*, e0006229, doi:10.1371/journal.pntd.0006229.

Annex *Strongyloides stercoralis* prevalence (STG-PR) and 95%CI for the countries for which estimates were made

Country	WHO Region	ISO 3	STG-PR
Afghanistan	EMRO	AFG	6.7% (3.4% - 10%)
Albania	EURO	ALB	8% (4.1% - 11.9%)
Algeria	AFRO	DZA	4.9% (2.5% - 7.3%)
Angola	AFRO	AGO	9.8% (5% - 14.6%)
Antigua and Barbuda	AMRO	ATG	7.3% (3.7% - 10.8%)
Argentina	AMRO	ARG	5.1% (2.6% - 7.6%)
Armenia	EURO	ARM	6.5% (3.3% - 9.7%)
Australia	WPRO	AUS	0.01% (0.005% - 0.015%)
Austria	EURO	AUT	0.01% (0.005% - 0.015%)
Azerbaijan	EURO	AZE	5.8% (3% - 8.7%)
Bahamas	AMRO	BHS	5.7% (2.9% - 8.5%)
Bahrain	EMRO	BHR	1.9% (1% - 2.9%)
Bangladesh	SEARO	BGD	17.3% (8.8% - 25.7%)
Barbados	AMRO	BRB	8.6% (4.4% - 12.8%)
Belarus	EURO	BLR	6.5% (3.3% - 9.7%)
Belgium	EURO	BEL	0.02% (0.01% - 0.03%)
Belize	AMRO	BLZ	11.8% (6% - 17.6%)
Benin	AFRO	BEN	10.7% (5.5% - 16%)
Bhutan	SEARO	BTN	18.5% (9.4% - 27.6%)
Bolivia (Plurinational State of)	AMRO	BOL	10.4% (5.3% - 15.4%)
Bosnia and Herzegovina	EURO	BIH	9.4% (4.8% - 14.1%)
Botswana	AFRO	BWA	6.3% (3.2% - 9.3%)
Brazil	AMRO	BRA	11.2% (5.7% - 16.6%)
Brunei Darussalam	WPRO	BRN	11.9% (6.1% - 17.7%)
Bulgaria	EURO	BGR	6.3% (3.2% - 9.3%)
Burkina Faso	AFRO	BFA	9.5% (4.8% - 14.1%)
Burundi	AFRO	BDI	11.7% (6% - 17.4%)
Cabo Verde	AFRO	CPV	5.8% (3% - 8.7%)
Cambodia	WPRO	KHM	13.6% (6.9% - 20.3%)
Cameroon	AFRO	CMR	12.8% (6.5% - 19%)
Canada	AMRO	CAN	0% (0% - 0%)
Central African Republic	AFRO	CAF	11.9% (6.1% - 17.7%)
Chad	AFRO	TCD	8.2% (4.2% - 12.2%)
Chile	AMRO	CHL	11.2% (5.7% - 16.7%)
China	WPRO	CHN	6.6% (3.3% - 9.8%)
Colombia	AMRO	COL	18.4% (9.4% - 27.4%)
Comoros	AFRO	COM	9.9% (5% - 14.7%)

Congo	AFRO	COG	13.4% (6.9% - 20%)
Costa Rica	AMRO	CRI	15.7% (8% - 23.4%)
Croatia	EURO	HRV	8.6% (4.4% - 12.8%)
Côte d'Ivoire	AFRO	CIV	11.6% (5.9% - 17.3%)
Cuba	AMRO	CUB	9.5% (4.9% - 14.2%)
Cyprus	EURO	CYP	2.8% (1.4% - 4.2%)
Czech Republic	EURO	CZE	4.5% (2.3% - 6.7%)
Democratic People's Republic of Korea	SEARO	PRK	9.5% (4.8% - 14.2%)
Democratic Republic of the Congo	AFRO	COD	13% (6.6% - 19.4%)
Denmark	EURO	DNK	0% (0% - 0%)
Djibouti	EMRO	DJI	6.8% (3.5% - 10.1%)
Dominica	AMRO	DMA	14.2% (7.2% - 21.1%)
Dominican Republic	AMRO	DOM	10.3% (5.3% - 15.4%)
Ecuador	AMRO	ECU	14.5% (7.4% - 21.6%)
Egypt	EMRO	EGY	4.9% (2.5% - 7.3%)
El Salvador	AMRO	SLV	12.1% (6.2% - 18.1%)
Equatorial Guinea	AFRO	GNQ	13.5% (6.9% - 20.1%)
Eritrea	AFRO	ERI	8.3% (4.2% - 12.3%)
Estonia	EURO	EST	4.1% (2.1% - 6.2%)
Ethiopia	AFRO	ETH	10.2% (5.2% - 15.2%)
Fiji	WPRO	FJI	15.9% (8.1% - 23.7%)
Finland	EURO	FIN	0% (0% - 0%)
France	EURO	FRA	0.02% (0.01% - 0.03%)
French Guiana	AMRO	GUF	13.2% (6.7% - 19.7%)
Gabon	AFRO	GAB	12.9% (6.6% - 19.2%)
Gambia	AFRO	GMB	9.5% (4.8% - 14.1%)
Georgia	EURO	GEO	10% (5.1% - 14.8%)
Germany	EURO	DEU	0.1% (0% - 0.1%)
Ghana	AFRO	GHA	11.1% (5.7% - 16.6%)
Greece	EURO	GRC	0.4% (0.2% - 0.6%)
Grenada	AMRO	GRD	13.7% (7% - 20.4%)
Guatemala	AMRO	GTM	13.7% (7% - 20.4%)
Guinea	AFRO	GIN	13.1% (6.7% - 19.5%)
Guinea-Bissau	AFRO	GNB	12.7% (6.5% - 18.9%)
Guyana	AMRO	GUY	14.6% (7.5% - 21.8%)
Haiti	AMRO	HTI	12.3% (6.3% - 18.4%)
Honduras	AMRO	HND	13.8% (7.1% - 20.6%)
Hungary	EURO	HUN	4.9% (2.5% - 7.3%)
Iceland	EURO	ISL	0% (0% - 0%)
India	SEARO	IND	10.4% (5.3% - 15.4%)
Indonesia	SEARO	IDN	16.4% (8.3% - 24.4%)
Iran (Islamic Republic of)	EMRO	IRN	4.8% (2.5% - 7.2%)
Iraq	EMRO	IRQ	5.3% (2.7% - 7.9%)

Ireland	EURO	IRL	0% (0% - 0%)
Israel	EURO	ISR	0.1% (0% - 0.1%)
Italy	EURO	ITA	0.03% (0.01% - 0.04%)
Jamaica	AMRO	JAM	13.4% (6.8% - 20%)
Japan	WPRO	JPN	0.04% (0.02% - 0.06%)
Jordan	EMRO	JOR	4.4% (2.3% - 6.6%)
Kazakhstan	EURO	KAZ	3.3% (1.7% - 4.9%)
Kenya	AFRO	KEN	8.7% (4.5% - 13%)
Kiribati	WPRO	KIR	6% (3.1% - 8.9%)
Kuwait	EMRO	KWT	1% (0.5% - 1.5%)
Kyrgyzstan	EURO	KGZ	6.5% (3.3% - 9.6%)
Lao People's Democratic Republic	WPRO	LAO	13.4% (6.8% - 20%)
Latvia	EURO	LVA	5.1% (2.6% - 7.5%)
Lebanon	EMRO	LBN	6.4% (3.3% - 9.5%)
Lesotho	AFRO	LSO	9.3% (4.7% - 13.8%)
Liberia	AFRO	LBR	16.5% (8.4% - 24.6%)
Libya	EMRO	LBY	4.2% (2.1% - 6.3%)
Lithuania	EURO	LTU	5% (2.5% - 7.4%)
Luxembourg	EURO	LUX	0% (0% - 0%)
Madagascar	AFRO	MDG	13.4% (6.8% - 20%)
Malawi	AFRO	MWI	11.2% (5.7% - 16.8%)
Malaysia	WPRO	MYS	15.9% (8.1% - 23.7%)
Maldives	SERAO	MDV	0.1% (0.1% - 0.2%)
Mali	AFRO	MLI	7.7% (3.9% - 11.5%)
Malta	EURO	MLT	0.02% (0.01% - 0.03%)
Mauritania	AFRO	MRT	6.7% (3.4% - 10%)
Mauritius	AFRO	MUS	13.1% (6.7% - 19.5%)
Mexico	AMRO	MEX	7% (3.6% - 10.5%)
Micronesia (Federated States of)	WPRO	FSM	8% (4.1% - 11.9%)
Mongolia	WPRO	MNG	4.3% (2.2% - 6.4%)
Morocco	EMRO	MAR	5.9% (3% - 8.7%)
Mozambique	AFRO	MOZ	10.8% (5.5% - 16.1%)
Myanmar	SEARO	MMR	19.2% (9.8% - 28.6%)
Namibia	AFRO	NAM	6.4% (3.2% - 9.5%)
Nepal	SEARO	NPL	14.2% (7.2% - 21.1%)
Netherlands	EURO	NLD	0.1% (0% - 0.1%)
New Caledonia	WPRO	NCL	0% (0% - 0%)
New Zealand	WPRO	NZL	0.04% (0.02% - 0.06%)
Nicaragua	AMRO	NIC	15% (7.6% - 22.3%)
Niger	AFRO	NER	7.6% (3.9% - 11.4%)
Nigeria	AFRO	NGA	10.7% (5.4% - 15.9%)
Norway	EURO	NOR	0% (0% - 0%)
Oman	EMRO	OMN	3% (1.6% - 4.5%)

Pakistan	EMRO	PAK	7.5% (3.8% - 11.2%)
Panama	AMRO	PAN	15.7% (8% - 23.4%)
Papua New Guinea	WPRO	PNG	19.4% (9.9% - 28.9%)
Paraguay	AMRO	PRY	9.1% (4.6% - 13.6%)
Peru	AMRO	PER	12.5% (6.4% - 18.6%)
Philippines	WPRO	PHL	15% (7.7% - 22.4%)
Poland	EURO	POL	5% (2.5% - 7.4%)
Portugal	EURO	PRT	0.05% (0.06% - 0.07%)
Puerto Rico	AMRO	PRI	9.1% (4.6% - 13.5%)
Qatar	EMRO	QAT	0% (0% - 0%)
Republic of Korea	WPRO	KOR	6.8% (3.5% - 10.2%)
Republic of Moldova	EURO	MDA	6.2% (3.2% - 9.3%)
Romania	EURO	ROU	6.1% (3.1% - 9.1%)
Russian Federation	EURO	RUS	4.1% (2.1% - 6.1%)
Rwanda	AFRO	RWA	11.2% (5.7% - 16.8%)
Saint Lucia	AMRO	LCA	13.8% (7% - 20.5%)
Saint Vincent and the Grenadines	AMRO	VCT	10.8% (5.5% - 16.2%)
Samoa	WPRO	WSM	5% (2.5% - 7.5%)
Sao Tome and Principe	AFRO	STP	20.7% (10.6% - 30.8%)
Saudi Arabia	EMRO	SAU	2% (1% - 3%)
Senegal	AFRO	SEN	8.7% (4.4% - 13%)
Seychelles	AFRO	SYC	12.5% (6.4% - 18.7%)
Sierra Leone	AFRO	SLE	17% (8.7% - 25.3%)
Singapore	WPRO	SGP	6.2% (3.2% - 9.3%)
Slovakia	EURO	SVK	6% (3.1% - 8.9%)
Slovenia	EURO	SVN	7.3% (3.7% - 10.9%)
Solomon Islands	WPRO	SLB	19% (9.7% - 28.4%)
Somalia	EMRO	SOM	7.9% (4% - 11.7%)
South Africa	AFRO	ZAF	6.4% (3.3% - 9.6%)
South Sudan	AFRO	SSD	11% (5.6% - 16.4%)
Spain	EURO	ESP	0.2% (0.1% - 0.3%)
Sri Lanka	SEARO	LKA	11.7% (6% - 17.4%)
Sudan	EMRO	SDN	7.1% (3.6% - 10.6%)
Suriname	AMRO	SUR	14.5% (7.4% - 21.6%)
Swaziland	AFRO	SWZ	8.6% (4.4% - 12.9%)
Sweden	EURO	SWE	0% (0% - 0%)
Switzerland	EURO	CHE	0.01% (0.005% - 0.015%)
Syrian Arab Republic	EMRO	SYR	5.7% (2.9% - 8.5%)
Tajikistan	EURO	TJK	7.8% (4% - 11.6%)
Thailand	SEARO	THA	10.8% (5.5% - 16.1%)
The former Yugoslav Republic of Macedonia	EURO	MKD	6.7% (3.4% - 10%)
Togo	AFRO	TGO	11.3% (5.8% - 16.8%)
Tonga	WPRO	TON	5% (2.5% - 7.5%)

Trinidad and Tobago	AMRO	TTO	12% (6.1% - 17.9%)
Tunisia	EMRO	TUN	5% (2.6% - 7.5%)
Turkey	EURO	TUR	5.6% (2.9% - 8.4%)
Turkmenistan	EURO	TKM	3.9% (2% - 5.8%)
Uganda	AFRO	UGA	11.5% (5.9% - 17.1%)
Ukraine	EURO	UKR	6.6% (3.4% - 9.9%)
United Arab Emirates	EMRO	ARE	0% (0% - 0%)
United Kingdom of Great Britain and Northern Ireland	EURO	GBR	0% (0% - 0%)
United Republic of Tanzania	AFRO	TZA	10.9% (5.6% - 16.3%)
United States of America	AMRO	USA	0.01% (0.005% - 0.015%)
United States Virgin Islands	AMRO	VIR	0% (0% - 0%)
Uruguay	AMRO	URY	8.7% (4.4% - 12.9%)
Uzbekistan	EURO	UZB	4.7% (2.4% - 7%)
Vanuatu	WPRO	VUT	0.3% (0.2% - 0.4%)
Venezuela (Bolivarian Republic of)	AMRO	VEN	11.2% (5.7% - 16.7%)
Viet Nam	WPRO	VNM	13.1% (6.7% - 19.6%)
Western Sahara	AFRO	ESH	5% (2.5% - 7.5%)
Yemen	EMRO	YEM	6.2% (3.2% - 9.2%)
Zambia	AFRO	ZMB	10.5% (5.3% - 15.6%)
Zimbabwe	AFRO	ZWE	8.7% (4.4% - 13%)

ISO 3: International Organization for Standardization country code

General discussion

The title of a paper published in 2009 by Olsen et al. nicely summarized the attention that strongyloidiasis received by the medical and the research communities: "Strongyloidiasis-the most neglected of the neglected tropical diseases?" {Olsen, 2009}. Indeed, for *S. stercoralis* the neglect was so remarkable that the infection was not even included in the WHO list of the NTD when this concept of NTD has been established (around 2005) and was still omitted on several redefinitions later on {Hotez, 2020; Molyneux, 2021 }.

Since then, substantial progress has been made, and this PhD work, carried out in the last decade and presented here contributed to it.

In this PhD thesis, I gathered a set of papers addressing key aspects of strongyloidiasis in the non-endemic setting, with several important findings. In Chapter 2 (epidemiology), new estimates of global prevalence have been provided, highlighting that around 600 million people are likely infected people (a much higher number than previously thought){Buonfrate, 2020 }. Also, the epidemiology of the infection in northern Italy has been better understood, with the main observation that strongyloidiasis is more frequently diagnosed in people – both immigrants and Italians - with eosinophilia compared to those with normal eosinophil count {Buonfrate, 2016 }. In Chapter 3 (clinical features), we showed in a systematic review that about 50% of people with chronic infection reported at least one symptom (such as pruritus, abdominal pain or respiratory symptoms) and more than 70% present with eosinophilia {Buonfrate, 2021}. Both symptoms and eosinophilia tended to clear after treatment. In Chapter 4 (diagnosis), we could conclude that serology is the diagnostic test with the highest sensitivity, playing a major role both for screening and post-treatment follow-up {Buonfrate, 2022; Buonfrate, 2015 }. In the final Chapter 5 (therapy), we demonstrated in a pivotal trial that a single dose of 200 µg/kg ivermectin has to be considered as the treatment of choice for chronic strongyloidiasis, with estimated efficacy of 86% (95% CI 79 - 91) {Buonfrate, 2019}.

The main limitation of this work is the focus on the non-endemic setting. Although studies in endemic settings would be relevant because the largest burden of the infection relies there, they are often hampered by logistic and economic constraints. Moreover, the possibility of re-infection might pose a bias for randomized controlled trials (RCTs). It should also be considered that evidence supporting diagnostic and clinical management procedures is badly needed also in our setting, where awareness is low but the number of patients at risk is not negligible. Another limitation is the limited light that can be shed on hyperinfection/dissemination due to the very low number of cases.

The main question that still needs to be addressed is how to manage this life-threatening condition, whenever it emerges after a long period of clinical latency. As an RCT would be unfeasible, because of the low occurrence of complicated cases diagnosed, clinical management will likely further rely on expert's opinion and cases series, maintaining much uncertainty on the optimal care {Nutman, 2017}. This is another reason why individual diagnosis and treatment would better take place before any drug-induced immunosuppression occurs, hence a call for mandatory screening of individuals at risk of infection.
The major strength of this thesis is the wide range of topics addressed, which encompass all important aspects of this complex parasitic infection. It was an attempt to give practical solutions to frequent questions raised during clinical practice, such as: a) When should I suspect strongyloidiasis and who should be screened? b) Which diagnostic test(s) should be used for screening? c) How to treat and follow up a patient?

The focus is clearly on care in the non-endemic setting, but some findings on diagnosis and treatment could be useful for the global health.

The fundamental question still lingering is however why a parasite that can disseminate all over the body and kill the host is so neglected. The main reason is probably shared by the other NTDs: they affect mostly vulnerable populations, living in the most disadvantaged areas of the world {Buonfrate, 2020}.

In my opinion, the lack of a diagnostic gold standard has played a major role in this. Both in endemic and in non - endemic setting, the microscopic examination of faeces has been the traditional method used to detect intestinal parasites. Unfortunately, this method has an exceedingly low sensitivity for *S. stercoralis*, thus causing underdiagnosis at individual level, and underestimation of prevalence at a global level {Buonfrate, 2015}. Previous estimates of global prevalence of strongyloidiasis were based on an educated guess {Genta, 1989}, and the supposed 30-100 million infected people seemed nothing compared to the prevalence estimated for example for the other STH {Bethony, 2006}. Only the top of the iceberg was seen.

An important step was hence revising the estimates of global prevalence of strongyloidiasis. The other relevant findings and perspectives of my work are reported here below in relation to the main clinical questions.

a) When should I suspect strongyloidiasis? Who should be screened?

An important area to be addressed to answer this question was the definition of the clinical and laboratory features of chronic strongyloidiasis. I performed a systematic review on this topic {Buonfrate, 2021} and, despite the scarce number and high heterogeneity of papers that could be included, the findings pointed out clearly that around half of infected people have symptoms. Moreover, proper diagnosis and treatment is also needed to achieve relief from symptoms, not only to avoid progression into disseminated infection. Another output of this work, also emerged in other studies collected here, was the relevance of eosinophilia as a first-line diagnostic predictor, which was present in a large proportion of infected individuals and tended to clear after treatment. This finding also emerged from the Strong Treat RCT {Buonfrate, 2019}, where we found that eosinophilia was common among participants, who had a median eosinophil count of 789 cells per µL (IQR 485–1,204), significantly reducing to 371 cells per µL (IQR 220–540) already at day 17 after treatment {Buonfrate, 2019}. Further, in the case-control study that I carried out in Norther Italy, we observed that people with eosinophilia (the “cases” in that study) had a significantly higher risk of having strongyloidiasis compared to people with normal eosinophil count (“controls”) {Buonfrate, 2016}.

Taken all together, evidence from these studies suggests that eosinophilia can be helpful to raise our index of suspicion, in particular for individuals who have always lived in a non-endemic area and have no significant history of travel abroad, and would hence be considered at low epidemiological risk.
However, the answer to our question “when should I suspect strongyloidiasis?” cannot be simplified to just “people with eosinophilia”. This would miss a sizeable proportion of people from endemic areas, who could be infected without presenting with eosinophilia: in my case-control study, immigrants with eosinophilia were diagnosed with strongyloidiasis in 17% (36/214) cases, while those without eosinophilia were found to be infected in a smaller but still substantial proportion: 2% (3/172) {Buonfrate, 2016}.

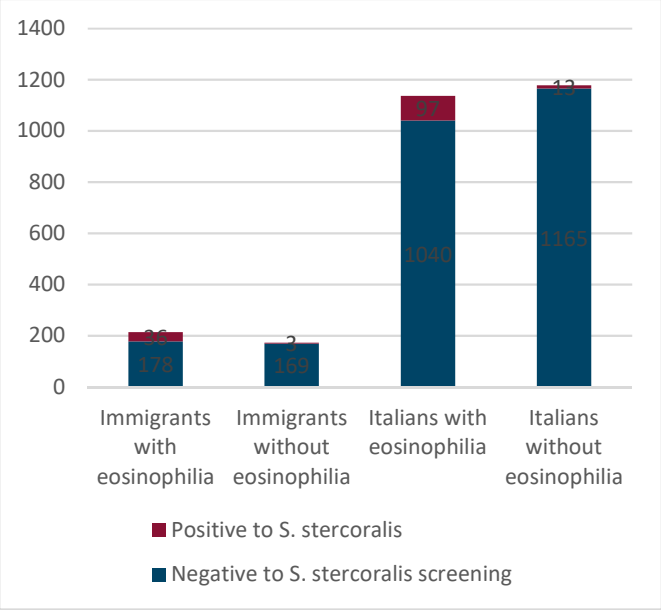


Figure 1. Proportion of *S. stercoralis* positive cases in immigrants and Italians with and without eosinophilia.

Indeed, the relevance of the country of origin was highlighted also in other studies, and some authors proposed universal screening for people coming from countries of high epidemiological risk {Zammarchi, 2020; Agbata, 2018} (a list of which is reported in Annex 1, from my work on global prevalence of strongyloidiasis). Universal screening might ease access to diagnosis for immigrants. The economic evaluations also supported this approach in terms of cost-benefit {Agbata, 2018; Zammarchi, 2020}.
In my experience, this happened many times when elderly Italians with no history of travel abroad came to our attention for unexplained eosinophilia.
Additional caution should be paid to candidates to immunosuppression and immunosuppressed patients. In a cost-benefit analysis, presumptive treatment demonstrated superior to the test-and-treat strategy {Joo, 2023}.

In summary, migrants who have been living in rural areas of endemic countries should be screened for strongyloidiasis irrespective of signs/ symptoms. People with low epidemiological risk should be tested in case of unexplained eosinophilia or "suggestive" symptoms. Presumptive treatment might be instead considered for immunosuppressed patients and candidates to immunesuppression, but this requires further research.

b) Which diagnostic tests should be used for screening?

In my work, the diagnostic issue is addressed on the one hand by highlighting the importance of the deployment of sensitive diagnostic tests for screening and individual diagnosis {Buonfrate, 2015; Buonfrate, 2018}.

First of all, considering the potential harm of an untreated infection and the good safety profile of the therapy, treating a variable proportion of false-positive individuals (depending on the specificity of the test) would be better than missing any *Strongyloides* case. Hence, in my scoping review {Buonfrate, 2022}, I point out that serology is the preferred method for screening, for its high sensitivity. Faecal-based tests, including molecular assays, are generally less sensitive and highly specific. They can be used for a better definition of

the presentation (they can confirm the infection) and are recommended for screening of immunosuppressed people, who might have false negative serology results.

In a systematic review with meta-analysis {Buonfrate, 2018}, real-time PCR eventually proved to have a sensitivity comparable to that of highly-sensitive parasitological methods – ranging between 64.4% (95% CI 46.2±77.7) and 56.5% (95% CI 39.2±72.4), depending on the panel of tests used to estimate the accuracy. PCR can be preferred to agar plate culture or Baermann method based on local expertise and resources, need of transport of samples (while parasitological methods need fresh stool, for PCR samples can be preserved either frozen or in ethanol). In that review, I also highlight that the sensitivity of a novel test can be overestimated if assessed against a single stool sample examined with microscopy (which has a very low sensitivity); hence, proper methods for estimating diagnostic accuracy should be designed and followed.

To sum up: serology is the preferred diagnostic method for screening. Immunosuppressed people may have false-negative serology {Buonfrate, 2022; Mascarello, 2011}, hence a sensitive faecal test (either PCR, agar plate culture or Baermann, according to local expertise/availability) should be done along with serology in this population. There is likely much room for improvement by developing and evaluating antigen-based assays, ideally at the point of care, that would better reflect the infection activity (at least in individuals previously exposed to treatment). This important point is specifically developed under the paragraph "Future insight" here below.

c) How to treat and follow up a patient?

A Cochrane review showed that ivermectin is the drug of choice for strongyloidiasis, as the other available options had either inferior efficacy (albendazole) or lower tolerability profile (thiabendazole) {Henriquez-Camacho, 2016}. What was missing, according to the authors' conclusion, was evidence about the dose of ivermectin: single or multiple doses?
With the Strong Treat randomized controlled trial (RCT) {Buonfrate, 2019}, we estimated at 86% (95% CI 79 - 91) and at 85% (95% CI 77 - 90) the efficacy of a single and multiple doses of ivermectin for the treatment of strongyloidiasis, respectively {Buonfrate, 2019}. Hence, we found clear indications about the treatment with a single dose of 200 µg/kg of ivermectin in case of uncomplicated strongyloidiasis {Buonfrate, 2019}. This was in line with previous RCTs, all carried out in endemic countries, which found no difference in efficacy between single and double doses of ivermectin {Suputtamongkol, 2011; Gann, 1994}.
Of note, if based on faecal tests only, efficacy would have resulted 100% (95% CI 90 – 100) in the single-dose group and 98% (95% CI 89– 100) in the multiple-dose arm, figure in line with those from previous RCT that used parasitological tests to estimate the efficacy of either single or double doses of ivermectin {Gann, 1994; Suputtamongkol, 2011}.
While there are RCT addressing the treatment in immunocompentent individuals, there is no high-quality evidence concerning immunosuppressed people, and specifically those with HTLV-1 coinfection {Henriquez-Camacho, 2016}.
The diagnostic issue has also an impact on the definition of response to treatment. Indeed, post-treatment monitoring with a poorly sensitive diagnostic test can result in overestimating drug efficacy in clinical trials, and erroneously classifying as cured patients who can still be at risk of developing the severe syndrome later in life. This was clear in a study by Dreyer et al {Dreyer, 1996}, who examined with Baermann method one

stool sample a week of 108 individuals. Over the 8 weeks of the study, the participants did not receive treatment for strongyloidiasis, and samples showed alternating positive and negative results from most of them. Moreover, the large majority of participants who tested positive on at least one sample, showed negative results in all following follow up tests (intermittent excretion). In conclusion, parasitological tests are not reliable to assess cure on their own, and should be better used in combination with more sensitive diagnostic tools.

A contribution to solve this issue was given in a diagnostic study, which retrospectively evaluated the dynamics of *Strongyloides* antibody levels detected by different serological assays {Buonfrate, 2015}. With this study, we showed that quantitative serology can be used for post-treatment monitoring, although the timeframe for follow up should be longer than that needed with faecal tests (from parasitological methods to PCR). While faecal tests tend to turn negative a few days after treatment (with the limitation that we might not know whether the test is negative because the infection was cleared or because the test is not sensitive enough to detect low intensity parasitemia), seroreversion takes months to occur, and at least three-four months are needed to demonstrate a decrease in antibody titre (in case quantitative results are provided) and six months to turn negative. The latter timeframe demonstrated its validity in a clinical context, as in the Strong Treat trial results at six and 12 months did not differ {Buonfrate, 2019}.

In conclusion: a single dose of 200 µg/kg ivermectin is the treatment of choice for uncomplicated strongyloidiasis in the immunocompetent individual. For post-treatment monitoring, tests that were positive at baseline should be repeated. Serology should be done not earlier than five to six months after treatment, while faecal tests can be repeated shortly (about one week) after treatment. Much research is still needed to define the optimal treatment in the immunosuppressed patient either with uncomplicated and severe strongyloiasis, as well as those patients not responding to the standard therapy. As this remains the most crucial knowledge gap in clinical care, the paragraph here below is specifically dedicated to future perspectives to address this issue.

Future insights

Management of people who do not respond to a single dose of ivermectin is currently undefined, with some experts recommending repeated doses or combination with albendazole. RCTs do not provide evidence about the best treatment strategy for immunosuppressed people with chronic, uncomplicated infection: some clinicians treat them with the single dose, but others prefer to give multiple doses. Due to the difficulty to conduct such a RCT, uncertainty about cases with undefined management, including hyperinfection, might be addressed with a prospective, international web-based registry of treatment, as it has been done for other rare infections (https://www.orpha.net/consor/cgi-bin/ResearchTrials_RegistriesMaterials.php?lng=EN). This might provide some basis for recommendations that, although of lower quality than evidence from RCT, would have a stronger rationale than expert's opinion only. Moreover, a registry of cases might help to quantify the risk of hyperinfection due to triggering factors, for instance giving some indications about the doses and length of treatment with steroids or other immunosuppressant medications that induce the severe syndrome, whose clinical management is also undefined.

Another future prospective would be to "validate" some of my findings in different epidemiological contexts, to understand whether recommendations retrieved from this work can be applicable in other settings, considering different needs, resources and acceptability. For instance, some of the studies that I carried out is currently under evaluation by a WHO working group working on guidelines for the control of strongyloidiasis in endemic areas. As per the other STH, preventive chemotherapy, the administration of treatment offered to populations without individual diagnosis, will probably be the main pillar of the WHO recommendations. Also, the choice of diagnostic tests to be used in tropical fields, where most cases occur,

should be based not only on the accuracy but also on local acceptability and feasibility. For example, deployment of real-time PCR might be hampered by cost constraints and lack of laboratory facilities in some endemic areas. Other diagnostics, such as serology, might use different procedures – collecting dried blood spots on filter paper eases storage and transport to reference laboratories. The good accuracy and feasibility of serology performed on dried blood spots was one main finding of a diagnostic study in Ecuador for the evaluation of accuracy, acceptability and feasibility for use in the field of different diagnostic tests for strongyloidiasis {Tamarozzi, 2023}. This study included a rapid immunochromatographic antibody test performed on whole blood from fingerprick, which was evaluated for the first time prospectively and in a clinical context. It is certainly worth to keep on improving and developing point-of-care tests, which could mark a turning point in some settings. For instance, they are much more feasible for use in the field compared to any other currently-available test, as they do not require laboratory facilities/parasitological expertise. Part of my work is in fact already moving towards the global health perspective, being involved in the upcoming WHO guidelines for the control of strongyloidiasis in endemic areas.

In conclusion, in the last decade major progress has been done to shed light on critical aspects of strongyloidiasis. Therefore, there is now sufficient data for evidence-based recommendations about screening, diagnostic procedures, and clinical management of strongyloidiasis in the non-endemic areas, at least for the immunocompetent population. Some important grey areas still remain (e.g: what is the risk of developing the severe form of strongyloidiasis for people under immunosuppressant agents? How to treat people who do not respond to ivermectin? How to manage cases of hyperinfection/dissemination? How to address the very specific challenges of the HTLV1-strongyloidiasis coinfection? and deserve further multicentric multicountry studies.

References

1. Olsen, A.; van Lieshout, L.; Marti, H.; Polderman, T.; Polman, K.; Steinmann, P.; Stothard, R.; Thybo, S.; Verweij, J.J.; Magnussen, P. Strongyloidiasis--the most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2009**, *103*, 967-972, doi:10.1016/j.trstmh.2009.02.013.
2. Hotez, P.J.; Aksoy, S.; Brindley, P.J.; Kamhawi, S. What constitutes a neglected tropical disease? *PLoS neglected tropical diseases* **2020**, *14*, e0008001, doi:10.1371/journal.pntd.0008001.
3. Molyneux, D.H.; Asamoa-Bah, A.; Fenwick, A.; Savioli, L.; Hotez, P. The history of the neglected tropical disease movement. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2021**, *115*, 169-175, doi:10.1093/trstmh/trab015.
4. Buonfrate, D.; Bisanzio, D.; Giorli, G.; Odermatt, P.; Fürst, T.; Greenaway, C.; French, M.; Reithinger, R.; Gobbi, F.; Montresor, A., et al. The Global Prevalence of Strongyloides stercoralis Infection. *Pathogens (Basel, Switzerland)* **2020**, *9*, doi:10.3390/pathogens9060468.
5. Buonfrate, D.; Baldissera, M.; Abrescia, F.; Bassetti, M.; Caramaschi, G.; Giobbia, M.; Mascarello, M.; Rodari, P.; Scattolo, N.; Napoletano, G., et al. Epidemiology of Strongyloides stercoralis in northern Italy: results of a multicentre case-control study, February 2013 to July 2014. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* **2016**, *21*, doi:10.2807/1560-7917.es.2016.21.31.30310.
6. Buonfrate, D.; Fittipaldo, A.; Vlieghe, E.; Bottieau, E. Clinical and laboratory features of Strongyloides stercoralis infection at diagnosis and after treatment: a systematic review and meta-analysis. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2021**, *27*, 1621-1628, doi:10.1016/j.cmi.2021.07.016.
7. Buonfrate, D.; Tamarozzi, F.; Paradies, P.; Watts, M.R.; Bradbury, R.S.; Bisoffi, Z. The diagnosis of human and companion animal Strongyloides stercoralis infection: Challenges and solutions. A scoping review. *Advances in parasitology* **2022**, *118*, 1-84, doi:10.1016/bs.apar.2022.07.001.

8. Buonfrate, D.; Sequi, M.; Mejia, R.; Cimino, R.O.; Krolewiecki, A.J.; Albonico, M.; Degani, M.; Tais, S.; Angheben, A.; Requena-Mendez, A., et al. Accuracy of five serologic tests for the follow up of Strongyloides stercoralis infection. *PLoS neglected tropical diseases* **2015**, *9*, e0003491, doi:10.1371/journal.pntd.0003491.
9. Buonfrate, D.; Salas-Coronas, J.; Muñoz, J.; Maruri, B.T.; Rodari, P.; Castelli, F.; Zammarchi, L.; Bianchi, L.; Gobbi, F.; Cabezas-Fernández, T., et al. Multiple-dose versus single-dose ivermectin for Strongyloides stercoralis infection (Strong Treat 1 to 4): a multicentre, open-label, phase 3, randomised controlled superiority trial. *The Lancet. Infectious diseases* **2019**, *19*, 1181-1190, doi:10.1016/s1473-3099(19)30289-0.
10. Nutman, T.B. Human infection with Strongyloides stercoralis and other related Strongyloides species. *Parasitology* **2017**, *144*, 263-273, doi:10.1017/s0031182016000834.
11. Buonfrate, D.; Formenti, F.; Perandin, F.; Bisoffi, Z. Novel approaches to the diagnosis of Strongyloides stercoralis infection. *Clinical microbiology and infection* **2015**, *21*, 543-552, doi:10.1016/j.cmi.2015.04.001.
12. Genta, R.M. Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. *Reviews of infectious diseases* **1989**, *11*, 755-767,
13. Bethony, J.; Brooker, S.; Albonico, M.; Geiger, S.M.; Loukas, A.; Diemert, D.; Hotez, P.J. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet (London, England)* **2006**, *367*, 1521-1532, doi:10.1016/s0140-6736(06)68653-4.
14. Zammarchi, L.; Tilli, M.; Botta, A.; Buonfrate, D.; Bartoloni, A.; Boccalini, S. Strategies for management of strongyloidiasis in migrants from Sub-Saharan Africa recently arrived in Italy: A cost-effectiveness analysis. *Travel medicine and infectious disease* **2020**, *36*, 101561, doi:10.1016/j.tmaid.2020.101561.
15. Agbata, E.N.; Morton, R.L.; Bisoffi, Z.; Bottieau, E.; Greenaway, C.; Biggs, B.A.; Montero, N.; Tran, A.; Rowbotham, N.; Arevalo-Rodriguez, I., et al. Effectiveness of Screening and Treatment Approaches for Schistosomiasis and Strongyloidiasis in Newly-Arrived Migrants from Endemic Countries in the EU/EEA: A Systematic Review. *International journal of environmental research and public health* **2018**, *16*, doi:10.3390/ijerph16010011.
16. Buonfrate, D.; Requena-Mendez, A.; Angheben, A.; Cinquini, M.; Cruciani, M.; Fittipaldo, A.; Giorli, G.; Gobbi, F.; Piubelli, C.; Bisoffi, Z. Accuracy of molecular biology techniques for the diagnosis of Strongyloides stercoralis infection-A systematic review and meta-analysis. *PLoS neglected tropical diseases* **2018**, *12*, e0006229, doi:10.1371/journal.pntd.0006229.
17. Mascarello, M.; Gobbi, F.; Angheben, A.; Gobbo, M.; Gaiera, G.; Pegoraro, M.; Lanzafame, M.; Buonfrate, D.; Concia, E.; Bisoffi, Z. Prevalence of Strongyloides stercoralis infection among HIV-positive immigrants attending two Italian hospitals, from 2000 to 2009. *Annals of tropical medicine and parasitology* **2011**, *105*, 617-623, doi:10.1179/2047773211y.0000000006.
18. Henriquez-Camacho, C.; Gotuzzo, E.; Echevarria, J.; White, A.C., Jr.; Terashima, A.; Samalvides, F.; Pérez-Molina, J.A.; Plana, M.N. Ivermectin versus albendazole or thiabendazole for Strongyloides stercoralis infection. *The Cochrane database of systematic reviews* **2016**, *2016*, CD007745,
19. Suputtamongkol, Y.; Premasathian, N.; Bhumimuang, K.; Waywa, D.; Nilganuwong, S.; Karuphong, E.; Anekthananon, T.; Wanachiwanawin, D.; Silpasakorn, S. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. *PLoS neglected tropical diseases* **2011**, *5*, e1044, doi:10.1371/journal.pntd.0001044.
20. Gann, P.H.; Neva, F.A.; Gam, A.A. A randomized trial of single- and two-dose ivermectin versus thiabendazole for treatment of strongyloidiasis. *The Journal of infectious diseases* **1994**, *169*, 1076-1079, doi:10.1093/infdis/169.5.1076.
21. Dreyer, G.; Fernandes-Silva, E.; Alves, S.; Rocha, A.; Albuquerque, R.; Addiss, D. Patterns of detection of Strongyloides stercoralis in stool specimens: implications for diagnosis and clinical trials. *Journal of clinical microbiology* **1996**, *34*, 2569-2571, doi:10.1128/jcm.34.10.2569-2571.1996.
22. Tamarozzi F, Guevara ÁG, Anselmi M, Vicuña Y, Prandi R, Marquez M, Vivero S, Robinzón Huerlo F, Racines M, Mazzi C, Denwood M, Buonfrate D. Accuracy, acceptability, and feasibility of diagnostic tests for the screening of Strongyloides stercoralis in the field (ESTRELLA): a cross-sectional study in Ecuador. Lancet Glob Health. 2023 May;11(5):e740-e748. doi: 10.1016/S2214-109X(23)00108-0. Epub 2023 Mar 24. PMID: 36972722.

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