Advances in the Diagnosis of Human Strongyloidiasis

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Abstract Strongyloidiasis is an intestinal parasitic infection
that is particularly relevant in immunosuppressed patients
because it can cause severe disseminated disease. This review
discusses the recent advances in the diagnosis of strongyloi-
diasis. We suggest clinical and epidemiologic criteria for the
diagnosis and screening of strongyloidiasis, taking into ac-
count different epidemiologic contexts. The state of the art of
the diagnosis of strongyloidiasis is discussed including para-
sitologic methods that are commonly used despite having low
sensitivity; serology, which has demonstrated better sensitiv-
ity (with some exceptions such as travelers or immunosup-
pressed patients), and molecular biology methods, which have
virtually 100 % specificity. Finally, we discuss different strat-
egies to follow up patients after treatment, highlighting the
importance of having accurate and reliable follow-up markers
when assessing treatment efficacy both in a clinical and re-
search context.

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Introduction

Strongyloides stercoralis is a unique nematode that is acquired
by humans when larvae penetrate intact skin following contact
with infected soil [1, 2•]. After a complex cycle, the female adult
becomes established in the small intestine, where it lays eggs on
a daily basis. *Strongyloides* eggs usually hatch in the intestine,
and are therefore only exceptionally found in the feces. The
presence of first-stage larvae in feces is diagnostic for strongy-
loidiasis. Most larvae develop into third-stage (infective) larvae
while still in the intestine, where they are able to penetrate the
bowel wall and initiate a new cycle [1]. This unique autoinfec-
tion cycle means that infected individuals will remain infected
for a lifetime if not appropriately treated [2•] (Fig. 1).

In immunosuppressive conditions, autoinfection may pre-
dominate and become overwhelming, with parasites at different
stages of development invading virtually every host organ and
tissue [3]. The result is the development of hyperinfection or
disseminated strongyloidiasis. The first is defined as the infec-
tion confined to the lungs and gastrointestinal tract but with
signs or symptoms of severe disease in relation to an elevated
number of larvae, whereas the latter, which is the most severe
form of hyperinfection, larvae might be found in any organ,
other than the respiratory or gastrointestinal tract [4••]. Dissem-
inated strongyloidiasis is fatal in over 50 % of cases, and is
particularly common in patients on immunosuppressive treat-
ment [4••, 5]. According to a recent systematic review, relative-
ly few cases of dissemination have been reported [4••], but we
believe that these cases are only the tip of the iceberg, as
apparent causes of death such as Gram-negative sepsis, pneu-
monia, and/or meningitis are not usually associated with a
parasitic origin in clinicians' minds. Consequently, it is crucial
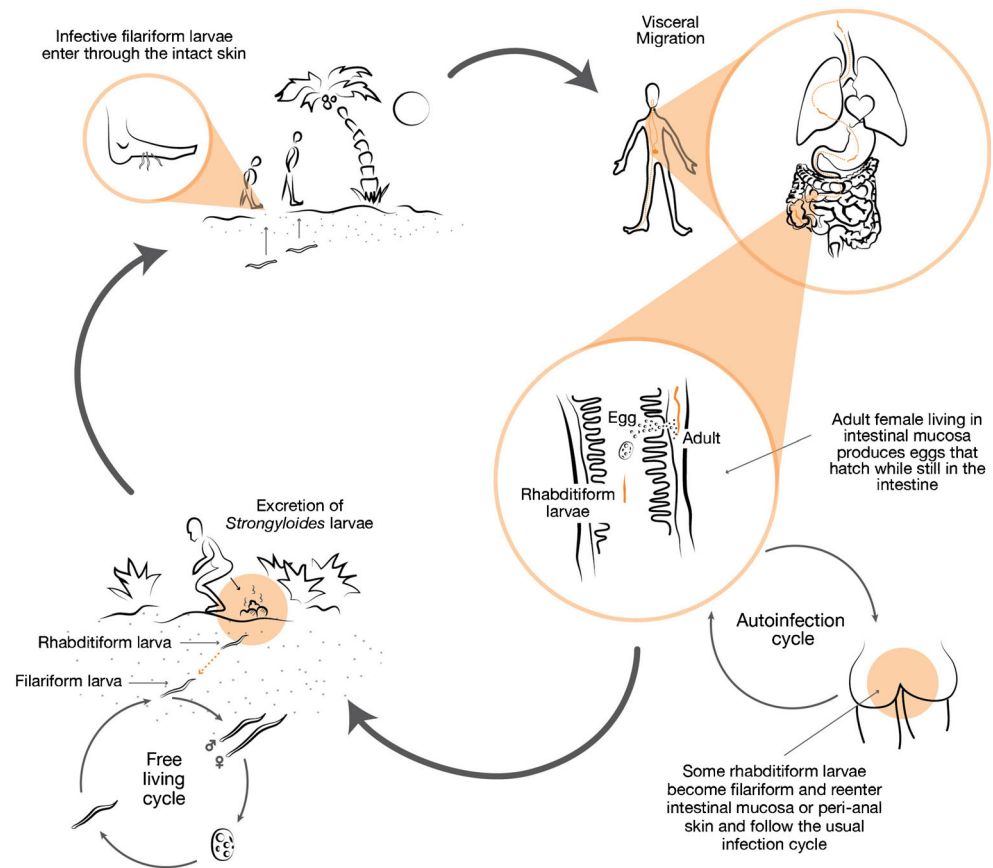
to detect and effectively treat infection as early as possible, because chronic strongyloidiasis can be fatal and indeed has been defined by some authors as a "time bomb" [6].

The global burden of strongyloidiasis has long been underestimated, mostly because of a lack of sensitivity of commonly used, fecal-based diagnostic methods [7••, 8, 9]. The traditional figure of 30–100 million infected people worldwide was recently questioned [10], and a new figure of over 350 million proposed [10, 11]. Diagnosis of strongyloidiasis is often delayed or overlooked because the infection is frequently asymptomatic or associated with non-specific gastrointestinal symptoms. Appropriate diagnostic methods are therefore crucial to improve our knowledge of the epidemiology of strongyloidiasis and ensure its inclusion in studies of soil-transmitted helminth (STH) infections. Moreover, improved diagnostics are essential for effective screening of immunosuppressed patients in populations at risk.

In this review, we discuss recent advances in diagnostic tests for strongyloidiasis.

Who should be Tested for Strongyloidiasis?

Community-based clinicians must be aware of the risks of strongyloidiasis and be able to clearly identify individuals

who should be tested. The guiding criteria will vary according to whether the context is clinical (diagnosis) or population based (screening).

An individual diagnosis of strongyloidiasis should be suspected even in asymptomatic individuals, but clinical suspicion can be raised by some clinical findings. With the exception of larva currens, which is virtually pathognomonic for strongyloidiasis, albeit very uncommon, signs and symptoms of chronic strongyloidiasis are usually scarce and non-specific [2•]. In highly endemic countries, *S. stercoralis* infection should be considered in any patient presenting with abdominal discomfort, and/or itching or skin rash [12]. Wheezing, dry cough, and related respiratory problems are also quite common [13]. Peripheral eosinophilia can support the suspicion of strongyloidiasis. Nonetheless, while eosinophilia has shown a high positive predictive value in some settings [14], in others, it has been found to be a poor predictor of infection [15]. In non-endemic countries, the above clinical criteria should be combined with consideration of the risk of exposure in the case of immigrants or travelers, regardless of the potential incubation period. In some regions of formerly endemic countries, such as Italy and Spain, individuals, and older individuals in particular, may harbor infections following exposure several decades

earlier [14]. An eosinophil count of over 500 cells/μL should be sufficient to raise suspicion.

Strongyloidiasis should be urgently ruled out in patients with clinical manifestations of hyperinfection syndrome or disseminated disease. In such cases, stool examination methods are highly sensitive owing to the massive parasite burden and, in severe cases, larvae can be found in other fluids such as sputum or cerebrospinal fluid [5, 7••].

The criteria for population-based screening of *S. stercoralis* infection in people at risk are still under discussion. In our view, all individuals who are, or are likely to become immunosuppressed (e.g., HIV- or HTLV1-infected individuals, oncologic patients, solid or bone marrow transplant recipients, candidates for corticosteroid or immunosuppressive therapy, including anti-tumor necrosis factor therapy), and who may have been exposed to the infection, should be screened and appropriately treated if necessary. The recommended method for screening is serology, if available. Presumptive treatment might be a more cost-effective alternative, but evidence is still lacking [16•].

There is no evidence to support routine screening of strongyloidiasis in immunocompetent patients. Even though eosinophilia has not been found to have a high positive predictive value for parasitic infections [17], a reasonable strategy could be to perform a complete blood work-up in high-risk individuals and screening tests in patients with a raised eosinophil count.

A summary of our recommendations for the diagnosis and screening of strongyloidiasis is given in Table 1.

How to Diagnose Strongyloidiasis (see Table 2)

Parasitologic Methods

Diagnosis of strongyloidiasis has traditionally been based on parasitologic methods aimed at detecting *S. stercoralis* larvae in feces rather than ova, in contrast to other intestinal helminths [7••]. However, as already mentioned, the sensitivity of most of these methods is very low [18] because of intermittent larval excretion [19, 20]. While simple and inexpensive, a direct fecal smear examination (DS) detects less than 30 % of chronic infections [7••]. The Kato-Katz method is a simple variation of the DS based on a microscopic examination aimed at measuring the intensity of infection by counting the number of eggs per gram [21]. It has been widely used in field studies of helminth infections [22, 23] because it quantifies the intensity of infection and is therefore very useful in public health interventions aimed at reducing the burden of STH infections [24]. However, it fails to detect low-density infections and, more importantly, it is not useful for detecting *S. stercoralis* [22]. The use of inadequate techniques for detecting *S. stercoralis* infections has largely contributed to the underestimation of the burden of strongyloidiasis in research studies.

Other methods, such as fecal concentration techniques, have been proposed to improve the sensitivity of the parasitologic diagnosis of strongyloidiasis. The formalin-ether concentration technique (FECT), described by Ritchie, is currently the most widely used test in clinical practice, although it also lacks sufficient sensitivity [19, 25, 26], particularly in

Table 1 Recommendations for screening and diagnosis of *Strongyloides stercoralis* infection in endemic and non-endemic areas

	Endemic areas	Non-endemic areas
Screening	Immunosuppressed individuals or candidates for immunosuppressive therapy	• Immunosuppressed individuals or candidates for immunosuppressive therapy Blood tests for migrants or individuals with a relevant travel history and specific testing for *S. stercoralis* infection in those with eosinophilia
Diagnosis	Strongyloidiasis should be considered: • in immunocompetent individuals with: - Abdominal discomfort - Itching or skin rash - Respiratory problems (wheezing, dry cough) - Unexplained eosinophilia in the presence of signs or symptoms of disseminated disease, i.e., worsening abdominal problems (abdominal pain, heavy diarrhea, ileus, obstruction, bleeding); severe and worsening pneumonitis and respiratory failure; sepsis; meningitis; febrile coma (eosinophilia is usually lacking)	Strongyloidiasis should be considered • in immunocompetent individuals with: - Abdominal discomfort - Itching or skin rash - Respiratory problems (wheezing, dry cough) - Unexplained eosinophilia PLUS - Relevant history of exposure to strongyloidiasis (travelers, migrants from endemic areas) OR - Older people from formerly endemic areas (e.g., Spain and Italy) in the presence of signs or symptoms of disseminated disease, i.e., worsening abdominal problems (abdominal pain, heavy diarrhea, ileus, obstruction, bleeding); severe and worsening pneumonitis and respiratory failure; sepsis; meningitis; febrile coma (eosinophilia usually lacking)

Table 2 Advantages and disadvantages of available diagnostic methods for Strongyloides stercoralis infection

	Technique	Advantages	Disadvantages	References
Parasitologic methods	DS	Simple and inexpensive; result on the same day	Very low sensitivity (detection rate <30 %); repeated test required to increase the sensitivity of the test	[7••, 19, 20, 33]
	FECT	Relatively simple and inexpensive; result on the same day	Better than DS but still low sensitivity; repeated test required to increase the sensitivity of the test	[19, 25–27]
	Baermann technique	Inexpensive; higher detection rate (3.6- to 4-fold) than FECT	Laborious and not available in clinical practice; repeated test required to increase the sensitivity of the test; fresh feces required; less sensitive than serology	[28, 30, 32•]
	Agar plate culture	Higher detection rate (1.6- to 6-fold) than FECT and DS; best stool method currently available; easy to implement	More expensive than other parasitologic methods; reading not possible before at least 72 h (time consuming); safety risk for laboratory staff; repeated test required to increase the sensitivity of the test; fresh feces required; less sensitive than serology	[25, 26, 29–31, 32•]
Serology	IFAT	High sensitivity; suitable for screening studies; only one sample required	Living infective larvae required; cross-reactivity with other nematode infections; relatively expensive compared with other techniques	[42, 55••]
	ELISA crude antigen	Good accuracy; suitable for screening studies; commercial test available; only one sample required	Cross-reactivity with other nematode infections; relatively expensive compared with other techniques	[45–51, 55••]
	ELISA-NIE (recombinant antigen)	Recombinant antigens can be produced in large amounts, with no need for infective larvae; only one sample required	Reported lower sensitivity compared with other ELISAs; cross-reactivity with other nematode infections; relatively expensive compared with other techniques	[53, 54, 55••]
	WB	Relatively good sensitivity depending on the study; only one sample required	Cross-reactivity with other nematode infections depending on the immunodominant antigen used; no filarial infections included to assess cross-reactivity; relatively expensive compared with other techniques	[43, 44]
	LIPS	High sensitivity and specificity and no cross-reactivity with other parasitic infections; useful for clinical diagnosis and prevalence studies; only one sample required	Not currently available in conventional laboratories; expensive owing to the high technology required	[53, 54, 55••]
PCR	18S rRNA, 28SrRNA	Specificity of almost 100 %	Sensitivity lower than serology and some stool-based methods; expensive; technology not available in limited resource settings	[58–61, 62••, 63, 64•, 65, 66]
Coproantigen	ELISA	High sensitivity; potentially inexpensive and simple to perform	Not validated in clinical practice or compared with serology	[56•, 57]

DS direct fecal smear examination, ELISA enzyme-linked immunoabsorbent assay, FECT formalin-ether concentration technique, IFAT immunofluorescence antibody test, LIPS luciferase immunoprecipitation system, PCR polymerase chain reaction, WB western blot

low-density infections [27]. Other more elaborate methods have been employed in recent years, including the Baermann method, which is based on the water tropism of *S. stercoralis* larvae [28], and agar plate culture (APC) [29]. Both methods have been shown to be superior to FECT and DS, with a 1.6- to 6-fold increase in detection rates [25, 26, 28–31, 32•]. Yet again, however, owing to low parasite loads and intermittent larval excretion, they have a high number of false negatives in chronic infections. Moreover, they are very laborious and require the presence of living larvae, making them logistically more complicated to use in clinical settings [7••]. The diagnostic sensitivity of APC and other parasitologic techniques can be increased through repeated stool examination [22, 23, 33, 34].

Serology

Immunologic methods have demonstrated better sensitivity than the parasitologic techniques discussed above. Most methods, however, have the drawback of cross-reactivity with other nematode infections, particularly filarial infections [35–37]. This is particularly important when evaluating results in endemic areas or in immigrants from areas with a high density of helminths. Immunologic methods thus hold promise as screening tools and for use in individual diagnoses where the possibility of co-infection is low [7••]. Serology has been found to be less sensitive in travelers returning from endemic countries than immigrants from these countries (73 % vs 98 %) [38]. One possible explanation is the shorter exposure time to the parasite in the former. Serology also seems to be less sensitive in immunosuppressed patients, possibly because of a reduced antibody response, although evidence in this regard is limited [39–41].

Several serum antibody tests have been tested over many years. Three tests in particular have shown high sensitivity and specificity: the immunofluorescence antibody test (IFAT), the enzyme-linked immunoabsorbent assay (ELISA), and immunoblotting [7••, 42–47]. ELISA is considered the easiest test, and is the most widely used technique for the diagnosis of strongyloidiasis, with sensitivity rates of between 73 % and 100 % [7••]. Several in-house ELISAs have been designed from crude antigenic extracts of filariform larvae, some of them from *S. stercoralis* [46–48] and others from *S. venezuelensis* or *S. ratti* [49–51]. In addition, two commercial kits are currently available: Bordier-ELISA from *S. ratti* antigen (Bordier Affinity products) and IVD-ELISA from *S. stercoralis* antigen (S-stercoralis serology Microwell ELISA Kit; IVD Research, Carlsbad, CA, USA) [45]. More recently, a recombinant antigen, NIE, [52], which has the advantage of being produced in large amounts, has also shown promising results in the NIE-ELISA test and the luciferase immunoprecipitation system (LIPS) in particular, as no cross-reactivity with other parasitic infections was found [53, 54].

A recent study compared five serologic tests (IFAT, IVD-ELISA, Bordier-ELISA, NIE-ELISA, NIE-LIPS) for the diagnosis of *S. stercoralis* infection [6, 55••]. Although the study had a retrospective design and used anonymized serum samples, the authors interestingly used a composite reference standard to establish the diagnosis of strongyloidiasis in addition to the classic gold standard based on a single parasitologic method. This approach allowed a more realistic classification of cases and controls. IVD-ELISA showed the highest concordance with the reference standard (Cohen's Kappa 0.9, confidence interval 0.86–0.95). IFAT was the most sensitive test (94.6 % confidence interval 91.2–96.9) followed by the commercial IVD-ELISA test. These tests, therefore, could be considered suitable for screening high-risk populations. The most specific, and accurate, test was NIE-LIPS, making it ideal for use in clinical trials. It is, however, only currently available for research purposes. Based on the findings of this comparative study, the two commercial tests appeared to work quite well in clinical practice and thus might be useful for screening and diagnosis in populations at risk. In the case of individual diagnoses, a higher optical density (OD) cut-off could be used to increase test specificity, particularly for immigrants from areas with a high incidence of other helminth infections.

ELISA tests have also been used to detect *S. stercoralis* coproantigen in fecal samples. Although results in human samples are limited [36], the method has proven to be very effective in detecting coproantigen in the feces of patients with *S. stercoralis* infection. Furthermore, no cross-reactivity with other parasitic infections was observed. However, it needs to be clinically validated before it can be used in routine practice.

Molecular Biology

Molecular polymerase chain reaction (PCR) techniques specifically developed for the diagnosis of strongyloidiasis have generally achieved 100 % specificity in several studies based on both simple and nested PCR [58–60]. The sensitivity reported to date was not superior to that of other parasitologic methods, in part probably because PCR inhibitors are commonly found in fecal samples and also possibly because the fecal samples used were very small.

Real-time PCR techniques targeting 18S rRNA and 28S rRNA have also demonstrated high analytic sensitivity and specificity [61, 62••, 63]. Whilst real-time PCR has shown very high specificity in human samples, it again has limited sensitivity, and has not proven to be diagnostically superior to other parasitologic techniques such as the Baermann method or APC [61, 63, 64•], particularly in low-density infections, supporting previous findings from animal models [65, 66]. However, recent improvements of this technique may increase the sensitivity in the near future.

Another important drawback of PCR techniques is that they are laborious and expensive, and are therefore not readily available outside research facilities.

The loop-mediated isothermal amplification (LAMP) assay is a molecular technique that performs highly specific and sensitive amplifications of nucleic acid to detect pathogens [67]. It has been used in the diagnosis of several parasitic infections, including malaria, leishmaniasis, and cysticercosis [68–70]. It has several advantages: it uses stable reagents, is simple to use, and does not require a complex infrastructure. Accordingly, it has already been used in low-resource settings [71, 72]. The LAMP assay has also been evaluated in preliminary studies for the detection of *S. stercoralis*, with very promising results [73•]. However, before it can be introduced on a widespread scale, it needs to be clinically validated and also requires more simplified or miniaturized extraction methods to facilitate use in remote areas [73•].

How to Follow Patients after Treatment

Accurate reliable methods for monitoring individuals with strongyloidiasis after treatment are of great importance for two main reasons. First, in clinical practice, the goal of treatment is to cure infection and prevent severe disseminated disease, which is a risk even in patients with low parasite burdens. Microbiologic tests should therefore be requested after treatment to detect treatment failure. Second, in the context of clinical research, follow-up markers are needed to assess the effectiveness of new anti-helminthic drugs and combinations of existing treatments.

Examination of a single stool sample using parasitologic methods is not sensitive enough to detect treatment failure and is not currently recommended in clinical practice [74]. Some authors have suggested analyzing stool samples collected on 2 consecutive days with the quantitative Baermann test. This has proven to be highly sensitive (>90 %) for post-treatment follow-up, but the lack of a reliable gold standard for comparison makes results difficult to interpret [75]. APC has been shown to be far more sensitive than standard DS, but the method produces similar results to those observed with the Baermann test and does not meet optimal follow-up criteria (Table 1) [7••, 20].

Serologic testing, if available, could be a suitable alternative for assessing cure, but only some ELISA and IFAT tests have been evaluated for this purpose. Whilst seroconversion does not always take place in patients with strongyloidiasis, serum levels tend to decrease after effective treatment [9, 47, 76–81].

Kobayashi et al. [9] observed that extremely high pretreatment OD levels in ELISA tended to remain positive at follow-up. It is possible that levels take longer to fall below the cut-off

for positivity in patients who have been infected for many years [83]. A post-treatment OD value of over 0.6 has been proposed for defining response to treatment. In the study by Kobayashi et al., 20 % of patients in whom OD values decreased inadequately and who were evaluated again with stool tests showed positive results. This suggests that persistence of positive serology in the absence of a consistent fall in OD might be indicative of treatment failure [9, 78]. This criterion is now used by most authors assessing serologic response to treatment [7••]. Biggs et al. [80] followed patients treated with two doses of ivermectin for 24 months, and found a significant cure rate (65 %) according to Kobayashi's criterion. Moreover, antibody titers decreased to some extent in 95 % of individuals.

LIPS and IFAT assays have also been evaluated for their ability to confirm a cure for *S. stercoralis* infection. While antibody titers tend to decrease after treatment, a reliable cut-off value for cure is lacking. Serologic follow-up for 1 year has been recommended to ensure a sustained decrease in serologic titers [80].

Finally, several authors who have investigated the value of eosinophil count as a marker of cure have reported a significant reduction in mean values after treatment [47, 78–81]. The fluctuating nature of eosinophil counts in helminth infections, however, poses a challenge. Moreover, although a normal eosinophil count following treatment does not confirm treatment response, persistent eosinophilia has been observed in non-responders [78].

When evaluating results in *S. stercoralis*-endemic countries, re-infection should be taken into account, because it can contribute to persistent serologic titers in a fraction of patients [82].

Assessment of treatment efficacy is heavily dependent on the method used to follow patients. Fecal-based methods (DS, formol-ether concentration, and stool culture) tend to overestimate treatment efficacy compared with serologic methods. One clinical trial comparing the efficacy of ivermectin and thiabendazole for treating strongyloidiasis [83], for instance, reported cure rates of 56.6 % for ivermectin and 52.2 % for thiabendazole based on serologic testing (in-house IFAT) but rates of 85.7 % and 94.6 %, respectively, for direct stool testing.

We support continued research into the value of new diagnostic and follow-up tools for strongyloidiasis, and currently recommend using a combination of both serology 6–12 months after treatment and stool methods (preferably Baermann and/or Koga agar culture) for the evaluation of a cure in patients with strongyloidiasis.

Conclusions

Strongyloides stercoralis has been the neglected member of the STH family for decades, mostly because of a lack of

diagnostic accuracy and difficulties in evaluating public health interventions. The global prevalence and clinical impact of strongyloidiasis are likely to be greatly underestimated. Furthermore, screening criteria are not well defined, and strongyloidiasis has largely been neglected by researchers evaluating treatment response in patients with STH infections in public health interventions.

Parasitologic methods based on stool examination, in particular the commonly used Kato-Katz method, lack sensitivity for diagnosis and follow-up. Several antibody tests have shown good sensitivity and specificity, but are still not ideal, and the usefulness of molecular biology methods remains to be tested.

In recent years, there has been a renewed interest in the study of strongyloidiasis, and several scientific groups and research institutions are advocating raising the profile of strongyloidiasis and giving it the attention it merits. *Strongyloides stercoralis* infection is slowly being contemplated in some studies on STH infections, and trials are underway to evaluate the most efficacious treatment regimen for eliminating infection. It is therefore extremely important to have an accurate case definition for strongyloidiasis based on robust diagnostic techniques and reliable follow-up.

In the absence of a reliable gold standard, recent research studies have used composite reference standards to provide a more accurate case definition and evaluate the sensitivity and specificity of serologic methods for the diagnosis of strongyloidiasis and evaluation of treatment response. High serologic cut-off values have been used in clinical trials to achieve a more specific case definition, but very possibly at the expense of sensitivity. In clinical practice, diagnosis of strongyloidiasis should be based on a combination of repeated parasitologic exams and serology (with a standard cut-off), if available.

Improving the diagnostic yield of easily implementable tests is crucial for overcoming the epidemiologic and therapeutic challenges posed by *S. stercoralis* infection worldwide. We envisage a short-term future in which strongyloidiasis will be taken into account in immunosuppressed patients at risk worldwide and included in preventive chemotherapy strategies for the control of STH infection.

Compliance with Ethics Guidelines

Conflict of Interest   Ana Requena-Mendez, Dora Buonfrate, Zeno Bisoffi, and Jose Muñoz declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent   This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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