REVIEW

Novel approaches to the diagnosis of *Strongyloides stercoralis* infection

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Abstract

Strongyloides stercoralis differs from the other soil-transmitted helminths because it puts infected subjects at risk of a fatal syndrome (in cases of immunosuppression for medical conditions, immunosuppressant therapies, or both). Chronic strongyloidiasis is often a non-severe condition, or is sometimes even asymptomatic, but diagnosis and effective therapy are essential in order to eradicate the infection and the life-long risk involved. Therefore, diagnostic methods need to be highly sensitive. Stool microscopy and the Kato–Katz technique are commonly used in prevalence studies, but they are inadequate for *S. stercoralis* detection. This is probably the main reason why the global prevalence has long been underestimated. Concentration methods, the Baermann technique and Koga agar plate culture have better, but still unsatisfactory, sensitivity. Serological tests have demonstrated higher sensitivity; although some authors have concerns about their specificity, it is possible to define cut-off values over which infection is almost certain. In particular, the luciferase immunoprecipitation system technique combined with a recombinant antigen (NIE) demonstrated a specificity of almost 100%. ELISA coproantigen detection has also shown promising results, but still needs full evaluation. Molecular diagnostic methods are currently available in a few referral centres as in-house techniques. In this review, on the basis of the performance of the different diagnostic methods, we outline diagnostic strategies that could be proposed for different purposes, such as: prevalence studies in endemic areas; individual diagnosis and screening; and monitoring of cure in clinical care and clinical trials.

Keywords: Diagnosis, follow-up, prevalence, screening, *Strongyloides stercoralis*
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Introduction

Strongyloides stercoralis is a soil-transmitted helminth (STH) that is endemic in many areas of the world. Recently, it has been estimated that at least 370 million people are infected [1]. In some areas, ≥60% of the local population are infected [2,3]. This nematode has some peculiarities that differentiate it from the other STHs. Humans acquire the infection by penetration of infective larvae from contaminated soil through intact human skin. The following phases of the life cycle (Fig. 1) are similar to those of other nematodes such as hookworms (which is acquired similarly) or *Ascaris lumbricoides* (which, in contrast, is acquired by ingestion, like most human parasites). *Strongyloides*, however, has a unique characteristic that explains most of the peculiar aspects of this parasite, which is that the infection can be life-long because of the 'autoinfective cycle' [4]: the eggs produced by the adult female hatch when they are still in the intestinal lumen, and newborn larvae can penetrate the last part of the bowel or the perianal skin, restarting the cycle inside the human body (Fig. 1). For this reason, this infection tends to be life-long, whereas infections with other STHs (namely: hookworm, *A. lumbricoides*, and *Trichuris trichiura*) are limited by the lifespan of the adult worm, unless a new infection occurs. Moreover, *S. stercoralis* infection can be fatal in a few days or weeks in immunosuppressed patients [4–6]: the larvae rapidly increase in number, and can disseminate throughout the human body, often causing bacteraemia by intestinal translocation [7]. Treatment is often ineffective at this stage, and it is therefore essential to diagnose and treat the infection at an earlier stage, in order to remove a 'time bomb' [8]. Consequently, a further

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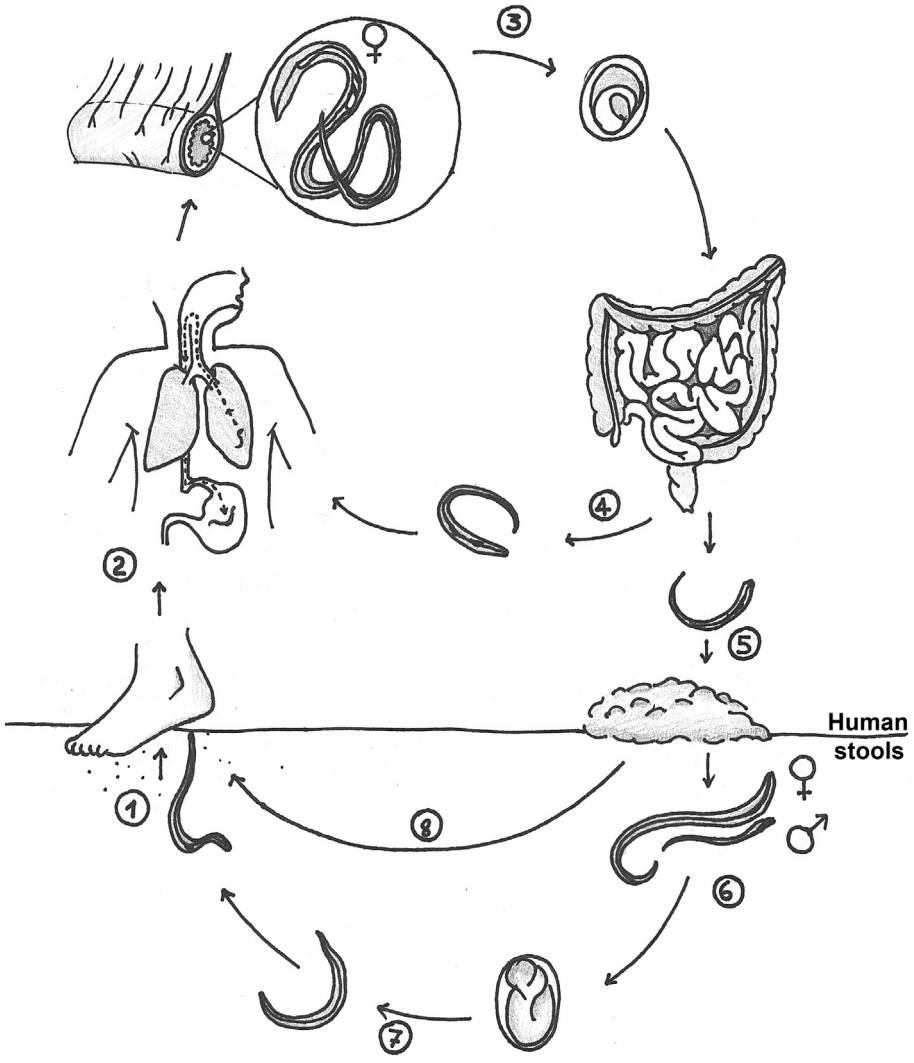

FIG. 1. Life cycle of *Strongyloides stercoralis*. 1, Filariform larvae (450 nm) penetrate human skin. 2, Larvae migrate to the lungs, trachea, and pharynx, and are swallowed. 3, Parthenogenetic adult females (2 mm) penetrate the small-bowel mucosa and release eggs. 4, Rabdiform larvae (250 nm) mature into autoinfective filariform larvae, and enter the circulation. 5, Rabdiform larvae are shed in stools. 6, Free-living 1-mm male and female adults develop in the soil. 7, Sexual reproduction in the soil (indirect development). 8, Filariform larvae develop in the soil—infective for humans (direct development).

difference from the other STHs is that, in this case, a simple decrease in the parasite load is not an acceptable goal. Only the complete eradication of the infection eliminates the future risk of a fatal complication [9,10]. This is why diagnostic methods for monitoring cure should be sensitive enough to detect even a very light, residual infection after a partially effective treatment. The same is true for the tests to be used in clinical trials, as otherwise a persisting infection at a low level after treatment may escape detection and therefore cause an overestimation of a drug's efficacy [11].

Unfortunately, the commonly used faecal-based methods have particularly low sensitivity. Examination of several stool samples, as well as concentration techniques, improve the performance of microscopy [12], which nevertheless remains insufficiently sensitive. The Baermann method and Koga agar plate culture (APC) are preferable, although they are cumbersome and not routinely used [13].

Alternative methods, from serology to molecular biology, have been developed and evaluated during the past few years [14].

The aim of this review is to outline what should be, based on the state of the art from the most recent studies, the optimal approach to the diagnosis of *S. stercoralis* infection for three main purposes: (a) prevalence studies in endemic areas, (b) individual diagnosis and screening, and (c) monitoring of cure in clinical care and in clinical trials.

We will not provide a lengthy, technical description of each diagnostic test and its accuracy: recent, excellent reviews on these are available in the references.

Prevalence studies

Until now, faecal surveys on the prevalence of STHs have often relied on stool concentration methods such as the Kato–Katz

[15–18], Mini-FLOTAC and McMaster [19] techniques. With standard methods, prevalence studies are affected by a lack of sensitivity. The larval output of *S. stercoralis* in stools is much lower than that of the eggs of hookworms, *T. trichiura*, or *A. lumbricoides* [20]. Moreover, if the peculiar autoinfective life cycle (Fig. 1) prevails, the larval load in stools may be undetectable [13,21,22]. Faecal surveys on STHs not aiming at *S. stercoralis* with specific techniques miss most infections, and, as a consequence, the global and local burdens of strongyloidiasis are grossly underestimated [1,2,9,23]. Prevalence figures would probably be different (but still far from reality) if multiple sampling were to be used [12], but this is logistically difficult to implement in epidemiological studies. Better diagnostic tools are needed for more correct estimation of *S. stercoralis* prevalence, and also in order to assess whether (and where) specific control measures are necessary [1,24]. Traditional methods that are specific for *S. stercoralis*, such as the Baermann technique or APC, provide more reliable prevalence figures [2], but are cumbersome and rarely used in epidemiological surveys [13].

Novel diagnostic approaches to prevalence studies are not necessarily based on 'new' technologies. An interesting improvement in the sensitivity of stool microscopy for *S. stercoralis* has been obtained with a very simple concentration method, i.e. spontaneous tube sedimentation (STS) [23]. This is a simple, innovative approach that does not require any sophisticated equipment, not even a centrifuge. Briefly, 2–5 g of stools is homogenized in 10 mL of saline solution and filtered through a surgical gauze into a 50-mL plastic tube, which is then filled with more saline solution, plugged, and shaken vigorously. The tube is left to stand for $\geq$45 min, after which the supernatant is removed, and a sample is taken from the bottom and put on a slide for microscopy. In a report from the Peruvian Amazon, the prevalence of *S. stercoralis* according to this method was 16%, whereas it was 22% with APC, and 0% with the standard Kato–Katz technique [24]. For the other STHs, the new method found the same prevalence as the Kato–Katz technique. Should this approach be corroborated by further studies, STS could become a good and inexpensive method to improve the rate of *S. stercoralis* detection in epidemiological surveys. However, in another study, in Brazil, the sensitivity of STS was lower than those of the Baermann technique and APC: 27.5% vs. 72.5% and 95%, respectively [25]. Another, simple modification of a classic method, formol–ether concentration (FECT), which is not itself ideal method for *S. stercoralis* detection, has been reported to have a sensitivity for *S. stercoralis* that is similar to those of the Baermann technique and APC [26]. The modification consisted of using wire meshes instead of gauze, and reducing the duration of exposure to formalin. The accuracy of traditional, faecal-based methods, according to recent papers, including a meta-analysis of studies conducted between 1980 and 2013, is summarized in Table 1.

Molecular diagnosis was introduced later in parasitology than in other fields of microbiology [27,28]. Its potential use for prevalence surveys in resource-constrained countries is still limited by cost and by the still low spread of its use outside research laboratories. However, a few prevalence surveys have been carried out with PCR. In a recent study in Kenya on human immunodeficiency virus-infected subjects, RT-PCR (sensitivity, 83.3%) was able to detect more *S. stercoralis* infections than the combination of wet preparation, the Kato–Katz technique, and FECT (sensitivity, 16.7%) [29]: also, none of the latter methods is adequate for *S. stercoralis* larva detection. In Bangladesh and in Tanzania, a lower sensitivity of PCR methods than of reference faecal methods was reported for the detection of low-intensity infections [30,31]. In detail, the study conducted on samples from Bangladesh showed a PCR sensitivity of 100% when it was assessed on samples with moderate-intensity and high-intensity infection, and a sensitivity of 16% when it was assessed on samples with low-intensity infection. The second study, conducted on samples from Tanzania, showed a PCR sensitivity of 17.4% as compared with the Baermann technique.

TABLE 1. Accuracy of conventional, faecal-based methods for *Strongyloides stercoralis* diagnosis according to recent, selected studies

Reference	Test(s) assessed	Sensitivity (%)	Specificity (%)	Study characteristics
[31]	Baermann	47	78.4	Accuracy assessed with PCR results as the reference standard
	Baermann	83.6	100	Accuracy assessed with a composite reference standard (PCR plus Baermann). Specificity assumed to be 100% for both methods
	Baermann	28.3	75.2	Accuracy assessed with a Bayesian approach to cope with the lack of a reference standard
[45]	Direct examination	21	100	Meta-analysis of papers published between 1908 and 2013. Specificity considered to be 100% by definition. Reference standard based exclusively on the results of conventional methods
	FECT	48	100	
	APC	89	100	
	Baermann	72	100	
[26]	C-FECT	48.1	100	Reference standard based on a combination of the three methods. Specificity considered to be 100% by definition. Study designed to assess the sensitivity of a modified FECT as explained in the text
	M-FECT	95.2	100	
	APC	94.2	100	

APC, agar plate culture; C-FECT, conventional formol–ether concentration; FECT, formol–ether concentration; M-FECT, Modified formol–ether concentration.

Surveys based on serology, on the other hand, have consistently reported a higher prevalence than those based on faecal diagnosis [3,32–34]. Serology was also used for a survey in the elderly population of Italy, in which a surprisingly high prevalence was found in subjects with eosinophilia [35]. Different serological assays were recently evaluated [36,37], and most of them showed a sensitivity ranging from 84% to 95% in chronically infected subjects (Table 2). Specificity was also good, and reached 100% above a given cut-off, at which sensitivity remained acceptable [36]. Therefore, the use of serology in community surveys is likely to provide a reliable estimate of the true prevalence. Recently, a serological test (NIE-ELISA) has been used on dried blood spots collected through finger prick on filter paper, and the results were comparable to those obtained with blood collected via venipuncture [38]. Standardization of this method (which has already been used for decades for antibody testing of several pathogens) for *S. stercoralis* infection would simplify the use of serology for epidemiological surveys in endemic countries.

Among the 'new' methods, a particularly useful tool for epidemiological surveys would be the antigen detection test in stools, which has the potential to be developed as a rapid immunochromatographic dipstick test [39]. However, further research on the accuracy of this technique is needed [13,14].

Molecular methods can also be applied to large-scale epidemiological and clinical investigation of intestinal parasites, as reported in a recent review [27]. Real-time PCR provides a valuable tool for survey studies; it is also ideally suited for automation, and provides the possibility of an integrated high-throughput approach for the detection of a wide range of parasites, bacteria, and viruses.

Individual diagnosis and screening

Clinical clues indicating *S. stercoralis* infection are poor and non-specific [14]. What matters more on an individual basis is to optimize the negative predictive value of the diagnostic approach; therefore, highly sensitive tests are needed. A particular case is that of individuals who are candidates for immunosuppressive treatment. In particular, screening for *S. stercoralis* with appropriate techniques should be included in all transplant screening protocols, both for potentially exposed recipients and for donors [40].

Serological methods are the most sensitive available diagnostic tools [13,14]. A variety of antigens have been used to develop serological tests. Two commercially available tests (Bordier ELISA and IVD-ELISA) use *Strongyloides ratti* and *S. stercoralis* larvae, respectively [41,42]. In recent years, a recombinant antigen named NIE (first identified in 2002) has been used to develop an ELISA test (NIE-ELISA) and a luciferase immunoprecipitation system (NIE-LIPS) [43,44]. A new commercially available, NIE-based ELISA test was recently compared with another ELISA test (SciMedx Strongyloides

TABLE 2. Accuracy of serological methods for *Strongyloides stercoralis* diagnosis according to selected studies published after 2005

Reference (year)	Test(s) assessed	Sensitivity (%)	Specificity (%)	Study characteristics
[36] (2014)	Bordier ELISA[a]	90.8	94.1	Study using a composite reference standard of faecal and serological tests (denominator for positives: samples with positive stool or ≥3/5 positive serological tests) to cope with the lack of a reference standard. Limits: retrospective study design
	IVD-ELISA[b]	92.3	97.4	
	NIE-ELISA[c]	70.8	91.1	
	IFAT[d]	94.6	87.4	
	NIE-LIPS[c]	83.8	99.6	
[37] (2014)	SciMedx ELISA[e]	85.5	82.6	Study mainly aimed at assessing percentage agreement of the three tests. Reference standard for positives: samples with ≥2/3 positive serological tests—faecal results not considered
	InBios-Strongy ELISA[f]	83.6	91.3	
	NIE-LIPS[c]	89.1	89.1	
[41] (2007)	AMC-ELISA[d]	93.3	95.0	Study using a panel of serum specimens from a population composed of patients with proven strongyloidiasis, healthy controls, and patients with various parasitic and other diseases. Faecal results used as a unique reference standard
	Dipstick[d]	91.1	97.7	
	Bordier ELISA[a]	83.3	97.2	
	IVD-ELISA[b]	88.9	97.2	
[42] (2010)	IVD-ELISA[b]	91.2	93.3	As above
[44] (2010)	Crude Ag-ELISA[d]	97.0	100	Specificity predetermined at 100% for all methods by the use of cut-off values obtained from ROC curves for 90 samples from patients with positive stools and ten healthy controls from a non-endemic area
	NIE-ELISA[c]	84.0	100	
	NIE-LIPS[c]	97.8	100	
[43] (2008)	NIE-ELISA[c]	97	95	Prospective design. Specificity assessed in a small group of healthy controls, but also confirmed (for LIPS) in samples from patients with filarial and other helminth infections
	NIE-LIPS[c]	97	100	
[47] (2007)	IFAT[d]	74.1	98.4	Accuracy (high titre) determined with latent class analysis to cope with the lack of a reference standard. Coprological methods used for the model not optimal for *S. stercoralis*
[49] (2006)	IFAT[d]	97.4	97.9	Reference standard for sensitivity: samples from patients with *S. stercoralis* larvae in stools. Reference standard for specificity: samples from controls with no risk of infection and negative stools. Cross-reactivity assessed separately
[55] (2011)	IFAT[d]	73	NA	Study on HIV-positive subjects, with average CD4 count of 373/μL

Ag, antigen; HIV, human immunodeficiency virus; IFAT, immunofluorescence antibody test; LIPS, luciferase immunoprecipitation system; NA, not available; ROC, receiver operating characteristic.
[a]Crude Ag (*S. ratti*), commercially available.
[b]Crude Ag (*S. stercoralis*), commercially available.
[c]Recombinant Ag, in-house.
[d]Crude Ag (*S. stercoralis*), in-house.
[e]Commercially available for study purpose only.
[f]Recombinant Ag, commercially available.

serology microwell ELISA, a test available for research use only) and with NIE-LIPS [37]. The last of these, which has not yet been made commercially available, was more accurate than the two commercial tests. Recent, selected studies on the accuracy of serological methods are summarized in Table 2; this demonstrates, in general, not only high sensitivity, as expected, but also specificity of >90% in most reports, although the results should be interpreted with caution, as they are influenced by the kind of reference standard used.

In fact, a methodological problem in assessing test accuracy is that a gold standard is not available. Faecal results alone do not provide an acceptable reference standard, owing to their poor sensitivity. For this reason, diagnostic studies on traditional, faecal-based methods (such as those reported in Table 1) tend to overestimate the test sensitivity. A recent meta-analysis on the evaluation of conventional parasitological methods found the highest sensitivity (89%) for APC, followed by the Baermann technique (72%), FECT (48%), and direct wet smear (21%), but the results were hampered by the reference standard used, which was also based on faecal methods [45].

If the larval output in stool is minimal, which is often the case in chronic infections, any faecal test would be likely to miss the infection, whereas a serological test give a positive result. If the accuracy of serology were to be evaluated against a faecal-based reference standard, discordant results (in particular: faecal-negative and serology-positive results) would be impossible to classify. This problem has been circumvented in several ways. Some researchers have estimated the accuracy of serology (along with the prevalence of *S. stercoralis* infection in selected areas) by using special statistical techniques, such as a Bayesian model [46] or latent class analysis [47]. More recently, the accuracy of five different serological tests was evaluated with a composite reference standard [48]. The tests evaluated were the two NIE-based assays and the two commercial ELISAs mentioned above, plus an in-house immunofluorescence antibody test (IFAT) [49], using for antigen preparation intact *S. stercoralis* filariform larvae obtained from a charcoal faecal culture [36]. The negative predictive value was almost 100% for all tests at low prevalence. This means that a single test is sufficient to rule out the infection, if it gives a negative result. This is not so for a recently acquired infection, e.g. in travellers, for which the sensitivity of serology was found to be much lower than for chronic infections in immigrants: 73% vs. 98% [50]. Acute strongyloidiasis in travellers [51] is a rare occurrence [8], but, in cases of clinical suspicion, the clinician should be reminded that the serology is not sufficient to rule out the infection if it gives a negative result. For patients with high pretest probability (e.g. a subject with a generalized itching and eosinophilia and a compatible exposure history, or the screening of a transplant candidate from a high-prevalence area), a single, negative serological test result will not safely exclude the infection, and a second serological test, preferably based on a different antigen, and/or a faecal-based test, should be added. The choice of the latter depends on local availability, and clear evidence supporting the use of a molecular method [52–54] rather than a 'traditional' one is still lacking. A potential alternative to excess testing might be presumptive treatment, as postulated by some [14], considering that the treatment agent of choice, ivermectin, is generally well tolerated. The cost-effectiveness of the two competing approaches has yet to be evaluated [14]. We think that presumptive treatment is justified if sensitive diagnostic tools are not available, or, even if they are and the results are negative, when a potentially exposed patient is symptomatic and other causes have been reasonably excluded. A particular case is the diagnosis of *S. stercoralis* in a subject who is already immunosuppressed, owing to medical conditions or treatments. Immunosuppression may cause diminished sensitivity of serum antibody detection [13,55]; therefore, serology should never be used alone in those subjects. Moreover, in cases of hyperinfection due to immunosuppression, the larval output is much higher, resulting in much better sensitivity of all faecal-based methods [13].

The use of molecular methods in parasitological diagnosis is still limited to a few reference laboratories, mostly in industrialized countries [52]. Only a few commercial kits are available, and the protocols used are not standardized. Major differences concern DNA extraction, the size and mode of preservation (if any) of the stool specimens, and the solutions used to neutralize potential PCR inhibitors. All of these, together with the critical issue of amplification, affect sensitivity, as mentioned above. Moreover, the criteria for the assessment of test accuracy are also variable (Table 3). Although the sensitivity is certainly lower than that of serology, it is also still unclear whether PCR and/or RT-PCR at least equal the sensitivity of the best faecal-based traditional methods, such as the Baermann technique or APC, as results from different studies are not in complete agreement [13,31,56,57]. On the other hand, the costs of RT-PCR are not necessarily higher than those of traditional microscopy [56], and may be even lower, considering the time saved [27,56].

Monitoring of cure in clinical care and clinical trials

The lack of a reference standard for diagnosis is a serious problem when the efficacy of a treatment is evaluated. A limited number of clinical trials on *S. stercoralis* treatment have been conducted during the last three decades, usually comparing thiabendazole, albendazole, and ivermectin. Although no

TABLE 3. Main characteristics of molecular biology techniques for *Strongyloides stercoralis* diagnosis according to recent studies

Reference (year)	Test assessed/ region targeted	Extraction/type of specimen	Sensitivity	Specificity (%)	Study characteristics
[28] (2011)	Fret-PCR/18S rRNA	QIAamp DNA stool MiniKit/ 100 mg unpreserved	LOD 4 × 10^2 copies/ reactions (40 larvae per gram)	100	Sensitivity calculated as LOD Specificity evaluated in a control group of negative stool samples from healthy adults plus stool samples positive for other parasites
[30] (2013)	RT-PCR/18S rRNA	Comparison of five methods: four manual and one automatic	10^{-2} dilution LOD	100	Sensitivity calculated as LOD in serial dilutions of *Strongyloides ratti* larvae spiked into stools Specificity calculated in 58 definitely negative samples
[52] (2009)	RT-PCR/18S rRNA, cytochrome *c* oxidase, sequence repeat	QIAamp DNA stool MiniKit/ 100 mg unpreserved; 2% PvPP	33/38[a] (87%) or 33/54[b] (61%)	100	For sensitivity, the denominator was: positive Baermann or single[a] or duplicate[b] APC. RT-PCR also detected 12 samples negative with both techniques Specificity was calculated by checking the oligonucleotide sequences on BLAST and in 145 definitely negative stool samples
[54] (2011)	Single and nested PCR/ ITS1-5.8S-ITS-2	QIAamp DNA stool MiniKit/ 3 g preserved sample (EtOH 70%)	Single PCR 100% Nested PCR 75%	100	Sensitivity calculated in 16 APC-positive samples Specificity calculated in stool-negative control group (35 APC-negative samples)
[59] (2014)	RT-PCR/18S rRNA	QIAamp DNA stool MiniKit/ 200 mg unpreserved	LOD 0.1 pg	100	Sensitivity calculated as LOD Specificity calculated in 13 definitely negative samples
[73] (2014)	LAMP/28S rRNA	QIAamp DNA stool MiniKit/ 100 mg unpreserved; 2% PvPP	LOD <10 copies	100	Sensitivity calculated as LOD Specificity calculated in 38 stool samples definitely negative for *S. stercoralis*
[87] (2011)	RT-PCR/18S rRNA	QIAamp DNA stool MiniKit	LOD 10 copies	100	Sensitivity calculated as LOD Specificity tested against DNA controls derived from a wide range of intestinal microorganisms

LAMP, loop-mediated isothermal amplification; LOD, limit of detection; PvPP, Polyvinylpolypyrrolidone.

systematic review or meta-analysis has been published so far, the evidence consistently shows that albendazole is unsatisfactory [58–60], and should no longer be considered as a treatment for this condition, although, in many countries, it is the only drug registered for this use. Thiabendazole and ivermectin have similar efficacies, but the much better tolerability of the latter has made it the drug of choice worldwide [36]. So far, all trials but one have been based exclusively on faecal methods. Ivermectin has been found to be highly effective (>90% in most trials), but the relative lack of sensitivity of faecal diagnosis is likely to have caused an overestimation of the drug efficacy [14]. The only trial that included serology in the assessment of cure [61] found much lower efficacy of ivermectin than previously reported. Serology should be mandatory for inclusion in a trial and as a marker of cure in combination with faecal methods. If serology is positive at inclusion, failure of it to become negative at 6–12 months of follow-up, or at least to show a consistent decrease in serological titre, should be regarded as a potential failure, even if faecal tests give negative results [13]. Several studies have reported that the serological titre usually tends to decrease after treatment [49,62,63]. For example, in a study in Italy on an in-house IFAT, 60% of the subjects showed either complete seroreversion or a decrease in antibody titre of at least two-fold within 4 months of treatment [49]. Another study in Australia on an in-house ELISA based on crude antigen showed, for 65% of the patients, either complete negativization or a consistent decrease in optical density within an average of 9 months after treatment [64]. In another and more recent study in Spain, using the commercially available IVD-ELISA, the same result was obtained for 81% of the treated patients after a 6-month follow-up [63]. Unfortunately, just as for diagnosis, a reference standard for cure is not available. We have recently shown, using a surrogate, composite reference standard for cure, that antibody titre (of IFAT, IVD-ELISA, Bordier ELISA, NIE-ELISA, and NIE-LIPS) tends to consistently decrease after effective treatment [11]. The difficulty is that negativization, or at least a consistent reduction in titre, takes much longer than with faecal-based tests, which hampers the use of serology for clinical trials in endemic countries, because of the risk of re-infection during the follow-up period. A randomized controlled trial is currently ongoing in Italy, Spain and the UK on single-dose ivermectin vs. multiple-dose ivermectin, combining serology with faecal methods for inclusion and for monitoring cure, with follow-up carried out 6 and 12 months after treatment (https://clinicaltrials.gov/ct2/show/NCT01570504). This trial is only including immunocompetent subjects who are not exposed to the risk of re-infection (immigrants from endemic areas or European subjects who were infected earlier in life when transmission was still occurring in Italy and Spain). The criterion for cure is a negative faecal test result at follow-up (comprising APC and/or RT-PCR), plus at least a two-fold reduction in antibody titre.

An assessment of the main diagnostic tools for the three main purposes is summarized in Table 4.

Possible new scenarios

Serology for *S. stercoralis* is likely to be further improved by the use of recombinant antigens. Recently, with the same NIE antigen

TABLE 4. Summary of diagnostic approaches to *Strongyloides stercoralis* diagnosis in epidemiological surveys, individual diagnosis and screening, inclusion in clinical trials, and monitoring of cure

Diagnostic tools	Prevalence surveys	Individual diagnosis and screening	Clinical trials and assessment of cure	References
STS	Potential use for faecal surveys instead of the Kato–Katz technique	Suitable for basic laboratories in endemic areas; conflicting results on sensitivity	Unsuitable	[23–25]
Formol–ether concentration	With simple modification, may approach Baermann or APC, but more studies are needed	Unsuitable (suboptimal sensitivity)	Unsuitable	[13,14]
Baermann, Koga APC	More reliable figures than with other faecal tests	Up to now the most accurate faecal tests, but do not exclude infection if negative; cumbersome, not routinely performed	Suitable, but will tend to overestimate cure rate if used alone	[2,13]
PCR, RT-PCR	Potentially excellent tools; figures comparable with those obtained with Baermann or APC according to some studies	Good, potentially cost-effective, allow simultaneous detection of multiple pathogens; low sensitivity for light infections according to some studies	May become reference faecal tests for inclusion in trials and cure monitoring, but more evidence is needed	[14,27,29–31,52,53,56,57,87]
Serology (IFAT, commercial ELISAs, NIE-LIPS)	Prevalence invariably higher than with any faecal method; may overestimate, because of false positives and cross-reactivity, but, with all tests, excellent PPV at higher cut-offs. The use of dried blood spots is a potential innovation for surveys	Most sensitive test, should be mandatory. NPV 100% at low prevalence (can be used alone if negative). Need for a second test for high prevalence or suspicion. Promising development with recombinant antigens. NIE-LIPS 100% specific. All serological tests lose sensitivity in immunocompromised patients (faecal test mandatory)	Should be mandatory. Use in combination with a faecal test. Cure determined by negativitization of faecal test plus negativization or consistent reduction in titre of serology	[3,11,13,14,32,33,35–37,43,44,61,63]

IFAT, immunofluorescence antibody test; LIPS, luciferase immunoprecipitation system; NPV, negative predictive value; PPV, positive predictive value; STS, sedimentation in tube.

mentioned above, a new, rapid serological assay has been developed, making use of novel, high-sensitivity diffractive optics technology, providing, in <30 min, results that were fully consistent with those of NIE-ELISA [65]. Incorporation of other recombinant proteins of *S. stercoralis* in the same platform in a multiplex format might further increase the accuracy of this method.

In molecular biology research, another interesting area is loop-mediated isothermal amplification (LAMP). It is characterized by the use of six different primers specifically designed to recognize eight distinct regions on a target gene, with amplification only occurring if all primers bind and form a product [66]. In the last few years, parasitologists have adapted the LAMP approach for the detection of several parasites, including *Echinococcus* [67–69], *Taenia* [70–72], *Loa loa* [73,74], *Opistorchis* [75], *Clonorchis* [76], filariae [77], and *Trichinella* [78]. The method appears to be promising for *S. stercoralis* too [79], although it still needs to be validated in clinical practice [13]. LAMP is cheaper than real-time PCR, has reasonably good sensitivity and specificity, and could be used as a diagnostic tool for field testing [80].

The recent finding that microRNAs (miRNAs) can be released from mammalian cells and tissues into the circulation has stimulated extensive interest in the potential use of these molecules as biomarkers [81–83]. miRNAs are small, non-coding RNA molecules (containing ~22 nucleotides) that function in RNA silencing and post-transcriptional regulation of gene expression. miRNA-based diagnostics are being developed for a number of diseases, and, although RT-PCR is the most common detection method at present, there is great interest in improving and diversifying detection technologies, which may provide more field-friendly tools, as suggested by a recent study reporting the experimental use of this approach for *Schistosoma* infection [84]. The authors show that miRNAs derived from *Schistosoma mansoni* are present in infected mouse and human serum, and offer advantages over endogenous miRNA as biomarkers of infection. To our knowledge, there are not yet any published studies regarding the investigation of *S. stercoralis*-derived miRNAs in host serum. It would be of great interest to investigate whether this could represent a novel, sensitive biomarker for the diagnosis of strongyloidiasis in humans.

A recent study focused on detecting genes essential for parasitism in *S. stercoralis*, using microarray technologies and next-generation sequencing [85]. According to the authors, a comprehensive genome project, comprising not only *S. stercoralis* but also *S. ratti*, *Strongyloides papillosus*, *Strongyloides venezuelensis*, and *Parastrongyloides trichosuri*, is nearing completion, and the annotated genomes of these parasites will be published in the very near future. If a subset of genes regulated preferentially in L3a could be identified, this might contain potential targets for intervention to prevent the most serious complications of human strongyloidiasis [85].

Conclusions

In low–middle-income countries, just as in developed nations, there is a trend for there to be a decrease in the number of

personnel who are well trained in the microscopic identification of parasites. Also, although not only, for this reason, diagnostic methods that are not dependent on skilled microscopists are needed; therefore, the application of molecular assays is expected to increase in parasitology, which is still lagging behind in this respect, as compared with virology or bacteriology. With the relatively recent advent of microarray technology, and the advances in lab-on-chip systems, potentially allowing the simultaneous and fully automated detection of multiple pathogens [86], this will become even more true. However, in the particular case of *S. stercoralis* infection, molecular diagnosis has yet to demonstrate an optimal sensitivity, which is particularly required for this parasite, for which even very light infections are relevant and must be detected and treated. Until then, molecular diagnosis is unlikely to completely replace the other diagnostic techniques, and serological assays, having so far shown the highest sensitivity, will remain the mainstay of *S. stercoralis* screening, as well as an essential tool for prevalence surveys and for cure assessment in clinical settings and in treatment trials.

Transparency declaration

The authors declare that they have no conflicts of interest.

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