Evaluation of a Rapid Enzyme-Linked Immunosorbent Assay for Diagnosis of Strongyloidiasis[▽]

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Diagnosis of strongyloidiasis using stool examination remains unsatisfactory due to the lack of sensitivity and fastidious techniques. In this work, we investigated the value of an anti-*Strongyloides* IgG enzyme immunoassay (EIA), using a panel of 207 sera retrospectively collected from patients with definitive diagnoses of strongyloidiasis ($n = 57$), other helminthic infections ($n = 46$), eosinophilia without parasitic infection diagnosis ($n = 54$), and digestive disturbances following a tropical journey ($n = 30$) and from 20 negative controls. By following a receiver operating characteristic (ROC) curve analysis, it was possible to optimize the test to reach a sensitivity of 91.2% and a specificity of 93.3%, with 92.8% of patients correctly classified. Considering the incidence of strongyloidiasis diagnosed in our own laboratory, the negative predictive value was calculated at 99.9%. In conclusion, this test is very rapid and easy to perform and may be valuable for diagnosis of strongyloidiasis both in cases where the infection is unrevealed by a parasitological stool examination and in patients at risk for severe clinical forms, such as patients receiving immunosuppressive therapy.

Strongyloidiasis is due to the intestinal nematode *Strongyloides stercoralis*. Due to poor sanitary conditions (lack of latrines) and because warm and moist climates promote the achievement of the life cycle of the parasite, the prevalence of the disease remains high in tropical and subtropical regions of the world (11). Thus, in temperate-climate countries, the infection is almost exclusively seen in patients originating from or having lived in these regions of the world. Usually, strongyloidiasis is responsible for mild abdominal troubles such as pains, alternation of diarrhea, and constipation (15). This infection may also be present with pruritus and crawling sensations under the skin. A peculiar form is the larva currens syndrome, where larvae migrate into the derma. It is considered that the intensity of the symptoms is correlated with the digestive parasitic burden. However, in cases of immunosuppression, such as that induced by human T-cell leukemia virus type 1 (HTLV-1) infection, corticosteroid treatment, or cytotoxic chemotherapy, an uncontrolled life cycle can take place, leading to the so-called hyperinfestation syndrome and even to dissemination of larvae through the body, the latter being associated with a very bleak prognosis (1, 5, 10, 12).

Thus, it is essential to diagnose strongyloidiasis in patients coming from areas of endemicity, notably patients with mild or no symptomatic forms, before the initiation of any kind of immunosuppressive treatment. Indeed, the endogenous auto-infection cycle of the parasite promotes the persistence of the parasite for decades, and strongyloidiasis has to be screened for even in cases where the patient stayed in an area of endemicity in the distant past (8). Eosinophilia is indicative of the disease but is frequently mild and nonspecific. To date, diagnosis of strongyloidiasis relies on the demonstration of *S. stercoralis* larvae in stool specimens (4). According to the infestation level, larvae can be detected either by examination of a fresh stool specimen or after the use of concentration techniques such as that described by Junod (6). However, Baermann's method, based on the thermo-hydrotropism of the larvae, is considered the most sensitive, even in the absence of a well-conducted study, demonstrating the superiority of this method over the others (4). In addition, the output of larvae is, as for other digestive nematodes, irregular, a phenomenon that can lower the sensitivity of the tests. Indeed, it has been recommended that at least four negative results for stool examinations are required to rule out the diagnosis of strongyloidiasis (3). Stool culture for *in vitro* reproduction of the environmental cycle and release of new larvae may be more sensitive but needs to be done with fresh stool, is more laborious, and adds the potential risk of laboratory-acquired contamination. To circumvent these limitations, serologic assays have been developed, but most of the surveys focused on homemade tests using antigen prepared from *S. stercoralis* larvae collected from infected patients (9, 13, 15, 17). In this work, we evaluate a rapid enzyme-linked immunosorbent assay (ELISA) using a large panel of serum samples collected from patients, including some with definitive diagnoses of strongyloidiasis, other helminthic infections, or eosinophilia without parasitic infection diagnosis. It was demonstrated that with a cutoff value adjusted using receiver operating characteristic (ROC) curve analysis, the test can reach sensitivity and specificity values of 91.2 and 93.3%, respectively.

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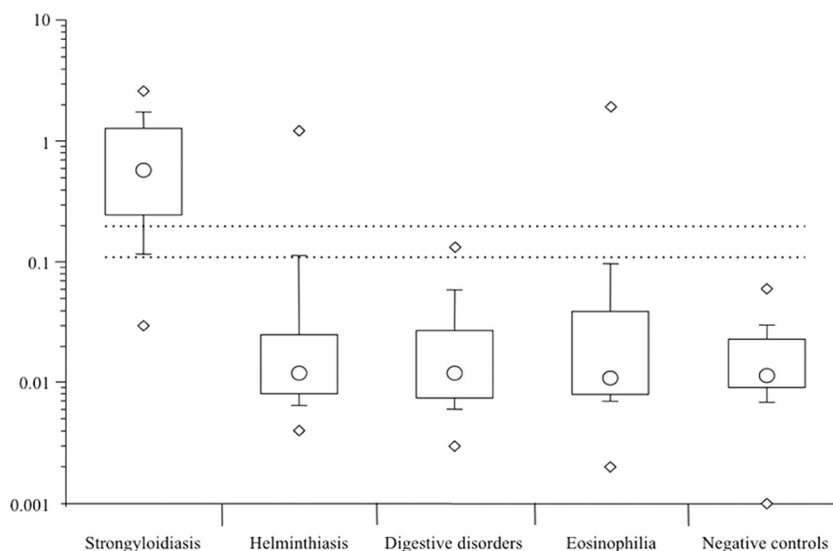
FIG. 1. Box plot of data from five groups of sera tested using the IVD ELISA for anti-*Strongyloides* IgG antibodies. Diamonds represent minimum and maximum values, central rectangles span the first quartile to the third quartile, bars above and below the box correspond to the 5th and 95th percentiles, respectively, and medians are represented by circles. Dotted lines represent the cutoff values recommended by the manufacturer (OD, 0.2) and used following a ROC analysis (OD, 0.11).

MATERIALS AND METHODS

Serum samples and patients. A collection of 207 sera retrospectively collected from 195 patients was assayed. All sera were stored at −20°C before being tested. For the majority of patients, all except those from group 5 (see below), biological data concerning parasitic stool examination and serological assays for cystic echinococcosis, schistosomiasis, filariasis, fasciolasis, and HIV infection were available.

Sera were divided into 5 groups as follows. Group 1 (57 sera and 48 patients) included patients with definitive diagnoses of strongyloidiasis based on demonstration of larvae in stool samples. Four patients had two samples collected at 2 (2 patients), 3 (1 patient), and 5 (1 patient) months before and after anti-*Strongyloides* therapy (with ivermectin in the first 3 cases and albendazole in the last case). Three patients had been tested for HTLV-1 antibodies and showed negative results. Group 2 (46 sera from 44 patients) comprised patients diagnosed with helminthiases such as filariasis ($n = 11$), schistosomiasis ($n = 22$), hymenolepiasis ($n = 5$), cystic echinococcosis ($n = 2$), trichuriasis ($n = 2$), enterobiasis ($n = 1$), toxocariasis ($n = 3$), and ancylostomiasis ($n = 1$). Group 3 included a panel of 30 sera collected from 30 returning travelers suffering from digestive trouble. For 9 of them, final diagnoses of protozoal digestive infection were documented (giardiasis [$n = 4$], sarcocystiasis [$n = 3$], and amoebiasis [$n = 2$]), with the results for other parasitologic investigations (parasitic stool examination and serologic tests) being negative. Group 4 corresponded to 53 patients (54 sera) with eosinophilia (eosinophil count, $>0.5 \times 10^9$/liter) for whom investigation (stool examination, serologic tests [mainly for schistosomiasis], and filariasis) failed to detect any parasitic disease. Group 5 corresponded to 20 sera collected from 20 pregnant women living in France without history of overseas travel.

EIAs. The test evaluated in this study (IVD Research, Carlsbad, CA) is CE marked but not approved by the FDA. It includes microtiter wells coated with the soluble fraction of *S. stercoralis* L3 filariform larval antigen (17). One hundred microliters of diluted sera (1/64) was dispensed into the wells and incubated for 10 min at room temperature. After the wells were washed three times with the provided washing buffer, 100 μl of protein A-peroxidase conjugate was added, and the mixture was incubated for 5 min at room temperature. The wells were then washed three times and slapped over a paper towel to remove excess moisture. One hundred microliters of tetramethylbenzidine was then dispensed into each well. After a 5-min incubation at room temperature, the reaction was stopped by the addition of 100 μl of 1 M phosphoric acid. In each assay, a negative control and a positive control provided by the manufacturer were included. Reading was done using a spectrophotometer at 450/650 nm to 620 nm (Asys Expert Plus microplate reader). The manufacturer recommends considering sera with optical densities (ODs) higher than or equal to 0.2 positive. This cutoff value was secondarily optimized by the establishment of a ROC curve (see below).

In cases of discrepancy between stool examination and enzyme immunoassay (EIA) results, a second EIA was performed using a kit commercialized by Bordier Affinity Products (Crissier, Switzerland). The sensitivity and specificity of the latter commercial ELISA have previously been calculated at 83% and 97.2%, respectively (17). In this test, *Strongyloides ratti* somatic larval antigens are used to coat the microwells. Sera were diluted 1:201 in Tris-buffered saline–Tween solution, distributed, and incubated for 30 min at 37°C in the wells. After a wash, a protein A-alkaline phosphatase conjugate was added and the plate incubated for 30 min. Then, after a wash and incubation with phosphatase substrate, addition of potassium phosphate stopped the reaction. Absorbance was measured at 405 nm. In each assay, a negative control, a weakly positive control, and a positive control provided by the manufacturer were included. An OD higher than that of the weakly positive control was considered a positive result.

Statistical analysis. Assay reproducibility was evaluated by calculating the coefficient of variation (the ratio of the standard deviation over the mean) of the positive control tested in each run. The performances of the test were evaluated by calculating specificity, sensitivity, efficiency (percentage of individuals correctly identified as having or not having strongyloidiasis), and positive and negative likelihood ratios (STATA/SE 10.0 for Macintosh) (5). In order to estimate positive predictive values (PPV) and negative predictive values (NPV), we retrospectively reviewed our charts to determine the annual incidence of strongyloidiasis diagnosed in our laboratory.

RESULTS

On the basis of 13 runs, the test was shown to be reproducible, with a calculated interassay coefficient of variation of 9.7%. Figure 1 illustrates the main results for each group of sera. With a cutoff value of 0.2, among the 57 sera of group 1 (confirmed strongyloidiasis), 47 sera were found positive, corresponding to a sensitivity of 84.2%. The specificity calculated using the 150 other sera reached 95.3%. By use of a ROC analysis, it was possible to improve the sensitivity of the test by lowering the OD cutoff value to 0.11 (Fig. 2). With this cutoff value, the sensitivity reached 91.2% while the specificity decreased to 93.3%, 92.8% of patients were correctly classified, and the positive and negative likelihood ratios were 13.68 and 0.094, respectively. In 2008, 13 strongyloidiases were diagnosed in our institution among 1,689 patients investigated for parasites in stools, corresponding to an incidence of 0.77%. With this value, the PPV and the NPV were 9.4% and 99.92%,
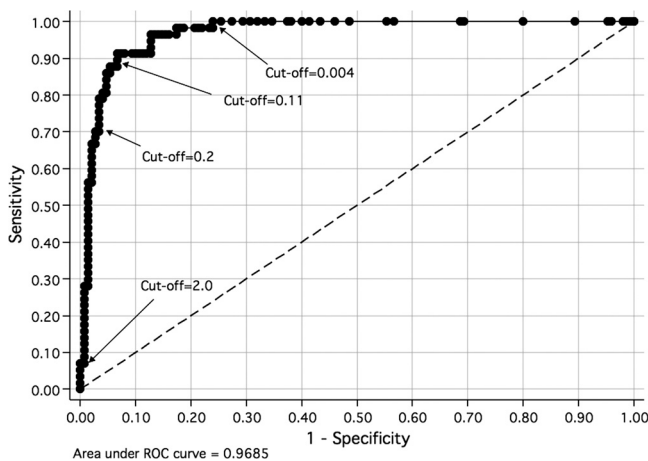
FIG. 2. ROC curve of cutoff values for the decision to diagnose strongyloidiasis on the basis of the OD value obtained with the IVD ELISA for anti-*Strongyloides* IgG antibodies.

respectively. Considering the potential indication of the test as a screening test, we decided to use the OD cutoff value of 0.11.

Regarding the post-anti-*Strongyloides* treatment, all patients treated within the interval between the assays showed decreases in the OD values of their respective serum samples, but none of these values became negative.

Discrepancies between EIA results (with the 0.11 OD cutoff value) and stool examination are reported in Table 1. Five sera from patients with confirmed strongyloidiasis were found negative. Among these patients, two were HIV positive, one had complicated diabetes mellitus, one had received a kidney transplant, and one suffered from liver cirrhosis. Four out of five also showed negative results with the anti-*Strongyloides* Bordier EIA.

Five sera from patients with other helminthiases (group 2) were considered false positive (with positive IVD EIA results and negative results for searches for larvae in stool samples), representing two cases of schistosomiasis and three of filariasis. Four of these sera were also positive for the Bordier test. A serum sample from group 3 representing a final diagnosis of giardiasis was found positive (OD, 0.132), while the Bordier test returned a negative result. Finally, five sera from group 4 (eosinophilia without parasitic infection diagnosed) were found positive. Two patients were not investigated using the Baermann method. Two showed positive results for the Bordier test. None of the sera from group 5 (negative-control group) was found positive.

DISCUSSION

Due mainly to immigration from tropical and subtropical regions, strongyloidiasis is one of the main digestive helminthiases diagnosed in microbiology laboratories of temperate-climate countries. On the other hand, the occurrence of immunocompromised status is increasingly observed with the extensive use of corticosteroid and other immunosuppressive therapies, notably for solid organ transplantations that are now proposed for more-elderly patients (7). Indeed, because of the high incidence of high blood pressure in the African population, as many as one-third of kidney transplantations are performed for patients originating from tropical areas (data not shown). Also, HTLV-1 infection, known to promote *Strongyloides* hyperinfection and recurrences, is highly endemic in West Africa (5, 10). Considering the time-consuming process and low sensitivity of stool examination for larvae, a reliable serological test would thus be of great value. The IVD assay tested in this work was easily performed in less than 30 min.

TABLE 1. Summary of discrepancies between IVD anti-*Strongyloides* EIA and stool parasitic examination results

IVD assay result and OD[a]	Stool examination result		Result for serologic test for underlying disease	Bordier assay result (OD)[b]
	Junod technique	Baermann method		
False negative				
0.07	Positive	ND[c]	HIV[+]	Negative (3.8)
0.05	Positive	ND	Liver cirrhosis	Negative (0)
0.03	Positive	ND	HIV[+]	Negative (8.4)
0.07	Positive	Positive	Diabetes mellitus	Negative (5.4)
0.07	Positive	ND	Renal transplantation	Positive (22.4)
False positive				
0.53	Negative[d]	ND	Serological schistosomiasis	Positive (13.7)
1.12	Negative	Negative	Filariasis	Positive (22)
0.42	Negative	Negative	Filariasis	Positive (44)
0.33	Negative	Negative	Filariasis	Positive (25)
0.133	*S. mansoni*	ND	Schistosomiasis	Negative (9.4)
0.132	*G. intestinalis*	ND	ND	Negative (2)
0.23[e]	Negative	Negative	Negative	Negative (5.8)
0.148[e]	Negative	ND	Negative	Negative (4.9)
1.948[e]	Negative	Negative	ND	Positive (100)
0.212[e]	Negative	Negative	Serological fasciolasis	Negative (2.9)
0.169[e]	Negative	ND	Negative	Positive (20.3)

[a] False positivity and negativity were determined using our modified cutoff value of 0.11.
[b] Results obtained with the Bordier EIA are reported for comparison; a value index of >10 is considered positive.
[c] ND, not determined.
[d] A urine parasitic examination revealed viable *Schistosoma haematobium* eggs.
[e] Patients with eosinophilia for whom parasites were not detected.

Compared to homemade EIAs, it eliminates the need for preparation of antigens, which is laborious and lacks standardization, which could have a negative impact on reproducibility. In a previous study by van Doorn et al., the sensitivity and specificity of this test were calculated, after elimination of sera from filariasis patients, at 89 and 97.2%, respectively (17). A second study used this test as a field-based diagnostic tool, comparing it to the Baermann method and stool culture on an agar plate (16). Interpretation of the results was difficult, as concordant data were lacking. Because of the known insensitivity of the Baermann method, the authors concluded in favor of the EIA because of its rapidity of performance, its high screening throughput, and its robustness and reliability on a day-to-day basis.

In our experience, when the cutoff value proposed by the manufacturer was used, the sensitivity reached only 84.2%, a value that may be insufficient for a screening approach, even though this test is probably more sensitive than a single stool examination (14). Negative results occurred for 9 patients having strongyloidiasis and concomitantly exhibiting an immuno-compromised status such as that involving HIV infection or immunosuppressive treatment for kidney transplantation. Since these patients will be particularly targeted by the test, we decided to lower the recommended cutoff value in order to obtain a sensitivity greater than 90%. This was done based on a ROC analysis, allowing the OD cutoff value to be lowered from 0.2 to 0.11, with an acceptable decrease in specificity from 95.3% to 93.3%. The specificity and sensitivity may be higher since in three cases considered false positive, the patients had eosinophilia and/or another helmintic infection(s), showed positive Bordier test results, and had been investigated with only a single Baermann test, which is considered insufficient to rule out diagnosis of strongyloidiasis (3). The eventuality of undiagnosed strongyloidiasis, alone or as part of a polyparasitism, cannot be ruled out. Similarly, a molecular approach testing PCR amplification from stools underlined the lack of sensitivity of stool examination for strongyloidiasis diagnosis (2). In our experience, very few patients, including those with eosinophilia, are in fact investigated with 3 Baermann tests (personal data). In addition, in this survey there were a couple of cases where the result for the first Baermann test was negative and then turned positive a few weeks later. In these cases, the result for the serological test was positive from the first date, suggesting, as already mentioned, that a single serological test might be more sensitive than several stool examinations (17). As already reported for other EIAs (13, 14), the high NPV value (99.9% in our case) makes this test very suitable for screening patients at risk for strongyloidiasis and having to undergo solid organ transplantation or an immunosuppressive therapy course.

Serologic assays for diagnosis of digestive helminthiases have long been considered to be of poor value due to their lack of specificity. Filariasis appeared to be the major cause of false-positive results in our study and that of van Doorn et al. (17). It is thus recommended that results for anti-Strongyloides tests be interpreted with caution for patients having histories of or suspected of having filariasis. Having said that, one can note that only 2 (both representing schistosomiasis) out of the 46 sera collected from patients with helminthiasis cross-reacted with the anti-Strongyloides test. Specificity among patients without history of travel was 100%.

In summary, the EIA tested in this work is a rapid, easy-to-perform, and valuable test that may be useful both for detecting strongyloidiasis undiagnosed using stool examination and for ruling out diagnosis of strongyloidiasis in patients at risk for severe clinical forms.

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