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SERODIAGNOSIS OF STRONGYLOIDIASIS. THE APPLICATION AND SIGNIFICANCE [1]

Yoshiya SATO (2, 3), Jun KOBAYASHI (2, 4) & Yoshiyuki SHIROMA (5)

SUMMARY

Parasitological diagnosis based on the faecal examination is frequently difficult in cases of chronic, low-level *S. stercoralis* infection. Even when a newly developed sensitive method (an agar plate culture) is applied, it is essential to examine faecal samples repeatedly to achieve a correct diagnosis. Additionally, it is important to note that a negative result does not necessarily indicate the unequivocal absence of the infection.

On the other hand, several serological tests which have recently been developed for strongyloidiasis have proven reliable when used to complement parasitological examination. We have developed two serological tests, ELISA and GPAT, to demonstrate *Strongyloides* infection and possible applications of the serological tests for diagnosis, mass-screening, epidemiological study and postchemotherapy evaluation of strongyloidiasis were reviewed based on our recent studies.

KEYWORDS: Strongyloidiasis; *Strongyloides stercoralis*; Serodiagnosis; ELISA; gelatin particle agglutination test (GPAT).

INTRODUCTION

Strongyloidiasis, which is relatively common in tropical and subtropical areas, is a parasitic disease resulting from an infection with a nematode *Strongyloides stercoralis*. One of the unique properties of the parasite is its ability to propagate in a host by internal autoinfection. It is probable that the autoinfection commonly occurs in human strongyloidiasis and that the phenomenon is responsible for pathogenicity in the parasitic infection. The parasite is usually non-pathogenic in an immunocompetent host, but due to the autoinfection, the infection often progresses to the fatal hyperinfected state under immunosuppressed conditions [27].

One of the current problems concerning strongyloidiasis is the difficulty to detect *S. stercoralis* larvae in stools, because the majority of recent cases involve chronic, low-level infection. Due to the opportunistic nature of the parasitic disease, to prevent severe or fatal infection it is essential to develop a reliable immunodiagnostic method to complement the parasitological diagnosis of chronic infection.

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(2) Department of Parasitology.
(3) Research Center of Comprehensive Medicine, School of Medicine, University of the Ryukyus, Nishihara, Okinawa 903-01, Japan.
(4) Laboratório de Imuno-Parasitologia, Gastrocentro, Universidade Estadual de Campinas, SP, Brasil.
(5) Izumizaki Hospital, Naha, Okinawa 901, Japan.
Correspondence to: Dr. Yoshiya Sato, Departament of Parasitology, School of Medicine, Univerrsity of the Ryukyus, 207 Nishihara, Okinawa 903-01, Japan.

TABLE 1

Results of re-examination on 123 persons with proven *Strongyloides* infection

Area	No. times examined	No. examined	No. reconfirmed (%)	No. reconfirmed (%) by:			
				Direct smear	Concentration	Faecal culture using: (Filter paper)	(Agar plate)
Osato	Once	90	54(60.0)	14(15.6)	22(24.4)	15(16.7)	52(57.8)
Gushikawa	Twice	33	29(87.9)	17(51.5)	15(45.5)	N.D.	28(84.8)

The inhabitants were found harboring the parasite in mass examination by repeated stool examination (an agar plate culture method). They were left for 5 months in Osato and 18 months in Gishikawa without any treatment and were re-examined to reconfirm the presence of faecal larvae. The faecal re-examinations were performed once in Osato and repeated 3 times in Gishikawa.
The presence of faecal larvae could not be reconfirmed in as many as 40% of the persons in Osato, and in 12% in Gushikawa even where the follow-up examination was repeated for 3 consecutive days.
If conventional methods other than the agar plate culture method were applied, the reconfirmation rate was only 45-51% even in the case of repeated examinations.

The parasitic infection has been known to be highly prevalent in Brazil and is one of the problems of medical importance, as an opportunistic pathogen, under the increasing use of immunosuppressive therapy and the presence of many AIDS cases [27].

EFFICACY OF FAECAL EXAMINATION

Parasitological diagnosis of strongyloidiasis is probably one of the most difficult problems in clinical parasitology. A filter paper culture (Harada-Mori method) has been a conventional method for detecting the larvae in stool samples. However, this method is not considered sensitive enough for diagnosis of chronic cases because in such cases the larvae are present only in very small numbers or frequently absent from stool specimens. In a previous investigation, JONES (1954) detected the larvae in only 27% of the faecal samples collected from 100 patients with proven *Strongyloides* infection [14]. Similarly, GROVE (1980) has shown that the positive rate of faecal larvae was 55% for 44 chronic strongyloidiasis patients upon initial axamination by the faecal culture method and that it was only 78% even after two or more attempts [11]. It was also our experience, as shown in Table 1, that a single stool examination has a detection rate of only 15-24% by direct faecal smear, filter paper culture and formalin-ether concentration methods, when 90 persons with proven *Strongyloides* infection were re-examined several months later without treatment. Even if the examinations were repeated daily for three consecutive days, the reconfirmation rate was about 50% by the direct smear and about 45% by the concentration method.

Recently, another method for faecal culture was developed in Okinawa, in which a faecal mass of about 3g was placed on the center of a primary agar plate and incubated for 3 days [1]. In this method, unique alignments of bacterial colonies on the surface of the agar plate, which formed along the tracks of wandering larvae, prompted us to suppose the presence of the larvae. Using this new method, we were able to more effectively diagnose chronic infection. The reconfirmation rate, however, was still less than 60%, if stool examination was performed only once, even when the agar plate culture method was applied (see Table 2).

Whatever the method used, at the present time, several faecal specimens collected on different days ought to be examined to obtain a correct diagnosis and

TABLE 2

Serodiagnosis of strongyloidiasis

Serologic method	Antigen used	Reference
Skin test	*S. stercoralis*	7, 18, 21)
	S. ratti	29)
	S. fuelleborni	3)
IFAT	*S. stercoralis*	5, 6, 9)
	S. stercoralis & *S. ratti*	12)
ELISA	*S. stercoralis*	2, 10, 16, 20)
	S. stercoralis & *S. ratti*	8, 17)
	S. ratti	4, 30)
IHAT	*S. stercoralis* & *S. ratti*	8)
GPAT	*S. stercoralis*	24, 26)

IFAT: indirect fluorescent antibody test; ELISA: enzyme-linked immunosorbent assay; IHAT: indirect hemagglutination test; GPAT: gelatin particle indirect agglutination test.

most importantly, failure to demonstrate larvae cannot be unequivocally interpreted as absence of the infection. This is especially true if the patient is to undergo immunosuppressive therapy.

SERODIAGNOSIS OF STRONGYLOIDIASIS

The serological diagnosis of strongyloidiasis has received relatively little attention because of low pathogenicity in immunocompetent hosts and perhaps also because of the difficulty to obtain an adequate antigen. Recently, there have been many reports concerning the serodiagnosis of strongyloidiasis. In Table 2, serologic methods applied for diagnosis are summarized. The ELISA test is currently used for serodiagnosis and has proven reliable in a number of laboratories. The indirect fluorescent antibody test has also been used with promising results in several laboratories, revealing that more than 90% of the patients were positive for the antibody [5, 6, 9, 12].

We have also prepared an antigen of *S. stercoralis* filariform larvae collected by the mass-culture of patients' stools and have clearly detected precipitin bands between the antigen extract and patient's sera by the gel-diffusion test (Ouchterlony method) [19]. Since it was difficult to obtain large amounts of antigen from the larvae, the micro-ELISA using a microtiter plate, which can be performed with a small amount of antigen, was further developed to diagnose many cases [20, 25]. The antibodies specific to the parasite were easily detectable among the patients by the assay (Fig. 1), indicating that *Strongyloides* infection well elicits humoral immune response in hosts and that serological testing may effectively complement other methods already in use for diagnosing the parasitic disease [25].

Most recently, we established another serodiagnostic method; an indirect agglutination test using newly developed gelatin particles (Fig. 2) [24]. The results obtained by the agglutination test were quite comparable to those of the micro-ELISA. The test is simple and rapid to perform and does not require specialized equipment. Additionally, the gelatin particles can be lyophilized to store for long periods after sensitization with antigen.

The ELISA method may provide an effective and a less labor-intensive method for mass-examination screening where large numbers of samples require testing. On the other hand, the gelatin particle indirect agglutination test (GPAT) can be performed rapidly and it seems to be convenient for diagnosis of few occasional cases suspected of *Strongyloides* infection. We have prepared a simple agglutination test kit [24] for strongyloidiasis to be used in routine medical examination in hospitals.

Specific cross-reactions between *S. stercoralis* and other species of animal origin have been exploited for serodiagnosis because *S. stercoralis* is difficult to maintain as an antigen source in the laboratory. When the antigenic composition of whole worm extracts from rodent *Strongyloides*, *S. ratti* and *S. venezuelensis*, was compared with that of *S. stercoralis*, strong cross-reactions with patient's sera were observed among the antigens, indicating that these rodent species could be

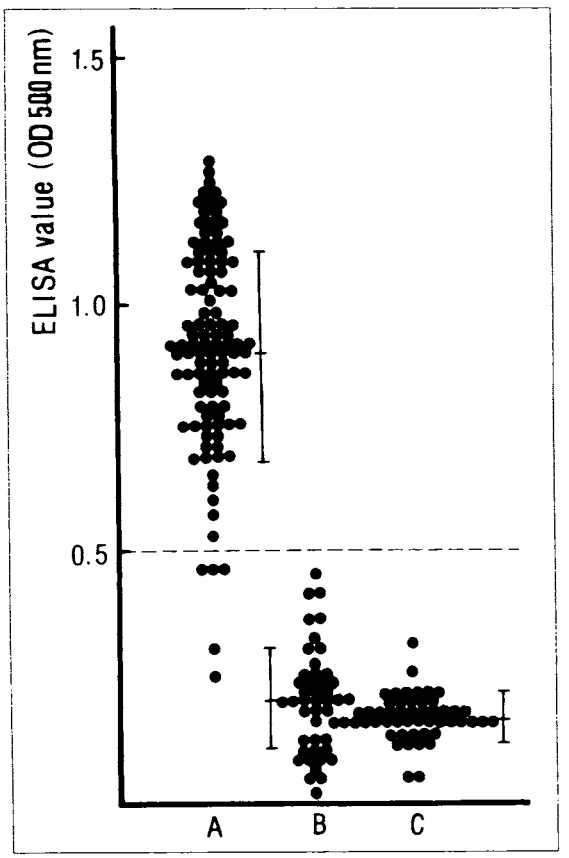

Fig. 1 - The distribution of ELISA values to *S. stercoralis* in inhabitants with or without *Strongyloides* infection. A: Sera of inhabitants in Okinawa, who were proven to be infected with *S. stercoralis* by faecal examination. B: Sera of inhabitants in Okinawa, in whose faeces the presence of *Strongyloides* larvae could not be demonstrated. C: Sera of inhabitants in Niigata, a non-endemic area in Japan for strongyloidiasis. The mean ELISA value was significantly higher in the *Strongyloides*-positive subjects than those of the two negative control groups. When the mean value +3S.D. in the uninfected controls in Okinawa (Group B) were assumed as a criterion of the positive antibody response (dashed line), false-negative results were observed only in 4.7% (5/107).

TABLE 3

Results of screening test for serum antibody to *S. stercoralis* and of subsequent faecal examination on the antibody positive persons in three areas, Okinawa, Japan.

Area	Method	Screening test	Faecal examination	Estimated infection rate
		No. posit./No. exam.(%)	No. posit./No. exam.(%)	
Gushikawa	ELISA	101/858 (11.8)	36/73 (49.3)	5.8
Nakazato	ELISA	144/849 (17.0)	50/93 (53.8)	9.1
Sashiki	ELISA	332/1,199 (27.7)	136/268 (50.7)	14.0
	GPAT	416/1,199 (34.7)	139/333 (41.7)	14.5

In the ELISA screening, about 12 to 28% of the inhabitants were considered to be positive for anti-*Strongyloides* antibody. The antibody positive persons received repeatedly the subsequent faecal examination by faecal culture (an agar plate culture) and faecal concentration method, and more than a half of them were found actually harboring the parasite.

In Sashiki, sera from 1,199 inhabitants were tested both by the ELISA and the GPAT. The positive rate of antibody by the GPAT was relatively higher than that by the ELISA. The actual demonstration rate of faecal larvae, however, was lower in the GPAT screening, and the presumptive overall infection rate was almost the same in the two screening tests. The serological tests seem to provide a sensitive and less labor-intensive method to complement the direct parasitological examination.

utilized as antigen for serodiagnosis instead of *S. stercoralis* antigen [22, 23]. Utilization of the rodent species provides a constant and safe source of antigen for routine serodiagnosis in the laboratory.

APPLICATION FOR MASS-SCREENING

As mentioned above, repeated examination of stool samples collected on different days is essential for the correct diagnosis of chronic *Strongyloides* infection. However, it is difficult to perform such repeated examinations in a mass-examination where large numbers of inhabitants must be examined simultaneously. Therefore, serodiagnostic studies also have applications in screening tests. However, practical use of the serologic test for prior screening in mass examination has not been previously tried. In mass surveys for detecting strongyloidiasis, we had applied the above serological tests as a screening test in three areas in Okinawa [25, 26]. As shown in Table 4, about 11 to 28% of inhabitants showed positive antibody response in the micro-ELISA. A conclusive diagnosis of strongyloidiasis can be made when *Strongyloides* larvae are detected in stools and/or other specimens. Therefore, in antibody positive persons given repeated stool examinations, about a half of them were found to be actually infected with the parasite. The mean overall infection rate estimated from the results was as high as 10%, which was about 5 times higher than that of past surveys in the same areas.

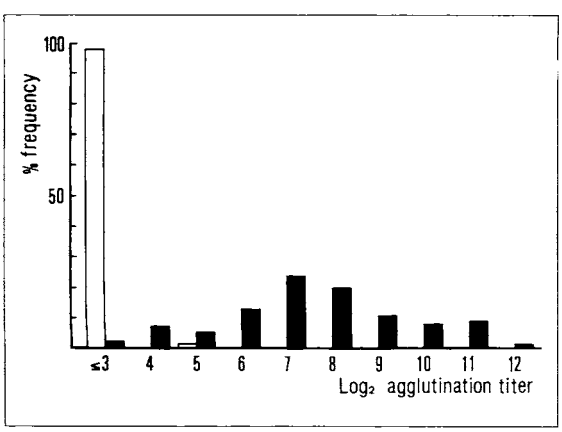

Fig. 2 - Percent frequency distribution of agglutination titers estimated with the antigen-gelatin particles in 92 patients with *Strongyloides* infection (■) and in 60 uninfected controls (□).

More than 97% of the patients gave positive agglutination responses at serum dilution of over 1:16, whereas in the control subjects the agglutination titer of 1:32 was detected only in one patient and the remaining 59 subjects showed negative results at the lowest serum dilution of 1:8.

The inhabitants in Sashiki were screened both by the ELISA and the GPAT. The positive rate of anti-*Strongyloides* antibody, as also represented in Table 3, was higher in the GPAT screening, whereas the detection rate of faecal larvae among antibody positive persons was lower in the GPAT screening. The estimated overall infection rate, however, was almost the same between the two screening tests, indicating that the indirect agglutination test should also be effective for screening for *Strongyloides* infection [26].

Thus, the serological tests were found to be useful for strongyloidiasis screening and for seroepidemiological purposes.

POSTCHEMOTHERAPY EVALUATION

Another possible use of the serological test is to assess the efficacy after treatment, because strongyloidiasis patients frequently fail to respond to anthelmintic treatment and also because it is difficult to confirm successful treatment by stool examination. In a previous study in which 7 patients were followed serologically for 2 to 4 months after thiabendazole treatment, GENTA & WEIL (1983) noted a significant fall in antibody titers to filariform larvae in all of the patients [9]. GROVE (1982) also followed 43 patients for 6 months after thiabendazole treatment and demonstrated a significant decrease in the blood eosinophil count and anti-*Strongyloides* serum antibody level in many patients who were negative in the follow-up faecal examination [13]. However, on the basis of the clinical and serological findings, he considered that perhaps one-third of the patients were still infected with the parasite.

In a mass-treatment of strongyloidiasis patients, we have compared the serum antibody titers specific for *Strongyloides* before and after treatment [28]. Fig. 3 represents the antibody ratios against the antibody levels before treatment in patients treated with pyrvinium pamoate. The antibody ratios were significantly low in the group found to be negative in the follow-up faecal examination, but not in the group treated unsuccessfully. In the latter group, the antibody ratio on follow-up examination was more than 0.6 in all but consisted of only two patients. From these results, if an antibody ratio of less than 0.6 was presumed as a criterion for successful cure, 42.1% and 46.2% of the patients in the group found to be negative for faecal larvae were interpreted to be equivocal for effective treatment. When further stool examinations were performed on the equivocal group 1 month later, 20% of them are additionally confirmed to be unsuccessfully treated.

The serological tests also seem to be reliable for postchemotherapy evaluation [15].

SIGNIFICANCE OF SEROLOGICAL TESTS

The following conclusions on the use and significance of serological tests can be drawn from the above results (Fig. 4).

In epidemiological mass-examination where a large group of individuals is to be examined, serological screening should be applied prior to stool examination. The micro-ELISA is convenient for such a mass-screening because large numbers of sera (96 sera per a microtiter plate) can be tested simultaneously in a relatively few hours. Once the positives are identified by the ELISA, they should be evaluated parasitologically to determine the actual presence of faecal larvae. Even if the results are negative in the parasitological examination, the seropositive individuals ought to receive further stool examination on separate ocasions.

In the case of clinical diagnosis of respective cases of suspected *Strongyloides* infection or when a risk factor for immunodepression exists, coprological examination is aplicable in advance of serological testing. Even if the reuslts are negative, these cases are to be further examined serologically to determine the possi-

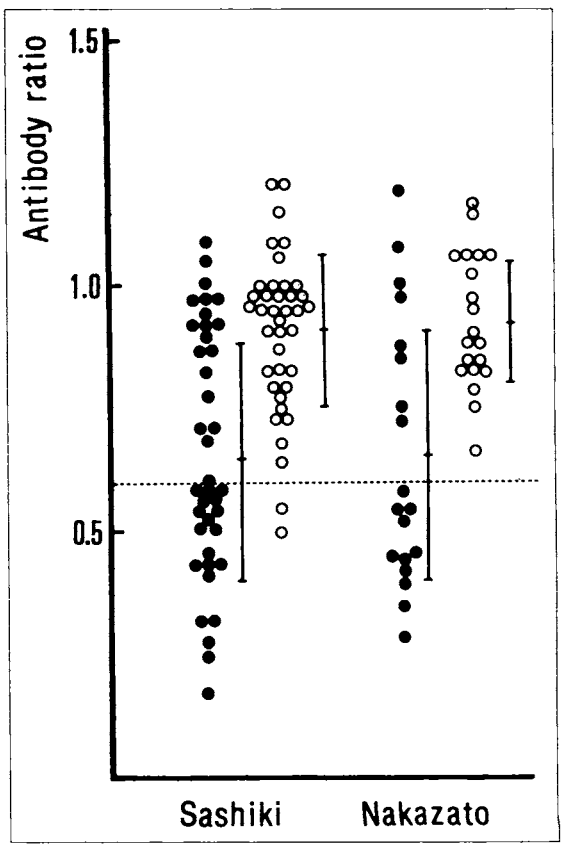

Fig. 3 - Comparison of ELISA values before and after pyrvinium pamoate treatment in strongyloidiasis patients. The data are represented as an antibody ratio against the antibody value before treatment, in relation to the results of follow-up faecal examination. A significant decrease in antibody ratio was observed in the group negative for faecal larvae (●), as compared to those in the group of unsuccessful treatment (○). The patients whose antibody ratios were more than 0.6 (dashed line) in the former group were interpreted to be equivocal for effective treatment.

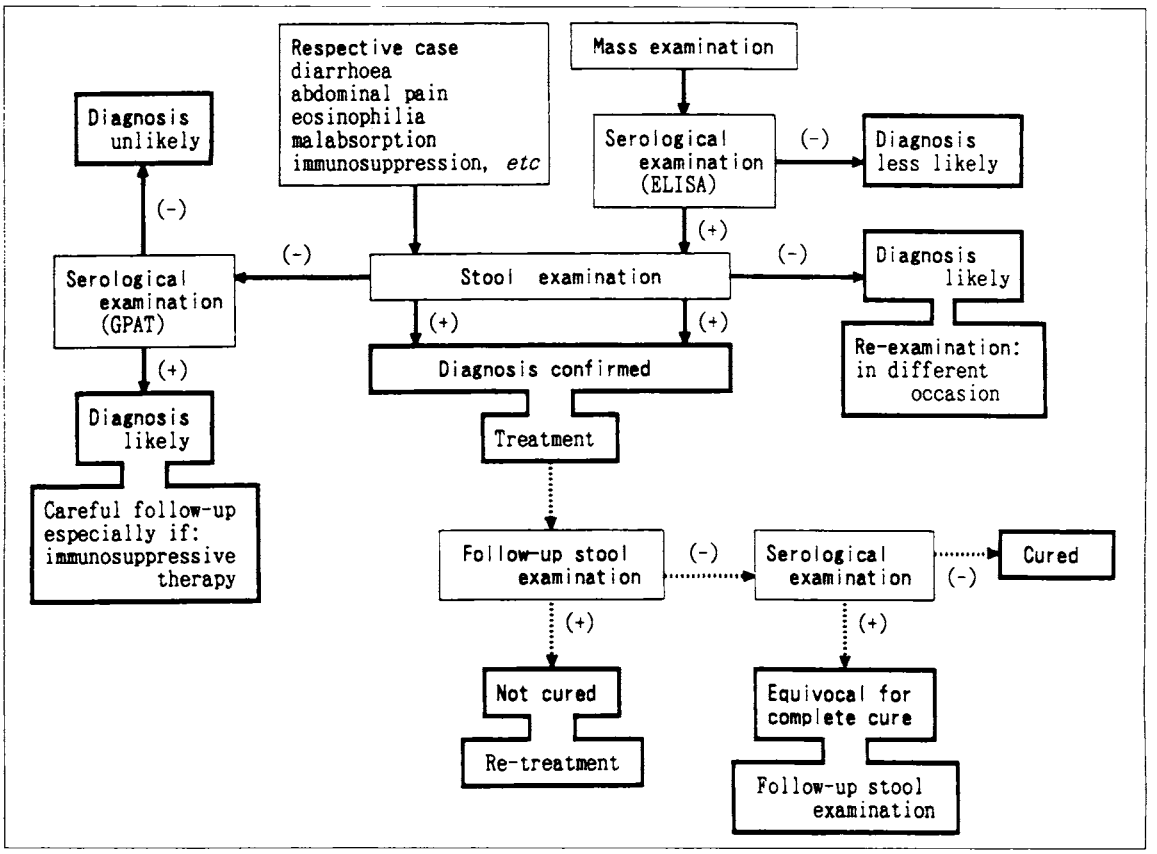

Fig. 4 - Flow chart for diagnosis and postchemotherapy evaluation of strongyloidiasis in endemic areas.
ELISA: enzyme-linked immunosorbent assay, GPAT: gelatin particle indirect agglutination test.

bility of occult infection. The GPAT, which can be performed within several minutes, is suitable for examination of such rare cases. When a positive antibody response to *S. stercoralis* is obtained, a careful observation is necessary to prevent a fatal hyperinfection. It is especially important during the time in which patients are in an immunosuppressed condition.

When the presence of an infection is confirmed, patients should be treated. The antibody response should also be monitored at appropriate intervals after treatment. When the antibody titers show no significant decrease after treatment, even if the coprological examination proves negative upon follow-up examination, the cases are interpreted to be equivocal for complete cure. In such cases, stool examinations should be repeated to confirm complete cure.

The above applications of serological testing may be effective for diagnosis, prevention and control of strongyloidiasis in endemic areas.

RESUMO

Aplicação e significado do diagnóstico sorológico na Estrongiloidíase

O diagnóstico parasitológico baseado no exame de fezes é muitas vezes difícil, principalmente nos casos de infecções crônicas ou leves pelo *S. stercoralis*. Mesmo utilizando o mais novo e sensível método (cultura em placas de ágar) é essencial examinar repetidamente as amostras fecais, para um diagnóstico correto. É importante ressaltar também que o resultado negativo não indica de modo inequívoco a ausência da infecção.

Por outro lado, vários testes sorológicos recentemente desenvolvidos para estrongiloidíase tem provado a sua eficácia quando usados para complementar exames parasitológicos. Para demonstrar infecção por *Strongyloides* desenvolvemos dois tipos de testes sorológicos - ELISA e GPAT - e, com base em nossos recentes estudos, apresentamos uma opinião sobre sua possível aplicação

para screening em massa, estudos epidemiológicos e avaliação pós-tratamento de estrongiloidíases.

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