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*Occult infection with **Strongyloides stercoralis** is crucial among immunocompromised patients leading to hyperinfection syndrome. As an alternative to routine stool examinations, the diagnostic value of an indirect immunofluorescent antibody test (IFAT) for detection of specific immunoglobulin G against S.stercoralis filariform larval surface antigens has been evaluated. The study was performed by using sera of 10 strongyloidiasis cases (group 1), 20 cases with parasitic infections other than **S.stercoralis** (group 2), 10 individuals living in Yasothorn Province without parasites detected from a single stool examination (group 3) and 10 negative control sera. Results revealed that all sera in group 1 gave positive IFAT. However, at the serum dilution titre of 1:64, 9 out of 10 cases in group 1, 8 cases in group 2 and 5 cases in group 3 had positive IFAT titre while all of the negative control group gave negative results of IFAT. Based on the results of a single stool examination among these groups, the sensitivity, specificity, positive predictive value and negative predictive value of the IFAT were 90%, 67.5%, 96.4% and 40.9%, respectively. The low negative predictive value might be due to the cross-reacting epitope(s) on the surface of nematode larvae since the IFAT titre could be reduced by preincubation of the sera with hookworm filariform larvae and Trichinella spiralis larvae. On the other hand, the presence of occult S.stercoralis infections in groups 2 and 3 could not be absolutedly excluded by a stool examination. Thus, IFAT may provide potential diagnostic implication in strongyloidiasis cases.*

Key words : *Strongyloidiasis, Imunofluorescence, Diagnosis.*

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การติดเชื้อพยาธิ Strongyloid ที่หลบซ่อนอยู่มีความสำคัญอย่างยิ่งในกลุ่มผู้ป่วยที่มีภาวะภูมิคุ้มกันต่ำ ซึ่งอาจก่อให้เกิดภาวะการติดเชื้อแบบแพร่กระจาย การประเมินคุณค่าของการตรวจสอบ antibody ชนิด immunofluoresent ที่จำเพาะต่อ antigen บนผิวของพยาธิ Strongyloid ระยะ filariform โดยวิธี immunofluoresent ทางอ้อม (IFAT) เพื่อเป็นอีกแนวทางหนึ่งในการตรวจการติดเชื้อพยาธิดังกล่าวนอกเหนือไปจากการตรวจอุจจาระโดยทั่วไป การศึกษาโดยใช้น้ำเหลืองของกลุ่มตัวอย่าง 4 กลุ่ม กลุ่มแรกเป็นผู้ติดเชื้อพยาธิ Strongyloid 10 ราย กลุ่มที่ 2 เป็นผู้ติดเชื้อปรสิตอื่น ที่ไม่ใช่พยาธิ Strongyloid 20 ราย กลุ่มที่ 3 เป็นผู้ที่ไม่พบการติดเชื้อปรสิตจากการตรวจอุจจาระครั้งเดียว ซึ่งอาศัยอยู่ในจังหวัดยโสธร 10 ราย และกลุ่มควบคุมซึ่งไม่มีการติดเชื้อปรสิตจำนวน 10 ราย ผลการศึกษาพบว่า น้ำเหลืองทุกรายในกลุ่มที่ 1 ให้ผลบวกในการทดสอบ อย่างไรก็ตามเมื่อทดสอบโดยเจือจางน้ำเหลือง 1 ต่อ 64 พบว่า กลุ่มที่ 1 ให้ผลบวก 9 ใน 10 ราย กลุ่มที่ 2 และกลุ่มที่ 3 ให้ผลบวก 8 ราย และ 5 ราย ตามลำดับ ส่วนกลุ่มควบคุมที่ไม่มีการติดเชื้อพยาธิให้ผลการทดสอบเป็นลบทุกราย เมื่อประเมินคุณค่าการทดสอบดังกล่าวพบว่า ให้ค่าความไว ความจำเพาะ ค่า positive predictive และค่า negative predictive ร้อยละ 90, 67.5, 96.4 และ 40.9 ตามลำดับ การที่ค่า negative predictive มีค่าต่ำอาจเกิดจาก epitope ที่คล้ายคลึงกันบนผิวของตัวอ่อนพยาธิตัวกลม เนื่องจาก titre ของ IFAT มีค่าลดลงภายหลังจากการดูดซับน้ำเหลืองด้วยตัวอ่อนระยะ filariform ของพยาธิปากขอและตัวอย่างของพยาธิ Trichinella หรืออาจเนื่องมาจากตัวอย่างในกลุ่มที่ 2 และ 3 มีพยาธิสตรองจิลอยด์หลบซ่อนอยู่ ซึ่งตรวจไม่พบจากการตรวจอุจจาระครั้งเดียว ดังนั้น IFAT น่าจะมีคุณค่าในการวินิจฉัยการติดเชื้อพยาธิ Strongyloid

Strongyloidiasis, a soil-transmitted helminthiasis caused by *Strongyloides stercoralis* infection, has been an important public health problem worldwide but is more prevalent in tropical countries.[1] Infection is acquired by cutaneous or mucus membrane penetration of the infective filariform larvae. After the larvae gain access into the host's circulation they traverse the lungs and later they are coughed up into the upper respiratory tract and are subsequently swallowed into the gatrointestinal tract where they develop into adult worms. The parasitic female worms that burrow into the submucosa of the small intestine produce their offspring by parthenogen esis.[2] Infection can be chronic persistent since autoinfection occurs in the host's intestine. Clinical manifestations vary from acute or chronic intermittent diarrhea though most infected individuals remain symptomless. The widespread use of chemotherapeutic and immunosuppresive drugs may have resulted in the increased incidence of disseminated strongyloidiasis.[3] Among immunocompromised patients, the parasites can multiply exceedingly and disseminate to other organs, resulting in a fatal outcome. Such a condition may be preventable by early detection and treatment.[1]

Diagnosis of strongyloidiasis is undertaken mostly by demonstration of rhabditiform larvae in stools. However, most of the infections may be left unrecognized since the shedding of the larvae in stool may be less than the detection threshhold of the methods currently used in routine coprological diagnosis such as the formalin-ether sedimentation technique and various cultivation methods.[1] To improve the sensitivity of routine stool examinations, it is neccessary to examine multiple samples or to exploit cultivation methods which are time consuming and labour intensive.[4] Therefore, recently several immunological diagnoses of strongyloidiasis have been developed, i.e. the enzyme-linked immunosorbent assay (ELISA) and western blot analysis using crude extract of the filariform larvae as antigens.[5-9] Here we attempted to assess the potential diagnostic value of the indirect immunofluorescent antibody test (IFAT) using intact filariform larvae as antigens.

Meterials and Methods

Sources of *S. stercoralis* antigens

Filariform larvae of *S. stercoralis* were derived from a patient attending the out-patient parasite clinic at Chulalongkorn Hospital. Approximately 50 grams of stool sample were cultured by the polyethylene tube method. Filariform larvae were harvested after 5 days of cultivation and were washed 5 times in 20 ml of sterile phosphate-buffered saline, pH 7.3 (PBS), and were stored at -20 C until use.

Stool examinations

Stool examination was performed by simple smear, formalin-ether sedimentation method and polyethylene tube culture for each individual.

Sera samples

Subjects for the sera were classified into 4 groups. These were group 1 with 10 strongyloidiasis patients (without other parasitic infections), group 2 with 10 parasite infected patients other than S.stercoralis, group 3 containing 20 individuals with negative results of stool examination and residing in an endemic area in Yasothorn Province and group 4 containing 10 medical technology students without parasitic infection and no history of exposure to *S. stercoralis*. Sera from groups 1-3 were obtained from another seroepidemiological study during May, 1994 at Lerng Nok Ta District, Yasothorn Province. Sera were kept at -20 C. Sera from group 4 were from the excess of blood samples from routine laboratory practice.

Indirect immunofluorescent antibody test (IFAT)

Indirect immunofluorescent antibody test was performed basically as described by McBride et al (10) except that all the procedures were done in 1.5 ml microcentrifuge tubes. Each se-

rum sample was analysed by 2-fold serial dilutions. Dilutions of the sera were made in PBS containing 1% bovine serum albumin and 0.01% sodium azide. Thirty 30 microlitres of each dilution was incubated at room temperature with approximately 100 filariform larvae of *S.stercoralis* which were fixed with acetone and air dried in a 1.5 ml microcentrifuge tube for 30 minutes. The larvae were washed 3 times, for 5 minutes each, in 500 microlitres of PBS. After removal of the supernatant by centrifugation at 5,000 rpm for 5 minutes, the larvae were dried at 60 C for 10 minutes. Thirty microlitres of a 1:100 dilution of fluoresceine isothiocyanate (FITC)-conjugated goat antihuman immunoglobulin G (IgG) (Zymed Laboratories, Inc, USA) was added to the larvae and they were incubated for 30 minutes. After 3 washes for 5 minutes each in PBS, 50 microlitres of 10% glycerine in PBS were added. The samples were applied to a glass slide with cover-slip and were visualized under an Olympus BX40 microscope with incident light of 450 - 490 nanometres at a magnifcation of X100. Positive results were surface fluorescence of the larvae which can be arbitrarily graded by the intensity of the reactivity. At least 20 larvae were examined for each test.

Preincubation of sera

Aliquots of 50 microlitres of the 1:64 PBS-diluted sera from 3 patients: one with strongyloidiasis, one with opisthorchiasis and one with hookworm infection, were incubated for 1 hour at room temperature with approximately 100 third stage larvae of Trichinella spiralis and approximately 100 filariform larvae of Necator americanus in separate microcentrifuge tubes. Removal of the larvae was done by centrifugation at 5,000 rpm for 5 minutes and the incubated serum was used for IFAT as described above.

Statistical analysis

The diagnostic value of the test was determined for sensitivity and specificity. The positive and negative predictive values of the test were expressed in per cent.

Results

Details of patients in groups 1, 2 and 3 are shown in Table 1. Cases in group 1 were all male, age range from 21-41 years old. All had *S.stercoralis* infection alone based on a single stool examination except No.555 who was coinfected with *Giardia lamblia* and No.662 who was coinfected with *Taenia sp.* Seven out of 10 patients in group 1 had eosinophil more than 5%. Group 2 consisted of 20 males aged 25-36 years old with parasitic infections other than *S.stercoralis.* The parasites detected were Hookworm, *Opisthorchis viverrini, Echinostoma sp., Taenia sp., Sarcocystis bovihominis* and *Giardia lamblia.* Eosinophilia was found in 8 cases. Parasites were not found in the stool samples of the cases in group 3. However, 4 out of ten cases in this group had eosinophilia. Group 4 was a negative control, without parasitic infection, and none showed peripheral blood eosinophilia (data not shown).

Table 1. Origin of the sera samples used in indirect immunofluorescent antibody test.

Sample No.	Sex	Age	%Eosinophil	Parasite Detected *
Group 1				
501	Male	41	11	*S. stercoralis*
503	Male	32	0	*S. stercoralis*
509	Male	28	0	*S. stercoralis*
550	Male	21	13	*S. stercoralis*
552	Male	33	15	*S. stercoralis*
555	Male	22	9	*S. stercoralis + G.lamblia*
557	Male	24	0	*S. stercoralis*
662	Male	21	9	*S. stercoralis + Taenia sp.*
672	Male	30	15	*S. stercoralis*
689	Male	26	12	*S. stercoralis*
Group 2				
539	Male	23	11	*O. viverrini*
542	Male	26	0	*O. viverrini + Hookworm*
554	Male	25	2	*O. viverrini*
560	Male	35	7	*Hookworm*
566	Male	25	6	*Taenia sp.*
567	Male	22	6	*O. viverrini*
568	Male	25	3	*O. viverrini*
607	Male	38	10	*S. bovihominis*
618	Male	28	4	*G. lamblia*
621	Male	28	19	*G. lamblia*
622	Male	39	3	*G. lamblia*
623	Male	34	9	*O. viverrini*
632	Male	40	3	*G. lamblia*
634	Male	32	1	*Hookworm*
637	Male	23	3	*O. viverrini + Hookworm*
640	Male	24	0	*O. viverrini*
653	Male	28	6	*G. lamblia*
654	Male	26	3	*O. viverrini*
657	Male	29	3	*Hookworm*
687	Male	23	1	*Hookworm + Echinostoma sp.*
Group 3				
500	Male	32	14	
504	Male	33	4	-
510	Male	29	18	-
511	Male	36	0	-
512	Male	32	0	-
513	Male	25	2	-
515	Male	31	6	-
517	Male	25	13	-
520	Male	33	0	-
537	Male	27	0	-

* stool examinations by direct smear, formalin-ether sedimentation technique and polyethelene tube culture.

\- denotes none parasite detected from a single stool specimen.

Table 2 shows the result of IFAT using *S.stercoralis* filariform larvae as antigens. Among group 1 sera, the IFAT was all positive, the highest titre being 1:514 (No.672) and the lowest 1:16 (No.557). The highest titre for which most of the samples (9 out of 10) gave positive IFA was 1:64 and this was considered to be a cut-off titre. Therefore, we tested sera from groups 2 and 3 using 1:64 dilution (Table 3). Eight out of 20 cases in group 2 and 5 out of 10 cases in group 3 had positive IFAT. Fifty and 60 per cent of the positive IFA sera in groups 2 and 3 had eosinophilia, respectively. On the other hand, all of the sera in group 4 (dilution 1:128, 1:64 and undiluted) gave negative IFAT (data not shown).

Since a considerable number of cases in groups 2 and 3 had positive IFA at titre 1:64, we investigated whether this was partly due to the presence of a cross-reacting antibody induced by other parasites (Table 4). Preincubation of the sera from hookworm infected patients (No. 657 and No. 560) with *T.spiralis* larvae and *S.stercoralis* filariform larvae was performed in two separate reactions. Reduction of IFA reactivity was observed in postincubation of serum. Similar results were found when sera from strongyloidiasis patients (No. 550 and No. 552) was tested. In contrast, serum from an opisthorchiasis case (No. 539) did not show any decrease in the reactivity of IFAT at titre 1:64. However, further dilution of the No.539 serum to 1:256 gave a significant reduction in the IFA reactivity after incubation with either *T.spiralis larvae* or *S.stercoralis* filariform larvae, from 3+ to 1+ and from 3+ to O, respectively (data not shown).

By computing the diagnostic value of the IFAT for strongyloidiasis diagnosis at the cut-off titre of 1:64 sensitivity, specificity, positive predictive value and negative predictive values, based on a single stool examination, were 90%, 67.5%, 96.4% and 40.9%, respectively.

Table 2. Indirect immunofluorescent antibody reactivity of sera from patients with strongyloidiasis (group 1) detecting IgG against filariform larvae of S.stercoralis *

Test serum No.	Reactivity of the following reciprocal serum dilutions:						
	16	32	64	128	256	514	1024
501	-	++++	++	0	0	-	-
503	-	++++	++++	0	0	-	-
509	-	-	++	0	0	-	-
550	-	++++	++++	++	0	-	-
552	-	++++	+++	++	0	-	-
555	-	++++	+++	++	0	-	-
557		+++	0	0	0	0	- -
662	-	++++	++++	0	0	-	-
672	-	++++	-	++++	++++	+	0
689	-	++++	+	0	0	-	-

* ++++, strongest reactivity; +, weakest reactivity; 0, no reactivity

- not done

Table 3 . Indirect immunofluorescent antibody reactivity of sera from patients with parasitic infections other than *S.stercoralis* (group 2) and stool negative individuals residing in Yasothorn Provice (group 3) detecting IgG against filariform larvae of S.stercoralis, using serum dilution 1:64

Group 2		Group 3	
Serum No.	IFA reactivity *	Serum No.	IFA reactivity
539	++++	500	+++
542	+++	504	0
554	++	510	++++
560	+++	511	++
566	0	512	++++
567	0	513	0
568	0	515	++++
607	++++	517	0
618	++++	520	0
621	++++	537	0
622	0		
623	0		
632	0		
634	0		
637	0		
640	0		
653	0		
654	0		
657	++++		
687	0		

* ++++, strongest reactivity; +, weakest reactivity; 0, no reactivity

Table 4. Indirect immunofluorescent reactivity of sera from individuals with strongyloidiasis (No.550 and 552), hookworm infection (No.560 and 657) and opisthorchiasis (No.539) before and after incubation with filariform larvae of hookworm and third stage larvae of T.spiralis

Test serum No.	Reactivity of serum dilution 1:64 *			
	Filariform larvae of Hookworm		Third stage larvae of T.spiralis	
	preincubation	postincubation	preincubation	postincubation
552	+++	+	-	-
560	+++	+	-	-
550	-	-	++++	++
657	-	-	++++	++
539	++++	++++	++++	++++

*++++, strongest reactivity; +, weakest reactivity; O, no reactivity

- not done

Discussion

Strongyloidiasis has been a significant parasitic infection, mainly because it is difficult to diagnose with high sensitivity based on stool examinations and because it can be activated to produce a hyperinfection syndrome in immunosuppressed patients. Furthermore, the efficacy of the antiparasitic drugs currently in use does not always completlely eradicate the infection. The prevalence of strongyloidiasis in developed countries varies from 0.4 - 4.0%. In contrast, among people residing in tropical countries the prevelance is much higher, up to 50%.[1] However, most of the surveys were based on a simple smear and concentration technique. The sensitivity of stool examinations can be reportedly increased to 90-100% if one spends an average of 9 hours of microscopy per specimen[4] or takes 7 stool specimens passed on 7 different days.[1] Therefore, the actual prevalence of strongyloidiasis would be higher due to the sensitivity of the techniques.

The applications of IFAT have been exploited for assisting diagnoses of several infectious diseases such as leptospirosis,[10] melioidosis[11] and toxoplasmosis.[12] The results of IFAT to detect specific IgG against the larval surface antigens in the present study revealed that all infected individuals gave a positive IFAT titre at 1:16. However, our preliminary study showed that at this titre most of the cases infected with other parasites also gave a positive IFAT. Therefore, we selected as 1:64 the cut-off titre since most of the infected cases still showed positive results. Based on this titre, the sensitivity, specificity, positive predictive value and negative predictive value were 90%, 67.5% 96.4% and 40.9%, respectively. It is noteworthy in this context that some cases in group 2 might contain occult *S.stercoralis* infection due to the low sensitivity of stool examinations performed in our the present study. It is also unlikely that protozoal infections in cases No. 607, 618 and 621 could induce nonspecific immunological cross-reactivity. Furthermore, several cases in group 3 had peripheral blood eosinophilia, suggesting undetected parasitic infections since all of them came from were in endemic area where the risk of being infected was high and none had history of allergy. Thus the actual specificity and negative predictive value would be much higher than examined here. Previous study by Grove and Blair[5] using live *S.ratti* and *S.stercoralis* filariform larvae for IFAT to detect all classes of Ig has reportedly shown sensitivity of 98% and high specificity. However, interpretation of the diagnostic value of the test must be done cautiously since the predictive value of a test is related to the prevalence of the condition it purports to detect.[13]

A recent study by Conway et al[8] showed that detection of serum IgG against *S.stercoralis* larval antigens by ELISA gave diagnostic sensitivities and specificities of 80% and 93.5%, respectively. After incubation of the sera with *Onchocerca gutturosa,* the sensitivity and specificity increased to 85% and 97.1%, respecitively.[9] In our study, the reactivity of IFAT using sera from patients infected with *S.stercoralis, Opsithorchis viverrini* and hookworm reduced after incubation of the sera with *Necator americanus* filariform larvae and *Trichinella spiralis* third stage larvae, implying that the larval nematode shares certain cross-reacting epitopes on the surface which can interfere with immunological diagnosis. Detailed studies on the surface structures of nematodes have shown that the cuticular elasticity and rigidity arise from extensive covalent cross-linking between cuticular collagens. The cuticle is covered with lipid layer of the epicuticle and the carbohydrate-rich surface coat which may help the parasite to evade host immune attack.[14] The collagen and some of the repetitive amino acid sequences in surface structures might be responsible for induction of T-independent B cell response, thus producing ineffective and cross-reactive antibodies.[15] For whatever the cross-reacting epitopes are, it is

obvious that the specificity of IFAT or ELISA could be improved by preincubation of the test sera with nematode larvae.

Immunoblot studies by Conway, et al, have shown that the larval proteins recognized most specifically by host immune responses have a molecular weight of approximately 41 KD. [16-17] If the gene encoding the protein is cloned and sequenced, specific diagnostic epitopes would be identified and the immunological diagnosis of strongyloidiasis would also be more specific and sensitive. Moreover, fine dissection of the specific Ig classes and subclasses induced by parasite specific antigen(s) during acute infection may be a useful adjunct in the immunological evaluation of patients with strongyloidiasis.[18-20] At present, however, our preliminary study of the IFAT in strongyloidiasis may provide a potential diagnostic implication after careful large-scale evaluation and an improved procedure of preincubation of the test sera.

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References

1. Genta RM. Global prevalence of strongyloidiasis : critical review with epidemiologic insights into the prevention of disseminated disease. Rev Infect Dis 1989 Sep; 11(15):755-67
2. Schad GA. Smith G, Megyeri Z, Bhopale VM, Niamatali S, Maze R. *Strongyloides stercoralis* : an initial autoinfective burst amplifies primary infection. Am J Trop Med Hyg 1993 May; 48(5):716-25
3. Igra-Siegman Y, Kapila R, Sen P, Kaminski ZC, Louria DB. Syndrome of hyperinfection with *Strongyloides stercoralis*. Rev Infect Dis 1981 May-Jun :3(3):397-407
4. Pelletier LL. Chronic strongyloidiasis in World War II Far East ex-prisoners of war. Am J Trop Med Hyg 1984 Jan; 33(1):55-61
5. Grove DI, Blair AJ. Diagnosis of human strongyloidiasis by immunofluorecence, using *Strognyloides ratti* and *S. stercoralis* larvae. Am J Trom Med Hyg 1981 Mar; 30(2):344-9
6. Neva FA, Gam AA. Comparison of larval antigens in an enzyme-linked immunosorbent assay for strongyloidiasis in humans. J Infect Dis 1981 Nov; 144(5): 427-32
7. Genta RM, Douce RW, Walzer PD. Diagnostic implications of parasite-specific immune responses in immunocompromised patients with strongyloidiasis. J Clin Microbiol 1986 Jun; 23(6):1099-103
8. Conway DJ, Atkins NS, Lillywhite JE, Bailey JW, Robinson RD, Lindo JF, Bundy DAP. Immunodiagnosis of *Strongyloides stercoralis* infection: a method for increasing the specificity to the indirect ELISA. Trans R Soc Trop Med Hyg 1993 Mar-Apr; 87(2):173-6
9. Lindo JF, Conway DJ, Atkins NS, Bianco AE, Robinson RD, Bundy DAP. Prospective evaluation of enzyme-linked immunosorbent assay and immunoblot methods for the diagnosis of endemic *Strongyloides stercoralis* infection. AM J Trop Med Hyg 1994 Aug; 51(2):175-9

10. McBride JS, Walliker D, Morgan G. Antigenic diversity in the human malaria parasite *Plasmodium falciparum*. Science 1982, 217, 254-7
11. Appassakij H, Silpapojakul K, Wansit R, Woodtayakorn J. Evaluation of the indirect immunofluorescent antibody test for the diagnosis of human leptospirosis. Am J Trop Med Hyg 1995 Apr; 52(4):340-3
12. Rugdech P, Anuntagool N, Sirisinha S. Monoclonal antibodies to *Pseudomonas pseudomallei* and their potential for diagnosis of melioidosis. Am J Trop Med Hyg 1995 Mar; 52(3):231-5
13. Walton BC, Benchoff BM, Brooks WH. Comparison of indirect fluorescent antibody test and methylene blue dye test for detection of antibodies to *Toxoplasma gondii*. Am J Trop Med Hyg 1966 Mar; 15:149-52
14. Vecchio TJ. Predictive value of a single diagnostic test in unselected populations. N Engl J Med 1966 May; 274: 1171-3
15. Maizels RM, Blaxter ML, Selkirk ME. Forms and functions of nematode surface. Exp Parasitol 1993 Nov; 77(3):380-4
16. Schofield L. On the function of repetitive domains in protein antigens of Plasmodium and other eukaryotic parasite. Parasitol Today 1991; 7:99-105
17. Conway DJ, Bailey JW, Lindo JF, Robinson RD, Bundy Dap, Bianco AE. Serum IgG reactivity with 41-,31-, and 28-kDa larval proteins of Strongyloides stercoralis in individuals with strongyloidiasis. J Infect Dis 1993 Sep; 168(3):784-7
18. Conway DJ, Lindo JF, Robinson RD, Bundy, DAP, Bianco AE. *Strongyloides stercoralis*: characterization of immunodiagnostic larval antigens. Exp Parasitol 1994 Sep; 79(2):99-105
19. McRury J, Messias ITD, Walzer PD, Huitger T, Genta RM. Specific IgE responses in human strongyloidiasis. Clin Exp Immunol 1986 Sep; 65(3):631-8
20. Genta RM, Lillibridge JP. Prominence of IgG4 antibodies in the human responses to Strongyloides stercoralis infection. J Infect Dis 1989 Oct; 160(4):692-9
21. Neva FA. Biology and immunology of human strongyloidiasis. J Infect Dis 1986 Mar; 153(3):397-406