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BRIEF COMMUNICATION

PARASITOLOGICAL AND IMMUNOLOGICAL DIAGNOSES OF STRONGYLOIDIASIS IN IMMUNOCOMPROMISED AND NON-IMMUNOCOMPROMISED CHILDREN AT UBERLÂNDIA, STATE OF MINAS GERAIS, BRAZIL

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SUMMARY

Parasitological and immunological diagnoses were part of a study conducted among 151 children, 83 immunocompromised (IC) and 68 non-immunocompromised (non-IC) aged from zero to 12, seen at the University Hospital, Universidade Federal de Uberlândia, State of Minas Gerais, Brazil, from February, 1996, to June, 1998. Three fecal samples from each child were analyzed for the parasitological diagnosis by Baermann-Moraes and Lutz methods. The immunological diagnosis to detect IgG and IgM antibodies was carried out by the indirect immunofluorescence antibody test (IFAT) with cryo-microtome sections of *Strongyloides stercoralis* and *Strongyloides ratti* larvae as antigens and by the ELISA test with an alkaline extract of *S. ratti* as the antigens. Of the 151 children 5 (3.31%) were infected with larvae of *S. stercoralis* (2 cases IC, 2.41%, and 3 cases non-IC, 4.41%). The IFAT-IgG detected 7 (8.43%) serum samples positive among IC, and 2 (2.94%) cases among non-IC. The ELISA-IgG test detected 10 (12.05%) serum samples positive among IC, and 1 (1.47%) case among non-IC. The IFAT-IgM detected 6 (7.22%) positive cases among IC, and 3 (4.41%) cases among non-IC. ELISA-IgM test detected 10 (12.05%) positive cases among IC, and 3 (4.41%) cases among non-IC. It was concluded that the immunological tests can help in the diagnosis of strongyloidiasis in immunocompromised children.

KEYWORDS: *Strongyloides stercoralis*; Strongyloidiasis; Diagnosis; Children; Immunocompromised.

Strongyloides stercoralis is one of the most difficult parasitic infections to diagnose. In normal hosts, it causes chronic or subclinical infections that can persist for decades without any adverse effect. However, in immunocompromised hosts *S. stercoralis* may cause a frequently fatal hyperinfection or disseminated disease[11]. Many cases of strongyloidiasis were described among a variety of conditions of immunosuppression, especially impairment of cell-mediated immunity, such as hematologic malignancies[10,19], lymphoma[21], renal transplantation[9] and HIV$^+$ (human immunodeficiency virus infection) [8].

The parasitological methods have low sensitivity even when repeated several times and have to be performed on fresh stools, rarely a standard procedure in hospital clinical laboratories[7,11]. Several studies support the view that detection of parasite-specific antibodies may be a useful complement to the traditional parasitological diagnosis of strongyloidiasis using the indirect immunofluorescence antibody test (IFAT)[4,12] and enzyme-linked immunosorbent assay (ELISA)[3,13,22].

The purpose of the present study was to detect *S. stercoralis* infection in immunocompromised (IC) and non-immunocompromised (non-IC) children aged from zero to 12 years of age seen at the University Hospital, Universidade Federal de Uberlândia, State of Minas Gerais, Brazil, from February, 1996, to June, 1998.

The study was conducted among 151 children, 83 IC (46 malignancies, 8 protein-calorie malnutrition degree II and III, 6 HIV$^+$, 3 acquired immune deficiency syndrome - AIDS, 3 renal insufficiency, 3 rheumatic fever, 3 falciform anemia, 2 nephrotic syndrome, 2 politraumatism, 1 renal transplantation, 1 cardiac insuficiency, 1 mellitus diabetes, 1 mucoviscidosis, 1 hepatic insufficiency, 1 Gaucher's disease, 1 necrotic enterocolite) that were clinically confirmed[18,23] and were being treated with immunosuppressive agents; and 68 non-IC hospitalized children with other non-immunosupressive infections. Fecal and serum samples were collected for both groups after previous written authorization from those responsible for the children. None of the patients of the two groups presented clinical or parasitological diagnosis of strongyloidiasis.

To parasitological diagnosis, three fecal samples from each child were collected in plastic vials without preservatives with intervals of 4 to 15 days and analyzed by the BAERMANN[1], MORAES[17] and LUTZ[15] methods. Six slides were prepared for each method for each of the 453

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Table 1
Frequency of intestinal parasites and comensal among immunocompromised and non-immunocompromised children seen at the University Hospital of Uberlândia, MG, Brazil, from February, 1996 to June, 1998

Parasites/comensal	**Immunocompromised** (n=83)		**Non-immunocompromised** (n=68)		**Total** (n=151)	
	Nº	%	Nº	%	Nº	%
G. lamblia	9	10.84	11	16.18	20	13.24
E. vermicularis	4	4.82	2	2.94	6	3.97
S. stercoralis	2	2.41	3	4.41	5	3.31
A. lumbricoides	4	4.82	1	1.47	5	3.31
E. coli	3	3.61	2	2.94	5	3.31
Hookworms	4	4.82	0	0	4	2.65
T. trichiura	1	1.20	0	0	1	0.66

Nº: number of children positives, %: percentage, (p>0.05).

Table 2
Identification of *S. stercoralis* positive cases from parasitological and immunological diagnoses among immunocompromised and non-immunocompromised children seen at the University Hospital of Uberlândia, MG, Brazil, from February, 1996 to June, 1998

Cases	Clinical Diagnosis	Parasitological Diagnosis	Immunological Diagnosis*					
		BM/L	IFAT				ELISA	
			IgG		IgM		IgG	IgM
			S.s.	S.r.	S.s.	S.r.	S.r.	S.r.
		Immunocompromised						
4	Rhabdomyosarcoma	-	-	-	-	-	160	80
8	Marrow's aplasia	-	20	-	80	40	80	160
15	Protein-calorie malnutrition	*S. stercoralis* (BM), *T. trichiura* and *A. lumbricoides* (L)	20	20	-	-	320	80
24	Acute lymphocytic leukemia	-	-	-	40	40	-	-
27	Marrow's aplasia	-	20	40	40	40	80	80
28	Renal transplantation	-	40	-	-	-	160	-
29	Encephalic tumor	-	-	-	40	40	-	80
31	Protein-calorie malnutrition	-	-	-	-	-	320	-
42	Sarcoma	-	-	-	-	-	-	160
44	Human immunodeficiency virus infected	-	20	20	-	-	80	-
45	Hodgkin's lymphoma	-	80	80	-	-	320	160
46	Acute lymphocytic leukemia	*S. stercoralis* (BM)	-	-	-	-	-	-
62	Nasofrontal tumor	-	-	-	-	40	-	80
74	Politraumatism	-	-	-	-	-	160	80
82	Rheumatoid arthritis	-	40	-	40	40	320	160
		Non-immunocompromised						
9	Cellulite, Osteomyelitis	*S. stercoralis* (L) and *G. lamblia* (L)	20	-	-	-	-	-
16	Revolver Accident	*S. stercoralis* (BM) and *G. lamblia* (L)	-	-	40	40	-	80
26	Septicemia	*S. stercoralis* (BM+L)	40	40	40	-	320	80
35	Medicamentosus allergy	-	-	-	40	-	-	80

BM: Baermann-Moraes, L: Lutz, IFAT: indirect immunofluorescence antibody test, S.s.: *S. stercoralis*, S.r.: *S. ratti*, - negative, * results in title.

samples. The total number of slides examined was thus 5,436. All the families of the children received the results of the laboratory diagnosis. The positive cases were given specific treatment.

To immunological diagnosis, *S. stercoralis* larvae were obtained from the feces of patients seen at the same University Hospital and *S. ratti* was obtained from the feces of experimentally infected rats (*Rattus rattus*). The larvae of each antigen were mixed with an equal part of finely ground charcoal, moistened with water, spread in an uniform layer on Petri dishes and incubated at 25 °C for 5 days. The filariform larvae were then harvested by the BAERMANN[1], MORAES[17] technique, concentrated by centrifugation at 1000g for 5 minutes and stored at –20 °C until the time of use.

For IFAT, *S. stercoralis* and *S. ratti* antigens were prepared according to COSTA-CRUZ *et al.*[4]. For ELISA, the alkaline extract was prepared with the addition of 1 ml of 0.15 M NaOH at 4 °C for 6 hours of agitation. Subsequently, adding 0.5 ml of 0.3 M of HCl until the pH reached 7.0. This preparation was centrifuged at 3000g for 15 minutes at 4 °C and the supernatant was submitted to LOWRY *et al.*[14] method for protein detection.

The immunofluorescence - IgG tests for *S. stercoralis* and *S. ratti* antigens utilized in the present work was done according to COSTA-CRUZ *et al.*[4]. The authors described 94.4% and 94.2% respectively of sensitivity and specificity to *S. stercoralis* and respectively 92.5% and 97.1% to *S. ratti* for IgG antibodies, considered titers positive when ³ 20. For IgM antibodies, IFAT was standardized previously by PEIXOTO[20] with 37.0% and 29.6% positive serum samples detected in patients with strongyloidiasis, by *S. stercoralis* and *S. ratti* antigens respectively; considered titers positive when ³ 40. The tests, in the present study, were performed simultaneously for each serum using each of the two antigens. The fluoresceinated anti-human IgG and IgM conjugate (Biolab, Brazil) were added at the ideal titers of 100 (for both antigens) and 20 (for both antigens), respectively.

ELISA was previously described by COSTA-CRUZ *et al.*[5] with a sensitivity of 93.4% and specificity of 96.9% for IgG antibodies. For IgM antibodies, ELISA was standardized previously by COSTA-CRUZ *et al.*[6] and 44.26% positive serum samples were detected in patients with confirmed strongyloidiasis. The cut off was determined as BASSI *et al.*[2] with two standard deviations. The results were expressed as titers, which were considered positive when ³ 80. The anti-human IgG and IgM labeled with peroxidase (Sigma) was added respectively at titers 4000 and 2000.

All positive serum sample IgM antibodies were tested using the "Kit Reumatex â " (Doles, Brazil) and FTA sorbent (Laborclin, Brazil).

The normality of distribution was tested for two proportions and X^2 at the significance level of 5%[16] to compare the results obtained with two groups.

From the 151 children studied, 139 (92.05%) were from the State of Minas Gerais (86, 56.95%, from Uberlândia) 11 (7.28%) from the State of Goiás and 1 (0.66%) from the State of Mato Grosso do Sul.

The distribution of intestinal parasites and comensal in IC and non-IC children is showed in Table 1. Of the 151 children studied 5 (3.31%) were infected with larvae of *S. stercoralis*. Two of these cases (2.41%) were IC, both were girls in the category of two to four years and 3 (4.41%) were non-IC, all were boys, one case being in the category of two years of age or under and two cases in the category of between ten and twelve. No statistical difference was observed by test for the two proportions (Z= 0.69, p>0.05) in the occurrence of *S. stercoralis* between the two groups.

The IFAT-IgG detected 9 (5.96%) positive serum samples, 7 (8.43%) IC and 2 (2.94%) non-IC. The 7 (8.43%) cases of IC were detected by the *S. stercoralis* antigen, 4 (4.82%) of these were also *S. ratti* antigen positives. The *S. stercoralis* antigen detected 2 (2.94%) positive cases

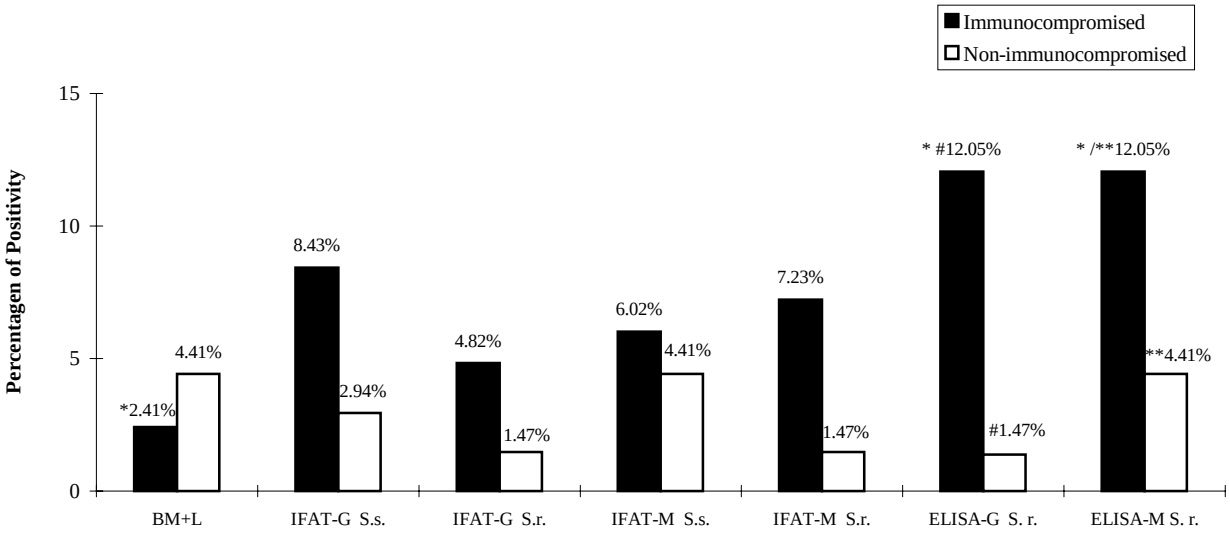

Fig. 1 - Comparison of parasitological (Baermann-Moraes-BM and Lutz-L) and immunological (IFAT-G and IFAT-M, *S.stercoralis*-S.s. and *S. ratti*-S.r., ELISA-G and ELISA-M) methods among 83 immunocompromised and 68 non-immunocompromised children seen at the University Hospital of Uberlândia, MG, Brazil, from February, 1996 to June, 1998. *Z=2.41; #Z=3.84 **Z=2.75 (p<0.05)

among non-IC and the *S. ratti* antigen only 1 (1.47%) case. The IFAT-IgM detected 9 (5.96%) positive cases, 6 (7.22%) cases among IC and 3 (4.41%) among non-IC. Of the 6 positive cases among IC 5 (6.02%) cases were detected by *S. stercoralis* and 6 (7.23%) cases by *S. ratti* antigens. The *S. stercoralis* antigen detected 3 (4.41%) cases among non-IC and the *S. ratti* antigen only 1 (1.47%) case. No statistical difference was observed using the test for the two proportions between the two antigens or the two groups ($p>0.05$).

The ELISA-IgG test detected 11 (7.28%) positive serum samples, 10 (12.05%) among IC and 1 (1.47%) among non-IC. The ELISA-IgM test detected 13 (8.61%) positive cases, 10 (12.05%) among IC and 3 (4.41%) among non-IC. Statistical difference was observed between the two groups in ELISA-IgG ($Z=3.84$, $p<0.05$) and ELISA-IgM ($Z=2.75$, $p<0.05$).

All IgM positive serum samples were negative in rheumatic factor research.

From the 151 children 19 (12.58%) were positive for *S. stercoralis*, considering the parasitological and immunological diagnoses, 16 (10.60%) were from the State of Minas Gerais [9 (5.96%) from Uberlândia] and 3 (1.99%) from the State of Goiás, aged from five months to 12 years, and of both sexes.

Positive children to *S. stercoralis* are shown in the Table 2. The comparison of positive cases to *S. stercoralis* by parasitological and immunological diagnoses is shown in Figure 1.

Considering immunosuppression as a risk factor of strongyloidiasis and the high positive rate of immunological tests among IC, the present study shows the value of IFAT and ELISA in the detection of IgG and IgM antibodies in diagnosis of human strongyloidiasis among immunocompromised children, contributing to the establishment of the diagnosis of this parasitosis and the possibility of early treatment of these children.

RESUMO

Diagnóstico parasitológico e imunológico da estrongiloidíase em crianças imunodeprimidas e imunocompetentes na cidade de Uberlândia, MG, Brasil

O diagnóstico parasitológico e imunológico da estrongiloidíase foi realizado em 151 crianças (83 imunodeprimidas -ID e 68 imunocompetentes -IC) de zero a 12 anos de idade internadas no Hospital de Clínicas da Universidade Federal de Uberlândia, Minas Gerais, Brasil, no período de fevereiro de 1996 a junho de 1998. Para o diagnóstico parasitológico três amostras de fezes de cada indivíduo foram processadas pelos métodos de Baermann-Moraes e de Lutz. O diagnóstico imunológico para a detecção de anticorpos IgG e IgM foi realizado através das reações de imunofluorescência indireta (RIFI) utilizando-se como antígeno cortes de 4 micra de larvas filarióides de *Strongyloides stercoralis* e *Strongyloides ratti* e do teste ELISA utilizando-se como antígeno extrato alcalino de larvas de *S. ratti*. Das 151 crianças, 5 (3,31%) estavam infectadas com *S. stercoralis* (2 casos ID, 2,41% e 3 casos IC, 4,41%). A RIFI- IgG detectou 7 (8,43%) amostras de soros positivas nas ID, e 2 (2,94%) nas IC. O teste ELISA-IgG detectou 10 (12,05%) amostras de soros positivas nas ID, e 1 (1,47%) nas IC. A RIFI-IgM detectou 6 (7,22%) casos positivos nas ID, e 3 (4,41%) nas IC. O teste ELISA-IgM detectou 10 (12,05%) casos positivos nas ID e 3 (4,41%) nas IC. Concluiu-se que os testes imunológicos podem contribuir para o diagnóstico da estrongiloidíase em crianças imunodeprimidas.

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XIII JORNADA PAULISTA DE PARASITOLOGIA

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TEMAS

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