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BRIEF COMMUNICATION

SEROEPIDEMIOLOGICAL STUDY OF HUMAN STRONGYLOIDIASIS WITH BLOOD SAMPLES COLLECTED ON FILTER PAPER, IN ABADIA DOS DOURADOS (MINAS GERAIS, BRAZIL)

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Strongyloides stercoralis (Bavay, 1876) infection is ranked in 5th place among the helminthiasis in the world with an estimated 35 million infected individuals, mostly living in tropical and semitropical countries[3]. The frequency of nonsymptomatic or disseminated forms has become a serious public health concern due to the lack of reliable tools for the diagnosis of this disease[5, 7].

The development of immunological tests for the diagnosis of strongyloidiasis can be helpful in epidemiological and clinical evaluations. Studies have been done trying to standardize a serological test for the diagnosis of this helminthiasis[15]. The main limitations found in developing such tests are the low antigen yielding which does not permit further fractionation and analysis[13], added to the difficulties in collecting blood samples for conducting seroepidemiological inquiries.

Immunological tests using the third-stage filariform larvae of *S. stercoralis* and *Strongyloides ratti* (Sandground, 1925), have shown comparable results[6,9]. Thus, the use of *S. ratti* has been preferred to overcome the problem in obtaining antigens in sufficient amount.

The use of blood samples collected on filter paper was proved to be useful for the serodiagnosis of parasitic diseases, as well as for seroepidemiological surveys in human and veterinary investigation. Thus this procedure has been applied with success in research on schistosomiasis[2], Chagas' disease[4,11,14,16], leishmaniasis[1,12] and toxoplasmosis[17].

Since the use of blood samples collected on filter paper for the diagnosis of human strongyloidiasis is very scarce, the objective of the present work is to utilize this procedure in a serological inquiry applying the indirect immunofluorescence antibody test (IFAT). In this test, the third stage filariform larvae of *S. ratti* was chosen as antigen because this parasite stage presented a sensitivity of 92.5% and specificity of 97.1%[6].

Abadia dos Dourados is a city located in the Alto Paranaíba region in State of Minas Gerais, Brazil, its population is about 6,424 inhabitants. In July 1996, 207 individuals who live in the rural area were selected at random. These individuals were later identified according to sex and age, after their agreement for the participation in this project. Blood samples were obtained by digital puncturing with disposable lancets, collected on filter paper (Klabin 80 g/m^2), as described[16] by SOUZA & CAMARGO, and stored in the Parasitology Laboratory of the Universidade Federal de Uberlândia at 4°C until November of 1996, when the blood elution was performed.

Filter papers were cut into 1.2 cm diameter discs using a metallic mold, and they were put individually in plastic bungs. On each bung 0.3 ml of phosphate buffered saline 0.01M, pH 7.2 (PBS) were added and kept during 18 hours at 4°C. The IFAT with the eluates was done according to COSTA-CRUZ et al.[6] using FITC-conjugated anti-human IgG at an ideal titer of 150. The analysis of the reactions was done using an immunofluorescence microscope (OLYMPUS BH-2 RFCA) at 400 X.

Of the 207 blood samples, 91 (44.0%) were from males and 116 (56.0%) from females. The age varied from two to 78 years. In the IFAT, 16 cases (7.7%) were seroreagents for strongyloidiasis. Table 1 shows the infection frequency of anti-*Strongyloides* antibodies in the inhabitants of the rural area in Abadia dos Dourados according to sex and age. Positive individuals of all age-groups were identified, with the youngest being seven years and the oldest being 74 years old. Applying the X^2 test, there was no statistical significant difference between sex and age ($p>0.05$).

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TABLE 1

Infection frequency of anti-*Strongyloides* antibodies (IgG-IFAT) according to sex and age among the inhabitants of the rural area in Abadia dos Dourados, State of Minas Gerais, Brazil, in July, 1996.

Age	Sex				Total	
	Male		Female			
Years	N° +/ total by sex	P	N° +/ total by sex	P	N° +/ total	P total
00 - 09	1/19	1.1	0/20	0	1/39	0.5
10 - 19	0/21	0	2/39	1.7	2/60	0.9
20 - 29	1/9	1.1	1/8	0.9	2/17	0.9
30 - 39	2/17	2.2	3/23	2.6	5/40	2.4
40 - 49	1/7	1.1	0/10	0	1/17	0.5
50 - 59	1/7	1.1	2/9	1.7	3/14	1.5
60 - 69	0/8	0	1/5	0.9	1/13	0.5
70 - 79	1/5	1.1	0/2	0	1/7	0.5
Total	7/91	7.7	9/116	7.8	16/207	7.7

N°+ = Numbers of seropositive cases P = Percentage

In view of the sensitivity (Sens) and specificity (Spec) of IFAT, we have calculated the true prevalence (PT) by a probabilistic model[8]. So, the obtained prevalence was corrected based on the following equation: True prevalence = (Obtained prevalence + Spec - 1)/(Sens + Spec - 1) or PT = (0.077 + 0.971 - 1)/(0.925 + 0.971 - 1) = 0.054. This correction seems plausible considering that the immunological test probably happen false negative results, as well as crossed reactions with others Nematoda infections. This model was also applied in the seroepidemiology of schistosomiasis *mansoni*[10] by HOSHINO-SHIMIZU et al.

Among several epidemiological factors which could be related to the incidence and prevalence of strongyloidiasis, the habit of walking barefoot seemed a preponderant factor, since 197 (95.2%) of the 207 examined individuals used to be barefooted. But applying the X^2 test there was no significant association between walking barefoot and the presence of seropositive cases of *Strongyloides*. Moreover, 14 of 16 seroreagent cases (87.5%) walked barefoot, 201 (97.1%) handled soil with unprotected hands corresponding to 13 positives cases, 187 (90.3%) usually defected directly on the soil, and 102 (49.3%) commonly drank unfiltered water. There is an association of this factors and its eventually explain the occurrence of strongyloidiasis in this region.

The collection of blood samples carried out on filter paper in seroepidemiological inquiries of strongyloidiasis is thought to be highly recommendable, since it is of low cost, easy storage and transportation and provides reliable data.

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REFERENCES

1. AFONSO-CARDOSO, S.R.; MACHADO, M.I.; COSTA-CRUZ, J.M. et al. - Leishmaniose tegumentar canina no município de Uberlândia, Minas Gerais. Diagnóstico clínico e sorológico de cães naturalmente infectados. **Rev. Cent. Cienc. bioméd. Univ. Fed. Uberlândia, 5**: 14-21, 1989.

2. ANDERSON, R.I.; SADUN, E.H. & WILLIAMS, J.S. - A technique for the use of minute amounts of dried blood in fluorescent antibody tests for schistosomiasis. **Exp. Parasit., 11**: 111-116, 1961.

3. BOUREE, P.; TAGOURDEAU, A.; BARTHELEMY, M. et al. - Anguillulose: analyse clinique, biologique et epidémiologique de 350 observations. **Nouv. Presse méd., 10**: 679-681, 1981.

4. CAMARGO, M.E.; SILVA, G.R.da; CASTILHO, E.A. & SILVEIRA, A.C. - Inquérito sorológico da prevalência de infecção chagásica, no Brasil, 1975/1980. **Rev. Inst. Med. trop. S. Paulo, 26**: 192-204, 1984.

5. CELEDON, J.C.; MATHUR-WAGH, U.; FOX, J. et al. - Systemic strongyloidiasis in patients infected with the human immunodeficiency virus. A report of 3 cases and review of the literature. **Medicine** (Baltimore), **73**: 256-263, 1994.

6. COSTA-CRUZ, J.M.; BULLAMAH, C.B.; GONÇALVES-PIRES, M.R.F. et al. - Cryo-microtome cuts of coproculture larvae of *Strongyloides stercoralis* and *Strongyloides ratti* as antigen sources for the immunodiagnosis of human strongyloidiasis. **Rev. Inst. Med. trop. S. Paulo, 39**: 313-317, 1997.

7. DREYER, G.; FERNANDES-SILVA, E.; ALVES, S. et al. - Patterns of detection of *Strongyloides stercoralis* in stool specimens: implications for diagnosis and clinical trials. **J. clin. Microbiol., 34**: 2569-2571, 1996.

8. GART, J.J. & BUCK, A.A. - Comparison of a screening test and a reference test in epidemiologic studies. II. A probabilistic model for the comparison of diagnostic tests. **Amer. J. Epidem., 83**: 593-602, 1966.

9. GROVE, D.I. & BLAIR, J. - Diagnosis of human strongyloidiasis by immunofluorescence, using *Strongyloides ratti* and *S. stercoralis* larvae. **Amer. J. trop. Med. Hyg., 30**: 344-349, 1981.

10. HOSHINO-SHIMIZU, S.; DIAS, L.C.S.; KANAMURA, H.Y. et al. - Seroepidemiology of schistosomiasis *mansoni*. **Mem. Inst. Oswaldo Cruz, 87** (suppl. IV): 303-306, 1992.

11. MINEO, J.R.; COSTA, J.M.; PIMENTA, C.V. et al. - Prevalência da infecção chagásica em crianças de cinco municípios da região Sudoeste de Minas Gerais, Brasil. **Rev. Cent. Cienc. bioméd. Univ. Fed. Uberlândia, 1**: 3-6, 1985.

12. NUNES, V.L.B.; YAMAMOTO, Y.I.; REGO-JÚNIOR, F.A. et al. - Aspectos epidemiológicos da leishmaniose visceral em cães de Corumbá, Mato Grosso do Sul. **Pesq. vet. bras., 8**: 17-21, 1988.

13. ROSSI, C.L.; TAKAHASHI, E.E.H.; TEIXEIRA, A.L.S. et al. - Avaliação de preparações antigênicas de *Strongyloides stercoralis* para o imunodiagnóstico da estrongiloidíase. **Rev. Soc. bras. Med. trop., 26**: 83-87, 1993.

14. SADUN, E.H.; DUXBURY, R.E.; WILLIAMS, J.S. & ANDERSON, R.I. - Fluorescent antibody test for serodiagnosis of African and American trypanosomiasis in man. **J. Parasit., 49:** 385-388, 1963.

15. SATO, Y.; KOBAYASHI, J. & SHIROMA, Y. - Serodiagnosis of strongyloidiasis. The application and significance. **Rev. Inst. Med. trop. S. Paulo, 37**: 35-41, 1995.

16. SOUZA, S.L. & CAMARGO, M.E. - The use of filter paper blood smears in a practical fluorescent test for American trypanosomiasis serodiagnosis. **Rev. Inst. Med. trop. S. Paulo, 8**: 255-258, 1966.

17. WALTON, B.C. & ARJONA, I. - Utilization of whole blood specimens on filter paper for the indirect fluorescent antibody test for toxoplasmosis. **J. Parasit., 57**: 678-679, 1981.

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