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AN IMMUNOEPIDEMIOLOGICAL STUDY OF SCHISTOSOMIASIS MANSONI IN PARAIBA'S VALLEY, SÃO PAULO, BRAZIL

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SUMMARY

Immunoepidemiological survey was carried out in Fazenda Santa Helena, located in the valley of the Paraiba do Sul River. Prevalence of schistosomiasis as determined by immunofluorescence test was 25.5%, and 29.8% by intradermal test. Stool examinations indicated 10.6% positive results, about three times higher than observed in the last 1977 inquiry. No significant influence of polyparasitism on the sensitivity or the specificity of immunological test was observed. Egg granulomata in liver section from infected hamster with **Schistosoma mansoni** infection showed to be a very satisfactory antigen for immunofluorescence tests.

INTRODUCTION

An epidemiological survey for **Schistosoma mansoni** infection was performed in Fazenda Santa Helena, a small community of about 500 inhabitants, located in the valley of the Paraiba do Sul River, São Paulo. This farm has an area of about 1,500 hectares and the main crops are corn, rice and coffee plus cattle breeding.

Besides stool examination for **S. mansoni** eggs, an immunological screening was performed with intradermal (IDT) and immunofluorescence (IFT) tests.

Almost two years before, in 1977, SUCEN (Superintendência de Combate de Endemias) had conducted a preliminarly study, which estimated 2.9% local-born people excreting parasite eggs. At that time, investigations had shown that schistosomiasis was not an occupational disease since infected snails were not found in the studied area. It had been acquired mostly on leisure time, by a close contact, as swimming, with central irrigation ditches in a neighbour farm, where snails were found highly infected with **S. mansoni** (unpublished data).

Such initial inquiry was now expanded to the whole community. Because of the low sensitivity of stool examination frequently reported [4,5,15,17] immunological tests were introduced in an attempt of assessing more accurately the extent of schistosomiasis prevalence.

MATERIAL AND METHODS

Immunofluorescence test (IFT) — Blood was collected on filter paper (Whatman no. 1) by finger prick and eluated as previously described [17].

IFT was performed in cryostat liver sections obtained from infected hamsters with **S. mansoni** [8].

Fluorescence conjugates to human IgM and IgG (Hyland Travenol Lab., USA) were used in dilutions which gave maximum reactivity. Specificity of conjugates was determined by immunoelectrophoresis.

Intradermal test (IDT) — Tests were performed on the forearm of individuals above 7

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years of age, according to PELLEGRINO & MACEDO [13].

Stool examination (SE) — Methods described by KATZ [9] and by HOFFMAN et al. [10] were used for the detection of **S. mansoni** and of other parasites. For the latter technique, three samples were tested from each stool specimen.

Chi-square test (x^2 test) — This statistical test was used to verify if the frequency of single or multiple parasitoses other than schistosomiasis differs significantly in cases with positive or negative immunological tests.

RESULTS

Tables I and II show that 25.5% of the cases yielded positive results in the IFT, and 29.8% of cases above 7 years of age, in IDT. However, in only 10.8% of the population positive results were observed by SE.

An agreement of 81.2% was observed in IFT and IDT.

TABLE I

Comparison of immunofluorescence test (IFT) with stool examination (SE) for **S. mansoni** infection

IFT \ SE	Positive	Negative	Total
Positive	28	70	98
Negative	2	284	286
Total	30	354	384

TABLE II

Comparison of intradermal test (IDT) with stool examination (SE) for **S. mansoni** infection in individuals above 7 years old

IDT \ SE	Positive	Negative	Total
Positive	25	62	87
Negative	2	203	205
Total	27	265	292

IgM antibodies anti-**S. mansoni** in abscence of IgG antibodies were found in 38 out of 98 (38.8%) positive serum samples in IFT, and in the remaining only IgG or both IgG and IgM were observed. Also, it was noted that about 26.6% (8/30) of patients excreting parasite eggs presented IgM antibodies and no IgG.

As showed in Fig. 1, prevalence of **S. mansoni** infection was higher in males (49%) than females (28.7%).

Other parasites such as **Ascaris lumbricoides, Ancylostoma duodenales, Enterobius vermicularis, Strongyloides stercoralis, Trichiurus trichiura, Hymenolepis nana, Giardia lamblia** and **Entamoeba histolytica** were found, concomitant or not with **S. mansoni** infection. Figure 2 illustrates the frequency of polyparasitism according to the immunological tests. The distribution of parasites unrelated to schistosomiasis was not significantly different among groups separated according to the immunological results, at the 5% level ($x^2 = 1.546$).

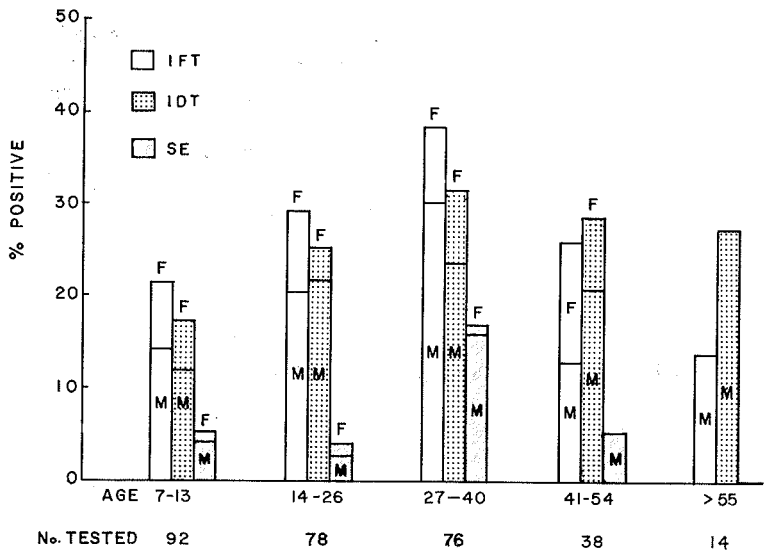

Fig. 1 — Prevalence of **S. mansoni** infection assessed by immunofluorescence test (IFT), intradermal tests (IDT) and stool examination (SE), according to age and sex, in 292 individuals from Fazenda Santa Helena, valley of the Paraiba do Sul River, São Paulo.

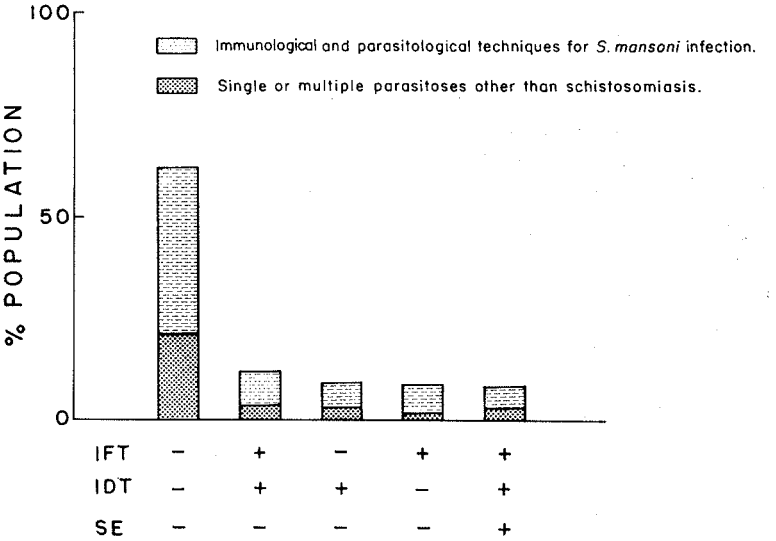

Fig. 2 — Frequency of parasitoses other than schistosomiasis according to immunological results, in residents of Fazenda Santa Helena, valley of the Paraiba do Sul River, São Paulo

DISCUSSION

In the present study, IF tests were performed on cryostat liver sections from **S. mansoni**-infected hamsters, showing granulomata to parasite eggs, since a similar sensitivity was observed to tests performed on worm sections [8]. Besides, infected livers were easier to obtain and could yield many more sections than worms, a relevant aspect when seroepidemiological purposes are envisaged.

Present parasitological data from stool examinations indicate that prevalence of schistosomiasis has increased about 3 times since last 1977 epidemiological inquiry. Also, positive IgM-IFT with negative IgG-IFT in several cases are suggestive of recently acquired **S. mansoni** infections, implying that transmission is currently occurring in that farm. The overall estimation of IgM- anti-**S. mansoni** antibodies in the population indicates an approximate 10% incidence rate in 1979.

Interference of non-specific IgM, as rheumatoid factor, could not be ruled out because of difficulties in obtaining serum for further studies. However, demonstration of positive IgM-IFT in abscence of IgG antibodies to **S. mansoni** among patients passing eggs, seems relevant.

These results point out the remarkable importance of using appropriate conjugates for serological diagnosis of schistosomiasis, so that both IgG and IgM antibodies can be detected. A lack of sensitivity of IFT, as referred by some investigators [15], might be due to the kind of conjugates used. IgA antibody was reported to be found only in recent infections but in low titers [8]. Thus, in the present work, the search of IgA antibodies was not possible due to little volume of blood eluates provided by filter paper, even in the dilution corresponding to 1/20.

It is interesting to note that, although in the studied population infection rates are not so high, patterns of age-prevalence of the parasitosis as revealed by SE, IDT and IFT were very similar to those observed in 1966 by CLARK (apud KAGAN [7]) in Rhodesia, where schistosomiasis is hyperendemic.

In spite of our low number of cases, it seems that IFT is less sensitive than IDT in age groups above 40 years. Similar observations have been already reported [19], but we feel that further studies must be done.

Percentages of positive results, of 92.6% for IDT and 93.3% for IFT, in patients passing eggs indicate a sensitivity close to that usually recorded [1,6,12,18,20]. Some loss in IFT sensitivity might derive from using filter paper eluates and

justify negative results in 2 parasitologically proven cases, but it could not be confirmed because of the already mentioned difficulties.

IDT is referred to be less sensitive in children [7] and adult females [12] which could explain negative results in a 11-years old boy and in a 25-years old woman. A negative IFT was also found for the latter one.

A 81.2% agreement was observed between IDT and IFT, a result comparable to that referred by WARREN et al [19], as well as between IDT and circumoval precipitin test [21].

Sensitivity of the liver egg-granulomata as antigen in the IFT seems reasonable and similar to that of worm or cercarial antigens [1,7].

A more difficult problem is posed in the evaluation of the antigen specificity, since SE, which gives a definite diagnosis, fails to detect eggs, especially in light infections. Results can be improved by repeating SE 4 or 5 times at different occasions [1,15,18], but this is impraticable for epidemiological purposes. Certainly the apparently low specificity we obtained, of 80.2% for IFT and 76.6% for IDT would be greatly improved if multiple stool examinations were carried out, as occurred in a previous seroepidemiological survey [4]. This fact has been confirmed for the circumoval precipitin test, with an increase in the specificity index from 66% to 96%, as a consequence of a rise in the sensitivity of the reference SE test by repeating this technique 3 or more times [15].

Furthermore, a problem to consider is the cross-reactivity of **S. mansoni** antigens with widespread avian and mammalian schistosomes in many areas of the world [11,14,16]. Thus, some of our positive results and some of the discrepancies observed between IFT and IDT might be due to such cross-reactivity, mainly because 3 different types of cercariae besides **S. mansoni** were observed in snails from the central irrigation ditches (unpublished data).

Polyparasitism was reported to reduce specificity and even sensitivity of immunological tests [2,3]. However, the polyparasitism observed in the studied population showed a similar distribution in cases with either both positive or negative immunological tests, as well as in cases presenting only one test positive. Thus, it seems that the presence of parasites other than **S. mansoni** had no marked influence on the results of immunological tests.

At the present time it seems that IFT with liver granulomata as antigen may constitute a good screening test for clinical and seroepidemiological purposes, and complementing IDT mainly in children and females. For further diagnosis corroboration, parasitological and other serological tests should then be performed.

RESUMO

Inquérito imunoepidemiológico de esquistossomose mansônica no Vale do Paraíba, São Paulo, Brasil

Inquérito imunoepidemiológico foi realizado na fazenda Santa Helena, localizada no Vale do Rio Paraíba do Sul, São Paulo.

A prevalência de esquistossomose determinada pela reação de imunofluorescência foi de 25,5% e de 29,8% pela reação intradérmica. O exame coprológico revelou 10,6% de infectados, cerca de três vezes mais do que observado no inquérito preliminar de 1977.

A influência de poliparasitismo na sensibilidade e na especificidade das reações imunológicas não foi significativo.

Resultados satisfatórios foram obtidos na reação de imunofluorescência com cortes de fígado de hamster infectado com **S. mansoni**, tendo granuloma e ovos do parasita como antígeno.

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