

## Do intestinal parasites interfere with the seroepidemiologic surveillance of *Schistosoma mansoni* infection?

B. ALARCÓN DE NOYA\*, C. COLMENARES, S. LOSADA, Z. FERMIN,  
G. MASROUA, L. RUIZ, L. SOTO AND O. NOYA

Sección de Biohelmintiasis, Instituto de Medicina Tropical, Universidad Central de Venezuela

(Accepted 23 October 1995)

### SUMMARY

In view of the known cross-reactivity of sera from patients with intestinal parasites to some *Schistosoma mansoni* antigens, field work was conducted in an area of Venezuela non-endemic for schistosomiasis using the routine immunoenzymatic assay (ELISA) with soluble egg antigen (SEA). False positive reactions represented 15.3% of the total population as determined by SEA-ELISA. SEA-immunoblotting of the false positive sera indicated that protein fractions of 91 and 80 kDa appear to be responsible for cross-reactivity. Sera from hookworm infected individuals produced a higher frequency and intensity of cross-reaction than other sera. SEA-fractions of 105, 54, 46, 42, 32, 25 and 15 kDa were the most specific.

### INTRODUCTION

The adequate control of any infectious disease depends substantially on appropriate diagnostic tests. As more efficient approaches to schistosomiasis control are implemented, the resulting decrease in prevalence and parasite load, limits the diagnostic effectiveness of classical stool examinations. For this reason, alternative methods should be developed. Immunodiagnosis of schistosomiasis is a growing field of research. Immunodiagnostic tests are a necessary tool in control programmes, especially in areas where the aim is the eradication of this parasitic disease [1].

Several serologic tests such as Circumoval Precipitin Test (COPT) and Indirect Immunofluorescent Assay (IFA) have been used for the detection of antibodies for individual diagnosis, but immunoenzymatic assays (ELISA) are preferred for mass diagnosis. This assay fulfils the requirements of low cost, reproducibility, objectivity and rapid results [2]. However, when ELISA with crude antigens of *S. mansoni* is compared

to COPT the former overestimates the prevalence of schistosomiasis infection [3, 4]. This reflects past infections since antibodies persist for long periods of time after cure [5]. False positive results could also be caused by cross-reactivity with other intestinal parasitic infections [6, 7]. This latter point becomes a problem when ELISA with crude *S. mansoni* antigens are used for epidemiologic surveillance in endemic areas where intestinal parasites are often prevalent.

The present work was carried out in a non-endemic area of schistosomiasis in Venezuela in order to test the validity of the seroepidemiologic approach of ELISA with Soluble Egg Antigen of *Schistosoma mansoni* (SEA). It attempts to identify, using Western blot with a luminescent substrate, the fractions of SEA that might be responsible for false positive reactions observed in individuals with intestinal parasites.

### MATERIALS AND METHODS

#### Study population

A group of 404 individuals from the Aripao municipality, Bolivar State, southern Venezuela, was

\* Address for correspondence: Dra. Belkisyolé Alarcón de Noya, Laboratorio de Estudios para Malaria, Universidad Central de Venezuela. Apartado Postal 4417. Caracas, 1010-A, Venezuela.

studied. This rural community, where intestinal parasitic infections and malaria are common, is *c.* 800 km from the schistosomiasis endemic area of Venezuela. The climate is tropical and the predominant activities are agriculture and fishing. Past history of schistosomiasis or residence outside the study area was recorded for each individual.

### Stool examination

A total of 346 stool samples were collected and preserved with Ralliet and Henry solution (5% formaldehyde and 2% acetic acid in saline solution [8] in 20 ml flasks. Each sample was sifted, centrifuged and the sediment was exhaustively examined for helminth eggs and protozoan cysts.

### Antigens

SEA was prepared from eggs obtained from livers of *S. mansoni*-infected mice ('JL' schistosome strain, kindly provided by Dr Italo Cesari, IVIC) and processed as previously described [9]. After manual homogenization of the eggs, they were ultracentrifuged at 100000 *g* for 2 h at 4 °C and the resulting supernatants used as SEA. Protein concentration was determined by the Lowry technique [10].

### Serology

ELISA was performed as described by Voller and colleagues [11]. Polystyrene plates (Immulon 2) were sensitized overnight with 5 µg/ml of SEA in carbonate/bicarbonate buffer pH 9.6. Blocking was performed using a solution of 0.05% PBS-Tween-20 with 1% bovine albumin. Serum samples diluted (1/200) in 0.05% PBS-Tween 20 with 0.25% bovine albumin were incubated 1 h at 37 °C and after washing, anti-human IgG-alkaline phosphatase conjugate (1/1000) was added and incubated 1 h at 37 °C. After further washings the reaction was developed with *p*-nitrophenylphosphate substrate (1 mg/ml). Cut-off values were established based on the average of the optical densities of 100 negative control sera, plus 3 standard deviations.

COPT was conducted based on the method of Oliver-González [12] with modifications described elsewhere [4]. All sera that gave positive reactions to ELISA were also assayed by COPT.

Table 1. 48 sera from the study area

With hookworms	With or without other parasites
12 sera ELISA positive (+)	12 sera ELISA positive (+)
12 sera ELISA negative (-)	12 sera ELISA negative (-)

Table 2. Age distribution of samples and SEA-ELISA results of the study population, Aripao, Edo. Bolivar, Venezuela

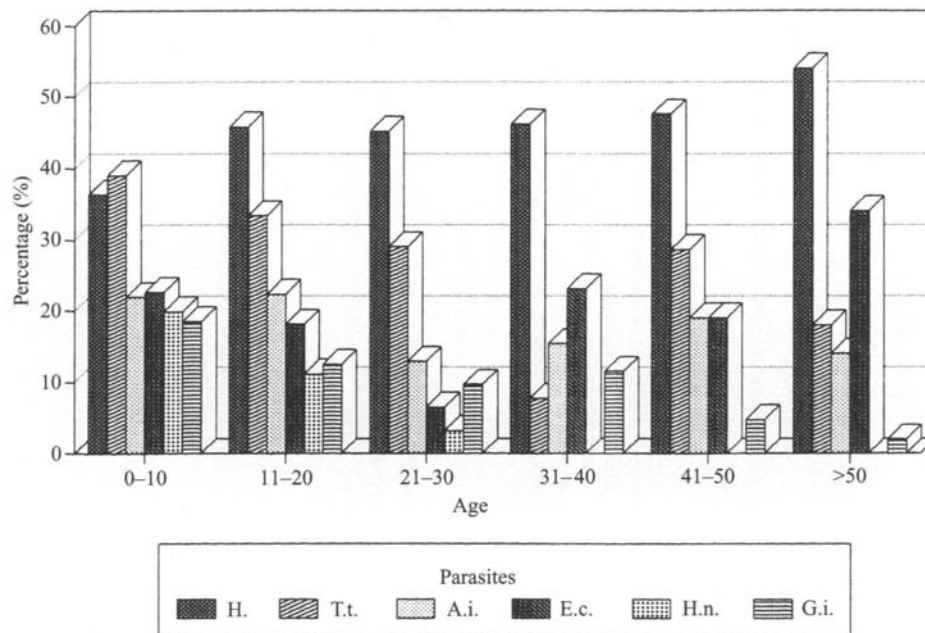
Age range	No. of faeces examined	No. of sera studied	SEA-ELISA Positive	
			No.	(%)
0-10	146	158	30	19.0
11-20	72	88	14	16.0
21-30	31	39	2	5.1
31-40	26	36	6	16.7
41-50	21	24	3	12.5
> 50	50	59	7	11.9
Total	346	404	62	

SEA was analysed by electrophoresis under dissociating (SDS-PAGE) and non-reducing conditions [13]. Antigen was diluted to 0.2 mg/ml in sampling buffer and 450 µl used per preparative gel. SDS-7 molecular weight standards (Sigma Chemical Co.) were used. The gel was electrophoresed at 200 V and 150 mA for 45 min. After electrophoresis the proteins were transferred onto nitrocellulose sheets (Schleicher and Schuell, 0.22 µm) at 500 v and 150 mA for 1.5 h [14]. Blocking was achieved with 5% non-fat milk in 0.1% PBS-Tween 20 for 1.5 h.

For immunoblotting 48 sera from the study area were chosen according to their ELISA results to SEA and are given in Table 1.

Three known schistosomiasis positive sera and three sera from subjects negative for intestinal parasites were also assayed in each blot (6 C+ and 6 C-).

Sera diluted (1/100) in blocking solution were incubated individually with SEA-nitrocellulose strips with constant agitation for 1.5 h. The second antibody, antihuman-IgG horseradish peroxidase, was diluted 1/1000 in blocking solution. Finally, the strips were washed and incubated with the fluorogenic substrate of the peroxidase enzyme (ECL Detection System Luminol, Amersham Laboratories) for 1 min



**Fig. 1.** Age distribution of parasitic infection in the study population, Aripao, Bolivar State, Venezuela. Parasites: H., Hookworm; T.t., *Trichiuris trichiura*; A.i., *Ascaris lumbricoides*; E.c., *Entamoeba coli*; H.n., *Hymenolepis nana*; G.l., *Giardia lamblia*.

and applied to photographic film (Kodak) for c. 15 sec.

## RESULTS

The majority of the study subjects were permanent residents of Bolivar State. Positive reactors to SEA were reinterviewed and except for 2 subjects, none had visited a schistosomiasis endemic area.

There were 404 individuals interviewed and bled. They provided 346 stool samples; 84.6% were positive for intestinal parasites. Sixteen parasite species were found, with the most frequent being, hookworm (43%), *Trichiuris trichiura* (31%), *Ascaris lumbricoides* (19.4%), *Giardia lamblia* (12.7%) and *Hymenolepis nana* (11%). *Schistosoma mansoni* eggs were not observed. The age distribution of the individuals who provided stool and serum samples is shown in Table 2. The prevalence of intestinal parasites according to age is shown in Fig. 1. Hookworm infection was present in all age groups. Polyparasitism was common, with 15.3% of the subjects infected with three parasite species, 28.6% with two and only 35.5% with one type of intestinal parasite.

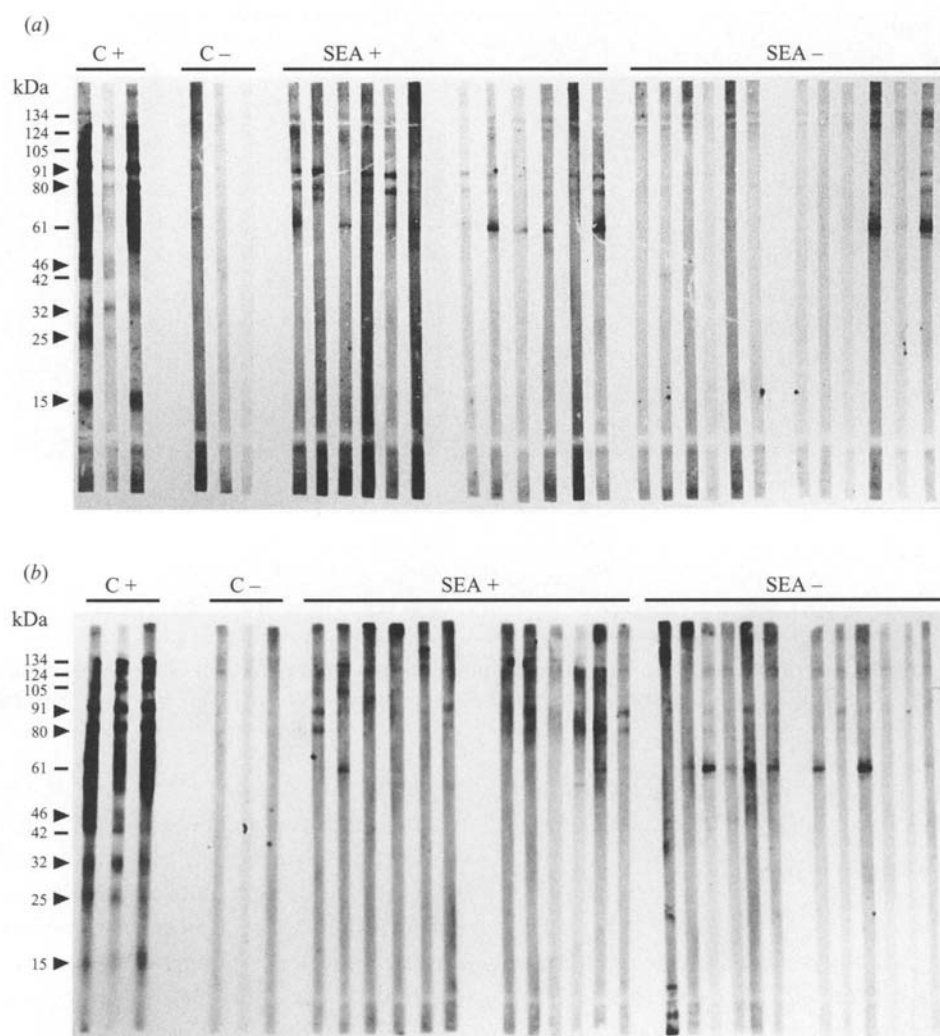
SEA-ELISA was positive in 15.3% of the population with the highest positive response observed in children of 0-10 years of age (19%) (Table 2). All sera assayed by COPT test were negative.

Immunoblots of false positive sera from hookworm

infected subjects and with subjects with other intestinal parasites are shown in Fig. 2a, b. From these blots, the frequency of global recognition was determined, and the results are shown in Fig. 3, which reflects the direct observation of the bands seen through the transilluminator (photographs miss important details). Positive controls recognized bands of 134, 124, 105, 91, 80, 74, 61, 54, 46, 42, 32, 25 and 15 kDa. Bands of 46, 32, 25 and 15 kDa were not recognized by sera of unexposed individuals who reacted in SEA-ELISA. Other SEA-fractions, such as 105, 54 and 42 kDa, were recognized weakly and with low frequency by the false positive sera (0.04, 0.08 and 0.08, respectively).

The 134, 124 and 61 kDa bands were recognized with frequencies higher than 0.5 by all groups of sera assayed. Bands of 91 and 80 kDa were also recognized by all groups of sera, but with stronger intensity by the SEA-ELISA positives. In fact, the SEA-ELISA negative sera recognized these bands with light intensity, similar to the negative control sera.

Nevertheless, when the false positive sera of hookworm-infected subjects were compared with false positive sera of those free from this infection, we observed the following: SEA antigens of 91 and 80 kDa were recognized by the SEA-ELISA positive from hookworm infected subjects with frequencies of 0.83 and 0.92, respectively. This frequency is significantly lower in people without hookworms (0.33,



**Fig. 2.** Immunoblots with SEA of false positive sera from hookworm infected subjects (*a*), from subjects infected with other intestinal parasites (*b*). Note the high intensity and frequency of recognition of the 91 and 80 kDa bands from hookworm-infected subjects.

0.50) and whenever these bands were recognized they were weak (Figs 2*a*, *b*, 3).

## DISCUSSION

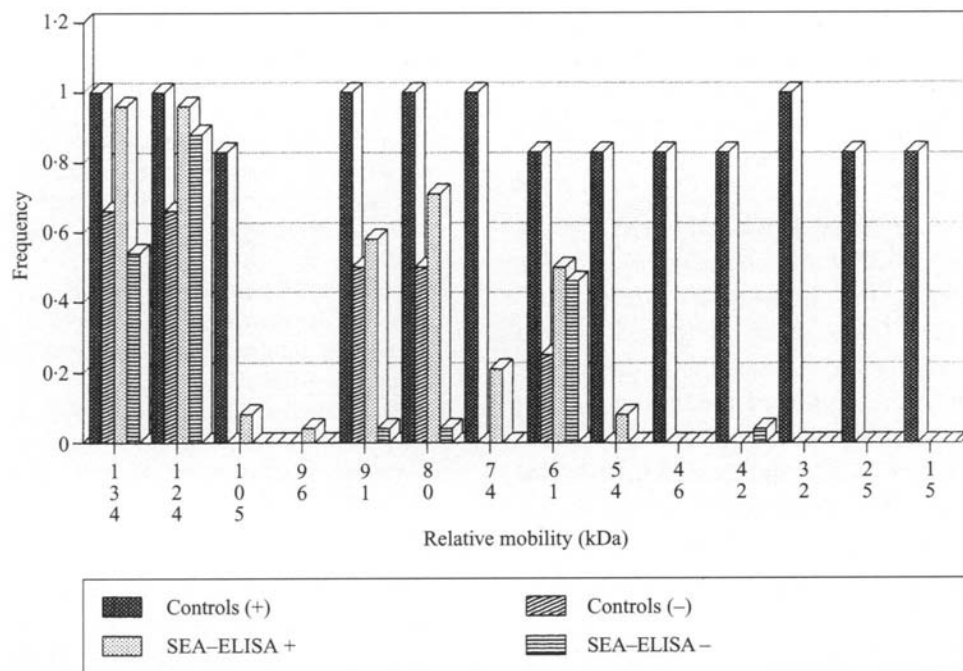
The cross-reactivity in serologic tests between *S. mansoni* and other parasites such as *Fasciola hepatica*, *Paragonimus westermani* and *Trichinella spiralis*, is well known [15–21]. In these particular cases, this does not necessarily represent a major diagnostic problem, since endemic areas of these parasites usually do not overlap. In contrast, cross-reactivity between *S. mansoni* and intestinal helminths such as hookworms, is a real impairment to diagnosis because these parasites frequently coexist.

Correa-Oliveira and colleagues [7] demonstrated the existence of antibodies in hookworm carriers

which cross-reacted with schistosomula antigens. Moreover, these sera were as lethal to schistosomula *in vitro* as were the sera from schistosomiasis patients.

It is important to clarify whether these cross-reactions interfere with the serological diagnosis of schistosomiasis and to identify the molecules of *S. mansoni* SEA involved in this reaction. Notably, 15% of the cross-reactivity might be expected in sera from non-endemic schistosomiasis areas where hookworms are prevalent (43%). Different studies in Venezuela have shown that *T. trichiura* and *A. lumbricoides* are the most prevalent intestinal nematodes [22, 23] and hookworm prevalence varies between the geographic areas.

In Venezuela, the existence of multiparasitism makes the interpretation of serologic results difficult even in subjects negative by examination of faeces. In



**Fig. 3.** Frequency of SEA recognition by immunoblot of sera from a non-endemic schistosomiasis area, Aripao, Bolivar State, Venezuela.

some areas annual treatment is given by the National Program for Control of Intestinal Parasites and antibodies against intestinal parasites can be present in subjects with transient negative stool examinations. Also, because of the limitations of the routine diagnostic techniques for the detection of parasites such as *Strongyloides stercoralis* and *Enterobius vermicularis* we cannot rule out possible infection with these parasite species. In Kenya, results from a comparative evaluation of SEA and CEF6 in a non-endemic area of schistosomiasis suggested that intestinal parasites did not seem to interfere with the serologic diagnosis of the *S. mansoni* infection [24]. However, the number of their hookworm-infected individuals was low and this might be the reason for the specificity of results among the subjects with intestinal parasites in that study.

In the present study the SEA-ELISA showed 84.7% specificity, as has been reported by others [4, 25–27]. Children reacted with less specificity to SEA than adults, and the reactivity diminished with age. A similar observation was reported by Ramos and Cesari [28] who observed that children gave more cross-reactivity to glycosylated components of SEA. It is conceivable that the higher cross-reactivity of children to SEA is due to their increased exposure to intestinal parasites. Demonstration of whether the glycosylated molecules are responsible for cross-reaction is currently undertaken.

The protein fractions of SEA most frequently recognized by false positive sera were the 91 and 80 kDa bands which also reacted strongly with positive control SEA-ELISA sera. Therefore, we infer that these might be the proteins responsible for false positive reactions observed with SEA. When the false positive sera were separated according to the presence or absence of concomitant hookworm infection, immunoblots to SEA showed that recognition of bands of 91 and 80 kDa was more frequent and more intense in the hookworm-infected subjects.

The percentage of hookworm-infected persons was high in the study area (43%), and cross-reactivity with *S. mansoni* egg antigen was observed in 15.3% of the entire group. It is important to note that not all cross-reactive subjects had hookworms, nor did all hookworm-infected subjects cross-react with SEA. Pre-absorption of the sera with hookworm antigen might ablate the cross reactivity in the sera from hookworm infected individuals. However, this procedure is cumbersome and the hookworm antigen is not easy to obtain. The level of antibodies to hookworms as well as length and intensity of infection may play an important role in the level of cross-reactivity.

The most specific fractions of SEA that were apparently recognized only by the schistosomiasis infected patients were the 46, 32, 25 and 15 kDa proteins. Besides being recognized by all the positive

control patients, the 105, 54 and 42 kDa fractions were recognized with a low frequency and intensity by the cross-reactive sera. Therefore, the present work confirms the potential diagnostic value of these highly specific molecules as it has been previously demonstrated by us [29].

The present work aimed to validate methodology for mass screening of *Schistosoma mansoni* infection to identify cases in low transmission areas. Non-specific molecules of SEA were recognized by sera from non-infected *S. mansoni* individuals, the specific molecules of SEA were confirmed and the luminescent substrate for immunoblot was used for the first time with this purpose. Considering these results in the complex area of schistosome immunodiagnosis, we conclude that in areas where schistosomiasis mansoni and hookworms coexist, a 15% positivity in SEA-ELISA can be attributed to false positive reactivity. There is the possibility that other non-helminthic agents could be also responsible for cross-reacting antibodies.

Through chemical synthesis or molecular engineering techniques, the production of specific SEA antigens would be ideal for the antibody detection of this disease and enormously facilitate the diagnosis of schistosomiasis by ELISA in areas where concomitant infection with other intestinal parasites occurs. Furthermore, since cross-protection with hookworm has been demonstrated [6] it is very important to evaluate the role of these antigens preventing *Schistosoma mansoni* infection.

## ACKNOWLEDGEMENTS

This work was supported by the 'Corporación Andina de Fomento' and the National Malaria Vaccine Project (MSAS-CVG-Gobernación del Edo. Bolívar-UCV). Thanks to the 'Consejo de Desarrollo Científico y Humanístico' for the financial support in the publication of these results. We are grateful to Jaime Torres, Neil Lynch and Susana Gonzalez for their assistance in the preparation of the manuscript. Also to the Photography Laboratory of the Science Faculty UCV, for their help.

## REFERENCES

- Alarcón de Noya B, Noya O, Balzan C, Cesari I. New approaches for the control and eradication of schistosomiasis in Venezuela. Mem do Inst Oswaldo Cruz 1992; **87**: 227–31.
- Mott KE, Dixon H. Collaborative study on antigens for a immunodiagnosis of schistosomiasis. Bull WHO 1982; **60**: 729–53.
- Hillyer GV, Ruiz Tiben E, Knight WB, Gomez de Rios I, Peley RP. Immunodiagnosis of infection with *Schistosoma mansoni*: comparison of ELISA, radio-immunoassay and precipitation tests performed with antigens from eggs. Am J Trop Med Hyg 1979; **28**: 661–9.
- Spencer L, Alarcón de Noya B, Noya O, Masroua G. Prueba de precipitación circumoval y ELISA con antígenos solubles en el diagnóstico de la esquistosomiasis. GEN 1991; **45**: 77–83.
- Alarcón de Noya B, Spencer L, Noya O. Pre and post-treatment immunodiagnostic evaluation in human schistosomiasis mansoni. Mem do Inst Oswaldo Cruz 1992; **87**: 271–6.
- Correa-Oliveira R, Oliveira GC, Golgher DB, et al. Analysis of antibody responses of *Schistosoma mansoni* infected patients against schistosomal antigens. Mem do Inst Oswaldo Cruz 1987; **82**: 179–81.
- Correa-Oliveira R, Dusse LMS, Viana IRC, Colley DG, Carvalho OS, Gazzinelli G. Human antibody responses against schistosomal antigens. Antibodies from patients with *Ancylostoma*, *Ascaris lumbricoides* or *Schistosoma mansoni* infections react with schistosome antigens. Am J Trop Med Hyg 1988; **38**: 348–55.
- Pessôa SB, Martins AV. Pessôa parasitologia médica. Guanabara Koogan SA, eds. Rio de Janeiro 1982: 832.
- Boros DL, Warren KS. Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from *Schistosoma mansoni* eggs. J Exp Med 1970; **132**: 488–507.
- Lowry OH, Rossenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin reagent. J Biol Chem 1951; **193**: 265–75.
- Voller A, Bartlett A, Bidwell D. Enzyme immunoassays for parasitic diseases. Trans Roy Soc Trop Med Hyg 1976; **70**: 98–105.
- Oliver-González J. Anti-egg precipitin in sera of humans infected with *Schistosoma mansoni*. J Infect Dis 1954; **95**: 86–91.
- Laemli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; **227**: 680–5.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Nat Acad Sciences 1979; **76**: 4350–4.
- Pelley RP, Hillyer GV. Demonstration of a common antigen between *Schistosoma mansoni* and *Fasciola hepatica*. Am J Trop Med Hyg 1978; **27**: 1192–4.
- Hillyer GV, Serrano AE. The antigens of *Paragonimus westermani*, *Schistosoma mansoni* and *Fasciola hepatica* adult worms. Am J Trop Med Hyg 1983; **32**: 350–8.
- Hanna REB, Hillyer GV. *Fasciola hepatica* and *Schistosoma mansoni*: Immunofluorescent antigen localization and cross-reactivity. Exp Parasitol 1984; **57**: 1–14.

18. Aronstein WS, Daton JP, Strand M. A *Schistosoma mansoni* surface glycoprotein cross-reactive with a T1 antigen of *Fasciola hepatica*. *Am J Trop Med Hyg* 1985; **34**: 889–97.
19. Aronstein WS, Dalton JP, Weiss JB, Strand M. Identification and characterization of a major *Schistosoma mansoni* glycoprotein antigen cross-reactive with *Fasciola hepatica*. *Am J Trop Med Hyg* 1985; **34**: 879–88.
20. Aronstein WS, Lewis SA, Norden AP, Dalton JP, Strand M. Molecular identity of a major antigen of *Schistosoma mansoni* which cross-reacts with *Trichinella spiralis* and *Fasciola hepatica*. *Parasitol* 1986; **92**: 133–51.
21. Linder E, Thors C, Lundin L, et al. Schistosome antigen GP50 is responsible for serological cross-reactivity with *Trichinella spiralis*. *J Parasitol* 1992; **78**: 999–1005.
22. Botero D. Persistencia de parasitosis intestinales endémicas en América Latina. *Bol Ofic Sanit Panam* 1981; **90**: 39–46.
23. Morales G, Pino LA, Rodriguez E. Estudio de las geohelmintiasis en humanos en las zonas marginales de la ciudad de Trujillo, Venezuela. *Bol Direc Malariol San Amb* 1984; **XXIV**: 71–8.
24. Doenhoff MJ, Butterworth AE, Hayes RJ, et al. Seroepidemiology and serodiagnosis of schistosomiasis in Kenya using crude and purified egg antigens of *Schistosoma mansoni* in ELISA. *Trans Roy Soc Trop Med Hyg* 1993; **87**: 42–8.
25. Hillyer GV, Gomez de Rios I. The enzyme-linked immunosorbent assay (ELISA) for the immunodiagnosis of schistosomiasis. *Am J Trop Med Hyg* 1979; **28**: 237–41.
26. Mc Laren M, Long EG, Goodgame RW, Lillywhite JE. Application of the enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of *Schistosoma mansoni* infections in St. Lucia. *Trans Roy Soc Trop Med Hyg* 1979; **73**: 636–9.
27. Alarcón de Noya B, Pujol FH. Diagnóstico inmunológico de la esquistosomiasis mansoni. *Interciencia* 1990; **15**: 95–101.
28. Ramos A, Cesari I. Evaluación de varias preparaciones solubles de huevos de *Schistosoma mansoni*, como antígenos para serodiagnóstico de la esquistosomiasis. *Acta Cient Ven* 1991; **42**: 202.
29. Noya O, Losada S, Alarcón de Noya B, et al. Effect of chemotherapy on immune response to egg antigens of *Schistosoma mansoni* in chronically infected children from areas of low transmission. *Parasite Immunol* 1995; **17**: 111–7.