

RE-INFECTION OF PATIENTS IN SCHISTOSOMIASIS MANSONI ENDEMIC AREAS AFTER SPECIFIC TREATMENT

I — Influence of age and worm burden

Naftale KATZ, Fábio ZICKER, R. S. ROCHA and V. B. OLIVEIRA

S U M M A R Y

312 *S. mansoni* patients living in two endemic areas (Calciolândia and Tuparecé) of the State of Minas Gerais have been followed-up, for a period of 18-24 months. These patients have been treated with a single intramuscular hycanthone dose of 2.5 mg/kg and considered as cured. Cure was assessed by repeated negative stool examinations performed six months after treatment. The percentages of re-infection after about two years were, for the two areas, 16.8 and 21.8, respectively. Children (under fifteen years of age) showed a reinfection rate significantly higher (34.5%) than that of the adults (6.9%). Quantitative feces examination by Kato-Katz method demonstrated that the arithmetical mean and the median of *S. mansoni* eggs in the feces of re-infected patients were 2-to-4 times smaller than those obtained before treatment. Approximately 20% of the patients cured and re-infected showed an increase of *S. mansoni* eggs per gram of feces, 60% of them presented a decrease, and the remaining 20% did not reveal any changes. In the control groups (I, patients from the same area but not treated because of other concurring diseases; II, patients from another endemic area — Comercinho de Bruno — and not treated either), both the percentages of increase and decrease in the number of eggs in the feces were around 20%, whereas the one concerning no alterations was 60%. A three-fold variation (increase or decrease) was always considered. The data obtained suggest a certain degree of resistance to re-infection in schistosome patients (higher in adults than in children) living in endemic areas after specific treatment, which may probably be due to inespecific and/or acquired and/or concomitant immunity.

I N T R O D U C T I O N

The fact of former *S. mansoni* patients, already treated and cured, getting re-infected in areas of active transmission is well known and undeniable. Nevertheless, detailed studies on rates of re-infection and factors likely to be involved in the phenomenon have, so far, been rarely conducted in Brazil^{3,15,16}.

On the other hand, SMITHERS & TERRY²² have drawn attention to the small num-

ber of papers published about immunity in man, emphasizing the need for further investigations of the populations living in endemic areas, which would greatly contribute for a better understanding of the relation man-parasite.

The present paper, first of a series, shows some aspects of the re-infection, with *Schistosoma mansoni*, in previously cured individuals

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living in endemic areas of Minas Gerais, Brazil, who had been followed up for a period of up to two years after specific treatment.

PATIENTS AND METHODS

This work has been carried out in three *S. mansoni* endemic areas of the State of Minas Gerais. The first one, Tuparecê, district of Medina, is located in the North and presents poor socio-economical level, precarious sanitary conditions and foci of *Schistosoma* infection with which its population is in permanent contact. The prevalence of the infection among the 744 local inhabitants was found to be 62.9% (based on a single feces examination), the arithmetical mean and the median of *S. mansoni* eggs being 701 and 288, respectively, and the percentage of hepatosplenic forms 5.7. 187 Patients were treated in this area.

The second area, Calciolândia, located in the west region of the State displays urban features with houses (all of them belonging to workers of a dairy industry provided with running water and hygienic water-closets that, nevertheless, empty their contents directly into the streams surrounding the locality. The infection prevalence was found to be 31.0% (619 patients examined), the mean and the median of *S. mansoni* eggs per gram of feces being 445 and 219, and the percentage of hepato-splenic forms, 0.9. The number of schistosome patients treated amounted to 219, all of them being factory workers or members of their family. The third area, Comercinho do Bruno, is similar, in hygiene and socio-economical conditions, to Tuparecê. The prevalence of schistosomiasis was 69.5% (among 1,299 cases), the mean and the median of eggs per gram of feces being 1,178 and 498, and the rate of hepato-splenic forms, 11.0%. 50 Patients were studied in this area.

The patients living in Tuparecê and Calciolândia were treated with a single intramuscular hycanthonone dose of 2.5 mg/kg body weight. Individuals weighing less than 20 kg, those over 65 years of age, pregnant women and other patients with severe disease or decompensated hepato-splenic forms of schistosomiasis, were excluded from treatment.

The 44 Tuparecê patients, showing counter-indication to the treatment, constituted

control group I and the 50 untreated patients from Comercinho do Bruno formed control group II.

Patients under 15 years of age were considered "children".

Repeated feces samples from the patients were examined, by Kato-Katz quantitative method¹³ (*) every six months, for up to 18-24 months after treatment. For diagnostic purposes, just one stool sample was examined. Six months after the end of treatment, three stool samples from each patient were examined on consecutive days and, from the 18th to the 24th month, just one sample. Two thick smears were prepared from each stool sample, the number of *S. mansoni* eggs per gram being them counted and the arithmetical mean of the data thus obtained, recorded.

From control group II, there were examined two smears from one stool sample, the patients being re-examined one year later.

Were considered as non-cured, the patients still presenting *S. mansoni* eggs six months after treatment and, as re-infected, the individuals who showed, after treatment, at least three negative tests followed by one or more positive ones after 18 to 24 months.

As the number of *S. mansoni* eggs per gram of feces varies when countings are performed at different occasions¹⁰, only a three-fold variation in their number, as compared with the data from the initial examination, was taken as criterion for the evaluation of the re-infection intensity.

Student's test has been used for evaluating the statistical significance of the data.

RESULTS

Among the 406 patients treated with hycanthonone, 161 (73.5%) from Calciolândia and 151 (80.7%) from Tuparecê were followed up for up to two years. The percentages of cure were, respectively, 94.2 and 95.7 for the adults and 89.7 and 79.0 for the children.

Table I shows the data concerning re-infection in those two areas. Re-infection of pa-

(+) Kit AK for parasitological stool examinations — AK Indústria e Comércio Ltda. 30000 BELO HORIZONTE, BRAZIL.

tients previously cured varied from 16.8% (Calciolândia) to 21.8% (Tuparecê), the difference between such data having no statistical significance. The percentage of re-infection in children (34.5%) was, however, significantly higher than that in adults (6.9%) ($p > 0.01$).

Comparing the mean and the median figures concerning *S. mansoni* eggs eliminated

before treatment with those obtained after re-infection, a decrease was observed in the number of eggs in the latter case. In fact, the mean and the median number of eggs in the feces of re-infected patients were 2-to-4 times lower than those observed before treatment. In control group I, the arithmetical mean remained unaltered whereas the median number increased (Table II).

T A B L E I
Re-infection of patients in two schistosomiasis mansoni endemic areas

Area	Patients re-infected/total number (% of reinfection)		
	Adults	Children	Total
Calciolândia	4/103 (3.9)	23/58 (39.7)	27/161 (16.8)
Tuparecê	8/ 70 (11.4)	25/81 (30.9)	33/151 (21.8)
Total	12/173 (6.9)	48/139 (34.5)	60/312 (19.2)

T A B L E I I

Arithmetical mean and median of the number of *S. mansoni* eggs, per gram of feces, before treatment and after re-infection, from patients still living in endemic areas

Area	Number of cases	Arithmetical mean		Median	
		Before	After	Before	After
Calciolândia	27	610	376	282	150
Tuparecê	33	836	340	650	150
Total	60	735	356	650	150
Control I	44	230	272	126	230

T A B L E I I I

Variation in the number of *S. mansoni* eggs in the patients feces before treatment and after re-infection

Area	Number of cases	Number of <i>S. mansoni</i> eggs per gram of feces		
		Increase	Decrease	No alteration
Calciolândia	27	7 (22.2)	14 (51.9)	6 (22.2)
Tuparecê	33	8 (24.2)	18 (54.6)	7 (21.2)
Total	60	15 (25.0)	32 (53.3)	13 (21.7)
Control I	44	10 (22.7)	9 (20.4)	25 (56.9)
Control II+	50	9 (18.0)	12 (24.0)	29 (58.0)

(): percentage of cases

+ : stool examination performed after 1 year

Table III shows the data from re-infected patients and controls regarding increase, decrease or no alteration in the number of *S. mansoni* eggs as compared with those obtained before treatment and taking into account a variation of, at least, three times. In control groups I and II (no specific treatment), about 60.0% of patients did not show any alterations, whereas 20% presented increase and the remaining 20%, decrease in the number of eggs per gram of feces. As to the treated patients, 53.3% of them eliminated a smaller number of eggs in their feces. Statistical analysis showed no significant difference between the data from the treated patients of the two endemic areas, but the difference between these data and those from the control groups, however, proved quite significant ($p > 0.01$).

DISCUSSION

KLOETZEL¹⁶ reported that *S. mansoni* infected children treated with Astiban (TWSb), but still living in endemic areas, got re-infected. Nevertheless, the mean number of eggs in their feces was, then, found to be four times smaller. Actually, considering the data obtained before treatment, the 83 followed-up children had eliminated, each of them, a mean number of 415 *S. mansoni* eggs per gram of feces, whereas, in the 47% patients still eliminating eggs, this number decreased to 32 four months later and, after re-infection, four years later, it increased to 135 (83% positive tests). Based on his findings, KLOETZEL suggested that treatment of patients living in endemic zones must be carried out, especially when re-infection is expected¹⁶.

The present investigation has shown that, two years after treatment, re-infection of children was very high. As a matter of fact, in the two localities where the infected individuals were treated and considered cured, over 30% of the patients under 15 years were eliminating eggs in their feces again, as contrasted with approximately 10% of the adults in the same circumstances. These findings confirm the cases observed by KATZ¹⁰ at another locality (Baldim) where, after similar lapse of time, 9% of the adults and 40% of the children had got re-infected.

When the number of *S. mansoni* eggs per gram of feces, indicative of the worm burden,

is taken into account, the results are more encouraging. Actually, while in the untreated group, 50% of the patients kept eliminating the same amount of eggs, over 50% of the patients treated and cured, presented, after re-infection, a lower number of *S. mansoni* eggs in their feces, the difference having proved statistically significant.

Three hypotheses may be raised to account for the decrease in the number of eggs and for the re-infection rates. Firstly, the phenomena could be explained by unspecific immunity, i.e., by increase of immunity with increase of the person's age. Secondly, they could be due to acquired immunity. Studies conducted by different investigators, such as, among others, SCOTT in Venezuela²⁰, CLARK in Rhodesia^{5,6}, FAROOK & SAMAN in Egypt⁸ and, in Brazil by, KLOETZEL^{15,16}, BARBOSA², ANDRADE & PRATA¹, PES-SOIA¹⁹ and KATZ¹⁰, demonstrated that, in groups of people living in endemic areas, the prevalence and/or the number of eggs per gram of feces is higher in youngsters and lower in groups of older people. Nevertheless, KLOETZEL & RODRIGUES DA SILVA¹⁷, surveying a population of Jacarepaguá infected in adulthood, concluded that decreases in prevalence and worm burden depend on the duration of infection and not on the patient's age.

It must be pointed out however, that decrease in the number of eggs, indicative of decrease in the number of worms parasitizing the inhabitants of the endemic areas, (CHEEVER⁴) should not definitely be traced back to acquired immunity, as it may be observed in cases when the probability of exposure markedly decreases and/or when the worm life span is shorter⁴. Nevertheless, according to COURA et al.⁷, patients that had left *S. mansoni* endemic areas in Brazil, about 6-to-30 years before, and were afterwards living in zones of no active transmission, still eliminated eggs in their feces.

The third hypothesis would be related to concomitant immunity. As well demonstrated by SMITHERS & TERRY²², some animal species parasitized by *S. mansoni* do not get re-infected while still bearing adult worms.

It seems that such immunity also occurs in man, although it does not completely prevent new infection^{9,14,18}. In fact, if concomitant immunity did not occur, it would be difficult to explain how inhabitants of highly endemic areas, exposed to daily contact with infected water, could survive¹⁹.

Probably, patients considered as cured continue harbouring small worm amounts, which would induce concomitant immunity. Indeed, as elegantly demonstrated by SILVA et al.²¹, treated patients (with repeated negative tests for *S. mansoni* eggs), when submitted to new treatment with schistosomicides, presented precipitating antibodies possibly resulting from the death of worms and consequent antigenic liberation.

It is worth while reminding that stool examination by KATO-KATZ technique¹³ can reveal up to 20 *S. mansoni* eggs per gram of feces, although displaying a coefficient of variation around 80%¹¹. Supposing 20 eggs per gram of feces to be its highest efficiency limit (KATZ et al.¹¹), 500-to-800 the largest number of eggs daily excreted by a couple of worms (CHEEVER⁴), and 200 g the total amount of feces daily excreted, it would be necessary 5-to-8 couples for providing a positive test. On the other hand, based on the data from control groups I and II (Table III), it was demonstrated a variation of increase or decrease in the number of eggs (considering a 3-fold interval) around 40%. This shows the number of eggs, per gram, from the remaining 60% to be unchanged, which seems to be in support of the probable occurrence of concomitant immunity in most human patients.

If, on one hand, the hypothesis of concomitant immunity can explain the fact of re-infection not taking place in patients already infected, as well as the low number of eggs in their feces, the higher frequency of re-infection in children can, on the other hand, support the first hypothesis.

It should be emphasized that the contact of children with infected waters is likely to be more frequent than that of adults, and that this may also account for the greater probability of re-infection in the former group. Notwithstanding, when comparing the answers to a questionnaire presented to two groups of patients previously treated and cur-

ed in Baldim, where just one group had got re-infected, it was found out that both groups had similar habits with regard to contact with natural waters²¹.

It seems a valid conclusion to say that unspecific, as well as acquired and/or concomitant immunity, influence the maintenance of low infection rates and worm burdens after specific treatment of patients living in *S. mansoni* endemic areas.

RESUMO

Reinfestação de pacientes em áreas endêmicas de *Schistosoma mansoni* após tratamento específico. I — Influência da idade e carga parasitária

Foram acompanhados durante 18-24 meses, 312 pacientes infectados pelo *S. mansoni* e residentes em duas áreas endêmicas (Calciolândia e Tuparecê) do Estado de Minas Gerais, que haviam sido tratados com hycanthone (2,5 mg/kg, dose única, i.m.) e considerados como curados. Os porcentuais de reinfecção, após este tempo, nas duas áreas, foram respectivamente de 16,8 e 21,8. As crianças (abaixo de 15 anos) apresentaram taxa de reinfecção maior (34,5%) que os adultos (6,9%). Os resultados dos exames de fezes quantitativos (método de Kato-Katz) demonstraram que houve diminuição de duas a quatro vezes na média do número de ovos de *S. mansoni* por grama de fezes, quando comparada com a anterior ao tratamento. Aproximadamente 20% dos pacientes curados e re-infectados mostraram aumento no número de ovos de *S. mansoni*, 60% uma diminuição e os restantes 20% não apresentaram alterações. Nos dois grupos controles (pacientes não-tratados), as porcentagens de aumento no número de ovos, diminuição e sem alteração foram de respectivamente 20,20 e 60%. Por esta avaliação foi sempre considerado uma variação de três vezes na quantidade de ovos de *S. mansoni* nas fezes.

Os resultados obtidos indicam um certo grau de resistência à reinfestação em pacientes esquistossomóticos tratados (maior em adultos que em crianças) residentes em zonas endêmicas, o que provavelmente pode ser imputado a imunidade inespecífica e/ou adquirida e/ou concomitante.

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