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AGE-ACQUIRED RESISTANCE AND PREDISPOSITION TO REINFECTION WITH *SCHISTOSOMA HAEMATOBIUM* AFTER TREATMENT WITH PRAZIQUANTEL IN MALI

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Abstract. The effect of age, previous intensity of infection, and exposure on reinfection with *Schistosoma haematobium* after treatment was studied in a cohort of 468 subjects six years of age and over living in an irrigation scheme area in Mali. Prevalence and intensity of *S. haematobium* infection were measured each year between 1989 and 1991, but the reinfection study period was restricted to the last year of the follow-up. Observations were made at the principal water contact sites where the number of *Bulinus truncatus* shedding furcocercous cercariae was recorded. A cumulative index of exposure taking into account time, duration and type of contact, and malacologic data was calculated for each subject. Univariate analysis showed that the reinfection risk decreased with age and increased with exposure and pretreatment intensity. These results were confirmed by fitting a logistic model that showed that this risk was seven times lower among those 15 years of age and older than among the 6–14-year-old children, while linear trends with exposure to infection and pretreatment intensity were significant. This study supports the concept of an age-acquired resistance to reinfection and is in favor of a predisposition to infection that raises the question of a genetic factor controlling susceptibility/resistance to *S. haematobium* infection.

Schistosomiasis control relies mainly on chemotherapy and requires proper quantification of factors that explain the variability in reinfection rate or intensity after treatment to define the optimum interval between treatments and to target the appropriate segment of the population.[1] Two key features of the epidemiology of schistosomiasis are the aggregated distribution among human hosts and the convex age-intensity relationship with a peak in the second decade of life. Mathematical modeling can generate useful insights into the statics of helminth populations, as well as their dynamics after chemotherapy. They show that the degree of parasite aggregation is a determinant factor in explaining the mean worm burden at equilibrium and the pattern of reinfection following chemotherapy.[2–4] This aggregation can be generated by predisposition to heavy or light infection (heterogeneity in immunocompetence due to genetic/nutritional factors, social/behavioral factors), environmental heterogeneity, and acquired immunity due to past experiences of infection.[4] Stochastic simulations of the impact of mass or selective chemotherapy, using a model incorporating density-dependent fecundity but no age-acquired resistance, have stressed the importance of the mechanism generating aggregation: the return to precontrol mean worm burden was most rapid when aggregation was generated by predisposition to infection.[3] Longitudinal reinfection studies, combined with a quantitative assessment of exposure to infection, are best suited to test the hypothesis of predisposition and its mechanism, and to assess the relative contribution of age-related change in exposure and age/time-acquired resistance in the age-related pattern of reinfection. Such studies carried out during the last decade on *Schistosoma mansoni* in Kenya and on *S. haematobium* in The Gambia and Zimbabwe provided evidence for an independent effect of age and exposure on reinfection.[5–7] In areas where exposure begins early in life, an age greater than 10–12 years was found to be associated with a lower reinfection intensity or incidence than that less than 10 years of age, a finding consistent with a slowly acquired age resistance. In *S. mansoni* infection, a positive correlation, after controlling for age, was found between pretreatment intensity of infection and reinfection intensity in Kenya and the rate of reinfection in Brazil, a relationship that could be interpreted in terms of predisposition.[8–10] In a previous reinfection study in Mauritania, we showed that gender, age, and pretreatment intensity of infection were independent predictors of the rate of reinfection with *S. haematobium* among a cohort of 6–20-year-old subjects.[11] However, in the studies looking at predisposition, water contacts were not accounted for, which made it impossible to distinguish between predisposition arising from behavioral factors and predisposition arising from genetic/nutritional factors.

A socioeconomic study of schistosomiasis, undertaken in a highly endemic area of Mali, allowed us to study reinfection with *S. haematobium* after treatment on a large cohort of individuals. An attempt to quantify exposure to infection made it possible to adjust the risk of infection after treatment for age, exposure, and pretreatment intensity of infection.

MATERIALS AND METHODS

Study area. Created in 1932, the Office du Niger is a public company maintaining an irrigation scheme spread over 550 km² in central Mali, north of the Niger River. A census completed in 1989 by the staff of the company enumerated 149 villages and 9,604 families (12 persons per family on the average) cultivating 421 km² fields. Children less than 15 years of age accounted for 43% of the population.

Malacologic investigations undertaken in 1981–1983 in the irrigation scheme showed that *Bulinus truncatus* and *B. pfeifferi* were present in lakes, primary and secondary canals, but were rarely found in tertiary or drainage canals.[12] Along the primary canals, the distribution of snails appeared to be associated with human water contact sites (WCS), which were easily identified by lack of vegetation and ero-

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sion of the banks. In addition, infected snails were almost always found in the primary canals where snail density increased in January-February; thus, intense transmission was expected from January to May in the primary canals. Although transmission during the second half of the year was not assessed in that study, a recent study found infected *B. truncatus* in the rainy and postrainy seasons (Traore M, unpublished data). In the early 1980s before any control measure, the crude prevalence of *S. haematobium* and *S. mansoni* human infections in the whole irrigation area reached 64% and 54%, respectively, while the prevalence of heavy infection reached 16% (≥ 50 eggs/10 ml of urine) and 25% (≥ 100 eggs/g of stool),[13] respectively.

Population. The three study villages, Maniale, Medina Coura, and Dogofry, were part of a larger sample of 14 randomly selected villages chosen to study the socioeconomic impact of schistosomiasis (Audibert M, Etard JF, unpublished data). The three study villages represented different ethnic populations (mainly Bambara in Maniale, Minianka in Medina, and a mixture of Bambara, Minianka, and Moorish in Dogofry). They do not differ in terms of water management in the fields and means of cultivation. Maniale is located near a secondary canal and the other villages are located near a primary canal. In December 1989, 30 families per village were randomly selected from a list of resident families.

Since children less than five years of age did not contribute to the cultivation process, only those six years of age and older were included in the socioeconomic study, and consequently in the present investigation. Before enrollment in December 1989, the eligible population was estimated to be 1,081.

Each family was identified by the village's name and number, the name and ethnic group of the householder, a family number, an ethnic code, and the year of settlement. Each individual in the eligible population received a number nested within the family number and was identified by name, sex, and age. Thus, a six-digit number uniquely identified a given eligible subject. This information was recorded on standard household forms.

Only those for whom parasitologic data were available (913 of 1,081 subjects; 84.5% of the eligible population) were enrolled. The main reason for the lack of coverage in urine examination was absence from the village. However, those not enrolled did not alter the age/sex structure of the study population: a log-linear analysis showed no difference with respect to age and gender between the eligible population and those enrolled.

Study design and treatment procedure. After an initial parasitologic survey in December 1989, the 14 villages in the socioeconomic study were resurveyed in December 1990 (second survey) and December 1991 (third survey). A mass treatment was offered in Maniale and Medina Coura immediately after the first survey and in Dogofry a year later. After the initial mass chemotherapy, selective chemotherapy was applied at subsequent yearly visits. The selection was based on a positive result in a parasitologic examination. A single dose of praziquantel (40 mg/kg) was given after subjects were weighed on a scale under direct supervision of the investigators. The participation rates for mass and/or selective treatment were at least 87% (Table 1). The reason for nonparticipation was primarily absence at the treatment sessions, which were held after urine examination. Occasionally, participants refused. The potential exposure to infection was assessed by conducting malacologic investigations and detailed observations on the same WCS after chemotherapy.

TABLE 1

Participation rate in treatment by village and infection status, Office du Niger, Mali 1989–1991

		Chemotherapy round			
		12/89		12/90	
Village	Status*	No.	% treated	No.	% treated
Maniale†	Infected	134	88	48	91.5
	Uninfected	203	87	245	5.5
	Missing	51	55	95	0
Medina†	Infected	89	96.5	50	92
	Uninfected	192	92.5	202	23.5
	Missing	50	48	79	1.5
Dogofry‡	Infected	99	0	106	92.5
	Uninfected	196	0	120	87.5
	Missing	67	0	136	31

* Missing = no urine specimen.
† Mass treatment in 12/89, selective treatment in 12/90.
‡ Mass treatment in 12/90.

Parasitologic investigations. The collection and processing of urine specimens were carried out by a village-based team. The microscopy reading was done in the laboratory of the health center of the sector or in the village if a convenient place could be found. The members of the field teams had extensive experience and did not change during the course of the study. After the arrival of the village-based team, a meeting was held in the evening with the village leader(s) and householders to explain the objectives of the visit and give appointments for the following morning. In the morning, each individual was given a 200-ml, screw-top, wide-mouth, plastic container and asked to provide a complete urine specimen. Each container was labeled with the participants' identification number. Urine specimens were collected between 10:00 AM and 3:00 PM. Two laboratory assistants processed the urine samples within 2 hr. Urine was mixed in a 10-ml syringe by aspirating and ejecting several times and then passed through a Whatman (Maidstone, United Kingdom) no. 1-type paper filter mounted in a Millipore® (Bedford, MA) 25-mm filter holder. In 1989 and 1990, 10 ml per sample were filtered. In 1991, up to 50 ml were filtered and in some instances several filters had to be used due to clogging. The volume of urine treated and the number of filters were recorded. After filtration, the filters were placed on plastic cups for staining with freshly prepared ninhydrin (3%) and drying. The containers, syringes, and filter holders were reused after thoroughly washing with a detergent. On the same day, two microscopists counted the number of *S. haematobium* eggs using light microscopy at a magnification of 10× after rehydration of the filter. The number of eggs per filter was recorded, and when several filters were used, the counts for an individual specimen were summed for all the filters used. *Schistosoma mansoni* eggs were sometimes found on the filters and counted separately. In 1991, the filters were dried after being read and kept in tubes with a dehydrant product for quality control. This quality control procedure was introduced because of the critical na-

ture of the reinfection data. In the same survey, hematuria was detected with reagent strip (Ecur Test;® Boehringer-Mannheim, Mannheim, Germany). Three types of laboratory quality control were implemented. First, to determine how microscopists were rounding their results, the distribution of the last digit of egg counts more than 19 eggs/10 ml was compared with an equal distribution of numbers between 0 and 9 to check for digit preference. Second, a random sample of 10% of the filters collected in 1991 were re-examined between January and May 1992 by a senior parasitologist, without knowledge of the results of the first examination. Third, in 1991, 15 filters were re-examined for disagreement with the results of hematuria detection. Kato-Katz examination of stool specimens was also performed at the first and second surveys for the socioeconomic study but not at third survey, which examined reinfection with *S. haematobium*.

Water contact observations. The water contact observations were restricted to the subjects who provided urine specimens at enrollment. All active WCS located along canals were clearly identified by the lack of vegetation and/or human activities. They were systematically observed. In Maniale, five WCS were located along the secondary or tertiary canal and three WCS located in the fields were also observed. In Medina, six WCS were located along the primary, secondary, or tertiary canal and one was located in a field. In Dogofry, seven WCS were located along the primary or secondary canal. Each WCS was observed once a week during a three-week period in December 1991 and during two two-week periods in March-April 1992 and June 1992. All observers were four-year Malian graduates from either the Ecole Normale Superieure (ENA, Bamako, Mali) or the Institut Polytechnique Rural (IPR, Katibougou, Mali) and had been previously identified for the socioeconomic study. One of them had received an additional one-year training in anthropology (Universite Aix-Marseille, France). The review of the WCS was made with them in the villages where they were trained on how to use standard forms to record water contact activities. One observer per village watched the WCS from 6:00 AM to 6:00 PM. The observers had a listing of the identification number, name, age, and gender of the villagers to be watched. For each water contact, the following information was recorded: time of arrival, type of activity, body surface exposed to the water, and time of departure. When a contact involved several activities with different body exposures, for example washing dishes and then bathing, effort was made to record each exposure separately. In addition to direct observations, the observers conducted unstructured interviews at night within each household at each observation period to check for presence of the individuals in the village. The observers did not know the infection status and were supervised by the senior investigator three times during the first period of observation and once during each of the two following periods. In Medina Coura and Dogofry, the observers had previously lived in the same village or in a nearby village for two years for the purpose of the socioeconomic study. When necessary, they were helped in the identification of the subjects by a village resident. In Maniale, the observer had previously worked in this village and knew everyone.

Malacology. Where aquatic vegetation was present, snails were collected using a fishing net mounted on a 2-m long handle. A 30-cm long claw was also used to collect snails on miscellaneous inert supports, such as plastic bags, pieces of cloth, and old utensils. Each WCS was sampled for 15 min by four persons (one person-hour/WCS). On the same day, the snails were classified according to their morphology and individually examined for furcocercous cercarial shedding by placing them in a 12.5-ml container filled with water from the site and exposing them to indirect sunlight for 2 hr.[14]

Statistical analysis. Three categories of intensity of infection were considered: 0, 1–49 (light infection), and 50 or more eggs per 10 ml of urine (heavy infection). Egg counts less than one per 10 ml were considered zero counts in the main analysis. Geometric mean densities were calculated after adding one to each egg count.

For each water contact, an elementary index of exposure was defined as the product of the following terms: duration of the contact in minutes, percentage of body surface exposed to water,[15] and a standard coefficient reflecting the diurnal variation of cercarial shedding, equal to the density probability at the hour of the contact, of a normal distribution with standardization parameters $\mu = 12.5$ hr and $\sigma = 1.5$ hr.[16] To account for the cercariacidal usage of soap while body washing, the exposure index was assumed to be zero. The elementary indices were then summed for all the observed contacts made by a given subject and multiplied by the proportion of *B. truncatus* found infected. This summation led to an individual cumulative index of exposure. Based on the distribution of this index, exposure was categorized into zero, low, and high exposure strata. The median of the distribution of the nonzero exposure subjects was used as the cutoff point to define the low and high exposure strata.

Reinfection analysis was restricted to the last year of the follow-up, 1990–1991, and included both previously uninfected and infected subjects in 1989 or 1990. A subject passing one or more eggs per 10 ml of urine was considered infected. Subjects never observed at any WCS during the three observation periods were not included in the main analysis because either they were frequently absent from the village or their exposure at other WCS was not measurable. Univariate risk ratios of reinfection were estimated and unconditional, as well as conditional, logistic regression was used to assess the effect of age on the 1991 infection status controlling for exposure and previous intensity of infection over the period 1989–1990. Orthogonal contrasts were used to test for linear and quadratic trends. Assessment of fit and regression diagnostics procedures followed the framework of Hosmer and Lemeshow.[17]

The results were then checked for consistency by considering egg counts between zero and one egg per 10 ml as active infections, by adjusting for the amount of urine filtered in the 1991 survey, by making different assumptions regarding the construction of the index of exposure, by restricting the analysis to the noninfected subjects in 1990, and by adjusting for *S. mansoni* infection in 1990.

Given the overdispersed (variance too large for a typical parametric distribution) distribution of the egg counts, intensity analysis required a distribution-free inference procedure. The Kolmogorov-Smirnov two-sample test, known to be sensitive to all types of differences between two cumulative distribution functions, was used.[18]

TABLE 2

Crude prevalence (P), prevalence of heavy infections (P_{50}, ≥50 eggs/10 ml), and geometric mean density of infection (GMD, eggs/10 ml) at each survey by village, Office du Niger, Mali, 1989–1991

	Eligible 12/89	Study population*											
		12/89				12/90				12/91			
Village	No.	No.	P	P_{50}	GMD	No.	P	P_{50}	GMD	No.	P	P_{50}	GMD
Maniale†	388	337	39.7	14.2	3.6	293	16.4	0.7	1.4	307	21.5	0.0	1.2
Medina†	331	281	31.6	6.0	2.3	252	19.8	0.8	1.4	247	26.7	0.4	1.3
Dogofry‡	362	295	33.5	8.1	2.4	226	46.9	15.9	4.3	222	40.6	2.3	1.7
Total	1,081	913	33.6	9.7	2.7	771	26.7	5.2	1.9	776	28.6	0.7	1.4

* Prevalence values are percentages.
† Mass treatment in 12/89, selective treatment in 12/90.
‡ Mass treatment in 12/90.

RESULTS

Impact of treatment. One year after the mass chemotherapy in Maniale and Medina Coura, the crude prevalence of infection was reduced by 59% and 37%, respectively, and mean density was decreased by 74% and 59%, respectively (Table 2). In contrast, in Dogofry, where no mass treatment was given at the first survey, the increase in prevalence and density during the first year was mainly due to the acquisition of infection among the 6–9-year old children (in this age group, prevalence increased from 43% to 71% and the mean density increased from 4.3 to 21.1 per 10 ml).

Selection of subjects for analysis. A complete parasitologic data set (three surveys) was available for 644 individuals (Figure 1). Fifteen subjects were excluded because they were found to be infected in 1990 but were not available during the treatment session. Data on exposure were missing for 161 subjects never seen at any WCS. The analysis included 468 subjects. Among these 468 subjects, 338 (72%) had water contact in each of the three observation periods, 121 in two of the periods, and nine in one. These 468 subjects had a total of 3,813 water contacts and 54 of them (11.5%) were found to be infected in 1991. Household interviews by observers showed that the 161 subjects never seen at the WCS were frequently absent from the village: only 48% of them were present in three observation periods.

Quality control. There was no last-digit preference by the microscopists (χ^2 = not significant). The second reading of 294 randomly selected filters showed an excellent agreement with the first reading using 1 egg/ml or more as the classification cutoff point (McNemar χ^2 not significant, kappa statistic [k] = 0.80). If one considered the second reading as a reference, the microscopists were able to detect 81% of the infections. Among the 15 double-checked filters, the first reading by the microscopists was in seven cases replaced by the result of the second reading by the senior parasitologist. These seven modifications were concerned with very light infections: six times with zero to either two or three eggs/10 ml and once with 0–10 eggs/10 ml.

Malacology. In Maniale and Medina Coura, the highest snail densities were observed in the tertiary canals (77% and 60%, respectively, of total *B. truncatus* captured in the village), while in Dogofry, 95% of the snails were found in the primary canal. Medina Coura showed the highest density (148 *B. truncatus* captured), followed by Maniale (87) and Dogofry (85). The proportion of infected *B. truncatus* was 0%, 3.4%, and 1.2% in Maniale, Medina Coura, and Dogofry, respectively.

Categorical analysis. The proportion of infected subjects after treatment decreased with age, and when 15 years was used as the cutoff point, the proportion was 10 times higher within the 6–14-year-old group than within the group 15 or more years of age (risk ratio = 0.09 [0.05–0.20]) (Table 3). This proportion increased with water exposure level from 4% at zero exposure to 24% in the high exposure stratum and with previous intensity of infection in 1989–1990 from 3% for the previously uninfected to 25% for the previously heavily infected subjects. Gender was not associated with a different risk of infection after treatment. The distribution of the exposure index differed by age group with a proportion of highly exposed subjects decreasing with age (Table 4).

Unconditional logistic regression analysis revealed that a young age was a strong risk factor for infection in 1991 after allowing for the three levels of exposure and the three levels of previous intensity of infection. Linear trends in exposure and previous intensity of infection were significant. Therefore, these variables were subsequently entered as continuous variables. No interaction terms were needed (Table 5). This model showed that the odds of infection after treatment were seven times lower among the subjects 15 years of age and older than among the 6–14-year-old children. An increase of one unit in exposure or in previous intensity of infection resulted in a two-fold increase of the odds of infection. The deviance demonstrated a good overall fit, but given many cells with a low expected frequency, the Hosmer-Lemeshow goodness of fit χ^2 based on deciles of the estimated probability was recommended. This test also showed a good overall fit. The regression diagnostics revealed one covariate pattern with a poor fit of high influence on the estimated coefficients. This covariate comprised 41 subjects 15 years of age and older who were highly exposed and previously not infected. None of them was found infected in 1991 but the related estimated probability was 4.3%. Since this covariate showed only a moderate leverage value, it can be assumed that the poor fit was responsible for the high influence. The exclusion of these 41 subjects slightly altered the risk odds ratios (ROR) and their standard error but the significance levels were not substantially modified. Among the predictors, exposure was most susceptible to measurement error. The introduction of a nondifferential misclassification rate of 25% between low and high exposure stratum altered only marginally the odds ratios.

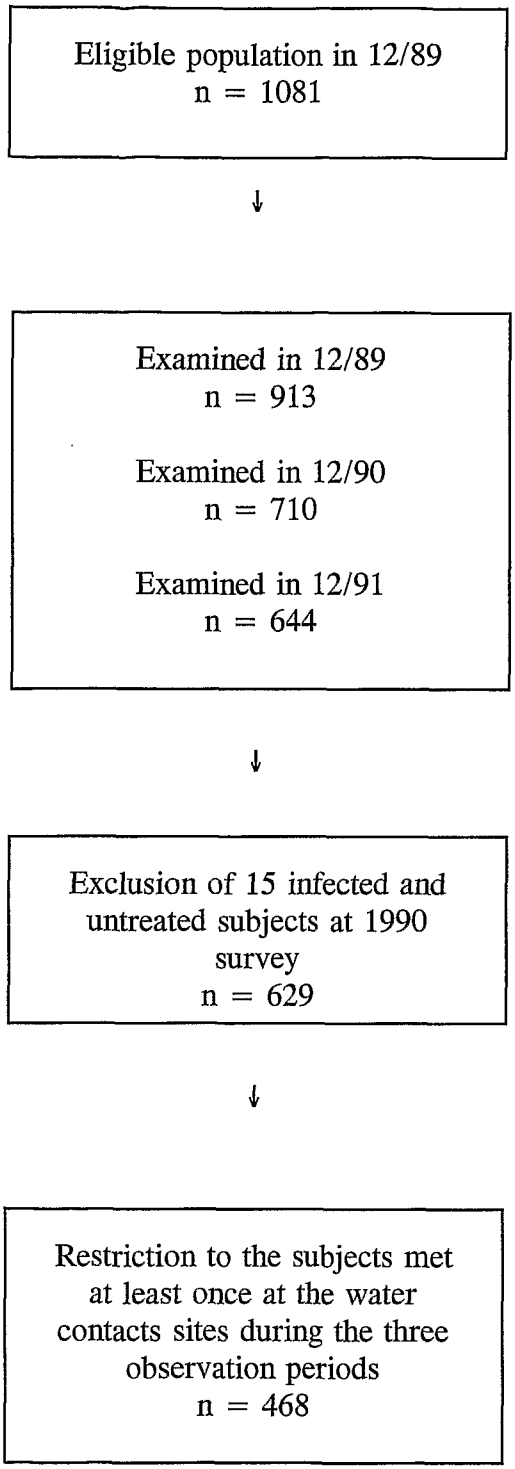

FIGURE 1. Process of selection of subjects for analysis, Office du Niger, Mali, 1989–1991.

The conditional maximum likelihood procedure, with exposure as stratification variable, led to estimates of the same order, although closer to the null value: ROR (age) = 0.17 [0.08–0.38], ROR (previous intensity) = 1.73 [1.16–2.56]).

Considering the nonzero egg counts less than one egg per 10 ml as an indication of an active infection resulted in more than a two-fold increase in the number of infected subjects

TABLE 3

Post-treatment Schistosoma haematobium *infection status in 1991 by characteristics of the 468 subjects, Office du Niger, Mali*

Variable	No. of subjects	No. (%) infected	Risk ratio	95% CI*
Age group (years)				
6–9	86	25 (29.1)	1.00	
10–14	84	21 (25)	0.86	0.52–1.14
15–19	49	6 (12.2)	0.42	0.19–0.96
>20	249	2 (0.8)	0.03†	0.01–0.11
Gender				
Males	219	24 (10.9)	1.00	
Females	249	30 (12)	1.09	0.66–1.81
Exposure				
Zero	217	9 (4.1)	1.00	
Low	125	15 (12)	2.89	1.30–6.42
High	126	30 (23.8)	5.74‡	2.82–11.70
Eggs 1989–1990§				
0	234	7 (2.9)	1.00	
1–49	165	30 (18.2)	6.08	2.74–13.50
≥50	69	17 (24.6)	8.24¶	3.56–19.04

* 95% Taylor series confidence interval.
† $\chi^2_{trend} = 67.2$, $P < 0.001$.
‡ $\chi^2_{trend} = 28.8$, $P < 0.001$.
§ Number of eggs per 10 ml: 0 if no eggs were found in 1989 and 1990, ≥50 if 50 or more eggs were found in 1989 and/or 1990, otherwise, 1–49.
¶ $\chi^2_{trend} = 33.7$, $P < 0.001$.

at the last survey (113 infections). It moved the adjusted RORs towards the null value but the effect of the predictors remained largely significant and the conclusions yielded by the main model were still valid (Table 6). Subsequently, the results were tested for robustness to different constructions of the exposure index. When the index allowed for the absolute number of infected snails instead of the proportion of infection, only marginal changes were observed. When the diurnal variation in cercarial shedding was not accounted for, the index of exposure slightly decreased but the contingency table used for the categorical analysis was not altered, resulting in the same model. When the index was not corrected for soap usage, the coefficients remained almost unchanged. It should be noted that 43% of the water contacts involving soap were made before 10:00 AM or after 4:00 PM, at a time where the cercarial density was set at a low value, and these contacts accounted for only 8% of all contacts. Two different ways of estimating an index of exposure for the 161 subjects never observed at the WCS, using the information from the 468 subjects with a recorded activity at the WCS, did not change the interpretation of the model. To increase the relevance of the effect of pretreatment intensity of infection on reinfection, a model restricted to the 343 subjects who did not pass eggs in 1990 was fitted. Although the fit of this model was the worst of all the fitted models, pretreatment intensity of infection had a significant impact on the risk of reinfection. To adjust for the potential effect of the volume of urine filtered in 1991, a variable coding for this volume (50 ml or less) was entered. The estimated odds ratios were not profoundly altered. Finally, an adjustment for *S. mansoni* infection in 1990, when Kato-Katz results were available (61% of the data set), decreased the effect of the three predictors but their respective contribution to the model was still significant.

Nonparametric intensity analysis. The egg output in

TABLE 4

Number of subjects by age and exposure stratum and comparison of the distribution functions of Schistosoma haematobium *egg counts in 1991 between the 6–14-year-old and ≥15-year-old age groups within each of the three strata of exposure, Office du Niger, Mali (468 subjects)*

Exposure stratum	Total	No. (%) of subjects by age group (years)*				Nonparametric test† (*P*)
		6–9	10–14	15–19	≥20	
Zero	217 (46.4)	28 (32.5)	37 (44)	15 (30.6)	137 (55)	0.43
Low	125 (26.7)	17 (19.8)	18 (21.5)	18 (36.7)	72 (29)	0.02
High	126 (26.9)	41 (47.7)	29 (34.5)	16 (32.7)	40 (16)	<0.01

*$\chi^2 = 42.0$, degrees of freedom = 6, $P < 0.0001$.
† Two-sample Kolmogorov-Smirnov statistic.

1991 has been plotted against age by stratum of exposure (Figure 2). Within the zero exposure stratum, the nonparametric test showed no difference between the cumulative distribution functions of the egg counts of the two age groups. A difference appeared at low exposure and was even more marked at high exposure (Table 4).

DISCUSSION

The categorical analysis demonstrated an independent effect of age, exposure, and pretreatment intensity of infection on reinfection with *S. haematobium* after chemotherapy. The intensity analysis gave consistent results.

The index of exposure was a surrogate measure of the true exposure and subject to measurement error. The true acquisition of cercariae through the skin is not measurable and assumptions must be made on the likelihood of cercarial penetration. The main assumption is that the cercarial penetration is proportional to the product of the time spent in the water and the percentage of the body surface exposed. Although it is a convenient assumption, made by other investigators and us in another study, there is no evidence that it should be valid.[6, 7, 19] We made no allowance for the absolute body size, but as argued by Wilkins and others,[6] the variation of body surface and urinary output with age are of comparable magnitude so that the age gradient in resistance will be unaffected by body size.

The different assumptions we made in the construction of the index of exposure altered the fit of the model to the data but did not change the interpretation of the effects of the three predictors we studied. Allowing for the absolute number of infected snails instead of the proportion of infected snails was not expected to exert a large impact since these two quantities were correlated. With regard to the coefficients for cercarial density we used, independent studies in other countries using different methods showed that the emergence of *S. haematobium* cercariae follows a bell-shape curve during the day with a peak just after noon and a very low infectivity early in the morning and in the late afternoon.[7, 17, 20] In the Gambian studies, it was outlined that corrections for cercarial densities were similar in all age/gender groups and had little effect on the relationship between age and observed susceptibility.[6] Our study led to the same conclusion. The harmful effect of the soap on cercariae was suggested in a study on the vitality of *S. mansoni* cercariae exposed to detergents in Cameroon.[21] This effect was incorporated in the Gambian studies.[6] Our regression coefficients were almost unaltered by the introduction of a soap factor, when the cercarial density was already allowed for, mainly because the usage of soap was correlated with the time of the contact.

Apart from the construction of the index of exposure, there were other potential sources of measurement error in exposure. Most of the observed WCS were located along primary or secondary canals where human activities took place and intermediate hosts were present, and it has been previously shown that infected snails were almost always found in well-defined WCS.[12] We are therefore confident that the main transmission sites of the villages have been observed. However, one cannot exclude the possibility of significant contacts away from the village. Assuming that absentees were likely to have unquantified contacts, the main analysis was restricted to the subjects observed at the WCS. Yet, the effect of the inclusion of the subjects who were never observed at any WCS was studied, but it required estimation of a value for the exposure index. To impute a null value would have assumed that the absentees were not exposed, which was not a tenable assumption. Rather, we preferred to infer a value from the observations made by subjects of the same age and sex.

Each WCS was observed for an average of seven days during the dry season (December, March, and June) but no observations were carried out during the rainy season. Thus, exposure could have been underestimated if transmission

TABLE 5

Adjusted risk odds ratios and 95% confidence intervals for infection with Schistosoma haematobium *in 1991, Office du Niger, Mali*

Variable*	Full model† (n = 468)	Exclusion of influential covariate‡ (n = 427)	25% misclassification in exposure§ (n = 468)
Age ≥15 years old	0.14 (0.06–0.32)	0.18 (0.08–0.43)	0.13 (0.06–0.29)
Exposure	2.30 (1.55–3.41)	2.47 (1.65–3.68)	2.27 (1.53–3.38)
Eggs in 1989–1990	2.14 (1.33–3.45)	1.98 (1.24–3.17)	2.12 (1.32–3.39)

* Referent category: 6–14 years old in 1990, zero exposure, and no eggs in urine in 1989–1990. Coding scheme: exposure: 1 = zero exposure; 2 = low exposure; 3 = high exposure; eggs in 1989–1990 (see footnote to Table 3): 1 = no eggs; 2 = 1–49 eggs; 3 = ≥50 eggs.
† Deviance = 16.96, degrees of freedom [df] = 14, $P = 0.26$; $\chi^2_{HL} = 6.0$, df = 8, $P = 0.64$. HL = Hosmer-Lemeshow.
‡ Deviance = 12.74, df = 13, $P = 0.47$; $\chi^2_{HL} = 3.99$, df = 13, $P = 0.86$.
§ Deviance = 10.80, df = 14, P = 0.70; $\chi^2_{HL} = 3.67$, df = 14, $P = 0.88$.

TABLE 6

Adjusted risk odds ratio and 95% confidence intervals estimated by altering the main logistic model shown in Table 5

Difference with main model	No.	Age*	Exposure	Eggs in 1989–1990	Goodness-of-fit χ^2 (*P*)	
					Deviance	Hosmer-Lemeshow
All egg counts >0 considered as infection	468	0.24 (0.14–0.39)	1.91 (1.44–2.55)	1.75 (1.23–2.47)	0.16	0.86
Index of exposure corrected for the absolute number of infected snails	468	0.14 (0.06–0.32)	2.23 (1.51–3.30)	2.13 (1.32–3.42)	0.13	0.75
Index of exposure not corrected for soap	468	0.14 (0.06–0.33)	2.28 (1.53–3.39)	2.16 (1.34–3.47)	0.17	0.60
Estimation of missing values for index of exposure by age/sex group mean	629	0.14 (0.07–0.28)	1.90 (1.37–2.64)	2.27 (1.53–3.37)	0.61	0.86
Estimation of missing values for index of exposure by regressing exposure on age and sex	629	0.14 (0.07–0.28)	1.86 (1.34–2.57)	2.29 (1.54–3.41)	0.60	0.82
Restriction to subjects excreting no eggs in 1990	343	0.16 (0.06–0.45)	2.73 (1.60–4.65)	3.65 (1.89–7.08)	0.16	0.36
Adjustment for amount of urine filtered in 1991	468	0.13 (0.06–0.31)	2.29 (1.54–3.39)	2.05 (1.27–3.32)	0.59	0.46
Adjustment for *Schistosoma mansoni* infection in 1990	287	0.19 (0.06–0.56)	1.74 (1.06–2.84)	1.96 (0.99–3.86)	0.40	0.54

* See footnotes in Table 5 for referent category and coding scheme of variables.

was expected during the second half of the year. However, there is no reason to think that the underestimation had differed by predictor level (age, exposure, and egg count category). The observers were well known by the study population and were able to identify each member of the cohort. Thus, we could rule out misclassification arising from incorrect allocation of exposure data on individuals.

If one assumes some degree of nondifferential misclassification of exposure, the observed adjusted measures of association are likely to be between the crude and the true measures.[22] This means that the true measures of association could lie between the observed adjusted measures and one, leading to a weaker age or pretreatment intensity effect than measured. However, we showed that the estimated coeffi-

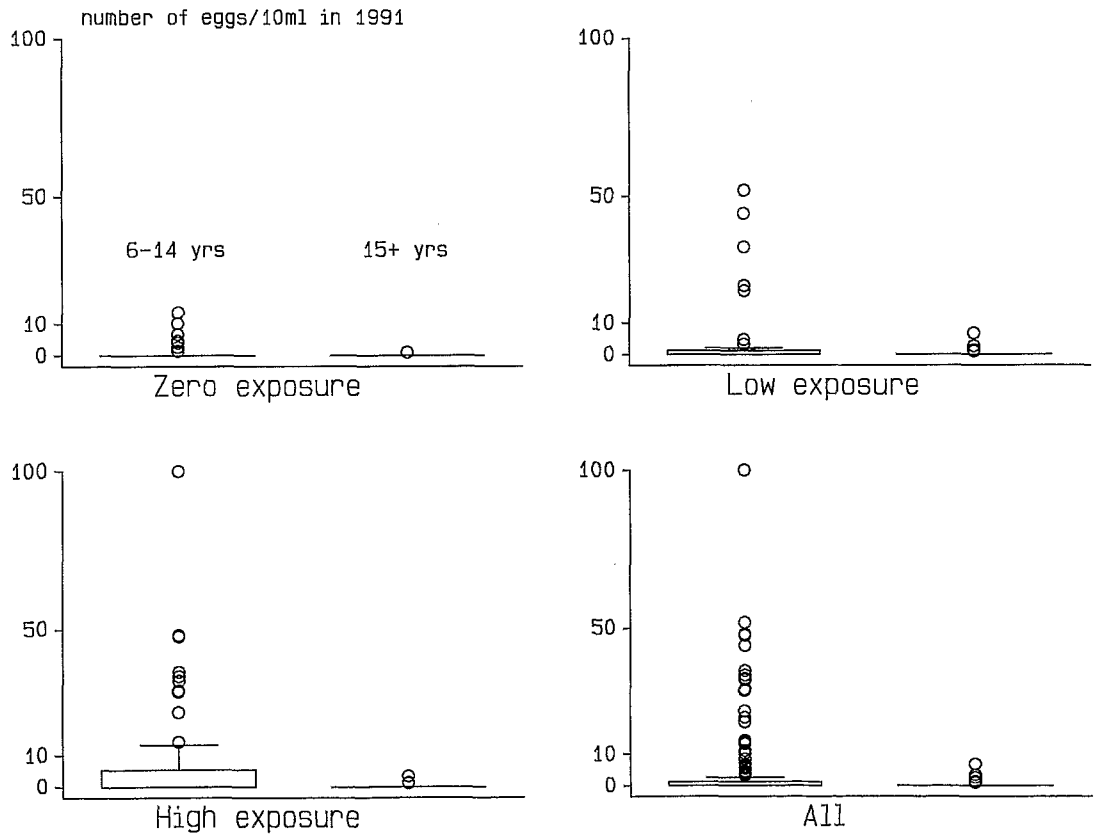

FIGURE 2. Box and whisker plot of egg output after treatment by age and exposure. The box represents the interquartile range (25th to 75th percentile) The upper whisker (T-shaped horizontal bar) is located at the 75th percentile plus 1.5 the interquartile range. yrs = years.

cients were robust to the introduction of a 25% nondifferential misclassification rate between low and high exposure strata.

The lack of ascertainment of cure prevented distinguishing strictly between residual infection and true reinfection. However, high cure rates ranging between 85% and 100% six months after treatment were expected with praziquantel.[23,24] Second, the analysis restricted to the 343 subjects passing no eggs in urine in 1990 reinforced the results yielded by the main model.

Only one urine specimen was used to classify the subjects as infected or uninfected. To limit misclassification of the pretreatment intensity of infection, the results of the parasitologic examinations performed in 1989 and 1990 were combined. To increase the sensitivity of this diagnostic procedure after treatment, a continuing effort was made to filter at least 50 ml of urine in 1991. However, a large amount of urine filtered increases the risk of clogging. To avoid an underestimation of the egg output when the filter got clogged, the remainder of the urine was passed through new filters until 50 ml was passed. In a classic study, it has been found that most of the clogged filters were due to white blood cells and often occurred in children who were uninfected or had light infections.[25] An issue of more concern was the risk of sedimentation of the eggs on the bottom of the container and lack of careful mixing of the urine before removal of the test sample. However, we were able to verify that the quantity of the urine specimen collected was independent of the characteristics of the individuals and that the volume of urine filtered did not act as a confounder. On the other hand, increasing to 50 ml the volume of urine filtered yielded positive counts of less than one egg per 10 ml. Considering these fractions of egg as active infections lowers the specificity of the diagnostic of reinfection. Indeed, their inclusion in the definition decreased the effect of the predictors.

If one assumes that some degree of two-way misclassification of infection status was present in the study population, the sensitivity and specificity of the diagnosis were not expected to differ by the level of predictors. The same diagnostic procedure was applied to all subjects. In a previous study, we found no difference in the six-month egg-negative rate by age, sex, or pretreatment level of infection.[11] As is recognized, nondifferential misclassification of disease will only bias the observed measure of association toward the null.

The 1991 infection status was not known by the observers; they were not part of the teams in charge of the parasitologic examinations and had no access to the data. Thus, we could rule out any differential observation rate among infected and uninfected individuals after treatment.

The age-related resistance demonstrated in the present study is consistent with previous results obtained in The Gambia and is in favor of a protective immunity.[6] Age-related physiologic changes could be acting and confounding the association, but there is little evidence to support this hypothesis.[6] The effect of skin lipids on the penetration of cercariae was suggested by Chandiwana and others[7] on the basis of experimental work done on rat skin.[26] Free fatty acids present on the skin are the probable natural stimulants for cercarial attachment and penetration.[27] A higher concentration of these lipids in the young age groups could then partially explain the higher reinfection rates in these age groups.[7] However, this hypothesis has never been tested in humans and one study has shown that the most effective stimulant was also the most lethal for cercariae.[28] The best evidence for an age-acquired protective immunity in human schistosomiasis stems from reinfection studies coupled with immunologic investigations carried out in The Gambia on *S. haematobium* infection and in Kenya and Brazil on *S. mansoni*.[29–32] A common pattern emerged from these studies: 1) immunity develops after natural contact with the parasite and is positively correlated with a specific IgE antibody response, and 2) the production of blocking antibodies in the early stages of the infection delays the development of a protective response.

Pretreatment intensity of infection as an independent risk factor for reinfection with *S. haematobium* after allowing for age and exposure has not yet been reported. The contribution of this variable to the model, whether restricted or not to subjects not passing eggs in 1990, was highly significant, and a trend in reinfection with increasing level of pretreatment intensity was even observed. This possible risk factor could be interpreted as a surrogate for predisposition to infection (subjects already infected being placed at a higher risk of reinfection). Since age and exposure were allowed for, the present study suggests that a genetic/nutritional factor controlling immunocompetence could generate predisposition. A study in Brazil using segregation analysis provided evidence for a codominant major gene controlling human resistance/susceptibility to infection with *S. mansoni*.[33]

In terms of control, the current search for a vaccine, which could also reduce morbidity by interfering with worm fecundity and egg viability, is a major goal.[34,35] However, a genetically-based heterogeneity in immunocompetence as a source of predisposition may question the control of schistosomiasis by mass vaccination and deserves further attention.[4] Until a vaccine is made available or the environment/sanitation dramatically improves in tropical countries, control will rely on chemotherapy. Given the aggregation and predisposition, targeting treatment to the core of susceptible children and young adolescents placed at risk of developing morbidity, and harboring most of the worms within a given community, seems a good strategy. Indeed, selective treatment of infected schoolchildren was implemented with satisfactory results in terms of intensity of infection up to three years after treatment.[11,36–39] The effect of chemotherapy on the acquisition of a natural immunity needs also to be considered. Numerical simulations suggested that in certain circumstances, mass treatment can increase the average worm loads in the older age groups above the pretreatment level.[3] Although it is doubtful that this could have an impact on morbidity, it raises the question of the level of protection of children after several years of reduced exposure, should they face an increase of transmission due to the interruption of control measures. The objective of schistosomiasis control is the prevention of morbidity, not the control of infection. The age-acquired immunity to reinfection, the potential impact of mass chemotherapy on the acquisition of this immunity, and the low sustainability of population-based chemotherapy militate in favor of treating only the symptomatic cases, while improving their detection.[40]

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