

## FAILURE OF MEBENDAZOLE IN TREATMENT OF HUMAN HOOKWORM INFECTIONS IN THE SOUTHERN REGION OF MALI

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**Abstract.** Preliminary studies indicated that single-dose (500 mg) mebendazole gave disappointing results in the treatment of hookworm infections (*Necator americanus*) in Mali. A placebo-controlled, randomized trial conducted with the participation of 103 infected subjects (background hookworm prevalence > 50%) confirmed that mebendazole (Vermox®) did not reduce parasite burdens significantly, as assessed through fecal egg counts. In contrast, a group of subjects treated with pyrantel (Combantrin®) experienced a significant reduction in fecal worm egg counts (overall, both sexes combined showed a 75% reduction). Male subjects carried significantly more intense infections compared with females, but there was no gender difference in response to treatment. A standard egg hatch assay showed that *N. americanus* from our subjects in Mali was more resistant to benzimidazoles compared with a laboratory-maintained strain that had not been exposed to anthelmintics in more than 100 generations (50% effective dose = 0.12 and 0.07 µg/ml of thiabendazole, respectively), suggesting that, among other possibilities, the development of resistance to the benzimidazoles by *N. americanus* may have contributed to the drug failure. Whatever the underlying explanation, our results indicate that single-dose treatment with mebendazole is an ineffective treatment for hookworm infections and despite its relatively cheap cost and wide availability, mebendazole should not be considered a drug of choice in the mass treatment of hookworm infections in this region of Mali.

While the overall prevalence of hookworm infections in Mali is approximately 8%, pronounced regional differences exist and the highest prevalence is found among communities living in the humid southern region of Sikasso.<sup>1</sup> This relatively high local prevalence prompted us to evaluate a possible regional strategy to control hookworms. Gastrointestinal nematode parasite infections are routinely treated with one of several drugs, with the most frequently used anthelmintics being various benzimidazoles, levamisole, and pyrantel.<sup>2-4</sup> None of these drugs are 100% effective at recommended doses in removing all worms from all subjects, but mebendazole and pyrantel, currently the two most popular drugs of choice, are reputed to have high efficacy (> 80%) against hookworms.<sup>2,5</sup> Mebendazole was selected for our study because of its efficacy against all the major human intestinal nematodes and because it has been shown previously to be highly efficacious for the treatment of *Necator americanus*.<sup>2,5,6</sup> the predominant hookworm affecting the communities in Sikasso. Moreover, mebendazole is relatively cheap,<sup>7</sup> an important consideration in the treatment of impoverished communities in Africa. The single-dose regimen (500 mg) was chosen, rather than the three-day regimen (200 mg/day), because it is known to have high efficacy in field trials,<sup>8-10</sup> it is recommended by the World Health Organization (WHO)<sup>7,11</sup> and other authorities, and because it is easier with the single treatment protocol to ensure compliance under local conditions.

In this paper, we summarize data from initial mass treatment that suggest the failure of mebendazole to reduce worm burdens in the treated subjects. We also report a placebo-controlled, randomized trial subsequently conducted to confirm the pilot observations and present laboratory data suggesting that resistance to mebendazole may have arisen among local hookworms.

### SUBJECTS, MATERIALS, AND METHODS

**Study site and subjects.** The study was carried out in the village of Massabla, 7 km from the district of Bougouni

(southeast Mali) in the Sikasso region (Third Region). The main activities in the village (1987 population estimate = 352) are cultivation of millet and cotton and some livestock husbandry. Earlier surveys indicated that the prevalence of *N. americanus* in this community was > 50% (De Clercq D, Sacko M, unpublished data).

**Quantification of hookworm infections.** Fresh, overnight stools were obtained from individuals who participated in the study and were examined on the same day by the Kato-Katz technique,<sup>12</sup> with one (preliminary surveys) or two (full trial) slides being prepared from the stools of each subject. Hookworm eggs were identified (all positive samples were cultured to distinguish hookworm species), and the intensity of infection was calculated as eggs per gram of feces (EPG) and then adjusted for stool consistency using WHO guidelines.<sup>13</sup>

**Treatment with anthelmintic.** During the two preliminary surveys (April and November 1993), the mebendazole used for treatment was obtained from Global Pharmaceuticals, Ltd. (London, United Kingdom), while for the full trial (December 1994), we received the drug (Vermox®) from Janssen Pharmaceutica (Beerse, Belgium). Pyrantel pamoate (Combantrin®) was a gift from Pfizer, Ltd. (Sandwich, United Kingdom). Placebo-treated subjects were given 1 gm of effervescent tablets of vitamin C. The trial was approved by the Direction of the National Institute for Research in Public Health (INRSP) in Bamako, Mali. Pregnant women and children less than two years of age were excluded from the study. All participants, irrespective of earlier treatment, were offered anthelmintic treatment when the study had been completed. Participants were fully informed during assemblies about the nature and objectives of the study, and about the personnel involved. Their consent was obtained prior to their acceptance for the trial.

**Study design—preliminary surveys.** In April 1993, stool samples were collected from 157 subjects living in Massabla

and one slide from each subject was examined for presence of helminth eggs. Since the prevalence of hookworms exceeded 50%, each person in the village, with the exception of pregnant women and children less than two years of age, was treated with a single dose of 500 mg of mebendazole. Six weeks after treatment, 56 treated subjects who were originally hookworm-positive were re-examined to establish drug efficacy.

Because of the surprisingly poor efficacy of mebendazole treatment, a second mass treatment was offered to all inhabitants in November 1993, at the end of the rainy season. Seventy-three subjects who had provided stools for examination in April 1993 were treated in November 1993 and re-examined in April 1994 (one slide per subject).

**Study design—full-randomized, placebo-controlled trial.** To resolve the uncertainty about the efficacy of mebendazole under local conditions, a full-randomized, placebo-controlled trial was initiated. In December 1994, 182 subjects provided stools for analysis of hookworm infections (preintervention survey) and these were examined by the Kato-Katz technique (two slides per subject).<sup>12</sup> The full trial was not double-blind, with those administering treatment, although not the subjects themselves, being aware of the treatment group to which each participant had been allocated. In addition to using mebendazole (single 500 mg dose of mebendazole), we treated one group of subjects with pyrantel (10 mg/kg dose of pyrantel pamoate), an anthelmintic with known efficacy against hookworms,<sup>3,14,15</sup> but with a different mode of action than mebendazole or other benzimidazole anthelmintics.<sup>4</sup> One group of subjects was given vitamin C tablets as a placebo. All subjects were divided by gender and then randomized into three treatments within single sex, age-stratified cohorts, with the three treatments being equally distributed across the age groups and both genders. During the second visit, six days after the initial preintervention survey, 170 of these subjects turned up for treatment. In January 1995 (four weeks after treatment), 95 subjects from among those originally identified as infected and subsequently treated presented for re-examination (post-intervention survey), and stool samples from each subject were again examined by the Kato-Katz technique (two slides per subject). An additional eight hookworm-infected persons were examined at both surveys but failed to appear for treatment. For analysis, these eight subjects were included in a fourth (no treatment) group.

**Egg hatch assay.** Fresh fecal samples were collected in Nottingham from hamsters infected with a strain of *N. americanus* that has been maintained in laboratory hamsters for more than 100 generations<sup>16</sup> and more than 20 years, and which had not been exposed to anthelmintics throughout this period. Stool samples were also collected from infected individuals from our study site in Mali. Both sets of fecal samples were obtained in the same week in July 1995, stored anaerobically,<sup>17</sup> and dispatched immediately to Belgium for testing. The two strains were compared for benzimidazole resistance using an egg hatch assay.<sup>18,19</sup> The eggs recovered from samples were incubated at 28°C for 48 hr in 11 concentrations of thiabendazole (TBZ) ranging from 0.02 to 2 µg/ml. Assays were conducted in triplicate for each sample in 96-well, flat-bottomed microtiter plates. The percentage of eggs that failed to hatch at each concentration, after cor-

rection for natural mortality and arcsin transformation, was used to construct a log dose response line, from which the 50% effective dose (ED<sub>50</sub>) was calculated for each sample.

**Statistical analysis.** The results of the field trial are presented as the mean adjusted EPG for each treatment group and by gender at both pre- and postintervention surveys. We also give the cure rates, which represent the number and percentage of individuals who were positive for hookworm eggs at the preintervention survey but showed no hookworm eggs at the postintervention survey. The quantitative fecal egg count data from the full trial were analyzed by a statistical system for generalized linear interactive modeling (GLIM 4, PC version; Royal Statistical Society, London, United Kingdom) as described previously using a model with negative binomial errors.<sup>20,21</sup> Host gender and drug treatment were entered as factors in the analyses, and age was entered as a covariate. To avoid pseudoreplication of data, we analyzed changes in fecal egg counts between the pre- and postintervention surveys of the full trial, rather than comparing the results from the two surveys with each other. The postintervention adjusted EPG were subtracted from the preintervention EPG for individual subjects. The lowest value (-1,680) was added to all data points to convert negative values to positive; thus, it is necessary to subtract this value from all the model coefficients to obtain the true estimates of change.

The results of each model are presented in two parts (A and B) in three tables. In part A, we present the outcome of step-wise deletion of sources of variation starting, in each case, with the most complex interaction and ending with the principal component under consideration. For models using binomial errors, the change in deviance is divided by the scale parameter and the resulting scaled deviance is distributed as  $\chi^2$ . For each analysis, the model in part A represents a rigorous statistical analysis of the source of variation. To obtain more information on the likely source of the variation in the data, we constructed minimum sufficient models, in which we entered only those sources of variation that had been identified as significant in analysis A. The data in part B presents estimates of the coefficients for such models. The GLIM procedure begins with an estimation of the coefficient for the first component of multicomponent factors and then the necessary adjustments for each of the other components comprising the factor.<sup>20</sup>

## RESULTS

**Preliminary surveys.** At the initial survey in April 1993, the overall prevalence of hookworm infections was found to be 69.6%. At the postintervention survey six weeks after treatment, a reduction of 28.5% (69.6% to 41.1%) in prevalence and 18.5% (157.7 to 128.6) in EPG were recorded. Following the second mass treatment in November 1993 and re-examination in April 1994, mebendazole was found to have been even less effective than during the earlier round of treatment (reduction in prevalence = 2.5% and EPG = 8.3%).

**Full-randomized, placebo-controlled trial.** The preintervention examination for the full trial revealed that 125 subjects of the 182 examined carried hookworms (68.7% prevalence).

TABLE 1

Composition of the subset of the study population carrying hookworms at the preintervention survey and re-examined subsequently, by treatment, age, and sex\*

Treatment	Sex	Number	Age (years)		
			Mean	SEM	Range
Mebendazole	Males	23	27.0	3.7	5-54
	Females	12	30.9	6.5	7-70
	Total	35	28.4	3.2	5-70
Pyrantel	Males	23	26.3	4.5	4-68
	Females	6	19.2	7.8	3-53
	Total	29	24.8	3.9	3-68
Placebo	Males	23	22.8	3.8	4-71
	Females	8	34.9	6.6	5-56
	Total	31	25.9	3.4	4-71
No treatment	Males	4	19.8	8.2	3-41
	Females	4	33.3	6.7	16-47
	Total	8	26.5	5.5	3-47

\* Statistical analysis by Kruskal-Wallis one-way analysis of variance by ranks (treatment on age) gives  $H = 1.21$ ,  $P =$  not significant.

The distribution of the 95 subjects by treatment, gender, and age who fully participated in the trial and the eight who were examined during both visits but did not return for treatment is summarized in Table 1. The groups given mebendazole, pyrantel, or placebo were reasonably well matched with identical numbers of male subjects (23) and smaller but comparable numbers of females (6-12) in each. Despite the range, there was no significant difference in mean or median age among any of the four groups.

Table 2 summarizes the mean adjusted EPG<sup>13</sup> and cure rates at both pre- and postintervention surveys by treatment group and gender. The statistical analyses are presented in Tables 3-5. Possible pre-existing differences between the treatment groups were examined through retrospective analysis of adjusted EPG from the preintervention survey because the original balanced distribution of ages and genders across treatments was altered by the failure of some subjects

TABLE 3

A Statistical analysis of factors affecting intensity of infections with *Necator americanus* (gender and four treatment groups, with age as a covariate) before drug intervention, through a two-way analysis of variance (ANOVA) with negative binomial errors\*

Source of variation	Change in deviance†	Degrees of freedom	Scaled deviance‡	P
Drug treatment	7,723	3	7.5	0.1 > P > 0.05§
Gender	14,771	1	14.3	<0.001
Drug treatment × gender	507	3	0.5	NS
Age plus all interactions	14,365	8	13.95	0.1 > P > 0.05§

B Minimum sufficient model following removal of all factors not contributing significantly to variation in the data, associated coefficients, and statistical evaluation

$$\text{Preintervention epg} = \beta_0 + \beta_1 \ddagger$$

Coefficient	Estimate	SEM	t	Degrees of freedom	P
$\beta_0$	414	78.95			
$\beta_1$	-222.0	115.2	1.9	28	0.05 > P > 0.025

\* NS = not significant; epg = eggs per gram of feces.

† Change in deviance following removal of combination specified in source of variation column from the full factorial model. We begin by removing age and all its interactions from the model and progressively remove the combinations in order from the base of the table towards the top.

‡ Scaled deviance = measure of contribution of factor specified under column labeled source of variation to explaining variation in the data, calculated by fitting ANOVA with negative binomial errors through GLIM<sup>12</sup>. It is distributed as  $\chi^2$ . For the preintervention analysis, the scale parameter was 1.030.

§ These effects were split into their individual coefficients to check whether significant ones were masked by the aggregated test, but no individual coefficient was significant.

¶ Where  $\beta_0$  = the constant, a reference point used by the model and equal to the mean for male subjects and  $\beta_1$  = the adjustment necessary for female subjects.

to participate in treatment and the follow-up survey. Analysis of variance showed that neither age nor any interactions involving age affected variation in the data significantly (Table 3A). Only gender had a highly significant effect on EPG before treatment, with female subjects carrying lower hook-

TABLE 2

Changes in intensity of infection between pre- and postintervention surveys and cure rates following treatment

Treatment	Mean adjusted epgs ± SEM			Reduction* (%)	Cure rate†	
	Preintervention survey	Postintervention survey			No.	%
1 Mebendazole						
All subjects	264.2 ± 91.5	281.5 ± 71.5	(+) 6.5	8	22.9	
Males	344.2 ± 135.8	378.8 ± 102.5	(+)10.1	4	17.4	
Females	111.0 ± 40.6	95.0 ± 32.4	(-)14.4	4	33.3	
2 Pyrantel						
All subjects	472.1 ± 164.6	117.9 ± 38.8	(-)75.0	13	44.8	
Males	512.3 ± 204.0	121.6 ± 48.5	(-)76.3	12	52.2	
Females	318.0 ± 159.7	104.0 ± 30.3	(-)67.3	1	16.7	
3 Placebo						
All subjects	327.9 ± 98.2	220.6 ± 42.9	(-)32.7	7	22.6	
Males	371.0 ± 126.1	265.6 ± 52.3	(-)28.4	4	17.4	
Females	204.0 ± 116.6	91.5 ± 51.6	(-)55.1	3	37.5	
4 No treatment						
All subjects	364.5 ± 220.9	220.5 ± 79.1	(-)39.5	2	25.0	
Males	504.0 ± 432.1	363.0 ± 103.5	(-)28.0	0	0	
Females	225.0 ± 167.7	78.0 ± 70.2	(-)65.3	2	50	

\* Positive and negative signs indicate relative reductions or increases in eggs per gram of feces (epgs), respectively.

† Number and percentage of subjects in treatment subset who had eggs during the preintervention survey but none at the postintervention survey.

TABLE 4

A Statistical analysis of factors affecting intensity of infection with *Necator americanus* after drug intervention\*

Source of variation	Change in deviance†	Degrees of freedom	Scaled deviance‡	P
Drug treatment	4,812	3	13.53	<0.005
Gender	6,473	1	18.2	<0.001
Drug treatment × gender	1,347	3	3.79	NS
Age plus all interactions	2,832	8	7.96	NS

B Minimum sufficient model, associated coefficients, and statistical evaluation

$$\text{Postintervention epg} = \beta_0 + \beta_1 + \beta_2 + \beta_3 + \beta_4\ddagger$$

Coefficient	Estimate	SEM	t	Degrees of freedom¶	P
$\beta_0$	309.0	54.75			
$\beta_1$	-141.5	48.38	2.92	28	<0.005
$\beta_2$	-122.7	61.74	1.99	27	0.05 > P > 0.025
$\beta_3$	-57.85	67.82	0.85	29	NS
$\beta_4$	-43.87	100.1	0.44	6	NS

\* NS = not significant; epg = eggs per gram of feces.

† See Table 3.

‡ Scale parameter = 355.6.

§ Where  $\beta_0$  = reference point corresponding to male subjects in treatment 1 (mebendazole). Adjustment for female subjects =  $\beta_1$ ; treatment 2, pyrantel =  $\beta_2$ ; treatment 3, placebo =  $\beta_3$ ; treatment 4, nontreated =  $\beta_4$ .

¶ Number of subjects - 2.

worm burdens than males. Reassuringly, the treatment groups did not differ significantly before treatment, either overall or individually, and there was no significant interaction between treatment and gender. Since there was no significant difference between the treatment groups, no age effect, and no interactions between these factors and gender, we removed them from the model and recalculated a minimum sufficient model with gender as the only factor to estimate the coefficients for the new model and their statistical significance. The results are shown in Table 3B and confirm that the effect of host gender was significant.

The outcome of treatment is summarized in Table 2, which presents mean EPG values for the subjects in each treatment by age and cure rates. The latter indicate that some subjects in all treatment groups ceased passing hookworm eggs by the postintervention survey, irrespective of treatment and gender. However while the overall cure rate in the groups given placebo, mebendazole, or not treated varied between 22.6% and 25%, the highest overall cure rate was seen in the group treated with pyrantel (44.8%).

The statistical analysis of the postintervention fecal egg counts is presented in Table 4. Although age was included in this analysis, neither age nor its interactions affected the data significantly (Table 4A). Highly significant main effects of gender and treatment were found, but no interaction was observed between gender and treatment, so male and female subjects responded comparably to treatment. We next fitted a minimum sufficient model, with treatment and gender as factors (Table 4B). This confirmed the highly significant effect of gender and indicated that only pyrantel significantly affected EPG ( $\beta_2$ ).

Finally, we analyzed the changes in EPG between the pre- and postintervention surveys. The results (Table 5A) indicate that neither age nor any interactions involving age had sig-

TABLE 5

A Statistical analysis of factors affecting changes in intensity of infection with *Necator americanus* following treatment\*

Source of variation	Change in deviance†	Degrees of freedom	Scaled deviance‡	P
Drug treatment	1,514	3	7.2	0.1 > P > 0.05§
Gender	30.56	1	0.15	NS
Drug treatment × gender	86.13	3	0.4	NS
Age plus all interaction	1,449	8	6.9	NS

B Minimum sufficient model, associated coefficients, and statistical evaluation

$$\text{Change in epg} = \beta_0 + \beta_1 + \beta_2 + \beta_3\parallel$$

Coefficient	Estimate	SEM	t	Degrees of freedom#	P
$\beta_0$	1,663	96.77			
$\beta_1$	371.5	152.3	2.44	27	<0.025
$\beta_2$	124.5	144	0.86	29	NS
$\beta_3$	161.3	2.33	0.69	6	NS

\* NS = not significant; epg = eggs per gram of feces.

† See Table 3.

‡ Scale parameter = 209.2.

§ The overall nonsignificance of this effect masks the significant coefficient of one of the treatments, as shown in 5B.

¶ Where  $\beta_0$  = reference point corresponding to all subjects in treatment 1 (mebendazole). Adjustment for treatment 2, pyrantel, both sexes =  $\beta_1$ ; treatment 3, placebo, both sexes =  $\beta_2$ ; treatment 4, nontreated, both sexes =  $\beta_3$ .

# Number of subjects - 2.

nificant effects. There was no significant interaction between drug treatment and gender and no main effect of gender; therefore, changes in EPG followed the same pattern in both genders. Drug treatment was only just outside the cut-off for significance in the full factorial model, but fitting the minimum sufficient model demonstrated that overall drug treatment masked the single significant effect of pyrantel (Table 5B), which caused a significant change in EPG between the pre- and postintervention surveys.

**Egg hatch assay.** A laboratory strain of *N. americanus*, which had not been exposed to any anthelmintics for more than 100 generations, was used as a reference strain to determine whether the field strain from Mali had become resistant to benzimidazoles. Our results indicated that the ED<sub>50</sub> for the Nottingham (laboratory) strain was  $0.069 \pm 0.0067$  µg/ml (mean ± SEM) of TBZ, while the Mali strain was almost twice as resistant with an ED<sub>50</sub> of  $0.117 \pm 0.0006$  µg/ml of TBZ.

## DISCUSSION

The results of the full-randomized, placebo-controlled trial reported herein confirmed the suspected poor efficacy of a single 500 mg dose of mebendazole treatment under local conditions. Statistical analysis of the trial outcome revealed that only two of the factors that we included in the analysis significantly influenced variation in our data sets.

The first factor was host gender. Male subjects during both surveys and in each of the treatments showed higher mean EPG than females. Host gender-dependent differences in susceptibility and resistance to infection are well recognized in the literature,<sup>22</sup> and it is usually, although not exclusively,

the case that males show higher prevalence and more severe infections compared with females.<sup>23</sup> Epidemiologic surveys of hookworm infection have likewise frequently concluded that prevalence and intensity are higher among males<sup>24-26</sup> and attributable to gender-dependent behavior associated with exposure; male subjects experience higher exposure while working on contaminated soils. Other explanations include hormonal differences between the sexes affecting susceptibility and immunocompetence.<sup>27</sup> In Massabla, male subjects are predominantly responsible for agricultural pursuits, including both arable crops and livestock husbandry as also for building dung-reinforced huts in the post rainy season. Most hookworm infections are probably acquired at this time, when the incorporation of human stools into the building materials, could account for higher exposure of male subjects to hookworm larvae.

The second factor was drug treatment; only pyrantel significantly reduced hookworm fecal egg counts. We can thus conclude that mebendazole was without significant effect in our study. The question then arises as to why this should have been the case, given the published track record of this drug in the treatment of hookworm infections.<sup>2,5,8</sup>

Several explanations are possible. One possibility is that in this region of Mali, *N. americanus* has developed resistance to the benzimidazoles and the parasites are no longer susceptible to treatment with mebendazole. To test for suspected resistance, we carried out a standard egg hatch assay, comparing the sensitivity of the eggs of *N. americanus* recovered from subjects who had failed to respond to treatment with mebendazole with that of eggs from hamsters infected with a laboratory strain of the parasite that had not been exposed to anthelmintics for more than 100 generations. The results indicated that the Mali strain of *N. americanus* was almost twice as resistant to benzimidazoles as the laboratory reference strain and by the criteria used to evaluate the sensitivity of nematodes affecting domestic animals (ruminants such as sheep and cattle) to benzimidazoles, the Mali strain would be considered resistant ( $ED_{50} \geq 0.1 \mu\text{g}$  of TBZ/ml).<sup>18</sup>

Benzimidazole resistance is widespread among nematode parasites of domestic animals, and there are pronounced regional differences with widespread resistance being reported in South Africa, New Zealand, and Australia.<sup>28,29</sup> Too frequent treatment, use of inappropriate doses, and a failure to alternate treatment with other drug classes are believed to be responsible for resistance to specific anthelmintics.<sup>30,31</sup> We are unaware of any other reports indicating that resistance to chemotherapy has arisen among human intestinal nematodes, but this may be because few surveys have specifically addressed this issue, practicable techniques for surveys are subject to error and bias, and resistance may have been overlooked. It is possible for resistance to go unnoticed for a long time among nematodes that do not produce easily recognizable disease states. Little is known about selection pressure for appearance of drug resistance among human nematodes but from studies of ovine parasites, it is recognized that only a few rounds of treatment at a time when most parasites are in their hosts selects strongly for resistance.<sup>32</sup> Mass treatment of a population in the dry season (when few larvae survive in the environment) could make a profound contribution to the evolution of resistance because the majority of organisms are exposed to the drug in ques-

tion. Although resistance may be slow to appear,<sup>33</sup> it has long been anticipated because of the widespread misuse of anthelmintics in treating human infections.<sup>32,34</sup> Mebendazole, because of its low price and because it has been readily available in the region since 1975, is the most frequently prescribed anthelmintic in the health center in Bougouni and the most common anthelmintic sold in local drug stores. Therefore, our results, suggesting that resistance has arisen locally, are not unexpected.

Among the other possibilities, drug formulation may have been responsible for lack of efficacy.<sup>35</sup> The contrasting efficacy of two formulations of mebendazole,<sup>36</sup> with similar high drug purity, was attributed to the efficiency of breakdown in aqueous fluid. However, the mebendazole used for the main trial in the present study was obtained from Janssen Pharmaceutica and our tests revealed that it did produce a very fine suspension in water, a requirement for high efficacy.<sup>35</sup> It is also possible that local *N. americanus* differ in sensitivity to mebendazole because of genetic drift among the parasites relative to other geographically distant hookworm populations. Exchange of genes between the local parasites and those elsewhere is unlikely because despite the limited movement of traders, the Massabla community is largely sedentary. Resistance to mebendazole may have arisen in the local strain of *N. americanus* in association with other genetic changes and not through direct selection in response to intensive use of mebendazole. Finally it is conceivable that local dietary factors may have altered the pharmacodynamic properties of mebendazole. Reducing food intake or fasting before drug administration increase the efficacy of benzimidazoles against gastrointestinal nematodes of ruminants,<sup>37,38</sup> but in the present study, the majority of subjects were treated in the late morning, i.e., approximately 12 hr after the main meal of the day, which typically is consumed in the evening after the daytime activities have been completed.

Our study does not exclude the possibility that more frequent dosing with mebendazole or treatment with higher doses would have had a greater effect in removing hookworms. However, our survey was specifically aimed at assessing the widely used single-dose treatment protocol at the dose at which it is generally implemented, and it is this particular combination that failed to remove hookworms in the circumstances in which it was tested, while pyrantel gave the expected efficacy. Whatever the underlying explanation for the failure of mebendazole in this study, our data serve to caution that it is unwise to rely on treatment with a single 500-mg dose of mebendazole when controlling hookworm infections in this region of Mali.

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