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Comparison of the efficacy of mebendazole, albendazole and pyrantel in treatment of human hookworm infections in the Southern Region of Mali, West Africa

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Abstract

A randomized, placebo-controlled trial of the efficacy of pyrantel (single dose 12·5 mg/kg bodyweight), mebendazole (single 500 mg dose) and albendazole (single 400 mg dose) in the treatment of hookworm infections (*Necator americanus*) was carried out in January 1998 in the Southern Region of Mali, West Africa, during the period of Ramadan (Islamic fast). Statistical analysis of the pre-intervention faecal egg counts showed that there was a significant pre-treatment chance bias, despite randomization of subjects into treatment groups, arising from the main effect of sex (heavier infections among males) and a sex × treatment interaction (the sex bias was not evident in the pyrantel-treatment group). The participants were re-examined 10 days after treatment, and after controlling for the drift in faecal egg counts in the placebo-treated subset, age, sex, fasting and intensity of infection, albendazole was clearly the most effective drug showing consistently efficacies in the range 92·1 to 99·7%, depending on the method of evaluation and the particular subset of the treatment group. Neither mebendazole nor pyrantel was as effective, with efficacies ranging from 60·9 to 89·8% and 4·8 to 89·7%, respectively. Fasting made no difference to drug efficacy. On the basis of our results the single 400 mg dose of albendazole is the treatment of choice for hookworm infections in this region of Mali. We emphasize the need for standardization of the methods used for trial designs, for calculation of summary data relating to drug efficacies and the accompanying statistical tests.

Keywords: hookworm infection, *Necator americanus*, chemotherapy, clinical trials, albendazole, mebendazole, pyrantel, Mali

Introduction

Hookworms rank amongst the most widespread of helminth parasites infecting people living in the tropics and subtropics (BUNDY, 1997; CHAN, 1997), wherever climatic factors and the living conditions of human communities are conducive for transmission (BUNDY & KEYMER, 1991). In Mali, the vast majority of hookworm infections are attributable to *Necator americanus*, and transmission is most intense in the Third Region, Sikasso, in the warm, humid south of the country (DE CLERCQ *et al.*, 1995). Here, transmission occurs because few people wear footwear and because human stools contaminate agricultural plots and are incorporated into building materials (DE CLERCQ *et al.*, 1997).

Although globally a range of drugs has been used to reduce hookworm infections in the past (JANSSENS, 1985), current treatment is predominantly based on the wide-spectrum Group 1 (benzimidazoles) and Group 2 (pyrantel/levamisole) anthelmintics which have few side-effects and are generally associated with high compliance rates among treated populations (WHO, 1997). The most recent benzimidazole to join those already in use is albendazole, and initial evaluation of this drug has indicated that it is extremely effective for the treatment of both *N. americanus* and *Ancylostoma duodenale* infections (STEPHENSON *et al.*, 1989; ALBONICO *et al.*, 1994; REYNOLDSON *et al.*, 1997). However, for gastrointestinal (GI) nematodes evaluation of drug efficacy is not straightforward because it is mostly based on assessment of changes in the intensity of faecal worm egg counts.

Quantitative faecal egg counts (FEC) on hosts infected with GI nematodes provide only an approximate indication of the intensity of the worm burden (ANDERSON & SCHAD, 1985) but nevertheless are frequently used to assess such infections because of convenience in relation to the alternative methods. Evaluation of anthelmintic drug efficacy in trials based on quantitative FEC, therefore, is fraught with many problems, not the least of which are the overdispersed nature of helminth FEC, the considerable daily variation in FEC and differences in FEC between the sexes and age-groups (ANDERSON & SCHAD, 1985; KEYMER & SLATER, 1987; DASH *et al.*, 1988; STEAR *et al.*, 1995a, 1995b). The distribution of FEC usually conforms to the negative binomial model (ANDERSON & SCHAD, 1985; DASH *et al.*, 1988), but can be so extreme that even this model cannot fully account for the data. In such cases simple arithmetic methods for presenting summary statistics, calculating reductions in FEC and analysis by multivariate statistics based on normal errors, are inappropriate (DASH *et al.*, 1988). Conclusions based merely on relative efficacies can be misleading, when for example bias in the proportion of each sex or age-group has not been taken into consideration, when control placebo-treated groups show drift or fluctuations in FEC with time (ANDERSON & SCHAD, 1985) and when the sexes respond differently to treatment. For these reasons, statistical models which take into account the negative binomial distribution of FEC and control for the other factors contributing to variation in FEC are all the more important in facilitating accurate evaluation of anthelmintic drug efficacy.

In the veterinary field a number of procedures have been developed to standardize presentation of drug efficacies and these are now widely employed (PRESIDENTE, 1985; DASH *et al.*, 1988; COLES *et al.*, 1992). The procedures are based on overall summary statistics, but involve calculations that control for the drift in FEC among untreated hosts (placebo groups) and take into account the overdispersed nature of the egg counts (by employing geometric means). Nevertheless, few trials with human GI nematodes have exploited this wealth of experience, and, in particular, placebo groups controlling for the well-recognized temporal variation in FEC have often been neglected (ALBONICO *et al.*, 1994). Frequently, efficacies have been summarized merely in terms of cure rates and little else (DESOWITZ *et al.*, 1970; FARID *et al.*, 1977). There has been little effort made to standardize trial designs, their analysis and the presentation of data in a format to enable legitimate comparisons between studies.

It is now widely established that many of the anthelmintic drugs used to control GI nematode infections in domestic ruminants have lost efficacy through the

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development of resistance among the parasites (JACKSON, 1993; GEERTS *et al.*, 1997; COLES, 1998). The World Association for the Advancement of Veterinary Parasitology (WAAVP) issued guidelines on the interpretation of FEC among which resistance is considered to be present if the percentage reduction in egg counts is less than 95% and the 95% confidence level is less than 90%. Resistance is also suspected if just 1 of these 2 criteria is met (COLES *et al.*, 1992). On this basis some of the anthelmintics commonly used for treatment of human GI nematode infections either never originally met the criteria required of them or have begun to lose efficacy through the appearance of resistance. In the latter context, 2 recent studies have reported drug failure in respect of mebendazole (DE CLERCQ *et al.*, 1997) and pyrantel (REYNOLDSON *et al.*, 1997). It is all the more important, therefore, to standardize the methods for assessing and interpreting drug efficacy so that even relatively small changes can be detected and evaluated early enough to provide warning of the possible need to revise treatment strategies.

In this paper we report a recent trial of the 3 anthelmintics currently most commonly employed for treatment of hookworms. The study took place in Mali where previous trials had indicated that a single 500 mg dose of mebendazole was not satisfactorily effective against the human hookworm *N. americanus* (DE CLERCQ *et al.*, 1997). Our particular objective was to assess a single 400 mg dose of albendazole as an alternative anthelmintic for people living in this region. Single-dose regimens were selected because they are considerably easier to implement reliably in the field, since compliance deteriorates rapidly when subjects are required to be present for treatment on more than one occasion. Our study design took into account the known sex difference in intensity of infection with hookworms in the region and our analysis controlled for all the known factors that could have affected drug efficacy, including fasting (ALI & HENNESSY, 1995). Finally our interpretation of the outcome of this trial is based on a comparative examination of drug efficacy by a wide range of analytical procedures, including those currently employed in the veterinary field (PANDEY & SIVARAJ, 1994).

Materials and Methods

Study site and subjects

The study was carried out in a village in the district of Bougouni (south-east Mali) in the Sikasso region (3rd Region). The main activities in the village are cultivation of millet, cotton and some livestock husbandry. Earlier surveys in this region of Mali indicated that the prevalence of *N. americanus* was >50% (DE CLERCQ *et al.*, 1997).

Study design

In 1998, in early January, 285 subjects (67·9% of the villagers) provided stools for examination: hookworm infection was confirmed in 151. The infected individuals were randomized into the 4 treatments within single sex, age-stratified cohorts, the 4 treatments being equally distributed across the age-groups and both sexes. The trial was not double blind, those administering treatment, although not the subjects themselves, being aware of the treatment group to which each participant had been allocated. During the second visit, 2 days after the initial pre-intervention survey, 148 of these subjects attended for treatment. Two of the absentees later provided stools for analysis at the post-intervention survey and were included in the placebo group for analysis. Ten days after treatment 127 subjects, from among those originally identified as infected and subsequently treated, provided stools for re-examination (post-intervention survey) and a further 18 provided stools over the course of the following 3 days. Five treated subjects did not provide stools at the post-intervention survey so the trial analysis was eventually based on 145 subjects.

Quantification of hookworm infections

Fresh, overnight stools were obtained from individuals who participated in the study and 2 slides were prepared from each specimen and examined on the same day by the Kato–Katz technique (KATZ *et al.*, 1972). Hookworm eggs were identified, the intensity of infection was calculated as eggs per gram of faeces (EPG) and then adjusted for stool consistency and host age using WHO (1963) guidelines. For formed stools, 1 egg detected on 1 of the 2 slides corresponded to an EPG of 12. The procedure was repeated for the post-intervention survey, but only those who had been treated were examined.

Treatment with anthelmintic

The anthelmintics compared in this study were Vermox (single dose of 500 mg mebendazole, Janssen Pharmaceutica, Beerse, Belgium), Combantrin (pyrantel pamoate at 12·5 mg/kg, Pfizer, Orsay, France) and Zentel (single dose of 400 mg albendazole, SmithKline Beecham Pharmaceuticals, Middlesex, UK). Placebo-treated subjects were given 1 g effervescent tablets of vitamin C (Upsa-C; Upsa Laboratories, Puel-Maimaison, France).

Ethical approval

The trial was approved by the Directorate of the National Institute for Research in Public Health (INRSP), in Bamako, Mali. The background and the purpose of the trial were explained at a meeting with the village elders and heads of families and their approval was obtained. Participation in the trial was subject to informed, voluntary consent of each individual or parents and/or head of family in the case of children. Free medical inspection was made available to all the villagers at the pre-intervention survey, when all participants were weighed. All females of reproductive age were tested for pregnancy with a kit based on monoclonal antibodies for human chorionic gonadotropin on strips for immersion in urine (Mum's the Word; Lagap Pharmaceuticals Ltd, Hampshire, UK) on the day of treatment and pregnant women were either excluded from the study or reassigned to the placebo group. Children aged <2 years were excluded from the study. All participants, irrespective of earlier treatment, were offered anthelmintic treatment when the study had been completed. Pregnant women established as carrying hookworm infection were left albendazole tablets with clear instructions to use only after childbirth.

Statistical analysis

The results of the full trial are presented as arithmetic means ± standard error of the mean (SEM) and geometric mean with 95% confidence limits (ELLIOTT, 1977) of the adjusted EPG for each complete treatment group, and then by sex. We also analysed the data according to whether individuals were fasting or non-fasting, at both pre- and post-intervention surveys.

The cure rates were analysed by maximum likelihood techniques based on log linear analysis of contingency tables implemented by the software package Statgraphics Version 7. Beginning with the most complex model, involving all possible main effects and interactions, those combinations that did not contribute significantly to explaining variation in the data were eliminated stepwise, beginning with the least significant. A minimum sufficient model was then obtained, for which the likelihood ratio of χ^2 was not significant, indicating that the model was sufficient in explaining the data.

The quantitative FEC data from the full trial were analysed by GLIM (a statistical system for generalized linear interactive modelling; GLIM 4, PC version, Royal Statistical Society 1993) as described previously, using models with negative binomial or normal errors (CRAWLEY, 1993; DE CLERCQ *et al.*, 1997). Host sex (2 levels)

and anthelmintic (drug) treatment (4 levels) were entered as factors. Age was entered as a co-variate although in some analyses subjects were allocated to age cohorts as indicated (4 levels: 1 = 3–9 years, $n = 43$; 2 = 10–19 years, $n = 35$; 3 = 20–39 years, $n = 33$; 4 = 40–71 years, $n = 34$) and age was then entered as a factor. The pre-intervention EPG conformed satisfactorily to a negative binomial distribution and the outcome of this analysis is presented in the text as χ^2 values with appropriate probabilities. Those from the post-intervention survey did not and were consequently square root transformed and analysed by 2-way ANOVA with normal errors.

Because of the within-subject nature of the data, we also analysed changes in FECs between the pre- and post-intervention surveys, rather than comparing the results from the 2 surveys with each other. For this purpose the post-intervention adjusted EPG were subtracted from the pre-intervention EPG for individual subjects. One individual, a 47-year-old man allocated to the placebo group, whose FEC showed an extreme change (from 1152 to 3336, representing an increase of 2184) was omitted from the analysis, because the iterations in GLIM diverged and the model could not be established when his values were included. Six hundred was added to all remaining data to convert negative values to positive. Analysis was by 2-way ANOVA with normal errors on square root-transformed data.

In a further analysis, to control for sex differences and variation in pre-intervention EPG, data were expressed as percentage change in EPG at the individual subject level for analysis by GLIM through a 2-way ANOVA (treatment × sex with age as a co-variate).

For models employing negative binomial errors the change in deviance is divided by the scale parameter and the resulting scaled deviance is distributed as χ^2. For models with normal errors the change in deviance is divided by the scale parameter and the result divided by the change in degrees of freedom (d.f.) following each deletion, to give a variance ratio, F.

Calculation of drug efficacy

Drug efficacy was evaluated by 7 procedures:

Procedure 1 was the cure rate, which represents the number and percentage of individuals who were positive for hookworm eggs at the pre-intervention survey but showed no hookworm eggs at the post-intervention survey.

Procedure 2 represents the mean of the change in EPG at the individual subject levels between the pre- and post-intervention surveys, thus for each subset of data

$$\{\sum^{i=n}{}_{i=1}[(T1_i - T2_i]\}/n \text{ where } i = i\text{th subject.}$$

Procedure 3 represents the mean percentage change in EPG at the individual subject levels between the pre- and post-intervention surveys, thus for each subset of data

$$\{\sum^{i=n}{}_{i=1}[(T1_i - T2_i)/T1_i] \times 100\}/n.$$

Procedure 4 was based on percentage change in mean EPG for each subset, thus

$$\{T1 - T2\}/T1 \times 100\%.$$

Procedure 5 (COLES *et al.*, 1992) was based on arithmetic means and calculated as

$$1 - \{T2/C2\} \times 100.$$

Procedure 6 (PRESIDENTE, 1985) was based on geometric means and was calculated as

$$(1 - \{T2/T1 \times C1/C2\}) \times 100.$$

Procedure 7 (DASH *et al.*, 1988) was based on arithmetic means and calculated as

$$(1 - \{T2/T1 \times C1/C2\}) \times 100.$$

$T1$ = EPG at pre-intervention survey, $T2$ = EPG at post-intervention survey, $C1$ = EPG in placebo group at pre-intervention survey, $C2$ = EPG in placebo group at post-intervention survey.

Results

Pre-intervention survey

The prevalence of infection in the village was 53%, but higher among male (63·2%) compared with female (43·6%) subjects. The overall arithmetic mean FEC was 94·0 ± 14·0 EPG, and again higher among males (133·6 ± 26·5) compared with females (57·8 ± 10·8).

The distribution of the 145 subjects who participated in the trial is summarized in Table 1, by treatment, gender and age. The mean egg count for both sexes combined was 176·3 ± 25 ($n = 145$) with a variance to mean ratio of 530·9 and the aggregation constant $k = 0{\cdot}333$. All the groups were well matched numerically with a range of 35–37 subjects per treatment. Although each group had more males than females, the percentage of males vs. females ranged from 54·3% to 61·1% males. There was no significant difference in age between treatments nor between sexes (Table 1).

Possible pre-existing differences between the treatment groups were examined by GLIM through retrospective analysis of adjusted EPG from the pre-intervention survey by a 2-way ANOVA with negative binomial errors (sex and treatment as factors, with age as

Table 1. Composition of the subset of the study population, carrying hookworms at the pre-intervention survey and re-examined subsequently, by treatment, age and sex

			Age	
Treatment	Sex	*n*	Mean (SEM)	Range
Placebo	Males	22	21·1 (2·9)	3–50
	Females	14	24·5 (4·9)	4–65
	Total	36	22·4 (2·6)	3–65
Pyrantel	Males	21	23·9 (4·5)	4–71
	Females	16	28·8 (4·8)	7–58
	Total	37	26·0 (3·3)	4–71
Mebendazole	Males	19	25·8 (4·7)	7–67
	Females	16	32·8 (5·3)	3–61
	Total	35	29·0 (3·5)	3–67
Albendazole	Males	22	21·5 (3·5)	5–51
	Females	15	27·9 (5·7)	3–70
	Total	37	24·1 (3·2)	3–70

Statistical analysis by 2-way ANOVA (treatment and sex on age) gave no significant main effects nor interaction.

a co-variate), because the original balanced distribution of ages and genders across treatments was altered by the failure of some subjects to participate in treatment and follow-up survey. As expected, the egg counts were significantly higher among male vs. female subjects ($\chi^2 = 12{\cdot}563$, d.f. = 1, 141, $P < 0{\cdot}0005$; overall arithmetic mean FEC, with treatment groups combined, for males = $209{\cdot}0 \pm 40{\cdot}4$, $n = 84$ and for females = $131{\cdot}3 \pm 22{\cdot}6$, $n = 61$). However, there was also a significant interaction between sex and treatment ($\chi^2 = 12{\cdot}861$, d.f. = 3, 140, $P < 0{\cdot}0005$) arising from the loss of the male bias in the pyrantel treatment group (Table 2). In the 3 remaining treatment groups the arithmetic mean EPG were higher among male subjects. There was no significant effect of age nor an overall significant difference between treatment groups, indicating that despite the interaction, which is taken into consideration in the analysis of post-intervention egg counts, the allocation of subjects to treatment groups was satisfactory.

Post-intervention survey

The post-intervention egg counts are given in Table 2 and the outcome of treatment is summarized in Table 3. Analysis of the cure rates (Procedure 1) by the maximum likelihood method gave a minimum sufficient model of treatment × presence of infection ($\chi^2 = 51{\cdot}659$, d.f. = 56, $P = 0{\cdot}640$), neither sex nor age (entered here as a factor) contributing significantly. Therefore, significant differences in cure rate were detected between the treatments. This can be seen in Table 3, where the cure rates (Procedure 1) are considerably higher after treatment with albendazole and lower in the remaining groups, with the lowest among placebo-treated subjects.

Statistical analysis of the egg counts recorded during the post-intervention survey was by GLIM with normal errors, and is given in Table 4. Clearly the 4 treatments differed markedly in their effects on FEC: there was a highly significant main effect of treatment on egg counts at the post-intervention survey. However, statistical analyses revealed that age and sex also played a significant role in explaining the variation in egg counts at the post-intervention survey. The age effect is not illustrated but this was controlled for by retaining age in subsequent evaluation of the remaining factors. The significant effect of host sex was expected from the sex difference identified at the pre-intervention survey and the poor efficacy of some of the treatments in the trial. It arose principally from the overall higher persisting egg counts among male versus female subjects in the placebo-, pyrantel- and mebendazole-treated groups (Table 2).

We also analysed the data at the level of absolute change between the 2 surveys (Procedure 2 in Table 3) and this revealed that only the main effect of treatment was significant ($F_{3,142} = 3{\cdot}847$, $0{\cdot}025 > P > 0{\cdot}01$). Although the interaction between sex and treatment was not significant, it was close to significance ($F_{3,139} = 2{\cdot}221$, $0{\cdot}1 > P > 0{\cdot}05$) but there was no main effect of sex.

However, to control for the differences in the initial intensity of pre-intervention EPG, the pre-existing sex bias and the pre-existing interaction between treatment and sex, we also calculated the percentage change in EPG for each subject (Procedure 3 in Table 3). Analysis of these data revealed that there was a highly significant main effect of treatment ($F_{3,143} = 5{\cdot}601$, $0{\cdot}005 > P > 0{\cdot}001$) but there was also a weak main effect of sex ($F_{1,141} = 4{\cdot}378$, $0{\cdot}05 > P > 0{\cdot}025$) and a weak interaction between treatment and sex ($F_{3,140} = 3{\cdot}036$, $0{\cdot}05 > P > 0{\cdot}025$). Host age did not play a significant role. From the summary statistics (Tables 2 and 3) it appears that in the pyrantel group females showed a greater relative reduction in EPG compared with males among whom little change was evident. Likewise, in the placebo group EPG increased among male subjects but dropped by 32·1% among females. However, these changes were masked in analysis of absolute changes by within-group variation (high SEM in all cases) and by the relatively consistent effect of albendazole among both sexes, becoming significant only when the intensity of, and sex difference in, pre-intervention EPG was controlled for.

Relative efficacy of the anthelmintics

Quite clearly albendazole was the most effective anthelmintic, as adjudged by all the criteria and procedures used to assess drug efficacy (Table 3). The effect of albendazole was consistent across both genders and all age cohorts. Only 6 subjects out of the 37 in this group still passed eggs at the post-intervention survey, 3 of each sex. Of these 6, egg counts fell in 5. One 6-year-old boy showed an increase in EPG from 18 to 48 while 2 other 6-year-old boys had lower counts but were also not totally cured. Among females those with remaining egg counts were aged 8, 12 and 47 years and all showed a drop in counts although the smallest drop was in the 8-year-old female (from 24 to 18).

Both of the other anthelmintics, mebendazole and pyrantel, were less effective by all of the summary statistics in Table 3 and their efficacies were compounded by variation in relation to host sex. Because of these confounding variables the relative efficacy of the 4 treatments could not be clearly established from the 2-way ANOVA of post-intervention EPG. However, analysis of the absolute change in EPG identified only treatment as a significant factor, enabling a minimum sufficient model based on 1-way ANOVA in GLIM in order to compare the 4 treatments directly against each other. We entered the square root transformed change in EPG by treatment into a GLIM model with normal errors and as expected this gave a highly significant result (model deviance = 3218·9, scale parameter = 22·99). Removal of treatment gave a change in deviance of 284·5 (scaled deviance = 3·871, d.f. = 3, 143, $P < 0{\cdot}025$). Examination of the model co-ordinates showed that in relation to the placebo-treated group the change in EPG was significant in all anthelmintic-treated groups (pyrantel $t = 1{\cdot}803$, d.f. = 36, $P < 0{\cdot}05$; mebendazole $t = 2{\cdot}514$, d.f. = 34, $P < 0{\cdot}01$; albendazole $t = 3{\cdot}384$, d.f. = 36, $P < 0{\cdot}001$). On the basis of the t values albendazole was clearly the most effective.

Examination of the drug efficacies, as calculated by Procedures 4–7 (Table 3), shows that for pyrantel and mebendazole none exceeded 90% among subsets corresponding to male, female and both sexes combined. At worst Procedure 4 gave a reduction of 4·8% for pyrantel among male subjects and at best Procedure 6 gave an efficacy of 89·8% for males treated with mebendazole. Overall, mebendazole showed a marginally higher efficacy than pyrantel by all of these 4 procedures. It was also slightly more effective in males as adjudged by Procedures 5–7, and substantially more effective by Procedure 4. Among female subjects pyrantel was more effective as adjudged by Procedures 4, 5 and 7, and mebendazole by Procedure 6. It is also worth noting that the significance of the values calculated by Procedures 4–7 could not be evaluated statistically because each was calculated from summary data.

The effect of fasting on anthelmintic efficacy

Several studies in ruminants have shown that the type of diet and the level of feed intake can markedly affect the bioavailability of orally administered benzimidazole drugs (ALI & HENNESSY, 1993, 1995; HENNESSY *et al.*, 1995; OUKESSOU & CHKOUNDA, 1997). A reduction in feed intake, and consequently a slower flow rate of digesta, extends the residence time of benzimidazoles in the host and enhances anthelmintic activity because of the longer duration of exposure of the parasite to the drug. Since the trial was carried out during the period of Ramadan in a predominantly Muslim region, a proportion of the villagers were fasting, providing an opportunity to compare the efficacy of drugs in fasting and non-

Table 2. Arithmetic and geometric mean values for adjusted faecal egg counts (*N. americanus*) following the pre- and post-intervention surveys

				Arithmetic mean EPG[a] ± SEM		Geometric mean EPG and 95% confidence limits[b]	
Group	Treatment	Subset	*n*	Pre-intervention survey	Post-intervention survey	Pre-intervention survey	Post-intervention survey
1	Placebo						
		All subjects	36	187·8 ± 47·2	260·8 ± 101·2	82·5 (51·8–131·1)	49·0 (22·8–104·1)
		Males	22	231·0 ± 74·9	390·7 ± 160·4	82·2 (40·8–164·7)	93·0 (36·1–237·2)
		Females	14	119·8 ± 24·1	56·6 ± 18·3	83·0 (45·5–150·6)	17·6 (4·8–58·7)
2	Pyrantel						
		All subjects	37	158·4 ± 38·0	86·3 ± 25·8	74·5 (49·1–112·8)	13·4 (5·7–29·8)
		Males	21	145·3 ± 39·2	138·3 ± 42·3	80·2 (46·4–138·1)	27·6 (8·6–83·9)
		Females	16	175·5 ± 72·8	18·0 ± 5·4	67·7 (32·9–138·0)	4·8 (1·2–14·6)
3	Mebendazole						
		All subjects	35	185·3 ± 67·2	58·3 ± 22·1	83·4 (56·3–123·4)	6·4 (2·4–14·9)
		Males	19	240·6 ± 120·5	88·7 ± 39·0	94·8 (52·2–171·7)	10·9 (2·8–36·2)
		Females	16	119·6 ± 34·5	22·1 ± 9·3	71·6 (41·1–124·4)	3·2 (0·5–11·1)
4	Albendazole						
		All subjects	37	174·5 ± 50·2	4·1 ± 1·7	87·1 (59·6–126·9)	0·7 (0·1–1·5)
		Males	22	220·4 ± 81·5	4·1 ± 2·5	104·1 (62·1–173·8)	0·6 (0–1·7)
		Females	15	107·2 ± 28·3	4·0 ± 2·2	67·0 (36·8–121·5)	0·8 (0–2·7)

[a]Eggs per gram of faeces.
[b]The geometric mean was calculated on $(x + 1)$, and then adjusted by subtraction of 1. The 95% confidence limits are shown as (lower limit and upper limit).

Table 3. Changes in intensity of infection with *N. americanus* between pre- and post-intervention surveys, cure rates and drug efficacies following treatment

			Procedure[a]						
			1		2	3	4	5	6	7
			Cure rate		Mean change in EPG	Mean % change in	% change in mean	Coles *et al.*, 1992	Presidente, 1985	Dash *et al.*, 1988
Group	Treatment	Subset	No.	%	± SEM	EPG ± SEM	EPG			
1	Placebo									
		All subjects	6	16·7	+73·0 ± 68·4	+207·5 ± 101·4	+38·9			
		Males	2	9·1	+159·7 ± 107·3	+360·0 ± 157·8	+69·1			
		Females	4	28·6	−63·2 ± 29·8	−32·1 ± 28·0	−52·8			
2	Pyrantel									
		All subjects	14	37·8	−72·1 ± 36·9	−25·1 ± 19·2	−45·5	66·9	70·0	60·8
		Males	6	28·6	−7·0 ± 27·3	−2·2 ± 31·0	−4·8	64·6	70·0	43·7
		Females	8	50·0	−157·5 ± 73·6	−55·2 ± 16·1	−89·7	68·2	66·6	78·3
3	Mebendazole									
		All subjects	18	51·4	−127·0 ± 57·3	−38·4 ± 24·8	−68·5	77·6	87·1	77·3
		Males	8	42·1	−151·9 ± 102·6	−35·0 ± 25·0	−63·1	77·3	89·8	78·2
		Females	10	62·5	−97·5 ± 34·0	−42·5 ± 46·4	−81·5	61·0	78·9	60·9
4	Albendazole									
		All subjects	31	83·8	−170·4 ± 50·2	−89·2 ± 7·4	−97·7	98·4	98·6	98·3
		Males	19	86·4	−216·3 ± 81·7	−87·0 ± 12·1	−98·1	99·0	99·5	98·9
		Females	12	80·0	−103·2 ± 28·2	−92·4 ± 5·2	−96·3	92·9	94·4	92·1

[a]Procedures used to calculate values are given in Materials and Methods. Where relevant, −ve and +ve signs indicate relative reduction or increase in EPG, respectively.

Table 4. Statistical analysis of factors affecting intensity of infection with *N. americanus* (sex and treatment, with age as a co-variate and square root transformed egg counts) after drug intervention, through a 2-way ANOVA with normal errors

Source of variation	Change in deviance[a]	Degrees of freedom	Scale parameter	Scaled deviance[b]	*P*
Drug treatment	2106	3	63·00	11·14	<0·001
Sex	813·7	1	54·89	15·39	<0·001
Drug treatment × sex	345·2	3	49·20	2·339	0·1 > *P* > 0·05
Age	551·7	1	51·43	10·727	<0·005

The full model deviance was 6494·1 with a scale parameter of 47·75.
[a] Change in deviance following removal of combination specified in 'Source of variation' column from the full factorial model. We began by removing age from the model, but since this proved significant, age was replaced to control for age in evaluation of subsequent factors. Then we progressively removed the combinations in order from the base of the table towards the top. The main effects were removed in turn to assess the change in deviance but then replaced before removing the next main effect.
[b] Scaled deviance = measure of contribution of factor specified under column labelled 'Source of variation' to explaining variation in the data, calculated by fitting ANOVA with normal errors through GLIM. In a model with normal distribution it is distributed as *F*.

fasting individuals (data not illustrated). Although most subjects participating in the trial were not fasting, small numbers of each treatment group, with the exception of placebo-treated females, were represented among the fasting subset and these would not accept treatment until after sunset. We found that albendazole was effective irrespective of whether subjects were fasting or not fasting. The pattern for pyrantel was for apparently better efficacy among non-fasting subjects by Procedures 4 and 7, and the reverse by Procedures 5 and 6. However, a 2-way ANOVA (pyrantel-only group) with fasting/non-fasting and host sex as factors, on percentage change in EPG, with normal errors, found no significant effects. A similar pattern was evident among the mebendazole-treated group and again a 2-way ANOVA (mebendazole-only group) found no significant effects. Therefore, despite the differences suggested by the trends in summary statistics, both sexes responded indistinguishably to treatment by pyrantel and mebendazole whether subjects were fasting or non-fasting. However, this analysis should be treated with caution because some of the subsets were small.

Dose of anthelmintic administered

We examined the relationships between the actual dose of pyrantel and mebendazole administered and the outcome of treatment. Pyrantel was administered so that on average each subject received 12·5 mg/kg bodyweight, but since the recommended dose was given as from one to six 125 mg tablets in relation to the relevant 10 kg weight band, there was some variation in actual dose administered. The mean dose given was 13·88 ± 0·35 mg/kg ($n = 37$) with a range from 10·56 to 22·73 mg/kg. Analyses of the relationships between the actual dose given and the post-intervention EPG, the change in EPG and the percentage reduction in EPG, by Spearman's correlation test all failed to establish any significant relationship.

A similar exercise was carried out with mebendazole which was given as a single 500 mg/dose tablet. The mean dose administered was 15·07 ± 1·49 mg/kg ($n = 35$) and the range was from 7·69 to 50·00 mg/kg. Again, we found no evidence that efficacy varied in relation to dose within this range; 100% clearance was achieved with both low and high doses in this range and there were significant failures of treatment right across the whole range.

Efficacy in relation to level of infection at pre-intervention survey

Most of the subjects in this study would be classed as carrying low-intensity hookworm infections, using the criteria adopted by others (NONTASUT *et al.*, 1989: low = <2000 EPG, intermediate = 2001–10 000 EPG, high = >10 000 EPG). Of the subjects who fully participated in the trial, only 1 had egg counts exceeding 2000 (a male in mebendazole group with EPG = 2340) and 3 had EPG between 1000 and 2000. All others had EPG <1000. Nevertheless, we tested the hypothesis that failure of drug efficacy may be related to differential activity in relation to magnitude of EPG. For this purpose the groups were separated on the basis of subjects showing an initial count <80 and ≥80 EPG. Again, albendazole was effective at all intensities of infection, perhaps marginally better when used to treat the high infections. However, for pyrantel and mebendazole there was considerable variation within the data subsets. These were analysed in 2 separate 2-way ANOVAs (one for mebendazole-only subjects and the other for pyrantel-only subjects) with normal errors (intensity of infection and host sex as factors, on percentage change in EPG). No significant effect or interaction was found.

Discussion

As in other studies, hookworm infections were clearly overdispersed in our population with the majority of subjects carrying light-intensity infections and only a few carrying substantially heavier parasite burdens (ANDERSON & SCHAD, 1985). Moreover, as previously found, there was considerable drift in FECs among the control group between the 2 surveys and, in our case, FECs showed opposite trends in male compared to female placebo-treated subjects (increasing and dropping with time, respectively). Both of these features of our data complicated interpretation, necessitating appropriate rigorous statistical analysis to ensure that conclusions were justified. In order to compensate for the former, the extreme values typical of data conforming to the negative binomial models of distribution veterinary parasitologists employ geometric means for FEC, and have developed associated procedures for calculating drug efficacy. All of these have some advantages and disadvantages as discussed by PRESIDENTE (1985), DASH *et al.* (1988) and COLES *et al.* (1992). To control for the latter we used Procedures 5, 6 and 7, which take into account drift in control groups, and presented data for both sexes separately as well as combined. Our statistical models took the pattern of distribution of the data, age, fasting, intensity of infection, sex bias and sex–treatment interactions into account when evaluating the main effects of treatment.

The main conclusion from this trial is that albendazole was a far superior drug to mebendazole and pyrantel in the treatment of *N. americanus* infections in southern Mali. By all the criteria that we used to assess drug efficacy albendazole was extremely efficient whether the subjects were male or female, young or old, fasting or non-fasting, carrrying low- or high-intensity infections. In comparison, both pyrantel and mebendazole showed disappointing efficacies, with significant numbers of subjects in each case failing to show a reduction in FECs and by WAAVP guidelines both would be suspected of lacking appropriate efficacy or of being subject to resistance in the region.

Our finding that there was a significant sex bias in

infections with hookworm was expected from earlier studies in the region, the basis of which has been reviewed (DE CLERCQ *et al.*, 1997). However, on this occasion our trial was conducted during Ramadan when a substantial proportion of the population were fasting. Those who were not fasting (most younger women and children) were treated between early morning and midday, whereas those who were fasting were treated during an evening visit to the village but after the inhabitants had taken their first meal soon after sunset. Although food availability is known to influence the efficacy of benzimidazoles (ALI & HENNESSY, 1993, 1995; HENNESSY *et al.*, 1995; OUKESSOU & CHKOUNDA, 1997), we found no evidence in our study that either mebendazole or albendazole showed any increased activity in persons who were fasting.

On the basis of this study albendazole in a single 400 mg dose is clearly the drug of choice in this region of Mali. However, locally, all 3 drugs are expensive (£1·70–1·82/course of treatment) and beyond the scope of most villagers. There is an urgent need to preserve the efficacy of albendazole for the future through rational use, because the group 3 anthelmintics, the avermectins/milbemycins, show little efficacy against *N. americanus* (BEHNKE *et al.*, 1993) and there are no novel families of anthelmintics sufficiently well developed to ensure their availability for the treatment of human GI nematode infections in the near future.

Finally, by drawing on the experience of the veterinary parasitologists, we hope to have demonstrated in this analysis that the standardization of the manner in which summary statistics of drug trials are calculated is an important consideration for the future. As we have shown, drug efficacies can vary enormously depending on how such statistics are calculated, on whether comparison is made to drift in placebo-treated groups and according to which subsets of data are analysed. This is particularly the case when some subjects have not been cured by treatment. Moreover, conclusions should be based on accompanying rigorous statistical interpretation of data, through models appropriate for the type of data collected.

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Announcements

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE
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The aim of the fund is to sponsor prospective Fellows from developing countries, who are at present unable to join because their country's fiscal rules prevent them from paying the subscription.

The fund, known as *The President's Fund for Overseas Fellows in Developing Countries*, is used to sponsor deserving candidates for full Fellowship of the Society, initially for a period of three years.

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The Royal Society of Tropical Medicine and Hygiene offers an annual prize of £300 for an account of work carried out of relevance to a tropical or developing country by a non-medical student of any nationality. The work (which may be laboratory-based and not necessarily carried out in a tropical or developing country) will add to the knowledge of human or veterinary health or hygiene in the tropics. Particular attention will be directed towards originality and quality in the award of the prize. It is anticipated that the prize will act as a stimulus for the pursuit of excellence in research carried out by undergraduates.

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1. Two prizes of £300 may be awarded annually in recognition of outstanding projects which increase knowledge of tropical medicine and hygiene in the broadest sense.
2. Candidates shall be nominated by their head of department, supervisor or Dean, with a supporting statement of up to 500 words.
3. The closing date for receipt of project reports is 31 December. The project should have been done or completed in the previous twelve months.
4. A committee of three shall choose the prize winners.
5. The announcement of the prize winners will be made at the March meeting of the Society.
6. The prizes will be presented by the President of the Society at the Annual General Meeting in June or July.

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