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Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar

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Objective To evaluate the efficacy of and resistance to mebendazole (500 mg) and levamisole (40 or 80 mg), alone or in combination, for the treatment of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections on Pemba Island — an area exposed to periodic school-based mebendazole treatment since 1994.

Methods A randomized, placebo-controlled trial was carried out in 914 children enrolled from the first and fifth grades of primary schools. Stool samples collected at baseline and 21 days after treatment were examined by the Kato–Katz technique to assess the prevalence and intensity of helminth infection.

Findings Efficacies of mebendazole and levamisole as single treatments against intestinal nematode infections were comparable with those in previous trials, but mebendazole treatment of hookworm infections gave significantly lower cure (7.6%) and egg reduction (52.1%) rates than reported in a study undertaken before the beginning of periodic chemotherapy (cure rate, 22.4%; egg reduction rate, 82.4%). Combined treatment with mebendazole and levamisole had a significantly higher efficacy against hookworm infections (cure rate, 26.1%; egg reduction rate, 88.7%) than either drug given alone. No difference in mebendazole efficacy was found in children who had been treated repeatedly compared with those who had not been treated previously.

Conclusion The overall efficacy of mebendazole against hookworm infections after periodic chemotherapy is reduced. The efficacy of benzimidazoles in chemotherapy-based control programmes should be monitored closely. Combined treatment with mebendazole and levamisole may be useful as a tool to delay the development of benzimidazole resistance.

Keywords Ascariasis/drug therapy; /Trichuriasis/drug therapy; Ascaris lumbricoides/drug effects; Trichuris/drug effects; Mebendazole/pharmacology/administration and dosage; Levamisole/pharmacology/administration and dosage; Placebos; Drug therapy, Combination; Treatment outcome; Ancylostoma/drug effects; Necator americanus/drug effects; Drug resistance; Randomized controlled trials; Comparative study; United Republic of Tanzania (*source: MeSH, NLM*).

Mots clés Ascaridiase larvaire/chimiothérapie; Trichocéphalose/chimiothérapie; Ascaris lumbricoides/action des produits chimiques; Trichuris/action des produits chimiques; Mébendazole/pharmacologie/administration et posologie; Lévamisole/pharmacologie/administration et posologie; Placebo; Polychimiothérapie; Evaluation résultats traitement; Ankylostome/action des produits chimiques; Necator americanus/action des produits chimiques; Résistance aux médicaments; Essai clinique randomisé; Etude comparative; République-Unie de Tanzanie (*source: MeSH, INSERM*).

Palabras clave Ascariasis/quimioterapia; Tricuriasis/quimioterapia; Ascaris lumbricoides/efectos de drogas; Trichuris/efectos de drogas; Mebendazol/farmacología/administración y dosificación; Levamisol/farmacología/administración y dosificación; Placebos; Quimioterapia combinada; Resultado del tratamiento; Ancylostoma/efectos de drogas; Necator americanus/efectos de drogas; Resistencia a las drogas; Ensayos controlados aleatorios; Estudio comparativo; República Unida de Tanzanía (*fuente: DeCS, BIREME*).

الكلمات المفتاحية : العدوى بالأسكاريس، المعالجة الدوائية للعدوى بالأسكاريس، العدوى بالديدان المُسَلَّكة، المعالجة الدوائية للعدوى بالديدان المُسَلَّكة، ديدان الأسكاريس (الصَّفَر) الخراطينية، التأثيرات الدوائية على ديدان الأسكاريس (الصَّفَر) الخراطينية، الديدان المُسَلَّكة، التأثيرات الدوائية على الديدان المُسَلَّكة، الميبندازول، فارماكولوجيا الميبندازول، طريقة إعطاء الميبندازول والجرعات، الليفاميزول، طريقة إعطاء الليفاميزول والجرعات، الأدوية الغُفْل، المعالجة الدوائية المشاركة، الديدان الشصية (الأنكليستوما)، التأثيرات الدوائية على الديدان الشصية (الأنكليستوما)، الديدان الفتَّاكة الأمريكية، التأثيرات الدوائية على الديدان الفتَّاكة الأمريكية، المقاومة للأدوية، الدراسات المُعَشَّاة المضبوطة بالشواهد، دراسة مقارنة، جمهورية تنزانيا المتحدة (المصدر: رؤوس الموضوعات الطبية، المكتب الإقليمي لشرق المتوسط) .

Introduction

Public health programmes to control morbidity associated with soil-transmitted helminth infections depend mainly on the delivery of anthelminthic drugs to primary-school children (*1*).

In theory, four single-dose drugs are available (albendazole, levamisole, mebendazole, and pyrantel); in practice, however, most control programmes only use the benzimidazoles (albendazole and mebendazole) because they are given as a single-dose tablet and children do not need to be weighed (*2*).

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Aside from reducing the load of worms, benzimidazole treatment also improves the nutritional status and cognitive development of children infected with *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms and reduces hookworm-associated anaemia in children and in women of childbearing age (*3–9*). A number of studies have shown the short- and long-term benefits of periodic treatment with a single dose of 500 mg mebendazole in endemic areas (*8, 10, 11*).

Levamisole also is used in helminth control programmes with good results, although it is less effective than mebendazole against *T. trichiura* and *Necator americanus* infections. Comparative trials of both drugs, alone or in combination, are few, however, and none have compared single-dose administration of 500 mg mebendazole and 40 or 80 mg levamisole (*12–16*).

At present, concern exists about the possible emergence of drug resistance to the anthelminthic compounds used to control intestinal nematodes in humans, particularly given the well-documented and widespread problem of anthelminthic resistance in livestock as a consequence of frequent periodic mass treatments (*17*). The efficacy of combined treatments that use anthelminthics with differing modes of action (e.g. mebendazole plus levamisole) need to be assessed, both to explore possible additive or synergistic effects and to identify a combination that could delay the occurrence of anthelminthic drug resistance to each class of drug (*18*).

Comparison of the efficacy of mebendazole in the treatment of *A. lumbricoides*, *T. trichiura*, and hookworm infection in an area where the school-age population has been exposed to the drug for the last five years with data collected before the beginning of the control programme and with other efficacy data collected elsewhere can give valuable information on possible changes in drug susceptibility of worms.

The primary purpose of this trial was to evaluate the efficacy of single-dose mebendazole in an area where this drug has been used widely for periodic chemotherapy targeted at schoolchildren. A secondary objective was to assess and compare the efficacy of mebendazole with that of levamisole given as one or two 40-mg tablets and the efficacy of the two drugs given in combination.

Materials and methods
Study area and study population
The study was carried out on Pemba Island, the smaller of the two islands of Zanzibar, United Republic of Tanzania. Important features of the island have been described in detail elsewhere (*19, 20*). Intestinal helminth infections affect most of the population and cause a heavy burden of disease in children and in women of childbearing age (*21*). The National Helminth Control Programme was initiated in Zanzibar in June 1994, and since then, mebendazole 500 mg has been given to schoolchildren as a single dose every 4–6 months (*21*).

The study was conducted in August 1999 among children enrolled in the first grade (Standard 1) and fifth grade (Standard 5) of 10 public schools on Pemba Island. The schools were chosen randomly from the 72 schools on the island. Children at Standard 1 had not yet been treated with mebendazole in school, whereas children at Standard 5 had been exposed to 15 rounds of mebendazole treatment.

Children were excluded from the trial if they did not have parental or guardian permission to participate, did not provide a stool sample, had significant comorbidities (e.g. severe diarrhoea, severe anaemia, or high fever), or had recently transferred to the school from an area outside Zanzibar.

Study design
The study was a randomized, placebo-controlled trial. Before children were enrolled in the study, parents or guardians of children in the selected schools were given a comprehensive explanation of the risk and benefits of the trial, and verbal consent was sought.

Children enrolled in the study were assigned randomly to one of four treatment groups to receive 500 mg mebendazole (Janssen, Belgium), 40 or 80 mg levamisole (Zeneca, UK), 500 mg mebendazole plus 40 or 80 mg levamisole, or placebo. Placebo pills resembled mebendazole in colour, size, taste, and shape.

On the day before the scheduled treatment date, children eligible to participate in the trial were given a container in which to bring a fresh stool sample the next day. On the day of treatment, the stools were collected and the children's weights were recorded. Randomization was blocked on weight, and a computer-generated programme was used to create two randomized treatment lists: one for children who weighed 15–20 kg, who were to receive one tablet of 40 mg levamisole, and another for children who weighed 21–60 kg, who were to receive two tablets (80 mg). One tablet of mebendazole 500 mg and one of placebo was given irrespective of body weight. Treatments given were placed in sealed, opaque envelopes and were coded with a number. Children were identified by these numbers only throughout the study.

Twenty-one days after treatment, all children were revisited to collect a further stool sample. Any child who failed to bring a stool sample was followed for up to 24 days. Parents and children were instructed to report to the teacher and refer to the nearest health centre with any severe adverse effects that occurred in the week after treatment. After completion of the study, children in the placebo group and children positive after the follow-up survey were treated with mebendazole 500 mg.

The study was approved by the Zanzibar Health Research Council, and by the ethical committees of WHO and the London School of Hygiene and Tropical Medicine.

Parasitology
After both surveys, egg counts in stool samples were assessed within six hours of the sample being produced at the Public Health Laboratory Ivo de Carneri. All laboratory investigations were blinded, so the technicians who examined the slides were unaware of the treatment the patients received. Stools were analysed using the Kato–Katz technique according to WHO guidelines (*22*). The slides were examined within one hour of preparation to avoid overclarification of hookworm eggs. Comparison of egg counts before and after treatment allowed calculation of the cure rate and the egg reduction rate. A random sample of 10% of the smears prepared for the Kato–Katz technique was read by two different technicians to evaluate the accuracy of the diagnosis and the precision of the egg counts. Slides were re-examined if the quality control showed a >10% difference in egg counts.

To describe the egg count distribution in the study population before and after treatment, intensity of infection was classified as "light", "moderate", or "heavy" on the basis of faecal egg counts. As no heavy infections with *A. lumbricoides* were present at baseline when analysed according to WHO cut-off points, categories for *A. lumbricoides* intensity of moderate and heavy infection were revised to include a meaningful number of children for comparison (Table 1). Categories for intensity of *T. trichiura* and hookworm infection are according to WHO guidelines (Table 1) (*23*).

Table 1. **Egg counts (eggs per gram) used to describe intensity of infection**

Causative pathogen	Intensity of infection (egg count per gram)		
	Light	Moderate	Heavy
A. lumbricoides	1–4999	5000–9999	⩾ 10 000
T. trichiura	1–999	1000–9999	⩾ 10 000
Hookworm	1–1999	2000–3999	⩾ 4000

Statistical analysis

Data were entered and analysed with EpiInfo software. Cure rates were calculated as the percentage of children with egg counts >0 before treatment who had negative egg counts after treatment. The percentage reduction in prevalence was calculated as $[(N^+/n) - (N_{21}^+/n)]/(N^+/n)$, where N^+ = the number of positive children at baseline, N_{21}^+ = the number of positive children 21 days after treatment, and n = the total number of children with samples from both day 0 and day 21. Both cure rates and percentage reductions in prevalence were calculated. As the sensitivity of the Kato–Katz method could be influenced by the intensity of infection, however, reductions in prevalence were comparable only when pre-treatment intensities of infection were similar.

The percentage reduction in eggs induced by treatment was estimated as $100[1 - \exp(-D)]\%$, where $D = \Sigma (\log_e(E_{21}) - \log_e(E_0))/n$, E_{21} = the egg count at 21 days (for each individual), E_0 = the count before treatment, and n = the number of children. Confidence intervals were based on the inter-individual variation in the differences in the logarithms of the egg counts. Proportions were compared with standard χ^2 tests. Geometric mean egg counts were estimated as $\exp[\Sigma(\log_e(c+1))/n] - 1$, where c = the count (eggs per gram) for a particular individual and n = the total number of samples. Geometric means were compared with analyses of variance by ANOVA if Bartlett's test of heterogenicity indicated homogeneity of variances and by the Kruskal–Wallis test if Bartlett's test was significant at the 5% level.

Results

Analysis of baseline data

Of 1137 children examined at the baseline, 904 (79.5%) returned stool samples at follow-up. Loss at follow-up was high compared with that in other surveys in Pembian schoolchildren because of an unexpected closure of schools on Pemba Island one week before the scheduled 21-day follow-up. The schools were closed for three weeks to allow children to help harvest an unusually large crop of cloves. The research staff thus walked from hut to hut through each village to trace missing children. Subsequent analysis showed that older, male children were more likely to be lost to follow-up, presumably because of their increased involvement in clove picking (12.2 vs 11.6 years, P<0.001; 54.5% boys vs 47.0% girls, P<0.05) (Table 2). Children subsequently lost at follow-up had similar baseline prevalence and intensity of *A. lumbricoides, T. trichiura,* and hookworm infections to children who were followed up.

Results are presented on the cohort of 904 children who returned a stool sample for the baseline and post-treatment surveys (Table 3). Their mean (SD; range) age was 11.5 (2.4; 7–18) years, and 45% were boys. The four groups were homogeneous at baseline for age and helminth infections, but differed in the proportion of males to females (Table 2). The prevalence of any helminth infection was 99.7%, with 38.3% and 54.9% of children harbouring double and triple infections, respectively. Table 3 gives the prevalence and intensity of *A. lumbricoides, T. trichiura,* and hookworms at baseline and 21 days after treatment in the four treatment groups.

Overall drug efficacy

Follow-up egg counts, cure rates, reductions in prevalence and egg reduction rates for the three nematode infections were statistically significantly better with all of the drug regimens compared with those at baseline, except for cure rates for hookworm infections with mebendazole and for *T. trichiura* infections with levamisole (although in both cases the mean egg counts were reduced substantially). Compared with placebo, all drug treatments produced significantly higher cure rates and egg reduction rates and lower prevalences at follow-up, except for the egg reduction rate for levamisole in *T. trichiura* infections (Table 3).

Both drugs had very high efficacy (98.5% and 99.1% egg reduction rates for levamisole and mebendazole, respectively) against *A. lumbricoides*. Mebendazole alone and in combination with levamisole had better efficacy than levamisole alone for *T. trichiura* infection (81% and 85% vs 41.5% egg reduction rates, P<0.001). Levamisole treatment produced a marginally significant reduction in prevalence of hookworm infection, which was greater than the reduction seen with mebendazole (8.9% vs 3.6%, P<0.05); the combination had better efficacy in reducing prevalence than either drug alone (23.6%, P<0.001). The egg reduction rate for hookworm infection was 88.7% for the combined treatment, but significantly less for either drug alone (61.3% for levamisole and 52.1% for mebendazole, P<0.001).

Figure 1 shows the distribution of intensities of infections, expressed as eggs per gram faeces, before and after treatment for *A. lumbricoides, T. trichiura,* and hookworm infections. Higher efficacy of treatment is indicated by an increase in negative and light infections and a decrease in moderate and heavy infections 21 days after treatment. Stratified analysis by intensity was performed on the cure rates and egg reduction rates for each species of helminth. For any treatment, lower cure rates were achieved in children with heavy infections compared with light infections: *A. lumbricoides* 93.3% vs 97.0% (P=0.07), *T. trichiura* 8.3% vs 19.3% (P=0.1), and hookworm 8.4% vs 16.3% (P<0.05). On the other hand, higher egg reduction rates were achieved in children with heavy compared with light infections for *T. trichiura* (93.8% vs 81.4%, P<0.05) and hookworm (91.3% vs 73.2%, P<0.01). Efficacy of treatment was not influenced by whether *A. lumbricoides, T. trichiura,* or hookworm infections presented as single or multiple infections. Although adverse effects were not investigated actively, no adverse events were reported after any single or combined treatment in the week following the administration of anthelminthics.

Drug efficacy in Standard 1 and Standard 5 children

Overall, 446 children were in Standard 1 classes and had a mean (SD) age of 9.5 (1.5) years, and 458 children were in Standard

Table 2. **Characteristics of the study sample at baseline**

Characteristic	Treatment							
	Mebendazole		Levamisole		Mebendazole + levamisole		Placebo	
	Total (n = 285)	Lost at follow-up (n = 49)	Total (n = 277)	Lost at follow-up (n = 67)	Total (n = 286)	Lost at follow-up (n = 70)	Total (n = 289)	Lost at follow-up (n = 47)
Mean age (years)	11.5 (2.3)[a]	12.3 (2.1)	11.7 (2.5)	12.1(2.3)	11.7 (2.4)	12.2 (2.5)	11.6 (2.5)	12.1 (2.3)
Sex (% boys)	48.4	53.1	54.2	56.7	43.0	55.7	42.6	51.1
Ascaris Prevalence (%)	61.4	69.4	60.3	62.7	62.9	65.7	58.1	63.8
Geometric mean (epg)[b]	134	292	99	117	129	149	100	122
Trichuris Prevalence (%)	90.9	91.8	94.6	97.0	93.7	95.7	93.8	93.6
Geometric mean (epg)	304	315	365	384	371	421	458	346
Hookworm Prevalence (%)	95.1	95.9	96.0	95.5	95.1	98.6	95.2	89.4
Geometric mean (epg)	429	352	454	392	401	504	475	316

[a] Values in parentheses are standard deviations.
[b] epg = eggs per gram.

Table 3. **Results before and 21 days after treatment with mebendazole, levamisole, mebendazole + levamisole, and placebo**

Causative pathogen	Drug	Prevalence[a]			Egg count (epg)[b]		Cure rate	Reduction in prevalence	*P*-value[c]	Egg reduction rate	*P*-value[c]
		n	Day 0	Day 21	Day 0	Day 21					
Ascaris	Mebendazole	236	59.7	3.0	114	0.2	96.5	95.0	<0.001	99.0 (98.2–99.4)[d]	<0.001
	Levamisole	210	59.5	5.7	94	0.4	91.2	90.4[e]	<0.001	98.5 (97.4–99.1)	<0.001
	Mebendazole + levamisole	216	62.0	1.4	131	0.1	98.5	97.7	<0.001	99.1 (98.4–99.5)	<0.001
	Placebo	242	57.0	51.2	96	63	22.5	10.2	NS[f]	33.9 (0.4–56.1)	NS
	Total	904	59.5		106						
Trichuris	Mebendazole	236	90.7	75.0	302	57	22.9	17.3[g]	<0.001	81.0 (71.9–87.1)[g]	<0.001
	Levamisole	210	93.8	90.0	359	210	9.6	4.1[h]	NS	41.5 (17.8–58.4)[h]	<0.01
	Mebendazole + levamisole	216	93.1	74.5	356	53	22.9	20.0	<0.001	85.0 (78.5–89.5)	<0.001
	Placebo	242	93.8	94.2	484	395	4.8	−0.4	NS	18.3 (−7.0–37.6)	NS
	Total	904	92.8		371						
Hookworms	Mebendazole	236	94.9	91.5	447	213	7.6	3.6[h,i]	NS	52.1 (36.0–64.2)[h]	<0.001
	Levamisole	210	96.2	87.6	476	184	11.9	8.9[h]	<0.001	61.3 (47.2–71.6)[h]	<0.001
	Mebendazole + levamisole	216	94.0	71.8	373	41	26.1	23.6	<0.001	88.7 (83.9–92.0)	<0.001
	Placebo	242	96.3	95.9	514	432	3.4	0.4	NS	16.0 (−8.0–34.6)	NS
	Total	904	95.4		451						

[a] Percentage positive for pathogen.
[b] Mean egg count expressed as geometric mean.
[c] *P*-value from day 21 to day 0.
[d] Values in parentheses are 95% confidence intervals.
[e] Compared with mebendazole plus levamisole *P*<0.05.
[f] NS, not significant.
[g] Compared with levamisole *P*<0.001.
[h] Compared with mebendazole plus levamisole *P*<0.001.
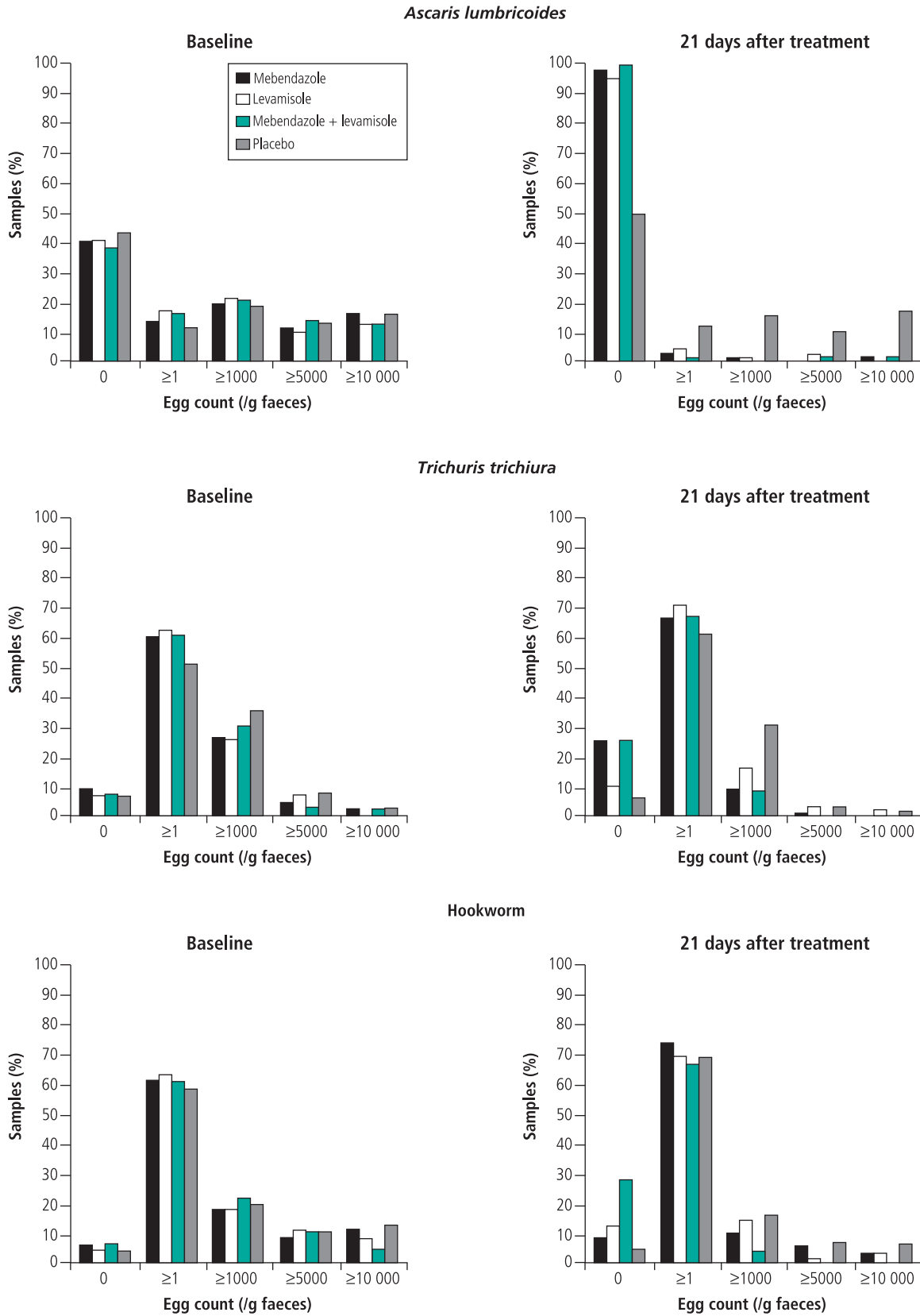[i] Compared with levamisole *P*<0.05.

5 classes and had a mean (SD) age of 13.4 (1.3) years. Boys accounted for 49.8% and 40.4% of children from Standard 1 and Standard 5 classes, respectively. Tables 4 and 5 give the prevalences and intensities of *A. lumbricoides*, *T. trichiura*, and hookworm infections at baseline and 21 days after treatment in the four treatment groups in Standard 1 and Standard 5 children, respectively. The baseline mean intensities of infection for all three species of helminths were significantly higher in the Standard 1 children than in the Standard 5 children (*P*<0.001). In addition, Standard 1 children at baseline

had higher prevalences of moderate and heavy infection for each helminth than Standard 5 children — (*A. lumbricoides*: moderate 14.1% vs 9.2%, heavy 18.8% vs 9.0%; *T. trichiura*: moderate 45.3% vs 22.3%, heavy 2.5% vs 0.2%; and hookworm: moderate 12.1% vs 6.3%, heavy 11.4% vs 5.0%).

In both Standard 1 and Standard 5 children, but particularly in Standard 1 children, mebendazole and its combination with levamisole were more effective than levamisole in *T. trichiura* infection (the efficacy of levamisole did not differ significantly from that of placebo). In Standard 1

Fig. 1. **Distribution of** *Ascaris lumbricoides*, *Trichuris trichiura* **and hookworm egg counts before and after treatment with mebendazole, levamisole, mebendazole + levamisole, and placebo**

Ascaris lumbricoides

Baseline   21 days after treatment

Trichuris trichiura

Baseline   21 days after treatment

Hookworm

Baseline   21 days after treatment

WHO 03.26

Table 4. **Results before and 21 days after treatment with mebendazole, levamisole, mebendazole + levamisole, and placebo in Standard 1 children**

Causative pathogen	Drug	Prevalence[a]			Egg count (epg)[b]		Cure rate	Reduction in prevalence	P-value[c]	Egg reduction rate	P-value[c]
		n	Day 0	Day 21	Day 0	Day 21					
Ascaris	Mebendazole	120	67.5	5.8	264	0.4	93.8	91.4[d]	<0.001	99.5 (98.8–99.7)[e]	<0.001
	Levamisole	98	70.4	4.1	254	0.4	94.2	94.2[f]	<0.001	99.5 (98.8–99.8)	<0.001
	Mebendazole + levamisole	108	67.6	0.1	248	0.1	100.0	99.9	<0.001	99.6 (99.1–99.8)	<0.001
	Placebo	120	65.0	61.7	192	152	17.9	5.1	NS[g]	20.9 (–44.1–56.6)	NS
	Total	446	67.5		237						
Trichuris	Mebendazole	120	95.0	76.7	534	69	22.8	19.3[h]	<0.001	86.9 (78.0–92.2)[h]	<0.001
	Levamisole	98	95.9	95.9	643	416	4.2	0[d]	NS	35.2 (–3.7–59.5)[d]	NS
	Mebendazole + levamisole	108	97.0	82.4	776	94	17.1	15.1	<0.001	87.9 (80.6–92.4)	<0.001
	Placebo	120	95.0	95.8	726	590	4.4	–0.8	NS	18.8 (–18.8–44.5)	NS
	Total	446	95.7		661						
Hookworms	Mebendazole	120	94.1	95.0	483	304	4.4	–1.0[i]	NS	37.0 (6.0–57.8)[d,i]	0.06
	Levamisole	98	99.0	90.8	827	278	9.2	8.3[d]	<0.01	66.3 (48.7–7.8)[d]	<0.001
	Mebendazole + levamisole	108	96.3	71.3	604	42	26.0	26.0	<0.001	92.9 (88.7–95.6)	<0.001
	Placebo	120	96.7	98.3	674	619	0.9	–1.7	NS	8.1 (–29.0–34.6)	NS
	Total	446	96.4		628.0						

[a] Percentage positive for pathogen.
[b] Mean egg count expressed as geometric mean.
[c] P-value from day 21 to day 0.
[d] Compared with mebendazole plus levamisole P<0.01.
[e] Values in parentheses are 95% confidence intervals.
[f] Compared with mebendazole plus levamisole P<0.05.
[g] NS, not significant.
[h] Compared with levamisole P<0.001.
[i] Compared with levamisole P<0.05.

children, the effect of mebendazole against hookworm infection was similar to that of placebo, although its reduction of intensity of infection approached statistical significance (egg reduction rate 37%, P = 0.06), while levamisole was more effective (egg reduction rate 66.3%, P<0.001) and the combination was the best treatment (egg reduction rate 92.9%, P<0.001). In Standard 5 children, the combination was still the better option (although to a lesser extent than in Standard 1 children) than either drug alone for the treatment of hookworm infection (egg reduction rate 81.9% vs 56.3% for levamisole and 60.4% for mebendazole, P<0.05). Overall, none of cure rate, percentage reduction in prevalence, or egg reduction rate differed significantly between Standard 1 and Standard 5 children for any helminth infection. Furthermore, no evidence was seen of a reduced cure or egg reduction rate for mebendazole relative to levamisole in Standard 5 children compared with Standard 1 children.

Discussion

This study confirms the extremely high prevalence of intestinal nematode infections in Zanzibar despite the periodic chemotherapy control programme that started in 1994. The objective of this programme, however, was reduction in intensity rather than prevalence of infection. In this respect, the significantly lower egg counts at baseline in Standard 5 children who had received 15 rounds of treatment with mebendazole compared with children in Standard 1 classes, who had never been treated, are encouraging. A possible explanation for this finding could be an intrinsic decline in intensity with increasing age. This is not supported by a previous study on untreated children in the same population, however, which showed that

between the ages of 9 (Standard 1) and 13 years (Standard 5), the mean intensity of ascariasis in fact increased, the intensity of hookworm infections remained stable, and the intensity of trichuriasis slightly decreased, although to a much lesser extent than between Standard 1 and Standard 5 children in the present study (21). The benefit of periodic chemotherapy is shown further in the significant reduction of moderate and heavy helminth infections — those most associated with morbidity — in Standard 5 children. From the public health perspective, it seems, therefore, that the helminth control programme is effective in reducing intensity, although the low efficacy of treatment of hookworm infections found in this trial is worrying.

High, and similar, levels of efficacy in the treatment of *A. lumbricoides* infection were achieved using mebendazole, levamisole, and their combination. The efficacy of each treatment was equally excellent in light and heavy *A. lumbricoides* infections, with egg reduction rates approaching 100%. This result is consistent with previous efficacy trials and is also similar — for mebendazole — to the results of an efficacy study carried out in Pemba in 1993 before the mebendazole-based control programme was started (14, 24–27).

Drug efficacies in *T. trichiura* infection were also similar to those seen in previous trials, as treatment with mebendazole reduced the prevalence of infection, while levamisole had a much smaller effect (14, 26–29). Reductions in egg counts were significant for mebendazole and for levamisole, although mebendazole and the combined treatment were both significantly better than levamisole alone. When compared with the efficacy trial performed in Pemba before the control programme started, the mebendazole cure and egg reduction

Table 5. **Results before and 21 days after treatment with mebendazole, levamisole, mebendazole + levamisole, and placebo in Standard 5 children**

Causative pathogen	Drug	Prevalence[a]			Egg count (epg)[b]		Cure rate	Reduction in prevalence	P-value[c]	Egg reduction rate	P-value[c]
		n	Day 0	Day 21	Day 0	Day 21					
Ascaris	Mebendazole	116	51.7	0.1	47	0.1	100.0	99.8[d]	<0.001	97.9 (95.8–99.0)	<0.0001
	Levamisole	112	50.9	7.1	39	0.5	87.5	86.1[e]	<0.001	96.1 (92.0–98.1)	<0.0001
	Mebendazole + levamisole	108	56.5	2.8	61	0.2	96.7	95.0	<0.001	98.0 (95.7–99.1)	<0.0001
	Placebo	122	49.2	41.0	48	26	28.3	16.7	NS	44.5 (3.8–68.0)	NS
	Total	458	51.7		48						
Trichuris	Mebendazole	116	86.2	73.3	167	46	23.0	15.0	<0.01	72.1 (51.1–84.1)	<0.001
	Levamisole	112	92.0	84.8	215	115	14.6	7.8[f]	NS	46.5 (12.6–67.2)[e]	<0.05
	Mebendazole + levamisole	108	88.9	66.6	163	30	29.2	25.1	<0.001	81.4 (68.0–89.1)	<0.001
	Placebo	122	92.6	92.6	325	266	5.3	0.0	NS	17.8 (−19.0–43.2)	NS
	Total	458	90.0		211						
Hookworms	Mebendazole	116	95.7	87.9	412	148	10.8	8.2[e]	<0.05	60.4 (45.2–76.4)[e]	<0.001
	Levamisole	112	93.7	84.8	293	128	14.3	9.5[e]	<0.05	56.3 (30.7–72.4)[e]	<0.01
	Mebendazole + levamisole	108	91.7	72.2	230	41	25.3	21.3	<0.001	81.9 (70.1–89.0)	<0.001
	Placebo	122	95.9	93.4	395	304	6.0	2.6	NS	23.0 (−11.5–46.8)	NS
	Total	458	94.3		327						

[a] Percentage positive for pathogen.
[b] Mean egg count expressed as geometric mean.
[c] P-value from day 21 to day 0.
[d] Compared with levamisole P<0.001.
[e] Compared with mebendazole plus levamisole P<0.05.
[f] Compared with mebendazole plus levamisole P<0.001.

rates were not significantly different (*24*). The poor efficacy of levamisole against *T. trichiura* has already been reported, although Ismail et al. showed an egg reduction rate of 73% (*2, 14*). The finding that egg reduction rates were significantly higher in both *T. trichiura* and hookworm "heavy" infections compared with "light" infections is consistent with trials with albendazole (*29, 30*).

Striking features of this study were the low cure (7.6%) and egg reduction (52.1%) rates when mebendazole was used against hookworms. Mebendazole efficacy was lower when compared with that in published studies and, more specifically, compared with that in the trial carried out in Pemba before exposure to periodic treatment, in which mebendazole had a cure rate of 22.4% and an egg reduction rate of 82.4% (*14, 24–27*). Furthermore, in a recent trial comparable with our trial in the neighbouring island of Mafia, in which there is no school-based deworming programme and use of benzimidazoles has been very limited, mebendazole efficacies were similar (cure rate 31.3%; egg reduction rate 78.1%) to the pre-treatment values on Pemba and very much higher than the current efficacy in Pemba (*31*).

A number of potential confounding factors were ruled out by the fact that both trials were carried out in school-children by the same staff and used the same methods and drug (mebendazole 500 mg from Janssen). It is notable that the cure rates and egg reduction rates for *Ascaris* and *Trichuris* were comparable between the two trials. Interestingly, levamisole also showed a lower efficacy against hookworms in the present study than in previous studies (*32–34*). A possible explanation is that levamisole has been reported to be more effective against *A. duodenale* than *N. americanus*, but the latter pathogen is the most prevalent species in Pemba (*20*). If mebendazole also was less effective against *N. americanus*, repeated use of mebendazole in Pemba could have created an increased

Necator:Ancylostoma ratio, which would have resulted in an overall lower efficacy against hookworms. Data from recent studies suggest that the *Necator:Ancylostoma* ratio is indeed higher now than it was before periodic anthelminthic treatment (Albonico, unpublished data, 2000) (*20*).

An alternative explanation for the apparent reduced efficacy of mebendazole compared with historical controls is the selection of drug resistance by the repeated treatment regimen. If a proportion of drug-resistant worms was present in the worm population in Pemba, we would have expected resistant worms to have accumulated in children after multiple rounds of treatment and reinfection and to have represented a higher proportion of worms than that found in Standard 1 children. In this case, a lower drug efficacy would have been expected in the Standard 5 children. In fact, the efficacy of mebendazole in Standard 5 children who had received multiple (15) doses of mebendazole was not lower than in Standard 1 children who had never been treated; this suggests that a mebendazole-resistant population of worms had not accumulated in the repeatedly treated Standard 5 children. Nevertheless, the possibility that drug resistance is emerging in Pemba cannot be excluded. In Mali, failure of mebendazole in the treatment of human hookworm infections has been reported (*35*). In that case, however, the population had not been exposed to periodic treatment with mebendazole, although mebendazole had been available in that community for some years; pre-exposure efficacy data were not available for comparison and the sample of children studied was rather small. These factors suggest a need for caution when interpreting these results. A subsequent drug efficacy study in Mali (*36*) showed reasonably good efficacy of mebendazole and even better results from albendazole, which show a lack of significant resistance in human hookworms to benzimidazoles in that area. Poor efficacy of pyrantel against hookworms has

been found in north-west Australia, but again it is not known if this represents a decline in efficacy because of emerging resistance (*37*).

The efficacy of the combined administration of mebendazole 500 mg and levamisole 40 or 80 mg was evaluated for the first time in this study and showed higher efficacy than either drug alone against hookworm infections. This is a promising result, because the combined administration of two different anthelminthic drugs could be used as the treatment of choice in this context. In addition, the adoption of combination therapy with drugs with distinct modes of action when used at early stages has been shown to delay the onset of anthelminthic drug resistance (*18*).

Although this study clearly shows that the efficacy of mebendazole in the treatment of hookworm infections in Pemba Island schoolchildren is lower than in previous studies, demonstration of anthelminthic resistance is still lacking. A need exists, therefore, for alternative methods by which to address this question, such as the in vitro egg hatch assay — a technique widely used in veterinary medicine (*38*) and recently adapted to human hookworms (*39*). This would require comparisons with strains of hookworms that had not been exposed to treatment. The early detection of drug resistance is of the utmost importance, because the in vivo and in vitro tests currently available in the veterinary field detect resistance when the proportion of worms carrying the drug resistance allele in a population already has reached >25% (*40*). The development of methods for the early detection of benzimidazole resistance in human nematodes would be of great value, therefore, and the polymerase chain reaction methods developed by veterinary scientists need to be evaluated for their applicability in human nematodes (*41*). ■

Résumé

Efficacité du mébendazole et du lévamisole seuls ou en association contre les nématodoses intestinales après traitement périodique ciblé par le mébendazole à Zanzibar

Objectif Evaluer l'efficacité du mébendazole (500 mg) et du lévamisole (40 ou 80 mg) et la résistance à ces composés administrés seuls ou en association pour le traitement des infections à *Ascaris lumbricoides*, *Trichuris trichiura* et les ankylostomes sur l'île de Pemba, une région où sont effectués depuis 1994 des traitements périodiques par le mébendazole en milieu scolaire.

Méthodes Un essai contrôlé randomisé contre placebo a été réalisé sur 914 enfants des premier et cinquième niveaux de l'enseignement primaire. Des échantillons de selles recueillis au début de l'étude et 21 jours après le traitement ont été examinés par la technique de Kato-Katz afin d'évaluer la prévalence et l'intensité des helminthiases.

Résultats L'efficacité du mébendazole et du lévamisole en traitement unique des nématodoses intestinales était comparable à celle observée lors de précédents essais, mais le traitement des ankylostomiases par le mébendazole donnait des taux de guérison (7,6 %) et de réduction du nombre d'œufs (52,1 %) significative-

ment plus faibles que lors d'une étude réalisée avant le début de la chimiothérapie périodique (taux de guérison : 22,4 % ; taux de réduction du nombre d'œufs : 82,4 %). Le traitement associé par le mébendazole et le lévamisole était significativement plus efficace contre les ankylostomiases (taux de guérison : 26,1 % ; taux de réduction du nombre d'œufs : 88,7 %) qu'un traitement par chacun des médicaments administrés séparément. Aucune différence d'efficacité du mébendazole n'a été observée entre les enfants ayant reçu une chimiothérapie périodique et ceux qui n'avaient jamais été traités auparavant.

Conclusion Il semble d'après les résultats que l'efficacité globale du mébendazole contre les ankylostomiases après chimiothérapie périodique soit diminuée. L'efficacité des benzimidazolés dans le cadre des programmes de lutte reposant sur la chimiothérapie doit être étroitement surveillée. Un traitement associant le mébendazole et le lévamisole peut être utile pour retarder le développement de la résistance à cette catégorie de médicaments.

Resumen

Eficacia del mebendazol y el levamisol, solos o combinados, contra las infecciones intestinales por nematodos después del tratamiento repetido focalizado con mebendazol en Zanzíbar

Objetivo Evaluar la eficacia del mebendazol (500 mg) y el levamisol (40 u 80 mg), solos o combinados, y la resistencia a ellos como tratamiento de las infecciones por *Ascaris lumbricoides* y *Trichuris trichiura* y de la anquilostomiasis en la Isla de Pemba, una zona en la que desde 1994 se llevan a cabo periódicamente tratamientos con mebendazol en las escuelas.

Métodos Se llevó a cabo un ensayo aleatorizado y controlado mediante placebo en 914 niños inscritos en alguno de los cinco

primeros años de escuela primaria. Las muestras de heces obtenidas en la situación de partida y a los 21 días de tratamiento fueron analizadas mediante la técnica de Kato-Katz a fin de evaluar la prevalencia y la intensidad de las infecciones por helmintos.

Resultados La eficacia del mebendazol y el levamisol como tratamientos únicos de las infecciones intestinales por nematodos fue comparable a la observada en ensayos anteriores, pero la administración de mebendazol contra las

infecciones por anquilostoma logró unas tasas de curación (7,6%) y de reducción del número de huevos (52,1%) significativamente inferiores a las notificadas en un estudio emprendido antes del comienzo de la antibioticoterapia periódica (tasa de curación, 22,4%; tasa de reducción del número de huevos, 82,4%). El tratamiento combinado con mebendazol y levamisol tuvo una eficacia significativamente mayor contra las infecciones por anquilostoma (tasa de curación, 26,1%; tasa de reducción del número de huevos, 88,7%) que cualquiera de los medicamentos por separado. No se observó ninguna diferencia en cuanto a la eficacia del mebendazol entre los niños tratados repetidamente y los que no habían sido tratados anteriormente.

Conclusión La eficacia global del mebendazol contra la anquilostomiasis se ve reducida después de la antibioticoterapia periódica. Debería vigilarse estrechamente la eficacia de los benzimidazoles en los programas de control basados en la antibioticoterapia. El tratamiento combinado con mebendazol y levamisol podría ser una valiosa arma para retrasar la aparición de resistencia a los benzimidazoles.

ملخص

فعالية الميبيندازول والليفاميزول إذا استخدما كلاً على حدة أو استخدما معاً ضد العدوى بالممسودات المعوية
بعد معالجة متكررة بالميبيندازول في زنجبار

الغرض: تقييم فعالية المعالجة بالميبيندازول (٥٠٠ ميلي غرام) والليفاميزول إذا استخدما كلاً على حدة أو استخدما معاً لمعالجة العدوى بالإسكاريس (الصفر) الخراطيني والمسلكة الشعرية الذيل والدودة الشخصية (الإنكلستوما) في جزيرة بيمبا، وهي منطقة يتم فيها تطبيق المعالجة بالميبيندازول المُرتَكزة على المدارس منذ عام ١٩٩٤.

الطريقة: تم القيام بدراسة معشاة مضبوطة بالأدوية الغفل ضمت ٩١٤ طفلاً من الصفوف الأول حتى الخامس من المرحلة الابتدائية، جمعت عينات البراز في بدء الدراسة ثم بعد مرور ٢١ يوماً من المعالجة وفحصت بطريقة كاتو–كاتز لتقييم مدى انتشار وشدة العدوى بالديدان.

الموجودات: إن فعالية الميبيندازول والليفاميزول عند إعطائهما كجرعة وحيدة لعدوى الممسودات المعوية كانت لا تقل عن فعاليتهما في دراسات أجريت سابقاً، إلا أن معالجة الديدان الشخصية (الإنكلستوما) بالميبيندازول أدت إلى نسبة شفاء (٧,٦٪) ومعدل تخفيض للبيوض للبيوض ٥٢,١٪ وذلك أقل

مما سبق أن أبلغ عنه في دراسة أجريت قبل البدء بالمعالجة الكيميائية الدورية (حيث كانت نسبة الشفاء ٢٢,٤٪ ومعدل تخفيض البيوض ٨٢,٤٪) أما المعالجة المشتركة بالميبيندازول والليفاميزول معاً فقد كان لها فعالية أعلى بكثير على العدوى بالديدان الشخصية (نسبة الشفاء ٢٦,١٪ ومعدل خفض البيوض ٨٨,٧٪) مما كان لاستعمال أحد الدوائين لوحده. لم يلاحظ أي فرق لفعالية الميبيندازول بين الأطفال الذين عولجوا مرات متكررة بالمقارنة بالأطفال الذين لم يعالجوا من قبل.

الاستنتاج: إن الفعالية الإجمالية للميبيندازول ضد العدوى بالديدان الشخصية (الإنكلستوما) قد انخفضت بعد المعالجة الكيميائية الدوائية. ولابد من رصد دقيق لفعالية لبرامج المكافحة المُرتَكزة على المعالجة الكيميائية بمركبات البنزيميدازول. وقد يكون للمعالجة المشتركة بكل من الميبيندازول والليفاميزول فائدة كوسيلة لتأخير ظهور المقاومة للمعالجة بالبنزيميدازول.

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