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Efficacy of Single Dose of 500 mg Mebendazole on Geohelminths amongst School Age Children in Bafoussam, Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VKP, LM, MN, CY, GRN, MM and BBCF designed the study, wrote the protocol and wrote the first draft of the manuscript. Author LM managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Background: The aim of this study was to evaluate the efficacy of mebendazole in the treatment of *Ascaris lumbricoides, Trichuris trichiura* and hookworm infections amongst school age children in Bafoussam.

Methodology: In this study, 948 school age children were recruited. Stool samples were collected from all participants before treatment. Twenty-one days later, specimen bottles were redistributed only to positive cases who received treatment. Parasitological analyses were performed using the concentration method of Willis and McMaster techniques. 500 mg mebendazole was administered

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to those who were positive for one of the above parasites. The prevalence, parasite load, cure rates and egg reduction rates were assessed.
Results: Baseline prevalence of infections and mean parasite loads were 1.69% and 6226.9 EPG (*Ascaris lumbricoides*), 0.74% and 50EPG (*Trichuris trichiura*), and 2.85% and 64.81 EPG (Hookworm) respectively. Post treatment control showed a decrease of prevalence and mean parasite load to 0.0% and 0 EPG (*A. lumbricoides*), 0.63% and 42.86 EPG (*T. trichiura*), and 0.84% and 14.81 EPG (Hookworm) respectively. Results of the study revealed that a single dose of 500 mg mebendazole resulted in cure rates of 100%, 70.37% and 14.29% and egg reduction rates of 100%, 68.57%, and 63.16%, for ascariasis, hookworm infection and trichuriasis respectively. Mebendazole is safe and no side effect was observed.
Conclusion: The results of this study suggested that mebendazole is mostly effective against *Ascaris* and hookworm infection but not for *Trichuris trichiura*.

Keywords: Mebendazole; Ascaris lumbricoides hookworm infection; Trichuris trichiura.

1. BACKGROUND

Globally, 2 billion people are infected with soil-transmitted helminths (STH). The World Health Organization (WHO) estimates 400 million school age children (SAC) to be chronically infected with geohelminthiasis [1]. These children are easily infected and re-infected because of their habits, personal and community hygiene. Severe infections may lead to bowel perforation and severe malnutrition as worms can deplete up to 60% of the nutritional supply of children [2].

Many studies carried out in developing countries in general and Cameroon in particular reported that, the most encountered species are *Ascaris lumbricoides*, hookworm (especially *Necator americanus*) and *Trichuris trichiura* [3,4,5,6]. There is considerable variation in the occurrence of infection [7]. There may also be considerable variation in prevalence of infection by regions, within countries, and even within different regions of the same country, largely depending on local environmental conditions. For example, the distribution of infection varies with altitude, probably as a result of temperature, humidity and ultra violet light on the survival of worm eggs and larva. In addition, worm load tend to accumulate slowly because each worm within a human host is the result of exposure to a single worm egg or larva. Since worms can live 1-3 years depending on the species and there seems to be no fully protective immunity, infection is thus a continuous process and disease can develop slowly [8].

Nevertheless, improvement in sanitation, water supply, housing conditions together with improvement of economic status in endemic areas have proven to be an effective strategy in controlling STH infections [9]. However, due to constraint at the national and individual levels, control infection using the above mentioned control methods have become unrealistic and it takes a very long time [10]. Nowadays, the availability of single dose broad spectrum and cost effective anthelminthics has helped in reducing infection burden in endemic communities. Studies have shown that periodic drug administration strategies has successfully lowered the intensity of infection of *Ascaris* [11,12,13] but their efficacy against *Trichuris trchiura* and Hookworm has been more variable [14]. The aim of this study was to evaluate the efficacy of single dose 500 mg mebendazole in reducing the prevalence (measure by cure rate) and intensity of infection (measure by reduction in egg count) in Bafoussam Western Region of Cameroon.

2. METHODOLOGY

2.1 Study Site

The area of study for this project was Bafoussam West Region of Cameroon, which has a land area of 13.000 km² and a population of 500,117. Bafoussam is about 300 km North-West of Yaoundé and 275km Northeast of Douala. It is situated on an altitude of 1450m above the sea between 5°23''-5°50'' latitude North and between 10°25''-10°-50'' longitude East. Temperature varies between 18°C and 23°C with a maximum of 21.7°C in March and a minimum of 9.5°C in July. The dry season runs from November-May and a rainy season from April-October [12]. The climate is gradually moving from subequatorial to a pseudo tropical conferring a high rainfall and a conducive environment for geohelminths most especially for hookworm infections [3,13].

2.2 Ethical Clearance

Before commencement of the study, the Cameroon Bioethics Initiative approved the overall protocol (Reference number: CBI/204/ERCC/CAMBIN). Written informed consent was obtained from the relevant authorities in this locality, and parents; while the pupils provided verbal consent. Laboratory samples were only taken from those children whose parents/guardians signed the consent forms.

2.3 Study Population

Nine schools were selected (using the simple randomized method) in Bafoussam to include areas that were either near or far from the town center for baseline and follow-up parasitological assessments. These were Nursery schools which included nursery I and nursery II children (St Damien, Diamdam II and Cispam), Primary schools including class I to class 6 pupils (Le prosery, St Mathias and Colebri) and Secondary schools from forms I to form 5 students (Socrate, Lyce Classique de Baffoussam and St Augustin). In this study, we included all schools who participated in the last de-worming program. In each classroom, a simple randomized selection without replacement was done by the lottery method for proper selection [14].

2.4 Study Design

Stool samples were collected within the period of six months from school age children and analyzed for the presence of STHs infections. Cure rate (prevalence) and egg reduction rate (intensity) were calculated to assess the efficacy of mebendazole on geohelminths. The intensity of worm infections was classified using the WHO [15] classification for egg count per gram (epg) which recommends the following ranges of egg counts corresponding to low, moderate and high intensities of infection respectively: *A. lumbricoides* (1–4999 EPG, 5000–49,999 EPG and 50,000 EPG), hookworms (1–1999 EPG, 2000–3999 EPG and 4000 EPG) and *T. trichiura* (1–999 EPG, 1000–9999 EPG and 10,000 EPG).

Before children enrolled in the study, parents or guardians were given a comprehensive explanation of the risk and benefits of the study and they agreed to sign the written consent form. Parasitological analyses were carried out immediately after the first collection of stool samples to determine those infected with geohelminths. To the later, one tablet of Mebendazole (500 mg) was administered. Twenty-one days after treatment, stool samples were recollected and examined to assess the efficacy of the treatment.

All those who participated brought two specimen bottles as recommended by the investigator. Parents were instructed to notify on the questionnaire given to them the different adverse effects that occur within the week after treatment. The analyses of the specimens were carried out in the Laboratories of Biology and Applied Ecology (LABEA) of the University of Dschang and Centre Medical d'Arrondissement (CMA) Lafe Baleng of Bafoussam.

2.5 Sample Collection

Nine hundred and forty eight stool samples were collected from the children before treatment and there was no drop out. Specimen containers were collected in a sequential manner, the respondents re-submitted their sample bottles the following morning with stool which was preserved immediately in 10% aqueous formaldehyde solution (10 g of stool for 3 ml) and the identification serial number placed on them [16,17]. These samples were then transported in a leak proof dark bag to the laboratory for analysis. Information such as sex, age and educational levels were annotated on each specimen bottles. During our first sample collection, the respondents were informed of the period of our next visit whereby, only those with positive results from laboratory analysis will be treated.

2.6 Macroscopic Examination of Stool Samples

The appearance of stool samples may be indicative of a particular disease. For example, semi- formed, black stool may indicate hookworm infection while unformed stool with blood and mucus may indicate schistosomal infection [18].

2.7 Parasitological Analysis

Flotation or Willis concentration technique as described by Euzeby, [19] was employed in this study. It consist of a qualitative test for the detection of nematode and cestode eggs whereby, eggs are separated from faecal material and concentrated by a flotation fluid of appropriate specific gravity of 1.20. This concentration technique increases sensitivity of stool microscopy to allow the detection of a small number of organisms. It is good for the concentration and identification of eggs of parasites especially if intensity of infection is low [20].

2.8 Identification of Helminths Eggs

The eggs or larvae of geohelminths were identified base on their morphology such as diameter or size, form, nature of the cover or membrane and colour. *A. lumbricoides* eggs were recognized based on their round, ova or elliptical shape with rough membrane. Fertilized eggs measured 45-75 µm by 35-50 µm while unfertilized eggs were a bit elongated and also had rough membrane137 measuring 75-85 µm by 35-50 µm. *Trichuris trichiura* were recognized based on their barrel-shaped eggs measuring 50 to 54 µm long and 22 to 23 µm wide with clear mucoid-appearing bipolar plugs. The hookworm eggs (55-75 µm) were oval or elliptical with the larvae coiled within, thus showing a clear zone between the embryo and the eggs shell [18,21].

2.9 Enumeration of Helminths Eggs

With the help of a Pasteur pipette 0.15 ml of a flotation fluid was used to fill the two chambers of a McMaster cells one after another. The counting chambers were allowed to stand for 5 minutes to allow the eggs to float to the surface and the debris to sediment. The suspension of the filtrate was examined under the microscope at 10x and 40x magnifications. All eggs within the engraved area of both chambers were identified and counted [19,21,22]. X was the number of eggs of parasite counted in each chamber. EPG (egg per gram of faeces) for each chamber of the cell was equal to 100 xs. However, since we used 2g of feces to fill the two chambers, the intensity retained will be the average of EPG obtained from the two chambers. EPG1= 100 x_1 and EPG2 = 100x_2

$$EPG = \frac{EPG1 + EPG2}{2}$$

2.10 Statistical Analysis

Data collected were analyzed using Statistical Packet for Social Science (SPSS) software version 20.0. The prevalence was analyzed using Chi Square test (X2). Egg count (intensity) values were transformed using Log_{10} (N+1) and compared with a Paired Sample T-test.

3. RESULTS

The mean age of the children (year ± SD) was 8.61±3.81 (range 3-21years) with 477 (4.40%) males and 471 (5.52%) females. Among the 948 children examined, 50 (5.27%) were infected with geohelminths which included *A. lumbricoides*, Hookworm and *Trichuris trichiura*. The most prevalent infection with 27 (2.27%) infected children was Hookworm infection, followed by *A. lumbricoides* with 16 (1.69%) infected children and *T. trichiura* with 7 (0.74%) infected children. Table 1 illustrates the distribution of the intensity of STH infections at day 0 before treatment to day 21 after treatment. The intensity of worm using the WHO (1987) criteria for egg count per gram (epg) estimation showed that a significant proportion of the children (946 [99.79%]) had egg count of < 5,000 epg compared to those that had > 5,000 epg (2 [0.21%]). None of the 23 infected children had > 5,000 epg post-treatment.

Mebendazole induced a significant (P<0.001) decrease in the overall prevalence (1.47% after treatment). Altogether, there was a significant reduction in prevalence (P<0, 01-0.001) for each of the parasites observed after treatment compared to before treatment except for *T. trichiura* (Table 2). Mebendazole was more effective for *A. lumbricoides* with a cure rate of 100%, followed by Hookworm (70.37%) and *T. trichiura* (14.29%). Even though the mean egg per gram of *A. lumbricoides* (1895.31±825.85epg) was greater than those of Hookworm (64.82±9.55epg) and *T. trichiura* (50.00±0.00epg) before treatment, mebendazole induced a significant reduction (P<0.001) of 100% and 77.16% for the mean egg count of *A. lumbricoides* (0.00 egp) and Hookworm (14.86 epg) respectively, and a non-significant decrease (P>0.05) of 14.29% for *T. trichiura* (42.86 epg) after treatment (Table 2).

Table 1. Intensity of infection according to species before treatment and after treatment (N=50)

Intensity of infection	***Ascaris lumbricoides* n (%)**		**Hookworm infection n (%)**		***Trichuris trichiura* n (%)**	
	Treatment					
	Before	**After**	**Before**	**After**	**Before**	**After**
Low	14(28%)	0	27(54%)	8(16%)	7(14%)	6(12%)
Moderate	1(2%)	-	-	-	-	-
Heavy	1(2%)	-	-	-	-	-
Total	16(32%)	0	27(54%)	8(16%)	7(14%)	6(12%)

Table 2. Pre- and post-treatment stool egg counts of *A. lumbricoides*, hookworm and *T. trichiura* among children treated with single doses of Mebendazole

Parasites	**Number and percentage of positive children**		**P-value**	**Cure rate (%)**	**Mean egg per gram**		**Egg reduction in egg count (%)**	**P-value**
	Before treatment	**After treatment**			**Before treatment**	**After treatment**		
Ascaris lumbricoides	16(1.69%)	0(0.00%)	0.000	100.00	1895	0	100	0.000
Hookworm	27(2.27%)	8(0.84%)	0.001	70.37	65	15	77	0.000
Trichuris trichiura	7(0.74%)	6(0.63%)	0.592	14.29	50	43	14	0.36
Total	50(4.7%)	14(1.47%)	0.000					

4. DISCUSSION

Cameroonian National Survey of helminth infections, conducted between 1985–1987, suggested that the prevalence of infections with *A. lumbricoides* and *T. trichiura* have decreased while the incidence of hookworm infection may have increased [5]. In the present study, we observed an overall prevalence of 5.27% amongst the school age children. A possible reason for this low prevalence may be due to the fact that the three major intervention methods for STH infection are employed in the study area. Anthelminthic drug treatment aimed at reducing morbidity by decreasing the worm burden amongst school age children. Improved sanitation aimed at controlling transmission by reducing soil and water contamination. Health education aimed at reducing transmission and re-infection by encouraging healthy behaviors [6].

In this study, Hookworm (2.27%) was the most prevalent followed by *A. lumbricoides* (1.69%) and *T. trichiura* (0.74%).The prevalence of the different parasites we observed are in line with those of Lindo and collaborators [23] in West India who says among children under the age of 12 years in an interior town of Guyana the most common intestinal helminth parasite were hookworm (28.2%), followed by *A. lumbricoides* (18.8%) and then *Trichuris trichiura* (14.1%). But our results diverged with those of Dennis and collaborators [5] who observed different trends of infection for these geohelminths (*A. lumbricoides*, *T. trichiura*, and hookworm) in Nhol (33.0%, 54.3%, and 26.6%, respectively) and Bawa (15.3%, 41.5%, and 18.7%, respectively) both in the West Region of Cameroon. A possible explanation is that epidemiological studies on the prevalence of infection of intestinal parasites differ by region, within countries, and even within different regions of the same country. This largely depends on the local environmental conditions [24]. However such comparisons between study areas must be interpreted very conservatively, given the multitude of factors (for example the distribution of infections varies with altitude, probably as a result of the effects of temperature, humidity and ultra-violet light on the survival of worm eggs and larvae) leading to dramatic differences in prevalence of geohelminth infections [25]. The high prevalence of hookworm infections found in this study may be explained by poor hygienic practices among the children despite the different major precautions established by the national program for schistosomiasis and helminthiasis.

In this investigation, the proportion of children who had egg count of <5,000 epg (low intensity) as to those that had > 5,000 epg (moderate and heavy intensities) was high. This implies that, in our study area the intensity of infection was unevenly distributed, with very heavy loads concentrated in a few individuals [26,27]. Authors such as Hall [28] and Stephenson [29] reported that morbidity has been traditionally considered as a result of heavy geohelminth infections; children with light infections were thought to suffer no ill effects. There is increasing evidence however, that even low or moderate intensity infections retards childhood growth and development.

Bennett and Guyatt [30] reported drug efficacy data as cure rate (CR) and egg reduction rate (ERR) for 500 mg single-dose mebendazole in treating *A. lumbricoides*, hookworm and *T. trichiura* infections. They concluded that marked variation is mainly observed when the CR is the analyzed endpoint. The efficacy of benzimidazole for *A. lumbricoides* infection has also been clearly apparent because expected efficacy levels are so high. In contrast, monitoring changes in drug efficacy against hookworms and *T. trichiura* is more unreliable because efficacy levels are relatively low and variable [30].

In the present study, **s**ingle dose of 500mg mebendazole was found to be very effective against *A. lumbricoides* with CR of 100%. This study has confirmed the findings of previous works that mebendazole at 500mg are highly effective at single dose treatment of infection with *A. lumbricoides* [31,32]. For most studies, conclusion drawn on drug efficacy are based on CR as the primary outcome measure, as there are insufficient valid studies, in the public domain, reporting the ERR [33]. However, Bennett and collaborators [30] concluded that the drug regimen was highly effective with median CR between 95-97% and median ERR of 99-100% specifically for *A. lumbricoides*. The efficacy of this treatment may equally be excellent in light to heavy *A. lumbricoides* infections [11,30,34]. After administration of the drug, no egg was excreted in the stools of the children 21 days after treatment. There was a significant reduction in mean egg count (100%). This result is consistent with previous efficacy studies and is also similar to the results of an efficacy study carried out in Pemba Island by Albonico and collaborators [35].

During our study, the distinction between the two species of hookworm was not realized and among the family of Ancylostomatidae, the species *Necator americamus* is the most encountered in Central Africa (Cameroon). However, there is some evidence to suggest that very heavy infection with *N. americanus* may require more than one dose to achieve complete cure [36,37]. Nonetheless, we observed very low parasite load for hookworm infection and as such we find high cure (70.37%) and egg reduction (77.16%) rates when mebendazole was administered. These results are opposite to those observed by Ismail et al. [11a]. They reported cure rate of 22.4% and egg reduction rate of 82.4%. This might be due to variation in intensity (epg) of infection since cure rate is sensitive to it [33]. Another possible reason may be the differences observe at the level of the information on the treatment regimen, sample size, age structure of the study population, study location and the diagnostic tests performed in evaluating both CRs and ERRs [30]. Mebendazole efficacy was high when compared with that in published studies and, more specifically, compared with that in the trial carried out in Pemba Island Zanzibar and in Mafia Island with corresponding cure rates of 22.4% and 31.3% and egg reduction rates of 82.4% and 78.1% respectively [38,39]. Our result corroborates that of Abadi et al. [40] in Ujung Pandang South Sulawesi Indonesia where mebendazole appeared to be effective against hookworm (cure rate 91.1% and ERR 98.3%).

The cure rate (14.29%) found for *T. tichiura* was markedly lower than that reported in other studies [11,31,41,42]. This was also the case for egg reduction rate (14.29%). The result obtained for CR convergence with that of Bennett et al. [30] who observed that *T. trichiura*, treated with single-dose mebendazole shows poor levels of drug efficacy, especially in terms of CR and equally in agreement with results of Levecke and collaborators, [43] who observe a significantly low ERR of 51% for *T. trichiura*. They observed that the ERR for *T. trichiura* varies across different study sites, which could be largely explained by the mean baseline egg per gram which is relatively low. Nevertheless, our results are relatively similar to those of Knopp et al. [44] who recorded a cure rate of 19% and but a high egg reduction rate of 67% for single dose Mebendazole. A possible reason for low cure rate maybe attributable to the presence/absence of the b-tubulin codon 200 polymorphism that has been linked to benzimidazole resistance or tolerance [45]. Even though the CR was low, it does not raise concern because its ERR was high (63.16%) since ERR is particularly more relevant for morbidity control and fears arise only when ERR is less than 50% [46].

5. CONCLUSION

From this study, it is obvious that there is low prevalence of intestinal helminth parasites in the study area due to the regular de-worming campaign put in place by the National program for schistosomiasis and intestinal helminthiases against geohelminths. The results confirm the therapeutic efficacy of a single dose of 500 mg mebendazole against *A. lumbricoides* and hookworms, and a low efficacy against *T. trichiura*. The prevention and control of mortality and morbidity associated with these infections and reduction based on regular chemotherapy, environ-mental sanitation, health education and community participation as implemented in this locality are to be encouraged.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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