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Current status of the efficacy and effectiveness of albendazole and mebendazole for the treatment of *Ascaris lumbricoides* in North-Western Indonesia

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ABSTRACT

Objective: To investigate the efficacy and effectiveness of albendazole and mebendazole in the treatment of *Ascaris lumbricoides (A. lumbricoides)* in the North-Western Indonesia. **Methods:** 229 primary school children who were positive for *A. lumbricoides* in their stool were recruited in the study. 123 children received single-dose of 400 mg albendazole and 106 children received single-dose 500 mg of mebendazole. After 1 week, their stools were examined for the cure rate (CR) and egg reduction rate (ERR). Egg culture was also performed and observation was made on week-1, -3, -4. **Results:** have shown a non-significant difference in CR 96.7% *vs.* 100%; and ERR of 99.3% *vs.* 100.0% for albendazole and mebendazole groups respectively ($P>0.05$). *In-vitro* egg culture has shown trends of decrease in the percentage of the unfertilized eggs and in $\geqslant 2$ cell eggs in both treatment groups ($P<0.05$). The embryonated eggs from the albendazole groups has shown an increase from 7.3% on week-1 to 13.8% on week-4, whilst the mebendazole group has shown a constant increase during the whole 4 weeks of culture from 7.5% to 28.3% ($P<0.01$). **Conclusions:** No evidence of drug resistance is noted so far from the area of North-Western part of Indonesia. In addition, although both drugs showed incomplete ovicidal effects, single-dose albendazole is better than mebendazole in sterilizing *A. lumbricoides* eggs.

1. Introduction

Albendazole and mebendazole is known to be efficacious for the treatment of *Ascaris lumbricoides (A. lumbricoides)*[1]. In spite of its high efficacy, there were concerns of the emergence of drug resistance with these agents in human and animals from Africa and Southern America[1-3]. So far, there is no report of drug resistance of albendazole or mebendazole for the treatment of *A. lumbricoides* from Asia. In spite of the lack of reported drug resistance, this issue in Asia remains unclear. An investigation to find out whether drug resistance exists in this region is therefore essential. Demographic similarities that all the regions share such as the tropical climate, lower standards of hygiene and nutrition should alert physicians in Asia for the possibility of drug resistance. This aspect is thus important as *A. lumbricoides* mostly affects school-age children. North-Western Indonesia where the island of Sumatera lies is very close to the main continent of Asia was chosen as a good target area to perform this study.

Therefore, this study was done to investigate if drug resistance towards albendazole and mebendazole for the treatment of *A. lumbricoides* is present in this region. The project was not only designed to study the drug efficacy in relation to the possible drug resistance but also to further study the drug effectiveness in sterilizing *A. lumbricoides* eggs. Both drug efficacy and drug effectiveness are important factors in relation to the prevention and eradication of the helminth.

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2. Materials and methods

The study was performed among primary school children in two districts within the province of North Sumatera, Indonesia. This area is located in the North-western part of Indonesia. The study areas are district of South Aek Nabara-Labuhan Batu and district of Kabanjahe-Tanah Karo. The choice of the two districts is made based on the previous survey that was reported by the department of health as the most endemic districts for *A. lumbricoides* infection in the province. The study has received ethical committee clearance from the School of Medicine of the University of North-Sumatera and the informed-consents have been given from the parents and the school principals.

Preparation day: The day when primary school children gave their stool samples. Stools were collected as early morning stool, or afternoon stool taken at home if the children could not pass motion in the morning. All stool samples were examined using Kato-Katz method[4]. Samples that were infected by single infections of *Trichruris trichiura,* hookworm or *Enterobius vermicularis* were excluded, and only stools with single infection of *A. lumbricoides* or mixed infections of *A. lumbricoides* with other soil-transmitted helminthes were selected. Two-hundred-twenty-nine children that were positive for *A. lumbricoides* were finally selected. The egg number from each stool sample was counted which is to be used as the baseline data for the cure rate and for egg reduction rate (ERR) and also to sub-classify the stool samples based on the number of eggs per gram feces (epg). The epg count is to be used to categorize the infection as mild (epg < 5 000), moderate (epg 5 000-49 999) and severe (epg > 50 000)[5].

Day-0: all infected children were randomly assigned using random-number table into two groups to receive either single dose of 400 mg of albendazole or single dose of 500 mg of mebendazole.

Day-1: is the day following the medication where stool samples were cultured to see the development of egg maturation.

Week-1: there were two tasks done on week-1:

(1) Counting of the egg number for calculation of the cure rate as well as for the ERR. The egg counting is done using fresh stool sample using Kato-Katz technique[6,7];

(2) Observation and counting of the egg maturation stages taken from the egg culture.

The observation and egg maturation counting from the egg culture was also performed by week-3 and week-4. The egg maturation stages are grouped into egg with one cell, two or more cells, and embryonated egg. The result of each grouped is expressed as percentage of the total egg number.

"Stool culture procedure"; into a plastic jar that has been given level mark, the examiner transferred stool using a spatula up to that level. The stool was later immersed in 30 mL of 2% potassium bichromate and left at room temperature (25-32 ℃) for 4 weeks. They were all placed in clean room where the jars were not covered by lid and kept open. The observation of the egg maturation and counting of the stage were done on week-1, -3, -4.

Observation of the egg maturation was not done by week-2. The reason that the observation was not done by week-2 is because eggs during *in-vitro* embryonation at room temperature showed that molt takes within the egg after 17-22 d. So we expect retardation in the egg development, and therefore we did not do the assessment of the culture by week-2.

Preparation of stool smear for differential egg counting was carried out using the same Kato-Katz technique as describe above. The egg maturation stages were grouped into: unsegmented eggs (1 cell); ⩾ 2 cell eggs (1 cell + 2 cells; 1 cell + 4 cell; 2 cells + 4 cells; 1 cell + 2 cells + 4 cells), and embryonated eggs. The result of each egg maturation stage was expressed in relative percentage of the total egg number.

Statistical analysis was carried out using Wilcoxon-rank-sum test and *Chi*-square test to see the difference between anthelminthic and the intensity of *A. lumbricoides* infection. In order to see the tendency whether there is increase or decrease of every step of the egg maturation during weeks of maturation, statistical calculation is performed by one-way analysis of variance (ANNOVA). The difference of the egg evolution between the two treatment groups is carried out using multivariate analysis of variance (MANOVA). All statistical tools come from SPSS version 14.0.

3. Results

All stools from the school children were first investigated for *A. lumbricoides* by Kato Katz examination. 229 stool samples fulfilled the criteria and they were divided based on the worm burden (epg) into mild infection (epg < 5 000), moderate infection (epg 5 000-49 999) and severe infection (epg > 50 000). 189 samples fulfilled the criteria of mild infection, 38 samples of moderate infection and 2 samples of severe infection. They were later randomized into two groups to receive the treatment. 123 children received albendazole and 106 children received mebendazole, and were further analyzed based on the worm burden:

The mild infection group showed that children who received albendazole were 105 out of 123 (85.4%) whilst mebendazole were 84 out of 106 (79.2%) (P>0.05). In the moderate infection group, 17 out of 123 (13.8%) who received albendazole showed no significant difference to 21 out of 106 (19.9%) (P>0.05). The severe infection group showed that

1 out of 123 (0.8%) of the albendazole group and 1 out of 106 (0.9%) of the mebendazole group had no statistical difference, (P>0.05). Based on the intensity of the infection as measured by the worm burden as a whole, both treatment groups showed no difference (P>0.05).

The calculation of the cure rate (CR) and the ERR was done using fresh stool samples taken on the preparation day and on week-1, and showed that there was no significant difference in the intensity of *A. lumbricoides* infection between the albendazole and mebendazole. The CR of albendazole and mebendazole groups are 96.7% and 100.0% respectively, but statistically they do not show significant difference (P>0.05). The ERR of albendazole is 99.3% while the ERR of mebendazole is 100.0% (P>0.05).

The stool cultures of the albendazole and mebendazole groups showed similar pattern. Both showed final increase in unsegmented eggs after 4 weeks of culture (P<0.05), and constant decrease pattern in ⩾ 2 cell eggs (P<0.05).

The embryonated eggs showed different pattern between albendazole and mebendazole groups. In albendazole group, there was an increase of the embryonated eggs during week-3 but dropped again a week later (P<0.05). In mebendazole group there was a significant constant increase of the embryonated egg number from the beginning till 4 weeks of culturing (P<0.05).

In order to see the evolution of egg maturation as a whole during 4 weeks of drug treatment, statistical analysis by multivariate analysis of variance was carried out and the result showed significant difference between albendazole and mebendazole (P <0.01).

For the embryonated eggs, both groups showed non-significant increase during week-1 and week-3. Nonetheless, during week-4 the embryonated eggs were significantly lower in the stool of children who received albendazole compared to children who received mebendazole(Table 1&2) (P<0.01).

Table 1

Percentage of *A. lumbricoides* eggs during their maturation in the culture (week 1-4).

		Week-1 (%)	Week-3 (%)	Week-4 (%)	P
Abendazole	Unsegmented	20.3	21.1	26.0	< 0.05
	⩾ 2 cell egg	15.4	10.6	9.8	< 0.05
	Embryonated	7.3	19.5	13.8	< 0.05
	Unfertilized egg	57.0	48.8	50.4	< 0.05
Mebendazole	Unsegmented	21.7	19.8	25.5	< 0.05
	⩾ 2 cell egg	25.5	17.9	15.1	< 0.05
	Embryonated	7.5	14.2	28.3	< 0.05
	Unfertilized egg	45.3	58.1	31.1	< 0.05

Annova is used to analyze the trend of every step of the egg maturation during weeks of maturation.

Table 2

The overall egg maturationl effect of albendazole and mebendazole during 4 week of culture.

		Week-1 (%)	Week-3 (%)	Week-4 (%)	P
Abendazole	1 cell & 2 cells	8.9	4.9	4.1	< 0.01
	1 cell & 4 cells	2.4	1.6	0.0	< 0.01
	2 cells & 4 cells	0.0	0.0	0.0	< 0.01
	1 cell, 2 cells, 4 cells	4.1	4.1	5.7	< 0.01
Mebendazole	1 cell & 2 cells	17.0	9.4	8.5	< 0.01
	1 cell & 4 cells	1.9	1.9	0.9	< 0.01
	2 cells & 4 cells	0.0	0.9	0.0	< 0.01
	1 cell, 2 cells, 4 cells	6.6	5.7	5.7	< 0.01

Multivariate analysis of variance is used to analyze the egg maturation effect of albenadazole and mebendazole, and the overall result shows that there is overall trend difference on the egg maturation between the two treatment groups.

4. Discussion

A. lumbricoides infection can usually be prevented by single dose of broad-spectrum anthelmintics such as albendazole or mebendazole given in low dose at prolonged intervals. School-age children are the most common to be affected by *A. lumbricoides* and administration of these drugs to school-age children can maintain worm burdens below pathogenic levels. Due to this fact, there is increased use of both drugs in many endemic areas which may lead to possible development of drug resistance. Recently, there is concern about emergence of drug resistance in human and animals from Africa[8], South-America[3,9] and Australia[10]. Although albendazole and mebendazole have been proven to be effective and safe, however issue of drug resistance should be put into special attention because anthelmintic

drug resistance is an irreversible problem. So far, reports about emergence of drug resistance to *A. lumbricoides* came only from some countries in Africa, South-America, Australia and no report on this issue came from Asia[11]. There was a report about low efficacy of these drugs in Asia but it was not to *A. lumbricoides* but to hookworm and other intestinal worm infections[12-13]. In spite of no report of drug resistance to *A. lumbricoides* from Asia, it is necessary to find out whether reduced efficacy of albendazole and mebendazole to *A. lumbricoides* has also occurred in Indonesia; a part of South-East-Asian countries.

The result of the CR^{+} of albendazole and mebendazole from our study proves that the CR of both drugs are higher than 95%, the level that is considered as efficacious against *A. lumbricoides* infection[11]. CR is 96.7% for albendazole and 100.0% for mebendazole but statistically they do not show significant difference. The ERR also showed good result which is 99.3% and 100% for albendazole and mebendazole respectively. This finding is similar with the previous report from that came from the close neighboring country. One recent study that was aimed not specifically to find out drug resistance has shown that in some countries in Asia the cure rates and the ERR are similar to our results[11]. This proves that reduced efficacy or drug resistance does not happen in this region.

The other important issue that needs to be put into account is that current antihelminthics have only temporary effect. Treatment although given in correct dosage gives only transitory effect and re-infection may occur[14-16]. It is therefore important to assess not only the drug efficacy in terms of the CR and ERR, but also to see possible embryonation of the egg *in-vitro* by mean of culturing the egg. The embryonated egg is infective and when it contaminates the environment and later ingested by animal or human, the gastric juice will break down the egg shell and produce release of larva[17]. In this study, there is significant reduction in the percentage of unfertilized egg after 4 weeks of culuture. This may be caused by the ovicidal action of the albendazole and mebendazole or due to self-death of the unfertilized egg. On the other hand, the relative percentage of the unsegmented eggs in both groups increased. Nevertheless, as they are expression of the relative percentage, the increase might be due to the inversion result of the decrease of the unfertilized eggs.

The result also shows that the embryonation of the egg was not abolished nor inhibited by both drugs. The appearance of the eggs containing 2 or more cells showed that the eggs reduced and developed into embryonated eggs. This shows that both albendazole and mebendazole do not abolish nor arrest the process of the embryonation. When we look at the pattern of the embryonated eggs; there is difference between between the albendazole and mebendazole groups. There is trend of embryonated eggs to increase after 3 weeks and dropped again after 4 weeks in the albendazole group. This decreasing number can happen because the eggs were being destroyed and there were less number of the eggs left to develop further into embryonated eggs. This may show that albendazole has ovicidal effect but its ovicidal action is delayed until 4 weeks after the treatment. On the other hand, the embryonated eggs in the mebendazole groups showed a constant increase during the 4 weeks of culture. This is an evidence that mebendazole does not inhibit the egg embryonation. Report from many studies shows similar result with our investigation that albendazole and mebendazole have incomplete ovicidal effects in human and in animal[18,19]. This result shows that in spite of good CR and ERR, the ovicidal effectiveness of the drugs is incomplete and is also potential to produce recurrence of the infection. Prolonged use, frequent recurrent infection and inappropriate dosage might lead to possible emergence of drug resistance. Discovery of new method of treatment is needed to anticipate this phenomenon either by discovery of single drug or combination of highly synergistic anthelmintic treatment[20-22].

When the whole egg maturation stages including the embryonated eggs were analyzed with multi-variate analysis of variance, both groups showed a significant difference. This difference might be caused by difference in the maturation of the embryonated eggs and not in the earlier stage of the egg maturation. The increase in the number of unsegmented eggs, and the decrease in $\geqslant$ 2 cells eggs showed relatively similar pattern both in the albendazole and mebendazole groups. The difference comes in the number of the embryonated eggs during 4 weeks of culturing. The result of embryonated egg analysis confirmed this finding. There was similar increase of the embryonated egg on week-3 in both groups, but they showed different pattern later on week-4. Embryonated eggs of the albendazole group declined whilst the mebendazole group showed a constant increase ($P<0.01$).

We conclude from our study that there is no evidence of drug resistance of both albendazole and mebendazole against *A. lumbricoides* in this region. In-spite of good CR and ERR, however the result from the *in-vitro* egg culture should be put into great account as single dose albendazole and mebendazole show incomplete ovicidal effects. The albendazole shows a better ovicidal effect but the effect appears only after 4 weeks of culture. Therefore, single dose albendazole has an important place in sterilizing *A. lumbricoides* eggs and in controlling environmental contamination.

Conflict of interest statement

We declare that we have no conflict of interest.

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