

## The Ethiopian SORT IT Course

# Trends and seasonal patterns in intestinal parasites diagnosed in primary health facilities in Northwest Ethiopia

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### Abstract

**Introduction:** Intestinal parasites have an insidious impact on human health. In response to high parasite frequencies in Northwest Ethiopia, mass drug administration (MDA) is provided for school children using albendazole/mebendazole (since 2007) and praziquantel (since 2015). The study objective was to assess trends and seasonal patterns of intestinal parasite infections in a context of MDA.

**Methodology:** This was a descriptive study collecting routine data from laboratory registers in two health centres in Denbia district, Amhara region. Stool test results (wet-mount direct microscopy) from patients attending these centres between 2013 and 2018 were included. Frequencies of different parasite species were evaluated within and across the years and stratified by age and gender.

**Results:** From a total of 8002 stool test results, the overall parasite frequency was 53.3%; this proportion remained constant. The most frequently diagnosed soil-transmitted helminths (STH) were *Ascaris lumbricoides* (16.9%) and hookworm (3.9%). STH frequency varied over the years, but was similar at the beginning (20.0%) and the end (22.0%) of the six-year period. STH infections were more frequent in winter (December–February; 20.4%) than in other seasons (16.0%). The most frequently diagnosed protozoa were *Entamoeba histolytica/dispar* (18.5%) and *Giardia lamblia* (12.2%). The frequency of *Giardia* steadily increased from 9.6% in 2013 to 15.3% in 2018. *E. histolytica/dispar* peaked in summer and *G. lamblia* in autumn.

**Conclusions:** Trends in routine laboratories may be a proxy for a status quo in the community. These findings suggest that higher MDA coverages and/or interventions beyond MDA are needed to reduce intestinal parasite-related morbidity.

**Key words:** Ethiopia; soil-transmitted helminths; intestinal protozoa; trends; routine laboratory; retrospective.

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### Introduction

Intestinal parasites have an insidious impact on human health because they cause nutrient deficiencies, anaemia, and impaired growth and cognitive development [1]. Intestinal helminths with a large burden on human health are the roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), hookworm (*Ancylostoma duodenale* and *Necator americanus*), and tapeworms (*Taenia spp.*) [2,3]. *Ascaris*, *Trichuris* and hookworm are often grouped together as soil-transmitted helminths (STH). The most important intestinal protozoa are *Giardia lamblia* and *Entamoeba histolytica/dispar* [4].

Three types of interventions can reduce the morbidity caused by intestinal parasites: improved

sanitation, identification and treatment of infected cases, and preventive chemotherapy (i.e. mass drug administration (MDA)). The WHO guidelines for STH control emphasise the most feasible of these interventions, i.e. MDA with mebendazole or albendazole to children and to women of childbearing age [2]. The global target is to eliminate STH as a public health problem in childhood by 2020, which is defined as a prevalence of STH infection of moderate and high intensity among school-age children of 1% or less. To achieve this target, it is required that 75% of preschool-age and school-age children in risk areas take preventive chemotherapy once or twice per year [2,5].

Ethiopia is a high-burden country for STH, and large parts of the country are also endemic for

schistosomiasis [6]. Between 2011 and 2018, at least ten STH surveys have been done in different regions of Ethiopia: the prevalence of any STH among children ranged between 12% and 68% [7-16]. MDA campaigns with albendazole and mebendazole for STH control in school-age children started in Ethiopia in 2007 and intensified in 2015 [5]; MDA with praziquantel started in 2015.

Very large numbers of stool samples are examined in primary health centres throughout Ethiopia. If the recording quality is adequate, routine laboratory registers can provide information that is complementary to what can be learnt from cross-sectional population-based surveys. In the routine laboratory registers, we expect to find shifts in the relative proportions of intestinal parasites within the years and over the years because of the following reasons: (1) increasing MDA coverage with anthelmintic drugs with different efficacy for different intestinal parasites, (2) seasonal variations in health seeking by rural populations, and (3) variations in temperature and rainfall affecting parasite life cycles in the soil and risk of infection [17]. The association between specific parasites and the age of the patients may also change over time, as MDA mainly targets children.

Information from routine laboratories over long periods of time is valuable because in high-risk areas for STH, people with digestive disorders often receive empirical treatment. Better insights in the changing aetiology of symptomatic intestinal infections in such settings will help clinicians to target empirical treatment more rationally. We therefore set out to assess long-term trends and seasonal variability in the patterns of intestinal parasite infections diagnosed in the laboratories of two primary health centres in Northwest Ethiopia between 2013 and 2018, in a context of MDA.

## Methodology

### *Study design*

This was a descriptive study based on routine data collected from the laboratories of two health centres.

### *Setting*

The study was conducted in Gorgora and Chuahit health centres located in West Denbia district, Amhara region, Northwest Ethiopia. Two prior studies in Amhara region found a relatively high prevalence of STH among children. The first was done in 2014 and reported a prevalence of 12% (any STH infection) in 212 children under five years old [18]. The second was done between 2011 and 2015 and reported a prevalence of 36% in 15455 children aged 6 to 15 years [7]. We

found no previous published information on the prevalence of infections with tapeworms and intestinal protozoa in the region.

West Denbia district has five health centres, three town health extension offices, and twenty health posts. We chose to do the study in Gorgora and Chuahit because these were the first health centres to start routine laboratory activities about 10 years ago. Stool samples are examined using the direct wet-mount technique. The cost of a stool test for the patient is 0.17 US\$; usually, only one test is done per patient. Date of testing, client characteristics (name, gender, age) and stool test results are handwritten in laboratory logbooks.

### *Study population and period*

The study population consisted of all clients who provided stool samples for laboratory examination between January 2013 and December 2018 and were registered in the laboratory logbooks of the health centres.

### *Sampling, data source, and variables*

Because the total number of stool examinations over six years is very high, we took a systematic sample consisting of all the results of the first week of every month. Date of testing, age, gender, and stool test results were extracted from the laboratory logbook. The findings related to *S. mansoni* are presented elsewhere [19]; findings for all other intestinal parasites are presented in the present paper.

### *Analysis and statistics*

Data was entered directly from the laboratory register into EpiData Entry software version 3.1 and analysed and visualised with SPSS software version 20 and Microsoft Excel. Descriptive statistics (proportions, means and medians) and graphs were used to summarise the data and to visualise trends. Frequency of infection was calculated as the number of stool tests with a positive result divided by the number of stool tests done, as documented in the laboratory register. Frequencies were calculated for any parasite, any soil-transmitted helminth, and per parasite species. The Wilson score method was used to calculate 95% confidence intervals (CI) for the proportions. To compare proportions stratified by gender and age groups and across seasons, we used the chi-square test with a level of significance set at 5%. Associations of infections with age group, gender, and season were also expressed using odds ratio (OR) with 95% CI. To evaluate linear trends over the years, we used linear regression (least squares estimation).

**Ethical considerations**

Ethical approval for the study was obtained from the Ethics Committee of the University of Gondar, Ethiopia. The study was also approved by the Ethics Advisory Group of the International Union against Tuberculosis and Lung Disease, Paris, France. As this was a record review study without patient identifiers, the issue of informed patient consent did not apply.

**Results**

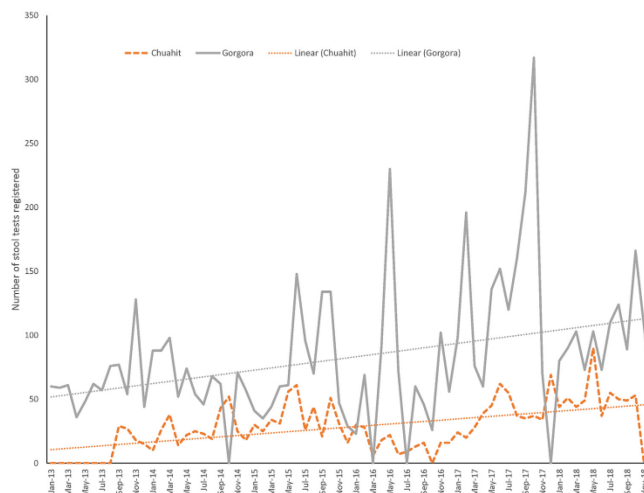
*Amount of stool tests performed*

A total of 8002 stool test results were registered during the six-year study period: 5959 in Chuahit (74.5%) and 2043 in Gorgora (25.5%). Because we collected the data of only one week per month, the estimated number of tests performed is four times higher, i.e. 32008. In Chuahit, the estimated number of samples tested per day was 17. From 2013-2016, two laboratory technicians were working in this health centre, and in 2016, two additional technicians were hired. In Gorgora, the estimated number of samples tested per day was 6. In this health centre, there was only one laboratory technician, who was replaced in 2015. Figure 1 gives an overview of the number of samples tested per week in each laboratory. In both centres, the number was variable and tended to increase over the years. There was no pronounced seasonal variation in the number of stool tests performed.

*Stool test results stratified by age and gender*

The median age of the patients providing stool samples was 24 years (interquartile range 14-35 years) and the proportion of men was 50.5%. The proportion of samples positive for at least one parasite was 53.3% (4265 out of 8002 samples; 95% CI: 52.2%-54.4%). The most frequent parasite species were *A. lumbricoides* (in 16.9% of the samples; 95% CI: 16.1%-

**Figure 1.** Number of registered stool tests during the first week of every month over a period of six years, Chuahit and Gorgora health centres.



P-value for linear trend Chuahit = 0.004 and for Gorgora = 0.004.

17.8%), hookworm (3.9%; 95% CI: 3.5%-4.3%), *E. histolytica/dispar* (18.5%; 95% CI: 17.7%-19.4%) and *G. lamblia* (12.2%; 95% CI: 11.5%-13.0%; (Table 1)).

There were significant differences in the stool test results according to the age groups. The proportion of infection (any parasite) was highest in the school-age children (56.5%) and lowest in the children under five years old (35.5%; P < 0.001). Taking the children under five as a reference group, the OR to be diagnosed with infection (any parasite) for school-age children was 2.3 (95% CI: 1.9-2.9) and the OR for people of 15 years or older was 2.1 (95% CI: 1.8-2.6). Detailed figures for each of the parasite species are given in Table 1.

When we stratified by gender, we found that most of the infections were significantly more frequent in men than in women, except for *A. lumbricoides*, which was slightly more frequent in women (Table 2).

**Table 1.** Stool test results stratified by age group.

Parasite species	< 5 years (n = 496)		5-14 years (n = 1535)		≥ 15 years (n = 5971)		Total (n = 8002)		P-value*
	N	%	N	%	N	%	N	%	
Any parasite	176	35.5	868	56.5	3221	53.9	4265	53.3	< 0.001
Any soil-transmitted helminth <sup>§</sup>	57	11.5	368	24.0	1197	20.0	1622	20.3	< 0.001
<i>Ascaris lumbricoides</i>	52	10.5	313	20.4	988	20.4	1353	16.9	< 0.001
Hookworm	7	1.4	69	4.5	236	4.0	312	3.9	0.008
<i>Taenia spp.</i>	9	1.8	15	1.0	44	0.7	68	0.8	0.04
<i>Hymenolepis nana</i>	6	1.2	24	1.6	38	0.6	68	0.8	0.002
<i>Strongyloides stercoralis</i>	1	0.2	8	0.5	33	0.6	42	0.5	0.7
<i>Trichuris trichiura</i>	0	0.0	3	0.2	15	0.3	18	0.2	0.8
<i>Enterobius vermicularis</i>	0	0.0	4	0.3	13	0.2	17	0.2	0.7
<i>Entamoeba histolytica</i>	52	10.5	200	13.0	1230	20.6	1482	18.5	< 0.001
<i>Giardia lamblia</i>	66	13.3	187	12.2	725	12.1	978	12.2	0.8

\* Pearson chi-square test if the expected cell counts were 5 or more; Fisher exact test otherwise; <sup>§</sup> *Ascaris lumbricoides* or hookworms or *Trichuris trichiura*.

**Table 2.** Stool test results stratified by gender.

Parasite species	Male gender (n = 4040)		Female gender (n = 3962)		Total (n = 8002)		P-value*
	N	%	N	%	N	%	
Any parasite	2287	56.6	1978	49.9	4265	53.3	< 0.001
Any soil-transmitted helminth <sup>§</sup>	785	19.4	837	21.1	1622	20.3	0.06
<i>Ascaris lumbricoides</i>	648	16.0	705	17.8	1353	16.9	0.04
<i>Hookworm</i>	167	4.1	145	3.7	312	3.9	0.3
<i>Taenia spp.</i>	45	1.1	23	0.6	68	0.8	0.009
<i>Hymenolepis nana</i>	43	1.1	25	0.6	68	0.8	0.04
<i>Strongyloides stercoralis</i>	27	0.7	15	0.4	42	0.5	0.07
<i>Trichuris trichiura</i>	6	0.1	12	0.3	18	0.2	0.1
<i>Enterobius vermicularis</i>	13	0.3	4	0.1	17	0.2	0.03
<i>Entamoeba histolytica</i>	845	20.9	637	16.1	1482	18.5	< 0.001
<i>Giardia lamblia</i>	527	13.0	451	11.4	978	12.2	0.02

\* Pearson chi-square test; all expected cell counts were 5 or more; § *Ascaris lumbricoides* or hookworms or *Trichuris trichiura*.

**Table 3.** Overview of the most frequently encountered parasite species by season.

Parasite species	Winter <sup>†</sup> (n = 1610)		Spring (n = 1942)		Summer (n = 2071)		Autumn (n = 2379)		P-value*
	N	%	N	%	N	%	N	%	
Any parasite	875	54.3	1033	53.2	1097	53.0	1260	53.0	0.8
Any soil-transmitted helminth <sup>§</sup>	393	24.4	391	20.2	390	18.8	447	18.8	< 0.001
<i>A. lumbricoides</i>	328	20.4	337.0	17.4	319.0	15.4	369	15.5	< 0.001
<i>Hookworm</i>	77	4.8	56.0	2.9	88.0	4.2	91	3.8	0.02
<i>E. histolytica</i>	252	15.7	376.0	19.4	463.0	22.4	391	16.4	< 0.001
<i>G. lamblia</i>	212	13.2	228.0	11.7	193.0	9.3	345	14.5	< 0.001

† Winter: December-February; spring: March-May; summer: June-August; autumn: September-November; \* Pearson chi-square test; all expected cell counts were 5 or more; § *Ascaris lumbricoides* or hookworms or *Trichuris trichiura*.

**Table 4.** Number of children treated with albendazole or mebendazole during the study period.

Year	Number of children treated (May)	Number of children treated (November)	Number of children registered in primary schools	Estimated coverage
2013	Not known	Not known	Not known	Not known
2014	Not known	Not known	Not known	Not known
2015	29235	35289	42450	76%
2016	29286	27254	38726	73%
2017	29251	28979	38309	76%
2018	31323	31199	41681	75%

The OR to be diagnosed with infection (any parasite) was 1.3 (95% CI: 1.2-1.4) for male compared to female patients.

*Trends in intestinal parasite infections over the years*

The trends in infection frequencies for each of the parasite species are shown in Figure 2. The patterns for the total population (panel A) and for the subgroup of school-age children (panel B) were similar. The frequency of *A. lumbricoides* decreased in 2016 and bounced back in 2017. *E. histolytica/dispar* made the inverse movement: the frequency increased in 2016 and decreased afterwards. The frequency of *G. lamblia* increased steadily over the years. In the overall patient population, the proportion of *G. lamblia* increased from 9.6% in 2013 to 15.3% in 2018 (P-value for linear trend = 0.006) and in school-age children, the proportion increased from 5.9 in 2013 to 17.9 in 2018 (P-value for linear trend = 0.001). The frequency of the other parasite species was lower and remained relatively constant during the study period. The frequency of co-infections with multiple parasite species decreased significantly in the total population (from 8.3% in 2013 to 3.5% in 2018; P-value for linear trend = 0.01) but not in school-age children (8.5% in 2013 and 6.1% in 2018; P-value for linear trend 0.3).

*Seasonal variation*

An overview of the most commonly found parasites stratified by season is given in Table 3. *A. lumbricoides* and hookworm were diagnosed more frequently in winter (December-February) than in the other seasons. In the case of *A. lumbricoides*, the OR for samples taken in winter compared to summer was 1.4 (95% CI: 1.2-1.7). *E. histolytica/dispar* reached its highest proportion in summer (June-August) and *G. lamblia* in autumn (September-November).

*Mass drug administration campaigns*

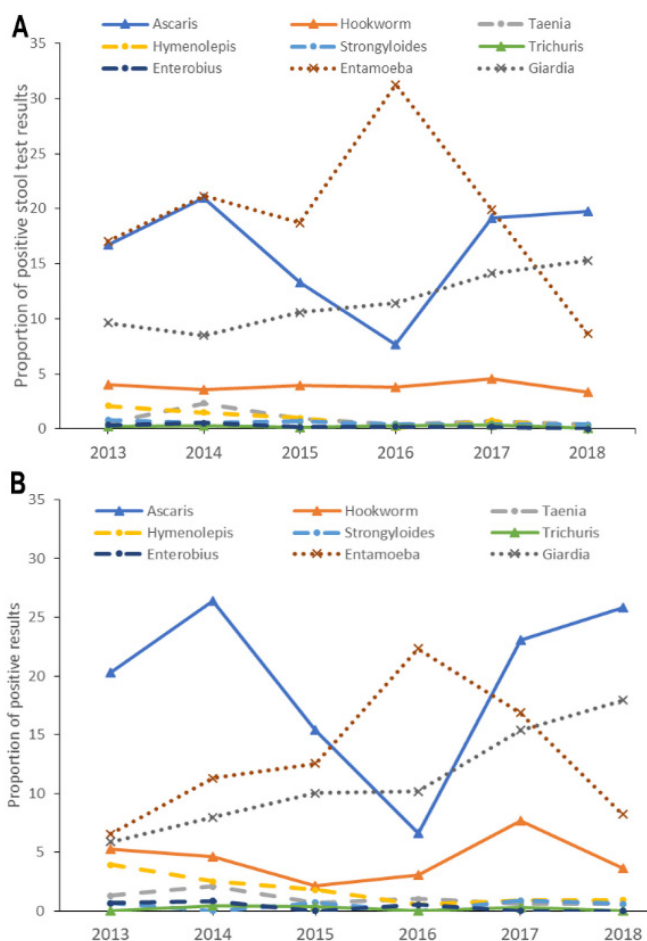
In West Denbia district, MDA campaigns have been organised twice every year, in May and in November. During these MDA campaigns, health extension workers give albendazole or mebendazole to all the children who attend primary school. The number of children treated in the first years of the study (2013-2014) was not known; this information was not available in the district health office. In the following years (2015-2018), approximately 30000 children were treated in each MDA campaign, which corresponds to a coverage of around 75% (Table 4).

**Discussion**

This study illustrates the large amount of data about routine stool testing that is available in the laboratories of primary health centres in Ethiopia and that allows to evaluate long-term trends in parasite infections. The most frequently diagnosed parasites were *Ascaris*, hookworm, *Entamoeba*, and *Giardia*. Despite the MDA campaigns with albendazole or mebendazole that are organised twice per year for school children, the frequency of STH remained high in the period from 2013 (23.5%) to 2018 (28.3%) in patients aged 5 to 14 years. The seasonal patterns and time trends of intestinal helminths and protozoa were markedly different.

These findings add to the body of evidence showing that the impact of MDA on the burden of STH in real-life conditions is less than what global advocacy

**Figure 2.** Trends in infection frequencies over the study period per parasite species.



Panel A: total population (n = 8002). Panel B: samples from children aged 5 to 14 years (n = 1535). Soil-transmitted helminths are indicated with solid lines, other helminths with dashed lines, and intestinal protozoa with dotted lines.

organizations claim [20]. The reported MDA coverage was moderate (around 75%) among school children in the region where our study took place, but field research in East Africa has shown that such official figures may overestimate real coverage [21]. There is an ongoing debate in the public health community about the effectiveness of MDA to control STH, and there has been a recent call to analyse the situation for specific helminths and for light- *versus* heavy-intensity infections, to revise the optimal age range of people targeted with MDA and the optimal prevalence threshold at which MDA is recommended, and to systematically collect information from diverse global areas [22].

An important limitation of this study is the use of wet-mount direct microscopy on one stool sample to diagnose intestinal parasites. This technique is known to have a low sensitivity, especially for low-intensity infections [23,24]. In addition, the sensitivity differs from parasite species to species. For example, in an Ethiopian study, the sensitivity of wet-mount microscopy compared to the formalin ether concentration technique was 83% for *Ascaris* and 33% for *Hymenolepis* [23]. As a result, we have probably underestimated the true frequencies of some of the intestinal parasites and protozoa. This hinders the comparison of our findings with those of surveys using more sensitive diagnostic techniques. On the other hand, the specificity of wet-mount direct microscopy is known to be high [23,24]. Moreover, the quality of the information in this study was consistent over time, because the same wet-mount technique was used throughout the six-year period and the staff turn-over at the laboratories was limited. A second limitation is the lack of corresponding clinical information. Because the patients in this study attended a health centre, provided a stool sample, and paid for the test, we assume that they were symptomatic but we have no details on the type and severity of their digestive problems. As the WHO target for MDA is to reduce the morbidity caused by STH, our description of symptomatic patients is relevant, but as most of the surveys describe parasite frequency in children of the general population, comparisons with other studies are difficult.

Our findings are in line with the overall picture of STH infection in Ethiopia and the region more generally [25]. In one cross-sectional study reporting from a similar setting in the laboratory of a primary health centre in South Gondar and using the formalin ether concentration technique, the overall frequency of helminth infections (21%) was similar to what we found (26%) [26]. Despite the differences in population and

diagnostic techniques, our findings are also in line with several community-based studies, which found moderate-to-high frequencies of intestinal parasites in different regions of Ethiopia: 52% of intestinal polyparasitism among residents around Gilgel Gibe Dam, and 48% of residents in Jimma town [27,28]. Another study, conducted in an urban setting in Southern Ethiopia, found similar prevalence levels (polyparasitism in 57%) [29].

There were no previous reports of long-term trends in the country, but one study in Southern Ethiopia did look at reinfection rates: three months after an MDA campaign in 2015, the prevalence of STH among school-age children was 37%, which corresponded to 93% of the prevalence before the intervention (39%) [15]. High reinfection rates could explain the lack of a clear MDA effect in our study as well.

There was seasonal variation in the stool test results, which may be explained by environmental and behavioural variation in the course of a year. Factors such as temperature, moisture levels, and light are known to affect the development and hatching rates of helminth eggs as well as the survival of infective larvae [30]. Such environmental factors may have differing effects on the prevalence of parasite species in human populations, depending on biological characteristics of the parasites, duration of their life cycles, and transmission dynamics [30,31]. The study region is characterised by a relatively stable temperature (lower (daily average around 18°C) in June-August and higher (21°C) in March-May) and a pronounced variation in precipitation (dry (11mm) in December-February and wet (264mm) in June-August) [from Climatemps.com; available: <http://www.gonder.climatemps.com/>. Accessed: 19 October 2019]. The frequency of *Ascaris* and hookworm peaked in December-February, when it is relatively cool and very dry. By contrast, *Entamoeba* peaked in June-August, when it is cool and very wet, and *Giardia* in September-November. Studies in other tropical countries such as Brazil and India have also found different seasonal trends in stool test results for different parasites [31,32].

The frequency of *Ascaris* diagnoses decreased in 2014 to 2016 and there was a marked increase in *Entamoeba* diagnoses in the same period. Although we do not have a direct explanation for this observation, it raises the question on whether the removal of certain parasites via MDA has unintended effects on other intestinal pathogens (bacteria, viruses, protozoa, and cestodes) occupying the same gastrointestinal niche. Studies in Bolivia, Bangladesh, and Ethiopia have suggested that deworming using mebendazole or

albendazole increases the risk of infection with intestinal protozoa and cestodes at the individual and the population level [33-35].

These findings have several implications for disease control. First, because the effect of MDA was smaller than we had anticipated and because no cross-sectional survey information is available for the study district in recent years, a closer monitoring of MDA coverage and effectiveness is needed in the future. Second, the information about seasonal variability can be used for the planning of interventions, e.g. the timing of MDA campaigns. A recent modelling study showed that the impact of MDA on *A. lumbricoides* could be substantially increased if it is given when the number of eggs and larvae (and the risk of reinfection) are at their lowest [30]. Third, the mirroring patterns of intestinal parasites and protozoa within and across the years are important, as these infections require different treatments.

## Conclusion

Despite the limitations of routine laboratory data, this study provides valuable information for the planning and monitoring of control activities for intestinal parasites. Trends in routine laboratories may be a proxy for a status quo in the community. These findings suggest that higher MDA coverages and/or interventions beyond MDA are needed to reduce intestinal parasite-related morbidity.

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