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-Chapter 6-

Impact of repeated mass treatment on human *Oesophagostomum* and hookworm infections in Northern Ghana

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Abstract

Oesophagostomum bifurcum is a common parasite of man causing disease in parts of northern Ghana and northern Togo. The impact of repeated mass treatment with albendazole on infection with *O. bifurcum* and hookworm is analysed and the results compared with those in a control area where no treatment was given.

At baseline, *O. bifurcum* and hookworm prevalences were 53.0% and 86.9% respectively (n = 1011 subjects). After twelve months, following two rounds of albendazole treatment, prevalences decreased significantly to 5.4% for *O. bifurcum*, and 36.8% for hookworm (n = 535 subjects). Twenty-four months after the baseline survey and following a total of four rounds of treatment, prevalences were further reduced to 0.8% and 23.4% for *O. bifurcum* and hookworm respectively (n = 478 subjects). Overall, there was a significant decrease in the geometric mean larval counts of O. *bifurcum* from 3.0 to 0.1 and of hookworm from 47.2 to 1.8. The fourth mass treatment was carried out in April 2003 by the Lymphatic Filariasis (LF) Elimination Programme. Overall, compliance to treatment varied from 70% to 80%.

In the control area, *Oesophagostomum* –prevalence increased from 18.5% to 37.0% and the intensity from 0.4 to 1.4. For hookworm, both prevalence (86.1% to 91.3%) and intensity (54.8 to 74.3) increased but not to a significant level.

The prospects of eliminating human oesophagostomiasis from the intervention area, while simultaneously achieving an important reduction of hookworm prevalences by albendazole mass treatment are discussed.

Introduction

Twenty years ago, *Oesophagostomum bifurcum* (*O. bifurcum*) infections in humans were considered rare zoonoses and the parasite was thought not to be able to complete its life cycle in man (Barrowclough & Crome, 1978). However, in parts of northern Ghana and in northern Togo, human-to-human transmission is common, mostly occurring during or shortly after the rainy season. In this endemic area, the prevalence of *O. bifurcum* infection, determined by the coproculture method is known to be high with approx. 250.000 persons infected and it has been estimated that a million more are at risk of being infected (Gigase *et al*., 1987; Polderman *et al*., 1991; Polderman *et al*., 1999). About 40% of the population may develop sub-clinical nodular pathology of which some may progress to clinical oesophagostomiasis (Storey *et al*., 2000a; Ziem *et al*., 2005a). Hookworm infection is also common in this area with village prevalences ranging from 48% to more than 90% (Pit *et al*., 1999a; Polderman *et al*., 1999; Yelifari *et al*., 2005).

The exact mode of transmission of *O. bifurcum* infection in humans is unknown, but the route of infection is assumed to be oral similar to other *Oesophagostomum* species of veterinary importance. In ungulates and pigs, ingested third stage larvae (L3-larvae) develop into a tissue stage in the intestinal wall and eventually return to the intestinal lumen to develop into egg-producing adult worms. Eggs excreted with host faeces and deposited into soil will, with adequate temperature and moisture, hatch and develop into infective third-stage larvae within 3-7 days, ready to infect a new host (Dash, 1973). Poor sanitation and unhygienic conditions prevailing in the endemic area may constitute major risk factors to acquiring new infections.

Symptoms and severity of clinical oesophagostomiasis are related to the development of nodular lesions in the colon wall or in the anterior abdominal wall and usually range from fever and mild abdominal pain to acute abdominal emergencies (Gigase *et al*., 1987, Haaf & van Soest, 1964).

Diagnosis of infection has to be made by coproculture since eggs of *O. bifurcum* and hookworm cannot be distinguished morphologically (Blotkamp *et al*., 1993). In small-scale trials conducted by Krepel and co-workers using albendazole, pyrantel pamoate, levamisole and thiabendazole, they concluded that albendazole, in a single 400–800 mg dose was most likely to give a parasitological cure of *O. bifurcum* infection (Krepel *et al*., 1993). In an earlier study to measure the shortterm impact of albendazole treatment, Ziem and co-workers showed that a single 400 mg dose of albendazole gives a parasitological cure of 98% for *O. bifurcum* but a much lower cure rate for hookworm (Ziem *et al.,* 2005b). Albendazole is currently the drug of choice for the treatment of intestinal nematodes in Ghana and is well tolerated when given as a single dose. In combination with ivermectin, it is now also used in the national Lymphatic Filariasis Elimination Program.

The vulnerability of *O. bifurcum* to albendazole treatment coupled with the limited distribution of infection gives good prospects for transmission control through repeated mass treatment. The aim of the present study was to explore the effects of repeated mass treatment, at the population level, with a single 400 mg dose of albendazole, on *O. bifurcum* and to compare the effects with the impact on hookworm transmission. The decline in prevalence and intensity of *O. bifurcum* and hookworm infection are evaluated after two and four rounds of mass treatments and the results compared with those in a control group where no mass treatment had been given. Subsequently, the remaining infections are analysed in relation to the subjects' history of compliance with treatment to differentiate between infections surviving treatment and continued transmission.

Subjects and methods

Study area

The study was conducted in the Garu district of the Upper East Region in northeast of Ghana and covers an area of about 150 km² along the Ghana-Togo Border. Fig. 6.1 shows the study area and the distribution of villages within the area.

The study area comprised 29 villages characterized by loose clusters of compounds scattered over the area. Approximately 18,000 inhabitants live in 1570 compounds and are from five tribes. The majority belong to the Bimoba (78%), with Kusasi (14%), Mamprusi (3%), Fulani (3%) and Busanga (2%) forming other tribal groups in the area. Most are subsistence farmers (89% of the adult males) or small-scale traders. The research area is located in the Guinea Savannah vegetation belt with savannah grasslands. The climate is typically sub-Sahalian with a long dry season and a single rainy season between May and September.

Subjects and sampling

Prior to the study, all villages and compounds in the study area were mapped with a Global Positioning System (GPS 12, Garmin International, Olathe, KS 66062 USA) and all villages, compounds and individuals were registered and assigned unique identification numbers, since civil registries are absent in the area. Twenty-four neighbouring villages (villages 1-24) were selected to be used as an intervention area. Similarly, 5 neighbouring villages (villages 25-29) were chosen to serve as a control. The division of the study area into intervention and control areas is shown in fig 1.8. (page 13). The tribal constitution and the geophysical aspect of those villages was the same as that of the intervention villages.

In September and October 2001, a cross sectional survey of 10% of the inhabitants of the area was conducted during which parasitological baseline data were obtained to determine the prevalence and intensity of infection with *O. bifurcum* and hookworm. In September-October 2002 and in the same months in 2003, follow-up surveys were carried out in the intervention area. In the control area, only one follow-up survey was conducted in September-October 2002. The September-October 2003 survey was postponed to the year after because the Lymphatic Filariasis (LF) Elimination Program carried out mass treatment in northern Ghana (including the control area), which disrupted the originally planned prospective study.

In each follow-up survey, 5% random samples of inhabitants, who did not participate in an earlier survey, were selected on compound basis in both the intervention and the control areas. Mass treatments with albendazole were carried out in the treatment area shortly after the baseline survey and subsequently in April 2002 and October 2002. In April 2003, the Lymphatic Filariasis (LF) Elimination Program gave further mass treatment which contained both albendazole and ivermectin.

Diagnosis and treatment of infections

Details on the program schedule of parasitological examinations and mass treatments are given in fig. 1.10 (page 20-21).

All selected compounds in the study and the control areas were visited and each inhabitant was given a labelled plastic container to produce a stool sample for laboratory examination. The samples were collected the next morning and immediately afterwards examined for helminths eggs, using a single 25-mg Kato smear, to determine the presence and numbers of 'hookworm-like' eggs.

Since eggs of hookworm and *O. bifurcum* are morphologically identical, differential diagnosis had to be based on coproculture. On the day the stool samples were received, 6-gram sub-samples were mixed with equal volumes of Vermiculite; the mixture was divided into three equal portions (2 gram) and cultured in three Petri dishes. Two of the cultures were examined 5–7 days later, at low power (x100) by two different microscopists. The third culture was a back up in case a culture was spoiled due to gross contamination with maggots or fungi. The third-stage larvae of *O. bifurcum* and hookworm were identified, differentiated and counted according to the procedure used by Blotkamp and co-workers (1993).

In the intervention area, all inhabitants were offered treatment with a single oral dose of 400 mg albendazole (Zentel®, GlaxoSmithKline, Mayenne, France) in late October 2001. In the control area, only individuals who were examined and found to be infected with *O. bifurcum* and/or hookworm were offered treatment; all other inhabitants did not receive treatment. In late April 2002 and in October 2002, two further rounds of mass treatment with 400 mg of albendazole were given in the study area and again no treatment was offered in the control area. Mass treatment in the intervention area was done through GPS-guided compound visits and treatment administration was directly supervised. During mass treatment children of age 3 years and below and pregnant women were excluded from treatment. In April 2003, another mass treatment of albendazole together with ivermectin was given; this time by the Lymphatic Filariasis (LF) Elimination Programme to all inhabitants in both the intervention and in the control area. During the LF treatment pregnant women and children below 3 years were excluded.

Data analysis

The parasitological and treatment data were entered using EpiInfo, version 6 (CDC, Atlanta, GA, USA). Statistical analysis was performed using SPSS statistical package, version 11.1 (SPSS Inc, Chicago, IL, USA). Individual larval counts, calculated as the sum of the number of larvae found in 2 cultures made from one 4 gram-stool sample, were recorded at baseline and during follow-up surveys.

Infection and larval counts were expressed as prevalence figures stratified by intensity-class at the individual level: as the number (or fraction) of subjects with "heavy infection" (larval count >100 larvae in two cultures), "moderate infection" (larval count 33-100 larvae in two cultures), "light infection" (larval count <33 larvae in two cultures) and "no infection" (no larvae in two cultures). The intensities of infection are also expressed as the geometric mean larval counts (gmlc) among all subjects examined (negatives included).

Differences in prevalence of infection between subjects infected with *O. bifurcum* and hookworm at baseline and after treatment and between the treated and control groups were analysed using the Chi-square test. The distribution of infection within the intensity classes at baseline and after treatment and between the intervention and the control groups was also analysed with Chi-square test with two degrees of freedom. The changes in the gmlc following treatment were analysed using non-parametric tests (Mann-Whitney). A *P*-value of less than 0.05 was used to imply statistical significance for all tests.

Ethical considerations

Ethical clearance for the study was obtained from the Ghana Health Service (GHS) and a witnessed informed consent procedure was followed as described previously (Ziem *et al,* 2005). Each time, both during the parasitological surveys and during mass treatments, the villagers were informed of the purpose of the project and asked for their consent to participate. The project was also approved by the Danish Central Scientific-Ethical Committee of Denmark.

Results

Data on the project timetable, the numbers of subjects living in the area and participating in the research as well as the number of persons treated during mass treatment are summarized in Fig. 1.10. (page 20-21). The number of subjects examined in the intervention area varied between 478 [243 (50.8%) males and 235 (49.2%) females] to 1011 [505 (50%) males and 506 (50%) females]. The ages ranged from 1-99 with median ages of 14-15 years. In the control area, the number of subjects examined varied from 173, [68 (39.3%) males and 105, (60.7%) females] to 303, [144 (47.5%) males and 159 (52.5%) females]. The ages ranged from 1-95 years with median ages of 14-17 years. The participants' ages did not significantly vary from one survey to another. Between 70% and 80% of eligible subjects in the intervention area was always treated.

Table 6.1 shows that the prevalence of *O. bifurcum* infection was high in the intervention area prior to treatment (53.0%) but significantly lower in the control area (18.5%), (χ^2_1= 112.3, $P < 0.001$). The gmlc was also higher in the intervention area than in the control area. For hookworm such differences between infection rates and intensities of infection in the intervention and control area did not exist. The prevalences were 86.9% and 86.1% in the intervention and the control area and the gmlc were 47.2 and 54.8 respectively.

One year later, after two rounds of mass treatment, both infection rates and the gmlc were considerably lower in the intervention area. The prevalence of *O. bifurcum* fell from 53.0% to 5.4%, (χ^2_1=341.8, $P < 0.001$), and that of hookworm from 86.9% to 36.8%, (χ^2_1 = 415.4, $P < 0.001$). The gmlc also fell significantly by a factor of 25-30 ($P = 0.001$) for both *O. bifurcum* and hookworm. Only 3 out of the 29 (10.3%) *O. bifurcum* positive subjects had moderate or heavy infections after the two mass treatments, while 144 (26.9%) of the 536 infected subjects were moderately or heavily infected prior to intervention. For hookworm, there was a significant shift from heavy to light infections (χ^2_2=150.3, $P < 0.001$) (Table 6.1).

In contrast, in the control area there was a significant increase in *O. bifurcum* prevalence (from 18.5 to 37.0, χ^2_1 = 20, $P < 0.001$) and intensity (from 0.4 to 1.4, P<0.001) but for hookworm, the increase in prevalence and intensity of infection was not significant (Table 6.1).

A year later, in 2003, after two more rounds of mass treatment, the numbers of *O. bifurcum* infected subjects were further reduced from 5.4% to 0.8%, (χ^2_1 =16.8, $P < 0.001$). Due to the small numbers of positive cases, comparison of the intensities of infection is not reliable but of the four infected cases, one was heavily infected, with a larval count of over 100. For hookworm the infection rate went down from 36.8 to 23.4%, (χ^2_1 =21.4, $P < 0.001$) with a significant reduction of gmlc from 1.8 to 0.9 ($P = 0.001$) but the frequency distribution of heavy, moderate and light infections remained unchanged (χ^2_2=1.52, $P < 0.46$).

Table 6.1. Prevalence and intensity of *O. bifurcum* and hookworm infections as determined by examination of copro-culture before intervention, after two rounds of mass treatment and after four rounds of mass treatment.

	Intervention area			Control area		
	n	% positive	gmlc[1]	n	% positive	gmlc[1]
	Baseline survey (September 2001)			Baseline survey (September 2001)		
Number examined	**1011**			**303**		
***O. bifurcum* positive**	**536**	**53.0**	**3**	**56**	**18.5**	**0.4**
Larval count <33	*392*	*73.1*		*56*	*100.0*	
Larval count 33-100	*94*	*17.6*		*0*	*0.0*	
Larval count >100	*50*	*9.3*		*0*	*0.0*	
Hookworm positive	**879**	**86.9**	**47.2**	**261**	**86.1**	**54.8**
Larval count <33	*242*	*27.5*		*52*	*19.9*	
Larval count 33-100	*184*	*20.9*		*70*	*26.8*	
Larval count >100	*453*	*51.5*		*139*	*53.3*	
	Following 2 rounds of treatment (September 2002)			Control area, one year later (September 2002)		
Number examined	**535**			**173**		
***O. bifurcum* positive**	**29**	**5.4**	**0.1**	**64**	**37.0**	**1.4**
Larval count <33	*26*	*89.7*		*51*	*79.7*	
Larval count 33-100	*2*	*6.9*		*13*	*20.3*	
Larval count >100	*1*	*3.4*		*0*	*0.0*	
Hookworm positive	**197**	**36.8**	**1.8**	**158**	**91.3**	**74.3**
Larval count <33	*139*	*70.6*		*33*	*20.9*	
Larval count 33-100	*40*	*20.3*		*38*	*24.1*	
Larval count >100	*18*	*9.1*		*87*	*55.1*	
	Following 4 rounds of treatment (September 2003)			Control area, no survey (September 2003)		
Number examined	**478**					
***O. bifurcum* positive**	**4**	**0.8**	**0.02**			
Larval count <33	*3*	*75*				
Larval count 33-100	*0*	*0*				
Larval count >100	*1*	*25*				
Hookworm positive	**112**	**23.4**	**0.9**			
Larval count <33	*82*	*73.3*				
Larval count 33-100	*17*	*15.2*				
Larval count >100	*13*	*11.6*				

Table 6.2. Prevalence of remaining infections of *O. bifurcum* and hookworm in the intervention area in relation to numbers of treatments given.

	Number examined	Subjects treated		Subjects not treated	
		Treated twice	Treated once	"Immi-grants"	Children age ≤3yrs
No of subjects	535	378	50	23	84
No *O. bifurcum* positive	29	16	4	5	4
% *O. bifurcum* positive	**5.4**	**4.2**	**8.0**	**21.7**	**4.8**
No hookworm positive	197	138	22	13	24
% Hookworm positive	**36.8**	**36.5**	**44.0**	**56.5**	**28.6**

The impact of treatment was not only assessed at the population level but could also be evaluated at the individual level since individual treatment records were kept. The association between the number of treatments received by individuals and the infection rate in September 2002 is presented in Table 6.2. The table shows that for *O. bifurcum* the infection rates are 4.2% for those treated twice and 8.0% for those treated once only. Newly arrived immigrants who had not been treated had an infection rate of 21.7%. Of those not treated because they were too young, four out of 84 appeared infected. For hookworm infections, the new immigrants who were not present during mass treatment were also the most infected group but the difference with those treated once or twice is much smaller. The infection rates in the fully treated individuals remained fairly high.

In Fig. 6.1, the evolution of the infection rates with age in young untreated children is given for both *O. bifurcum* and hookworm. The age-specific infection rates in children during the baseline survey in 2001 suggested a yearly increase of 15 to 20% for *O. bifurcum* and hookworm. For *O. bifurcum,* the young children quickly lost their infection and no new infections appear to have been acquired in the intervention area. With hookworm, the infection rates in the 2-3 year old children went down as well after 2 rounds of treatment, but in children of less than two years of age, 7 out of 23 were infected in September 2002 after 2 rounds of mass treatment, and 7 out of 34 were infected in 2003, after 4 rounds of mass treatment.

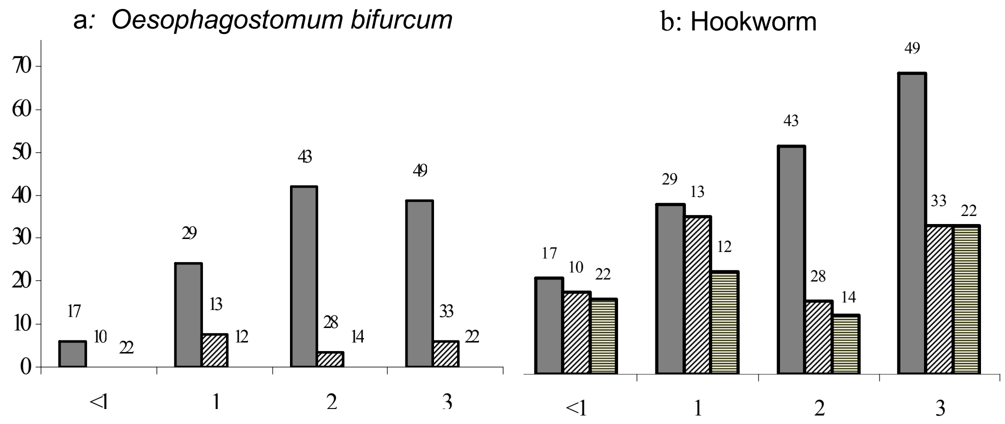

Fig. 6.1 Prevalence of *O. bifurcum* (fig. 6.1a) and hookworm (fig. 6.1b) infection among children age of three years or younger at baseline and after treatment.
Solid bars indicate prevalence of infection in 2001.
Diagonally hatched bars indicate prevalence of infection in 2002.
Horizontally hatched bars prevalence of infection in 2003.

Discussion

Control of soil transmitted helminthiasis by population-based mass treatment tends to be jeopardized by rapid re-infection due to the persistence of reservoirs of infection in untreated subjects and immigrants, long survival of eggs or larvae in the environment, poor cure rates of the drugs used and low compliance with treatment.

Effective morbidity control and significant reductions in the parasite load in an infected population can be achieved, even when cure rates are less than complete, when infected and untreated immigrants enter the treatment area or when compliance is less than perfect. A relatively greater impact of treatment may be expected if the transmission period is short. This is the case in northern Ghana, where hookworm transmission is thought to take place mainly during and shortly after the rainy season (Krepel *et al*., 1993). During the baseline survey of the present study, the prevalence and intensity of hookworm infections were high, but a satisfactory reduction in prevalence was obtained after mass treatment. This is likely to be due to the combined effects of a stable rural population, a detailed registration of inhabitants, the fact that over 70% of the eligible population was given treatment, and undoubtedly to the short transmission season. Nevertheless a quarter of the population remained infected.

For *O. bifurcum*, the results were significantly better than for hookworm. First of all, the albendazole cure rate against *O. bifurcum* has been shown to be extremely high and much better than that for hookworm (Krepel *et al*., 1993; Ziem *et al*., 2005b). Secondly, prevalence and intensity of *O. bifurcum* at the onset of the study were definitely lower than that of hookworm. Thirdly, the geographic distribution of endemic human oesophagostomiasis is very limited (Pit *et al*., 1999a; Yelifari *et al*., 2005) and importation of new infections from non-treated immigrants into the intervention area may have been fairly uncommon; a large proportion of the infected area was covered by mass treatment.

In spite of the marked reduction of *O. bifurcum* infection rates, even among those who received two rounds of treatment with albendazole (cure rate >98%; Ziem *et al*., 2005b), as many as 4.2% remained infected (Table 6.1). This could be due to refusal to swallow the drug, to continued transmission, explained by a remaining reservoir of free-living infective larvae, or by the importation of infection with subjects coming in from outside the intervention area. Because a system of directly observed treatment was used during mass treatments, the first explanation would seem unlikely. The role of migration can hardly be overestimated as there is a lot of movement between villages to visit relatives, markets and schools, and for similar reasons people from villages outside the intervention area may visit and settle for varying periods in the intervention area. Such movements may play an important role in the dissemination of soil transmitted helminths- and other infections.

Following two further rounds of treatment between September 2002 and September 2003, 4 (0.8%) and 112 (23.4%) subjects remained infected with *O. bifurcum* and hookworm respectively (Table 6.1). This important further reduction of *O. bifurcum* infection in September 2003 indicates that in the transmission season of 2003 (June-September 2003), very few new infections were acquired or brought into the intervention area. This is in sharp contrast to the events during the transmission season of 2002. The principal difference between both transmission seasons would seem that in April 2003, treatment was given in a much wider area than before: all villages in northern Ghana and also in most in neighbouring northern Togo were treated by the LF-programme. In that way, importation of cases from beyond the study area and the creation of nuclei of transmission around cases of imported infection in inwards migrating persons was avoided. Theoretically the addition of ivermectin to the standard dose of albendazole, as used by the LF, may also have had a beneficial effect. This effect, however, was probably small since the efficacy of albendazole alone (98%) could hardly be improved by adding ivermectin.

In hookworm infection, the picture is rather different and less clear as the efficacy of albendazole treatment is much lower compared to that of *O. bifurcum.* For hookworm, it is impossible to reliably distinguish between infections that survived treatment, infections imported from outside the intervention area and infections that were newly acquired. The infection rate in the second year decreased by no more than 36% (from 36.8 to 23.4%) while the intensity of infection among those infected remained almost the same. To some extent the further reduction of the hookworm-prevalence observed in the second year of mass treatments (Table 6.2) may be associated with the addition of ivermectin. In *Trichuris* infections, for instance, the efficacy of the albendazole-ivermectin combination has been shown to be significantly better than albendazole only (Ottesen *et al*., 1999; De Rochars *et al*., 2004).

The comparatively high infection rate in hookworm, even after 4 rounds of mass treatment, can at least partly be explained by the use of the comparatively more sensitive stool culture. In the present *Oesophagostomum*-hookworm research in northern Ghana it has been clearly demonstrated that infection rates measured with Kato or coproculture were similar prior to intervention, when worm loads were high. Examination of Kato-smears after mass treatment, however, when worm loads were much smaller, resulted in lower detection rates than those obtained with coproculture. Based on coproculture the prevalence of hookworm-infection was 36.5%, in September 2003; based on examination of a single 25-mg Kato smear it was only 27.9% (Ziem *et al*., submitted).

To measure whether transmission is still continuing, a study on incidences would have been appropriate. However, since no individuals were followed in the present study, incidence could not be measured. Similar information can be obtained by the analysis of series of cross sectional infection rates in different age-classes. Figure 6.1 shows that in *O. bifurcum*, the infections were quickly lost in young children in the intervention area and no new infections were acquired. In hookworm, the infections found among 2 and 3 years of age might reflect infections acquired before the onset of mass treatment, but those of the younger children, of 1 and 2 years of age can only reflect ongoing transmission.

In conclusion, the data suggest that in the case of *O. bifurcum*, the transmission cycle may be broken and elimination might be achieved if a similar reduction of infection can be achieved in the entire endemic area. Careful follow-up studies in the next few years and expansion of intervention to the surrounding endemic area at large, will show whether this hypothesis is valid. Efforts to achieve control on a much larger scale are now in progress, in close collaboration with the Lymphatic Filariasis Elimination Programme, both in Ghana and in Togo.

The data also indicate that the transmission cycle of hookworm is considerably more robust and continuously repeated treatment will be needed to consolidate the low prevalence and intensity of infection achieved after the four rounds of treatment in 2001-2003.

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