Prevalence and intensity of *Onchocerca volvulus* infection and efficacy of ivermectin in endemic communities in Ghana: a two-phase epidemiological study

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Summary

Background **Ivermectin has been used for onchocerciasis control since 1987. Because of the long-term use of this drug and the development of resistance in other nematodes, we have assessed *Onchocerca volvulus* burdens, effectiveness of ivermectin as a microfilaricide, and its effect on adult female worm reproduction.**

Methods **For the first phase of the study, 2501 individuals in Ghana, from 19 endemic communities who had received six to 18 annual rounds of ivermectin and one ivermectin naive community, were assessed for microfilarial loads 7 days before the 2004 yearly ivermectin treatment, by means of skin snips, and 30 days after treatment to assess the ivermectin microfilaricidal action. For the second phase, skin snips were taken from 342 individuals from ten communities, who were microfilaria positive at pretreatment assessment, on days 90 and 180 after treatment, to identify the effects of ivermectin on female worm fertility, assessed by microfilaria repopulation.**

Findings **487 (19%) of the 2501 participants were microfilaria positive. The microfilaria prevalence and community microfilarial load in treated communities ranged from 2·2% to 51·8%, and 0·06 microfilariae per snip to 2·85 microfilariae per snip, respectively. Despite treatment, the prevalence rate doubled between 2000 and 2005 in two communities. Microfilaria assessment 30 days after ivermectin treatment showed 100% clearance of microfilaria in more than 99% of people. At day 90 after treatment, four of ten communities had significant microfilaria repopulation, from 7·1% to 21·1% of pretreatment counts, rising to 53·9% by day 180.**

Interpretation **Ivermectin remains a potent microfilaricide. However, our results suggest that resistant adult parasite populations, which are not responding as expected to ivermectin, are emerging. A high rate of repopulation of skin with microfilariae will allow parasite transmission, possibly with ivermectin-resistant *O volvulus*, which could eventually lead to recrudescence of the disease.**

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Introduction

Onchocerciasis, commonly known as river blindness, is caused by the filarial nematode *Onchocerca volvulus*, and is transmitted by a blackfly vector from the genus *Simulium*. In 1995, WHO estimated that 17·7 million people were infected with *O volvulus*, of whom 270 000 were blind and another 500 000 were visually impaired.[1] In addition to visual impairment, onchocerciasis is associated with serious pathological changes of skin. Surveys show that the WHO figures are an underestimate of the extent of disease—37 million people are now thought to be infected, with 90 million at risk in Africa and more than 400 000 infected in Central and South America.[2]

Almost three decades of onchocerciasis control in Ghana by the Onchocerciasis Control Programme in west Africa (OCP) has brought great relief to millions of people living in infected communities, by restoration of previously abandoned villages and substantial reduction of onchocerca induced blindness and other clinical symptoms of the disease. With the close of the programme at the end of 2002, and the transfer of all subsequent onchocerciasis control activities to the participating countries,[3] the need for these countries to sustain the successful achievements is of great importance. Since the Ghana Onchocerciasis Control Programme assumed full responsibility for onchocerciasis control, one of its priorities has been to assess disease frequency, parasite burden, and field efficacy of ivermectin as a microfilaricide and its effect on suppression of reproduction in adult worms. This information is important for the planning and management of disease control. The control of onchocerciasis with ivermectin was initiated in OCP areas in 1987, either in conjunction with vector control or as the sole control agent. Ghana was one of the first countries to start large-scale ivermectin treatment under the OCP, and yearly ivermectin mass treatment still continues.

Ivermectin is the only drug available for mass treatment of onchocerciasis, through community directed treatment. It is given as a single annual dose of 150 µg/kg bodyweight. At this dose, it eliminates skin microfilariae and maintains very low skin microfilaria counts for up to 9 months.[4–7] At the standard dose, ivermectin does not kill substantial numbers of adult *O volvulus*, but temporarily blocks release of intrauterine microfilariae by the adult female worms.[8,9] With yearly ivermectin treatments over several years, people infected with adult worms normally show sustained suppression of skin microfilaria repopulation for 6–9 months. Subsequently,

low skin microfilaria counts are reported, resulting in a striking reduction in disease transmission.[10]

Despite these successes, genetic selection by ivermectin of *O volvulus* and persistence of skin microfilariae after several treatments with this drug have been reported.[11–17] Drug resistance to ivermectin has become a major obstacle to the control of nematode parasites of livestock worldwide.[18] Furthermore, ivermectin has been used for the past 19 years and the parasite seems to persist in some communities despite several yearly treatments. Assessment of the parasite burden and the efficacy of ivermectin as a microfilaricide and adult worm steriliser are therefore very important. This information is necessary for strategies to sustain the achievements of the OCP. Our aim was to investigate *O volvulus* endemicity, the field efficacy of ivermectin, and the effect of ivermectin on adult female worm reproduction.

Methods

Study design

A two-phase epidemiological study was undertaken in conjunction with the activities of the Ghana Onchocerciasis Control Programme, between Oct 4, 2004, and June 30, 2005. The study included ivermectin treatment and skin snipping to assess the parasitological profile at various time points before and after ivermectin treatment, to characterise *O volvulus* responses in terms of skin microfilaria clearance and repopulation rate by adult female worms after treatment. For the first part of the study, 2501 individuals were randomly selected from 19 onchocerciasis endemic communities that had satisfied the inclusion criteria, and an additional community, Begbomdo, which had never received ivermectin at the start of the study. A minimum of 50 participants were randomly selected from every community. The ivermectin-treated communities had received between six and 18 rounds of ivermectin (Mectizan, Haarlem, Netherlands) treatment before the study started. The first phase of the study was assessment of skin microfilaria load of all participants and post-treatment skin assessment on day 30 after ivermectin treatment. The second phase was follow-up at days 90 and 180 after ivermectin treatment for 342 participants, who were microfilaria positive at the start of the study and selected from ten of the 20 communities assessed in the first phase of the study.

The objectives and schedules of the study were explained to all eligible individuals, and those who participated signed a consent form. Ethical approval was obtained from the ethics review committees of Noguchi Memorial Institute for Medical Research, Ghana, and McGill University, Canada.

We decided to do this study in the savannah areas of Ghana, which are known to be associated with the severe form of onchocerciasis. We analysed available data and annual reports of the Ghana Onchocerciasis Control Programme, and obtained a list of 22 districts that had up-to-date records on ivermectin treatment history and coverage. Of these districts, 17 had average ivermectin treatment coverage of 50% or more over the previous 5 years. Treatment coverage was defined as percentage of the total community population who received ivermectin. Although children younger than 5 years, pregnant and early lactating women, and those who were sick were not given ivermectin, their numbers were included when treatment coverage was calculated. Inclusion criteria to select districts were first, good documentation on yearly community ivermectin treatment coverage and community and individual ivermectin treatment history, and second, at least one survey over the previous 6 years, with available information on microfilaria prevalence or community microfilarial load (CMFL), or both. We identified four districts that fully satisfied these criteria, which were located in two regions of Ghana—Brong-Ahafo and the Northern Region. Districts in the Brong-Ahafo included Kintampo in the Lower Black Volta River basin, Atebubu and Nkoranza in the Pru River basin, and the Gonja East District in the Daka River basin in the Northern Region.

To select the study communities, we obtained further information from the disease control officers of the four districts. Additional inclusion criteria for selection of study communities were average ivermectin treatment coverage of 55% or more for the previous 5 years, uninterrupted yearly ivermectin treatment over the previous 6 years, and an ivermectin treatment within the previous 10–12 months. With these criteria, 19 communities were selected. Begbomdo in the East Gonja district was selected from hundreds of communities investigated to find one that had truly never received ivermectin.

To ensure that we obtained an adequate number of participants from every community, two methods were adopted for recruitment depending on the size of the community. The first method, consisting of random sampling of individuals, was adopted for communities with adult populations (older than 18 years) of more than 300 people. Seven of the 20 communities had adult populations of more than 300. The second method, consisting of sampling the entire community, was used for communities with adult populations of less than 300.

For large communities, the investigators obtained community census data and met the chief and elders of the community to inform them of the proposed study and explain the methods and study design. Households were randomly selected in these communities. In all communities, households with at least one adult aged 18–65 years were assigned sequential numbers. From a database of all households enumerated, a random list of household numbers was generated with a computer-based random-number generator. Households were then recruited sequentially from the randomised list.

We confirmed the treatment history of all participants from the recruited household by checking the ivermectin

treatment records and by interviewing the participants and community ivermectin distributors. All adults aged 18–65 years in recruited households who were willing to participate in the study were assessed for eligibility. Individuals who met the inclusion criteria were enrolled. The inclusion criteria were permanent resident of the community for at least the previous 6 years, available ivermectin treatment history for at least the previous 6 years, ivermectin treatment received within the previous 11–12 months, and no contraindication for ivermectin. 10–15% of eligible people declined to participate in the study.

In smaller communities, two investigators met the chief and elders to inform them of the study and explain the methods and study design. For all communities, a day was scheduled by the chief to enable sampling of the entire community. All adults aged 18–65 years who were willing to participate in the study were assessed for eligibility with a standardised questionnaire by interview. Inclusion criteria were as for the larger communities. 8–12% of eligible people declined to participate in the study.

For the second phase, communities were selected on the basis of the following selection criteria—at least ten rounds of yearly ivermectin treatment received, average community ivermectin treatment coverage 55% or more over the previous 5 years, and the community should be accessible by road throughout the study period, especially during the rainy season. Of the 20 communities studied in the first phase, ten met all these criteria (nine communities with histories of 10–18 yearly ivermectin treatments, plus the ivermectin-naive community). 342 people from these ten communities were recruited for the second phase of the study. All subjects were microfilaria positive at pretreatment assessment and had no interruption of yearly ivermectin treatment over the previous 5 years.

Sampling procedures

Participants from all communities were prepared for skin snipping at four different time points—7 days before the yearly ivermectin treatment and 30, 90, and 180 days after treatment. Two skin snips were taken from the iliac crest of every person with a 2 mm Holth-type corneoscleral punch. Skin snips (average weight 1·0 mg) were placed in 96-well microtitre plates containing a few drops of physiological saline solution, and microfilariae were

	Rounds of ivermectin treatment	Number examined	Nodule prevalence at 7 days pretreatment	Microfilaria prevalence at 7 days pretreatment	CMFL at 7 days pretreatment (microfilariae per snip)	Microfilaria prevalence at 1 month post-treatment	CMFL at 1 month post-treatment (microfilariae per snip)
Atebubu district							
Akrakuka	12	139	7·2%	21·6%	0·91	0	0
Asubende	19	79	5·6%	13·9%	0·62	0	0
Baaya	18	161	6·9%	8·7%	0·28	0	0
Beposo	18	124	4·0%	4·8%	0·39	0	0
Faoamang	18	225	3·8%	9·7%	0·34	0	0
Hiampe	18	238	5·1%	5·0%	0·21	0	0
Mantukwa	17	176	3·3%	14·3%	0·48	0	0
Mempeasem	18	66	2·1%	6·1%	0·31	0	0
Senyase	18	187	6·0%	4·3%	0·36	0	0
Kintampo district							
Dwere	7	66	4·5%	7·5%	0·09	0	0
Gomboi	7	64	3·1%	7·8%	0·27	0	0
Kyingakrom	17	61	22·9%	50·8%	2·85	1·63%	0·0070
New Longoro	17	174	16·1%	35·8%	1·42	1·72%	0·0120
Nkoranza district							
Ayerede	12	139	2·9%	2·2%	0·06	0	0
Nyemeberekyere	6	142	12·7%	21·1%	0·96	0	0
East Gonja district							
Bankaba	6	72	9·7%	15·0%	0·97	0	0
Chabon	6	110	21·8%	51·8%	2·51	2·72%	0·0110
Jagbenbendo	12	150	19·3%	43·3%	2·12	0·70%	0·0047
Wiae	10	52	15·4%	38·5%	1·20	0	0
*Begbomdo	1	76	40·8%	72·4%	15·95	3·95%	0·0277

Community ivermectin treatment history includes the ivermectin treatment given during the study (day 0). *Begbomdo community was ivermectin naive before study treatment.

Table 1: **CMFL and nodule and microfilaria prevalence rates for *O volvulus*, before treatment and 1 month after ivermectin treatment, in 20 onchocerciasis-endemic communities in Ghana**

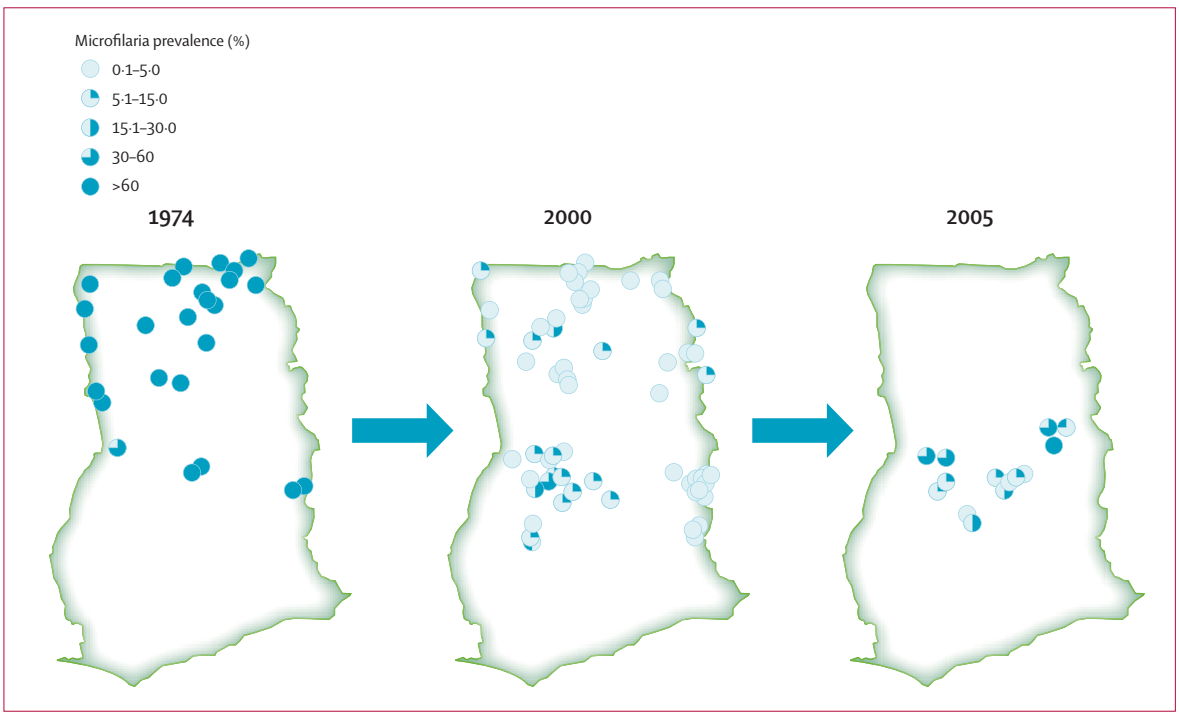

Figure: **Microfilaria prevalence rates in some endemic communities over the past three decades of onchocerciasis control**
Microfilaria prevalence in 1974 and 2000 is adapted from reference 20. The circles for 2005 represent only the microfilaria prevalence rates found in the study areas.

harvested under a dissecting microscope after 24 h incubation. Average microfilarial counts for the two sites were scored as microfilariae per snip.

A week before ivermectin treatment, all participants were examined to establish the presence and number of onchocerca nodules, and skin snips were taken to assess the pretreatment microfilaria load of individuals and to measure the microfilarial prevalence of every community. Ivermectin was then given to all adults at a standard dose of 150 µg/kg bodyweight. At day 30 after ivermectin treatment, microfilaria load was assessed again in people who were microfilaria positive before treatment to establish the microfilaricidal action of ivermectin. For the second part of the study, participants were skin snipped again on days 90 and 180 after ivermectin treatment, to identify early signs of skin microfilaria repopulation by adult worms and any further build-up of skin microfilariae at day 180.

Indices and statistical analyses

Three parasitological indices were calculated to assess the degree of endemicity in the study communities. Nodule and microfilariae prevalence were calculated as the percentage of participants having nodules and microfilaria in skin snips, respectively. Intensities of infection in the communities were also assessed with CMFL, the reference index used in the OCP, as the geometric mean of individual microfilaria loads (including zero counts) in people aged 20 years or older. The calculation was done with the log (x+1) transformation, where x is the individual microfilaria load.[19] The microfilaria densities in the ten communities examined in the second part of the study were compared by use of a Kruskal-Wallis non-parametric test. Pair-wise comparisons of microfilaria densities were also done with the non-parametric Mann-Whitney *U* test. Differences were regarded as significant at $p<0·05$.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 2501 patients assessed for skin microfilariae, 487 were positive at pretreatment assessment, giving an overall microfilaria prevalence of 19·5%. The prevalence, however, varied greatly between communities (table 1). 12 (63%) treated communities had microfilaria prevalence of 15% or less and nodule prevalence of less than 8%, whereas five (26%) treated communities had microfilaria prevalence of more than 35% and nodule prevalence of 15·4–22·9%. CMFL of the treated communities showed a somewhat similar pattern, with 14 (74%) of the treated communities having CMFL of less than 1 microfilaria per snip. However, five (26%) communities had CMFL of 1·20–2·85 microfilariae per snip. The ivermectin-naive community had a high microfilaria prevalence, nodule prevalence, and CMFL (table 1). Of the treated communi-

ties, Kyingakrom had the highest endemicity, despite having received 17 rounds of ivermectin treatment.

The Gonja East district (Bankaba, Chabon, Jagbenbendo, Wiae) had higher endemicity than the other districts, with a mean microfilaria prevalence rate of 37·2%, nodule prevalence of 16·6%, and CMFL of 1·7 microfilariae per snip. The Kintampo district (Dwere, Gomboi, Kyingakrom, New Longoro) had a 25·5% microfilaria prevalence, 11·7% nodule prevalence, and CMFL of 1·2 microfilariae per snip. Nkoranza District (Ayerede, Nyemeberekyere) had a microfilaria prevalence of 11·7%, nodule prevalence of 7·8%, and CMFL of 0·5 microfilariae per snip, whereas Atebubu District (Akrakuka, Asubende, Baaya, Beposo, Faoamang, Hiampe, Mantukwa, Senyase) had the lowest mean microfilaria prevalence of 9·3%, nodule prevalence of 4·3%, and CMFL of 0·43 microfilariae per snip.

Some communities selected for this study were also assessed for microfilaria prevalence during 2000, in the epidemiological study undertaken by the National Onchocerciasis Control Team.[20] Comparison of microfilaria prevalence rates of some communities studied in both 2000 and 2005 (figure) showed that New Longoro and Kyingakrom (with ivermectin treatment coverage in 2000 of 61·8% and 60·0%, respectively) had an increase in microfilaria prevalence from less than 15·1% in 2000, to 35·8% and 50·8%, respectively, in 2005. These results represent an increase of more than 100% in microfilaria prevalence rates over 5 years, despite yearly community treatment with ivermectin.

The microfilaricidal effect of ivermectin was assessed 1 month after treatment, and we noted an overall reduction of more than 99% in skin microfilaria load. Of the infected participants assessed 1 month after treatment, there was 100% reduction in skin microfilaria loads in more than 99%. Furthermore, of the 20 communities, 15 had complete skin microfilaria clearance in all infected individuals. New Longoro was the community with the highest microfilaria prevalence at day 30 after treatment, with a prevalence of 1·7% (CMFL 0·012 microfilariae per snip).

The ivermectin-naive community (Begbomdo) can be used as a baseline to compare both microfilaria densities and repopulation of the skin by microfilaria as a proportion of pretreatment skin microfilaria densities in the nine treated communities in the second part of the study. The mean microfilaria density was significantly higher in Begbomdo 7 days before treatment than in all nine treated communities (p=0·001). However, at day 30 after treatment, there was no difference in microfilaria counts between the previously naive community and the treated communities, showing the sustained efficacy of ivermectin against microfilaria in both naive and ivermectin-treated populations. Skin microfilaria counts were significantly higher in Begbomdo than in five of the communities that had received several rounds of ivermectin at both day 90 (highest p=0·034) and day 180 (highest p=0·013). However, the other four communities, Jagbenbendo, Kyingakrom, New Longoro, and Waie, showed no significant difference in skin microfilaria counts compared with the naive community at day 90 and day 180 after treatment. Microfilaria counts were significantly higher in Kyingakrom and Jagbenbendo, at both day 90 and day 180, than in all other treated communities (highest p=0·016), whereas New Longoro and Wiae had significantly higher skin microfilaria counts than did the five communities that showed good responses to ivermectin (highest p=0·05) at either day 90 or day 180 after treatment.

Microfilaria assessments at days 90 and 180 after treatment for the ten selected communities were also used as a measure of skin microfilaria repopulation, by adult female worms, in terms of percentage of pretreatment counts. To have a power of test of 80% with a mean pretreatment microfilaria count of 13·9 microfilariae per snip and an expected reduction of 80% in counts at day 180 after treatment, a sample size of 297 was necessary. Of the 342 individuals recruited for the second phase of the study, skin snips were taken from 301 on all occasions. Three had died of causes unrelated to onchocerciasis or treatment. Ten were not prepared to continue the study because of discomfort caused by skin snipping, 16 had gone on brief visits to other communities and were not available for the day 90 sample collection, and another 12 were not available for assessment at day 180.

Day 90 assessment showed an increase within expectations (<6% of pretreatment count)[11] in skin microfilaria repopulation in six communities, including the ivermectin-naive community (table 2). However, there were unexpectedly large increases in skin microfilaria repopulation, between day 30 and day 90, in four communities (Kyingakrom, Jagbenbendo, New Longoro, and Waie; table 2). The day 180 assessment showed an alarming pattern of skin microfilaria repopulation in two communities, Kyingakrom and Jagbenbendo, with repopulation densities higher than 30% of pretreatment counts.

The geometric mean microfilaria densities noted at day 30 after treatment in the four communities that showed poor responses (0–0·04 microfilariae per snip) provide unequivocal confirmation that the participants consumed the administered ivermectin. The treatment histories (17–19 rounds) and coverage do not significantly differ between communities in the Atebubu District, which showed expected responses to ivermectin treatment, and the two communities in the Kintampo District (New Longoro and Kyingakrom), which showed poor parasitological responses to treatment. With the exception of 2001, when the treatment coverage of New Longoro and Kyingakrom was 58·6% and 52·8%, respectively, the ivermectin treatment coverage over the previous 5 years in all nine treated communities was between 65·0% and 87·5% of the total population.

Table 3 shows the distribution of patients with no microfilaria and various microfilarial densities at 7 days before treatment and days 90 and 180 after treatment. Of particular importance is the proportion of individuals in different communities which showed moderate to high microfilaria counts at day 90. In Jagbenbendo and Kyingakrom, about 10–15% of participants had skin microfilaria counts between 10 and 40 microfilariae per snip by day 90, although almost all had been free of microfilaria at day 30 after treatment. The proportion of individuals with high skin microfilaria counts had doubled by day 180 in these communities. These results show a rapid repopulation of skin and a repopulation to high counts in some individuals.

Discussion

We have shown that ivermectin remains a potent microfilaricide, since 1 month after treatment almost all patients had complete clearance of microfilariae. However, the subsequent early repopulation of skin with microfilaria observed in individuals in some communities, suggests possible emergence of *O volvulus* adult populations that are becoming resistant to ivermectin.

The use of ivermectin to control onchocerciasis in Ghana was initiated in the Pru and Black Volta River basins in 1987.[3,11] Since then, an increasing number of communities have been enrolled into the treatment programme, resulting in a substantial reduction in disease

	Average number of treatments of participants (SD)	Number of participants*	Microfilaria density per snip at day 7 before treatment	Microfilaria density per snip at day 30 after treatment (% of pretreatment)	Microfilaria density per snip at day 90 after treatment (% of pretreatment)	Microfilaria density per snip at day 180 after treatment (% of pretreatment)
Asubende	14·4 (3·7)	12 (14)	2·40	0	0	0·53 (22·10%)
Baaya	13·5 (2·7)	21 (24)	1·38	0	0	0·17 (12·31%)
Beposo	13·8 (1·5)	10 (12)	2·24	0	0	0·37 (16·50%)
Hiampe	12·3 (3·8)	18 (22)	2·97	0	0·07 (2·16%)	0·48 (16·16%)
Senyase	13·1 (3·4)	10 (13)	1·69	0	0 (0)	0·27 (15·97%)
Kyingakrom	13·2 (2·1)	38 (42)	6·44	0·03 (0·47%)	1·36 (21·12%)	3·47 (53·88%)
New Longoro	12·5 (2·9)	62 (70)	5·91	0·04 (0·67%)	0·42 (7·11%)	1·33 (22·50%)
Jagbenbendo	10·4 (1·6)	65 (70)	8·01	0·01 (0·12%)	0·91 (11·36%)	2·91 (36·32%)
Wiae	8·6 (1·4)	25 (30)	3·41	0	0·29 (8·50%)	1·01 (29·61%)
Begbomdo†	1 (0)	40 (45)	30·09	0·04 (0·13%)	0·87 (2·89%)	3·79 (12·60%)

*Figures in parentheses show number of patients selected for the follow-up study, and those not in parentheses show individuals who participated in the entire study. †This community was ivermectin naive at the start of the study.

Table 2: **Geometric mean densities (microfilaria per skin snip) of *O volvulus* before treatment and at different times after treatment in ten onchocerciasis-endemic communities in Ghana**

	Pretreatment geometric mean density (microfilariae per snip)	Number of participants*	Number of individuals with microfilariae per snip of:									
			<3 at day –7	3–9 at day –7	10–40 at day –7	0 at day 90	<3 at day 90	3–9 at day 90	10–40 at day 90	0 at day 180	<3 at day 180	3–9 at day 180	10–40 at day 180
Asubende	2·40	12 (12)	6 (50%)	4 (33%)	2 (17%)	12 (100%)	0	0	0	7 (58%)	2 (16%)	3 (25%)	0
Baaya	1·38	21 (21)	19 (90%)	1 (5%)	1 (5%)	21 (100%)	0	0	0	18 (86%)	2 (10%)	1 (5%)	0
Beposo	2·24	10 (10)	6 (60%)	3 (30%)	1 (10%)	10 (100%)	0	0	0	7 (70%)	2 (20%)	1 (10%)	0
Hiampe	2·97	19 (19)	10 (53%)	6 (32%)	3 (16%)	16 (84%)	3 (16%)	0	0	10 (53%)	8 (47%)	0	0
Senyase	1·69	13 (10)	10 (79%)	2 (14%)	1 (7%)	13 (100%)	0	0	0	7 (70%)	3 (30%)	0	0
New Longoro	5·94	65 (62)	20 (31%)	26 (40%)	16 (25%)†	49 (75%)	8 (13%)	8 (12%)	0	26 (42%)	20 (32%)	9 (15%)	7 (11%)
Kyingakrom	6·44	40 (38)	13 (33%)	11 (26%)	9 (22%)‡	21 (52%)	7 (18%)	6 (15%)	6 (15%)	9 (22%)	9 (22%)	8 (19%)	12 (30%)
Jagbenbendo	8·01	68 (65)	21 (31%)	18 (26%)	24 (35%)§	38 (56%)	12 (18%)	11 (16%)	7 (10%)	18 (28%)	13 (20%)	21 (32%)	13 (20%)
Wiae	3·41	28 (25)	14 (50%)	9 (32%)	5 (18%)	23 (82%)	3 (11%)	2 (7%)	0	14 (57%)	5 (20%)	3 (12%)	3 (11%)
Begbomdo¶	30·09	42 (40)	5 (12%)	3 (7%)	11 (26%)‖	27 (64%)	6 (14%)	9 (21%)	0	14 (35%)	2 (5%)	17 (43%)	7 (18%)

Data are number (%) unless otherwise indicated. *Number of patients in parentheses shows those who fully participated in the study (ie, including day 180). †Three (4%) patients had more than 40 microfilariae per snip at day –7. ‡Seven (18%) patients had more than 40 microfilariae per snip at day –7. §Five (8%) patients had more than 40 microfilariae per snip at day –7. ¶This community was ivermectin naïve at the start of the study. ‖23 (55%) patients in this ivermectin-naive community had more than 40 microfilariae per snip at day –7.

Table 3: **Distribution of microfilaria counts at day –7, day 90, and day 180 in the study communities**

transmission and parasite burden.[10] By the close of the OCP, ivermectin was established as a successful approach for elimination of onchocerciasis as a public-health problem in control areas, but proved unlikely to eliminate disease transmission in west Africa.[3] Therefore, for the successes achieved by that programme to be sustained after it closed, the efficacy of the drug in elimination of skin microfilaria and prevention of early skin microfilaria repopulation by the adult female worms needs to be sustained until a new control measure, such as a macrofilaricide, is discovered and developed.

The greatest effect of ivermectin on skin microfilariae is seen 4 weeks after treatment.[21] Skin microfilaria assessment at day 30 after treatment showed very large reductions in skin loads in all communities, with no microfilariae in any patients in 15 communities. This result is consistent with various studies that were undertaken when ivermectin was introduced for onchocerciasis treatment,[22] and shows that after 19 years of use, the drug is still a highly effective microfilaricide. The sustained effectiveness of ivermectin against microfilariae is an important component in ensuring that onchocerciasis does not emerge as a public-health problem.

Apart from the immediate microfilaricidal action of ivermectin, another very important effect is its suppression of parasite transmission. Arguably, the most important effect of the drug against onchocerciasis is the extended inhibition of microfilariae production by the adult female worms,[6,23,24] leading to the absence or very low amounts of skin microfilaria repopulation for 6 to 9 months after treatment. Furthermore, the effect of ivermectin on suppression of reproduction by the adult worms had previously been shown to rise with increasing rounds of ivermectin treatment.[10] *O volvulus* populations in ivermectin-naive communities are expected to resume reproduction after a single treatment with ivermectin, earlier than those in multiply treated communities, because of the cumulative effect of this drug on *O volvulus*.

Awadzi and colleagues[11] have proposed that for *O volvulus* to be regarded as responding adequately to ivermectin, the build-up of skin microfilariae at day 90 after treatment should be less than 6% of pretreatment count. At day 90 after ivermectin treatment assessment, we noted virtually no repopulation of skin microfilariae by the adult female worms in five of ten communities. However, four of the repeatedly treated communities had skin microfilaria densities similar to the previously ivermectin-naive community, despite having received between ten and 17 rounds of treatment. Furthermore, the degree of repopulation as a proportion of pretreatment counts was also high in these four communities. On the basis of previous findings,[24,25] a normal repopulation response 6 months after ivermectin treatment, is for skin microfilaria counts to be less than 20% of pretreatment count, although one study[26] suggested that after several rounds of ivermectin, microfilaria counts should be 1–3% of pretreatment counts at 6 months (and 5–7% by 12 months after six or more treatments). The assessment of microfilaria count at day 180 showed a moderate skin repopulation of less than 20% of pretreatment count in five communities, including the ivermectin-naive community. A sixth community, Asubende, which showed a good response at day 90, had a microfilaria repopulation percentage at day 180 that was a little above the suggested cut-off for a normal response.

When both the day 90 and day 180 data are considered, the results obtained for Kyingakrom, Jagbenbendo, New Longoro, and Waie are of concern. In these communities, skin microfilaria repopulation had started before day 90 and had increased sharply by day 180. Some individuals in Jagbenbendo and Kyingakrom already had substantial microfilaria counts by day 90 after treatment, despite being virtually free of microfilariae at day 30 after treatment. The proportion of individuals with high counts had doubled in these communities by day 180. The rapid rate of skin microfilaria repopulation in these communities after treatment would explain the distribution towards high microfilaria loads reported at the assessment of microfilaria load 7 days before the start of our study.

When all these results are considered together, there is evidence of substantial differences in responses between communities. Some communities, including the ivermectin-naive community, responded as expected to treatment. The low microfilarial loads after treatment in the six communities that responded as expected suggest that the adult female worms had not yet resumed a high rate of microfilaria production because of the suppressive effect of ivermectin on adult worms. These good responses were reflected in the fairly low pretreatment microfilaria prevalence rates and CMFL of less than 1 microfilariae per snip found in these communities. There is, therefore, the possibility that if the adult worms in these communities continue to respond adequately, the microfilaria prevalence will continue to fall to near zero with further treatment, provided repopulation with *O volvulus* that is more tolerant of ivermectin does not arise. However, some communities that responded poorly showed early repopulation of skin microfilariae of 7·1% or more of pretreatment counts at day 90, and substantially higher microfilarial counts, of up to 53·9% of pretreatment counts at day 180. A proportion of individuals in these communities who responded poorly to treatment showed moderate to high skin microfilaria counts by day 90, with the proportion increasing further by day 180.

There was no apparent association between age or sex and response to ivermectin in terms of skin repopulation. The results show that the suppressive effect of this drug on adult female worms, to prevent releases of microfilariae into the skin, was much less than was expected. This finding suggests that a resistance is developing to ivermectin, which is manifested by some adult worms not responding adequately to treatment, and thus they rapidly regain fecundity. This failure to suppress reproduction between yearly treatments, the control practised in most of

Africa, might result in the progressive return of clinical symptoms. The moderately high parasite burden and high pretreatment microfilaria prevalence rates, noted in four repeatedly treated communities, could be the result of a rapid repopulation of the skin by microfilariae and continuation of transmission between treatments.

The greater than doubling in microfilaria prevalence rates in New Longoro and Kyingakrom between 2000 and 2005 could be explained by a loss of effectiveness of ivermectin in suppression of reproduction in adult worms, resulting in transmission of drug-tolerant parasites. Another striking difference between communities responding well and those responding poorly was the proportion of participants with nodules. We noted that in communities with 10 years or more of ivermectin treatment that were responding well, less than 30% of individuals with nodules were microfilaria positive. However, in communities that were responding poorly, more than 70% of individuals with nodules were still microfilaria positive, indicating the parasites in the nodules were still producing microfilariae.

Since community microfilaria load is the most important factor in assessment of the endemicity of onchocerciasis, the CMFL values that we recorded in New Longoro, Jagbenbendo, and Kyingakrom, could be regarded as acceptable, since onchocerciasis is considered a public-health problem only when the CMFL exceeds 5 microfilariae per snip.[27] However, since Jagbenbendo had received 12 rounds of yearly ivermectin treatment with average treatment coverage of 71·5% (over the previous 5 years) and New Longoro and Kyingakrom have each received 17 yearly treatments with an average treatment coverage of 66·5%, we think that the observed CMFL values, coupled with a high microfilaria and nodule prevalence is an alarming situation, and suggests a developing ivermectin resistance. This concern is increased by the early skin microfilaria repopulation at days 90 and 180.

The poor responses by adult female worms and subsequent early skin microfilaria repopulation will increasingly affect disease transmission, because this rapid repopulation makes microfilariae available in the skin for a long period (about 9 months) after treatment, for vector uptake and parasite transmission. Entomological studies undertaken in parallel with this study showed blackflies having L3 *Onchocerca* larvae in New Longoro and Kyingakrom (unpublished data).

These findings provide evidence that ivermectin resistance is developing in *O volvulus* in some communities in Ghana, and is manifesting itself as a more rapid return to high microfilarial counts after treatment. The drug seems to be becoming less effective in suppression of reproduction in adult parasites than it was in previous years. This reduction in effectiveness could be happening in other areas of west Africa, but there are few data from other countries in the region. In South and Central America, where ivermectin is given every 6 months for onchocerciasis control, the emergence of drug resistance (manifested as a reduction in the effects of ivermectin on adult worm reproduction) could be less apparent and impair the control programme to a lesser extent than in west Africa, because a high degree of microfilaria suppression could still be maintained with this twice-yearly treatment. However, the selection pressure for resistance to develop might be greater with more suppression of *O volvulus*. These considerations suggest a need for monitoring the responses of *O volvulus* to ivermectin, and the likely need to make changes in the control programmes in those communities in which the persistent suppression of skin microfilarial repopulation has been lost.

Because drug resistance has a genetic basis, monitoring of genetic markers associated with ivermectin selection and resistance, such as Eng[28] and Bourguinat (unpublished) and their colleagues proposed, should be undertaken. Bourguinat and co-workers' study was done in Cameroon on the same individuals before any use of ivermectin and after up to 13 rounds of treatment, eliminating the possibility that genetic differences could have arisen because the samples came from different locations and individuals. Substantial changes were seen in a gene that has been associated with ivermectin resistance in the nematode parasite of livestock, *Haemonchus contortus*. Nevertheless, further validation is needed on possible genetic markers for ivermectin resistance. Evidence of microfilariae in the skin at day 90 after treatment, in patients definitely known to have been treated and who have been in a yearly treatment programme for several years, might suggest that the response to treatment is poor and should be further investigated in that community.

The possible emergence of ivermectin resistance that we and others[11,25] have shown, suggests the urgent need for a new macrofilaricide or alternative means of control, such as a vaccine against onchocerca.[29–31] Until such new measures become available, control in individuals and communities showing evidence of resistance to ivermectin in *O volvulus* could include treatment to deplete *Wolbachia* bacteria,[32] focal use of vector control, or nodulectomies. All these adjunct treatments have disadvantages and are of limited use for mass treatment. However, if, as these findings suggest, ivermectin resistance might still exist only in focal areas, such measures could be very important to restrict the spread of resistance in *O volvulus*. The gains that have been achieved by mass ivermectin treatment in control of onchocerciasis could be jeopardised if communities in which treatment has been highly effective become repopulated by ivermectin-resistant *O volvulus* from areas in which poor responses to this drug have not been noticed because of an absence of monitoring, or where appropriate changes in the control programmes have not been made to maintain suppression of the parasite. The lack of attention to the possibility that ivermectin resistance can develop and might spread could, in the long term, threaten all gains that have been achieved through the onchocerciasis control programmes.

Contributors

MYO-A, JOG, DAB, and RKP designed the study. Patients were recruited by MYO-A, JOG, and DAB. The field work was done by MYO-A and supported by a technician. Laboratory work was done by MYO-A and JKLE. Statistical analysis was done by MYO-A, JKLE, and RKP. MYO-A and RKP wrote the report and other authors added their contributions and comments.

Conflict of interest statement

We declare that we have no conflict of interest.

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