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Waning Trends in Prevalence and Intensity of *Wuchereria bancrofti* Transmission Over Ten Chemotherapy Rounds in Ghana

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Abstract: Ghana's lymphatic filariasis (LF) elimination was intensified by Global Programme to Eliminate Lymphatic filariasis in 2000. LF transmission was interrupted in some endemic district, yet, persistent in others. This study investigated some characteristics of *Wuchereria bancrofti* that potentially contribute to persistent LF transmission in Ghana's endemic districts after prolonged mass chemotherapy (≧15yrs) expected to interrupt transmission. Participants' finger-prick blood was screened for antigenaemia and positives followed for night blood to detect microfilariae. The antigenaemia/microfilaraemia prevalence, implications of *W. bancrofti* transmission, and microfilariae clearance using scoring method (SM) were assessed. Exactly 2973 individuals were screened for antigenaemia and 33 found positive. Infected individuals were followed for night blood to detect microfilaraemia in 8 communities from the 4 study districts. The antigenaemia/microfilaraemia prevalence in Antseambua, Asemkow, Badunu, Navio (Sanwu) Central, Atampiisi Bongo, and Balungu Nabiisi were 1.3/0.0, 0.8/0.3, 0.8/0.0, 1.2/0.0, 1.7/0.0, and 3.1/0.9 respectively but none in Ampeasem and Obrayebona. Microfilariae density for thick blood films/Sedgwick counts was relatively higher in Western Region (283.9/410.0) compared to Upper East Region (44.5/25.0). Microfilariae clearance/reinfection was observed (using SM). Currently, prevalence thresholds suggest competent vectors can sustain LF transmission in Ahanta West District (hotspot) and cause possible reinfection/resurgence in Bongo (control district).

Keywords: Lymphatic filariasis; mass drug administration; prevalence; antigenaemia; microfilaraemia; hotspot; control; transmission; Ghana

Introduction

Lymphatic filariasis (LF) is a parasitic disease caused by the filarial worms, *Brugia malayi*, *B. timori*, and *Wuchereria bancrofti*, spread through the infective bites of mosquito vectors. It has been a little over two decades since LF was recognised as a disease of public health importance in Ghana, before the commencement of interventions on a national level by the Global Programme to Eliminate Lymphatic Filariasis (GPELF) [1-5]. The two strategies of the GPELF were to interrupt transmission of LF through large-scale annual mass drug administration (MDA); and morbidity management and disability

prevention (MMDP) [6, 7]. The target of the GPELF was to achieve elimination of LF disease in Ghana by the year 2020. However, due to several factors and operational challenges, the target of LF elimination was not met. The complex dynamics of *Wuchereria bancrofti* infection in endemic foci, prolonged doling out of the LF drug regimen during MDA, and MDA non-compliant individuals among others has led to the creation of hotspot districts in Ghana [8-10]. The LF hotspots have been the main reason responsible for the sustenance of LF despite the numerous rounds of MDA conducted in LF endemic foci in Ghana [8, 9, 11]. After failing to meet the target of elimination in 2020, the GPELF is now working to achieve elimination of LF with a new roadmap having a 10-year extension set as the deadline for LF disease elimination by the WHO [12, 13]. The goals for the new neglected tropical diseases (NTD) roadmap include ensuring that (i) 4 years after stopping MDA and giving essential package of care to all known LF patients in all endemic countries, sustained infections of the disease will be below target thresholds and with about 80% of the endemic countries meeting the criteria for validation of elimination of LF as a disease of public health importance; (ii) that implementation of post-MDA or post-validation surveillance is done in all endemic countries; and that the total population that need MDA is reduced to zero [13]. Strictly adhering to the goals set in the new NTD roadmap for elimination of the disease will help the GPELF achieve its initial target of elimination by the deadline. Already, it has been noticed that the number of hotspot districts in Ghana keeps decreasing after the first transmission assessment survey (TAS). Studies have reported a high initial average baseline LF prevalence in Ghana to be 8.7% (range = 0% - 45.7%) [11, 14]. Currently, the average LF or microfilariae prevalence is just a little above the targeted threshold of 1%, below which interruption of disease transmission is expected in districts that are still having MDA. However, reliable data is required for the decision to stop MDA. Such decisions are driven by *W. bancrofti* parasite clearance in the blood of infected persons in LF endemic areas. The immunochromatographic test (ICT) card and/or filarial test strip (FTS) band has been used to determine *W. bancrofti* antigenaemia positive individuals. The intensities of these bands can be used as a surrogate measure to estimate *W. bancrofti* parasite clearance. This could be exploited to assess parasite clearance in the blood of indigenes after MDA. These analyses utilised a free downloadable R software and packages. This simple scoring method could be employed by intervention programmes and national NTD programme managers to critically assess available LF intervention data for the decision to stop MDA. It may serve as a quick analytical tool to complement all LF MDA assessments, including transmission assessment surveys (TAS), for decision-making (continue or stop MDA) especially in resource limited settings. This is because it does not require technical expertise, expensive equipment (microscopes, thermocyclers, ELISA plate readers, etc.) and high-through-put assays (ELISA, PCR, etc.). Also, intervention programmes with low budgets or limited funding can patronize this simple scoring method when conducting any LF MDA assessment. This method will also be very useful in LF Hotspot areas. In this commission the *W. bancrofti* and circulating filarial antigen clearance trends was assessed using a new antigenaemia scoring method and the standard WHO criteria, in some control and hotspot districts. The implications for LF transmission are also discussed.

Methods

Study sites

This study was conducted in the Ahanta West and Mpohor districts of the Western Region and the Bongo and Kassena Nankana West districts of the Upper East Region of Ghana (Figure 1).

Selection criteria

Inclusion criteria

The selected hotspot communities and districts had at least 10 rounds of LF MDA; antigenaemia prevalence of ≥ 2.0%; microfilaraemia prevalence of ≥ 1.0%. Communities selected as controls had participated in at least one of the LF MDAs; and have both antigenaemia and microfilaraemia prevalence below 2.0% and 1.0% respectively or being zero percent.

Exclusion criteria

Communities and districts were excluded if their antigenaemia and/or microfilaraemia prevalence were below 2.0% and 1.0% respectively, and control communities as well as control districts must not be selected if their antigenaemia and/or microfilaraemia prevalence was above the respective thresholds mentioned above. Also, children below the age of 5 years, lactating mothers and pregnant women were all not recruited.

Figure 1. Map of study area showing the control and hotspot districts. The base layer for this map was accessed from the "Humanitarian Data Exchange (HDX)" website [https://data.humdata.org/dataset/dc4c17cf-59d9-478c-b2b7-acd889241194/resource/4443ddba-eeaf-4367-9457-7820ea482f7f/download/gha_admbnda_gss_20210308_shp.zip]. The software for drawing this map is QGIS 3.40 [https://www.qgis.org/download/]. The terms of use / license information for the base layer image or shapefile is [https://data.humdata.org/about/license/legacy_hrinfo]. **Ahanta West and Mpohor.**

These are adjoining districts with Mpohor found at the northern border of Ahanta West. Agrarian activities constitute the major occupation in these districts. The total land size for the two districts is 1,273.4 km² (Ahanta West = 554.9 km² and Mpohor = 718.5 km²). This is located between latitudes 4º.45" N, and 5°4′3857" N; and longitudes 1°52′6288" W and 1º.58" W. Ahanta West is positioned at the southernmost point of Ghana and the entire West African sub-region spanning 52.8 km of the Gulf of Guinea coastline. These two districts are found within the South-Western Equatorial Climatic Zone, the high rainforest vegetation zone (Ahanta West); and the Tropical Climate Zone (Mpohor) of Ghana. The Ahanta West had higher population / population density (153,140 / 276.0 persons/km²) than Mpohor (52,473 / 73.03 persons/km²). The two districts also have mean rainfall; temperature; and relative humidity ranging between 1,500 mm - 1,700 mm; 20 ºC - 34 ºC; and 70% - 85% respectively. AW has 5 rivers and 5 lagoons. Mpohor has no lagoon but has a total of 8 rivers / streams [15-17].

Bongo and Kassena Nankana West

The total land size of these two districts is 1,237 km² (Bongo = 425 km² and Kasena Nankana West = 812 km²). It is approximately found between latitudes 10° 97′ N and 11° 5′ 24" N; and longitudes 000° 27′ 0" W and 001° 10′ 0" W. They are found in the Guinea Savannah Zones or Woodlands. These districts had total population sizes of 120,254 (Bongo) and 90,735 (Kasena Nankana West) with population densities of 282.9 persons/km² and 111.8 persons/km² respectively. The rainfall and temperature in these districts also range between 600 mm - 1,400 mm, 12 ºC – 45 ºC. These districts are found in the Guinea, Sahel and Sudan Savannah vegetation, lies within the Northern Savannah Zone and Interior Continental Climate Zone [15, 18-21].

Community engagement

Prior to sampling activities, meetings were held with the directors of the District Health Directorates, chiefs, elders and opinion leaders to discuss the scope of the study. Public announcements were then made in each community after all stakeholders had approved the study.

Sample size estimation

Sample sizes for each selected district was calculated from their respective demographic data using Epi-info software 7 (CDC, Atlanta, GA, USA). The initial parameter used was an expected maximum antigen prevalence of 2%. Below this threshold, the WHO guideline recommends MDA stoppage in areas having *Anopheles* mosquitoes as the primary vectors for the transmission of lymphatic filariasis disease [22]. Even at 99% confidence level only 52 individuals were to be recruited per site for the study. This number of subjects was anticipated to be inadequate to detect ample number of *Wuchereria bancrofti* positive individuals that would be significant for good analysis due to prolonged MDA rounds (over 10 MDAs) which had significantly reduced the level of parasitaemia in the human population. To increase the chances of getting several *W. bancrofti* positive study participants, a 50% prevalence was assumed to purposefully recalculate the samples sizes using demographic data of the communities selected for the study (Table 1).

Table 1. Sample size calculation with Epi-info software 7 (CDC, Atlanta, GA, USA) assuming 50% prevalence.

Region	District	Community	Community Population Size	Sample Size
Western	[H]Ahanta West	Antseambua	215	162
		Asemkow	938	389
	[C]Mpohor	Ampeasem	734	348
		Obrayebona	1972	496
Upper East	[H]Kassena Nankana West	Badunu	1159	422
		Navio (Sanwu) Central	733	348
	[C]Bongo	Atampiisi Bongo	1207	455
		Balungu Nabiisi	1450	428

[C] = control districts; [H] = hotspot districts.

Wuchereria bancrofti screening

Finger-prick and venous blood (from antigenaemia positive individuals) were obtained from recruited persons and screened for the presence of *W. bancrofti* parasites using the methods described below.

Blood sampling

Sterile lancet was used to puncture the tip of the third or fourth finger selected for bleeding. The first drop of blood was expressed while applying gentle pressure and wiped using a dry piece of cotton ball. About 100 µl expressed blood was collected using a calibrated capillary tube.

Circulating filarial antigen (CFA) screening

Immunochromatographic test (ICT)

The 100 µl finger-prick blood collected from the volunteer was added to the ICT kit and results read after 10 minutes. The test was said to be positive for *W. bancrofti* parasites when both the control (C) and test (T) pink lines are observed in the viewing window of the ICT card. The test was negative if only the C pink line was seen in the viewing window of the ICT card. The test was, however, considered invalid if only the T pink line or no pink line shows in the ICT card viewing window.

Filariasis test strip (FTS)

About 75 µl finger-prick blood collected with the fixed volume micropipette was used for the Alere™ Filariasis Test Strip (FTS) following manufacrturer's protocol, and results read after 10 minutes.

When the control (C) and test (T) pink lines shows after 10 minutes, the test was deemed positive for *W. bancrofti* parasites. The test was negative if only the C pink line appears and was invalid if only the T pink line or no pink line was seen.

Surrogate determination of Wuchereria bancrofti clearance after treatment using Immunochromatographic test (ICT) cards or filariasis test strip (FTS)

The ICT/FTS was used to detect antigenaemia positive individuals before treatment (baseline screening). All ICT/FTS positive persons were followed for night blood collections. The night blood samples were examined for *W. bancrofti* parasites. Individuals with parasitaemia were then followed up for *W. bancrofti* parasite detection at 3-month interval for 9 months after treatment with LF MDA drug regimen (albendazole and ivermectin). The intensity of the pink band on a positive ICT card or FTS is assessed to estimate *W. bancrofti* parasite clearance from blood of indigenes post treatment. This is done as a semiquantitative assessment using a scoring method based on intensity of the "T-line" (positive test band) relative to the "C-line" (control band) [23, 24].

Immunochromatographic test (ICT) cards or filariasis test strip (FTS) scoring method

All antigenaemia positive cards were independently scored by six randomly selected persons following given criteria. The average score is then used to estimate *W. bancrofti* parasite clearance. A test is considered valid if only the "C-line" is visible (i.e. test = negative) or both the "C-line" and "T-line" are visible (i.e. test = positive). However, a test is invalid if the "C-line" is not visible. Once a test was deemed valid, the simple scoring criteria ranging 0 - 3 was used to estimate *W. bancrofti* clearance in the blood. A valid test is assigned a score of "0" if the only band visible was the "C-line"; "1" if the intensity of the "T-line" is lower than that of the "C-line"; "2" if the intensity of both "C-line" and "T-line" are almost equal or equal; and "3" if the intensity of "C-line" is lower than that of the "T-line".

Wuchereria bancrofti clearance assessment using Mann-Kendall's time-series trend analysis, Sens slop, and Pettitt's point-of-change estimations

After LF MDA or treatment, it is expected that microfilariae (mf) load in the blood of infected individuals will clear over a period. To confirm the trends in parasite clearance from the average scores from each of the positive test, the Mann-Kendall's time-series trend analysis was exploited. This was a package from R programming. This programme was to predict whether there was *W. bancrofti* parasite clearance, repopulation, and/or reinfection in the blood of mf positive individuals over a given period after treatment. This was complemented by the Sen's slope and Pettitt's point-of-change analysis to confirm the trends predicted by the average scores from the ICT/FTS positive band intensities (see Appendix A).

Estimation of parasitaemia

Venous night blood was collected from *W. bancrofti* antigenaemia positive volunteers between 21:00 hr and 02:00 hr GMT. About 2 ml blood was taken from the veins and then injected into an ethylenediaminetetraacetic acid (EDTA) tube, and then stored in a cool box.

Wuchereria bancrofti parasite counts using Sedgwick-Rafter counting chamber

About 100 µl capillary night blood was added to 900 µl 3% acetic acid. This was applied to the Sedgwick Rafter Counting Chambers and observed under the Leica Galen III compound microscope. The concentration of *Wuchereria bancrofti* parasites in the observed blood was then estimated by counting the total number of mf in the grids of the Sedgwick Rafter Counter and dividing by 100 after

which the quotient is multiplied by 1000 to express the value as mf/ml [22]. The mf density for the study districts was also estimated as the total number of mf observed with Sedgwick Rafter Counter multiplied by 10 and dividing the product by the total number of Sedgwick counter (or total number of individuals) found to be *W. bancrofti* positive after microscopy (Table 3).

Prevalence was estimated as the number of individuals positive for mf (after screening with the Sedgwick Rafter Counter) multiplied by 100 and the product was divided by the total number of individuals screened with the Sedgwick Rafter Counter for the detection of *W. bancrofti* mf [22].

Wuchereria bancrofti parasite counts using thick blood film

Night blood obtained from volunteers who consented to be part of the study was also used for thick blood film slides. To get the density of mf in 1 ml of *W. bancrofti* infected blood used for thick blood film preparation, the total number of mf observed during microscopy was multiplied by 16.7 and the product was divided by the total number of slides found to be *W. bancrofti* positive after microscopy to express the value as mf/ml [22]. The mf prevalence was estimated as the number of individuals whose slides were positive for mf multiplied by 100 and the product was divided by the total number of individuals screened for *W. bancrofti* mf [22].

Wuchereria bancrofti parasite counts using nucleopore filtration parasitaemia estimation

About 1 ml of night blood sampled from subjects recruited for the study was added to 9 ml of 3% acetic acid solution and passed through a 25 mm diameter Nucleopore Track-Etch filter membrane with a pore size of 5 μm [25, 26]. The mf trapped on the membrane was placed on a clean glass slide upside down. The total number of parasites was then counted with the aid of a Leica Galen III compound microscope. This gives the number of *W. bancrofti* mf observed per ml (i.e., mf/ml) of blood.

Antigenaemia prevalence

Circulating filarial antigens in the human population was screened using the Binax Now® Filariasis Immunochromatographic Test (ICT) cards, or Alere™ Filariasis Test Strip (FTS). The antigenaemia prevalence was estimated as the number of individuals found to be *W. bancrofti* antigen-positive (i.e., ICT-positive or FTS-positive) multiplied by 100 and the product was divided by the total number of individuals screened with ICT or FTS [22]. This value gave the *W. bancrofti* antigenaemia prevalence for the study area (Table 2).

DNA extraction from blood and packed cells (whole blood having no serum)

A slightly modified protocol of the QIAGEN DNeasy Blood & Tissue Kit was used for the extraction of genomic DNA from the blood samples collected from the volunteers. The modifications made included: (i) increasing proteinase K volume by 50% and doubling the incubation time; (ii) adding equal volume of lysis buffer as the volume of the lysate; (iii) incubating lysate at 100 °C; (iv) adding ethanol as much as half the volume of the entire lysate; (v) adding one additional Buffer AW1 wash step; (vi) tripling the incubation time before eluting DNA; and (vii) eluting DNA at half the recommended volume as specified in the manufacturer's protocol.

Wuchereria bancrofti detection using conventional polymerase chain reaction (PCR)

Conventional polymerase chain reaction (PCR) was used for the detection of *Wuchereria bancrofti* parasites using the extracted DNA from the blood samples of subjects recruited. This PCR amplified a *SspI* (small, acid-soluble spore protein I) region of the *W. bancrofti* genome which have repeated sequence unique to this filaria parasite. The expected band size after amplification of the DNA is 188 bp [27-29]. The constituents of the master mix included 2X GoTaq (2X Green GoTaq (Flexi Buffer), 0.2 mM of each dNTP, 25 mM $MgCl_2$, 1.25 Units of GoTaq DNA polymerase), 0.4 μM each of NV1 [5'- CGT

GAT GGC ATC AAA GTA GCG - 3'] – (21-mer), NV2 [5' – CCC TCA CTT ACC ATA AGA CAA C - 3'] – (22-mer) primers and nuclease free water (Invitrogen™ LS4387936). A volume of 5 µl DNA template was added to the master mix and amplified in a thermal cycler. The cycling conditions included a 2 min pre-heating temperature of 95 °C, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min for denaturation, annealing and elongation respectively, followed by another elongation temperature of 72 °C for 7 min and finally a 4 °C.

The amplicon was run on a 2% (w/v) agarose gel prepared with ethylenediaminetetraacetic acid (EDTA The electrophoresis was run with and observed under a Mupid-2 plus Submarine electrophoresis system and ultraviolet (UV) light (Cleaver Scientific Ltd, Clear View, UV Transilluminator) respectively to visualize the expected band size of 188 bp for *W. bancrofti* detection (Figure 2).

Figure 2. : Wuchereria bancrofti detection using NV1 and NV2 primers. The base layer for this map was accessed from the "Humanitarian Data Exchange (HDX)" website [https://data.humdata.org/dataset/dc4c17cf-59d9-478c-b2b7-acd889241194/resource/4443ddba-eeaf-4367-9457-7820ea482f7f/download/gha_admbnda_gss_20210308_shp.zip]. The software for drawing this map is QGIS 3.40 [https://www.qgis.org/download/]. The terms of use / license information for the base layer image or shapefile is [https://data.humdata.org/about/license/legacy_hrinfo].

Wuchereria bancrofti detection using real-time polymerase chain reaction (RT-PCR)

The extracted genomic DNA was also used as template for real-time polymerase chain reaction (RT-PCR) using "Long DNA Repeats" (LDR) primers as described by Rao's working group [28]. Briefly, a 5 µl DNA template was added to a master mix consisting of a final concentration of 1X TaqMan One-Step Real-Time PCR Master Mix, 0.45 µM each of LDR1 [5'-ATT TTG ATC ATC TGG GAA CGT TAA TA-3'] and LDR2 [5'-CGA CTG TCT AAT CCA TTC AGA GTG A-3'] primers, 0.125 µM TaqMan probes with sequence [5'-ATC TGC CCA TAG AAA TAA CTA CGG TGG ATC TCT G-3'], and Nuclease Free Water (Ambion®, P/N: AM9932, L/N: 1608217) to run the PCR. This reaction was run with the ABI Prism 7300 instrument having the SDS software (Life technologies/Applied Biosystems Listing No. 200114) and calibrations of the manufacturer. The samples were run in duplicates and the cycle threshold (Ct) values were as instructed by the manufacturer. Amplified samples were seen as Sigmoid curves (Figure 3). These curves were seen at different Ct values with a given range (i.e., 23 – 33 Ct values).

Figure 1: *Detection of* Wuchereria bancrofti *using real time polymerase chain reaction (RT-PCR). The base layer for this map was accessed from the "Humanitarian Data Exchange (HDX)" website [https://data.humdata.org/dataset/dc4c17cf-59d9-478c-b2b7-acd889241194/resource/4443ddba-eeaf-4367-9457-7820ea482f7f/download/gha_admbnda_gss_20210308_shp.zip]. The software for drawing this map is QGIS 3.40 [https://www.qgis.org/download/]. The* terms of use / license information for the base layer image or shapefile is [*https://data.humdata.org/about/license/legacy_hrinfo*].

Results

Table 2. Wuchereria bancrofti antigenaemia and microfilaraemia prevalence.

Region	District	Community	*Wuchereria bancrofti* Screening			Prevalence	
			Number Observed	ICT Positive	Mf Positive	ICT (%)	Mf (%)
Western	[H]Ahanta West	Antseambua	159	2	-	1.3	-
		Asemkow	370	3	1	0.8	0.3
	[C]Mpohor	Ampeasem	349	0	-	-	-
		Obrayebona	510	0	-	-	-
Upper East	[H]Kassena Nankana West	Badunu	385	3	-	0.8	-
		Navio (Sanwu) Central	332	4	-	1.2	-
	[C]Bongo	Atampiisi Bongo	413	7	-	1.7	-
		Balungu Nabiisi	455	14	4	3.1	0.9

[C] = control districts; [H] = hotspot districts; and (%) = values expressed as percentages.

Table 3. Wuchereria bancrofti microfilariae concentration and density estimation.

Region	District	Community	Microfilariae Concentration		Microfilariae Density	
			Thick Blood Film (mf/ml)	Sedgwick Count (mf/ml)	Thick Blood Film (mf/ml)	Sedgwick Count (mf/ml)

Region	District	Community				
Western	[H]Ahanta West	Antseambua	-	-	-	-
		Asemkow	283.9	410.0	283.9	410.0
	[C]Mpohor	Ampeasem	-	-	-	-
		Obrayebona	-	-	-	-
Upper East	[H]Kassena Nankana West	Badunu	-	-	-	-
		Navio (Sanwu) Central	-	-	-	-
		Atampiisi Bongo	-	-	-	-
	[C]Bongo		16.7	30.0		
			83.5	-		
		Balungu Nabiisi			44.5	25.0
			-	20.0		
			33.4	-		

[C] = control districts; and [H] = hotspot distric.

Wuchereria bancrofti clearance and repopulation/reinfection estimation

A total of 33 (Antseambua = 2, Asemkow = 3, Ampeasem = 0, Obrayebona = 0, Badunu = 3, Navio Central = 4, Atampiisi Bongo = 7, and Balungu Nabiisi = 14) people in all communities who tested antigenaemia and microfilaraemia positive were followed over a period of 9 months (3 months interval) post infection to assess *W. bancrofti* parasite clearancein all the study areas.

Discussion

This study looked at the trends in LF transmission to contribute data on when it is appropriate to halt MDA, due to the emergence of hotspots in LF endemic communities in Ghana. The immunochromatographic test (ICT) card and/or filarial test strip (FTS) band was used to determine *W. bancrofti* antigenaemia positive individuals.

Stopping MDA is dependent on the outcomes of a study satisfying the WHO criteria of antigenaemia and microfilaraemia thresholds that are respectively below 2.0% and 1.0% where *Anopheles* and *Culex* mosquitoes are principal vectors of LF. In all Hotspot districts, antigenaemia and microfilaraemia were below the recommended WHO threshold suggesting MDA was no longer needed in these districts. However, stopping MDA will not be the best decisionas. Entomological surveys in the same study area have suggested the presence of vectors competent to transmit even at very low parasitaemia [10, 30]. Data from parasitological survey show microfilaraemia concentrations and densities found in the Control districts to be high enough to appreciably sustain active transmission of *W. bancrofti*. This implied that presence of mosquito vectors for *W. bancrofti* transmission in these districts will sustain LF transmission following vector bites in the district. Briefly, entomological data in the study area shows high densities of mosquito vectors such as *Anopheles gambiae* s.l, and *Mansonia* mosquitoes known for *W. bancrofti* transmission in the study districts and in Ghana [30-35]. For instance, in the Western Region of Ghana, mosquito vectors efficient in transmitting *W. bancrofti* at very low parasitaemia such as *An. melas* [32] and *Mansonia* mosquitoes [36, 37] were recorded in the Mpohor and Ahanta West districts. However, only *An. melas* was found to be transmitting *W. bancrofti* in the Ahanta West District during the study   with an annual transmission potential of about 7.4 [10]. Also, in the Upper East Region of Ghana, the principal vector (*An. gambiae* s. l.) and secondary vectors (*Mansonia africana* and *Ma. uniformis*) for *W. bancrofti* transmission, were collected [38]. The *An. gambiae* s. l. mosquitoes in Upper East Region has been reported to exhibit phenomenon of "limitation" which is the ability of vector(s) to efficiently transmit the parasites at very low parasitaemia [35].

Prolonged MDA activities by the GPELF in Ghana has significantly reduced levels of microfilaraemia. This had led to interruption of *W. bancrofti* transmission in most endemic districts in Ghana. However, for such competent mosquito vectors to be found in Hotspot districts, there will be the potential for residual disease transmission. In all the study districts, there were vectors capable of maintaining *W. bancrofti* transmission regardless of numerous MDA rounds conducted. Several factors account for persistent LF transmission in any endemic area. These may include non-compliance to MDA [39]; low epidemiological coverage [40, 41] which may include both geographical coverage [11] and therapeutic coverage [11, 14]; high baseline mf prevalence [42] and efficacy of MDA drug regimen [43] among others. All these factors contribute to the creation of 'hotspot' districts. In our study, issues of non-compliance, epidemiological coverage, and efficacy of drug regimen were not presently contributing much to sustenance of LF after several MDA interventions. Parasitaemia has been low in endemic areas showing evidence of drug efficacy and education to reduce non-compliance in the endemic areas. With time, there was also evidence of good epidemiological coverage [11, 14]. However, high baseline mf prevalence may still be a relevant factor in the hotspot districts remaining in Ghana. For example, while there was no baseline antigenaemia and microfilaraemia prevalence data for Mpohor, Bongo and Kassena Nankana West district, the baseline

antigenaemia (immunochromatographic card test, i.e. (ICT)) and microfilaraemia (microscopy) prevalence data for Ahanta West District was respectively 32.80 and 19.46 (Ghana Neglected Tropical Disease Programme – unpublished data). This was evidence of high baseline antigenaemia and microfilaraemia prevalence. This was contributing to the creation of Hotspot districts aided by some combination of factors that ensured the gradual decline of microfilaraemia after numerous MDA rounds. In addition, the main factors seen to be driving the incidence or creation of Hotspot districts seem to be i) high microfilariae concentrations and densities (high intensity of infection in the human population), and ii) presence of competent vectors that exhibit "limitation" and therefore can transmit *W. bancrofti* when parasite levels in the blood of indigenes are very low.

The intensity of infection was rather high in the Ahanta West District compared to the Kassena Nankana West District. However, they were all quite high enough for competent vectors in these districts to maintain *W. bancrofti* transmission. As expected, Mpohor District had no *W. bancrofti* transmission as a Control District. However, Bongo, that had passed transmission assessment survey (TAS) and therefore stopped MDA, had a relatively high antigenaemia prevalence above the WHO recommended threshold for interrupting LF transmission. Consequently, a resurgence in this district is envisaged if LF surveillance procedures are not reviewed and/or revised to randomly include a few extra communities that were not selected as sentinel and cross-check sites. This will help the intervention programme managers make informed decisions regarding re-starting treatment in this district if similar results are found. In this study, a surrogate method that will generate useful results to support the crucial decision-making to either continue or stop MDA at no extra cost to intervention programmes was demonstrated. This method simply exploited the use of average scores from the ICT/FTS band intensities of antigenaemia and/or microfilariaemia positive individuals screened for *W. bancrofti* parasites. This was used to estimate the possible status of parasite clearance, repopulation, and/or reinfection in antigenaemia and microfilariaemia positive individuals. This simple scoring method is augmented with some statistically sound R programme packages (Mann-Kendall's time-series analysis, Sen's slope and Pettitt's test) to provide additional vital information on the *W. bancrofti* clearance, repopulation, and/or reinfection trends in the human population treated (see Appendix). A negative value from the Mann-Kendall's time-series analysis suggested active parasite clearance and a positive value meant either reinfection or repopulation of mf in the blood. A Sen's slope value (negative or positive) together with its confidence interval augments the Mann-Kendall's time-series trend analysis. The Pettitt's test also supports the Mann-Kendall's time-series analysis. The value given after running the Pettitt's test confirms the downward or upward trend in the average ICT/FTS scores being analysed. Issues of Hotspot districts are unique and the rules of engagement for the stoppage of MDA and other GPELF activities must be reviewed to successfully interrupt *W. bancrofti* transmission in such districts. This review may include i) setting a lower prevalence threshold than the existing one specifically for Hotspot districts; and incorporating the ICT/FTS average scoring intensity method and related analysis for an enhanced decision to continue or stop MDA backed by additional but vital data. This is because there is spatial heterogeneity associated with *W. bancrofti* transmission in Hotspot districts. This requires that each Hotspot settings is given a tailor-made intervention after a critical assessment of the LF transmission dynamics in such settings. The ICT/FTS band intensity scoring method offers additional critical information about the *W. bancrofti* transmission dynamics such as antigenaemia and microfilaraemia clearance, repopulation and/or reinfection status in the treated individuals of any Hotspot settings.

Conclusion

The current single-dose ABZ and IVM combination therapy drug regimen for LF MDA is still efficacious and has reduced parasitaemia significantly in the blood of people in LF endemic districts leading to interruption of transmission in most endemic districts of Ghana [8, 44]. However, some individuals in the Hotspot districts have mf concentrations and densities high enough to be transmitted by competent vectors such as *Anopheles* mosquitoes, which are efficient even at low

parasitaemia. These vectors known to be exhibiting the phenomenon of "limitation" are found in the study districts. This is of concern because Bongo District, which had stopped MDA have antigenaemia prevalence above the recommended WHO threshold for stopping MDA, thus there is the potential of having LF resurgence in this district. There is the need for tailor-made solutions to accelerate elimination of LF in Hotspot districts due to the unique nature of the factors that ensures the maintenance of residual disease transmission in these settings. The semiquantitative ICT/FTS antigenaemia band intensity scoring method and its related analyse package can be used as a surrogate for estimating *W. bancrofti* parasite clearance, repopulation, and/or reinfection in the human population.

Recommendation

Results from parasitological survey data should always be complemented with entomological data and the ICT/FTS band intensity scoring method to decide whether to stop MDA after assessing antigenaemia and microfilaraemia prevalence in an endemic area. Also, the WHO threshold for stopping MDA should be reviewed for Hotspot areas. The threshold value should be lower than the existing one to ensure adequate rounds of MDA that will interrupt LF disease transmission and hence MDA stoppage. This will help reduce the number of Hotspot districts created through prolonged MDA. Further, due to issues of spatial heterogeneity in Hotspot districts, there is the need to review the criteria for LF MDA activities and disease interruption using custom-made solutions to expedite the goal of elimination. This will help intervention programme managers to meet the 2030 deadline set for the new NTD Roadmap

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the Noguchi Memorial Institute for Medical Research, Accra, Ghana (CPN 077/13-14).

Informed Consent Statement: Informed consent for participation and publication was obtained from all identifiable human participants. Consent forms were completed by all participants.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Conflict of interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Meaning
ABI	Applied Biosystems Instrument
ABZ	Albendazole
AW	Ahanta West
CDC	Center for Disease Control and Prevention
CFA	Circulating Filarial Antigen
CHPS	Community-Base Health Planning and Service
DDCO	District Disease Control Officer
DEPC	Diethylprocarbonate
DHMT	District Health Management Team
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
FTS	Filarial Test Strip
GMT	Greenwich Mean Teime
GPELF	Global Programme to Eliminate Lymphatic Filariasis
ICT	Immunochromatographic Test
1D	Identification
IVM	Ivermectin
IVM/VC	Integrated Vector Management / Vector Control
KNW	Kassena Nankana West
LDR	Long DNA Repeat
LF	Lymphatic Filariasis
MDA	Mass Drug Administration
MMDP	Morbidity Management and Disability Prevention
MOFA	Ministry of Food and Agriculture
NAMRU	Naval Medical Research Unit
NMIMR	Noguchi Memorial Institute for Medical Research
NTD	Neglected Tropical Diseases
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcription Polymerase Chain Reaction
TAS	Transmission Assessment Survey
USA	United States of America
UV	Ultraviolet

WHA	World Health Assembly
WHO	World Health Organisation

Appendix A

Appendix A.1

Mann-Kendall time series trend analysis

> #*Running Mann-Kendall time series trend analysis using R programme packages*

> library("mice")

> library("Kendall")

> library("trend")

> library("expss")

> library("finalfit")

> library("dplyr")

> library("tidyr")

> library("broom")

> sapply(pkgs,require,character.only=TRUE)

dplyr tidyr broom

 TRUE   TRUE   TRUE

> pkgs<-c("dplyr","tidyr","broom")

> library("rootSolve")

> require(R2wd)

> library("rtf")

###

Appendix B.1

> *#The Wuchereria bancrofti clearance, repopulation/reinfection in Atampiisi Bongo human*

population using time-series trends

> atb<-read.csv("C:\\Users\\JHNOSEI\\Desktop\\Atampiisi wb trends.csv",header=TRUE)

> a<-data.frame(atb)

> a

Table Al. *Wuchereria bancrofti* trends in Atampiisi Bongo

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
1	P1	2	2	2	1
2	P2	1	2	0	1
3	P3	2	2	2	1
4	P4	2	2	Travelled	Travelled
5	P5	2	2	2	Travelled
6	P6	2	2	2	1
7	P7	2	2	0	1

Appendix B.2

> *#The "mice" package is run to detect missing data values (i.e. Travelled) to allow the software*

insert predicted values (in the programme's memory for this table) after running the "complete"

command

> h<-mice(a)

Warning message:

Number of logged events: 1

> *#The "complete" command is used to fill in missing values with the most likely values after some*

R background simulations

> a=complete(h)

>

> #*Estimating missing values to fill in all "Travelled" values using time-series trends or simulations*

> TS<-ts(a$X2nd.Follow.Up,frequency=1,start=c(a$X2nd.Follow.Up,7))

> TS

Time Series:

Start = 2

End = 8

Frequency = 1

[1] 2 1 2 3 2 2 1

attr(,"levels")

[1]   0   2 Travelled

>

> #*The "MannKendall(TS)" command is executed to runs the time-series trend analysis for the data*

under consideration

> MK=MannKendall(TS)

> #*The "Summary(MK) summarizes the Mann-Kendall's time-series trend analysis*

> summary(MK)

Table A2. Time series summary for Atampiisi Bongo.

Score =	Var(Score) =	denominator =	tau =	2-sided pvalue =
-2	34.66667	17.14643	-0.117	0.86513

>

Appendix B.3

> #*The Sen's slope estimates the approximate gradient for the Mann-Kendall's time-*

series trend analysis

> sens.slope(TS)   Sen's slope

data: TS

Table A3. Sen's slope estimations for Atampiisi Bongo.

z =	n =	p-value =	alternative hypothesis: true z is not equal to 0	95 percent confidence interval:		sample estimates: Sen's slope
-0.16984	7	0.8651		-0.5000	0.3333	0

Appendix B.4

> #*The Pettitt test gives the point in the data where the change begins or occurs*

> pettitt.test(TS)   Pettitt's test for single change-point detection

data: TS

Table A4. Pettitt's test estimations for Atampiisi Bongo.

U* =	p-value =	alternative hypothesis: two.sided	sample estimates:	probable change point at time K
5	1.364			6

> a[6,]

Table A5. Pettitt's test point-of-change values.

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
6	P6	2	2	2	1

>

> #*Notice that where applicable, all three parameters (Mann-Kendall, Sen's Slope, and Pettitt's Test), are considered to give a better explanation for the time-series trends.*

Appendix C.1

> #*Wuchereria bancrofti clearance, repopulation/reinfection in Balungu Nabiisi human population using time-series trends*

> bln<-read.csv("C:\\Users\\JHNOSEI\\Desktop\\Balungu wb trends.csv",header=TRUE)

> b<-data.frame(bln)

> b

Table Al. *Wuchereria bancrofti* trends in Balungu Nabiisi

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
1	P8	2	2	Travelled	Travelled
2	P9	2	2	2	1
3	P10	2	2	0	1
4	P11	2	1	0	0
5	P12	2	Travelled	Travelled	Travelled
6	P13	2	2	Travelled	2
7	P14	2	2	2	Travelled
8	P15	2	2	2	1
9	P16	2	Travelled	Travelled	1
10	P17	2	2	2	0
11	P18	2	2	0	0
12	P19	2	2	2	Travelled
13	P20	2	2	2	1
14	P21	2	0	Travelled	0

> i<-mice(b)

Number of logged events: 61

> b=complete(i)

> *#Estimating missing values*

> TS1<-ts(b$X2nd.Follow.Up,frequency=5,start=c(b$X2nd.Follow.Up,10))

> TS1

Time Series:

Start = c(1, 2)

End = c(3, 5)

Frequency = 5

 [1] 1 2 1 1 3 3 2 2 3 2 1 2 2 3

attr(,"levels")

[1]   0   2 Travelled

>

> MK=MannKendall(TS1)

> summary(MK)

Table A2. Time series summary for Balungu Nabiisi

Score =	Var(Score) =	denominator =	tau =	2-sided pvalue =
20	288	76.31514	0.262	0.26289

> sens.slope(TS1)   Sen's slope

data: TS1

Table A3. Sen's slope estimations for Balungu Nabiisi

z =	n =	p-value =	alternative hypothesis: true z is not equal to 0	95 percent confidence interval:	sample estimates: Sen's slope
1.1196	14	0.2629		0.00 0.167	0

> pettitt.test(TS1)   Pettitt's test for single change-point detection

data: TS1

Table A4. Pettitt's test estimations for Balungu Nabiisi

U* =	p-value =	alternative hypothesis: two.sided	sample estimates:	probable change point at time K 4
30	0.3187			

> b[4,]

Table A5. Pettitt's test point-of-change values

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
4	P11	2	1	0	0

> TS2<-ts(b$X3rd.Follow.Up,frequency=14,start=c(b$X3rd.Follow.Up,14))

> TS2

Time Series:

Start = c(2, 2)

End = c(3, 1)

Frequency = 14

 [1] 2 2 2 1 4 3 4 2 2 1 1 4 2 1

attr(,"levels")

[1]   0   1   2 Travelled

>

> MK=MannKendall(TS2)

> summary(MK)

>c

Table A6. Time series summary

Score =	Var(Score) =	denominator =	tau =	2-sided pvalue =
-13	293	78.08329	-0.166	0.48327

> sens.slope(TS2)   Sen's slope

data: TS2

Table A7. Sen's slope estimations

z =	n =	p-value =	alternative hypothesis: true z is not equal to 0	95 percent confidence interval:	sample estimates: Sen's slope
-0.70105	14	0.4833		-0.2000 0.0000	0

> pettitt.test(TS2)   Pettitt's test for single change-point detection

data: TS2

Table A8. Pettitt's test estimations

U* =	p-value =	alternative hypothesis: two.sided	sample estimates:	probable change point at time K NA, 7, 8, NA, 9
19	0.9573			

> b[7,]

Table A9. Pettitt's test point-of-change values

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
7	P14	2	2	2	Travelled

> b[8,]

Table A10. Pettitt's test point-of-change values

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
8	P15	2	2	2	1

> b[9,]

Pettitt's test point-of-change values

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
9	P16	2	Travelled	Travelled	1

> #*Wuchereria bancrofti clearance, repopulation/reinfection in the Badunu human population using time-series trends*

>   bnu<-read.csv("C:\\Users\\JHNOSEI\\Desktop\\Badunu   wb trends.csv",header=TRUE)

> d<-data.frame(bnu)

> d

Table Al. *Wuchereria bancrofti* trends in Badunu

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
1	P22	2	2	2	1
2	P23	2	2	0	1
3	P24	2	2	2	0

> #*Estimating time-series trends*

> TS3<-ts(d)

\> MK=MannKendall(TS3)

\> summary(MK)

Table A2. Time series summary for Badunu

Score =	Var(Score) =	denominator =	tau =	2-sided pvalue =
-37	311.6667	82.61356	-0.448	0.041431

\> sens.slope(TS3)   Sen's slope

data: TS3

Table A3. Sen's slope estimations for Badunu

z =	n =	p-value =	alternative hypothesis:	95 percent confidence interval:		sample estimates: Sen's slope
-2.0392	15	0.04143	true z is not equal to 0	-0.1667	0.0000	0

\> #*Wuchereria bancrofti clearance, repopulation/reinfection in the Navio (Sanwu)*

Central human population using time-series trends

\>   nvc<-read.csv("C:\\Users\\JHNOSEI\\Desktop\\Navio   wb

trends.csv",header=TRUE)

\> e<-data.frame(nvc)

\> e

Table A1. *Wuchereria bancrofti* trends in Navio (Sanwu) Central

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
1	P25	2	2	Travelled	Travelled
2	P26	2	2	2	1
3	P27	2	2	Travelled	Travelled
4	P28	2	2	Travelled	Travelled

> k<-mice(e)

Number of logged events: 3

> e=complete(k)

>

> *#Estimating missing values*

> *#No variable has been stated for time-series simulation hence time series is run*

for entire data frame "e"

> TS4<-ts(e)

> MK=MannKendall(TS4)

> summary(MK)

Table A2. Time series summary for Navio (Sanwu) Central

Score =	Var(Score) =	denominator =	tau =	2-sided pvalue =
-28	538	124.8199	-0.224	0.2444

> sens.slope(TS4)   Sen's slope

data: TS4

Table A3. Sen's slope estimations for Navio (Sanwu) Central

z =	n =	p-value =	alternative hypothesis: true z is not equal to 0	95 percent confidence interval:		sample estimates: Sen's slope
-1.1641	20	0.2444		0.0000	0.0000	0

> *#Wuchereria bancrofti clearance, repopulation/reinfection in the Antseambua*

human population using time-series trends

>   ant<-read.csv("C:\\Users\\JHNOSEI\\Desktop\\Antseambua   wb

trends.csv",header=TRUE)

> f<-data.frame(ant)

> f

Table Al. *Wuchereria bancrofti* trends in Atseambua

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
1	P29	2	1	2	1
2	P30	2	2	2	0

>

> #*Estimating time-series trends*

> TS5<-ts(f)

> MK=MannKendall(TS5)

> summary(MK)

Table A2. Time series summary for Atseambua

Score =	Var(Score) =	denominator = 34.85685	tau =	2-sided pvalue =
-9	93		-0.258	0.40679

> sens.slope(TS5)   Sen's slope

data: TS5

Table A3. Sen's slope estimations for Atseambua

z =	n =	p-value =	alternative hypothesis: true z is not equal to 0	95 percent confidence interval:		sample estimates: Sen's slope
-0.82956	10	0.4068		-0.2500	0.0000	0

> #*Wuchereria bancrofti clearance, repopulation/reinfection in the Asemkow human population using time-series trends*

>   asm<-read.csv("C:\\Users\\JHNOSEI\\Desktop\\Asemkow   wb trends.csv",header=TRUE)

> g<-data.frame(asm)

> g

Table Al. *Wuchereria bancrofti* trends in Asemkow

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
1	P31	2	2	Travelled	2
2	P32	2	2	Travelled	2
3	P33	2	2	2	2

> m<-mice(g)

Number of logged events: 3

> g=complete(m)

>

> #*Estimating missing values*

> TS6<-ts(g)

> MK=MannKendall(TS6)

> summary(MK)

Table A2. Time series summary for Asemkow

Score =	Var(Score) =	denominator =	tau =	2-sided pvalue =
-4	194.6667	63.16645	-0.0633	0.82975

> sens.slope(TS6)   Sen's slope

data: TS6

Table A3. Sen's slope estimations for Asemkow

z =	n =	p-value =	alternative hypothesis:	95 percent confidence interval:		sample estimates: Sen's slope
-0.21502	15	0.8298	true z is not equal to 0	0.0000	0.0000	0

> pettitt.test(TS6)   Pettitt's test for single change-point detection

data: TS6

Table A4. Pettitt's test estimations for Asemkow

U* =	p-value =	alternative hypothesis: two.sided	sample estimates:	probable change point at time K
13	1.509			1

> g[1,]

Table A5. Pettitt's test point-of-change values

#	Participant	Baseline	X1st.Follow.Up	X2nd.Follow.Up	X3rd.Follow.Up
1	P31	2	2	Travelled	2

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