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PCR and Mosquito dissection as tools to monitor filarial infection levels following mass treatment

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Abstract

Background: Entomological methods may provide important tools for monitoring the progress of lymphatic filariasis elimination programs. In this study, we compared dissection of the vector, *Culex quinquefasciatus*, with the polymerase chain reaction (PCR) to assess filarial infection levels in mosquitoes in the context of a lymphatic filariasis elimination program in Leogane, Haiti.

Methods: Mosquitoes were collected using gravid traps located in 4 sentinel communities with *Wuchereria bancrofti* microfilaria prevalence that ranged from 0.8% to 15.9%. Captured mosquitoes were divided between dissection, to enumerate *W. bancrofti* larvae (L1, L2, L3) and desiccation for later analysis by PCR. PCR was conducted on DNA extracts from pooled mosquitoes (1–15 pooled females) utilizing a competitive PCR system with primers specific for the *Ssp I* repeat. PCR products were analyzed with a hybridization ELISA using probes specific for a control sequence and the *Ssp I* repeat.

Results: The prevalence of mosquito infection with *W. bancrofti* ranged from 0%–3.66% by dissection (L1–L3) and point estimates of infection prevalence, as assayed by PCR, ranged from 0.25% – 9.16%. Following mass treatment, *W. bancrofti* infection prevalence dropped significantly as determined by PCR and dissection in 2 of the 4 sentinel sites (Leogane and Barrier Jeudi, P = 0.04 and P = 0.005, respectively). Although transmission declined in the other two sites, larval recoveries were low and these changes were not statistically significant.

Discussion: Our results suggest that a single round of mass treatment can have an impact on transmission of lymphatic filariasis. The use of entomologic methods as a tool to monitor filariasis programs and the statistical limitations of mosquito trapping are discussed.

Background

Nearly 1.2 billion people are at risk of developing lymphatic filariasis and over 128 million are estimated to have circulating microfilariae or one of the various clinical conditions associated with filarial infection [1]. With tools currently available, lymphatic filariasis, in principle, could be eliminated. Combined drug treatment regimens using annual treatment with microfilaricidal drugs are the method of choice for combating filarial infection and

elimination programs using this strategy have commenced in more than 30 countries [2].

Demonstrating the success of filariasis programs depends on careful monitoring of infection levels in human populations as well as vectors following the introduction of the drug intervention. Due to reluctance on the part of human populations to submit to regular blood examinations, assessment of infection in vectors offers advantages for the monitoring of infection after a mass treatment program has been implemented. Two methods of detecting infection in vector populations that are now being widely used are dissection and PCR. Dissection has been the gold standard for measuring infection level in mosquitoes; however, dissection becomes increasingly costly and laborious in areas where mosquito infection prevalence drops below 1%. Furthermore, in preliminary studies, PCR was more sensitive for detecting filarial parasites in mosquitoes than conventional dissection and microscopy [3]. The ability to adapt PCR techniques to detect microfilariae in pools of mosquitoes suggests that current PCR methods should be adequate for testing large numbers of mosquitoes in the context of filariasis elimination programs. PCR has already been demonstrated to be a successful tool for monitoring onchocerciasis programs [4,5]. The availability of such a tool permits rigorous surveillance for resumption of transmission following completion of control activities.

This study was designed to monitor mosquito infection levels in conjunction with a mass treatment program for filariasis in the commune of Leogane, Haiti. The goal of this program in Leogane was to develop an effective elimination strategy that could be adapted to other settings in Haiti and elsewhere. The drug intervention consisted of annual treatment with diethylcarbamazine (DEC) and albendazole and was monitored by collecting data in 4 well-characterized sentinel sites. We assessed filarial infection rates of vectors trapped in the 4 sentinel sites and compared PCR with dissection as monitoring tools.

Methods

Study site

The study was conducted in the plains of the Commune of Leogane, located west of Port au Prince. To monitor the impact of the mass treatment program, sentinel sites were chosen based on estimated community antigen prevalence inferred from rapid card-based immunochromatographic testing (ICT) of local school children (Amrad ICT, New South Wales, Australia). Microfilaria prevalence and intensity were assessed in the sentinel sites prior to the intervention. Mass treatment using single dose diethylcarbamazine (6 mg/kg) and albendazole (400 mg) was carried out at the end of October 2000. Details of the drug distribution will be reported elsewhere. Research activities in the sentinel sites were reviewed and approved by the CDC and Notre Dame Institutional Review Boards, and the Ethics Committee of Hopital St. Croix.

Mosquito Trapping

The main vector for *Wuchereria bancrofiti* in Haiti is *Culex quinquefasciatus* [6]. Some transmission occurs year-round. The trapping of mosquitoes was done using CDC gravid traps (Model 1712, J.W. Hock Co., Gainesville, FL), 5 to 6 per sentinel site. Collections were done in each sentinel site for 4 consecutive nights every other week. Mosquitoes were collected from traps each morning within 30 minutes of dawn. After transport to the laboratory at Hopital St. Croix, mosquitoes were knocked down by placing trap containers in a -20°C freezer for ten minutes or by introduction of a triethylamine-soaked cotton pad, and subsequently separated by species and sex; undesired specimens were noted and discarded (males and non-vector species). Female *Culex* mosquitoes were divided 1:1 for assay by PCR or dissection for the first 100 mosquitoes caught at each trap; the remainder was used for PCR. The samples to be used for PCR assay were desiccated and sorted until shipment to CDC for processing. Because preliminary PCR experiments showed inhibition to be an occasional problem when pool sizes of 25 or greater were used, mosquitoes to be desiccated were sorted into batches of no more than 15 mosquitoes and then placed atop a cotton plug, which was above 1/4 inch of drierite desiccant in a polypropylene screw cap tube. This ensured desiccation and reduced any concerns about fungal contamination during storage and transport.

Mosquito Dissection

Mosquitoes were dissected on a glass slide using a set of dissection needles to separate the head, thorax, and abdomen. A saline solution was dropped onto each segment. These three body segments were then teased apart and examined stereoscopically to reveal larval stage worms or microfilaria. Mosquito infection status was noted as well as the corresponding location of the parasites. Ten percent of dissected mosquitoes harboring 3rd stage larvae were microscopically examined at 100× to verify filarial species. A maximum of 50 mosquitoes were dissected per trap per day. Dissection results were entered into an Epi-Info database. Infective mosquitoes were defined as those containing L3 larvae in any of the body segments. Mosquitoes carrying microfilaria, L1, L2, or L3 larvae were defined as infected.

Extraction of DNA

DNA extraction for PCR amplification was performed as described by Chanteau et al. [7]. Briefly, the dried pools of mosquitoes were homogenized with a pellet pestle (Kimble/Kontes, Vineland, NJ, USA) and 500 μl of NIB buffer (5 M NaCl, 1 M Tris pH 8, 0.5 M EDTA, 0.07% β-mercap-

toethanol, 0.5% NP-40) in a 1.5 ml eppendorf tube (Marsh Bio Products, Rochester, NY, USA). The extracted DNA was then bound to silica beads (Sigma-Aldrich, St. Louis, MO, USA). The beads were washed to remove inhibitors. After washing, the silica beads were dried and the DNA was then eluted into 100 μl of 1X Tris-EDTA (1% Tris, 0.02% EDTA), pH 8.

PCR Amplification

The PCR for the competitive ELISA analysis was conducted using NV-1 and NV-2 oligonucleotides specific for the *Ssp I* repeat as previously described by Bockarie and others [8]. Amplification was performed with an Applied Biosystems 9700 PCR thermocycler (Branchburg, NJ, USA) using 36 cycles with Amplitaq gold (Applied Biosystems Branchburg, NJ, USA) in a reaction volume of 50 μl. Included in the assays was 100 fg of internal control plasmid [9] (generously provided by Dr. Steven Williams of Smith College) to verify amplification. Primer NV-2 was biotinylated to facilitate binding of the product to a streptavidin (Sigma-Aldrich) -coated microtiter plate (Immulon II Thermo Labsystems, Franklin, MA, USA). UV treated distilled water without internal control was used as a negative control. Positive control DNA was derived from parasites obtained from microfilaria infected individuals. Filarial DNA was extracted using a DNAzol (Invitrogen, Carlsbad, CA, USA) extraction method as described by the manufacturer.

PCR-ELISA

PCR products were detected by ELISA as per protocols described by Fischer and others [9] with slight modifications described below. PCR product was added to streptavidin-coated plates. The initial volume of the PCR product (50 μl) was diluted to a total volume of 220 μl with hybridization buffer. Samples were tested at 100 μl per well and were hybridized with fluorescein-labeled probes specific for wild type or internal control sequences. Probes were hybridized to plate bound PCR products at 55°C for 30 minutes and then incubated alkaline phosphatase-labelled anti-fluorescein Fab fragments (Roche Diagnostics, Indianapolis, IN). Plates were sealed with Falcon pressure sensitive film (Falcon Becton Dickinson, Franklin Lakes, NJ, USA) at all assay stages to eliminate any potential contamination between wells. Plates were read on a Uvmax plate reader at 405 nm (Molecular Devices, Sunnyvale, CA, USA) after 1 hr development with substrate. A positive sample was defined as previously described, as 5 times the uncorrected optical density (OD) of a sample containing no template DNA, but with 100 fg of internal control [8]. The controls were designed to allow for cutoff determination, as well as to determine the success of the PCR thermocycler run. Amplified high positive control DNA (0.1 μg of extracted *W. bancrofti* DNA) yielded an almost immediate response by ELISA and low positive control DNA (0.01 μg concentration) provided a low range positive against which to compare samples. Both sample positive controls were run with and without 100 fg of internal control to monitor the level of competition between internal control and *W. bancrofti* template DNA. Negative controls included water with and without 100 fg of internal control and DNA extracted from a pool of 15 parasite-negative lab-reared mosquitoes (2 μl of extracted product, prepared in parallel with Haitian samples). UV treated, distilled, deionized water was used to replace the volume of mosquito DNA usually present. This sample was run in triplicate, along with all other controls. In conjunction with the PCR controls, the microtiter plates had a plate blank on each plate that was 6 wells of sample buffer hybridized with either wild probe or internal control. Positive controls exhibited a high degree of reproducibility with the cutoff as established. The high positive (0.1 μg *W. bancrofti* DNA) was 97% reproducible and the low positive was positive for 86% of the assays.

Statistical Analysis

Prevalence rates from dissection data were computed and compared using Sudaan version 8.0 (SUDAAN Statistical Software Center, Research Triangle Park, NC, USA), which takes into consideration the correlation of multiple collections from the same location over time. The t-test was used for rate comparisons. The Pearson correlation coefficient was used to estimate the association of dissection based prevalence rates with PCR based rates. PCR point estimates were computed and compared using Poolscreen 2.0 generously provided by Dr. Tom Unnasch and Charles Katholi (The University of Alabama, Birmingham) [10].

Results

Baseline microfilaria prevalence in the human population ranged from 0.8–15.9% in the sentinel sites (Table 1). Median microfilaria level ranged from 5 to 11 microfilaria per 20 μl for microfilaria-positive persons. Microfilaria intensity did not differ significantly by sentinel site (Table 1). Mass treatment with DEC/albendazole took place at the end of October 2000 and tablets were taken by 72% of the at risk population [11]. By 9 months following treatment, microfilaria prevalence declined to 3%, 4.3%, 0.4% and 6% in Barrier Jeudi, Masson/Mathieu, Mapou and Leogane, respectively.

To monitor the impact of mass treatment on filarial infection level in mosquitoes, trapping was initiated in August of 2000, 3 months prior to mass treatment. Because the number of mosquitoes trapped and percentage of infected mosquitoes varied from month to month, results were pooled into 3-month intervals for analysis. As seen in Figure 1, total mosquito infection levels, as detected by dissection, declined from pre-treatment to the first post-treatment interval in all 4 sites; however, these decreases

Table 1: *Wuchereria bancrofti* Baseline Microfilaria Prevalence[1]

Community	Microfilaria (%)	Median[2]
Barrier Jeudi	78/1157 (6.7)	8
Masson/ Mathieu	125/1140 (11.0)	11
Mapou	5/588 (0.8)	5
Leogane	98/617 (15.9)	7

1 Microfilaria prevalence was determined by microscopic examination of a stained 20 μl thick film. Blood was collected nocturnally. 2 Median microfilaria count per 20 μl for microfilaria-positive persons.

Table 2: *W. bancrofti* Larval Recoveries in Dissected Mosquitoes Before and After Treatment

Locale	Time Interval	#Dissected	Mosquitoes w/larvae	%infected[1]	Mosquitoes w/L3	%infective
Masson/ Mathieu	Aug-Oct	334	7	2.1 (0.85,4.27)	4	1.20 (0.33,3.04)
Masson/ Mathieu	Nov-Jan	449	7	1.56 (0.63,3.19)	6	1.34 (0.49, 2.89)
Masson/ Mathieu	Feb-April	674	5	0.74 (0.24,1.72)	1	0.14 (0,0.82)
Mapou	Aug-Oct	379	2	0.53 (0.06,1.89)	1	0.26 (0.01,1.46)
Mapou	Nov-Jan	815	0	0 (0,0.45)	0	0 (0,0.45)
Mapou	Feb-April	370	2	0.54 (0.07,1.94)	2	0.54 (0.07,1.94)
Barrier Jeudi	Aug-Oct	328	12	3.66 (1.90, 6.30)	8	2.44 (1.06,4.75)
Barrier Jeudi	Nov-Jan	722	5	0.69** (0.23,1.61)	0	0* (0,0.51)
Barrier Jeudi	Feb-April	902	5	0.55* (0.16,1.17)	2	0.22 (0.03,0.80)
Leogane	Aug-Oct	2033	52	2.56 (1.92,3.34)	18	0.89 (0.53,1.40)
Leogane	Nov-Jan	1385	22	1.59 (1.00,2.40)	10	0.72 (1.00,2.40)
Leogane	Feb-April	1068	16	1.50* (0.86,2.42)	8	0.75 (0.86,2.42)

[1]Prevalence (95% confidence interval) * = P < 0.05 ** = P < 0.01 Compared to Baseline

were statistically significant in only 2 of the sites, Leogane and Barrier Jeudi. With the exception of Mapou, larval infection rates were lower in the second than in the first 3-month time interval following treatment. The numbers of L3 larvae recovered declined significantly in only one of the 4 sentinel sites (Figure 1, Table 2). If we adopt a more conservative definition of what constitutes an infective mosquito and restrict our analysis to those with L3 found in the head, no change over time was observed. In fact, only 16 out of 9459 mosquitoes dissected had L3 in the head (Table 3).

W. bancrofti infection of *Culex* mosquitoes was also assessed by PCR for a total of 1704 pools (median pool size = 7) collected over the study period. Because pool sizes ranged from 1 to 15, we used Poolscreen 2.0 to generate point estimates and confidence intervals for the level of filarial infection in mosquitoes. As seen in Table 4, point estimates of *W. bancrofti* infection prevalence declined following mass treatment in all 4-sentinel sites. Because of the relatively wide confidence intervals, significant declines in infection prevalence were observed in only Barrier Jeudi and Leogane for both time periods following treatment, mirroring the results obtained with dissection.

We also compared changes in infection prevalence for mosquito pools of constant size (n = 15) collected in the Leogane sentinel site. The other three sentinel sites did not have enough pools of this size to conduct this analysis (Table 4). Infection prevalence in Leogane decreased from 4.5% pre-treatment to 0.55% in the first three months following treatment and to 0.12% in the second time period (Figure 2). These numbers agree closely with those generated when all pools were included in the analysis. There was a moderately strong correlation between larval infection rates as determined by dissection and PCR (Figure 3; r = 0.72, p = 0.008).

Discussion

Careful monitoring of filarial transmission is necessary, both to make programmatic decisions about stopping mass treatment as well as to assist with certification of elimination. Serologic tools for assessment of exposure to

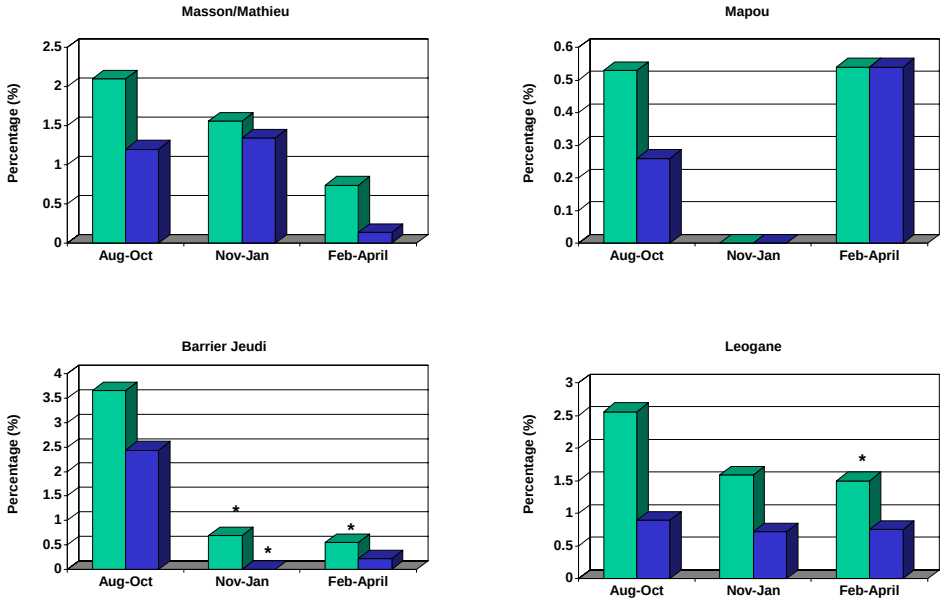

Figure 1
Wuchereria bancrofti in *Culex* mosquitoes before and after mass treatment. Dissection results were pooled in 3-month intervals and are plotted for each of the four sentinel sites. The percentage of mosquitoes harboring any stage larvae is plotted in the lighter bars. The percentage of mosquitoes carrying L3 larvae is plotted in the darker bars. Significant changes from the August-October pre-treatment interval are indicated with an asterisk. Note that different scales are used for each sentinel site.

Table 3: *W. bancrofti* L3 Stage Larvae Recoveries in Dissected Mosquitoes Before and After Treatment

Locale	Time Interval	#Dissected	#Mosquitoes w/ L3 in Head	% L3 in Head (Confidence Limits)	Mosquitoesw/L3	%infective
Masson/Mathieu	Aug-Oct	334	1	0.3 (0.01, 1.66)	4	1.20 (0.33,3.04)
Masson/Mathieu	Nov-Jan	449	2	0.45 (0.05, 1.60)	6	1.34 (0.49, 2.89)
Masson/Mathieu	Feb-April	205	0	0 (0, 1.78)	1	0.14 (0,0.82)
Mapou	Aug-Oct	379	0	0 (0, 0.97)	1	0.26 (0.01,1.46)
Mapou	Nov-Jan	815	0	0 (0, 0.45)	0	0 (0,0.45)
Mapou	Feb-April	370	1	0.27 (0.01, 1.50)	2	0.54 (0.07,1.94)
Barrier Jeudi	Aug-Oct	328	4	1.22 (0.33, 3.09)	8	2.44 (1.06,4.75)
Barrier Jeudi	Nov-Jan	740	0	0 (0, 0.50)	0	0* (0,0.51)
Barrier Jeudi	Feb-April	902	0	0 (0, 0.40)	2	0.22 (0.03,0.80)
Leogane	Aug-Oct	2033	1	0.05 (0, 0.27)	18	0.89 (0.53,1.40)
Leogane	Nov-Jan	1385	3	0.22 (0.04, 0.63)	10	0.72 (1.00,2.40)
Leogane	Feb-April	1068	4	0.37 (0.10, 0.96)	8	0.75 (0.86,2.42)

Table 4: The Prevalence of *W. bancrofti* in Mosquito Pools

Site	Timepoint*	#Mosquitoes	#pools[1]	Point Estimate[2]	Confidence Interval[2]	%infective
Masson/ Mathieu	Aug-Oct	409	96	0.0716	.0451, .0106	1.20 (0.33,3.04)
Masson/ Mathieu	Nov-Jan	450	86	0.0161	.0060, .0335	1.34 (0.49, 2.89)
Masson/ Mathieu	Feb-April	868	122	0.0171	.0088, .0293	0.14 (0,0.82)
Mapou	Aug-Oct	490	77	0.0329	.0175, .0552	0.26 (0.01,1.46)
Mapou	Nov-Jan	881	129	0.0104	.0044, .0201	0 (0,0.45)
Mapou	Feb-April	507	111	0.0326	.0173, .0547	0.54 (0.07,1.94)
Barrier Jeudi	Aug-Oct	456	104	0.0916	.0630, .1269	2.44 (1.06,4.75)
Barrier Jeudi	Nov-Jan	693	106	0.0089 *	.0030, .0196	0* (0,0.51)
Barrier Jeudi	Feb-April	1003	122	0.0226 *	.0219, .4722	0.22 (0.03,0.80)
Leogane	Aug-Oct	5280	394	0.0441	.0371, .0520	0.89 (0.53,1.40)
Leogane	Nov-Jan	1531	184	0.008 *	.0039, .0144	0.72 (1.00,2.40)
Leogane	Feb-April	1583	173	0.0025 *	.0006, .0065	0.75 (0.86,2.42)

* [1]Median Pool size = 7; [2]Derived with Poolscreen 2.0 software; * Indicates a Significant Difference

the parasite are still under development and testing. Assessing infection in humans can be considered a "lagging indicator" because the prepatent period may extend for months after infection. In contrast, entomologic measures of filarial infection of vectors provide "real-time" estimates of filarial transmission. We compared dissection and PCR to determine their utility as monitoring tools for filariasis elimination programs.

For monitoring mosquito infection status within the context of a filariasis elimination program, mosquito dissection has been the gold standard against which other methods are compared. Our dissection results show a marked decrease in infection level from the pre-treatment period to the end of the post-treatment study period in 3 of the 4 sites that we monitored (Figure 1). The exception was Mapou, which started and ended the study with almost the same infection prevalence in the mosquito populations we collected. This can be attributed to the smaller than desired sample of mosquitoes captured (379 pre-treatment and 370 during the second post-treatment time interval) and the low infection prevalence that we observed there.

Dissection and PCR assays of captured mosquitoes generated similar, but not identical results. We observed a positive correlation between larval infection rates determined by these two methods (Figure 3). The proportion of positive pools detected by PCR was, as expected, higher than that obtained by dissection, even after using the PoolScreen program to calculate a point estimate of infection prevalence. This can be accounted for by the increased sensitivity that is provided by the PCR assay, especially for detection of early larval stages that are more difficult to detect by dissection. Independent of the method used, mass treatment led to a reduction in levels of *W. bancrofti* in trapped mosquitoes as assessed by dissection and PCR. The reductions evident via dissection were observed in all 4 sentinel sites studied, representing an overall decrease of 60.8% from the pre-treatment levels to the final study interval 6 months post-treatment. The decreases in filaria-infected mosquitoes were comparable to the decreases in microfilaria prevalence seen in the human populations in the sentinel sites.

Although dissection is a very effective way to monitor infection prevalence in vector populations, it is a very laborious method for monitoring infection prevalence that requires highly trained technicians and can be cost intensive. Dissection is most suitable for monitoring mosquito infection levels when the infection level in the mosquito population is greater than 1%. This will be true in early stages of filariasis programs. The decision to use dissection rather than PCR may also be influenced by the trapping method. Household collections may yield substantially higher rates of infected mosquitoes than the gravid traps that were employed in our study. When infection prevalence declines below 1%, the potential for missing mosquitoes infected with the earliest larval stages and for misidentifying the species of filarial larvae found within mosquitoes increases. Another concern pointed out by Burkot and Ichimori [12] is that, as infection level declines, increasing numbers of mosquitoes must be dissected in order to demonstrate a significant decline in infection prevalence. This is especially true for infective larvae. In our study, the numbers of mosquitoes carrying infective larvae, as detected by dissection, were insufficient to allow for an adequate statistical analysis of

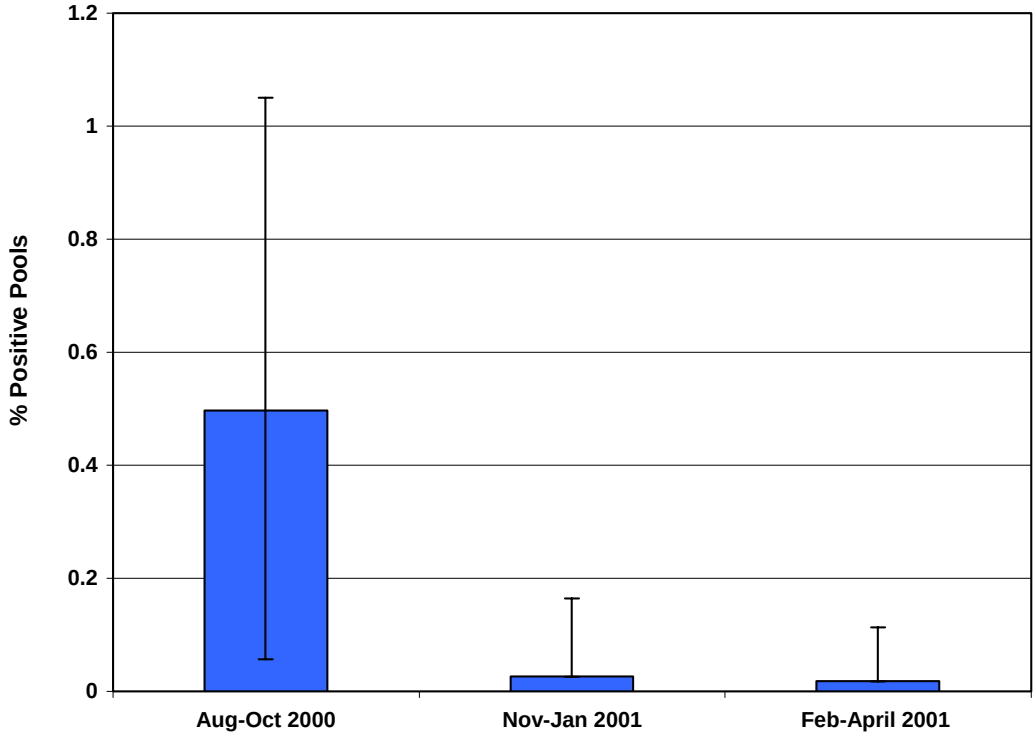

Figure 2
Point estimate of *W. bancrofti* infection prevalence in Leogane. Only pools of 15 mosquitoes were used for this analysis, Leogane was the only site with sufficient numbers of pools of this size. The point prevalence for each time interval was estimated using Poolscreen 2.0 software.

changes in infection prevalence. Monitoring transmission levels based on levels of infective larvae may not be feasible within the scope of lymphatic filariasis elimination programs in settings like Leogane where *Culex* is the vector because of the relative rarity of identifying infective mosquitoes. Lot quality assurance methods may provide an alternative sampling strategy that may prove useful for demonstrating with the smallest possible number of mosquitoes that filarial infection is below a pre-established threshold [13].

PCR assays of mosquitoes permit larger number of samples to be evaluated for infection prevalence. PCR is both specific and highly sensitive for the detection of parasite DNA. The primary limitations are the cost and the need for a well-equipped lab. In addition, PCR methods are also limited by a lack of robust statistical methods to compare and analyze data obtained from unequal pool sizes. Although confidence intervals of infection before and after treatment were overlapping for 2 of the sentinel sites, it is important to point out that confidence intervals were not overlapping for the two locations where dissection data indicated significant declines in larval prevalence.

We used a competitive PCR system in order to control for the presence of inhibitors that could prevent amplification and detection of parasite DNA. The disadvantage of the competitive PCR is the need to use an ELISA assay to quantify the product and the competitor, adding to the cost of the assay. Work underway is focused on trying to develop a standardized PCR methodology for all filariasis programs to employ [14]. Simple standardized methods

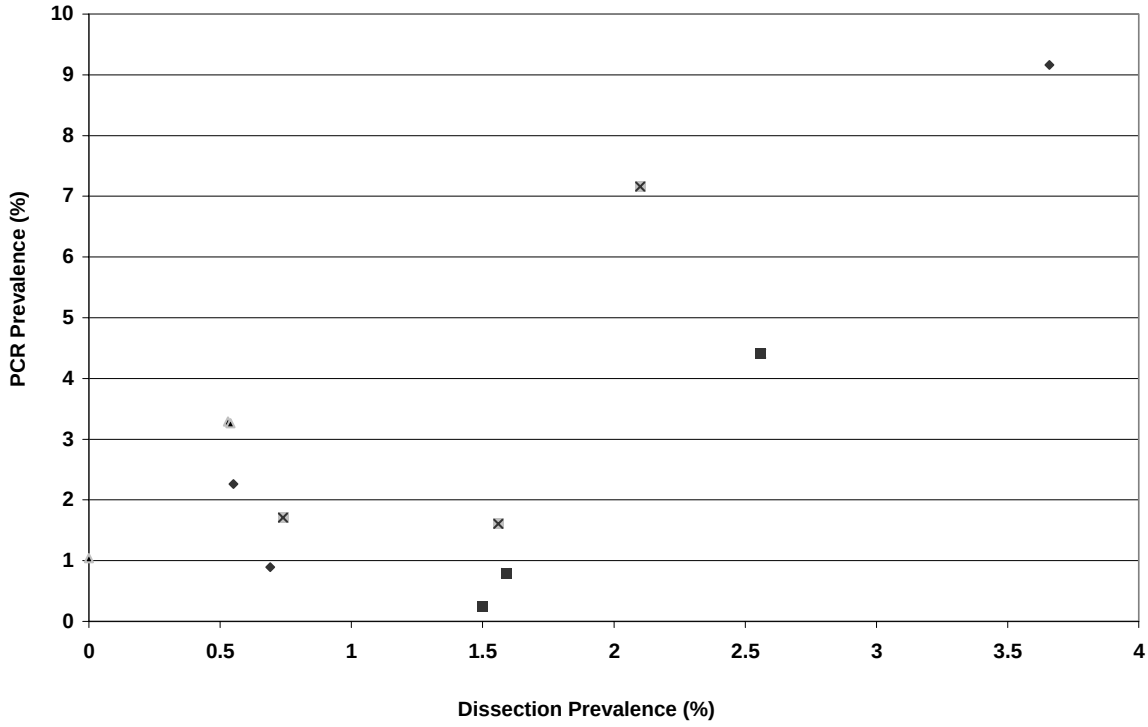

Figure 3
Scatter plot of *W. bancrofti* infection prevalence in mosquitoes as determined by dissection and PCR (r = 0.72). R-value calculated using Pearson correlation coefficient. Barrier Jeudi is represented with the diamond, Leogane is represented with the square, Mapou is represented by triangles, and the X represents Masson/Mathieu.

would permit comparisons of infection levels across epidemiologic settings as has been done with PCR assays for *O. volvulus* in black flies for Onchocerciasis control programs [4,5]. PCR methods for onchocerciasis work reliably for pools of up to 100 fly heads, an important advantage in terms of cost and throughput. As similar methods are validated for lymphatic filariasis, it is important to bear in mind that assay sensitivity will decline as filarial infection prevalence decreases, independent of the method used to detect *W. bancrofti*. Consequently, quality control considerations will become more important as the program progresses. WHO should consider distribution of controls to laboratories involved in PCR testing to permit inter-laboratory comparisons of results. Periodic proficiency testing represents an additional possibility.

A main concern of mass treatment programs is whether or not the decreases in transmission will be sustainable over time and will eliminate completely transmission of microfilaria to mosquitoes. Although infection levels were decreased, transmission of *W. bancrofti* was not interrupted by a single cycle of mass treatment in our study. This is not surprising given the intensity of transmission in this setting and the modest coverage in the first round. Additional follow up will be necessary to determine how many cycles of mass treatment will be necessary to achieve interruption of transmission in Leogane. These results will have important implications for the length of lymphatic filariasis elimination programs.

Competing Interests
None declared

Authors' Contributions

PJL and TGS designed and coordinated the study, JNO collected samples and oversaw dissections, DSG conducted PCR assays, data analysis, data entry, and JMR performed the statistical analysis.

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