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Evaluation of PCR-ELISA as a tool for monitoring transmission of *Wuchereria bancrofti* in District of Gampaha, Sri Lanka

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Comments
Lymphatic filariasis constitutes one of the major problem in tropical countries. This is a good study and the authors emphasized on the methods that used for diagnosis of lymphatic filariasis in *Cx. quinquefasciatus* mosquito, which is helpful to the management of vector control programmes in the country.
(Details on Page 386)

ABSTRACT

Objective: To compare *Wuchereria bancrofti* (*W. bancrofti*) infection rates of *Culex quinquefasciatus*, using dissection and PCR-ELISA in two consecutive time periods (from 2007 to 2008 and from 2008 to 2009). **Methods:** Mosquitoes were collected in 30 sentinel and 15 non-sentinel sites in 15 Medical Officer of Health areas of Gampaha District known for the presence of *W. bancrofti* transmission in two consecutive time period of 2007 to 2008 and 2008 to 2009. Captured mosquitoes were dissected to determine the *W. bancrofti* larvae (L_1, L_2, L_3). PCR was carried out using DNA extracted from mosquito pools (15 body parts/pool) utilizing the primers specific for Wb-SspI repeat. PCR products were analyzed by hybridization ELISA using fluorescein-labeled wild type specific probes. The prevalence of infected/infective mosquitoes in PCR pools (3 pools/site) was estimated using the PoolScreen™ algorithm and a novel probability-based method. **Results:** Of 45 batches of mosquitoes dissected, *W. bancrofti* infected mosquitoes were found in 19 and 13 batches, with an infection rate of 13.29% and 3.10% with mean larval density of 8.7 and 1.0 larvae per mosquito for two study periods in the Gampaha District. Total of 405 pools of head, thorax and abdomen were processed by PCR-ELISA for each year. Of these, 51 and 31 pools were positive for *W. bancrofti* in the two study periods respectively. The association of dissection based prevalence rates with PCR based rates as determined by the Pearson correlation coefficient were 0.176 and 0.890 respectively for the two periods. **Conclusions:** Data indicate that PCR-ELISA is more sensitive than the traditional dissection techniques for monitoring transmission intensity.

KEYWORDS
Wuchereria bancrofti, *Culex quinquefasciatus*, Dissection, PCR-ELISA, Sri Lanka

1. Introduction

Lymphatic filariasis (Lf) is one of the leading causes of disability, affecting 128 million individuals annually in more than 80 countries worldwide[1]. According to the current global estimation, over one billion people are at risk of developing Lf[2-4]. Historical data indicate the presence of Lf in Sri Lanka since 13th century[5]. At present, only bancroftian filariasis is found in the country, distributed mainly in eight districts in the Southern, Western, and North-Western Provinces inhabited by a population of approximately 10 million[3]. The Lf rate of endemic population in Gampaha District as assessed by thick blood film test for the year 2005 was 0.03%. In Sri Lanka, *Culex quinquefasciatus* (*Cx. quinquefasciatus*) acts as the vector of Lf and transmission usually occurs throughout the year[6].

The World Health Organization (WHO) initiated the Global Programme to Eliminate Lymphatic Filariasis in 2000 with the aim of eliminating Lf by 2020[1,3,7]. The program is mainly based on mass administration of antifilarial medication diethylcarbamazine citrate (DEC) (together with albendazole) to endemic populations (MDA), in order to achieve a human infection rate sufficient to make the transmission of Lf unsustainable. The WHO figures show

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that approximately 2 billion people living in 48 countries had been covered by the MDA programme in 2010[7]. In Sri Lanka, a 5-year MDA programme was implemented in 8 filaria-endemic districts in 2001. The programme, which consisted of annual administration of a combination of DEC (6 mg/kg) and albendazole (500 mg), was successful in reducing the Lf transmission in certain endemic areas[8,9]. Currently, the MDA is identified as the most cost-effective tool to interrupt the transmission and control of the spread of Lf[10]. To determine the successfulness of the MDA programme following implementation in endemic areas, it is vital to monitor the human and mosquito populations for Lf. Presently, three methods are mainly used to monitor the human populations. They include measuring the levels of filarial antigenemia, microfilarial load, and antifilarial antibodies[11]. All these methods are invasive and usually resisted by many individuals. Under these circumstances, screening of the vector populations for filarial parasites is a preferable alternative to monitor the successfulness of MDA programmes. Dissection and PCR-ELISA techniques are widely used to determine the Lf infection in mosquitoes[12,13]. While dissection has been the gold standard, PCR-based molecular xenomonitoring of vector populations for filarial DNA is becoming increasingly popular today. The latter method is highly sensitive, specific and robust[12,13]. Furthermore, screening of mosquito pools speeds up the processing of large numbers of specimens and thereby maximizes the efficiency of monitoring[12-13]. Several studies have successfully used a repeated DNA segment (Ssp I repeat) in the *W. bancrofti* genome for pool screening of Lf vectors[12,13]. So far, National Anti Filariasis Campaign (AFC) has been relying on the mosquito dissection technique to monitor the mass chemotherapy programme in Sri Lanka. In this study, we reported the comparison of adult mosquito dissection with a PCR and a PCR-ELISA assay, to determine their applicability as monitoring tools for the Lf elimination program in Sri Lanka. The goal of this study was to develop a highly effective methodology for the monitoring of Lf transmission levels in the country.

2. Materials and methods

2.1. Study sites

The present study was carried out in 15 Medical Officer of Health (MOH) areas in Gampaha District of the western province of Sri Lanka. The land area of Gampaha District is 1 387 square kilometres which is 2.1% of whole land area of Sri Lanka and has the latitude of 7.091 666 7° N, and longitude of 79.994 166 7° E. Gampaha is the district with the second highest population density. Its population density is 1 539 persons per square kilometre (Census, 2001). In order to monitor the filarial infection level in mosquito vectors, dissection and PCR-ELISA were used; in-door resting mosquito collection was carried out once during the year of 2007 to 2008. For further confirmation of the results study was repeated once, during the following year (2009). Three study sites were selected from each MOH area, which included two sentinel sites; (each from an urban and a semi-urban area) and one non-sentinel rural site. In each MOH area, all study sites were selected to represent the high-risk areas categorized by the national AFC, based on the prevalence rates of mosquito infection and the number of microfilaria positive patients. The ethical approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya.

2.2. Mosquito collection

Adult *Cx. quinquefasciatus* mosquitoes were collected in 15-30 households in each study site once a year. Initially, an index household was selected based on the presence of a microfilaria carrier (already treated for microfilaria) among the household residents within the past 5 years. The data were obtained from filariasis positive patient data records of the AFC. Rest of the households in each site was randomly selected within an area of 300 m radius from the index household. Indoor-resting adult mosquitoes were collected between 7:00 am and 11:00 am using mechanical aspirators from walls and hanging objects within houses.

2.3. Mosquito processing

Collected mosquitoes were knocked down by placing the trap containers in a -20 °C freezer for 10 min. Subsequently, they were separated based on species and sex using morphological and taxonomic keys described in the workshop on mosquito identification at MRIa, 2002[14]. Female *Cx. quinquefasciatus* mosquitoes were divided into batches of 15-20 mosquitoes for the analysis by PCR and dissection. Mosquito batches for dissection were analyzed immediately and the rest were desiccated and stored at -20 °C until further analysis. In this study, a minimum of three samples of pooled female *Cx. quinquefasiatus* mosquitoes (1-5) per site were analyzed. At least one pool of collected mosquitoes per site was dissected and examined for developing larvae while the rest of the mosquitoes were used for PCR assay.

2.4. Dissection of adult mosquitoes

The head, thorax and abdomen were dissected separately on a glass slide with 3 drops of normal saline and examined stereoscopically for developing larval stages. The data pertaining to mosquito infection status and the corresponding location were recorded. Only 10% of mosquitoes harboring different larval stages (L_1, L_2 and L_3) were examined under 100× magnification to verify larval stage. Dissection results were entered into an Epi-Info database. Mosquitoes carrying L_1 and L_2 stage larvae were defined as infected. Infective mosquitoes were defined as those containing L_3 larvae in any of the body segments examined.

2.5. Extraction of DNA and PCR amplification

Filarial genomic DNA was extracted as described by Chanteau *et al*[15]. The PCR amplification of the *Wuchereria bancrofti* (*W. bancrofti*) Ssp I repeat was performed as described by Williams *et al.* with modifications to facilitate the quantitative ELISA-based assay[15]. Each amplification reaction was performed in a final volume of 50 μL containing 10 mmol Tris-HCl (pH 9.2), 1.5 mmol $MgCl_2$, 75 mmol KCl, 1.25 mmol of each deoxynucleotide triphosphate (dNTP), 10 pmol each of the NV1 and NV2 primers and 2 units of Taq polymerase[16]. NV2 primer (reverse) was biotinylated at the 5′ end to enable the amplicon to bind to a streptavidin-coated microtiter plate. The PCR cycle consisted of an initial denaturation at 94 °C for 5 min, and then 35 cycles, of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, and final extension

of 72 °C for 10 min. The PCR controls included two negative controls and two positive controls as previously described by Kamel *et al*[17]. The PCR products were run on 15 g/L agarose gel and visualized by ethidium bromide staining.

2.6. PCR-ELISA assay

PCR products were detected by ELISA as described by Fischer and colleagues with minor modifications[18]. The PCR product (40 μL) was diluted in streptavidin-coated plates to a total volume of 220 μL with hybridization buffer [6× SSPE (0.8 mol sodium phosphate (pH 8.3), 3 mmol EDTA, 5× Denhardt's solution (0.01% ficoll, 0.01% polyvinylpyrolidone, 0.01% bovine serum albumen (BSA), 0.1% sodium sarcosine, 0.025 SDS and 0.05% NaN_3)]. Hundred microlitre aliquots of the solution were hybridized at 55 °C for 30 min with fluorescein-labeled probes specific for the wild type sequences. These were then incubated for 30 min at 37 °C with alkaline phosphatase labelled anti-fluorescein Fab fragments (Roche Diagnostics, Indianapolis, IN). The ELISA reading was recorded at 405 nm following a one-hour incubation at 37 °C using a Vmax microplate reader (Molecular Devices, Sunnyvale, CA). A positive sample was defined as 5 times the uncorrected optical density (OD) of a sample containing no template DNA[17]. Negative controls included water with and without DNA extracted from a pool of 15 parasite-negative lab-reared mosquitoes (2 μL of extracted product, prepared in parallel with study samples). This sample was run in duplicate, along with all other controls. In conjunction with the PCR controls, the microtitre plates also had a blank on each plate containing sample buffer hybridized with wild probe. The controls were designed to determine the success of the PCR amplification.

3. Results

3.1. In-door mosquito collection

Adult *Cx. quinquefasciatus* mosquitoes were collected from nearly 74.8% of selected households. A total of 8 415 mosquitoes were collected, ranging from 1 to 45 per household in each site during 2007-2008, and 6 711 mosquitoes ranging from 1 to 23 per household during the second study period of 2008-2009 respectively. The average mosquito densities per man-hour were 10.40 and 14.91 respectively for the two consecutive study periods in the district of Gampaha. In general, higher densities of adult *Cx. quinquefasiatus* mosquitoes were observed along the coastal areas over the inland areas. Collection of adult mosquitoes from a total of 1 350 households in the Gampaha District decreased from 650 to 555 households between the first and the second year of study (Figure 1). Further, total number of adult mosquitoes collected decreased from 8 415 to 5 012 from the total of 1 350 housholds. Some sites represented less than 45 mosquitoes per 60 households and the numbers were not sufficient for dissection and PCR-ELISA analysis. Therefore, during the second period of study, in addition to the mosquito collections from the 20 households additional collections were carried out in further 10 households randomly selected within the same area.

3.2. Dissections

A total of 926 *Cx. quinquefaciatus* mosquitoes were dissected from 45 batches, of which 19 batches included infected mosquitoes from the first study period. For the second study period it was 1 678 from 45 batches after of which 13 included infected mosquitoes respectively. The features of L_3 larva was illustrated in Figure 2. The image was observed under 100× magnification of light microscopy with image processing system under oil immersion technique. The mean larval density (L_1, L_2 or L_3) per positive mosquito was estimated as 8.7 and 1 per positive mosquito in the district of Gampaha during the two study periods of 2007-2008 and 2008-2009 respectively. The infection rates based on one infected mosquito per batch was lower than the average infected mosquito rate for the district of Gampaha and the difference was statistically significant ($P<0.05$). However, the difference of infective and infected mosquito rates were not statistically significant.

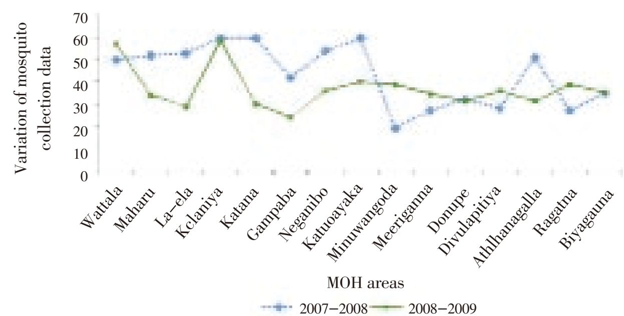

Figure 1. Variation of mosquito collection data among MOH. Comparison of finding of adult mosquitoes per 60 households within each MOH area during the two study periods.

Figure 2. *W. bancrofti* L_3 larvae resulted from *Culex* mosquito dissection from Meegahawaththa site (Kelaniya MOH area) (100×).

3.2.1. Infection rate in the parous vector population

Infection rate in the parous vector population resulted by dissection were 13.29% and 3.10% for the years 2007-2008 and 2008-2009 respectively in the Gampaha District.

3.2.2. Infective rate in the parous vector population

Percentage of infective mosquitoes in the parous vector population for the district of Gampaha as estimated by the dissection was 5.29% and 2.03% for the years 2007-2008 and 2008-2009 respectively.

Table 1
Estimation of *W. bancrofti* infection in vector mosquitoes by PCR.

Study period	Proportion of positive body fraction pools of mosquito				
	Head pools	Thorax pools	Abdomen pools	Sites	MOH areas
2007-2008	24/405	25/405	2/405	19/45	12/15
2008-2009	13/405	14/405	3/405	15/45	5/15

Table 2
Estimation of *W. bancrofti* infection in vector mosquitoes by PCR-ELISA.

Study period	Proportion of positive body fraction pools of mosquito				
	Head pools	Thorax pools	Abdomen pools	Sites	MOH areas
2007-2008	24/405	25/405	2/405	21/45	12/15
2008-2009	13/405	14/405	4/405	15/45	5/15

3.3. Larvae per parous mosquito

Value of larvae per parous mosquito was nine for the years 2007-2008 and one for the years 2008-2009 as determined by dissection.

3.4. DNA extraction from pooled mosquitoes

During the study periods (2007-2008 and 2008-2009) 405 pools of mosquito body parts (equal number of head, thorax and abdomen pools) were subjected to DNA extraction and further purification by Sephedax G-50 column. From these 405 body pools, 48 and 31 pools in the years 2007-2008 and 2008-2009 respectively gave clearly visible DNA band in the 15 g/L agarose gel.

Infection rates for head and thorax pools did not differ significantly from each other, but the overall infection rate of head and thorax pools were significantly greater than that of abdomen pools ($P<0.05$). Amplified products of positive control DNA (0.1 μg) yielded an immediate response by ELISA, while the low concentrations (0.01 μg) provided a low range positive signal.

3.5. Comparison of infection and infective rate of vector mosquitoes by molecular biological method

Sensitivity of detection rate of the PCR-ELISA was higher than PCR alone. However, the minimum infection rate determined by PCR and PCR-ELISA (2.27%) ($P<0.05$) was identical. Further more, results of PCR and PCR-ELISA varied slightly, when the numbers of PCR-ELISA-positive pools were considered in relation to site level (Tables 1 and 2) during the study period of 2008-2009. During the same study period, PCR analysis revealed that 7.65% (31/405) mosquito pools were positive for *W. bancrofti* (Table 1). On the other hand, PCR-ELISA assay indicated *W. bancrofti* positive mosquitoes in 7.90% (32/405) of the mosquito body pools. However, it did not influence the distribution rates of *W. bancrofti* infected vector mosquitoes in relation to the site as well as to the MOH area. Further, transmission characteristics varied considerably from one MOH area to the other, with different transmission levels within the same year as well as within the two different collection periods (Figures 3 and 4). Further more, it was revealed that even though infection rate declined in year 2008-2009 when compared to 2007-2008 (Figure 3); infective rate of the transmission active sites had increased for the same years (Figure 4).

3.6. Comparison of infection prevalence rates from dissection data and PCR analysis.

Prevalence rates from dissection data and PCR analysis were computed and compared using Epi-Info, version 3.2 (developed by CDC and Prevention in collaboration with the global program on AIDs and the WHO) which takes into consideration the correlation of multiple collections from the same location over time. Mosquito infection prevalence rates as assayed by dissection and PCR-ELISA for two consecutive study periods are given in Figure 5. The association of dissection based prevalence rates with PCR based rates as determined by the Pearson correlation coefficient were 0.176 and 0.890 for the 2007-2008 and 2008-2009 study periods respectively. The t-test was used for rate comparisons. Accordingly, probability associated with a student's paired t-test, with a two-tailed distribution for the infection prevalence rate resulted by dissection and PCR-ELISA for the two different study period was 0.394 and 0.023 respectively.

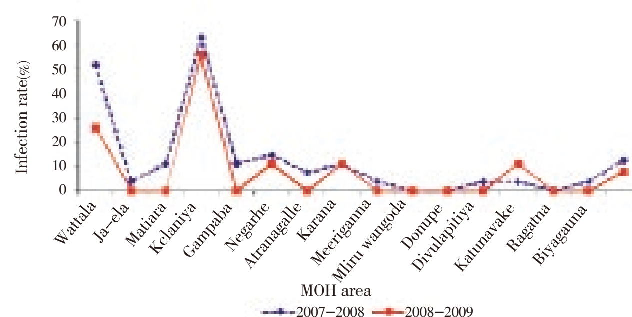

Figure 3. Comparison of infection rates between the year 2007-2008 and 2008-2009.
Infection rates are compared with respect to the MOH area as determined by the pool screen PCR-ELISA for two study periods.

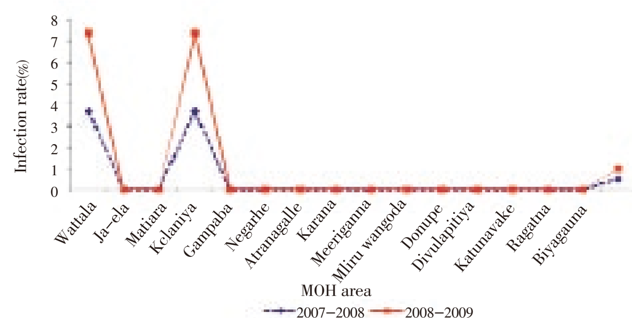

Figure 4. Comparison of infective rates between the year 2007-2008 and 2008-2009.
Infection rates are compared with respect to the MOH area as determined by the pool screen PCR-ELISA for two study periods.

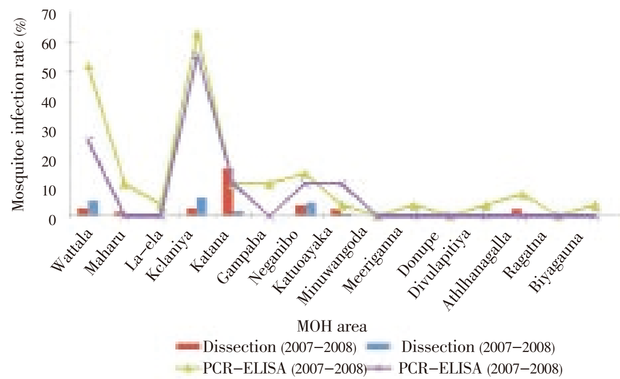

Figure 5. Variation of infection rates.
Comparison of mosquito infection rates determined by dissection and PCR–ELISA for two study periods; 2007–2008 and 2008–2009.

4. Discussion

International Task Force for Disease Eradication established in 1988 has identified Lf as one of the six eradicable diseases. The control of filariasis is important especially in the third world countries as it causes serious economic and social consequences by affecting many young working adults. Chemotherapy is considered as the most potential and cost–effective tool to interrupt the transmission of Lf[19,20]. Routine and extensive sentinel surveys carried out during the program has shown a steady decline of microfilaria rates to a level below 1%[21]. In addition, special surveys carried out among high–risk groups (inmates of prisons), demonstrate a microfilaria positive rates of 1.22% to 1.29%. MDA continued in 2005 too, targeting approximately 9.5 million people for the fourth consecutive time. The percentage of new cases of filarial infection in the Gampaha District increased from 0.18% in 1994 to 2.03% in 1999, but since then it has decreased significantly to 0.03% in 2005. However, infection rate of the vector *Cx. quinquefaciatus* mosquito has increased from 0.46% in 2001 to 1.52% in 2006[22].

In any disease eradication programme, it is important to evaluate the effectiveness of implemented strategies in a timely and reliable manner. With regard to vector–borne diseases, an approach of evaluating the transmission potential of vector populations is a good alternative to assess the disease status in a particular population[12,13,23]. The implementation of such a method for the assessment of a control programme would be easy and successful due to the very low level of direct community participation required. Moreover, the monitoring of the early phases of control programmes for diseases such as Lf would be more meaningful when vector populations are targeted rather than assessing the human populations because of the long duration taken to develop the disease in humans. Therefore, evaluation of the dynamics of infection rates in mosquito populations would be one of the most preferred methods to monitor the successfulness of MDA programme for Lf in endemic areas.

In the present study, adult *Cx. quinquefasciatus* mosquitoes were collected from 74.8% of examined households in the study area during two consecutive study periods. Further, when the findings of these two studies were compared with AFC data, the average mosquito density per man hour was 10.40, 14.91 and 21.80 respectively. According to the survey carried out during two study periods, it was observed that finding of adult *Culex* mosquitoes were much higher than the presence of breeding sites (data were not given). This may be due to higher productivity (higher number of adult mosquito released per breeding site at one time) of each breeding site even though number of breeding sites were low in each site. Therefore, the above finding further highlights the importance of implementing proper control measures to reduce the vector population. Further, results also showed noticeable decrease in number of mosquito collected and entomological finding of breeding places (data was not given) in the second year of study period (2008–2009). This could be due to community awareness programme conducted to control the prevalent dengue infection in the Gampaha district during that study period.

In this study, dissection results showed a marked decrease in infection level from the year 2007–2008 study periods to the end of the next study period of 2008–2009 in 5 of the 15 MOH areas that were monitored. The exception was found in Wattala, Kelaniya and Negambo, which showed increased infection prevalence in the collected mosquito populations. It can be due to unavoidable and uncontrolled breeding site prevalence in these MOH areas. For example, most sites in these MOH areas have at least one vegetation–blocked drains or canals.

In this study, dissection and PCR–ELISA assays on captured mosquitoes generated similar, but not identical results. A positive correlation was observed between larval infection rates determined by these two methods. The proportion of positive pools detected by PCR–ELISA 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–ELISA assay, especially for detection of early larval stages which is more difficult by dissection. However, independent use of the method and mass treatment led to a reduction in levels of *W. bancrofti* in collected in–door resting mosquitoes as assessed by dissection and PCR–ELISA. Similar to our results, study in Ghana reported that dissection and PCR–pool screen of *Cx. quinquefasciatus* mosquitoes generated results having no significant difference[24]. Further, it was also observed that the decreases in filaria–infected mosquitoes in our study were comparable to the decreases in microfilaria prevalence seen in the human populations in the sentinel sites. Similarly, an observational study in northern Uganda has documented a sharp decline in *W. bancrofti* infection in humans, as well as reduction in transmission potential by the *Anopheles* vector (as assessed by the traditional dissection) in a previously highly Lf endemic area of Uganda[25].

Furthermore, a rather inexpensive PCR–based ELISA assay was used in this study to detect *W. bancrofti* infection in pools of *Cx. quinquefasciatus* mosquitoes. Since PCR alone did not give idea of the quantification of the intensity of infection and increase the sensitivity of infection detection it was used PCR–ELISA rather than use of PCR alone[25]. The efficacy of the massive administration of a single dose of DEC in reducing microfilaria prevalence and intensity is well proven[22,26], but the ultimate success of this strategy depends on several factors such as drug distribution and compliance.

There are several limitations of using the traditional, manual dissection of mosquitoes to monitor the transmission potential of Lf in vector populations subsequent to a MDA programme. Firstly, manual dissection is highly time and labour–intensive as large numbers of mosquitoes are

required to be dissected to achieve a significant difference in the mosquito infectivity rates between the pre and post implementation phases[23]. It has previously been shown that the number of mosquitoes needed to be dissected was quite higher to determine the efficacy of the MDA programme for Lf based on L_3 infection rates[25]. The dissection has to be essentially a real-time monitoring measure of mosquito infectivity as it cannot be done on preserved samples. And also 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[27]. Further more, identification of infective larvae (L_3 larvae) is very difficult under 10× magnification and without a relatively experienced technical officer. Another concern pointed out is that, when the infection level declines, increasing numbers of mosquitoes must be dissected in order to demonstrate a significant decline in infection prevalence[24]. This is especially true for infective larvae. In this study, the numbers of mosquitoes carrying infective larvae, as detected by dissection, were insufficient to allow for an adequate statistical analysis of changes in infection prevalence. Monitoring transmission levels based on levels of infective larvae may not be feasible within the scope of Lf elimination programs in settings like Sri Lanka where *Cx. quinquefasiatus* is the vector because of the relative rarity of identifying infective mosquitoes.

PCR based assays can be effectively used to overcome these limitations. The cost of performing PCR and related assays such as PCR-ELISA evaluated in this study is comparable to performing dissection. Our cost analysis showed that an individual dissection of 15 mosquitoes cost approximately 3.00 US dollar. The estimated cost for PCR and PCR-ELISA analysis of a pool containing a similar number of mosquitoes was 3.40 US dollar and 4.40 US dollar respectively. Even if dissection appears less time consuming than PCR based assays when analyzing a few samples, the latter out performs when it comes to large scale community-based assays. Our analysis showed that only 2.30 h were required for the analysis of a batch of 15 mosquitoes by dissection whereas it was 7 and 12 h for PCR and PCR-ELISA assays respectively. However, when the numbers of analyzed mosquitoes were increased to 480, the time taken for dissection, PCR and PCR-ELISA was 72, 24 and 30 h respectively. In this regard, the ability to process pools of mosquitoes rather than analyzing individual mosquitoes by PCR facilitates rapid turnover in community assays. Furthermore, PCR based assays are highly specific and sensitive. Hence, they can be used to detect even the early larval stages in mosquitoes that are more difficult to be detected by dissection. These results are encouraging in terms of the potential precision and sensitivity of pool screening given the fact that more than one infected mosquito may be contained in pools of variable numbers. According to our results, PCR-ELISA assay based on the detection of *W. bancrofti*-specific Ssp I repeat region using pools of captured mosquitoes was more sensitive than the PCR alone, making it a better method for the monitoring of vector populations for Lf transmission. In fact, PCR has already been demonstrated to be a successful tool for the monitoring of onchocerciasis programs[27]. The availability of such a tool permits rigorous surveillance for resumption of transmission following completion of MDA programmes for Lf. According to latest publications countries like Egypt, France Polynesia, Thailand, Haiti and Papua New Guinea were used successfully PCR detection of mosquito infections in various field studies[27]. However, PCR-based techniques to detect infections have not been previously evaluated for mosquito vectors in Sri Lanka.

The detection of filarial DNA in vectors by PCR assays does not necessarily indicate active transmission of Lf in a given area. This is because PCR assays detect any stage of the parasite present in the vector, not just the L_3. This can be overcome by developing stage specific PCR assays. According to the findings of the present study, the infection levels have not decreased to expected levels, the level below that is necessary to maintain transmission suppression. Based on the failed status of the MDA programme in Gampaha district (MDA coverage 73.6%) than other parts of the country, which exceeded more than 80% coverage, this can be further justified. This was further confirmed by the prevalence of residual infections in Gampaha District detected in routine screenings of humans by the MOH of Sri Lanka[28,29]. Therefore, the information generated from this study will lead to a better understanding of the transmission of filariasis and be useful for management and control of Lf and vector control programmes in the country, which will be of immense national importance.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Lymphatic filariasis constitutes one of the major problem in tropical countries. Different methods were used to identify the infection rate among mosquitoes including dissection, PCR and ELISA techniques.

Research frontiers

The study was conducted to estimate the infection rate of mosquito *Culex quinquefasciatus* with *Wuchereria bancrofti* larvae by using dissection, PCR and ELISA methods.

Related reports

A total of 15 126 mosquitoes *Culex quinquefasciatus* were collected during two years (2007-2009) of study. The infection rate with Lf was 13.29% and 3.10% for 2007-2008 and 2008-2009 respectively.

Innovations and breakthroughs

The study refereed to the infection rate to the infection rate

of Lf in two consecutive time periods in District of Gampaha, Sri Lanka.

Applications

The study showed the significance of Lf infection in Sri Lanka and the sensitivity and specificity of PCR and ELISA in comparison to dissecting method.

Peer review

It is a good study and the authors emphasized on the methods that used for diagnosis of Lf in mosquito *Culex quinquefasciatus*.

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