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A surveillance system for lymphatic filariasis after its elimination in Sri Lanka

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ABSTRACT

Lymphatic filariasis (LF) has been declared eliminated in Sri Lanka in September 2016. To maintain elimination status, a surveillance system to detect hidden endemic foci or LF resurgence is of highest priority. In this paper, we have reported an investigation of LF transmission in Trincomalee district where a surveillance program was not carried out due to 30 years of civil unrest. Proposed surveillance system included, measurement of anti-filarial IgG4 in urine of schoolchildren in areas where LF transmission could exist and assessment of circulating filarial antigen (CFA) and microfilaria (mf) in all urine antibody positive schoolchildren, their family members and 10–15 neighbours of each urine antibody positive household. Spatial distribution of the anti-filarial antibody titers in urine in a high antibody suspected area was analyzed using GPS logger data. Among 2301 school children from 11 schools studied, 41 (1.8%) urine antibody positives were found. The antibody positive rates of the schools ranged between 0 and 4.0%. Nine of the 630 (1.4%) examined became positive for CFA but were negative for mf. Although there were no mf positives, positive CFA and antibody results indicated the existence of *Wuchereria bancrofti* in Trincomalee. Highest antibody titres in an area correlated with the prevalences of urine antibodies and CFA. Spatial analysis showed LF transmission foci. Therefore, a combination of the non-invasive methods, urine ELISA and GPS mapping, will be a new effective surveillance system to identify hidden LF transmission foci.

1. Introduction

Lymphatic filariasis (LF) is a debilitating and disfiguring disease. Individuals living in poor socioeconomic condition are mostly distressed by LF [1]. Loss of productivity and compromised quality of life were the major outcomes of chronic LF [2]. The World Health Assembly in 1997 has identified LF as a potential disease which needs to be eliminated [3]. Global Program to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 with the target to eliminate LF by 2020 [3]. GPELF has achieved tremendous success in its elimination efforts. Number of countries required Mass Drug Administration (MDA) as a strategy to eliminate LF has steadily declined since the inception of the GPELF. At present, only 52 countries of 73 required MDA [1]. Since 2000, about 6.7 billion treatments have been distributed among > 850 million people [1].

In Sri Lanka, the disease was endemic in Southern, Western, and North Western provinces (Fig. 1). As one of the first countries, Sri Lanka initiated LF elimination program as per WHO guidelines [4]. Sri Lanka national Anti-Filariasis Campaign (AFC) started National Program to Eliminate LF (PELF) in 2002 and distributed five consecutive annual rounds (2002–2006) of Mass Drug Admiration (MDA) with the combination of diethylcarbamazine (DEC) and albendazole in all endemic districts. After repeated annual rounds of MDA, microfilaraemia (mf) prevalence in all sentinel and spot check sites dropped significantly and passed Transmission Assessment Surveys (TAS) by AFC in 2013 [4]. Considering the results of TAS WHO has validated the elimination of LF in Sri Lanka as a public health problem in September 2016 [4]. However, Rao, et al. 2017 has documented several TAS failed foci [4]. At

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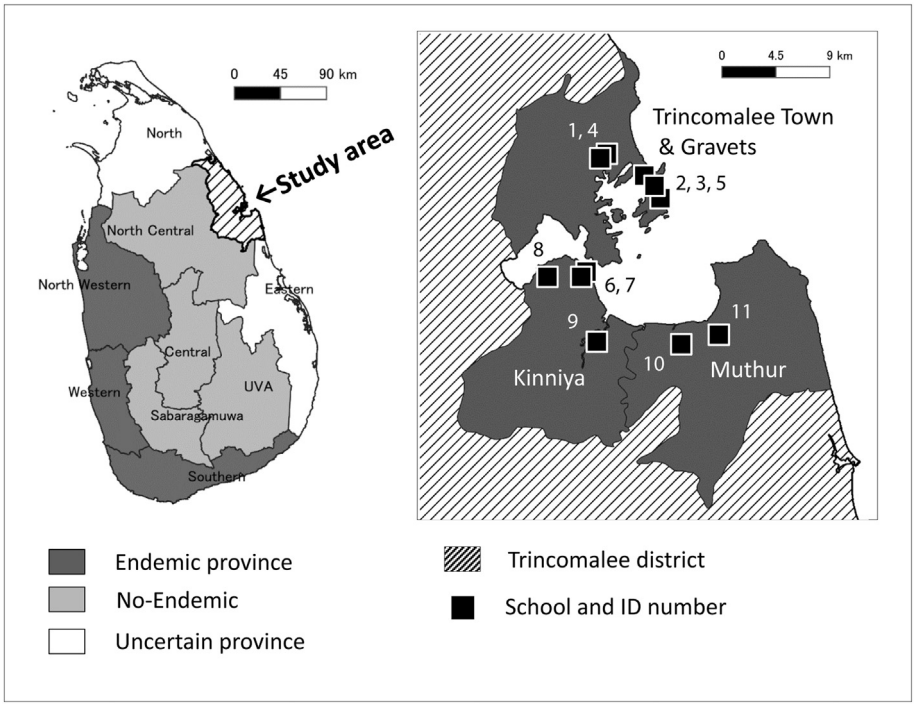

Fig. 1. Map of Sri Lanka showing endemic provinces at the inception of national PELF: 2001 [6], the current study area and the locations of the 11 schools studied.

post elimination stage, careful surveillance of LF is vital to maintain the elimination status. Surveys performed during 1960's have revealed the presence of LF endemic foci in some areas of Eastern province [5] where LF had not been investigated due to the prolonged civil war. In this study, we have applied a combination of methods, screening school children for anti-filarial IgG4 using urine ELISA, and examination of subject reside around the urine ELISA positives with a point of care test kit—Filariasis Teat Strip (FTS)—to detect CFA in blood. The antibody levels (IgG4 titer) of the school children and their positions obtained by the GPS loggers were spatially analyzed to identify foci where LF transmission is possible.

2. Materials and methods

2.1. Study area

The present study was conducted in Trincomalee district situated on the Eastern coast of Sri Lanka (Fig. 1), about 267 km Northeast from Colombo. Although there were endemic foci of LF before 1960 in Trincomalee [5], national PELF of Sri Lanka at its inception has identified eastern province as an uncertain area for LF transmission [6].

2.2. School survey

For school selection, socioeconomic condition of the area and availability of vector mosquito and breeding sites were given priority [7]. Such data were collected from the key informants (Regional Directors of Health and Education). Schools and schoolchildren were selected by survey sample builder 2.3 (http://www.ntdsupport.org/resources/transmission-assessment-survey-sample-builder). However, key informant's data were considered in selecting 10 schools for the survey (Table 1, four in Trincomalee Town and Gravets—School ID 1, 2, 3, 5; three in Kinniya—6, 7, 8; two in Muthur—9, 10; one in Sampur—11). Further, all children of Grade I to Grade VI (5–13 year-old) of either gender were enrolled for the primary screening of LF using urine base immunodiagnosis [8–14].

2.3. Enmzyme linked immunosorbent assay (ELISA) with urine samples

Urine samples (5 mL) were collected from each child and mixed with sodium azide at 0.1%. IgG4 antibodies to *Wuchereria bancrofti* recombinant antigen, SXP1 were detected by ELISA as described elsewhere [9,12]. Briefly, a 96 well micro titer plate was coated with SXP1. After blocking the plate, urine samples (100 μL/well) were applied directly to the plate and incubated overnight at 25 °C. The plate was washed and to it added peroxidase-conjugated monoclonal anti-human IgG4 antibody (Southern Biotechnology Associates, Inc., Birmingham, AL) and incubated for 1 h at 37 °C. ABTS (KPL, Gathersburg, MD) was used as peroxidase substrate. The absorbance was measured at 415 nm with 492 nm as a reference. Serially diluted positive standards were available for each plate. The unit in this system ranged from 0 to 7, 290 U, and the cutoff value was 7.08 U.

2.4. Survey of residents who were living closer to the urine ELISA positive schoolchildren

Target sampling was done using urine ELISA positive cases. All family members of the urine ELISA positive case and 10–15 individuals from neighbourhood were enrolled. Blood samples were collected from the subjects by finger prick at 21.00 h to 23.00 h. One part (60 μL) was used for microfilaria examination from a thick smear and following Giemsa staining. Another part (75 μL) was used for CFA detection by Alere™ Filariasis Test Strip (FTS) according to the manufactures manual. A scoring system for test results was applied as follows: no test line, 0; the test line weaker than the control line, +1; the test line equal to the control line, +2; the test line stronger than the control line, +3; and test with no control line as invalid. Blood sample from the CFA positive individual was examined by Nucleopore® membrane filtration method in a different occasion [15].

2.5. Identification of house locations by the GPS logger analysis

The area with the highest number of antigen positive cases was selected for this investigation (Fig. 3). The area mentioned above has been identified by KIs as a catchment area of a different school (School

Table 1
Number of school children and villagers examined, prevalence of anti-filarial IgG4 and prevalence of CFA found in each school and locality in Trincomalee district.

Locality	School ID	No. of urine sample examined	No. positive for anti-filarial IgG4 (%) [CI]	Highest IgG4 Unit	No. of blood sample examined	No. positive for CFA (%) [CI]	FTS score
Trincomalee Town & Gravets	1	101	4(3.96) [1.55–9.74]	303	76	1(1.32) [0.23–7.09]	2
	2	298	10(3.36) [1.84–6.07]	547	120	3(2.5) [0.85–7.09]	1,2,2
	3	169	3(1.78) [0.61–5.10]	86	62	1(1.61) [0.28–8.58]	3
	4	356	6 (1.69) [0.78–3.63]	123	n.d[a]	–	–
	5	74	0 (0) [0.00–4.93]	6	n.d[b]	–	–
Sub-total		998	23(2.30) [1.54–3.43]	547	258	5(1.94) [0.83–4.46]	
Kinniya	6	292	4(1.37) [0.53–3.47]	22	85	0(0) [0.00–4.32]	0
	7	276	2(0.73) [0.20–2.60]	40	50	0(0)[0.00–7.13]	0
	8	156	3(1.92) [0.65–5.50]	23	59	0(0) [0.00–6.11]	0
Sub-total		724	9(1.23) [0.65–2.34]	40	194	0(0) [0.00–1.94]	
Muthur and Sampur	9	152	3(1.97) [0.67–5.64]	103	74	1(1.35) [0.24–7.26]	2
	10	247	2(0.81) [0.22–2.90]	186	43	1(2.33) [0.41–12.07]	2
	11	180	4(2.22) [0.87–5.57]	502	59	2(3.39) [0.93–11.54]	2,3
Sub-total		579	9(1.55) [0.82–2.92]	502	178	4(2.25) [0.88–5.64]	
Total		2301	41(1.78) [1.31–2.41]	547	628	9(1.43) [0.75–2.70]	

[a] n.d = not done; only GPS data logger study has been carried out.
[b] n.d = not done; there were no target (urine Ab positive) for sampling.

ID-4) which we did not include in the earlier survey (Table 1). Therefore, School ID-4 was selected as target for GPS logger (GT-600, Mobile Action Technology, Inc., Taiwan) distribution. Further, the selection of School ID-4 as the target for GPS logger survey was justified due to- i) Assessment of anti-filarial antibody titers in some more number of children from the target area ii) Location of the school was closer to the high antigen and antibody prevalent area and iii) The school was easily accessible with high enrolment of targeted age group. All children from grade I to grade VI were enrolled for GPS logger distribution and collection of sample of urine (5 mL) for urine ELISA. GPS loggers were collected from every child and every child's tracking data (waypoint/20 s) were retrieved on the next day at school. The house of every child was identified from these tracking data, and the urine ELISA data of each child was tagged to the corresponding house.

2.6. Mapping of ongoing LF transmission using schoolchildren's house location

For all children, an adjacency matrix W_W (normalized by row sum of adjacency matrix W_B) according to the adjacency indicated by the Delaunay triangle was defined. Using each adjacency matrix, each child's antibody titer was converted to local Moran statistic (*li*), the Local Indicators of Spatial Accumulation (LISA) [16]. W_W and *li* were calculated using the spdep library of R software. When the degree of space accumulation is the same, *li* has the same positive value for both high and low antibody titer accumulation (transmission is possible spot/transmission is unlikely spot). Therefore, each child was applied to the Moran scatter diagram. The positive *li* value of the children distributed in the third quadrant of the Moran scatter diagram was converted from positive to negative, and the negative *li* values distributed in the 2nd and 4th quadrants were converted to 0. The horizontal axis of the Moran scatter plot shows the log antibody titers of each child, the vertical axis the average value of the log antibody titers of the children connected by Delaunay triangle network with each child, and the origin is the mean value of both axes. Finally, this converted *li* value given in point was interpolated as a raster (transmission is possible spot) map by 2D kernel density processing (averaging radius of 250 m, output pixel spacing of 10 m) in QGIS (Quantum GIS Wien 2.8).

2.7. Data management and analysis

Correlation analysis was performed using non-parametric correlation methods to study association between IgG4 titers and CFA prevalence. GPS coordinates including latitude and longitudes were taken for each house with personal digital assistants (PDA) (HP iPAQ 211, Hewlett Packard, Palo Alto, CA, USA). The GPS track data of students were exported with @trip PC (Mobile Action Technology, Inc., Taiwan), and mapped in QGIS (Quantum GIS Wien 2.8).

2.8. Ethical considerations

Ethical clearance for this study was obtained from the Ethical Review Committee of Faculty of Medicine, University of Ruhuna. For the urine and blood sample collection, oral and written informed consents were obtained from the all participants. In case of children, informed consent was obtained from their parents/guardian.

3. Results

3.1. Anti-filarial IgG4 prevalence in school children

Among 2301 school children examined from 11 schools, 41 were positive with anti-filarial IgG4 in their urine (Table 1). The antibody positives were found in 10 of the 11 schools. Antibody positive rates in each school varied from 0 to 3.96% with an overall of 1.8%. Although the difference was not statistically significant among the three localities, the positives rate was highest in schools of Trincomalee Town & Gravets (2.3%) and lowest in those in Kinniya (1.2%) (Table 1). Fig. 2 showed antibody titer dispersion of all schoolchildren. A congestion of antibody titers is observed just under the cut-off line.

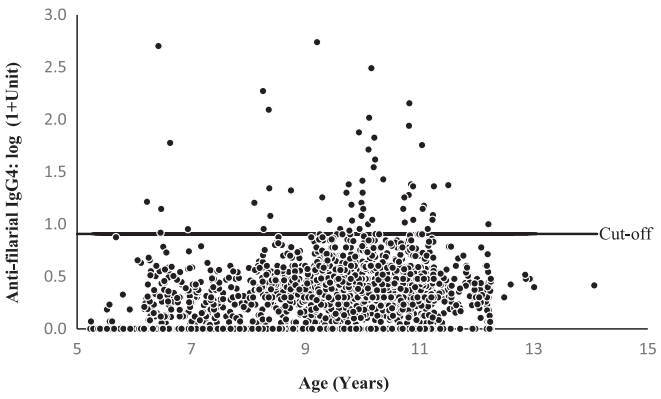

Fig. 2. Anti-filarial IgG4 levels of 2301 schoolchildren analyzed by age.

3.2. Circulating filarial antigen (CFA) prevalence among subjects who were living adjoining to the urine ELISA positive schoolchildren

The age of subjects varied from 5 to 84 years old with a median age of 31 years old. Of all the participants, 28.3% (178 of 630) were school aged children. In total, 630 individuals were tested for CFA and two showed invalid results due to the reduced flow of serum through the test strips. Nine of 628 individuals (1.4%) were found positive for CFA: five of 547 individuals in Trincomalee Town and Gravets, four of 178 individuals in Muthur and Sampur but no positives among 194 individuals in Kinniya (Table 1). FTS were negative in all 41 urine antibody positive schoolchildren.

3.3. Correlation analysis between antibody prevalence/titer and CFA prevalence

Comparison between anti-filarial antibody prevalence (Ab) and CFA prevalence in nine localities examined did not show any significant association. Highest antibody titer recorded in each area have shown a positive correlation with Ab prevalence ($r^2 = 0.404$; $p < .05$) and CFA prevalence of the same area ($r^2 = 0.694$; $p < .01$).

3.4. Survey for microfilaria (mf)

Six-hundred and twenty-eight 60 μL night thick blood films were negative for mf. Examination of all 9 CFA positive in a separate survey using 60 μL thick smear and Nuclepore® membrane filtration found negative for mf.

3.5. Estimation of LF transmission foci

The transmission is possible-spots (TP), transmission is not known-spots (TN) and transmission is unlikely-spots (TU), estimated were shown in Figs. 3 and 4. The size of the catchment area of the school distributing the GPS loggers was about 1.5 km square, and 5–7 places around 200 m in radius from this range could be estimated as a TP spot. The histograms of anti-filarial IgG4 levels of each geographical accumulation group showed that antibody titers distributed from zero to positive in the TP spots, whereas almost all members showed zero antibody titer in TU spots (Fig. 3). These results suggest LF transmission is active only in limited areas (hidden foci) in the catchment area and no transmission in other areas.

4. Discussion

WHO has declared the successful elimination of LF as a public health problem in Sri Lanka in 2016 [4]. However, the finding of ongoing transmission in Trincomalee for the first time in recent history has provided most awaited data to AFC. AFC of Sri Lanka has already started its interventions covering the areas we have highlighted in our present study. Therefore, results of present study and subsequently AFC using them indicated that the urine based ELISA can be used to delimitate LF transmission. The new epidemiological approach was complimented by CFA testing and GPS logger mapping. One of the WHO recommended approaches to delimitate LF is based on CFA [17,18]. Antibody based methods are yet to be recognized by the WHO. Thus we used CFA testing, however, in the current study a different sampling method was implemented successfully. The approach mentioned above is new to the literature.

When a national PELF has reached its cessation, the mf and CFA positive rates recorded may be ultra-low. As a result, sensitivity of mf and CFA detection also is reduced, and may no longer be adequate to meet growing demand of improved diagnostic technique for detection and verification of the transmission. Urine based ELISA used in the

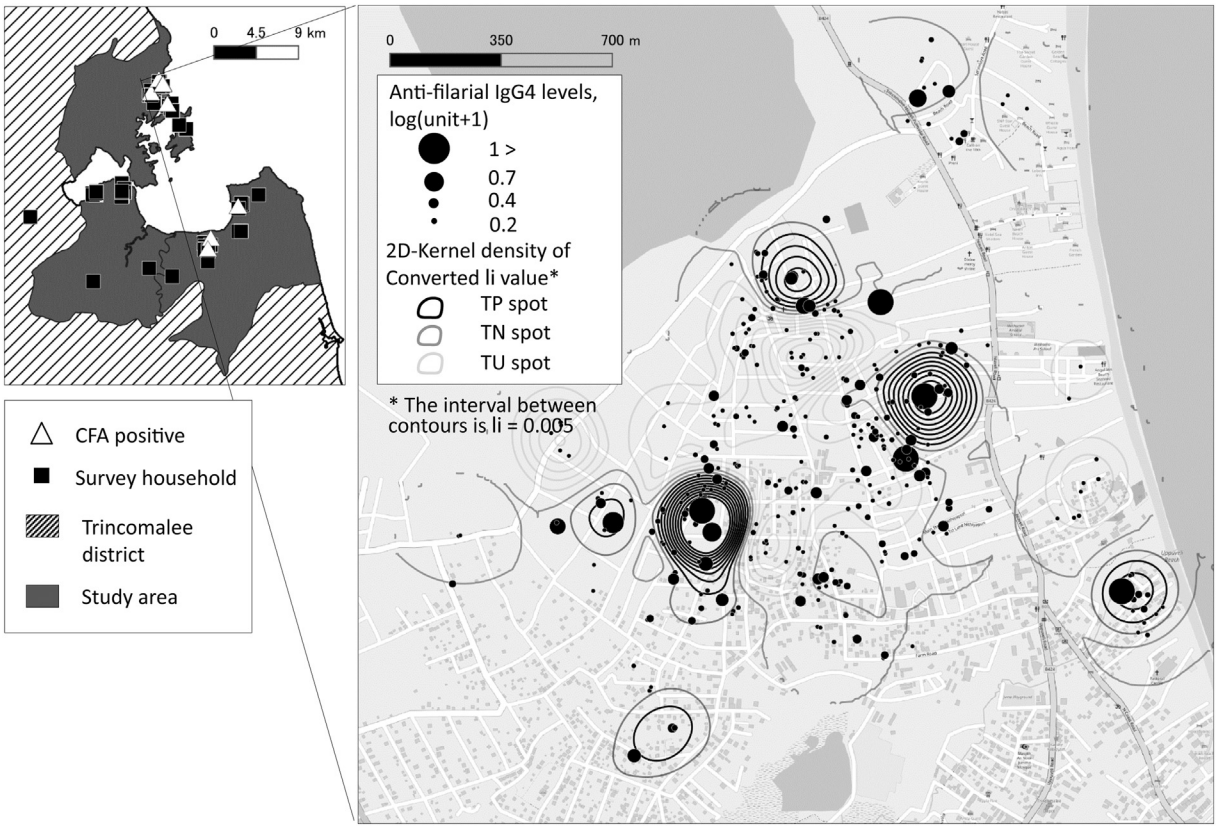

Fig. 3. Locations of surveyed households, the household with CFA positive and mapping of the LF transmission possible (TP), transmission unlikely (TU) and transmission not known (TN) spots.

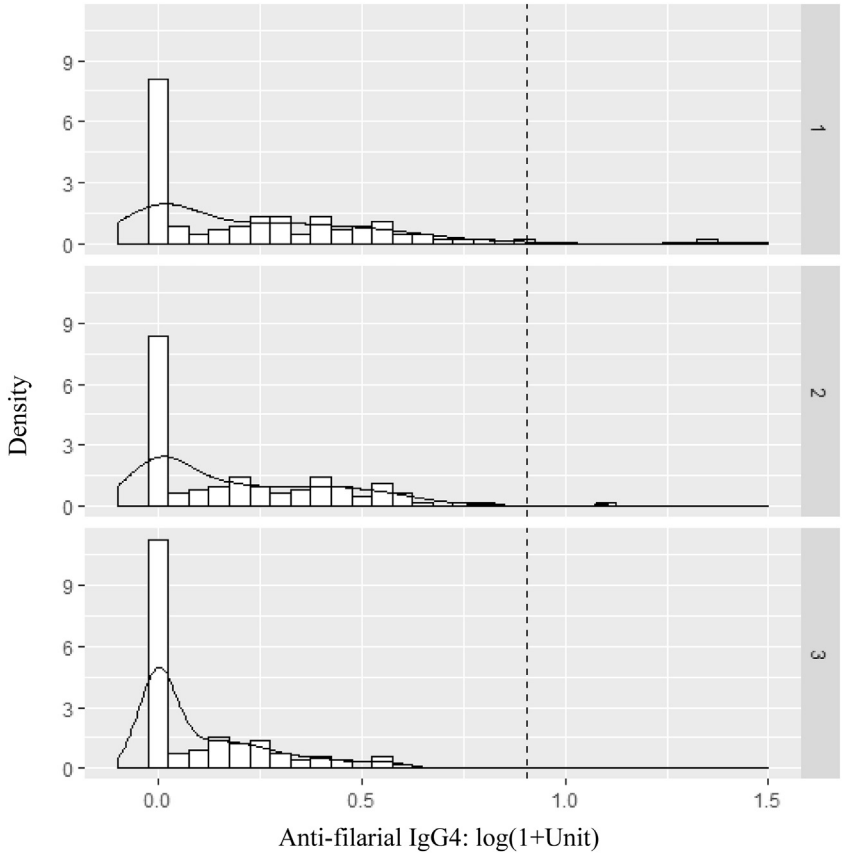

Fig. 4. Antibody titer histogram of TP/TN/TU spot in the catchment area.

present study has been shown to have a high sensitivity and specificity [8–11] and higher positive rates than ICT [11]. Investigations in Sri Lanka also reported urine ELISA and hydrocele rates as determined by clinicians correlated well which indicates the usefulness of the ELISA as a supportive technique to study the distribution and prevalence of LF in low endemic areas in Sri Lanka [9,13]. This technique has been used in Sri Lanka for > 15 years for detecting anti-filarial antibody and hidden foci in many areas [9,11,13,14]. Several other investigations have also highlighted the usefulness of antibody detection technique for prevalence study, post MDA surveillance and verification [9,17,19–25]. Comprehensive assessments are necessary at the final phase of elimination and post elimination period to track on-going transmission and hidden foci. Antibody detection among young subjects can be one of the useful techniques to detect on-going LF transmission. Young children have been used as sentinel population [13,14]. In the present study we have selected school children as a sentinel group to detect on-going transmission and its foci. The presence of anti-filarial IgG4 positives and some with high titers (≥100 U) among the school children have indicated the exposure to an ongoing LF transmission.

Anti-filarial IgG4 developed in an exposed person as an early response also indicated active infection—having worm/s (mf or adult) in the system. It is difficult to conclude whether the anti-filarial IgG4 positive people had their exposure in their native areas or elsewhere. Almost a half of the positive subjects (19 of 41, 46.3%) had no travel history to a known endemic district such as Colombo, Gampaha, Kalutara, Puttalam, Kurunegala, Galle, Matara and Hambantota. Further, travelling was minimal among school children aged between 5 and 8 years old. Therefore these results emphasized that schoolchildren have contracted the parasite by an indigenous transmission.

Long term follow-up survey carried out elsewhere (relatively known low endemic area) in Sri Lanka has shown dense accumulation of urine antibody titers just under the cut-off level before the completion Mass Drug Administration of national PELF [13]. Distribution of urine antibody titers and its dense acumination just under the cut-off level in current study is highly comparable with the above mentioned known low endemic area. All said facts are in support of using urine based antibody test in LF delimitation.

Examination of CFA, which indicates current adult worm infection, showed positives among subjects living close to the positive school children and most of the CFA positive subjects (8 of 9) had higher FTS score (≥2). The adult worm and mf release antigen in the blood circulation which is not affected by periodic variation [26]. Detection of CFA by ICT test is more sensitive than mf detection in night blood by microscopy [27]. All the CFA positive subjects were negative for mf in this study. An individual having CFA or antibody positive results without any mf in the circulation could be possibly explained by a cryptic infection such as having one adult worm, having one sex either male or female, having non-fecund worm or no established infection. However, we are not clear about the exact reason behind this phenomenon.

Due to said complexity in immunology-based diagnostics, the direct correlation between anti-filarial IgG4 prevalence and CFA prevalence have revealed no significant association and three localities reported having over 1.5% of prevalence margin. However, anti-filarial IgG4 titer has shown positive significant correlation with both anti-filarial IgG4 prevalence ($r^2 = 0.404$; $p < .05$) and CFA prevalence ($r^2 = 0.694$; $p < .01$). The fact mentioned above revealed that when the reported antibody titer in an area is higher, possibility of having established infection and ongoing transmission is also higher. It is a well-known fact that the LF transmission is not effective, and need at least 15 infective bites to get an established infection [28]. This indicates that LF transmission can operate at a very low level without having established infection in an area. Therefore, to delimitate ongoing transmission in an area where LF is not reported, urine based

anti-filarial IgG4 test could play a major role. In August 2018, anti-filarial IgG4 in urine was tested in 108; 6 to 10 years students of *Kandawala Central Collage* in Colombo district by FRTSU. The catchment area (*Rathmalana*) of the school was a known endemic site and received its final MDA in 2006. The Urine ELISA prevalence of said school was zero (Ekanayake, et al., manuscript in preparation). A recent study conducted by AFC in Colombo district for post-MDA verification, found that schools selected in three (3) out of four (4) sentinel sites had zero CFA prevalence [29]. *Katukurunda* and *Borella* have recorded zero CFA prevalence and both villages were in close proximity to catchment area of *Kandawala Central Collage*. This indicates that the anti-filarial IgG4 in urine successfully monitors LF transmission by itself.

Due to limited time and resources, only a school is selected for special analysis. In the school selection higher possibility of having ongoing LF transmission was considered. Spatial analysis revealed accumulation of persons with high antibody levels in Trincomalee Town & Gravets located within ~200 m radius (Fig. 3). The accumulation of filarial antibody positive cases in a small area indicated potential LF transmission foci. GPS logger used in the present study worked well in identifying small foci of LF transmission (TP-spots). Similarly urine ELISA has identified clearly the areas where LF transmission is unlikely (TU-spots). Such a device would be valuable in studying distribution of LF in other unsurveyed areas and locate foci with ongoing LF transmission rapidly and economically to adopt necessary control measures.

In conclusion, examination of schoolchildren with urine ELISA for LF antibodies and supplemented with CFA detection of villagers reside closer to urine ELISA positives have detected ongoing LF transmission in Trincomalee district. Surveys with GPS logger followed by spatial analysis helped to identify TP spots readily. Urine ELISA has many advantages over CFA tests, such as, non-invasiveness, higher sensitivity and specificity and easy sample collection. Therefore, we would like to propose a new epidemiological approach using urine ELISA with GPS mapping to delimitate LF. A similar epidemiological approach can be applied in other areas to delimitate LF transmission and take necessary actions.

Conflict of interest statement

None.

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