Impact of polystyrene beads as a mosquito control measure to supplement lymphatic filariasis elimination activities in Socotra Island, Yemen

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تأثير خَرَزات البوليستيرين بوصفها أحد تدابير مكافحة البَعوض اللازمة لاستكمال أنشطة التخلص من داء الفيلاريات في جزر سُقُطْرَى، اليمن

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الخلاصة: من المعلوم أن التخلص من داء الفيلاريات اللمفية قد تم اعتباره هدفاً رئيسياً في العالم قاطبة. أما في اليمن فقد أصبحت جميع الوحدات التنفيذية بالبر الرئيسي، تفي بمعايير منظمة الصحة العالمية الخاصة بوقف المعالجة الدوائية الجُمُوعية بعد خمس جولات، ولكن هذه المعايير لم تتحقق بَعْدُ في جزيرة سُقُطْرَى. وقد قام الباحثون بتقييم نجاعة تطبيق خَرَزات البوليستيرين المديدة على جموع البَوَاعض، وأثر ذلك على انتقال الفيلاريات اللمفية. وتم إجراء مسوحات للبشر والبَعوض في أربعين أسرة تم انتقاؤها عشوائياً في هديبو (عاصمة سُقُطْرَى) قبل استخدام خَرَزات البوليستيرين الممتدة وبعدها. وقد أسفرت خَرَزات البوليستيرين المديدة عن تقليص كثافة البعوض بنحو 80٪، وتخفيض معدل انتشار المكروفيلاريات بنحو 64.3٪. وترى غالبية الأُسَر التي أُجريَ اللقاء معها (98٪) أن خَرَزات البوليستيرين المديدة تُجدي في تقليص جموع البعوض. ويستنتج الباحثون أن استعمال خَرَزات البوليستيرين المديدة يعتبر مكملاً فعّالاً للمعالجة الدوائية الجُمُوعية لإحراز مرمى استئصال الفيلاريات اللمفية.

ABSTRACT Lymphatic filariasis (LF) is targeted for worldwide elimination. In Yemen, all mainland implementation units met the WHO criteria for stopping mass drug administration (MDA) after 5 rounds. However, in Socotra Island these criteria were not met. Our study evaluated the efficacy of applying expanded polystyrene beads (EPBs) on the *Culex* population and the effect on LF transmission. Human and mosquito surveys were conducted in 40 randomly selected households in Hadibo (capital of Socotra) before and after application of EPBs. The EPBs intervention resulted in a reduction in mosquito density of 80% and a 64.3% reduction in microfilaria prevalence. The majority of interviewed households (98%) thought EPBs considerably reduced the mosquito population. After the intervention all collected pools tested negative. Application of EPBs is an effective supplement to MDA for achieving the goal of LF elimination.

Impact des billes de polystyrène en tant que mesure de lutte contre les moustiques en complément des activités d'élimination de la filariose lymphatique sur l'île de Socotra (Yémen)

RÉSUMÉ L'objectif pour la filariose lymphatique est une élimination à l'échelle mondiale. Au Yémen, toutes les unités chargées de la mise en œuvre sur la partie continentale du pays ont rempli les critères de l'Organisation mondiale de la Santé permettant d'interrompre la distribution massive de médicaments après cinq campagnes. Toutefois, dans l'île de Socotra, ces critères n'ont pas été remplis. Notre étude a évalué l'efficacité de l'utilisation de billes de polystyrène expansé sur la population de moustiques *Culex* et son effet sur la transmission de la filariose lymphatique. Des enquêtes étudiant les moustiques et interrogeant les habitants ont été menées dans 40 ménages sélectionnés aléatoirement à Hadibo, la capitale de Socotra, avant, puis après l'utilisation de billes de polystyrène expansé. L'intervention utilisant des billes de polystyrène expansé a entraîné une réduction de la densité des moustiques de 80 % et une baisse de la prévalence des microfilaires de 64,3 %. La majorité des ménages interrogés (98 %) pensaient que les billes de polystyrène expansé avaient considérablement réduit la population de moustiques. Après l'intervention, tous les points d'eau analysés étaient négatifs. L'utilisation de billes de polystyrène expansé représente un complément efficace à la distribution massive de médicaments pour atteindre l'objectif d'élimination de la filariose lymphatique.

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Introduction

Lymphatic filariasis (LF), a vector-born disease caused mainly by the parasite *Wuchereria bancrofti*, is a major health problem that affects more than 120 million people in 83 tropical and subtropical countries [1–3]. The Global Programme to Eliminate Lymphatic Filariasis was launched in the year 2000 based on the principles of interruption of transmission, and alleviation and prevention of disability due to lymphatic filariasis [2]. Currently, the Global Programme depends largely on repeated annual cycles of mass drug administration (MDA) of albendazole and either diethylcarbamazin or ivermectin to interrupt the transmission of *W. bancrofti* [4].

In most LF endemic countries, *W. bancrofti* is transmitted by *Culex* mosquitoes, which typically breed in stagnant and organically polluted water [5]. It appears unlikely that MDA solely would be sufficient for sustained interruption of transmission in highly endemic areas of *Culex* transmission of LF, due to their high vectorial efficiency [6]. Therefore, application of expanded polystyrene beads (EPBs) to pit latrines and soakage pits has been recommended for prolonged suppression of vector potential [7]. The floating layers of EPBs have been shown to prevent breeding of *Culex* mosquitoes because they create a physical barrier to egg-laying adults and suffocate larvae and pupae [8]. A study in Zanzibar has showed that the *Culex* mosquito population decreased by about 98% after applying EPBs to all the wet pit latrines, without any change in a nearby untreated community [7]. One round of MDA with diethylcarbamazin resulted in decreasing the proportion of mosquitoes with third-stage larvae (L3) resulting in an overall 99.7% decrease in the number of infective bites per year in the treated area [7].

In Socotra Island, Yemen, LF is endemic and more prevalent in Hadibo, the capital of the island, which is a semi-urban area. *Culex quinquefasciatus*, the main vector transmitting LF in the Hadibo area, breeds exclusively in outdoor cesspits that are not well covered [Al-Kubati and Gad, unpublished data, 2005]. In 2000, the baseline antigen rate reached 40% in the Hadibo area [9]. Five rounds of MDA (ivermectin plus albendazole with > 80% average drug coverage rates of the total population) were completed in 2006 [10]. Monitoring microfilaria (Mf) post MDA showed slow progressive reduction (36.2%) in Mf prevalence from 5.8% in 2003 to 3.7% in 2006. Such finding indicate the need for further MDA rounds to reach the target of LF elimination (Mf < 1%).

Consequently, we aimed to evaluate the efficacy of applying EPBs on the *Culex* population and the impact on LF transmission. In particular, we measured mosquito density and used molecular xenomonitoring [11] to assess *Culex* infection rates before and after applying EPBs in the Hadibo area.

Methods

Study area

The study was conducted in the Hadibo area, at the northern coast of Socotra Island, starting November 2007. The island (with a population of approximately 48 000 according to the 2005census) [12] has an area of 3625 km^2, is located in the Indian Ocean (12° 26′ N & 54° 42′ E) at the mouth of the Gulf of Aden. The climate is tropical (temperature variation between 22 °C and 31 °C in the coastal area, and relative humidity of 50%–75%) with rainfall of about 170 mm/year during winter (October–February). During June–September, monsoonal winds make the island inaccessible except by plane.

Hadibo is inhabited by about 17 168 people (2005census) and there is no government environmental sanitation. Each house has its individual latrine pits, which in the majority of cases are inadequately covered or not well closed. The density of *Culex* mosquitoes is high and they breed mainly in these cesspits and wet pit latrines.

Study sample

Human and mosquito surveys were conducted in 40 houses who complained of high mosquito density, selected from 127 houses with inadequately covered cesspits (based on a list of houses at the Roll Back Malaria Programme on the island), before and after application of EPBs. Houses with well-closed cesspits and dry pits (300) were excluded from the study.

Data collection

The following procedures were carried out: household members in 40 household selected were tested for Mf; a questionnaire for the density of indoor mosquitoes was filled for all 127 households with inadequately covered cesspits (breeding sites); and mosquitoes were collected before the use of EPBs. Mosquito collection and questionnaire surveys were repeated 2 week after the use of EPBs to allow for the mosquito development cycle. For comparison, the Mf survey was repeated 1 year later in the annual Mf monitoring.

Mf surveys

A total of 421 thick blood smears (60 µL) were collected about 1 month before the 6th round of MDA and before the use of EPBs. Thick blood smears (n = 530) were collected 12 months later and after application of EPBs. Smears were collected at night (between 21:00 and 02:00), air dried, Giemsa-stained and examined microscopically. For quality control, smear slides were examined twice and Mf counted. Results are expressed as the percentage of individuals with Mf and as Mf density.

Mosquito collection and EPBs intervention

To measure the vector density, adult mosquitoes were collected from the 40 selected houses. Two trained staff members, from the malaria control

programme in Socotra, carried out the spray capture of mosquitoes (total catch) using the spray sheet collection method [13]. Indoor-resting mosquitoes were collected from 1 bedroom per house on 1 × 2 m white cotton sheet after knock-down by insecticide. The collection was done in the early morning (around sunrise) during November 2007. Mosquitoes collected from each room/house were counted, pooled and stored in small boxes.

EPBs (2 mm) were applied to all 127 recorded mosquito-breeding places (cesspits of all houses with inadequate covering) to form a 2-cm thick layer, which required 10 L/m^2 of water surface. Mosquito collection was repeated in the same bedrooms after 2 weeks of the EPBs intervention and compared with the pre-intervention data.

Household questionnaire

A pretested questionnaire was used to determine whether the household members of the targeted houses would notice a difference in the density of adult mosquito populations before and after the EPBs intervention. Heads of all 127 households were interviewed 1 night before each mosquito collection. The questionnaire included questions about their observation of: mosquito density before the use of EPBs, type of mosquitoes (if known), places of high density, breeding sites and their observation after the EPB application in terms of decrease in mosquito density, level of decrease, period of decrease post-EPBs use, reduction in of nuisance level, resurgence and the householder's evaluation of the benefits of EPBs use.

Detection of *Wuchereria bancrofti* DNA

Genomic DNA was isolated from a total of 40 mosquito pools, 20 randomly selected before and after EPBs application from the original 40 houses, according to the method of Ramzy et al. [14]. *W. bancrofti* DNA was detected in the isolated DNA using the ligase detection reaction real-time polymerase chain reaction (PCR), which targets the LDR DNA sequence, according the method of Rao *et. al.*, 2006 [15]. Briefly, the LDR real-time system uses two primers (LDR1: ATTTGATCATCTGGGAACGTTAATA & LDR2: CGACTGTCTAATCCATTCAGAGTGA) and TaqMan probe (ATCTGCCCATAGAAATAACTACGGTGGATCTCTG) designed by Primer Express software (Applied Biosystems, Foster City, CA). The primers were used unlabelled, but the probe was labelled with the reporter dye FAM (6-carboxyfluorescein) at the 5' end and the quencher dye TAMRA (6-carboxytetramethylrhodamine) at the 3' end. The real-time PCR reaction was performed with 12.5 µL of TaqMan master mix (Applied Biosystems) along with 450 nmol/L of each primer and 125 nmol/L probe in a final volume of 25 µL. Two microliters of gDNA isolated from mosquito pools were mixed with the PCR master mix in 96-well MicroAmp optical plates (Applied Biosystems). Thermal cycling and data analysis were done with an ABI Prism 7000 instrument using SDS software (Applied Biosystems). Water was used as a negative control and DNA from *W. bancrofti* Mf served as a positive control sample in all real-time PCR runs. All real-time PCR reactions were carried out in duplicate, and cycle threshold values for each sample were determined according to the manufacturer's instructions.

Statistical analysis

The PoolScreen2 software [16], available upon request from the Department of Biostatistics and Division of Geographic Medicine, University of Alabama at Birmingham, United States of America, was used to estimate the maximum likelihood of *W. bancrofti* infection in collected mosquitoes at the 95% confidence level.

Reduction levels (RL) for Mf prevalence and Mf load were calculated as below (T = treatment).

$$RL = \frac{\text{pre-T value} - \text{post-T value} \times 100}{\text{pre-T value}}$$

Data were analysed with *SPSS* for Windows, version 11.0.1). The Mann–Whitney 2-tailed rank sum test was used for comparisons of means limited to 2 groups.

Results

Pre-intervention mosquito density

Culex quinquefasciatus was present in all houses investigated. The total number of mosquitoes collected was 4 268 adults with a mean of 107 mosquitoes per room per night; the number of pre-intervention mosquitoes collected ranged from 22 to 420 mosquitoes per room per night.

Impact of EPBs on adult *Culex quinquefasciatus* population

The total number of mosquitoes collected from all rooms decreased to 837 adults (19.6% relative to pre-intervention) with a mean of 21 per room per night; the number ranged from 3 to 68 mosquitoes per room per night. The EPBs intervention resulted in an overall reduction in mosquito density of 80% ($P = 0.02$). Through follow-up and observation, the reduction appeared to last for more than 3 months after the application of the EPBs.

Questionnaire data

All 127 households were interviewed both before and after the intervention. The questionnaire data indicated that 97% of the household members noted a decrease in the density of the mosquito population; 96% thought the intervention resulted in a 50% reduction in mosquito density. Of the interviewed household members, 98% considered that the use of polystyrene beads was

Table 1 Opinion of 127 households interviewed to evaluate impact of expanded polystyrene beads (EPBs) on mosquito density and nuisance

Opinion	High mosquito density before EPBs	Density reduction after EPBs	Estimated % of mosquito reduction	Time of noted reduction after EPBs application	Nuisance reduction	Evaluation of polystyrene use
Agree No. (%)	127 (100)	123 (96.8)	50%: 122 (96) 25%: 1 (0.8)	2 days: 2 (1.6) 1 week: 114 (89.8) 2 weeks: 9 (7.1)	123 (96.8)	Good: 124 (97.6)
Disagree No. (%)	0	2 (1.6)	0	0	2 (1.6)	0
No comment No. (%)	0	2 (1.6)	4 (3.2)	2 (1.6)	2 (1.6)	3 (2.4)

a good intervention for reducing mosquito populations (Table 1).

Impact of vector control on filariasis transmission

Mosquito infection

Mosquitoes collected before the EPBs intervention were sorted (n = 4268), and males and mosquito species other than *Culex quinquefasciatus* were discarded (n = 176 mosquitoes). A total of 450 randomly selected, mosquitoes were tested in 20 pools (a pool/house) by the LDR real-time PCR. Fifteen (15) of the tested pools contained 25 mosquitoes and the other 5 pools contained different numbers (15, 15, 16, 10 and 19 mosquitoes). Three pools had positive PCR results (Table 2). The minimum mosquito infection rate (providing that a positive pool contained one infected mosquito) was calculated as 0.66%. The Poolscreen algorithm estimated the maximum likelihood of mosquito infection rate as 0.73% (95% CI: 0.14%–2.10%).

Following application of the EPBs, after discarding males and mosquito species other than *Culex quinquefasciatus* from the mosquitoes collected from the 20 houses, a total of 281 mosquitoes were tested by the LDR real-time PCR in 20 pools (1–25 mosquitoes/pool). All pools were negative. The Poolscreen algorithm estimated the maximum likelihood of mosquito infection rate as 0.00% (95% CI: 0.00%–0.68%).

Human infection

Mf prevalence decreased from 1.4% before the EPBs intervention to 0.5% 12 months after the EPBs application (64.3% reduction). In the same manner, the Mf density decreased from 53 mf/mL to 28 mf/mL (47.12% reduction).

Discussion

The current strategy for LF elimination, based on interruption of transmission, depends mainly on drug distribution. However, a WHO consultation [17] encouraged integrated vector control measures, especially in areas where dramatic reduction in the prevalence of infection following multiple MDA rounds is not observed. In the present study, we supplemented MDA with application of EPBs as a measure for mosquito control.

In our study application of EPBs resulted in a large decrease of *Culex* mosquitoes (19.6% relative to pre-intervention). The decrease in the mosquito population was maintained over 3 months, which would lower human exposure to filariasis transmission. Of great interest is our observation that Mf prevalence among household members decreased by 64.3% consequent to supplementing MDA with the application of EPBs. In previous years, Mf prevalence had decreased by 38.3 % (from 6% to 3.7%) and 35.1% (from 3.7% to 2.4%) following yearly MDA in Socotra. Thus, the use of EPBs as a vector control measure had a synergetic effect on reducing LF transmission leading to timely disease elimination.

Despite the progress made towards LF elimination in Socotra, several factors work against achieving LF elimination using only 5 MDA rounds, including the relatively high infection rates [40% antigenaemia by

Table 2 Impact of expanded polystyrene beads (EPBs) on mosquito infection as determined by the LDR real-time PCR

Period	No. of mosquitoes	No. of mosquito pools examined[a]	No. of positive mosquito pools	Minimum MI rate (%)[b]	Maximum likelihood of MI (95% CI) (%)
Pre-EPBs application	450	20	3	0.66	0.73 (0.14–2.10)
Post-EPBs application	281	20	0	0.00	0.00 (0.00–0.68)

[a]Pre-EPBs mosquito pools contained 10–25 mosquitoes/pool, and post-EPBs mosquito pools contained 1–25 mosquitoes/pool.
[b]Minimum MI rate providing that a positive pool contains at least 1 positive mosquito.
PCR = polymerase chain reaction; MI = mosquito infection; CI = confidence interval.

the immunochromatographic (ICT) filariasis test] prior to starting MDA. Therefore, the findings of our study suggest that while the national programme focus will be primarily on achieving high rates of MDA coverage, integration of other vector control measures will be necessary. However, wide application of EPBs as an effective vector control measure faces some challenges. For example, finding all the wet cesspits infested with *Culex quinquefasciatus* is not easy. Furthermore, periodic emptying of cesspits and the presence of strong seasonal wind may remove the polystyrene beads and would likely reduce the effectiveness of a single EPBs treatment. Nevertheless, our finding that 98% of the participants interviewed appreciated the use of EPBs for mosquito control is encouraging and indicates that the community would willingly support the wide use of such a control measure.

Conclusion

Our study in Hadibo area (known for high LF infection rate before MDA) clearly showed that application of EPBs in major cesspits together with MDA resulted in a dramatic reduction of Mf prevalence (to < 1%). Wide application of such an effective strategy in Socotra would ensure successful LF elimination in the island.

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