

Research Note

Duration of detection of anti-*BmR1* IgG4 antibodies after mass-drug administration (MDA) in Sarawak, Malaysia

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Abstract. The detection rates of brugian filariasis in three regions of Sarawak namely Central, North and South after three courses of mass drug administration (MDA) from year 2004 to 2006 was investigated. A recombinant *BmR1* antigen-based IgG4 detection test, named Brugia Rapid and night blood smear for microfilaria (mf) detection were used. All three regions recorded a sharp fall in mf positive rates after a year post-MDA. Meanwhile Brugia Rapid positive rates declined more gradually to 3.8% and 5.6% of the pre-MDA levels in the Central and North regions, respectively. This study showed that in filariasis endemic areas in Sarawak, anti-filarial IgG4 antibodies to *BmR1*, as detected by the Brugia Rapid test, were positive for one to two years after mf disappearance.

Lymphatic filariasis (LF) is a mosquito-borne disease caused by three species of lymphatic filaria namely *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. With at least 120 million people infected and more than 40 million people incapacitated by LF in 83 countries, WHO had launched a Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 1997, with the ambitious goal of eliminating LF in all disease-endemic areas by the year 2020 (Ottesen, 1997). The GPELF aims to interrupt transmission with a programme that is largely based on annual mass drug administration (MDA) with anti-filarial medications that reduce microfilaria (mf) rates in communities to levels below those needed for sustained transmission by mosquitoes (Ottesen, 2000). Of the 81 endemic countries, 52 (64.2%) are actively conducting the MDA programmes, and of these 37 countries have completed ≥ 5 rounds of MDA (Addiss, 2010).

Diagnostic tools are important to GPELF because they affect decisions regarding where to distribute MDA, how to measure its effects, how to define targets and endpoints for stopping MDA and how to monitor populations for resurgence to LF transmission following suspension of MDA (WHO, 2005). In the case of brugian filariasis, no rapid antigen detection test is available to monitor the effectiveness of MDA in filariasis endemic areas. Brugia Rapid kit (current manufacturer: Reszon Diagnostics Int. Sdn. Bhd; <http://www.reszonics.com>), which detects IgG4 antibodies against *BmR1* recombinant antigen, is an alternative test to be used in the programme. The use of Brugia Rapid to detect brugian filariasis infection has been shown to be highly sensitive and specific for detection of brugian filariasis in laboratory and field studies (Rahmah *et al.*, 2001, 2003, 2005; Lammie *et al.*, 2004; Fischer

et al., 2005; Jamail *et al.*, 2005; Melrose *et al.*, 2006).

Preliminary studies on post-treatment positivity of *BmR1*-based ELISA among microfilaria (mf) positive individuals in a low endemic area of Malaysia, showed that the assay became negative six months to about two years post treatment with the full course of diethylcarbamazine (DEC) (Rahmah *et al.*, 2001, 2005). Post-treatment samples from endemic areas in Indonesia (which has higher infection prevalence than Malaysia) showed that the dipstick detected 78% of one year post treatment patients, 53% and 60% of two year post treatment patients and 71% of three year post treatment patients (Rahmah *et al.*, 2003). Interpretation of post-treatment patients are complicated by the fact that re-infection can occur and that there is no gold standard to determine whether all adult worms are killed by the treatment.

Therefore, it is important that more data is obtained on the period of persistence of anti-filarial IgG4 antibodies post-treatment when tested by Brugia Rapid test especially in the context of the once a year mass treatment in the GPELF; and this would probably vary between areas of different levels of LF endemicity. Thus the present study, performed between May 2003 and April 2006, was aimed at evaluating the duration of positivity of Brugia Rapid after MDA in Sarawak. This information may be useful to assist in the decision making processes in the pre-certification and surveillance post-certification phases of GPELF in other brugian filariasis areas with similar LF endemicity.

This study was conducted in 16 selected brugian filariasis endemic districts from three different regions of Sarawak, i.e. Miri, Limbang, Lawas and Marudi in the Northern region; Sarikei and Sibu from Central region; and Saratok from the Southern Region. Based on the previous records, these districts had higher prevalence of filariasis than the others in the state of Sarawak (Jamail *et al.*, 2005). The highest mf prevalence then was observed in the Northern region (4.09%; 155 positives of 3793 slides), followed by the Southern and Central regions with mf positive rate of 0.57% in both regions (21 positives of 3669 and 19

positives of 3337, respectively) (Jamail *et al.*, 2005) These areas are replete with dense forest, freshwater swamps and palm oil plantation.

The initial rounds of MDA began in 2004 and consisted of oral administration of DEC (6 mg/kg of body weight) combined with albendazole (400 mg) tablets given annually. The participants in this study were individuals that had been earlier identified as mf positive from a previous survey (before-treatment) (60%, n = 145) as well as their close contacts (40%, n = 65). The latter comprise mostly family members of the infected individuals who live in the same household. All individuals over the age of two years were asked to participate in the study. Informed consents were obtained from all adults or, in the case of children, from their parents. All participants are native people of Sarawak, with majority of them of Malay ethnicity (53.81%) followed by Iban (32.38%) and others are from indigenous population (13.81%). A resident of the endemic area is defined as someone who has lived in the community for at least 10 years and who has not been absent for more than six months. Children less than 10 years have been confirmed as resident that have lived in the community since birth. The study was reviewed and approved by the ethical board of the Universiti Sains Malaysia and the Ministry of Health Malaysia.

Sample collection was performed by field teams consisting of a public health assistant, a technician and local health workers who visited the houses in the evening. Field personnel recorded demographic data and MDA compliance information. Pre-treatment and post-treatment finger blood samples (300 µl) were collected between 8:00 PM and midnight into tubes containing ethylenediamine-tetra-acetic acid (EDTA). After a finger was pricked, individual blood samples were divided into two aliquots; one aliquot (60 µl) for thick night blood smear (NBS) and the another aliquot (35 µl) for Brugia Rapid test. The NBS were taken to the district health facility where the slides were promptly stained by Giemsa stain and examined microscopically for the presence of mf by the laboratory personnel of the local primary

health center. Brugia Rapid was performed on-site by the field personnel, according to the procedure in the manufacturer's product insert.

Data were entered and prevalence rates were calculated using Microsoft Excel. Statistical analysis was performed using PASW/SPSS for Windows version 17.0 (SPSS, Inc. Chicago, IL). The chi-square test was applied for comparison of proportions. Fisher's exact test was used if otherwise indicated.

A total of 210 inhabitants in the three regions of Sarawak participated in this study. The study population consisted of about equal proportion of the two gender i.e. 108 males (51.4%) and 102 females (48.6%). The average age of all participants was 33.6 years (95% confidence interval (CI) = 30.8–36.5). The median age (range) in years of males and females, respectively were as follows – Central region: 51 (12–80 years) and 22 (7–83 years); Northern region: 31 (2–80 years) and 30 (4–80 years); Southern region: 35 (2–72 years) and 29 (4–78 years). There was no significant age difference between the number of males and females in each of the three regions.

No significant difference was observed in prevalence between males and females

with both Brugia Rapid and mf tests ($P > 0.05$). Figure 1 shows that the prevalence of Brugia Rapid positive rates for all age-specific groups before MDA were more than 50% with the highest prevalence of 82% (41/50) in the age group 10-19 years. Meanwhile the mf prevalence rate was less than 30% in each of the age-specific groups. There was a significant difference in age-specific prevalence of positive results between Brugia Rapid and mf ($P < 0.001$) in each of the three regions. Brugia Rapid detects anti-*BmR1* IgG4 antibodies to both mf and adult worms, thus the prevalence of Brugia Rapid would be expected to be higher than mf prevalence.

Post-MDA data collection was successfully obtained from subjects in the Central and Northern regions until third MDA i.e. from 2004 to 2006. However due to various logistics challenges, the only data from the Southern region was those after the first MDA. Figure 2 shows the drop in mf and Brugia Rapid positive rates in Central and Northern regions. The prevalence of both Brugia Rapid and mf pre MDA was taken as 100% and prevalence thereafter were calculated with reference to the pre-MDA level. A similar data analysis approach has been reported in a study on bancroftian filariasis in Egypt (Ramzy *et al.*, 2006).

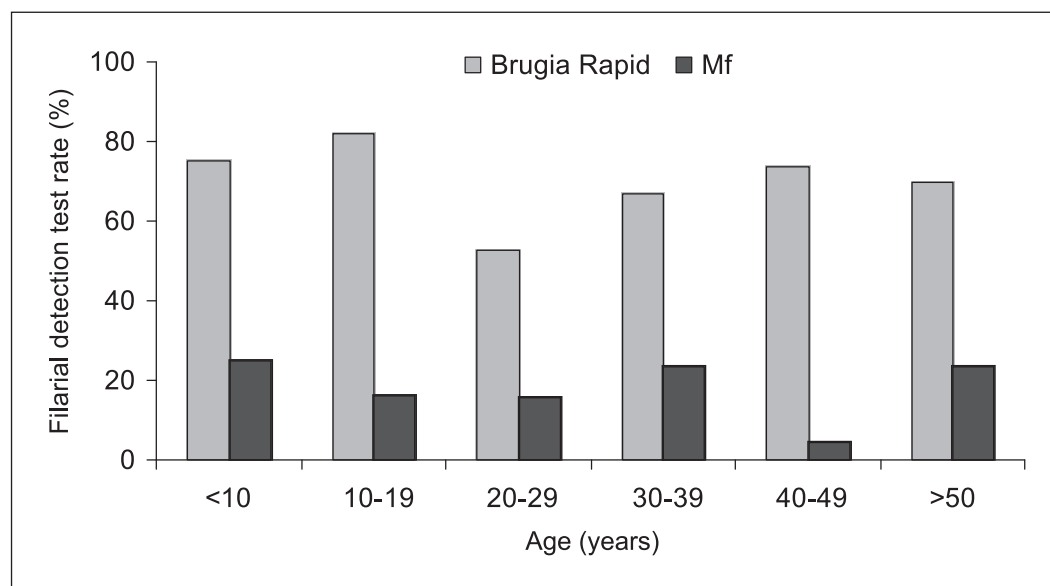


Figure 1. Baseline (before MDA), age-specific prevalence rate in the three regions of Sarawak

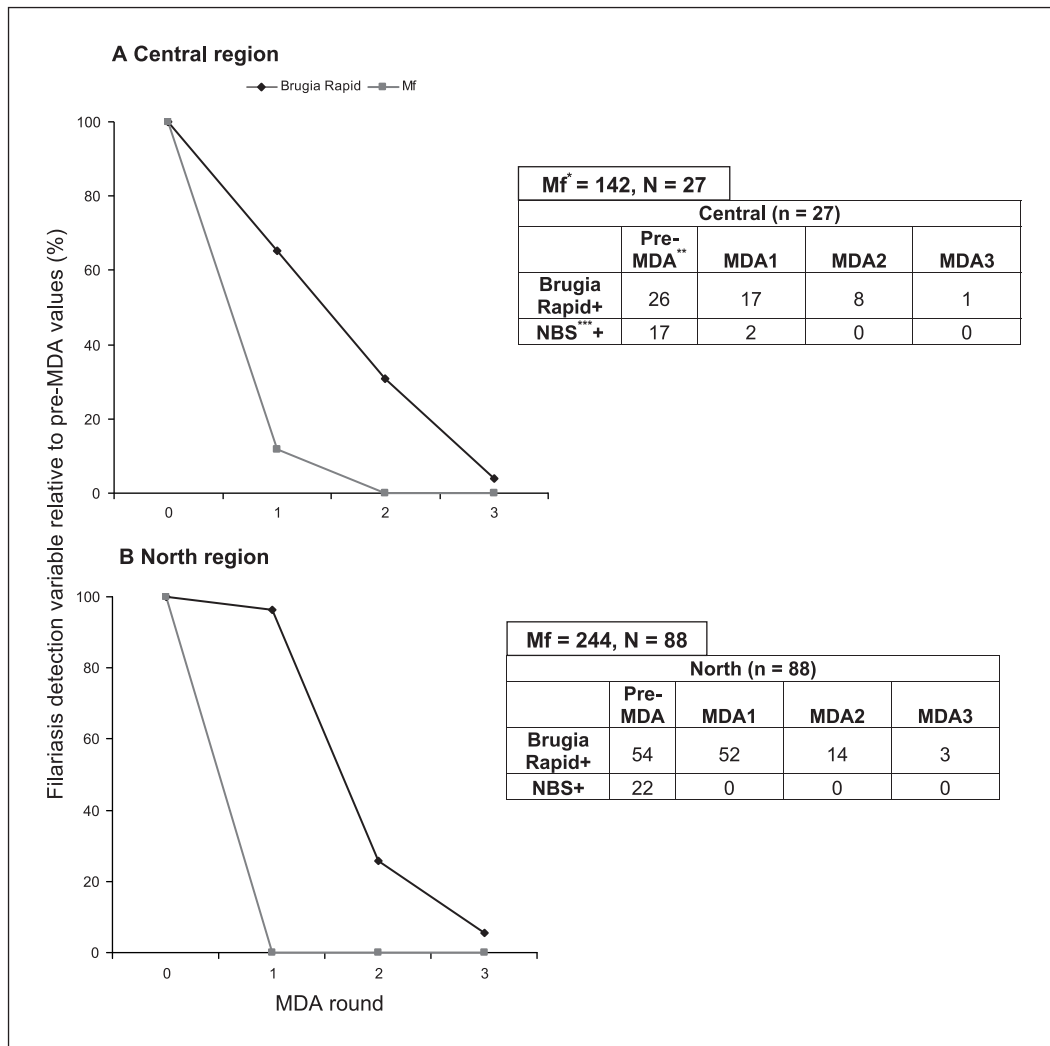


Figure 2. Relative effect of MDA on filariasis detection in (A) Central region, (B) North region. Data shown are percentage relative to baseline value measured pre-MDA. Data from Southern region not shown since there was only one mf+ pre-MDA and the Brugia Rapid results post-MDA were only available at year one

*Mf = microfilaria

**MDA = mass drug administration

***NBS = night blood smear

Mf positive rates fell more sharply than Brugia Rapid positive rates in all the three regions. In the Southern region there was only one mf+ individual (1/95), and the individual became mf negative after a year post-MDA. Microfilaria are sensitive and easily killed by DEC used in the MDA, whereas adult worms usually do not die immediately upon treatment. More than one DEC dose may be needed for complete adulticidal effect if they

are many adult worms nests in the body. As mentioned above, Brugia Rapid detects anti-*BmR1* antibodies to both mf and adult worms, thus Brugia Rapid positivity will be expected to persist for a longer period after treatment compared to mf. Thus the above observation, whereby Brugia Rapid positivity declined more gradually compared to that of mf was consistent with this scenario.

When analyzed by year using by 'chi-square for trend', the IgG4 antibody prevalence test by Brugia Rapid decreased significantly in both Central and Northern regions ($P < 0.001$ and < 0.001 , respectively). After the third MDA, the Brugia Rapid positive rate in Central region was 3.8% compared to 5.6% in the North. After mf dropped to zero, it took two years for Brugia Rapid to decrease to the low level in the North compared to one year in the Central region. This is consistent with the fact that the average mf prevalence/person in the Northern region was 11.1 mf as compared to 8.4 mf in the Central region. Similarly total mf count in Northern and Central regions were 244 and 142, and this difference was found to be statistically significant ($P = 0.012$). This showed that the average infection density (and hence average number of adults worms) in the North was higher compared to the Central region. In the Southern region, after the first MDA, Brugia Rapid positivity rate reduced to 53.5% of the pre-MDA prevalence.

This study shows that in the low to slightly moderate endemic areas of Sarawak, anti-filarial IgG4 antibodies to *BmR1*, as detected by the Brugia Rapid test, were positive for one to two years after mf disappearance. A proportion of the individuals may have become negative by Brugia Rapid six months or less post-MDA, but were not able to be captured by this study. The results of this study corroborated those of earlier studies on post-treatment of infected individuals in Malaysia which showed that the *BmR1*-based assay became negative six months to about two years post treatment (Rahmah *et al.*, 2001, 2005). More data from brugian filariasis areas with higher endemicity are needed to help formulate clear guidelines for certification and surveillance thereafter to ensure continued success of the GPELF.

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