

Identification of the vectors of lymphatic filariasis in the Lower Shire Valley, southern Malawi

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Abstract

An investigation of lymphatic filariasis vectors in Malawi is reported. *Anopheles funestus*, *A. arabiensis*, and *A. gambiae sensu stricto* had high rates of filarial infection (2.2–3.1%) and carried infective larvae. *Anopheles funestus* was the predominant species collected (77.6%) and was the primary vector during the study period of April to May 2002.

Keywords: lymphatic filariasis, *Wuchereria bancrofti*, transmission, *Anopheles funestus*, *Anopheles gambiae*, *Anopheles arabiensis*, *Culex quinquefasciatus*, Malawi

Introduction

Lymphatic filariasis (LF) is a leading cause of long-term and permanent disability worldwide (WHO, 1995). Many species of anopheline and culicine mosquitoes can transmit the causative agents, *Wuchereria bancrofti* and *Brugia* spp. In sub-Saharan Africa, the nocturnally periodic form of *W. bancrofti* is transmitted by night-biting *Culex* and *Anopheles* species, primarily *C. quinquefasciatus* and the malaria vectors *A. gambiae sensu lato* and *A. funestus s.l.* (Sasa, 1976; McMahon & Simonsen, 1996). Determining which of these potential vectors are responsible for LF transmission is essential prior to vector control, as well as for monitoring the efficacy of a drug-based, transmission-reducing intervention.

Nielsen *et al.* (2002) recently reported on a previously neglected highly endemic focus of filariasis transmission in southern Malawi, where microfilariae (mf) prevalence and circulating filarial antigen (CFA) levels reached 22.2% and 79.1%, respectively, in the population, with unexpectedly high CFA rates in children. *Anopheles gambiae sensu stricto*, *A. arabiensis*, *A. funestus*, and *C. quinquefasciatus*, all known vectors of *W. bancrofti* in Tanzania and Kenya, are present in the region (Donnelly & Townson, 2000; Spiers *et al.*, 2002) but their role as vectors in this area is unknown. With a mass drug administration (MDA) control programme for LF scheduled to begin in late 2002 within the Lower Shire Valley, this study set out to determine the role of local mosquito species as vectors of LF.

Materials and Methods

Mosquitoes were sampled from Belo village in Chikwawa district, southern Malawi (16°01'12''S, 34°49'04''E), where the prevalence of *W. bancrofti* infection, detected by the immunochromatographic test (ICT) for adult CFA, was previously reported to be 79.1% in all age groups and 77.9% in individuals aged > 15 years (Nielsen *et al.*, 2002). Mosquitoes were collected by pyrethrum knock-down from 2–3 houses per day between 06:30 and 08:00, 3 times per week from 15 April to 20 May 2002. In total, 54 houses from across the study village were sampled. The purpose of the work was explained to each householder recruited to the study. Permission to enter the house was sought and the right to refuse or withdraw at any time was respected. Temperature and barometric data were recorded for each house using a handheld Global Positioning System receiver (Magellan, CA, USA). Female mosquitoes were identified morphologically using the taxonomic keys of Edwards (1941), Gillies & Coetzee (1987), and Service (1990). After provisional species identification all female mosquitoes were separated into

head, thorax, and abdomen in a drop of saline and examined for the presence of *W. bancrofti*. Infection and infectivity status were recorded. Following dissection, all specimens identified as *A. gambiae s.l.* were stored over desiccant for later species identification by polymerase chain reaction (PCR). *Anopheles funestus s.l.* is also a species complex but as only *A. funestus s.str.* has been identified in the Lower Shire (Nora Besansky, personal communication) morphological identification of *A. funestus* specimens was sufficient. DNA was extracted following the Collins *et al.* (1987) protocol from mosquitoes identified as *A. gambiae s.l.* This DNA was used as a template for the *A. gambiae* species identification PCR of Scott *et al.* (1993). Since the extraction procedure yields both filaria and mosquito DNA, all *A. gambiae s.l.* found to be infected by dissection were subjected to a second PCR analysis to amplify *W. bancrofti* specific sequences following an adaptation of the protocols of Lenhart (2002) and Farid *et al.* (2001).

Results

The number, species, infection and infectivity status of all mosquitoes collected and dissected are shown in the Table. The number of mosquitoes harvested each day ranged from 29 to 435 (mean ± SD, 198.6 ± 117.1). At the time of collection, daily mean temperature and atmospheric pressure ranged from 20.8°C to 28.2°C and 1017 mbar to 1027 mbar, respectively. Infection rates with all filarial stages and infective L₃ larvae were highest in *A. gambiae s.str.* (3.1% and 3.1%, respectively) followed by *A. arabiensis*, (3.0% and 2.3%, respectively) and *A. funestus* (2.2% and 1.6%, respectively). However, these differences were not statistically significant ($P > 0.05$) (*t* test for proportions, significance adjusted for multiple tests by Bonferroni procedure). A single *Mansonia* was found infected with 2 infective filarial larvae (see below). No filarial infections were found in any *Culex* species.

A total of 9 *A. arabiensis*, 2 *A. gambiae s.s.*, 1 *A. gambiae s.l.* and 1 *Mansonia* sp. were found to be infected with filarial parasites and were processed for *W. bancrofti* detection by PCR. All were confirmed as infected with *W. bancrofti* except 1 *A. gambiae s.str.* and the single *A. gambiae s.l.* and *Mansonia* specimens.

Discussion

This is the first study to investigate transmission of LF in southern Malawi. During the study period the predominant mosquito species was *A. funestus* (77.6%) followed by *A. arabiensis* and *A. gambiae s.str.* (10.8% and 2.3%, respectively). Another study in this area found that *A. gambiae* and *A. arabiensis* are more abundant at other times of the year (Spiers *et al.*, 2002). Thus, while infective females of all 3 anopheline species were found in the present study, their relative importance as vectors, in terms of their annual transmission potentials, remains to be determined.

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Table. Checklist and filaria infection status of mosquito species collected by pyrethrum knock-down in Belo village, Malawi, April–May 2002

Species	Collected n (%)	Mean no./house	Infected ^a n (%)	Mean no. infected/house	Infective ^b n (%)	Mean no. infective/house
<i>A. funestus</i>	2159 (77.63)	40	48 (2.2)	0.89	35 (1.6)	0.65
<i>A. gambiae</i> s.str.	64 (2.30)	1.2	2 (3.1) ^d	0.04	2 (3.1)	0.04
<i>A. arabiensis</i>	299 (10.75)	5.5	9 (3.0) ^e	0.17	7 (2.3)	0.13
<i>A. gambiae</i> s.l. ^c	2 (0.07)	0	1 (50)	0.02	0	0
<i>M. uniformis/africana</i>	87 (3.13)	1.6	1 (1.1)	0.02	1 (1.1)	0.02
<i>C. univittatus</i>	155 (5.57)	2.9	0	0	0	0
<i>C. neavei</i>	11 (0.4)	0.2	0	0	0	0
<i>C. quinquefasciatus</i>	3 (0.11)	0.1	0	0	0	0
<i>C. annulioris</i>	1 (0.04)	0	0	0	0	0
Total	2781	51.5	61 (2.2)	1.13	44 (1.6)	0.84

^aInfection defined as microfilariae, L₁, L₂ or L₃ stage filaria larvae in mosquito carcass.

^bInfectivity defined as L₃ (infective) stage filaria larvae in mosquito head, thorax or mouthparts.

^cThese specimens were identified morphologically as belonging to the *Anopheles gambiae* complex but did not PCR-amplify for specific identification.

^dOne confirmed as *Wuchereria bancrofti* infection by PCR.

^eAll confirmed as *Wuchereria bancrofti* infection by PCR.

Since very few *Culex* were found (6% of the total catch), of which only 3 were *C. quinquefasciatus*, the role of this species as a vector in the area also remains to be determined. *Culex quinquefasciatus* is known as an important vector of filariasis elsewhere in East Africa (Maxwell *et al.*, 1990; Bogh *et al.*, 1998; Pedersen *et al.*, 1999). Whether the LF refractory phenotype is expressed in this region requires further investigation (Omar & Zielke, 1978).

Of the remaining species found, *Mansonia* transmits *W. bancrofti* in parts of Asia but has not been identified as a vector in Africa (Sasa, 1976; Service, 1990). Time constraints prevented specific identifications for all *Mansonia* although both *M. uniformis* and *M. africana* were found in the sample. In areas of Africa, both species are known to transmit the animal filariae *Dirofilaria immitis* and *D. repens* (Service, 1990), allowing speculation on the identity of the filarial parasites found in the single infected *Mansonia*. However, there is little doubt that the overwhelming majority of filarial infections found in the *Anopheles* mosquitoes are *W. bancrofti*. These *Anopheles* species are highly anthropophilic and are unlikely to transmit animal filaria. Furthermore, developing stages were found in the thoracic musculature of the insects whereas *Dirofilaria* develop in the Malpighian tubules.

Because anophelines show a facilitation pattern in infections with filaria (Southgate & Bryan, 1992), any reduction in the mf level of the human population following drug distribution is likely to lead to a decrease in transmission of *W. bancrofti* by these vectors. Moreover, these findings provide unequivocal evidence that insecticide-treated bednets, currently being promoted vigorously for malaria prevention throughout Malawi, will protect sleepers against another major mosquito-borne disease.

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Book Review

Manson's Tropical Diseases, 21st edition. Gordon C. Cook & Alimuddin Zumla (editors). London/Philadelphia: Saunders/Elsevier Science Ltd, 2003. xviii + 1848 pp. Price £122.00 hardback; £22.00 softback specifically for developing countries. ISBN 0-7020-2640-9 hardback; 0-7020-2790-X softback.

This 21st edition of *Manson's Tropical Diseases* is a remarkable volume in many ways. Following the long tradition originally set out by Patrick Manson in 1898 in his monograph entitled *Tropical Diseases: A Manual of the Diseases of Warm Climates*, this modern treatise maintains the tradition and has an updated feel about it; many of the 87 chapters have been entirely rewritten, indeed 11 are *de novo*, and balance both disease-specific and system-based approaches. The attractive hardcover, depicting a mosquito, provides an appropriate visual theme and throughout this volume there are many photographic images (more than 1000) carefully selected to illustrate typical clinical conditions; most of these are produced in black and white but over 50 have been duplicated in vivid colour (slightly more than in the 20th edition). In addition there are numerous well-selected illustrations, graphs, and clear clinical management diagrams, all very useful for teaching purposes as well as for general reference. With extensive cross-referencing throughout all chapters, there are very few instances of undue duplication, but perhaps what is most remarkable is the low cost of this volume. At £122 the book is very reasonably priced and well within the affordability of educational institutes, medical surgeries, and the individual pockets of clinicians and/or scientists who have chosen to pursue a career in tropical medicine. The softback version, specifically available for developing countries, is very generously priced. Indeed those with an interest in travel medicine, or general infectious disease, will not be disappointed with a copy of this acknowledged 'bible' of tropical medicine.

The 87 chapters are arranged within a framework of 12 sections that include: Underlying factors in tropical medicine, Symptoms and signs, System-oriented disease, Related specialities in the tropics, Environmental/genetic disorders, Viral infections, Rickettsial infections, Bacterial infections, Mycotic infections, Protozoan infections, Helminth infections, and Ectoparasites, with 5 subsequent appendices. With an assembled international authorship of 119, many of whom have spent their working lives within the tropics, much of their clinical and scientific acumen has been wisely passed on. Personally, I have had occasion to consult this volume on several areas outside my immediate interests and have not been disappointed in the guidance this book offers. The new chapters include treatises on: Primary Care, Epidemiology, Traditional medicine, Genetics, Economics, Ethics and tropical diseases: some global considerations, Tropical oral health, and Sources of information on tropical medicine. The latter contains a wealth of information which,

after consultation, could carefully streamline further searching on both hardcopy and electronic resources. The associated web-based resources, e.g. e-TALC, were particularly informative and good initial points to work from.

At the start of each section there is a brief taster of what each subsequent chapter offers; it is outside the goal of this review to consider all 87 but I would wish to highlight a few. The first chapter on *History of tropical medicine, and 'medicine in the tropics'* should be considered essential reading for those wishing to understand how this discipline originated and evolved, giving rise to the London and Liverpool Schools; both of which still remain of international renown. The chapter on *Epidemiology of disease in the tropics* brought home the shocking burdens of HIV and related AIDS conditions, tuberculosis and malaria as well as those from diarrhoeal-related diseases. Indeed the latter disease spectrum forms the major source of ill health for those travelling abroad and was duly highlighted in a dedicated chapter entitled *Travel medicine*. The often expanded chapters on the former diseases were particularly informative. On considering the tradition of practical advice within *Manson's*, I was disappointed by *Ethics and tropical disease* as I thought the balance between theory and practice was misplaced. Ethical issues are indeed complicated and there are many disparities to correct but a pragmatic approach needs to be fostered otherwise, as even on simple issues, we could fall into a trap of procrastination.

In the light of Manson's keen knowledge and expertise in helminthology and protozoology, the 21st edition still provides much to keep the medical parasitologist happy and *Clinical laboratory diagnosis* describes many of the simpler diagnostic tests possible even within a resource impoverished setting. The results of research on emerging and worsening drug resistance and new treatments for several diseases were presented and where appropriate alternative potential therapeutic targets or novel drug regimes were explored. Amongst others, the chapters on *Ophthalmology in the tropics and subtropics*, *Dermatological problems*, *Malaria*, *Tuberculosis*, *Animal toxins*, and *Sexually transmitted infections (excluding HIV)* were particularly thorough and very impressive.

To close, this latest edition of *Manson's Tropical Diseases* sets another very high standard within its long history and although rather heavy in weight, will soon be seen on the book shelves of many a grateful reader who seeks authoritative advice. The book is clearly an invaluable addition to the existing literature and owes greatly, without doubt, to the extensive experience and expertise of the present editors and to the distinguished panel of authors.

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