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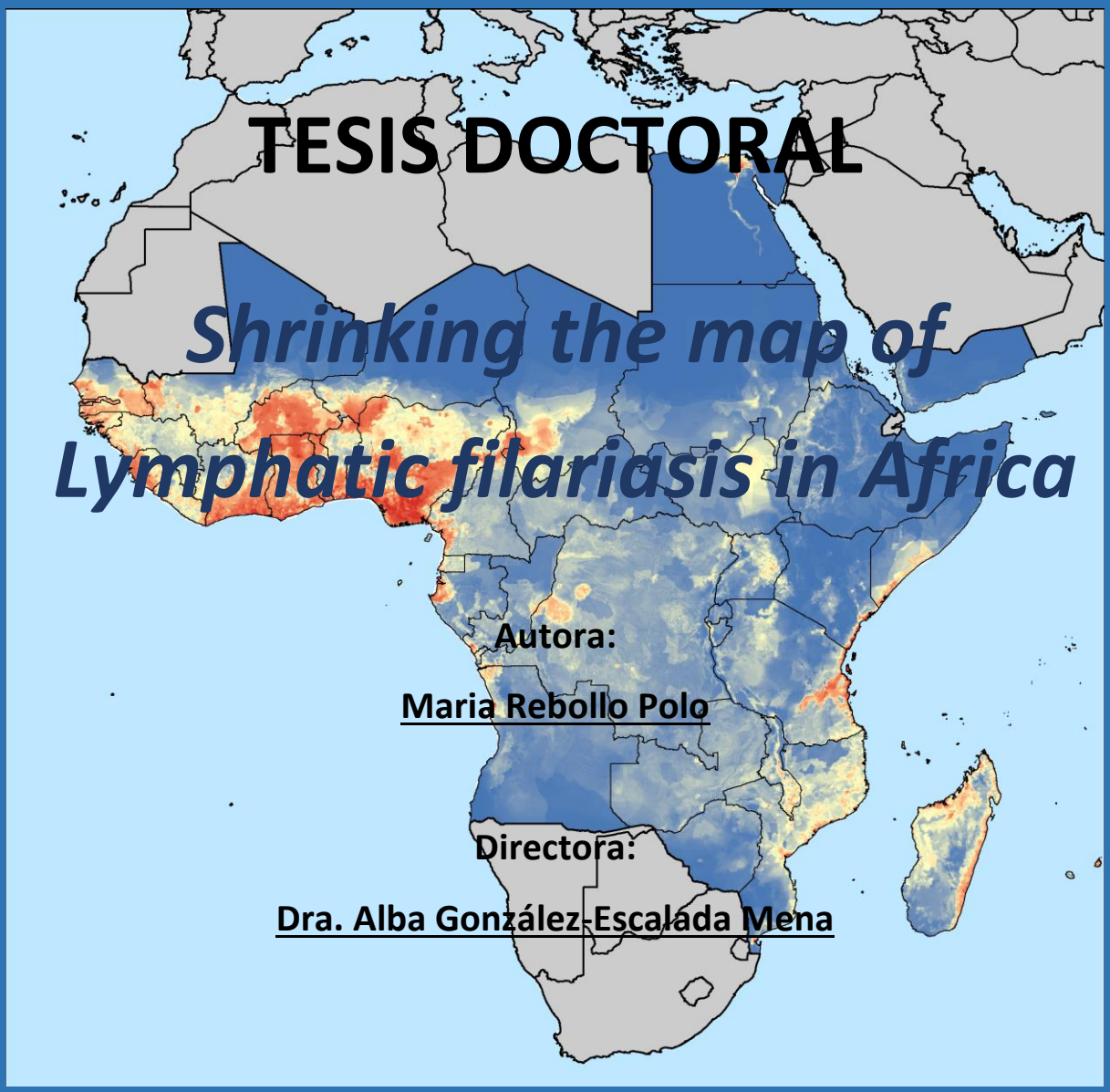

TESIS DOCTORAL

Shrinking the map of Lymphatic filariasis in Africa

Autora:

Maria Rebollo Polo

Directora:

Dra. Alba González-Escalada Mena

Programa de Doctorado en Ciencias de la Salud

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To Africa

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Resumen (Español)

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En enero de 2012, socios de la industria, de la investigación, gobiernos, organizaciones no gubernamentales de desarrollo, financiadores e instituciones académicas, firmaron la Declaración de Londres (DL) y se comprometieron a mantener y expandir los programas de intervención de enfermedades tropicales desatendidas (ETD). El objetivo final es facilitar el control, la eliminación o la erradicación de 10 enfermedades incluyendo la filariasis linfática (FL). Sin embargo, cuando se firmó el DL, los datos disponibles para el programa de FL en África (correspondientes a las cifras de tratamientos de 2009 y 2010) distaban de ser optimistas, con solo seis países en proceso de cunmplir con los objetivos de eliminacion. En ese momento, solo 85 millones de personas estaban siendo tratadas para la FL de los 405.9 millones de personas estimadas por la Organización Mundial de la Salud (OMS) que requieren tratamiento para FL en 34 países de África. No está claro cómo se estimó la carga de morbilidad y la población en riesgo que necesita tratamiento en África. Por lo tanto, la cuestión central que se aborda en esta tesis es: **¿pueden las herramientas más recientes de diagnóstico, epidemiología y modelado matemático ayudar al Programa Global para Eliminar la Filariasis Linfática (GPEFL) al proporcionar estimaciones más precisas de la carga de FL en África?** Obtener una estimación más precisa del número de personas que requieren tratamiento para la FL permitiría que el objetivo de eliminación de esta enfermedad para el 2020 fuese más realista. Al reducir el número estimado de personas que necesitan tratamiento y reducir el mapa de endemicidad de FL, la meta 2020 se vuelve mas alcanzable y los esfuerzos pueden canalizarse en aquellas zonas geográficas donde realmente son necesarios.

Introducción

Epidemiología de la Filariasis Linfática

La filariasis linfática (FL) es una infección transmitida por mosquitos, y es una causa importante de morbilidad aguda y crónica de miles de seres humanos en áreas tropicales y subtropicales de Asia, África, el Pacífico occidental y algunas partes de las Américas.

Más del 20% de la población mundial vive en áreas en riesgo de infección por las especies de nematodos responsables de la FL. Las regiones del mundo que asumen la mayor carga de enfermedad por la FL son el sur y sudeste de Asia, África, el Pacífico occidental, el este del Mediterráneo y algunos países de Centroamérica y Sudamérica.

Un mapa de riesgo basada en datos ambientales, desarrollado usando modelos de árboles de regresión, muestra que las condiciones ambientales adecuadas para la transmisión de FL se dan a lo largo de las regiones forestales y sabanas de África occidental, África oriental y Madagascar y focos restringidos en África central y meridional. Las condiciones ambientales idóneas para la transmisión también ocurren en áreas tropicales del sur y sureste de Asia y el Pacífico, así como en grandes áreas de América Central y del Sur, incluidos los estados del sur de América, donde la enfermedad fue eliminada a principios del siglo XX. Las predicciones de idoneidad ambiental en regiones templadas son consistentes con distribuciones históricas documentadas antes de la intervención a gran escala y la eliminación local en Japón, Corea del Sur y el sur de China, la costa norte de Australia y la costa sureste de Estados Unidos

En la actualidad, la OMS estima que más de 120 millones de personas están infectadas por la FL en 80 países endémicos. El 91% de las infecciones son causadas por *Wuchereria bancrofti*, mientras que las infecciones por *Brugia malayi* y *Brugia timori* representan el otro 9%. *B. timori* solo es conocido por ser endémica en Timor y las islas Flores del archipiélago indonesio.

Se estima que la presencia de microfilarias en el sistema linfático es causa de linfedema, patología genital (especialmente hidroceles) y elefantiasis en aproximadamente 41 millones de hombres, mujeres y niños. Otros 76 millones tienen infección oculta, la mayoría de las veces con microfilarias en sangre y afectación interna de sus sistemas linfático y renal.

Los principales vectores que transmiten FL a los humanos incluyen especies de los géneros Anopheles, Aedes, Culex, Mansonia y Ochlerotatus.

El Programa Global de Eliminación de la Filariasis Linfática

La disponibilidad de regímenes de tratamiento seguros, de dosis única y con fármacos capaces de reducir la microfilaremia a niveles cercanos a cero a partir del primer año, junto con una mejora notable de las técnicas diagnósticas ha despertado optimismo en la comunidad científica sobre la factibilidad de lograr la eliminación de la enfermedad a nivel global. En 1997, la Asamblea Mundial de la Salud adoptó la Resolución WHA 50.29 haciendo un llamamiento a la eliminación de la FL como un problema de salud pública a nivel mundial. La OMS, en colaboración con otras agencias internacionales de salud pública y el sector privado, formando una 'Alianza Global', lanzó una campaña mundial para eliminar la FL hacia el año 2020. El objetivo principal del Programa Global para la Eliminación de la Filariasis Linfática (PGEFL) es romper el ciclo de transmisión de la enfermedad entre los mosquitos y los seres humanos, principalmente a través de la administración masiva de albendazol en combinación con ivermectina o dietilcarbamazina (DEC). La FL sigue siendo una causa importante de morbilidad en muchas partes del mundo. Se ha realizado un esfuerzo global por parte del PGEFL y se han logrado avances significativos, pero se cree que la enfermedad aún afecta a más de 100 millones de personas en todo el mundo. La ausencia de un reservorio no humano de *Wuchereria bancrofti* y solo un reservorio animal muy pequeño para *B. malayi* (que probablemente juega poco o ningún papel en la transmisión a los humanos) significa que la transmisión puede interrumpirse eliminando el reservorio de microfilarias a través del tratamiento comunitario, reduciendo el contacto humano-vector o un enfoque integrado que combine los dos. Sin embargo, incluso cuando la transmisión se ha interrumpido, los gusanos adultos pueden seguir induciendo patología linfática y la consiguiente morbilidad. Por lo tanto, reducir o controlar la morbilidad causada por la infección es el segundo gran objetivo del PGEFL .

En resumen, el PGEFL ha desarrollado una estrategia respaldada por dos pilares principales: primero, interrumpir la transmisión de la enfermedad (para que no ocurran nuevas infecciones) y segundo, e igualmente importante, controlar la morbilidad para prevenir la discapacidad (y aliviar el sufrimiento de las poblaciones afectadas).

Para lograr la interrupción de la transmisión, las principales actividades recomendadas son la quimioterapia preventiva (QP) y el control vectorial (CV).
La QP consiste en la distribución de medicamentos a una gran proporción de individuos elegibles para recibir medicación anti-helmíntica. La estrategia es la administración masiva de medicamentos (AMM) con periodicidad (típicamente anual para los programas de FL), diseñada para que la carga de la infección de la enfermedad se reduzca con el tiempo a niveles que permitan la interrupción sostenible de la transmisión, y por ende la eliminación.

La gestión de la morbilidad y la prevención de la discapacidad (GMPD) consiste en un paquete de medidas preventivas y curativas para proporcionar la mejor atención clínica disponible a nivel nacional y el apoyo psicosocial que necesitan los pacientes. Cuando se logra la interrupción de la transmisión de FL, el paquete GMPD debe integrarse en los sistemas de salud para garantizar la atención sostenible de los pacientes durante su vida o hasta que ya se resuelvan las morbilidades debidas a la infección filárica.

Quimioterapia preventiva para las helmintiasis humanas

El uso de medicamentos antihelmínticos, ya sean solos o en combinación, como una herramienta de salud pública contra las infecciones por helmintos se conoce como quimioterapia preventiva (QP). Cinco enfermedades tropicales desatendidas son susceptibles de QP: FL, Tracoma, Oncocercosis (Oncho), Esquistosomiasis (SCH por sus siglas en ingles) y geo-helmintiasis (helmintiasis transmitidas por contacto con el suelo) (STH por sus siglas en ingles).

- Filariasis linfática (FL): causada por una infección con los nematodos *Wuchereria bancrofti*, *Brugia malayi* y *B. timori.*
- Oncocercosis (Oncho) - causada por la infección con el nematodo *Onchocerca volvulus.*
- Esquistosomiasis (SCH) - SCHi (esquistosomiasis intestinal) causada por infección con los trematodos *Schistosoma mansoni*, *S. mekongi*, *S. japonicum* y *S. intercalatum*, y SCHu (esquistosomiasis urinaria) causada por infección con *S. haematobium*.

- Helmintiasis transmitida por contacto con el suelo (STH) - causada por infección con los nematodos *Ascaris lumbricoides* (lombriz intestinal), *Ancylostoma duodenale* y *Necator americanus* (anquilostoma) y *Trichuris trichiura* (lombriz intestinal).
- Tracoma - causado por la bacteria *Chlamydia trachomatis*

La administración masiva de medicamentos (AMM) consiste en la distribución en medicamentos a toda la población elegible a través de campañas periódicas. AMM es la estrategia utilizada en el PGEFL. Existen una serie de criterios de exclusión para no recibir tratamiento que son específicos para cada uno de los fármacos y esquemas de tratamiento utilizados. La población que no cumple los criterios de exclusión se denomina *población elegible*. La población total que vive en un área donde tiene lugar la transmisión de la enfermedad es la población llamada en *riesgo* y todos son diana de tratamiento quimiopreventivo. La población total que requiere QP, y es la población diana para el tratamiento, se utiliza como el denominador para estimar la cobertura efectiva de MDA. Esta estrategia también se conoce como *intervenciones de tratamiento dirigido a la comunidad* por los programas de control de la oncocercosis, mientras que habitualmente se llama AMM en los programas de eliminación de la FL. Para que la AMM sea efectiva en la interrupción de la transmisión, se debe llegar al menos al 65% de la población objetivo la cual debe consumir (tragar) los medicamentos. Eso es equivalente al 80% de la población elegible en los programas de FL.

Cuando la población objetivo para el tratamiento no es toda la población, la intervención se denomina *tratamiento dirigido* en lugar de AMM. El tratamiento dirigido generalmente ocurre cuando el objetivo de la PC es controlar la morbilidad en lugar de interrumpir la transmisión. En esos programas de control la población que tiene mayor riesgo de morbilidad o es más vulnerable, como niños en edad escolar o en edad preescolar, mujeres en edad fértil o grupos profesionales expuestos a la enfermedad a través de su trabajo (también llamado grupos de alto riesgo).

Se usan diferentes fármacos contra las NTDs susceptibles de QP:

- Ivermectina (IVM): es el fármaco de elección contra la Oncocercosis. En los países donde la FL y la Oncocercosis son endémicas, ivermectina es el fármaco de elección también para FL. Cuando se usa ivermectina para FL, se distribuye en combinación con albendazol 400 mg. La

ivermectina se distribuye como una dosis única por peso o altura. La ivermectina tiene principalmente un efecto microfilaricida, pero no macrofilaricida (no produce la muerte del gusano adulto). Las mujeres embarazadas, las mujeres que amamantan en la primera semana después del nacimiento, los niños <90 cm de altura (aproximadamente equivalente a 15 kg / peso corporal) y los gravemente enfermos no son elegibles para recibir este tratamiento. La ivermectina es donada por la compañía farmacéutica Merck (www.merck.com) durante el tiempo que sea necesario hasta alcanzar la eliminación de la oncocercosis y FL (donde FL es endémica con Oncho). La ivermectina es donada con el nombre de Mectizan® a través del programa de donación de Mectizan (www.mectizan.org)

- Dietilcarbamazina (DEC): Combinado con albendazol, es el fármaco de elección para eliminar la FL en países que no son endémicos para la Oncocercosis. El fabricante Eisai es el donante de dicho tratamiento. La dosis de DEC se administra por grupo de edad:

- Praziquantel: utilizado para controlar la morbilidad por esquistosomiasis. La dosis se administra según la altura.

- Albendazol: se usa para prevenir la morbilidad debida a STH. Los comprimidos son de 400 mg y se pueden administrar a cualquier persona de 2 años o más. Los niños de 12 meses a 24 meses pueden recibir 200 mg de albendazol. El albendazol también se usa en combinación con IVM o DEC para eliminar FL a través de AMM. Para este propósito, se administra a personas mayores de 4 años como una comprimido de 400 mg combinada con la dosis adecuada de DEC o IVM.

  El albendazol administrado una o dos veces al año es la estrategia de MDA recomendada cuando la ivermectina no se puede utilizar debido al alto riesgo de eventos adversos graves (SAE) que pueden ocurrir en las áreas co-endémicas con *Loa loa*.

- Mebendazol: se usa en QP para controlar la morbilidad de STH en la población de riesgo. El mebendazol se puede administrar a partir de los 12 meses de edad. Los comprimidos vienen en dosis de 500 mg y se administran en monodosis una o dos veces al año según la estrategia recomendada por la OMS según la prevalencia de la enfermedad.

- Azitromicina: es el fármaco de elección para eliminar el tracoma como un problema de salud pública. Es donado por Pfizer bajo el nombre de Zithromax a través de la Iniciativa Internacional de Tracoma (ITI).

Quimioterapia preventiva contra la FL

Ivermectina o DEC más albendazol en AMM es la estrategia de elección para eliminar la FL.

La AMM frente a la FL generalmente implica el suministro anual de una dosis combinada de medicamentos a todas las personas elegibles que viven en todas las áreas endémicas durante al menos 5 años. Los tratamientos administrados durante la MDA reducen la densidad de los parásitos que circulan en la sangre de las personas infectadas y la prevalencia de la infección en la comunidad a niveles tan bajos que la transmisión no puede mantenerse, haciendo posible por tanto la eliminación de la enfermedad. Cuando el nivel de infección se ha reducido por debajo de los umbrales críticos de interrupción de la transmisión, se considera que el AMM ya no es necesario. El umbral de prevalencia de individuos infectados con FL por debajo del cual la transmisión de FL se considera no sostenible, y por tanto las intervenciones ya no serían necesarias, es del 2% para las áreas de transmisión por *Anopheles* y *Culex* y del 1% para las áreas de transmisión por *Aedes*.

La AMM realizada anualmente necesita alcanzar una proporción suficiente de individuos para ser efectiva en la interrupción de la transmisión de FL. La cobertura de AMM es la proporción de personas que ingieren los medicamentos sobre aquellos a los que se dirige el tratamiento (población elegible).

Progreso hacia la eliminación de la filariasis linfática

En 2001, la OMS declaró que 327 millones de personas necesitaban QP frente a la FL en 38 países africanos.

En 2002, la OMS amplió este número a 477 millones en 39 países. Para ese mismo año, solo 9 países estaban llevando a cabo AMM, con solo 9.9 millones de personas cubiertas, lo que

representaba solo el 2.1% de la población total que se estimaba necesitaba QP frente a la FL en África.

Un paso esencial en la implementación de programas para eliminar la FL es definir **dónde** se debe realizar la AMM. En 2000, se inició el primer programa de mapeo de la FL en África, de manera que en 2001, 4 países habían identificado las áreas endémicas para la FL, y por tanto necesitadas de ser tratadas: Benin, Burkina Faso, Ghana y Togo.

En 2009, once países endémicos de África aún no habían completado el mapeo de la FL (Angola, Camerún, República Centroafricana, Côte d'Ivoire, República Democrática del Congo, Etiopía, Liberia, Nigeria, Sudán del Sur, Zambia y Zimbabwe), y dos paises no habian ni siquiera iniciado esta actividad (Chad y Eritrea).

Para ese mismo año, se estimaba que 405.9 millones de personas en 39 países de África necesitaban QP. Sin embargo, de acuerdo con el informe de progreso de PGEFL para el periodo 2000-2009, publicado en 2010, la evidencia de transmisión activa de FL en muchos de los 39 países endémicos era débil y algunos incluso podrían no requerir AMM. Cinco países - Burundi, Cabo Verde, Islas Mauricio, Ruanda y Seychelles - fueron revisados en 2011 y fueron reclasificados como no endémicos, reduciendo el número de países endémicos de África a 34. La inclusión de Sudán del Sur después de la independencia en 2011 elevó el número a 35.

En el año 2009, diecinueve países implementaron AMM en África. En ese momento, solo 85 millones de personas (20.9% del total que requería MDA) fueron elegidas para MDA y solo 66 millones de personas fueron tratadas. Quince países aún no habían comenzado el MDA, mientras que 13 todavía no habían alcanzado la cobertura geográfica completa. El hecho de que los programas de MDA en las poblaciones urbanas suelan lograr una cobertura baja en comparación con entornos más rurales contribuye a la baja cobertura en algunas áreas. Las personas que viven en las ciudades tienden a estar más ocupadas, lo que dificulta la movilización social; las poblaciones son heterogéneas, con estructuras sociales, económicas y religiosas complejas; además los habitantes de zonas urbanas le dan una mayor prioridad a la privacidad lo cual conlleva una menor participación en intervenciones comunitarias de salud pública.

Para 2009, solo 6 de los 34 países que requerían AMM para FL en África habían logrado una cobertura geográfica del 100% (Burkina Faso, Comoras, Ghana, Mali, Malawi, Togo), encaminándose al logro de alcanzar la eliminación de la FL para 2020.

Con más de 320,9 millones de personas que necesitan QP que aún no recibían AMM en el año 2009, el escenario presentado por estos datos resultaba ciertamente pesimista para África en términos de lograr de manera realista el objetivo de eliminación en 2020. Estos datos incluyen países enteros que aún no habían iniciado la AMM, como Gambia y Gabón, y también incluyen otros países con alta carga de FL, como Etiopía y la República Democrática del Congo, donde solo un pequeño porcentaje de la población que requería tratamiento la estaba recibiendo.

Por otro lado, en el 2009, en Benin, Burkina Faso, Nigeria, Ghana y Tanzania (incluida Zanzíbar en la República de Tanzania) algunas áreas geográficas ya habían interrumpido la AMM después de haber llevado a cabo 5 rondas o más. Sin embargo, la OMS continuaba erróneamente contabilizando la población de dichas áreas como parte del total necesitado de AMM al no haberse declarado oficialmente la interrupción de la transmisión.

A pesar de que PGEFL es uno de los programas de salud global que se ha expandido más rápidamente en la historia de la salud pública, en el año 2011 en África, solo 17 de los 35 países endémicos estaban implementando MDA.

En 2014, de los 73 países considerados endémicos para la filariasis linfática por la OMS, 16 habían completado las rondas indicadas de AMM (al menos 5 alcanzando coberturas terapéuticas) y estaban realizando la vigilancia epidemiológica para validar la eliminación. Otros 23 países habían administrado AMM en todas las áreas endémicas y también estaban en camino de lograr la eliminación. Los países restantes no habian podido alcanzar una cobertura geográfica del 100%. Sin embargo, de los 13 países que todavía no habían comenzado la AMM, 12 de ellos se encontraban en África, mientras que de los 23 que aún no lograron alcanzar el 100% de cobertura geográfica en las campañas de AMM, 16 se encontraban en África. Por lo tanto, el continente africano representa la carga no tratada más grande de FL en el mundo y el impedimento más grande para alcanzar el objetivo de eliminación de la FL para el año 2020.

No está clara cual fue la metodología empleada por la OMS para determinar el número de individuos que requieren QP frente a la FL y si ese número sigue siendo exacto debido al panorama epidemiológico cambiante desde 2000. En la actuallidad se disponen de mejores herramientas epidemiológicas (modelos matemáticos y geoespaciales) y de herramientas diagnósticas más sensibles y específicas para *Wuchereria bancrofti* que en principio no tienen reacción cruzada con antígenos de otras especies de filaria, espacialmente *Loa loa*.
Podemos pensar que ciertos factores pueden haber contribuido a la interrupción, o al menos a la reducción, de la transmisión de la FL en algunos países:

1) el control vectorial (VC) en zonas endémicas de malaria, que se ha intensificado en los últimos años y que ha permitido alcanzar altas coberturas poblaciones con herramientas como las telas mosquiteras impregnadas de insecticida y el rociamiento intradomicilario con insecticidas residuales;
2) en las áreas de transmisión por *Anopheles*, la transmisión urbana puede ser menos frecuente de lo estimado;
3) la QP puede haber interrumpido la transmisión de la FL tras completar 5 o más rondas de MDA.
Todos estos factores pueden haber reducido de manera significativa el número estimado de individuos que requerirían QP frente a la FL.

En este proyecto de tesis, nos hemos preguntado si teniendo en cuenta todo lo anterior es posible obtener una estimación más precisa del número de personas que necesitarían tratamiento para la FL en África y cual es la posición real del continente en la hoja de ruta para alcanzar el logro de la eliminación de FL en el año 2020.

Por medio de una serie de artículos publicados en revistas científicas de impacto internacional y que conforman esta tesis doctoral, he publicado los resultados de la investigación realizada de 2012 a 2016 como respuesta a la pregunta de si las nuevas herramientas disponibles epidemiológicas, de diagnóstico y de modelado podían proporcionar una medición más precisa de la brecha real entre el número de personas que requieren AMM frente a la FL y el número de personas que estarían recibiendo AMM en África.

Hipótesis y Objetivos

Nuevas herramientas de diagnóstico, epidemiológicas y de modelado permitirán obtener estimaciones más precisas del número de personas que requieren quimioterapia preventiva contra la filariasis linfática en África y reducirán el mapa de endemicidad de la filariasis linfática en el continente.

1. La población que requiere quimioterapia preventiva contra la filariasis linfática en países con alta carga de enfermedad, y en los que aún no han comenzado la administración masiva de anti-helmínticos, es menor que la estimación actual de la OMS debido a la baja idoneidad ambiental para la transmisión de enfermedades. Estudio de caso: Etiopía.
2. El control vectorial ha interrumpido la transmisión de la filariasis linfática en las áreas de transmisión por *Anopheles* que aún no han comenzado la administración masiva de anti-helmínticos, en lugares donde la cobertura de las intervenciones de control vectorial ha sido alta. Estudio de caso: Gambia.
3. Cinco o más rondas de tratamiento masivo con anti-helmínticos habría permitido lograr la interrupción de la transmision de la FL en ciertas regiones en Africa. Las personas que viven en estas regiones donde la transmisión se ha interrumpido no requieren más tratamiento y por tanto deben deducirse del número total de personas que requieren quimioterapia preventiva. Estudio de caso: Zanzibar.

Para dar respuesta a las hipótesis formuladas he diseñado un estudio con los siguientes objectivos:

• **Objetivo general:** determinar si los métodos actuales epidemiológicos, de diagnóstico y modelado matemático y geospacial pueden ayudar a reducir el mapa de la transmisión de la filariasis linfática en África y reducir las estimaciones del número de personas que requerirían quimioterapia preventiva.

- **Objetivo Específico 1**: reestimar la población que requiere tratamiento para filariasis linfática en Etiopía.

- **Objetivo específico 2**: demostrar la interrupción de la transmisión de la filariasis linfática en Gambia debido a las actividades de control de vectores para la malaria.

- **Objetivo específico 3**: demostrar la interrupción de la transmisión de la filariasis linfática en Zanzíbar después de haber implementado más de 5 rondas de tratamiento masivo con anti-helmínticos.

Materiales, Métodos y Resultados

Artículo 1

Rebollo, M.P[1,6]., H. Sime[2], A. Assefa[2], J. Cano[3], K. Deribe[4], A. Gonzalez-Escalada[6], O. Shafi[7], G. Davey[4], S. J. Brooker[3], A. Kebede[4], and M. J. Bockarie[1]. 2015. 'Shrinking the Lymphatic Filariasis Map of Ethiopia: Reassessing the Population at Risk through Nationwide Mapping', *PLoS Negl Trop Dis*, 9: e0004172.

Filiacion de los autores:

[1] Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom,

[2] Ethiopian Public Health Institute, Addis Ababa,Ethiopia,

[3] London School of Hygiene & Tropical Medicine, London, United Kingdom,

[4] Brighton and Sussex Medical School, Falmer, Brighton, United Kingdom,

[5] School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia,

[6] Faculty of Infectious and Tropical Diseases, Rey Juan Carlos University, Madrid, Spain,

[7] Federal Ministry of Health, Addis Ababa, Ethiopia

Artículo 2

Rebollo, M. P[1,4,*]., S. M. Sambou[2], B. Thomas[1], N. K. Biritwum[3], M. C. Jaye, L[1]. Kelly-Hope[1], A. G. Escalada[4], D. H. Molyneux[1], and M. J. Bockarie[1]. 2015. 'Elimination of Lymphatic Filariasis in the Gambia', *PLoS Negl Trop Dis*, 9: e0003642.

Filiacion de los autores:

[1] Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom,

[2] Ministry of Health and Social Welfare, Banjul, The Gambia,

[3] Ghana Health Service, Accra, Ghana

[4] Rey Juan Carlos University, Madrid, Spain

* Current address: Neglected Tropical Diseases Support Center, Task Force for Global Health, Decatur, Georgia, United States of America

Artículo 3

Rebollo, M. P[1,5,*]., K. A. Mohammed[2], B. Thomas[1], S. Ame[2], S. M. Ali[3], J. Cano[4], A. G. Escalada[5], and M. J. Bockarie[1]. 2015. 'Cessation of mass drug administration for Lymphatic Filariasis in Zanzibar in 2006: was transmission interrupted?', *PLoS Negl Trop Dis*, 9: e0003669.

Filiación de los autores:

[1] Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom,

[2] Ministry of Health, Zanzibar, United Republic of Tanzania,

[3] Public Health Laboratory-IdC, Chake-chake, Pemba, Zanzibar, Tanzania,

[4] London School of Hygiene and Tropical Medicine, London, United Kingdom,

[5] Rey Juan Carlos University, Madrid, Spain

* Current address: Neglected Tropical Diseases Support Center, Task Force for Global Health, Decatur, Georgia, United States of America

El mapeo de la filariasis linfática (FL) es esencial para delinear las áreas endémicas y determinar la población en riesgo que debe ser tratada mediante administración masiva de medicamentos (AMM). Antes del presente estudio, solo 116 de los 832 woredas (distritos) en Etiopía habían sido mapeados para la FL. El objetivo de este estudio fue completar el mapa de endemicidad de la FL a nivel nacional para determinar el número de personas que deberían ser objeto de AMM en 2016.

En la década de los 50 en Gambia, la prevalencia de infecciones por *Wuchereria bancrofti*, causante de la filariasis linfática (FL), era una de las más altas en África. Sin embargo, las encuestas epidemiológicas realizadas en 1975 y 1976 revelaron una disminución drástica en la endemicidad de la FL a pesar de que nunca se había realizado tratamiento masivo con anti-helminticos. La disminución de la prevalencia se atribuyó en parte a una reducción significativa en la densidad del mosquito a través del uso generalizado de redes mosquiteras. Sobre la base de los hallazgos en otros paises que revelaban que el control vectorial puede interrumpir la transmission de FL, en 2013 establecimos la hipótesis de que el incremento rápido en el uso de redes mosquiteras contra la malaria había logrado la interrumpción de la transmisión de la FL en Gambia.

La Organización mundial de la salud ha hecho un llamamiento a la eliminación de la filariasis linfática (FL) a través de la administración anual masiva de medicamentos (AMM). En 2006, Zanzibar detuvo la AMM frente a la FL después de cinco rondas de tratamiento, y tras que encuestas llevadas a cabo en sitios centinela revelaran que no había personas con microfilaremia. La pregunta que trato de responder en este proyecto de investigación es si la transmisión de FL se interrumpió realmente en 2006 cuando se detuvo la AMM.

Se realizo una encuesta transversal basada en un muestreo intencional por conglomerados en dos etapas (woreda y comunidades), para un mapeo integrado de FL y podoconiosis, en siete estados regionales y dos administraciones de la ciudad de Addis Abeba. En cada woreda o distrito se seleccionaron dos comunidades utilizando la estrategia de mapeo de la Organización Mundial de la Salud (OMS) para la FL basada en el muestreo de 100 individuos por comunidad y dos comunidades seleccionadas a propósito por woreda deacuerrdo a criterios clínicos de sospecha de presencia de la enfermedad (casos de elefantiasis o hydrocele). En total, 130 166 personas

fueron examinadas en 1315 comunidades en 658 woredas. En total, 140 personas resultaron positivas para la circulación de antígenos FL mediante test rápido inmunocromatográfico (ICT) en 89 comunidades. Según las directrices de la OMS, 75 de las 658 woredas encuestadas en las nueve regiones resultaron ser endémicas para FL con una población proyectada para 2016 de 9 267 410 residentes en áreas de transmisión activa de FL. Combinando estos resultados con otros datos, se estima que 11 580 010 personas en 112 woredas estarán expuestas a la infección en 2016 y por lo tanto requerían AMM. En línea con los estudios de esta tesis doctoral y con el objetivo de reducir el mapa FL, en enero de 2012 realice encuestas de evaluación de la transmisión siguiendo la metodología recomendada por la OMS (método llamado TAS por sus siglas en ingles) para verificar la ausencia de transmisión FL en Gambia y en las principales islas de Zanzíbar: Unguja y Pemba. En Zanzibar, 3275 niños fueron evaluados yen Gambia 3180. En Zanzibar 89 resultaron ser positivos para antígenos circulantes de *Wuchereria bancrofti*; 70 en Pemba y 19 en Unguja. En Gambia no se encontró ningún positivo. Con un total de 89 niños positivos en Zanzibar el umbral de transmisio'n ha sido sobrepasado demostrándose que la transmisión de la FL sigue siendo activa. Sin embargo en Gambia demostramos que la transmisión de *W. bancrofti* se ha interrumpido en todos los 21 distritos del país.

Mi estudio consiste en el mapeo nacional de FL en Etiopía y demuestra que el número de personas que viven en áreas endémicas de FL es un 60% más bajo que las estimaciones actuales manejadas por la OMS.

La transmisión de FL ha sido interrumpido en Gambia a través del uso extensivo durante décadas de redes mosquiteras para el control de la malaria. La creciente evidencia del impacto de las actividades de control del vector de la malaria en la transmisión de parásitos ha sido respaldada por la OMS a través de una declaración de posición en 2011 sobre el manejo integrado de vectores para controlar la malaria y la FL.

Mis estudios indicaron la transmisión de FL en Zanzíbar en 2012. Además, presento evidencia de estudios previos de que la transmisión de FL también era activa en la isla de Unguja poco

después de suspender AMM en 2006. Basándose en los resultados de este estudio, el gobierno de Zanzíbar decidió reanudar AMM contra FL en ambas islas en 2013.

Discusión

Lograr la eliminación de una enfermedad requiere una serie de pasos epidemiológicos así como de intervenciones de salud pública. El primer paso es comprender dónde está presente la enfermedad (distribución geográfica de la enfermedad o "mapeo") utilizando herramientas de diagnóstico sensibles y específicas y métodos epidemiológicos sólidos. El correcto diagnóstico e identificación de las áreas endémicas donde la transmisión es activa permite rentabilizar los recursos mediante la dirección de las intervenciones sobre dichas áreas.

Desde el lanzamiento del PGEFL, muchos países endémicos para FL de África se han embarcado en Programas Nacionales para la Eliminación de FL mediante AMM anual con dosis única de DEC o ivermectina, más albendazol, con el objetivo de disminuir la microfilaremia circulante hasta que se alcance un umbral de prevalencia por debajo del cual se considera que la transmisión no es posible, lográndose así la extinción natural de la enfermedad. A pesar de que la administración anual de AMM es más económica que la mayoría de las intervenciones de salud pública, requiere recursos significativos. El coste financiero del tratamiento por persona por año se ha estimado en 0.48 USD promedio mientras que el costo económico global para tratar a un individuo, incluyendo costes de logística, ascendería a 4.98 USD por persona tratada por año. Por lo tanto, a pesar del compromiso de las organizaciones sin ánimo de lucro, las compañías farmacéuticas y la OMS, en el año 2010 solo 6 países de África habían podido implementar la AMM en el 100% de las áreas endémicas.

La identificación de áreas donde existe transmisión activa de la FL permitirá orientar los escasos recursos hacia el tratamiento de aquellos que viven en áreas donde existe transmisión de la enfermedad y evitar el gasto innecesario de tratamiento en áreas donde no existe trasmisión activa. El uso de nuevas técnicas de diagnóstico y herramientas de evaluación epidemiológica más precisas está permitiendo reducir la extensión geográfica del mapa de FL en África, demostrando que el continente puede haber estado siempre más cerca del objetivo de eliminación de lo esperado debido, entre otros factores, a la baja idoneidad ambiental para la FL en muchos países, las actividades de control frente al vector de la malaria, común para la FL, y la sobreestimación de la carga de FL en algunos países debido a la reactividad cruzada de los antígenos de *Loa loa* en las pruebas diagnósticas rápidas usadas con anterioridad.

El PGEFL no es un programa estático, a medida que nuevos conocimientos y nuevas herramientas se vuelven disponibles, es crucial mapear y volver a estimar la carga de FL y redefinir dónde deben enfocarse los recursos para lograr la eliminación.

Existen nuevas y mejoradas herramientas de diagnóstico rápido fundamentales para alcanzar el objetivo de reducir el mapa de FL a cero países endémicos para 2020. Estas herramientas de diagnóstico rápido son esenciales para los procesos de mapeo y evaluación de impacto. La información obtenida en el mapeo y evaluación de impacto se utiliza para determinar donde iniciar tratamientos contra la FL y cuándo se puede detener la MDA después de la interrupción de la transmisión.

Cuando se inició este proyecto en 2013, 13 de los 73 países donde se sabía que FL eran endémica no habían determinado la distribución geográfica de la enfermedad en sus territorios. En África, aproximadamente la mitad (48.5%) de las 464 millones de personas expuestas a FL residía en los cuatro países con mayor carga de enfermedad por FL: la República Democrática del Congo (RDC) (49 millones), Etiopía (30 millones), Nigeria (109 millones) y Tanzania (45 millones). Con excepción de Tanzania, ningún de los otros países en África habían completado la cartografía para FL en el año 2013. Gambia y Gabón todavía se consideraban endémicos para FL. No estaba claro si la transmisión FL era activa en las grandes ciudades urbanas de África occidental. Así mismo, la enfermedad estaba volviendo a surgir en Zanzíbar en la República Unida de Tanzania. En Etiopía, solo 112 de los 817 distritos del país habían sido mapeados hasta la fecha para la enfermedad.

Este capítulo de discusión es una presentación contextualizada de 3 artículos que se publicaron como resultado del estudio de doctorado. En ellos se presentan los resultados y conclusiones de la investigación dirigida a reducir el mapa de la FL en África y producir una estimación más adecuada del número de personas que requieren quimioterapia preventiva. Esto también dará como resultado la aceleración del progreso hacia el logro de los objetivos de 2020 de la eliminación de las FL dirigiendo el tratamiento exclusivamente a personas que viven en distritos donde la transmisión de FL es activa.

En esta tesis he demostrado que la población en riesgo de FL en África es inferior a la estimada en Etiopía (de 30 a 5.8 millones de personas), Gambia (de 1.2 a 0 millones de personas),

mientras que en el caso de Zanzíbar la transmisión de FL es todavía activa, al contrario de lo que pensaba en Ministerio de Sanidad de Zanzíbar el cual había detenido la AMM de forma prematura. Por lo tanto el número de personas en riesgo pasaría de 0 a 1.2 millones de personas en esta región insular de Tanzania.

	País	***Etiopía***	***Gambia***	***Zanzíbar***
Año 2012	**Población total**	68,445,633	1,412,054	1,027,674
	Zonas endémicas	30,000,000	1,412,054	1,027,674
	Zonas no endémicas	38,445,633	0	0
Año 2018	**Población total**	79,722,590	1,662,686	1,235,919
	Zonas endémicas	5,893,309	0	1,235,919
	Vigilancia Post eliminación	0	1,662,686	0
	Zonas no endémicas	73,829,281	0	0

En el listado de países que la OMS publicó en el 2014 se enumeran 25 países africanos que no estaban en la trayectoria correcta para poder alcanzar la meta de 2020, ya que no habían iniciado la AMM o no habían alcanzado una cobertura geográfica del 100%. Volver a estimar las áreas endémicas de FL en esos países utilizando las herramientas descritas en esta tesis puede ayudar a tener una población diana más realista e identificar las áreas y países que realmente están atrasados de cara a intensificar los esfuerzos y acelerar la eliminación para 2020.

Conclusiones

- La población en riesgo para la filariasis linfática en Etiopía, determinada a través de este estudio, es 60% más baja que las estimaciones de la OMS para Etiopía antes de este proyecto que ascendían a 30 millones de personas.
- 112 distritos en todo el país son endémicos de la enfermedad. La población que requiere tratamiento en estos distritos endémicos es de 11 580 010.

- Las encuestas de evaluación de la transmisión realizadas en Gambia demuestran la falta de transmisión activa de la filariasis linfática en todo el país debido a las medidas de control vectorial implementadas frente a la malaria, que comparte las mismas especies vectoras que la filariasis linfática.
- Gambia no tiene transmisión activa de filariasis linfática y 1.2 millones de personas han sido declaradas libres del riesgo de contraer la enfermedad.
- Aunque la administración masiva de anti-helmínticos (AMM) frente a la filariasis linfática se detuvo en 2006 y la transmisión se declaró interrumpida, las encuestas de evaluación de la transmisión llevadas a cabo en Zanzíbar mostraron que cinco rondas de AMM en las islas no lograron interrumpir la transmisión de la filariasis linfática.
- La población de Zanzíbar, 1 235 919 personas, ha sido declarada en riesgo de ser infectadas por el parásito de la filariasis linfática y por tanto todavía requieren quimioterapia preventiva.

Introduction

Introduction

Epidemiology of Lymphatic Filariasis

Lymphatic Filariasis (LF) is mosquito-borne infection responsible for acute and chronic morbidity in humans in tropical and subtropical areas of Asia, Africa, the Western Pacific and some parts of the Americas (1).

Over 20% of the world's population lives in areas where they are at risk of infection with filarial parasites (Figure 1). The regions of the world bearing most of the burden of Lymphatic Filariasis include South and Southeast Asia, Africa, the Western Pacific, the East Mediterranean and some Central and South American countries (2, 3).

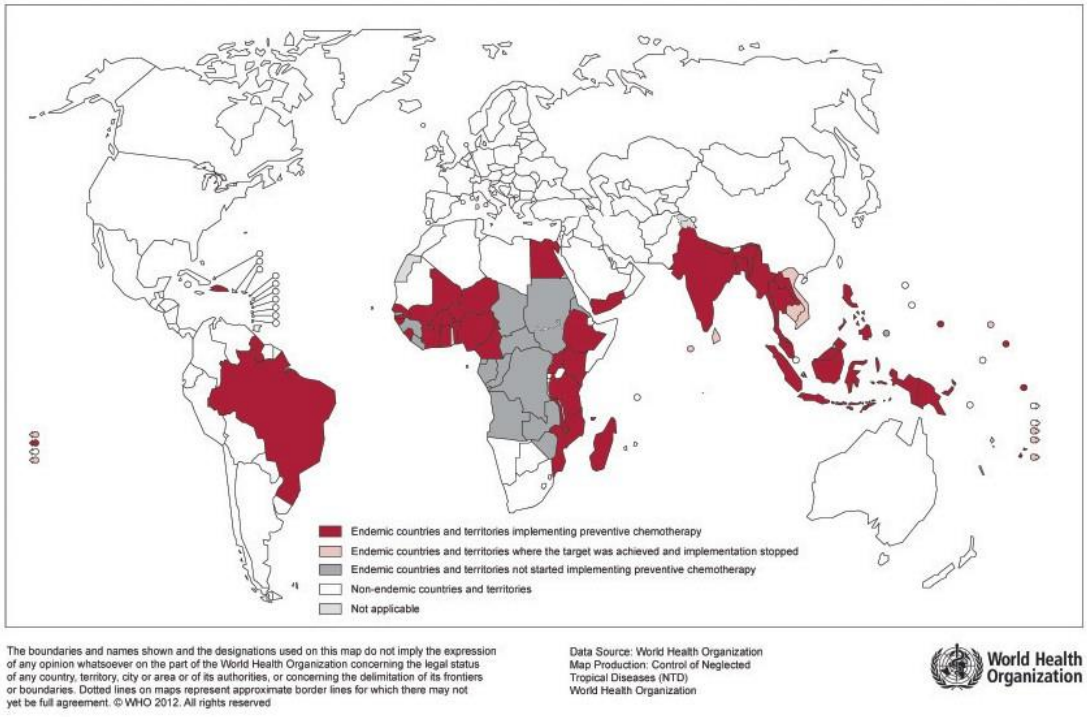


FIGURE 1. DISTRIBUTION AND STATUS OF PREVENTIVE CHEMOTHERAPY (PC) FOR LYMPHATIC FILARIASIS WORLDWIDE, 2011. WHO

A recent environmental risk map (3), developed using boosted regression tree modelling, shows that the environmental conditions suitable for LF transmission occurs throughout the forest and savannah regions of west Africa, coastal east Africa and Madagascar and restricted foci in central and southern Africa (Figure 2). Suitable environmental conditions also occur across tropical areas of south and southeast Asia and the Pacific as well as large areas of Central and South America, including the southern States of America, where the disease was eliminated at the beginning of 20th century (4). Predictions of environmental suitability in temperate regions are consistent with documented historical distributions prior to large scale intervention and local elimination in Japan, South Korea, and southern China (5-9), the north coast of Australia (10) and southeastern coast of United States (4).

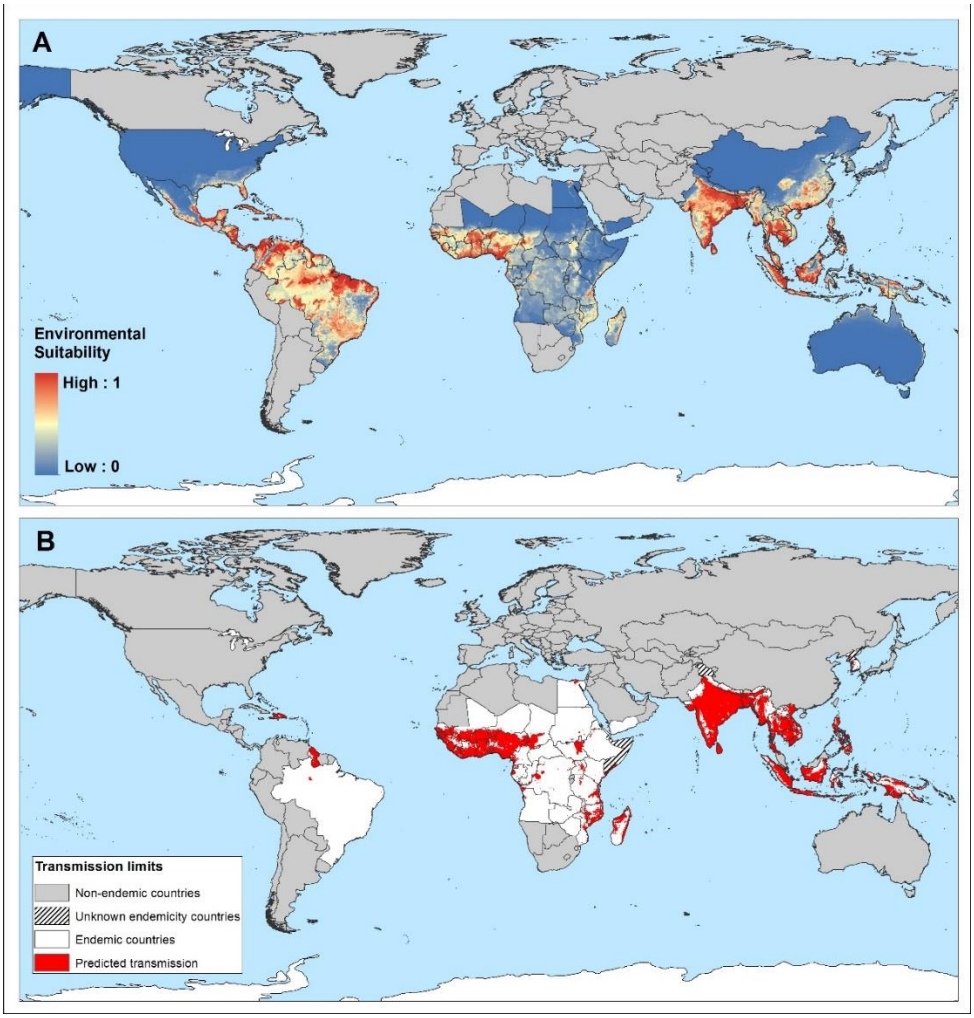


Figure 2.GLOBAL ENVIRONMENTAL SUITABILITY (A) AND LYMPHATIC LIMITS (B) OF FILARIASIS TRANSMISSION AS PREDICTED BY MACHINE LEARNING BASED MODELLING APPROACHES (CANO & REBOLLO, 2014) (3)

Presently, over 120 million people living in 80 endemic countries, 91% are caused by *Wuchereria bancrofti* while *Brugia malayi* and *Brugia timori* infections account for the other 9% (11-13). *B. timori* is only known to be endemic in Timor and the Flores islands of the Indonesian archipelago (14). The microfilariae in the lymphatic system can cause lymphoedema, genital pathology (especially hydrocoeles) and elephantiasis in some 41 million men, women and children (13). A further 76 million have hidden infection, most often with microfilariae in their blood and hidden internal damage to their lymphatic and renal systems. The principal vector that transmit LF to humans include species of the genera *Anopheles, Aedes, Culex, Mansonia* and *Ochlerotatus* mosquitoes.

TABLE 1. PERIODICITY AND DISTRIBUTION OF ORGANISMS THAT CAUSE HUMAN LYMPHATIC FILARIASIS.

Source: www.dpd.cdc.gov/dpdx

Organism	**Periodicity**	**Distribution**	**Main vector**
Wuchereria bancrofti	Nocturnal periodic	Worldwide, including Africa, Indonesia, Melanesia, Micronesia, Middle East, South America, South Asia	*Anopheles, Culex*
	Nocturnal sub-periodic		*Aedes*
	Diurnal sub-periodic	South-East Asia Polynesia	*Aedes*
Brugia malayi	Nocturnal periodic	India, Indonesia, South-East Asia	*Anopheles, Mansonia*
	Nocturnal sub-periodic	Indonesia, South-East Asia	*Mansonia*
	Diurnal sub-periodic	Thailand	*Mansonia*
Brugia timori	Nocturnal periodic	Alor, Flores, Indonesia, Roti, Timor	*Anopheles*

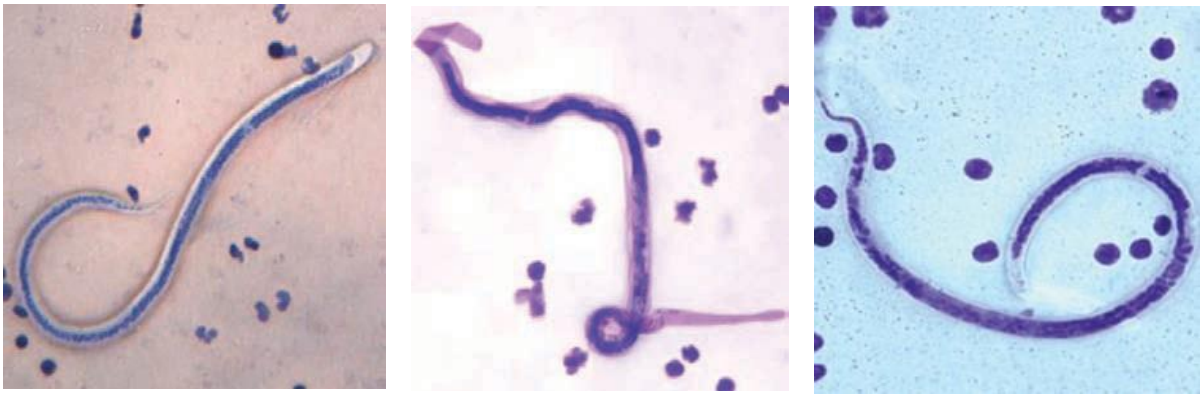


FIGURE 3. IMAGES OF MICROFILARIAE OF THREE FILARIAL WORMS IN BLOOD FILMS STAINED WITH GIEMSA, WUCHERERIA BANCROFTI (LEFT), BRUGIA MALAYI (CENTRE) AND B. TIMORI (RIGHT)

Biology and life cycle of LF parasites

The life cycles of these parasites in humans consist of adult worms living in the afferent lymphatic vessels while their progeny, the microfilariae, circulate in the peripheral blood.
The life cycle includes an obligatory maturation stage in a blood-sucking insect and a reproductive stage in the tissues or blood of a definitive mammal host (biphasic life cycle). *Wuchereria bancrofti* seems to infect exclusively humans (Figure 4), whereas *Brugia* spp (Figure 5) are zoonotic in many situations, being able to infect cats and dogs predominantly in human peri-domestic environment (15-17).

Adult male and female LF worms live in the lymphatic vessels (Figure 3). Microfilariae (which are specialized embryos not larvae) are produced by the female worm, circulate in the blood or invade the skin, and are ingested by the vector (Figure 4 & 5). Larval development but not multiplication occurs within the muscles of the vector. Only a portion of the microfilaria will make its way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles. The infective L3 stage migrates to the proboscis and is transmitted to the new host during feeding (18). The infective stage (L3) is deposited onto the skin during a blood meal, while the mosquito is feeding. Through the open puncture parasite penetrates the skin into the new host. Once the third-stage filarial larvae penetrates onto the human skin, they transform into the fourth larval stage (L4). The L4 larvae migrate through the subcutaneous tissues to the lymphatic system and develop into female and male adult worms residing in the lumen of the lymphatics vessels. The adult worm develop inside the human body

and live in the lymphatic system for an average time of 4-6 years. The adult worms measure 80 to 100 mm in length for a female worm and 0.24 to 0.30 mm in diameter, and about 40 mm by .1 mm for the male. The lymphatic-dwelling filariae are dioecious and undergo ovoviviparous reproduction resulting in the release of microfilariae (L1) from adult females. The microfilariae produced by the adult worm measure between 244 and 296 μm by 7.5 to 10 μm. During their lifetime, adult filarial worms reproduce and release millions of microfilariae in to the peripheral blood.

Microfilariae typically shows nocturnal periodicity, however the South Pacific microfilariae has been described as having absence of periodicity. While the adult worm reside in the lymphatic system, the microfilariae moves between lymph and blood. The females release thousands of microfilariae daily into the peripheral blood circulation where they become available to female mosquitoes to be ingested during a blood meal.

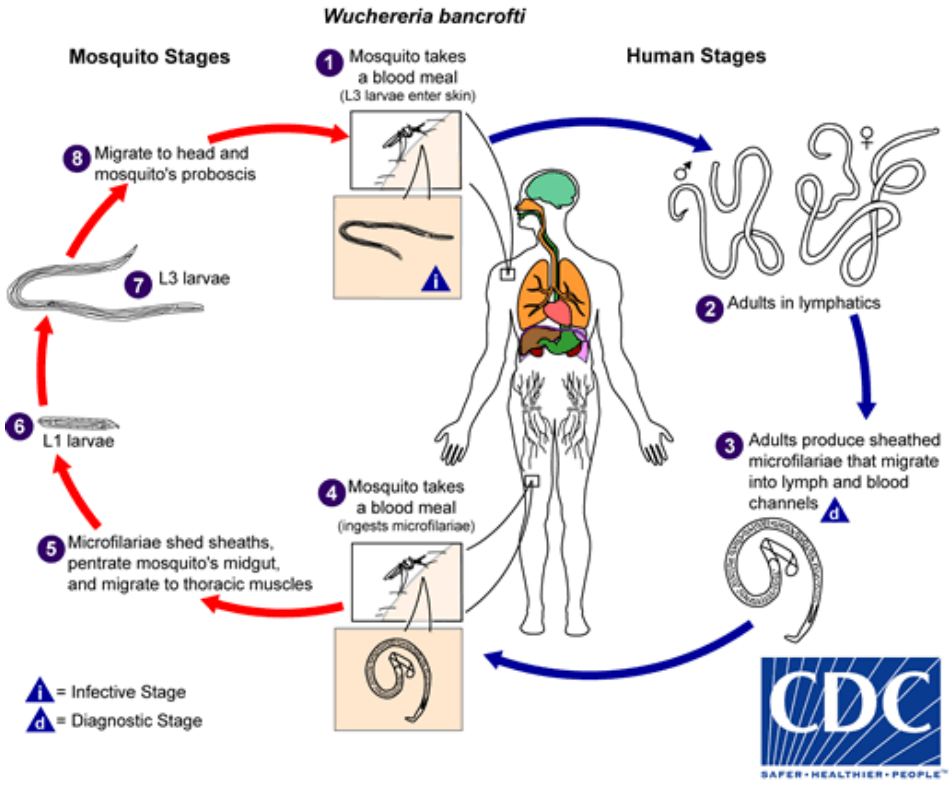


FIGURE 4. LIFE-CYCLE OF WUCHERERIA BANCROFTI

Source: http://www.dpd.cdc.gov/dp

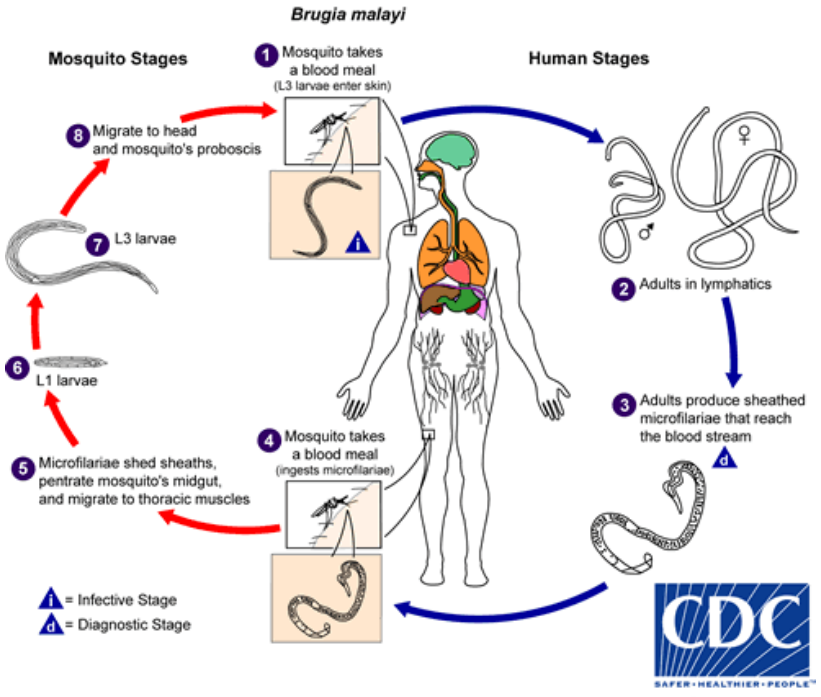


FIGURE 5. LIFE-CYCLE OF BRUGIA SPP.

Source: http://www.dpd.cdc.gov/dp

History of Lymphatic Filariasis

The discovery by Patrick Manson in India in 1877, that mosquitoes act as vectors of Lymphatic Filariasis was the first time an insect was associated with the transmission of a parasite of human disease (19). This gave rise to renewed hopes about a new, possibly easy, way of eradicating the mosquito-borne diseases by exterminating the vectors.

Vector control is particularly attractive for Lymphatic Filariasis. Unlike malaria, mosquitoes are very inefficient carriers of the filaria parasite for several reasons. First, parasites ingested by the mosquito does not multiply inside the insect. Second, infection is only maintained through continuous exposure to infectious mosquitoes. Hairston and Meillon (1968) investigated the transmission dynamics of Lymphatic Filariasis in Rangoon, Burma, and determined that approximately 15 500 infective bites of *Culex quinquefasciatus* were needed to produce one new infected person (20).

Wuchereria bancrofti

The history of the discovery of LF has been described in detail by Sasa in 1976 (14). In 1862 Jean-Nicholas Demarquay, a Cuban surgeon, discovered a worm-like "creature" in hydrocele fluid(14). In 1866 Brazilian, Otto Wucherer, found the same organism in chylous urine (21) but it was not until 1872 that microfilariae were discovered in blood (21).

1876, Brisbane physician and parasitologist, Joseph Bancroft, discovered a female adult worm in an abscess on the arm of a butcher (14) and further examples were found in South America and China (14). The name Wuchereria bancrofti was formally adopted in 1921 (14).

***Brugia* Species**

In 1927 Lichtenstein and Brug discovered a microfilaria in the Dutch East Indies (now Indonesia) which was morphologically different from *W. bancrofti* and called it *Filaria malayi* (Brug, 1927; Lichtenstein, 1927; Brug, 1928; Brug and de Rook, 1930).

This in fact may not have been the first description of Brugia. In 1905 Ashburn and Craig described a case from the Philippines, which had microfilariae that differed from *W. bancrofti*, which they called "*Filaria philippinesis*" (Ashburn and Craig, 1905). Their material was reviewed by Manson-Bahr in 1941, who believed that the microfilariae were identical to Ashburn and Craig's *F. malayi*. Rao and Maplestone first described the adult worm in 1940 (22). The pioneering work of Brug was acknowledged in 1958 when Buckley proposed the new genus *Brugia* and *F. malayi* was re-named *Brugia malayi* (23). The zoonotic feature of *Brugia* was discovered in 1939 when microfilariae, later identified as those of *B. malayi* were discovered in a Kra monkey (24). Edeson and Wharton (1958) (25) and Laing et al. (1960) (26) broadened the range of vertebrate host to include other monkey species, domestic cats and dogs.

In the 1960's another filarial species was discovered in Portuguese Timor and given the name *Microfilaria timori* (27). Partono (1977) infected Mongolian gerbils with the new species and obtained adult worms. He confirmed that the new species belonged to the genus *Brugia* and called it *B. timori* (28).

Mosquito vectors

Both *W. bancrofti* and *B. malayi* are unique among the various mosquito-transmitted parasites because the LF parasite is able to develop in five different genera of the mosquito. White (1989) (29) lists over 80 different species of mosquitoes that have been implicated in LF transmission.

Three main zones of transmission are recognized: the South Pacific islands and some limited areas of South East Asia, where *Aedes* vectors predominate; West Africa, Papua New Guinea, Vanuatu and Solomon islands where *Anopheles* mosquitoes are principal vectors; China, South East Asia, Egypt, East Africa, the Caribbean and Latin America where the infection is transmitted mainly by *Culex* species. The taxonomic group of mosquitoes involved in filariasis transmission may determine the effectiveness of any control strategy aimed at interrupting transmission.

W. bancrofti filariasis has different vectors depending on the geographical distribution. The main vectors are *Culex, Anopheles, Aedes, Mansonia* and *Coquillettidi*a. The main species are the following:

- *Culex (C. quinquefasciatus, C. bitaeniorhynchus, C. annulirostris,* and *C. pipiens);*
- *Anopheles (A. arabinensis, A. bancroftii, A. farauti, A. funestus, A. gambiae, A. koliensis, A. melas, A. merus, A. punctulatus* and *A. wellcomei*);
- *Aedes (A. aegypti, A. aquasalis, A. bellator, A. cooki, A. darlingi, A. kochi, A. polynesiensis, A. pseudoscutellaris, A. rotumae, A. scapularis,* and *A. vigilax);*
- *Mansonia (M. pseudotitillans, M. uniformis);*
- *Coquillettidia (C. juxtamansonia).*

Culex mosquitoes

Mosquitoes of the *Culex pipens* complex, especially *Cx. quinquefasciatus,* are urban vectors of nocturnally periodic *W. bancrofti* in Asia, Africa the West Indies, South America and Micronesia. In Egypt, the biotype *molestus* of *Cx. pipiens* is involved (30) whereas *Culex pallens* is the vector of *W. bancrofti* in the sub-tropical zone of China (31) and Japan (32).

Culex annulirostris transmits *W. bancrofti* in some coastal parts New Guinea (33), where the main vectors are members of *Anopheles punctulatus* group (29).

Cx. quinquefasciatus breeds in a wide variety of stagnant water habitats; water barrels, wells, tanks, privies, fresh pools, ponds, provided the water has been sufficiently polluted, also in pools and canals near houses. It breeds most abundantly in polluted water in domesticated situations such as pools, drains and septic tanks (34). Distribution of *C. quinquefasciatus* is increasing with urbanization and human activity and many rural pockets that were relatively free of this mosquito are becoming colonized (35).

Cx. quinquefasciatus is mainly a night biting mosquito although it has occasionally been found to bite freely in darkened rooms during the daytime. It bites both indoors and outdoors with peak activity at 22:00-23:00 hours. Although humans are usually emphasized as the primary source for blood meals, when both humans and poultry are available poultry are more heavily attacked than humans (36, 37). However, blood-meal assays performed on indoor resting catches usually show a very high percentage of positives for humans in comparison to animals and birds (38). In the South Pacific, *Cx. quinquefasciatus* is widespread but very restricted locally, since it has not been able to establish itself in the wild and appears to depend on its association with human beings for breeding places (39).

Cx. quinquefasciatus from Guinea (40), Liberia (41) and Senegal (42) have proven to not be susceptible to infection by local strains of *W. bancrofti*, although West African *Cx. quinquefasciatus* are susceptible to *W. bancrofti* from India (41), Sri Lanka (43) and Tanzania (44). McCaroll *et al.* (45) reported evidence from Sri Lanka that elevated levels of esterases associated with organophosphate resistance in *Cx quinquefasciatus* appear to reduce filaria infection rates. However, Curtis (46) pointed out that Tanzanian *Cx. quinquefasciatus* with high levels of elevated esterases are highly susceptible to development of *W. bancrofti* to the L3 stage in experimental feedings (44).

Aedes mosquitoes

Aedes mosquitoes are involved in the transmission of *W. bancrofti* and *B. malayi* in South Asia and the Pacific regions. Chow (47) lists 15 species of *Aedes* as vectors of Lymphatic Filariasis. The diurnal subperiodic form of *W. bancrofti* occurs only in the South pacific region, where the most important vector is *Ae. polynensiensis*. Other important *Aedes* vectors are *Ae. niveus, Ae. poecilus, Ae. samoanus, Ae. togoi* and the *Ae. scutellaris* group. *Aedes polynesiensis* is the most important vector of the subperiodic form of *W. bancrofti* in the Polynesian region wherever it occurs (48). It breeds in artificial and natural containers of rainwater, such as coconut shells, fallen coconut leaf bracts, discarded tins, old automobile tires, drums etc., as well as tree holes, canoes and crab holes made in sandy beaches. It has also been found breeding in the leaf axils of Pandanus. The females are exophilic, day-biting voracious mosquitoes which feed mainly on humans. It is exophagic and exophilic, feeding mainly in the open and resting in shaded vegetation and crab holes. It sometimes enters houses to bite but prefers to rest out of doors (49).

Aedes vigilax is a vector in New Caledonia and the Loyalty Islands (50), where *Ae. polynesiensis* is totally absent. It is an exophilic, day-biting species which breeds in brackish water, but also occurs in rock holes and fresh water pools. It rests outdoors during the day in grass and low vegetation. *Aedes niveus* is the vector of noctunal subperiodic *W. bancrofti* in West Thailand. It has also been shown to be the secondary vector of nocturnally periodic *W. bancrofti* in Mountain Province of Luzon, Philippines (51, 52). *Aedes poecilus* is an important vector of nocturnal periodic *W. bancrofti* in the abaca growing areas of the Philippines (53). It is an endophagic and exophilic species that bites mostly before midnight and occasionally may bite outdoors in the day. It breeds in the axils of the Abaca (*Musa textilis*) and banana. *Aedes samoanus* transmits subperiodic *W. bancrofti* in American Samoa and Western Samoa (54). It is endophagic and exophilic; females bite mainly at night with a peak during the third quarter of the night. It breeds mainly in leaf axils of *Freycinetia*. *Aedes kasseli*, a member of the *Aedes scutellaris* group has been incriminated as a vector of subperiodic *W. bancrofti* in the Tonga archipelago (55).

Ochlerotatus togoi (formally known as *Aedes togoi)* is a vector of nocturnally periodic *B. malayi* in China (31), Japan (32), and Korea (56). It is endophilic and bites mainly during the day with a

peak shortly after sunset. It breeds in rock holes with brackish water as well as in artificial containers with rainwater.

Mansonia mosquitoes

The main vectors of Brugian filariasis are six species of *Mansonia*. The nocturnal subperiodic filarial parasite is only known to occur in Brunei, Malaysia and Philippines, where it is transmitted mainly by *M. annulata*, *M. bonneae, M. dives* and *M. uniformis*. *Mansonia annulifera* and *M. indiana* are minor vectors in Malaysia (57). *Mansonia annulata* is also a vector of periodic *B. malayi* in Indonesia and Thailand (54). It breeds at the edge of swamp-forest and larvae attach to the roots of certain trees and grasses. It is exophagic and exophilic. Biting occurs during the day with peak activity soon after sunset. The breeding sites, feeding and resting habits of *M. bonneae* are similar to *M. annilifera*. *Mansonia dives* has an ecology similar to *M. annulata* and *M. bonneae* but it distributes more widely in West Malaysia where it has been identified as a vector of the periodic *B. malayi* (58). Chang *et al*. (59) estimated the productivity of *M. bonneae* and *M. dives* females to be 1.6 million per hectare per annum in swamp forest habitats vegetated with any of the host-plants studied.

Mansonia indiana and *M. uniformis* have similar ecology. They breed mainly in open swamps and tanks, particularly associated with water lettuce (*Pistia statiotes*) and water hyacinths (*Eichornia crassipes*). They are predominatly zoophilic and exophagic but readily enter houses to feed on humans. The peak biting activity is during the early hours of the night. *Mansonia uniformis* is the most widely distributed species of the *Mansonia* mosquitoes. It is a vector of *periodic B. malayi* in Sri Lanka, India and Thailand (54).

Anopheles mosquitoes

In many rural areas, the vectors of Lymphatic Filariasis are *Anopheles* mosquitoes, usually the same species that also transmit malaria. Nelson (60) lists 26 *Anopheles* species as vectors of bancroftian and brugian filariasis. Eighteen species are vectors of *W. bancrofti*, three of *B. malayi* and five species transmit both parasites. Recent transmission and distribution records

include *An. gambiae* s.l from the island of Grande Comore, and *An. flavirostris* from Sabah, Malaysia. *Anopheles barbirostris* is the only known vector of *B. timori* (61).

In Africa, where no *Brugia* parasites of humans occur, the most important vectors of *W. bancrofti* are *Anopheles funestus* and members of the *Anopheles gambiae* complex — including the freshwater breeding *An. gambiae s.s.* and *An. arabiensis* as well as *An. melas* and *An. merus* which breed mainly in saltwater. The ecology and behaviour of the *An. gambiae* complex have been reviewed by (62). *Anopheles nili* and *Anopheles hancocki* are minor vectors in Liberia, West Africa (55).

Anopheles vectors of *W. bancrofti* in Asia include *Anopheles jeyporiensis candidiensis* and *Anopheles minimus* in China; *An. flavirostris* in Philippines and *Anopheles balabacensis*, *Anopheles maculatus*, *Anopheles letifer* and *Anopheles whartoni* in Malaysia. *Anopheles candidiensis* breeds in slow running streams, irrigation channels and ditches, with grass edges. It is anthropophagic and endophelic with peak biting after midnight. The breeding habitats and feeding behaviour of *An. minimus* are similar to *An. candidiensis.* The breeding places of *An. flavirostris* are similar to *An. minimus.* However, *An. flavirostris* is a highly exophilic and zoophilic species. *An. balabacensis* is highly anthropophagic; it breeds in seepage pools along the jungle streams and temporary pools in the jungles along the foothills. *An. maculatus* is an exophagic species that breeds in streams, drains and seepages. *An. letifer* breeds mainly in flat areas, usually under the shade. It is exophilic and bites both indoors and out of doors. The bionomics of *An. whartoni* resembles that of *An. letifer*.

The *Anopheles punctulatus* group of mosquitoes, including *An. punctulatus, An. koliensis* and *An farauti*, are the principal vectors of periodic *W. bancrofti* in Papua New Guinea, Irian Jaya (Indonesia), Solomon Islands and Vanuatu (63-65). *Anopheles farauti* has a wider distribution in the South Pacific than *An. punctulatus* and *An. koliensis*; it occurs mainly in the coastal areas. It can breed in fresh or brackish water and permanent swamps or temporary pools. *Anopheles koliensis* occurs mainly in the subcoastal areas. It generally breeds in temporary pools, in grasslands and in pools around the edges of jungles. *Anopheles punctulatus* prefers breeding in

sun-lit water, road ruts and drains. Charlwood *et. al*, (66) have written a comprehensive review of the ecology and behaviour of the *An. punctulatus* group.

Anopheline vectors of periodic *B. malayi* include *Anopheles campestris* and *Anopheles donaldi* in Malaysia and *Anopheles lesteri* and *Anopheles sinensis* in China. *An. campestris* is anthropophagic and endophagic (67). It breeds in only coastal plains. *An. donaldi*, on the other hand, breeds in almost any collection of ground water. It feeds on human inside houses, but it is mainly a zoophilic and exophagic species (68). *An. sinensis* breeds in a variety of water collections, mainly rice fields. It is zoophilic and prefers to rest in cowsheds (69).

Vector-parasite relationships

Understanding the quantitative aspects of transmission of filarial parasites by mosquitoes is essential for the rational planning of control measures. A very important determinant of transmission efficiency is the relationship between parasite yield, the success rate of ingested microfilariae becoming infective L3 larvae in the mosquito vector, and density of microfilaraemia (Mf) in the human host (70). For filariasis transmission to be interrupted, vector density or microfilaria intensity needs to be driven below a threshold that ensures no new infection occurs (71). Two types of vector-parasite relationships, *limitation* and *facilitation*, have been shown to be epidemiologically important. The relevance of these different relationships to filariasis elimination lies in the predicted importance of low-density Mf in sustaining transmission in different epidemiological settings. There are indications that for a given vector density low levels of Mf can initiate a resumption of transmission after efficient control where culicine mosquitoes are vectors, whereas interruption can be sustained in areas of anopheline transmission (72).

Limitation, associated with culicine transmitted filariasis, occurs when the proportion of ingested microfilariae which survive to become L3 larvae decreases as more microfilariae are ingested. In the case of *facilitation*, which is associated with *Anopheles* mosquitoes, L3 yield increases as the number of microfilariae increases from very low up to some intermediate number, declining at higher microfilarial densities**.** Of less epidemiological value is a third relationship called

proportionality — when the parasite yield is a constant ratio, neither increasing nor decreasing as microfilaria intake increases.

The concept of limitation and facilitation arose from experimental studies (73-76). However, later mathematical analysis (77-82) showed that the limitation system has only one positive stable equilibrium (E). There will be a threshold, for interruption of transmission, which depends on vector density, but there is no threshold that depends on parasite density (83). *Facilitation* on the other hand could lead to an unstable breakpoint at some positive microfilarial density (C). When microfilarial density is higher than the breakpoint, the system tends towards the higher of the two stable equilibria (E).

In *facilitation* relationship, the lower point below which transmission will be interrupted can be achieved either by reducing density of parasites or density of mosquitoes. Brengues & Bain (74) demonstrated facilitation with *An. gambiae* and *W. bancrofti* in Burkina Faso. Possible examples of *facilitation* related to reduction in vector densities have been found during malaria control programmes in Solomon Islands (84-86) and Togo (87, 88). In both situations vector control operations of insufficient efficiency to interrupt transmission of malaria led to the elimination of bancroftian filariasis transmitted by *Anopheles* vectors. Bockarie *et al.* (89), working in an area of intense perennial transmission of *W. bancrofti* by *An. punctulatus* in the East Sepik Province of Papua New Guinea attributed failure to detect infective mosquitoes for many months, following mass treatment with DEC (diethylcarbamazine) in combination with ivermectin, to the phenomenon of *facilitation*. *Brugia malayi* transmitted by *An. sinensis* was virtually eradicated in at least two instances in China following repeated mass treatments with DEC (90, 91). *Limitation* has been shown to occur in *Cx. quinquefasciatus* with strains of *W. bancrofti* in Tahiti (79, 80, 82, 92) and Tanzania (80, 93). The practical implications of *facilitation* and *limitation* have been discussed in detail by Bryan & Southgate (94-96), Bryan *et al* (94) and Southgate & Bryan (97).

Disease: clinical manifestations

The bast majority of LF cases present themselves as asymptomatic microfilaremia with absence of signs and symptoms. Lymphatic dysfunction may develop in some individuals infected with *Wuchereria bancrofti*, causing lymphedema and elephantiasis (typically in the lower extremities) and, hydrocele or scrotal elephantiasis. Acute manifestations include fever episodes of lymphangitis and lymphadenitis. Individuals who arrived recently into the endemic areas may characteristically present afebrile episodes of lymphangitis and lymphadenitis. Pulmonary tropical eosinophilia syndrome, with nocturnal cough and wheezing, fever, and eosinophilia is seen as a manifestation of LF in Asia.

While LF infection can occur early in life affecting children as young as 3 years old (98), the clinical manifestations usually appear later in life if they do. However subclinical damage starts at an early age (99). Lymphatic Filariasis is unlikely to cause lymphoedema or hydrocoele in children under 10–15 years of age (100, 101). Lymphatic Filariasis causes a variety of acute and chronic clinical signs and symptoms. Two forms of acute disease may present clinically: acute dermatolymphangioadenitis (ADLA), usually due to secondary bacterial infection, which requires antibiotic therapy, and acute filarial lymphangitis, due to death of adult worms, which is self-limiting. Chronic manifestations include lymphoedema, hydrocoele, chyluria and tropical pulmonary eosinophilia.

Asymptomatic infection

In *W. bancrofti* and *B. malayi* endemic areas, the majority of infected individuals are not found with overt clinical manifestations even those with large numbers of circulating microfilariae in the peripheral blood. However despite the apparent absence of damage, virtually all infected individuals have some degree of subclinical disease and damage. Proteinuria and hematuria are found as signs of low-grade renal damage, present in 40% of the microfilaraemic individuals. After treatment the clearance of microfilariae from the blood will result in reversing these clinical manifestations.

Asymptomatic individuals observed by ultrasound examination of scrotal lymphatics and lymphoscintigraphy to visualize the anatomy of lymphatic vessels have shown abnormal, dilated and tortuous lymphatics with abnormal lymph flow and lymphangiectasia (102).

Unfortunately, a low number of infected individuals will progress from this asymptomatic stage to either acute or chronic stages. One-third of those with LF infections would have clinical disease (3.33 % of the total at-risk population) (103). However the other two-thirds of individuals infected with LF actually have subclinical morbidity; half of these cases would progress to overt clinical disease in their lifetimes. Cases of clinical disease occur in the following proportion: 62.5 % hydrocele, 37.5 % lymphedema (104).

Acute manifestations

Adenolymphangitis (ADL) is a clinical acute manifestation of LF characterized by fever attacks followed by inflammation of the lymph nodes (adenitis) and/or lymph vessels (lymphangitis) (105).

The body areas that are more frequently affected include inguinal, axillary, and epitrochlear lymph nodes, Limb, breast and male genitalia (105).

The clinical symptoms include local pain, tenderness, warmth and lymphadenitis and/or lymphangitis and the course of this acute clinical episode may last for days up to 6 weeks and result in prolonged inability to work.

Two types of acute ADL episodes are distinguished: due to the filarial parasite itself or due to secondary bacterial or fungal infections (105). Evidence, both from clinical and immunohistopathological and bacteriological studies of tissue from lymphoedematous limbs of affected patients, has suggested that bacterial or fungal superinfections of limbs with compromised lymphatic function play the primary role in triggering episodes of ADL which themselves actually cause or exacerbate the chronic obstructive changes in the lymphatic of affected patients. The acute process usually starts in the skin and then spreads along the lymphatics to the lymph nodes. Based on those observations Olszewski et al. proposed that the syndrome be renamed dermatolymphangioadenitis (DLA) (106).

Therefore it is generally agreed upon that it is possible to distinguish two forms of acute attacks:

1. Filarial fever-lymphadenitis and retrograde lymphangitis when there is no clear entry site for bacterial infections such as any injury and it is associated with the filarial parasite itself.

2. Cellulitis associated with a visible site of entry for bacteria. Oedematous infiltration of the surrounding subcutaneous tissues, or even formation of abscesses, which may turn ulcerate and lead to scarring may occur. The ulcer in filariasis cellulitis is clean, and produces a serosaniguinous fluid.

The presence of Lymphoedema is a common occurrence on these acute episodes. Usually, the lymphooedema persists after the episodic attack, particularly with repeated attacks, leading to chronic lymphedema. Typically, each attack of fever and lymphadenitis lasts for several days resolving spontaneously after rest. Repeated episodes of ADL have been shown to be important in the progression of the chronic disease. There is a direct relationship between the number of acute attacks and the grade of lymphedema. Bancroftian filariasis is associated with more frequent acute attacks than brugian filariasis.

Acute dermatolymphangioadenitis (ADLA) or acute attacks

Due to the presence of adult filarial worms the lymphatic systems suffer inflammation of the, resulting in lymphangitis and lymphadenitis. As a result, damage in the lymphatic vessels occur, even in asymptomatic people, and lymphatic dysfunction, and subsequent recurrent bacterial infections. These secondary infections provoke ADLA, commonly called ‘acute attacks’. Repeated acute attacks produce the progression of lymphedema. The bacteria find an entry point in damaged skin (for example small blister) and invade the damaged lymphatic vessels unable to perform their regular defense role, producing these acute attacks. ADLA, produce local pain, inflammation and fever and the clinical presentation is similar to erysipelas or cellulitis.

Chronic manifestations

The major signs of chronic disease in filariasis are hydrocele, chyluria, lymphoedema and elephantiasis. Hydrocele and swelling of the testis are the most frequent, followed by elephantiasis of the lower limb, the scrotum, the entire arm, the vulva and the breast (in that descending order of frequency) (107).

Although the chronic clinical manifestations do not produce pain when there is no associated adenolymphangitis, the chronic manifestations affect the capacity of the patient to work and take care of themselves including strong stigma and discrimination. (108).
Though not all infections lead to disability, the health burden due to LF is considerable, estimated at 2.74 million disability-adjusted life years (DALYs) (1.73m-4.00m) (109).

Lymphoedema and elephantiasis

The enlarged lymph vessels become less efficient at transporting lymph from the periphery, which in the legs is always oriented against gravity. Insufficient fluid transport will lead to fluid extravasation, particularly in the lower limbs, and eventually to lymphedema (LE).

A more advanced form of lymphoedema is known as elephantiasis and it is associated with repeated acute attacks or ADLA. It affect primarily women in their lower limbs. It is often difficult to differentiate lymphedema due to filariasis with other causes of lymphedema such as venous disease, HIV/AIDS-associated Kaposi sarcoma, heart failure, malnutrition, podoconiosis and the scientific community have reached no consensus on the its classification and clinical differential diagnosis. Elephantiasis can be a major impediment to conduct a normal life and conduct daily activities. Not all cases of elephantiasis are caused by filariasis and elephantiasis is not the most common symptom of Lymphatic Filariasis. Due to the visibility of elephantiasis it has been known since early times. The disease was well known to physicians and medical writers from very early times. Ancient Hindu medical workers knew of the condition and it is referred to in Sanskrit texts dating back to 600 BC.

Hydrocoele

Scrotal hydrocoele is characterized by damage on the lymphatic vessels resulting on accumulation of fluid in the cavity of the tunica vaginalis. The death of adult filarial worm produce filarial hydrocoele. After the break of the lymphatic vessels in the scrotal cavity chylocoele liquid accumulate producing chylocele. A system for classifying hydrocoele has been proposed (110) that may allow international comparisons.

Different geographical endemic areas have different typical clinical manifestations of LF. In Tanzania, the most common clinical form of the disease is hydrocele, while lymphoedema and

elephantiasis are less common forms of manifestation of the disease Tropical pulmonary eosinophilia and chyluria have been reported frequently from India, Brazil and Malaysia. Microfilariae positive and microfilariae negative infected individuals have been shown to have the same risk of having clinical manifestations of the disease (111).

Socioeconomic burden of Lymphatic Filariasis

Lymphoedema and hydrocoele have very deep consequences in the life of the patients affected, leading to long-term disability, often disfigurement, and serious psychosocial and economic consequences in all areas of their lives. In addition, LF has a direct economic cost to both patients and health systems. The direct cost of managing acute and chronic manifestations are a burden while indirect losses due to diminished productivity are also of great importance. The cost to patients of treating ADLA episodes ranges from US$ 0.25–1.62, almost 2 days' wages in some countries, while the cost of hydrocoele surgery, depending on the country and source of care, is US$ 5–60 (98). ADLA was estimated to be responsible for losses of US$ 60– 85 million per year in India (112) and US$ 38 million per year in the Philippines (13). It has been described that patients loss jobs and marriages due to the disability and disfigurement of chronic manifestations (113), that includes feeling shame, being depressed and not living a fulfilling emotional life (114).

For men, genital damage is a severe disability, leading to physical limitations and social stigmatization (115). For women, shame and taboo are associated with lymphoedema and especially elephantiasis. Lack of access to education in affected children is also frequent. Patients are susceptible to depression and poor mental health (116). Lymphatic Filariasis often affects not only the patient but also the family, especially if the patient is the major income earner.

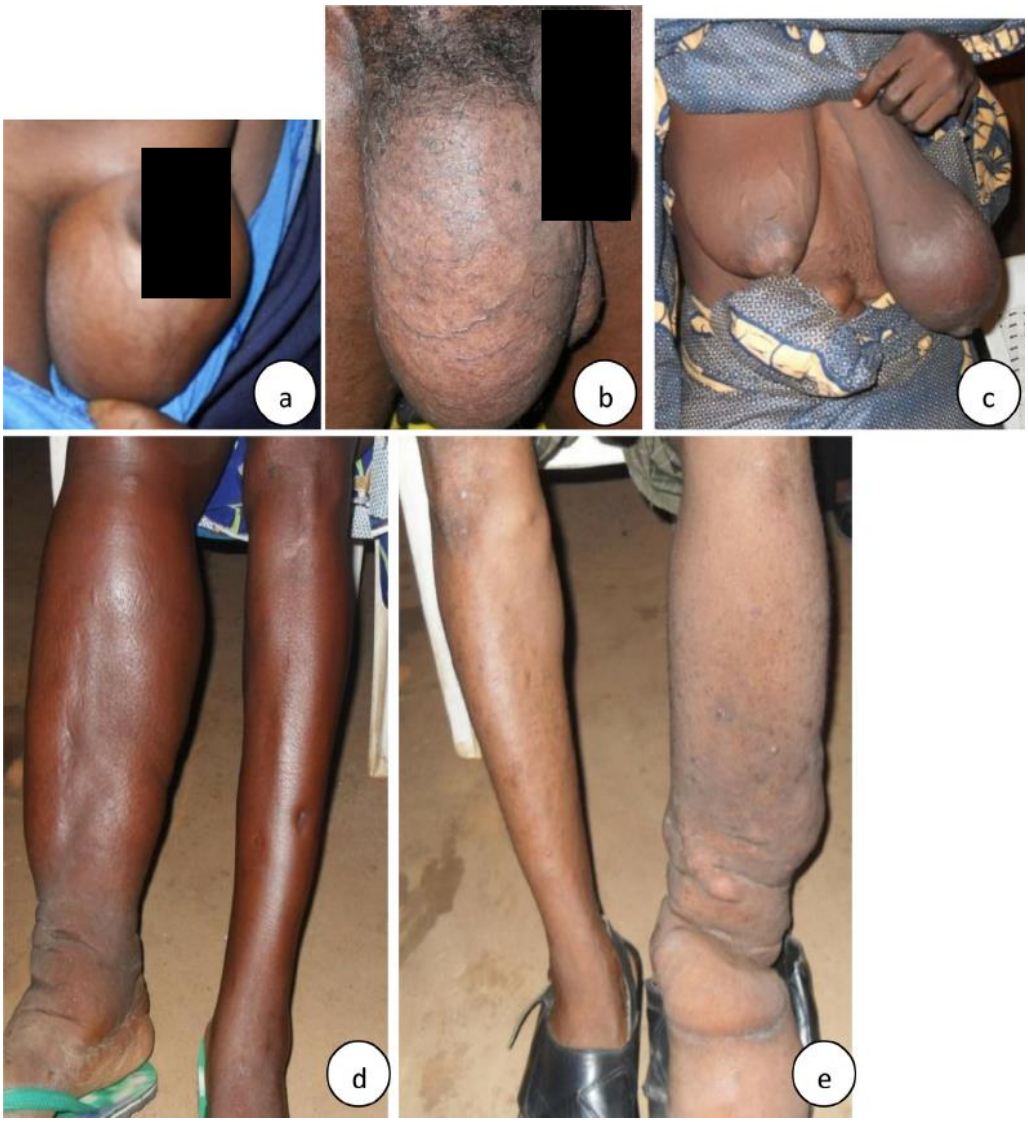
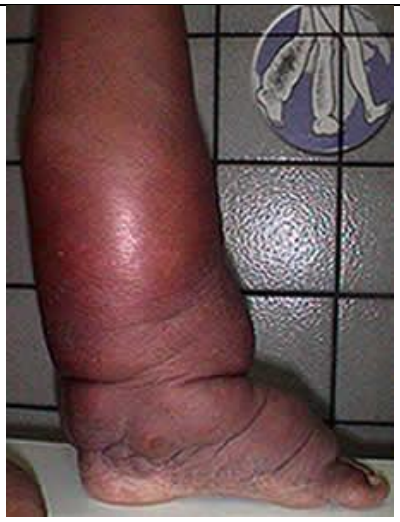
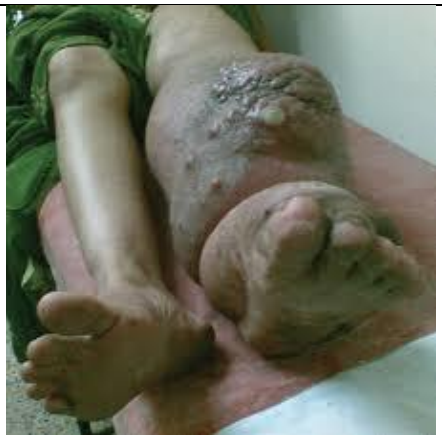
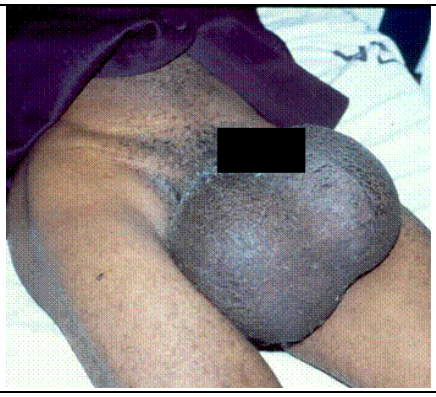


FIGURE 6.CLINICAL MANIFESTATIONS OF LYMPHATIC FILARIASIS BY W. BANCROFTI. A) HYDROCELE IN THE EARLY STAGE (FUCULITIS) IN A YOUNG BOY. B) HYDROCELE IN ITS ADVANCED STAGE IN ADULT MALE. C) ELEPHANTIASIS OF THE BREAST IN ADULT FEMALE. D) ELEPHANTIASIS OF THE LEG (STAGE II) IN ONE OF THE PARTICIPANTS. E) ELEPHANTIASIS OF THE LED IN THE ADVCANCED STAGE IN ADULT MALE.

Source: Lymphatic Filariasis and associated morbidities in rural communities of Ogun State, Southwestern Nigeria. Article (PDF Available) in Travel Medicine and Infectious Disease 12(1) · March 2013 with 210 Reads DOI: 10.1016/j.tmaid.2013.02.006 · Source: PubMed

TABLE 2.CLINICAL MANIFESTATIONS AND TREATMENT

Clinical manifestation	Image	Treatment
Acute dermatolymphangioadenitis		Antibiotics, antipyretics, analgesics
Lymphoedema and elephantiasis		Hygiene, antibacterial creams, antifungal creams
Hydrocoele		Surgery

The Global Programme to Eliminate Lymphatic Filariasis

The availability of safe, single-dose, two-drug treatment regimens capable of reducing Mf to near zero levels for one year or more, along with remarkable improvement in techniques for diagnosing the infection has resulted in a great sense of optimism for a global strategy to eliminate the disease (117). In fact, an independent International Task Force for Disease Eradication concluded that Lymphatic Filariasis was one of only six infectious diseases considered to be "eradicable" or "potentially eradicable" (118). According to this, the World Health Assembly (WHA) in 1997 adopted Resolution WHA50.29 calling for the elimination of Lymphatic Filariasis as a public health problem globally. The WHO, in collaboration with other international agencies in public health and private sector, forming a 'Global Alliance' (119), launched a global campaign to eliminate Lymphatic Filariasis by the year 2020 (120). The main goal of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) is to break the cycle of transmission of the disease between mosquitoes and humans, mainly through mass drug administration of albendazole in combination with either ivermectin or di-ethyl carbamazine citrate (DEC) (119, 121, 122).

LF continues to be a significant cause of morbidity in many parts of the world despite the fact that the disease has been targeted for elimination worldwide by 2020 (123). A massive global effort by the GPELF has been made and significant progress been achieved but the disease is still believed to impact over 100 million people worldwide (124). The absence of a non-human reservoir of *Wuchereria bancrofti* and only a very minor animal reservoir for *B. malayi* (that probably plays little or no role in transmission to humans) means that transmission can be interrupted by either eliminating the reservoir of microfilariae through community-wide treatment, or reducing human-vector contact or an integrated approach combining the two. This is known as transmission control (125). However, even when transmission has been interrupted, the adult worms may continue to induce lymphatic pathology and consequent morbidity. Reduce morbidity from Lymphatic Filariasis is the secondary objective of GPELF.

The GPELF has developed a strategy supported by two major pillars: first, interrupting transmission of the disease (so that no new infections occur), and second (and equally important) controlling morbidity to prevent disability (to alleviate the suffering of affected populations).

To achieve transmission interruption, preventive chemotherapy (PC) and vector control (VC) are the main recommended activities.

PC consists of the distribution of medicines to a large proportion of eligible individuals. The strategy is mass drug administration (MDA) with periodicity (typically annual for LF programs), designed so that the burden of disease infection is reduced over time to levels that result in the elimination of sustainable transmission (126).

Morbidity management and disability prevention (MMDP) consists of a package of preventive and curative measures to provide the best clinical care available at the country level and the psychosocial support needed by the patients. When LF transmission interruption is achieved, the MMDP package must be integrated into the health systems to ensure the sustainable care of patients during their life time or until they no longer require care (98, 127).

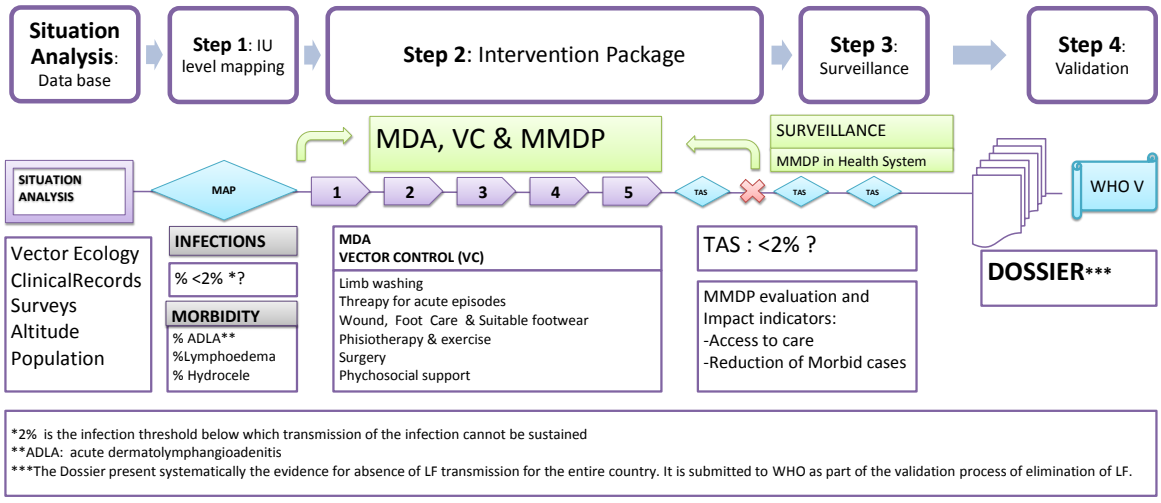


FIGURE 7. STRATEGY OF THE GLOBAL PROGRAMME TO ELIMINATE LYMPHATIC FILARIASIS (GPELF)

Preventive chemotherapy for human helminthiasis

The use of anthelminthic drugs, either alone or in combination, as a public health tool against helminth infections is referred to as Preventive chemotherapy (PC). Five neglected tropical diseases are amenable to PC: LF, Trachoma, Onchocerciasis (Oncho), Schistosomiasis (SCH) and Soil transmitted helminthiasis (STH).

- Lymphatic Filariasis (LF) – caused by infection with the nematodes *Wuchereria bancrofti, Brugia malayi* and *B. timori.*
- Onchocerciasis (Oncho) – caused by infection with the nematode *Onchocerca volvulus.*
- Schistosomiasis (SCH) – SCHi (intestinal schistosomiasis) caused by infection with the trematodes *Schistosoma mansoni, S. mekongi, S. japonicum* and *S. intercalatum*, and SCHu (urinary schistosomiasis) caused by infection with *S. haematobium*.
- Soil-transmitted helminthiasis (STH) – caused by infection with the nematodes *Ascaris lumbricoides* (roundworm), *Ancylostoma duodenale* and *Necator americanus* (hookworm), and *Trichuris trichiura* (whipworm).
- Trachoma – caused by the bacteria *Chlamydia trachomatis.*

Mass drug administration (MDA) consists on the distribution on PC tablets to entire eligible population through campaigns. This strategy has also been called Community directed treatment interventions (CDTi) by the Onchocerciasis control programs, while it is frequency call MDA in the LF elimination programs. MDA is the strategy used in the GPELF. Exclusion criteria apply to the distribution of the different drug packages based on drug safety (126, 128, 129). The population that does not meet the exclusion criteria is called ***eligible population***. The total population living in an area where the disease transmission is active is call ***at risk population*** and they all become targeted by PC. The total population requiring PC and being targeted for treatment is used as the denominator to estimate effective coverage of MDA. For MDA to be effective on interrupting transmission at least 65% of the targeted population must be reached and swallow the drugs. That is equivalent to 80% of the eligible population on LF programs.

When the targeted population for treatment is not the entire population the intervention is called ***targeted treatment*** instead of MDA. The targeted treatment typically occurs when the objective of the PC is controlling the morbidity rather than interrupting transmission. In those control

programs target the population that have the highest risk of morbidity or is more vulnerable, such as children in school age (SAC) or preschool age (Pre-SAC), women of child bearing age (WCBA) or professional groups exposed to the disease through their work (also called high risk groups).

Different drugs are used to target NTDs amenable to PC (Table 3):

- Ivermectin (IVM). It is the drug of choice to target Onchocerciasis. In countries where LF and Onchocerciasis are co-endemic, IVM is the drug of choice for LF too. When used for LF, IVM is distributed in combination with Albendazole 400mg. IVM is distributed as a single dose by weight or height by MDA (height is measured with the help on a pole) (Table 3). IVM has mainly a microfilaricide effect, not resulting in the death of the adult worm. Pregnant women, lactating women in the first week after birth, children <90 cm in height (approximately equivalent to 15 kg/body weight), and the severely ill are ineligible for treatment. IVM is donated by Merck (www.merck.com) pharmaceutical company for as long as it is needed to eliminate Onchocerciasis and LF (where LF is coendemic with Onchocerciasis). Ivermectin is donated under the name of Mectizan® through the Mectizan donation program (www.mectizan.org).

- Dietilcarbamazine (DEC). Combined with Albendazole it is the drug of choice to eliminate LF in countries that are not coendemic with Onchocerciasis. Up to 2.2 billion tablets are donated to eliminate LF by the manufacturer Eisai from 2012 to 2017. DEC dose is administered by age group (Table 3).

- Praziquantel. Used to control morbidity due to Schistosomiasis. Dose is administered by height with the help of a dose pole (Table 3).

- Albendazole. It is used to prevent morbidity due to STH. Tablets are 400 mg and can be administered to any person 2 years of age or older. Children 12 months to 24 months can receive 200 mg of Albendazole. Albendazole is also used in combination with IVM or DEC to eliminate LF through MDA. For this purpose, is administered to individuals older than 4 years old as a 400 mg tablet combined with the adequate dose of DEC or IVM.

Albendazole alone once or twice a year is the MDA strategy recommended where Ivermectin cannot be used due to the high risk of severe adverse events (SAE) that may occur in *Loa loa* coendemic areas.

TABLE 3. DRUGS USED IN PREVENTIVE CHEMOTHERAPY AGAINST HELMINTHIASIS, AND DOSES ACCORDING TO HEIGHT AND AGE.

Drug	**Height / Age**	**No. Tablets**
Ivermectin	90-119 cm	1 tablet (3 mg)
	120-139 cm	2 tablets (6 mg)
	141-159 cm	3 tablets (9 mg)
	>159 cm	4 tablets (12 mg)
DEC	2-5 años	1 tablets (100 mg)
	6-15 años	2 tablets (200 mg)
	>15 años	3 tablets (300 mg)
Praziquantel	94-109 cm	1 tablets (600 mg)
	110-124 cm	1 ½ tablets
	125-137 cm	2 tablets
	138-149 cm	2 ½ tablets
	150-159 cm	3 tablets
	160-177 cm	4 tablets
	>177 cm	5 tablets

- Mebendazole: it is used in PC to target STH at risk population. Mebendazole can be administered from 12 months of age. Tablets are 500 mg and it is administered in mono dose once or twice a year according to WHO recommended strategy for the disease prevalence.

- Azithromycin. It is the drug of election to eliminate trachoma as a public health problem. It is donated by Pfizer under the name of Zithromax through International trachoma initiative (ITI).

Preventive chemotherapy against Lymphatic Filariasis

Ivermectin or DEC plus Albendazole in MDA are the drug combination used to eliminate LF through MDA interventions.

MDA for LF generally involves annual provision of a combined dose of medications to all eligible persons living in all endemic areas for at least 5 years. Treatments delivered during MDA reduce the density of parasites circulating in the blood of infected persons and the prevalence of infection in the community to such low levels that transmission cannot be sustained, and new infections eventually cease (130-132). When the level of infection has been reduced to below target thresholds, MDA is considered no longer required. The prevalence threshold of LF infected individuals below which LF is considered not sustainable and interventions no longer required is 2% for *Anopheles* and *Culex* transmission areas and 1% for *Aedes* transmission areas (133).

MDA conducted annually needs to reach a sufficient proportion of individuals in order to be effective on interrupting transmission of LF (134). Coverage of MDA is determined by the proportion of individuals who swallow the drugs from those targeted for treatment.

- **Geographical coverage**: number of geographical areas under treatment divided by number of geographical areas requiring preventive chemotherapy. Each geographical area under treatment is called Implementation Unit (IU). IUs for LF are typically the administration unit level 2. That administrative unit level 2 has different denominations depending on countries: district, county, woreda, LGA, etc.

$$Geographical\ coverage = \frac{Number\ of\ IUs\ receiving\ PC}{Number\ of\ IUs\ requiring\ PC} x100$$

- **Programme coverage**: Proportion of individuals ingesting the drugs from those targeted and eligible. In the LF programmes, the population targeted is the total population while in other programmes like SCH and STH the target population is often only a subset of the total population. It is an indicator of the effectiveness of the intervention.

$$Programme\ coverage = \frac{Number\ of\ individuals\ receiving\ PC\ in\ the\ IUs}{Number\ of\ individuals\ targeted\ and\ eligible\ in\ the\ IU} x100$$

- **Drug coverage or National coverage**: Number of individuals ingesting the drugs from those living in areas where the treatment intervention is required. This indicator enables to understand if sufficient people is being treated to be able to interrupt transmission. At least 65% of the population living in endemic areas needs to be treated in order to consider the MDA round was effective to achieve transmission interruption in 5 MDA rounds.

$$Drug\ coverage = \frac{Number\ of\ individuals\ ingesting\ the\ drugs}{Number\ of\ individuals\ living\ in\ the\ areas\ requiring\ PC} x100$$

Progress towards the elimination of Lymphatic Filariasis

By 2014, of 73 countries listed by WHO as being endemic for Lymphatic Filariasis, 16 countries have completed interventions and were conducting surveillance to validate elimination. An additional 23 countries had delivered MDA in all endemic areas and were also on track to achieve elimination. The remaining countries had not been able to achieve 100% geographical coverage; 13 of these had yet to initiate preventive chemotherapy or submit evidence that MDA is not required (124, 135).

Of the total population requiring preventative chemotherapy, 57% live in the South-East Asia Region (9 countries) and 37% live in the African Region (35 countries). However, from the 13 countries yet to start MDA, 12 of them were in Africa, while from the 22 yet to achieve 100% geographical coverage 16 were in Africa (135). Therefore, the African continent represents the largest untreated burden of LF in the world and the biggest impediment to achieve the goal of LF elimination by 2020.

In 2000, thirty-nine countries were considered endemic for LF in Africa. An early attempt to develop a risk map for LF in Africa was published by Lindsay and Thomas in 2000, based on data from 32 studies using frequentist logistic regression and coarse-resolution environmental data (136). More recently in 2014, Cano and Rebollo used boosted regression tree models to delineate the ecological niche for LF in Africa (3, 137) predict the geographical occurrence and distribution of LF in Africa. Lately, through a combination of Bayesian geostatistical modelling and mathematical modelling, it has been predicted the intensity of transmission across Africa (138). All these predictive risk maps showed that LF in Africa occurs over a large area extending from the west to the east primarily across the middle region of the continent. A high degree of heterogeneity in the probability of LF occurrence on the continent was displayed in their results. For example, a large zone of high probability of LF occurrence was demonstrated in the Western Africa region. On the other hand, in Central and Eastern Africa and in Madagascar, large areas of medium probability occurrence were interspersed with smaller areas of high probability, especially along the coasts. Importantly, all WHO considered LF-free countries were shown to have fairly low probabilities of infection. In their estimates, the population requiring PC for LF in 2010 in Africa was calculated to be either 804 million or 542 million using different thresholds of presence of disease (3, 136).

In 2001, the WHO stated that 327 million people were considered to be in need of PC for LF in Africa in 38 countries (139).

In 2002, the WHO expanded this number to 477 million in 39 countries (140). By that same year just 9 countries were carrying out MDA with only 9.9 million people being covered, representing only 2.1% of the total population needing PC for LF in Africa (140).

An essential first step in implementing programmes to eliminate Lymphatic Filariasis is to define where MDA should be conducted. In 2000, the first LF mapping was initiated in Africa, and by 2001, 4 countries has already completed mapping (Benin, Burkina Faso, Ghana and Togo) (141). By 2009 eleven endemic countries in Africa had not yet completed mapping (Angola, Cameroon, Central African Republic, Côte d'Ivoire, Democratic Republic of the Congo (DRC), Ethiopia, Liberia, Nigeria, South Sudan, Zambia, and Zimbabwe), and two had not yet begun mapping (Chad and Eritrea) (139, 142).

By that same year, 405.9 million people in 39 countries in Africa were estimated to require PC. However, according to GPELF's progress report for 2000–2009 (143), the evidence for active transmission of LF in many of the 39 endemic countries was weak and some probably did not require MDA. The statuses of 5 countries (Burundi, Cape Verde, Mauritius, Rwanda and Seychelles) were reviewed in 2011 and were reclassified as non- endemic, reducing the number of endemic countries in Africa to 34 (inclusion of South Sudan following independence in 2011 now makes 35) (144).

TABLE 4.PROGRESS TOWARDS CONTROL AND ELIMINATION OF LYMPHATIC FILARIASIS IN AFRICA (2009)

Source from table 11, WHO Progress report 2000-2009

http://www.who.int/lymphatic_filariasis/resources/9789241500722/en/

Country	Population requiring PC	LF Mapping status	MDA status	Type of MDA
Angola	12 090 000	In progress	Not started	IVM+ALB
Benin	5 282 204	Completed	Started	IVM+ALB
Burkina Faso	15 411 849	Completed	Started	IVM+ALB
Burundi		Not endemic	MDA not required	
Cameroon	14 305 000	In progress	Started	IVM+ALB
Cape Verde		Not endemic	MDA not required	
Central African Republic	3 300 000	In progress	Not started	IVM+ALB
Chad	7 270 000	Not started	Not started	IVM+ALB
Comoros	514 110	Completed	Started	DEC+ALB
Congo	2 600 000	Completed	Not started	IVM+ALB
Côte d'Ivoire	14 000 000	In progress	Started	IVM+ALB
Democratic Republic of Congo	49 140 000	In progress	Not started	IVM+ALB
Equatorial Guinea	420 000	Completed	Not started	IVM+ALB
Eritrea	3 577 000	Not started	Not started	DEC+ALB
Ethiopia*	30 000 000	In progress	Started	IVM+ALB
Gabon	1 290 600	Completed	Not started	IVM+ALB
Gambia*	1 200 000	Completed	Not started	DEC+ALB
Ghana	11 587 953	Completed	Started	IVM+ALB
Guinea	6 067 135	Completed	Not started	IVM+ALB
Guinea-Bissau	1 311 741	Completed	Not started	IVM+ALB
Kenya	3 031 878	Completed	Started	DEC+ALB
Liberia	3 600 000	In progress	Not started	IVM+ALB
Madagascar	17 948 748	Completed	Started	DEC+ALB
Malawi	12 887 248	Completed	Started	IVM+ALB
Mali	13 798 000	Completed	Started	IVM+ALB
Mauritius		Not endemic	MDA not required	
Mozambique	15 538 610	Completed	Started	IVM+ALB
Niger	11 465 194	Completed	Started	IVM+ALB
Nigeria	70 650 902	In progress	Started	IVM+ALB
Rwanda		Not endemic	MDA not required	
Sao Tome Principe	410 000	Completed	Not started	DEC+ALB
Senegal	5 314 600	Completed	Started	IVM+ALB
Seychelles		Not endemic	MDA not required	
Sierra Leone	5 319 758	Completed	Started	IVM+ALB
Togo	1 191 720	Completed	Started	IVM+ALB
Uganda	13 264 445	Completed	Started	IVM+ALB
United Republic of Tanzania *	37 369 939	Completed	Started	IVM+ALB
Zambia	8 780 000	In progress	Not started	DEC+ALB
Zimbabwe	6 000 000	In progress	Not started	DEC+ALB

*Countries included in this doctoral thesis

Nineteen countries were implementing MDA in Africa in 2009. At that time, only 85 million people (20.9% of the total requiring MDA) were targeted for MDA and only 66 million people were treated. Fifteen countries had not yet started MDA, while 13 had not yet achieved full geographical coverage (139, 142). Contributing to low coverage in some areas was the fact that MDA programmes in urban populations typically achieve low coverage compared to more rural settings. People who live in cities tend to be busier, making social mobilization more difficult; populations are heterogeneous, with complex social, economic, and religious structures; and urban dwellers place a higher priority on privacy (39).

Despite GPELF being one of the most rapidly expanding global health programs in the history of public health (145), by 2011 in Africa, only 17 of the 35 endemic countries were implementing MDA (144).

By 2009, only 6 out 34 countries requiring MDA for LF in Africa had achieved 100% geographical coverage (Burkina Faso, Comoros, Ghana, Mali, Malawi, Togo) and were on track to achieve LF elimination by 2020 (139).

With more than 320.9 million people in need of PC not yet receiving MDA by 2009, these data presented a very pessimistic scenario for Africa in terms of realistically achieving the 2020 goal. These data included entire countries that had not yet initiated MDA, like the Gambia and Gabon, and also included other high LF burden countries, like Ethiopia and DRC, where only a small percentage of the population requiring treatment was receiving it (139).

On the optimistic side, some areas in Benin, Burkina Faso, Nigeria, Ghana, and Tanzania (including Zanzibar in the Republic of Tanzania) had already stopped MDA after 5 rounds or more. However, the population living in those areas was still being counted in the WHO treatment gap.

TABLE 5. INDICATORS OF PROGRESS BY 2009

Source: from Lymphatic Filariasis: progress report 2000–2009 and strategic plan 2010–2020

http://www.who.int/lymphatic_filariasis/resources/9789241500722/en/

Indicator (2009)	**No. of countries**
Countries stopped MDA	**0**
Countries completed 5 or more rounds of MDA with 100% geographical coverage	**2 countries** Togo, Burkina Faso
Countries implementing MDA with 100% geographical coverage	**6 countries** Burkina Faso, Comoros, Ghana, Mali, Malawi, Togo
Countries implementing MDA in only in part of the geographical area considered in need of treatment	**19 countries** Benin, Burkina Faso, Cameroon, Comoros, Côte d'Ivoire, Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Sierra Leone, Togo, Uganda, United Republic of Tanzania
Countries where MDA not yet started	**15 countries** Angola, Central African Republic, Chad, Congo, Democratic Republic of the Congo, Equatorial Guinea, Eritrea, Gabon, Gambia, Guinea, Guinea-Bissau, Liberia, Sao Tome and Principe, Zambia, Zimbabwe
Countries with mapping in progress	**10 countries** Angola, Cameroon, Central African Republic, Côte d'Ivoire, Democratic Republic of the Congo, Ethiopia, Liberia, Nigeria, Zambia, Zimbabwe
Countries not started mapping	**2 countries** Chad, Eritrea
Countries unlikely to require MDA	**5 countries** Burundi, Cape Verde, Mauritius, Rwanda, Seychelles

It is unclear how the number of individuals requiring PC for LF was historically determined by the WHO and whether that figures remain accurate due to the changing epidemiological landscape since 2000. Improved diagnostic methods, epidemiological tools and mathematical models are now available, including more sensitive and specific diagnostics for *Wuchereria bancrofti* that may not cross-react with *Loa loa*. Additional factors may have interrupted or helped to reduce transmission in some countries:

a) Vector control (VC) may have interrupted transmission in some regions where malaria vector control activities have had high coverage;
b) in *Anopheles* transmission areas, urban transmission may be less frequent than estimated; and
c) MDA may have interrupted transmission after completing 5 or more rounds of MDA resulting in some countries scaling down treatment programmes in some districts.

These factors should all be considered by reducing the denominator of the estimated number of individuals that require PC against LF.

For this thesis, I asked the question of whether these factors have reduced the number of people requiring MDA for LF in Africa and if a more accurate assessment can be made of where Africa is on the road map to achieve LF elimination.

In a number of papers contained in this thesis, I have published the results of research conducted from 2012 to 2016 to answer a critical question: ***whether the newly available epidemiological, diagnostic and modelling tools can provide a more accurate measurement of the true gap between the number of people requiring MDA for LF and the number of people receiving MDA***.

Hypotheses & Objectives

Hypotheses

New diagnostics, epidemiological and modelling tools will enable the re-estimation of the number of people requiring preventive chemotherapy against LF in Africa and will shrink the map of LF in the region.

1. The population requiring PC in **high burden countries** in areas yet to start MDA is smaller than the current estimate based on low environmental suitability for disease transmission. Case study: Ethiopia.

2. **Vector control** (VC) has interrupted transmission of LF in *Anopheles* transmission areas that are yet to start MDA where coverage of VC interventions has been high. Case study: The Gambia.

3. **Regions scaling back on MDA due to 5 or more years of successful treatment has resulted in a reduction of the denominator of number of people requiring PC.** These regions may have interrupted transmission and need to be deducted from the total number of people requiring PC. Case study: Zanzibar.

Objectives

- **General objective**: To determine if current diagnostic, modelling and epidemiological methods can shrink the map of Lymphatic Filariasis in Africa and reduce estimates of the number of people requiring preventive chemotherapy.

    - **Specific Objective 1:** To re-estimate the population requiring treatment for LF in Ethiopia
    - **Specific Objective 2:** To demonstrate transmission interruption of LF in the Gambia due to vector control activities against Malaria

- **Specific Objective 3:** To demonstrate transmission interruption of LF in Zanzibar due to more than 5 rounds of MDA.

Materials, Methods & Results

Materials, Methods and Results

- **Research Article 1. Rebollo, M.P.**, H. Sime, A. Assefa, J. Cano, K. Deribe, A. Gonzalez-Escalada, O. Shafi, G. Davey, S. J. Brooker, A. Kebede, and M. J. Bockarie. 2015. 'Shrinking the Lymphatic Filariasis Map of Ethiopia: Reassessing the Population at Risk through Nationwide Mapping', *PLoS Negl Trop Dis*, 9: e0004172.

- **Research Article 2. Rebollo, M. P.**, S. M. Sambou, B. Thomas, N. K. Biritwum, M. C. Jaye, L. Kelly-Hope, A. G. Escalada, D. H. Molyneux, and M. J. Bockarie. 2015. 'Elimination of Lymphatic Filariasis in the Gambia', *PLoS Negl Trop Dis*, 9: e0003642.

- **Research Article 3. Rebollo, M. P.**, K. A. Mohammed, B. Thomas, S. Ame, S. M. Ali, J. Cano, A. G. Escalada, and M. J. Bockarie. 2015. 'Cessation of mass drug administration for Lymphatic Filariasis in Zanzibar in 2006: was transmission interrupted?', *PLoS Negl Trop Dis*, 9: e0003669.

Paper 1. Shrinking the Lymphatic Filariasis Map of Ethiopia: Reassessing the Population at Risk through Nationwide Mapping

Rebollo, M.P[1,6]., H. Sime[2], A. Assefa[2], J. Cano[3], K. Deribe[4], A. Gonzalez-Escalada[6], O. Shafi[7], G. Davey[4], S. J. Brooker[3], A. Kebede[4], and M. J. Bockarie[1]. 2015. 'Shrinking the Lymphatic Filariasis Map of Ethiopia: Reassessing the Population at Risk through Nationwide Mapping', *PLoS Negl Trop Dis*, 9: e0004172.

Authors affiliation:

[1] Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom,

[2] Ethiopian Public Health Institute, Addis Ababa,Ethiopia,

[3] London School of Hygiene & Tropical Medicine, London, United Kingdom,

[4] Brighton and Sussex Medical School, Falmer, Brighton, United Kingdom,

[5] School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia,

[6] Faculty of Infectious and Tropical Diseases, Rey Juan Carlos University, Madrid, Spain,

[7] Federal Ministry of Health, Addis Ababa, Ethiopia

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Shrinking the Lymphatic Filariasis Map of Ethiopia: Reassessing the Population at Risk through Nationwide Mapping

Maria P. Rebollo[1☯¤], Heven Sime[2☯], Ashenafi Assefa[2], Jorge Cano[3], Kebede Deribe[4,5], Alba Gonzalez-Escalada[6], Oumer Shafi[7], Gail Davey[4], Simon J. Brooker[3], Amha Kebede[2], Moses J. Bockarie[1]*

1 Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom, **2** Ethiopian Public Health Institute, Addis Ababa, Ethiopia, **3** London School of Hygiene & Tropical Medicine, London, United Kingdom, **4** Brighton and Sussex Medical School, Falmer, Brighton, United Kingdom, **5** School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia, **6** Faculty of Infectious and Tropical Diseases, Rey Juan Carlos University, Madrid, Spain, **7** Federal Ministry of Health, Addis Ababa, Ethiopia

☯ These authors contributed equally to this work.
¤ Current address: Neglected Tropical Diseases Support Center, Task Force for Global Health, Decatur, Georgia, United States of America
* moses.bockarie@lstmed.ac.uk

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Abstract

Background

Mapping of lymphatic filariasis (LF) is essential for the delineation of endemic implementation units and determining the population at risk that will be targeted for mass drug administration (MDA). Prior to the current study, only 116 of the 832 woredas (districts) in Ethiopia had been mapped for LF. The aim of this study was to perform a nationwide mapping exercise to determine the number of people that should be targeted for MDA in 2016 when national coverage was anticipated.

Methodology/Principal Finding

A two-stage cluster purposive sampling was used to conduct a community-based cross-sectional survey for an integrated mapping of LF and podoconiosis, in seven regional states and two city administrations. Two communities in each woreda were purposely selected using the World Health Organization (WHO) mapping strategy for LF based on sampling 100 individuals per community and two purposely selected communities per woreda. Overall, 130 166 people were examined in 1315 communities in 658 woredas. In total, 140 people were found to be positive for circulating LF antigen by immunochromatographic card test (ICT) in 89 communities. Based on WHO guidelines, 75 of the 658 woredas surveyed in the nine regions were found to be endemic for LF with a 2016 projected population of 9 267 410 residing in areas of active disease transmission. Combining these results with other data it is estimated that 11 580 010 people in 112 woredas will be exposed to infection in 2016.

Conclusions

We have conducted nationwide mapping of LF in Ethiopia and demonstrated that the number of people living in LF endemic areas is 60% lower than current estimates. We also showed that integrated mapping of multiple NTDs is feasible and cost effective and if properly planned, can be quickly achieved at national scale.

Author Summary

About 1.4 billion people are believed to be living in areas where Lymphatic filariasis (LF) is actively transmitted. However, the distribution of this disfiguring mosquito-borne parasitic disease and the true population at risk that can be targeted for treatment have not been defined for all endemic countries. By 2013, Ethiopia had not delineated the majority of the endemic implementation units that can be targeted for MDA. Here, we present the results of a nationwide mapping exercise conducted in 2013 to determine the number of people that should be targeted for treatment in 2016 when nationwide treatment coverage is expected. We adopted a two-stage cluster purposive sampling method for the integrated mapping of LF and podoconiosis in seven regional states and two city administrations. Using a WHO mapping strategy for LF, based on sampling 100 individuals per community ICT positive individuals (ICT+) and two purposely selected communities per district, we examined 130 166 people in 1315 communities in 658 districts. Only 140 people were found to be positive for LF antigen in 89 different communities. According to WHO guidelines, 75 of the 658 districts surveyed in the 9 regions were found to be LF endemic. Including the 37 endemic *Woredas* identified enprior to this study, 112 *woredas* across the country are known to be endemic for the disease with 11 580 010 people exposed to infection. However 6 190 482 of those resided in *woredas* where our survey results were borderline with only one ICT positive individual identified. We have demonstrated that the number of people living in areas of active LF transmission is at least 60% lower than current WHO estimates of 30 million. We also showed that integrated mapping of multiple NTDs is feasible and cost effective. However, the sensitivity of the diagnostic test used for LF is less than 100% and the identification of a single ICT positive adult may not provide evidence of disease transmission. Based on these limitations, and in addition to the restricted geographical representation of just two sites within a *woreda*, we recommend conducting research in the 45 *woredas* with borderline results (one ICT+) to shrink the denominator even further.

Introduction

Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease (NTD) associated with debilitating conditions that affect at least 40 million people worldwide [1]. Chronic LF is manifested in the form of acute dermatolymphangioadenitis, lymphoedema, elephantiasis of the limbs and hydrocele. These disfiguring conditions are stigmatizing and affect mobility leading to impairment in educational and employment opportunities. In Africa, the causative agent of LF, *Wuchereria bancrofti*, is transmitted by many species of mosquitoes belonging to the *Anopheles*, *Culex* and *Mansonia* genera. *Anopheles* species are the main vectors LF in Ethiopia [2]. LF was recognised in 1993 as an eradicable disease [3, 4] and systematic efforts aimed at eliminating the disease were initiated in 1997 through a World Health Assembly Resolution

(WHA 50.29) calling for the elimination of the disease as a public health problem in all endemic countries. To accelerate the elimination process, pharmaceutical companies, donors, governments and other partners renewed their commitments in 2012 through London Declaration, to control, eliminate or eradicate ten NTDs including lymphatic filariasis by 2020 [5]. The renewed commitment was to complete mapping, ensure the continued supply of drugs for preventive chemotherapy, advance research and scale up the implementation of control interventions. Only communities that have been shown to be endemic through mapping are targeted for treatment.

In March 2011, the endemicity status of 9 countries historically considered to be endemic but with no current evidence for active transmission (Burundi, Cape Verde, Costa Rica, Mauritius, Rwanda, Seychelles, Solomon Islands, Suriname, and Trinidad and Tobago) was reviewed. The outcome of the review led WHO Strategic and Technical Advisory Group on Neglected Tropical Diseases to reclassify all 9 of them as non-endemic [6].

WHO currently estimates that 1.4 billion people live in areas where LF is actively transmitted, nevertheless in 2013, 13 of the 73 countries where LF is known to exist had not delineated all or the majority of the endemic implementation units (IU) for mass drug administration (MDA) to a defined population at risk, including Ethiopia [1]. Moreover as more sensitive diagnostic and transmission monitoring tools become available and mapping progresses, the country specific estimates for the number of people living in areas with active transmission will be reassessed to determine the amount of medicines required for community-wide treatment [7].

According to WHO estimates, in Africa, about half (48.5%) of the 464 million people exposed to LF reside in the four high burden countries of Democratic Republic of Congo (DRC) (49 million), Ethiopia (30 million), Nigeria (109 million) and Tanzania (45 million) [8]. With the exception of Tanzania, the high burden countries in Africa are yet to complete mapping of all implementation units. In Ethiopia only 116 of the 832 districts in the country have so far been mapped for the disease [9].

The four sequential steps, recommended by WHO for the implementation of MDA-based interventions, begin with mapping [10]. Mapping is the systematic epidemiological assessment of all geographical areas to determine if a disease is actively transmitted in each area. The implementation unit (IU), typically the district or second administrative division of government, is the basic survey unit that will inform if MDA is required. In Ethiopia, the IU corresponds to the *woreda* (district).

Historically, the known distribution of LF in Ethiopia was restricted to the lowlands of the south-western regions, especially in Gambella Region [11–14]. In 2008, the Carter Center and Addis Ababa University conducted surveys in 112 districts in western Ethiopia where onchocerciasis is also endemic [15]. The main objective of the 2008 mapping surveys was to determine the presence or absence of LF infection in districts that were under ivermectin treatment in order to add albendazole to the preventive chemotherapy package on those that were endemic for LF. As recommended by WHO, the selection of villages was biased towards finding LF infection. The WHO Operational Guidelines for Mapping of Bancroftian Filariasis in Africa informed the sampling procedures and the *woreda* (district) was defined as the unit for determining LF endemicity. About 100 people were tested in each selected *woreda*, and *woredas* where one or more positives were found were classified as endemic. Thirty-four of the 112 districts, with a population of 1 547 685 in 2007, were found to be endemic and MDA against LF commenced in Ethiopia in 2009 [16].

To date, less than 2 million people have been targeted for treatment representing 5.3% national coverage with an estimated 30 million people at risk as the denominator. In this paper we present the results of the nationwide mapping exercise carried out in 2013 to inform the

number of people that should be targeted for MDA in 2016 when national treatment coverage is expected. We also discuss the significance of the reduction of the denominator, and the impact of shrinking the LF map in Ethiopia and other high-burden countries to accelerate the achievement of the 2020 goal of LF elimination.

Materials and Methods

Study area

With 30 million people estimated to be living in areas where LF is transmitted, Ethiopia ranks fourth in Africa with regard to number of people at risk of infection [8]. Located in the horn of Africa, Ethiopia is a landlocked country with a surface area of some 1.1 million square kilometres. The estimated population for 2014 is 86.6 million with over 45% of its people aged 15 years or older [17]. Population estimates used in this study are based on the projections by the Central Statistical Agency of the government of Ethiopia published in 2013, to the *woreda*, level for the years 2014, 2015, 2016 and 2017 (http://www.csa.gov.et/images/general/news/pop_pro_wer_2014-2017_final).

Ethiopia is characterised by great geographical diversity; ranging from the deserts along the eastern border to the tropical forests in the south to extensive Afromontane in the northern and south-western regions. The topographic features range from the highest peak at 4 550 metres above sea level to 110 metres below sea level. This wide diversity of terrain has given rise to a huge variation in climate, soils and natural vegetation that produces unique biological niches suitable for different flora and fauna.

The federal administrative divisions in the country are presented as 9 regions and 2 city administration councils. The regions are sub-divided into zones and *woredas* but city administrations are only divided into *woredas*. In total, there are currently 832 *woredas* in Ethiopia which are further divided into well-defined communities called *kebeles* [17]. Prior to the current study, the endemicity and distribution of LF in Ethiopia had only been established for 116 districts in parts of the regions of Gambella, Benishangul-Gumuz, Southern Nations Nationalities and Peoples (SNNPR), Amhara and Oromia[15]. Of the remaining 716 districts, only the 710 *woredas* outside the national capital, Addis Ababa, were targeted for mapping during this survey. Addis Ababa was excluded from this survey because of the difficulties associated with mapping urban areas using purposive sampling (rural to urban migration and population movements inside the capital make it difficult to select areas of higher risk). Further investigation will be required using more appropriate methods to assess transmission in non rural areas like the national capital (for example xenomonitoring and school cluster random sampling used during transmission assessment surveys).

Study design and sampling

The details of the survey design, procedures and experiences of conducting the survey are provided elsewhere [18]. In brief, a two-stage cluster purposive sampling was used to conduct a community-based cross-sectional study of mapping two diseases, LF and podoconiosis, in 7 regional states (Tigray, Affar, Amhara, Oromiya, Somali, Southern Nations Nationalities and Peoples (SNNP), and Harari) and 2 city administrations (Addis Ababa and Dire Dawa Administration Councils). Surveys were not conducted in the Benishangul-Gumuz and Gambella regions because they had previously been mapped for LF in 2008. Surveys for podoconiosis and LF were conducted simultaneously using a coordinated mapping strategy between June and October 2013. Initially, *kebeles* (communities) considered to be at high risk for LF were identified based on health facility records of morbidity associated with LF, presence of diseased individuals and suitability for vector mosquito breeding. Two *kebeles* in each *woreda* were

purposely selected using the WHO mapping strategy for LF based on sampling 100 individuals per community and two purposely selected communities per IU (i.e. *woreda* in Ethiopia) [10]. The primary sampling unit for the LF survey was the *kebele* and consenting individuals were selected systematically with a random start from those 15 years or older. Social mobilization was conducted one day prior to the survey using health extension workers. During the awareness campaign, an attempt was made to inform every adult in the community through a house to house visit that a survey was to be conducted, and that they were invited to participate. We have previously described in detail the survey protocols and sampling procedures used in the study including the lessons learnt from the integrated mapping of LF and podoconiosis in Ethiopia [18].

LF diagnosis

The presence of LF infection was determined by the immunochromatographic card test (ICT) markedted as Binax NOW Filariasis card test (Alere Inc., Scarborough, ME) which detects circulating filarial antigen (CFA) as described in the WHO guidelines [10] and in a recent review [7]. Fingerprick blood from individuals were transferred to an ICT card using a calibrated capillary tube and results were read 10 minutes after closing the card, following manufacturer's instructions. Recombinant *W*. *bancrofti* antigen was used as a positive control to confirm the quality of the ICT cards. The field team spent several days outside the main towns and ICT kits were refrigerated while in storage in central points in hospitals and regional laboratories across the country. ICT test results were recorded on the ICT card and entered in a database on a smartphone platform (see below).

Questionnaires and clinical examinations

The survey forms used included questions regarding general demographics and LF control activities. In addition to collecting information on study site, region, zone, *woreda* (district) and *kebele* (community), questions were asked about sleeping under a bednet the previous night, deworming and treatment with ivermectin and or albendazole in the past year. Participants were not clinical examined for hydrocele but were asked to self-report if they had hydrocele. Signs of lymphedema in the lower extremities were recorded by trained nurses during physical examination in the interviews. For individuals with lymphedema, an algorithm was used to differentiate between LF and podoconiosis as described elsewhere [18].

Data entry and analysis

Motorola Atrix HD smartphones with GPS capabilities, long life batteries and an android application were used for data collection for the coordinated mapping of LF and podoconiosis, as detailed elsewhere [18]. Data were then downloaded in Excel format and imported to STATA 13.0 (Stata Corporation, College Station, TX) for further cleaning and analysis. Maps of infection distribution were generated using ArcGIS 10.2 (ESRI, California). Hierarchal data were collected using separate surveys for community level information and for individual level information. The different survey data sets were later linked to produce a complete analytic database. The community survey forms included population counts and information about community-wide treatment of LF and other deworming activities in the past year. Detailed description and results of the demographic and podoconiosis surveys have been published separately [18].

An endemic district for lymphatic filariasis was defined using a threshold for LF infection of at least one infected individual in either of the two selected communities in each *woreda*. This definition is consistent with the WHO definition of endemic IUs (being those where any

subunit of the district has an antigenemia or microfilaremia rate of 1% or greater). The relationship between infection and risk factors was investigated using univariate logistic regression, adjusted for clustering at the community level. As one of the objectives of this study is to determine the population at risk in 2016, when national MDA coverage is anticipated, we used the populations projections provided by the Federal Democratic Republic of Ethiopia Central Statistical Agency (http://www.csa.gov.et/images/general/news/pop_pro_wer_2014-2017_final). Using the government projection figures at the Woreda level encouraged communication between the WHO country office, the Federal MOH, Regional Health Bureaus and the NTD programmes in preparing requests for medicines for MDA.

Ethical approval and consent procedures

Ethical clearance for the study was granted by the Research Ethics Committee of the Liverpool School of Tropical Medicine (Research Protocol 12.22), the Institutional Review Board of the Medical Faculty, Addis Ababa University and the ethics committee at the Ethiopian Public Health Institute (EPHI). Details on the consent procedures are provided elsewhere [18]. Briefly, individual written informed consent was obtained from each participant aged 18 years and above. Consent for younger survey participants, 15 to 18 years of age was obtained from their parents/guardian and the participant themselves provided informed assent. The response rate was 98.6%. For those with lymphoedema, health education was given about how to manage their condition and prevent any disability.

Results

Overall, 130 116 people were examined in 1315 communities in 658 *woredas* (Table 1). Fifty-two *woredas*, mostly in the Somali region, could not be surveyed because they were not easily accessible due to major logistical challenges encountered by the survey teams. The median age

Table 1. The number of implementation units (IUs) per region and populations at risk for LF determined by immunochromatographic card tests (ICT) in 11 regions/zones in Ethiopia. The 75 endemic IUs identified during the 2013 mapping surveys included 45 with borderline ICT results based on one ICT+ only. The updated number of endemic IUs, including results from previous surveys, and the projected population at risk in 2016 are presented in the last two columns. Populations for 2016 are based on population projection estimates provided by the Federal Democratic Republic of Ethiopia Central Statistical Agency: http://www.csa.gov.et/images/general/news/pop_pro_wer_2014-2017_final.

Region	No. of IUs	Population of IUs	No. of IUs mapped	No. of people examined for LF	No. positive for LF	No. of endemic IUs (1 ICT+)	Population of endemic IUs (1 ICT +)	No. of endemic IUs (>1 ICT+)	Population of endemic IUs (> 1 ICT+)	No. of endemic IUs (all surveys)	Total Population at risk for LF
Tigray	47	5151998	46	9 164	8	4	469503	1	111993	5	581496
Afar	33	1769002	32	6 289	1	1	41360	0	0	1	41360
Amhara	153	20769985	140	27 721	19	13	2458242	3	240171	19	2973020
Oromia	308	34575008	243	48 003	62	19	2166826	13	1353217	36	3990525
Somali	72	5598002	49	9 583	0	0	0	0	0	0	0
Benshangul G	21	1033999	0	0	0	0	0	0	13	13	604592
SNNPR	158	18719008	128	25 354	49	7	1036930	13	1371534	30	3176186
Gambella	13	422002	0	0	0	0	0	0	0	7	194499
Harari	9	240000	9	1 800	1	1	17621	0	0	1	18332
Dire Dawa	8	453000	7	1 402	0	0	0	0	0	0	0
Addis Ababa	10	3352000	4	800	0	0	0	0	0	0	0
Total	**832**	**92084004**	**658**	**130 166**	**140**	**45**	**6 190 482**	**30**	**3 076 928**	**112**	**11 580 010**

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of individuals was 34 years (Inter-quartile range (IQR): 25–46), but this ranged from 30 (IQR: 23–40) years in Afar Region to 39 (IQR: 27–50) years in Somali Region, with age range between 15 and 100 years. Many survey participants had been resident in their respective regions for more than 30 years (median: 30, IQR:22–45) but very few had attained education levels beyond the primary school grade. The mean number of individuals surveyed per community was 99 (sd: 9.3).

LF endemicity and distribution

In total, 140 people were found to be positive for CFA by ICT performed in 89 different communities (Table 1). The median age of CFA-positive individuals was 35.8 (IQR: 25–41.5) years with range between 15 and 80 years. Fig 1 shows the location of all 1315 communities that were surveyed and the distribution of the 89 communities with CFA positive individuals. Communities with CFA positive individuals were observed in all the regions surveyed with the exception of Somali and the two city administration councils of Addis Ababa and Dire Dawa. Only one community was shown to be positive in the Afar Region. Communities with positive individuals were equally distributed in the northern and southern parts of the country. The CFA positivity rates for males and females in the endemic *woredas* were 0.8% (60/7443) and 1.1% (80/7429), respectively.

Based on WHO guidelines, whereby *woredas* with any CFA positive individual are classified as endemic [10], 75 of the 658 *woredas* surveyed in the nine regions were found to be endemic (Fig 2), including 45 *woredas* where only one ICT positive was observed (in yellow). The estimated population for these 75 endemic *woredas* is 9 267 410, based on the projected population for 2016 as indicated previously.

Fig 3 shows the updated endemicity map for Ethiopia incorporating data from previous published mapping surveys [15]. The estimated total population at risk of LF for the 112 endemic districts in Ethiopia is 11 580 010, including 6 190 482 from the 45 *woredas* where only 1 ICT positive individual was identified during the survey, being therefore on the margin of the endemicity threshold. This number includes the 75 newly-discovered endemic *woredas* and the 37 previously known to be endemic.

Relationship between reported interventions and CFA positivity

Individuals who were residing in the endemic *woredas* were interviewed about interventions that might impact exposure to LF and the likelihood of becoming CFA positive. In the 75 newly-identified endemic *woredas*, out of 14 676 people who responded to questions about previous treatment with an LF medication, only 499 (3.4%) reported that they had received treatment for LF. We found no significant association between CFA positivity rates and reported previous treatment for LF. Similarly, there was no association between reported deworming and a CFA negative outcome (OR 1.14, 95% confidence interval (CI) 0.77–1.69). We also failed to demonstrate an association between bednet usage and being negative for CFA (OR 1.04, 95% CI 0.74–1.45).

Relationship between LF morbidity and CFA positivity

The risk of presenting with lymphedema at any stage was almost double (OR 1.80, 95% CI 1.68–1.93) for those living in an endemic *woreda*. Hydrocele was self-reported by individuals and not verified by health personnel which represents a potential limitation in accuracy of any association found. Nevertheless, we found almost twice the risk (OR: 1.91, 95%CI: 1.4–2.5) of reporting hydrocele among males living in endemic compared to non-endemic areas.

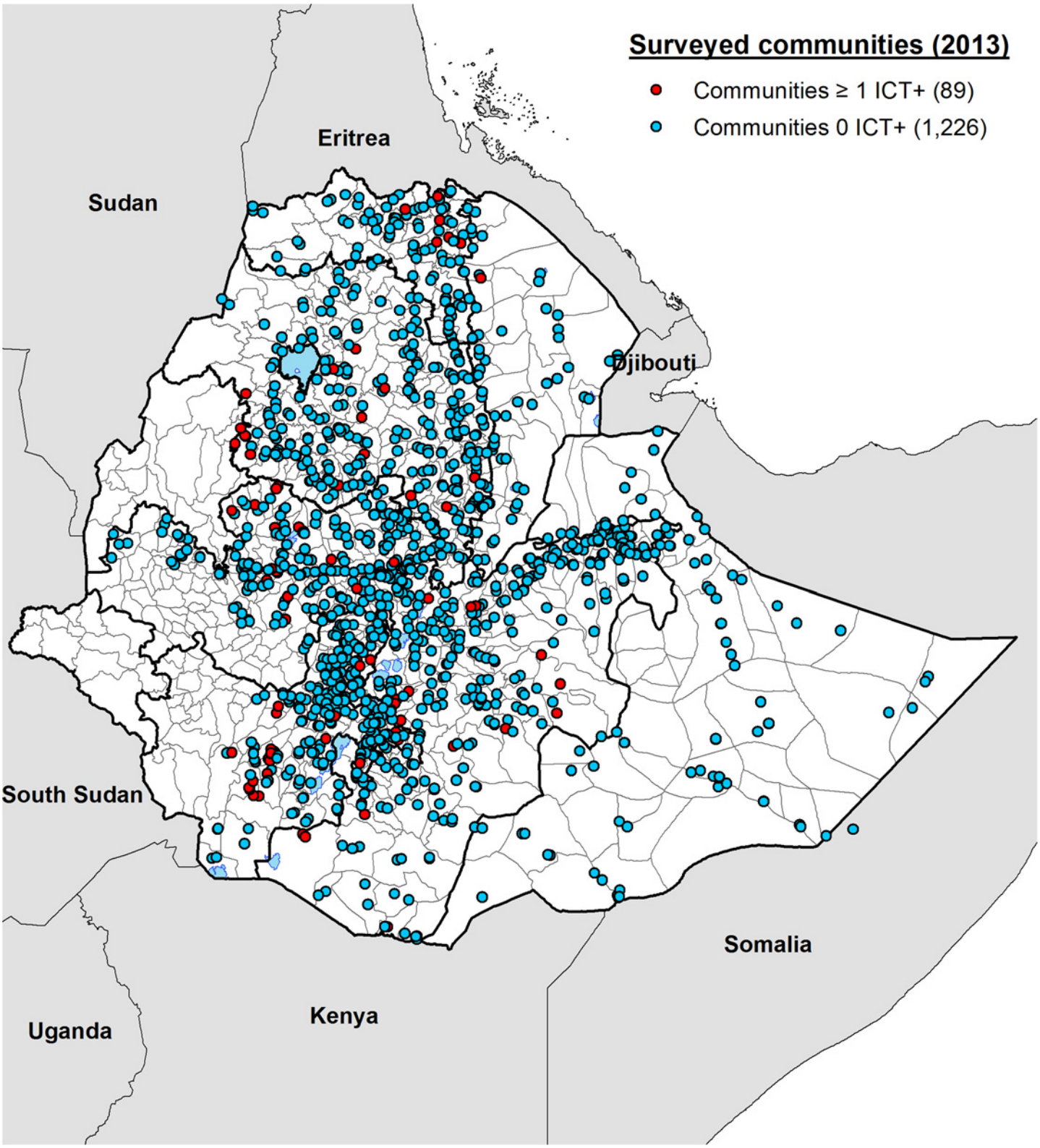

Fig 1. Map showing the locations of 1315 communities surveyed in Ethiopia during the mapping project in 2013. Eighty-nine communities in which one or more persons out of 100 individuals tested were found to be positive for circulating filarial antigen (CFA) are shown in red. Communities where no positive individuals were identified after testing approximately 100 adults are marked in blue.

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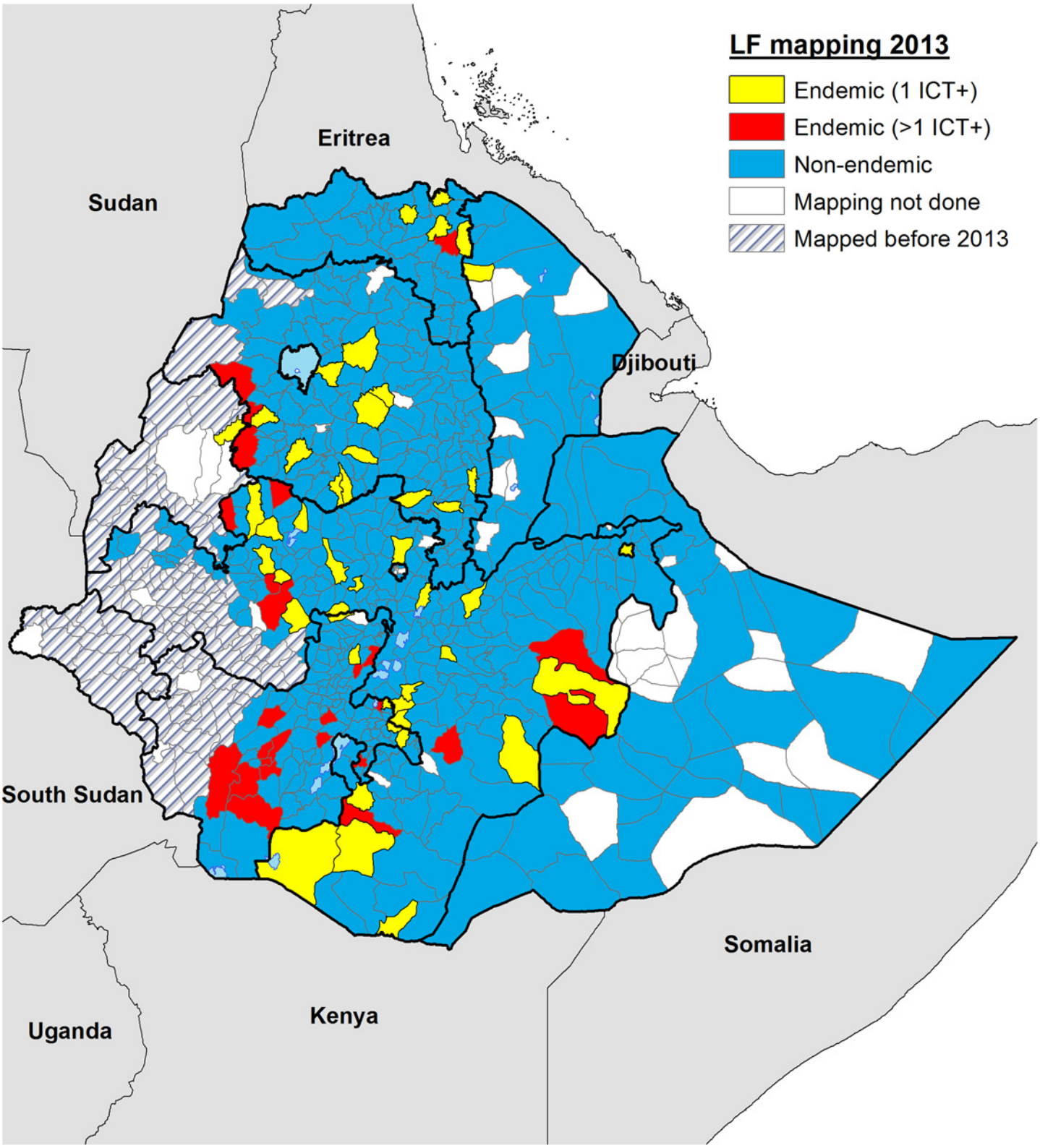

Fig 2. Map showing the distribution of endemic and non-endemic implementation units (IUs) or *woredas* identified during the 2013 mapping surveys in Ethiopia. After completing surveys in 658 implementation units, 75 were classified as endemic based on finding at least one infected person after testing approximately 200 individuals in two different communities. Forty-five of the endemic IU, shown in yellow had borderline endemicity with only one CFA positive person identified (1 ICT+). The 30 IUs with two or more CFA positive individuals (>1 ICT+) are shown in red.

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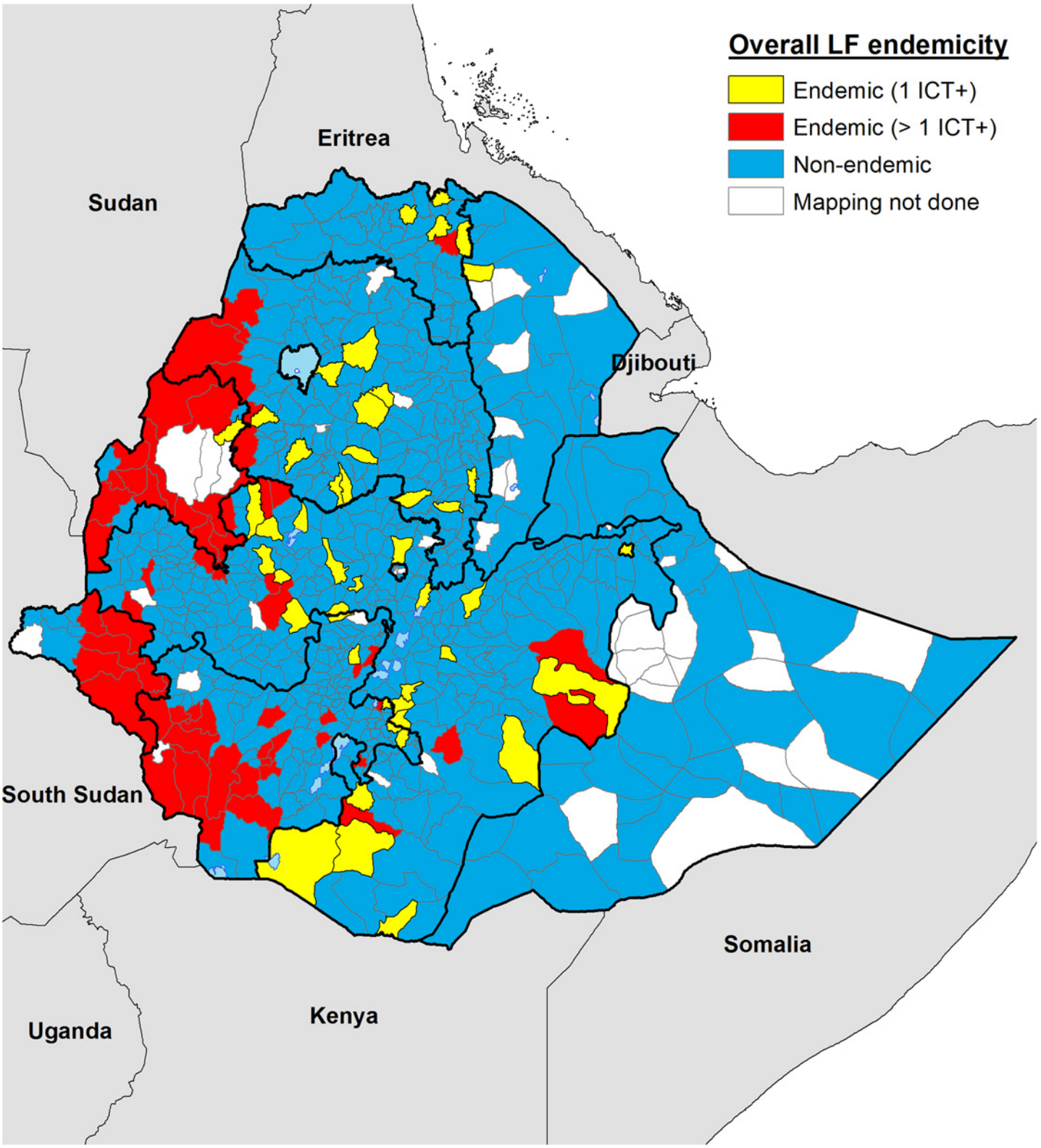

Fig 3. Current LF endemicity map for Ethiopia for all IUs surveyed to date. Endemic IUs are shown in red (>1 ICT+) and yellow (1 ICT+) depending on the ICT results. Non-endemic IUs are shown in blue leaving only 58 districts yet to be mapped.

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Discussion

Our surveys increased the number of *woredas* mapped for LF in Ethiopia from 116 to 774 representing 93% of the mapping required. With only 58 *woredas* out of 832 remaining to be mapped, the nationwide mapping of LF in Ethiopia indicates that 112 districts across the country are endemic for the disease. The projected population that will require treatment in these endemic districts in 2016, when national MDA coverage is anticipated, is 11 580 010. The population at risk for LF, determined through our surveys, is 60% lower than the current WHO estimates [8]. Our findings are important in providing well-informed nationwide estimates of the distribution of LF and demonstrating the value of coordinated mapping of multiple diseases [18] that can result in significant cost savings for national NTD programmes that currently rely on disease-specific mapping protocols [19].

Historically, LF has been reported as having limited distribution in the southwest of the country. The disease was first detected in a native Ethiopian in 1937 [13] but it was never reported as widely distributed outside the Gambella area, where examination of 1 ml of diurnal blood in 82 adults in 1971 revealed a 24% microfilaraemia rate[13]. A more recent survey in Gambella (1993) confirmed the high transmission intensity of *W. bancrofti* in this area, with an average microfilaraemia rate of 20.7% in the population surveyed at two communities adjacent to the Baro river [20]. Hydrocele and elephantiasis were however uncommon in the Gambella area and studies carried out in 1976 [21] ruled out the involvement of *W. bancrofti* in the occurrence of elephantiasis in the highlands, where this condition had previously been reported [22, 23]. Entomological investigations in the Gambella area showed that *Anopheles gambiae s.l* (probably *An. arabiensis*) and *An. funestus* were the main vectors, with no evidence for the involvement of the culicine mosquitoes despite the high biting rates [13]. A total of 3 228 *Mansonia* mosquitoes, vectors of LF in West Africa [24], were dissected but none was found to be infected. However, the *W. bancrofti* infectivity rates in *An. gambiae s.l* (0.24%) and *An. funestus* (0.35%) were lower than what was reported in other parts of East Africa at the time, including Kenya [25], and Tanzania [26], suggesting that the intensity of LF transmission in the Gambella area was relatively low despite the high MF rate shown in adults. The low infectivity rates for the vectors in Ethiopia were probably related to the low intensity of infections observed because 9 of the 20 infected persons were low density MF carriers with less than 6 mf/ml of blood. Only 3 of the 20 infected persons harboured more than 100 mf/ml of blood [13]. In his review, Southgate [27] concluded that *Anopheles* mosquitoes are poor vectors when their blood-meal source is presented by low density MF carriers.

Cano and co-workers [28] have recently modelled the spatial limits of LF transmission for Africa using *boosted regression trees* (BRT) approach over a suite of environmental and climatic data and available mapping data, including data generated by this study,. The environmental suitability for LF was shown to be very low for over 80% of Ethiopia and moderate for narrow bands in the western and south western parts of the country [28, 29]. Recent predictive maps of LF prevalence in Africa, performed using Bayesian geostatistics modelling approach, presents Ethiopia as one of the lowest burden countries in Africa with an estimated infection rate of 2.8% for 2000 and disease distribution limited to less than 20% of the country [28–30].

It is not clear how the WHO estimate of 30 million people at risk for LF in Ethiopia was derived. Based on the high proportion of low density MF carriers in endemic areas, consistent prediction of transmission probability in less than 20% of the landmass by predictive models and the relative inefficiency of the LF vectors in Ethiopia [28–30], it is highly unlikely that 30 million people are at risk for LF in Ethiopia. The wide distribution of malaria, which is transmitted by LF vectors, and lymphedema associated with podoconiosis may have historically suggested a wider distribution of the disease than demonstrated by the current study. Additional

entomological studies to improve understanding of mosquito distribution and LF transmission dynamics would be beneficial to inform public health policies.

There are three important limitations of the mapping methods used on this research. Firstly, mapping LF has the objective of identifying areas where transmission is active and therefore preventive chemotherapy is required. In 45 out of 658 IUs surveyed during this exercise, only one positive individual for CFA was identified. Fig 2 shows in yellow the location of these 45 districts with 'borderline' results. ICT cards are not 100% sensitive and specific, furthermore antigenemia may remain positive even after infection has been cleared and individuals can move from one district to another, therefore it is arguable whether one positive individual by ICT equates to active transmission in an IU. Operational research in these 45 borderline results districts is therefore required to assess the use of more robust tools to determine whether transmission of LF is truly active and MDA necessary. Greater geographical representation will be required in the districts in which purposive sampling has failed to identify a significant number of positive individuals. Demonstrating that MDA is not needed in any of these 45 IUs would potentially represent important cost savings in terms of cost of drugs, distribution, supervision, coverage surveys, impact assessments and surveillance post MDA. Secondly, although we found twice the risk of presenting with clinical symptoms for those living in endemic *woredas*, due to the similarities between LF and podoconiosis morbidity, selection of sites for mapping may have failed to identify areas where LF transmission is active. Thirdly, we cannot completely rule out ongoing transmission in other communities within a *woreda* just because no CFA-positive individuals have been found in the two communities selected for the mapping survey. The selection of these communities relies on the evidence or suspicion of ongoing transmission, normally based on the report of clinical cases or environmental suitability for transmission. In low endemicity settings, few clinical cases are expected and therefore hot spots of LF transmission can easily be overlooked by the health public system. According to our results, confirmation using more statistically robust methods and a larger sample size with greater geographical representation will be required in those districts declared non-endemic to certify that they are completely free of LF before WHO can provide certification that Ethiopia has eliminated LF. Moreover, the sensitivity of the diagnostic test used for LF in this study is less than 100% and the identification of a single ICT positive adult may not provide evidence of disease transmission. Based on these limitations, in addition to the restricted geographical representation of just two sites within a *woreda*, we recommended conducting operational research in the 45 *woredas* with borderline results (one ICT+) to shrink the denominator even further. This recommendation was endorsed by the WHO AFRO Regional Programme Review Group (RPRG) in 2014 and submitted to the Federal Ministry of Health through the WHO country office for action.

The present study was conducted as part of an integrated mapping project for LF and podoconiosis [18] and the nationwide survey was completed within three months. During that period we visited 1315 communities and examined 130 166 individuals in 658 *woredas* across Ethiopia. A detailed description of the integrated mapping of lymphatic filariasis and podoconiosis and the lessons learnt from the exercise have been published separately [18]. Mapping the overlap of multiple NTDs in an implementation unit is critical for informing strategies for interventions, morbidity management and disability prevention. This is particularly important in areas in which LF may be co-endemic with loiasis, malaria, onchocerciasis or podoconiosis. The Ethiopian government planning budgets for disease-specific mapping of LF and podoconiosis in 710 planned districts, including diagnostics, training, field work, data management, supervision, were $1 212 209 and $1 211 664 respectively, but the actual financial cost of our coordinated mapping of LF and podoconiosis was only $1 291 400 for 658 districts [18]. This significant reduction in total cost for mapping both diseases was achieved through savings in

the areas of team training, supply chain management and travel. The total cost of uncoordinated disease specific mapping for the two diseases was 1.9 times as high as the integrated survey approach [18].

In conclusion, with 93% of the *woredas* in Ethiopia mapped, the LF map of Ethiopia is almost complete. In the process, we demonstrated that the number of people living in LF endemic areas is 60% lower than previous estimates. We also showed that integrated mapping of multiple NTDs is feasible and cost-effective and if properly planned, can be quickly achieved at national scale. This is very encouraging for accelerating the mapping of NTDs in Africa, estimating the true population at risk of LF, scaling up to reach 100% geographical coverage of MDA and banishing lymphatic filariasis to history.

Supporting Information

S1 Checklist. STROBE Checklist.
(DOC)

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Author Contributions

Conceived and designed the experiments: MJB MPR. Performed the experiments: MPR HS KD AA OS AK. Analyzed the data: MPR JC. Contributed reagents/materials/analysis tools: MPR JC. Wrote the paper: MJB MPR JC SJB GD AGE OS AK.

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Paper 2. Elimination of Lymphatic Filariasis in The Gambia

Rebollo, M. P[1,4,*]., S. M. Sambou[2], B. Thomas[1], N. K. Biritwum[3], M. C. Jaye, L[1]. Kelly-Hope[1], A. G. Escalada[4], D. H. Molyneux[1], and M. J. Bockarie[1]. 2015. 'Elimination of Lymphatic Filariasis in the Gambia', *PLoS Negl Trop Dis*, 9: e0003642.

Authors affiliation:

1 Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom,

2 Ministry of Health and Social Welfare, Banjul, The Gambia,

3 Ghana Health Service, Accra, Ghana

4 Rey Juan Carlos University, Madrid, Spain

* Current address: Neglected Tropical Diseases Support Center, Task Force for Global Health, Decatur, Georgia, United States of America

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Elimination of Lymphatic Filariasis in The Gambia

Maria P. Rebollo[1¤], Sana Malang Sambou[2], Brent Thomas[1], Nana-Kwadwo Biritwum[3], Momodou C. Jaye[2], Louise Kelly-Hope[1], Alba Gonzalez Escalada[4], David H. Molyneux[1], Moses J. Bockarie[1]*

1 Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, **2** Ministry of Health and Social Welfare, Banjul, The Gambia, **3** Ghana Health Service, Accra, Ghana, **4** Rey Juan Carlos University, Madrid, Spain

¤ Current address: Neglected Tropical Diseases Support Center, Task Force for Global Health, Decatur, Georgia, United States of America
* moses.bockarie@lstmed.ac.uk

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Abstract

Background

The prevalence of *Wuchereria bancrofti*, which causes lymphatic filariasis (LF) in The Gambia was among the highest in Africa in the 1950s. However, surveys conducted in 1975 and 1976 revealed a dramatic decline in LF endemicity in the absence of mass drug administration (MDA). The decline in prevalence was partly attributed to a significant reduction in mosquito density through the widespread use of insecticidal nets. Based on findings elsewhere that vector control alone can interrupt LF, we asked the question in 2013 whether the rapid scale up in the use of insecticidal nets in The Gambia had interrupted LF transmission.

Methodology/Principal Finding

We present here the results of three independently designed filariasis surveys conducted over a period of 17 years (1997–2013), and involving over 6000 subjects in 21 districts across all administrative divisions in The Gambia. An immunochromatographic (ICT) test was used to detect *W. bancrofti* antigen during all three surveys. In 2001, tests performed on stored samples collected between 1997 and 2000, in three divisions, failed to show positive individuals from two divisions that were previously highly endemic for LF, suggesting a decline towards extinction in some areas. Results of the second survey conducted in 2003 showed that LF was no longer endemic in 16 of 21 districts surveyed. The 2013 survey used a WHO recommended LF transmission verification tool involving 3180 6–7 year-olds attending 60 schools across the country. We demonstrated that transmission of *W. bancrofti* has been interrupted in all 21 districts.

Conclusions

We conclude that LF transmission may have been interrupted in The Gambia through the extensive use of insecticidal nets for malaria control for decades. The growing evidence for the impact of malaria vector control activities on parasite transmission has been endorsed

by WHO through a position statement in 2011 on integrated vector management to control malaria and LF.

Author Summary

The prevalence of lymphatic filariasis (LF), in The Gambia was among the highest in Africa in the 1950s when about 50% of the adult population was positive for microfilaraemia. However, surveys conducted in 1975 and 1976 revealed a dramatic decline in LF endemicity in the absence of systematic treatment with anti-filaria medicines. This decline in LF prevalence in all villages was partly attributed to a significant drop in human-mosquito contact through a sustained reduction in rainfall in the 1960s and 1970s and the widespread use of insecticidal nets to protect against malaria. We asked the question in 2013 whether the rapid scale up in the use of insecticidal nets for malaria control in Gambia had resulted in the interruption of LF transmission. In this paper we present the results of three independently designed filariasis surveys conducted over a period of 17 years (1997–2013), and involving over 6000 subjects in 21 districts across all administrative divisions in the country. In 2001, tests performed to detect circulating filarial antigens (CFA) in serum samples collected between 1997 and 2000, in three divisions, failed to show positive individuals from two that were previously highly endemic for the LF. Results of the second survey conducted in 2003 indicated that five of the 21 districts were slightly endemic for LF with CFA rates of 1% or 2% but MDA was never implemented in The Gambia. The results of our final survey conducted in 2013 were unequivocal in confirming the absence of transmission of LF in all 21 districts surveyed using WHO recommended statistically robust and validated tool known as transmission assessment survey (TAS). The Gambia achieving a non-endemic status for LF represents a significant step in the efforts to shrink the filariasis endemicity map and demonstrates the value of cross sector approaches in disease control.

Introduction

The Gambia is among 73 countries currently considered endemic for lymphatic filariasis (LF) by the World Health Organization [1]. LF, a neglected tropical disease (NTD), is a debilitating mosquito-borne nematode infection that affects 120 million people in low and middle income countries where 1.4 billion people are exposed to the parasites [1]. *Wuchereria bancrofti*, the causative agent of LF in The Gambia, is responsible for over 90% of the LF infections worldwide; *Brugia malayi* and *Brugia timori* account for the remaining infections and have a distribution restricted to the southeast Asian region [2]. The LF parasites are carried by various species of mosquito vectors from the genera *Anopheles*, *Aedes*, *Culex* and *Mansonia* but in sub-Saharan Africa, *Anopheles* species are the principal vectors[3]. There is no evidence that *Culex* species play any significant role in West Africa where the malaria vectors, *An. gambiae* s.l and *An. funestus*, are also the vectors of *W. bancrofti* [3–9]. In 1997, LF was targeted for elimination when the World Health Assembly adopted Resolution WHA 50.29 calling for the elimination of the disease as a public health problem globally [10]. In 2000, WHO, in collaboration with pharmaceutical companies and implementing partners launched the Global Programme to Eliminate LF (GPELF) as a disease specific intervention initiative to interrupt transmission and alleviate morbidity[11]. The GPELF has two strategic objectives for achieving this goal: 1)

interruption of parasite transmission through mass drug administration (MDA) using albendazole in combination with either ivermectin or diethylcarbamazine citrate (DEC) and 2) morbidity management and disability prevention (MMDP) by providing access to care for those who suffer clinical manifestations of LF in endemic areas.

By 2012, 56 of the 73 countries where LF is considered endemic had started implementing MDA to eliminate the disease [1]. Among countries implementing MDA, only 13 countries had completed at least five rounds of MDA and moved to post-MDA surveillance phase [1]. Nevertheless, the scale up in the use of insecticidal nets and vector control in Africa, where 17 countries including The Gambia are yet to start MDA, has significantly reduced mosquito densities in many of these countries and contributed to a significant reduction in malaria prevalence [12, 13] and a possible decline in filariasis endemicity [3, 7, 14, 15]. In Solomon Islands where LF was transmitted by *Anopheles* mosquitoes, anti-mosquito measures to control malaria resulted in the interruption of LF transmission in the absence of MDA [16–18].

The Gambia had historically high prevalence of LF as described by Hawking [4, 5] in his historical reviews of the distribution of filariasis in Africa where he stated that the prevalence of *W. bancrofti* among adults in the Gambia in the 1950s [19, 20] was about 50%—one of the highest in the world. Prevalence surveys conducted in 17 villages across the country in 1975 and 1976 reported village specific microfilaraemia (MF) rates for people ≥15 years ranging from 2.9% to 26.9%; and the apparent decline after 25 years was mainly attributed to a reduction in mosquito density[21]. Nevertheless, MF positive children (<15 years) were present in many villages in the Upper River, Western and Lower River divisions where LF was still highly endemic.

WHO estimates that 1.2 million people require preventive chemotherapy against LF in The Gambia [22]. In line with the growing momentum to shrink the map of LF endemicity, we performed transmission assessment surveys (TAS) in The Gambia in May and June 2013 to determine if the widespread use of insecticidal bed nets and other vector control efforts over the past decades had eliminated transmission of *W.bancrofti* in the absence of MDA [12, 14]. The increasing momentum to take the "neglected" out of NTDs is driving public and private partners including drug companies, donors, and governments committed to what is now referred to as the 2012 London Declaration to shrink the NTD map and eliminate or eradicate by 2020 ten NTDs including LF [23]. The commitments made to date have been centred on ensuring the supply of drugs needed to implement preventive chemotherapy with limited emphasis on alternative intervention strategies such as vector control through the use of long lasting insecticidal nets (LLINs) or indoor residual spraying. Demonstrating the interruption of active transmission of *W.bancrofti* in The Gambia, in the absence of MDA, could have wide ranging public health and policy implications with regard to alternative strategies and synergies between malaria and NTD control programmes. In this paper we report the application of a new WHO methodology, the Transmission Assessment Survey (TAS), to validate the interruption of transmission in the absence of MDA against the disease in The Gambia after two mapping surveys carried out 15 years earlier suggested a dramatic decline in LF endemicity following a rapid decline in transmission and the possibility that elimination in the country could be achieved. The results presented here were collected over a period of 17 years (1997–2013), from three independently designed surveys, involving over 6000 subjects in 21 districts across all administrative divisions in the country.

Materials and Methods

Study area

The Gambia is a country in West Africa situated on either side of the Gambia River which flows through the country's centre and empties into the Atlantic Ocean. It lies between latitudes

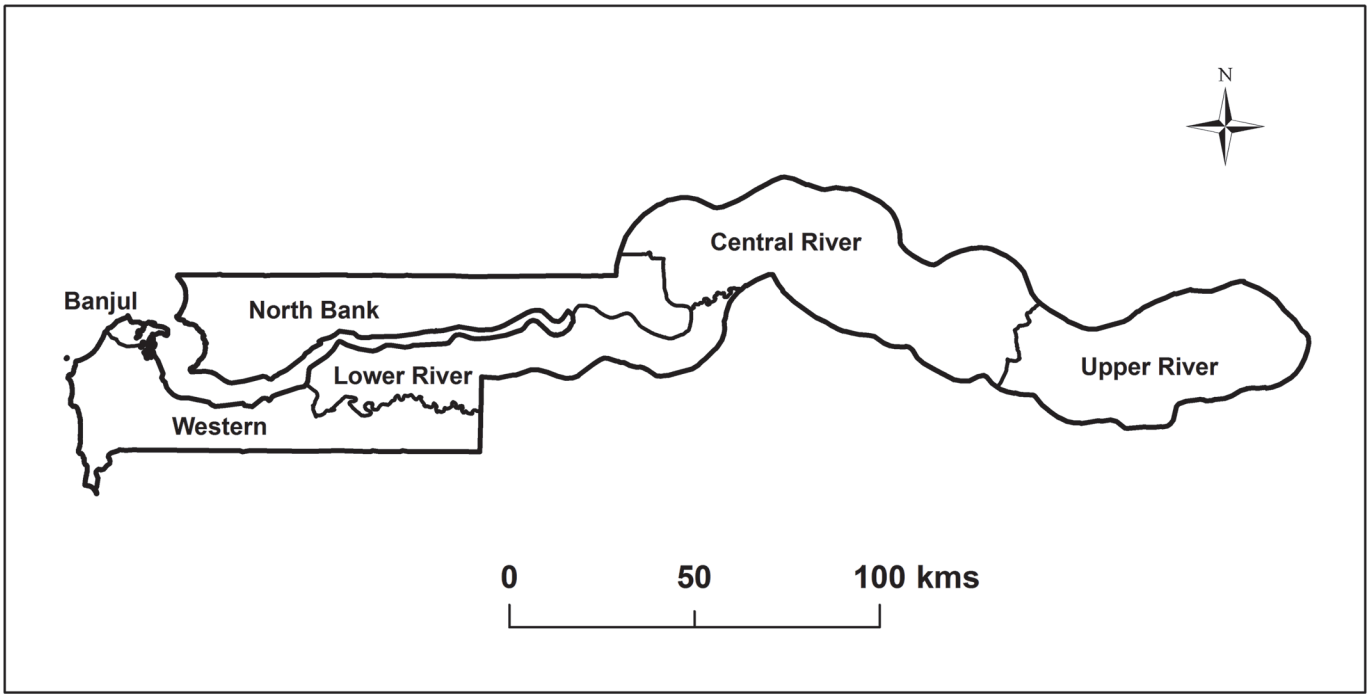

Fig 1. Map of The Gambia showing the historical boundaries for the six administrative divisions recognised before 2000.

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13° and 14°N, and longitudes 13° and 17°W, and stretches inland for approximately 400 km. The Gambia is less than 48.2 km wide at its widest point, with a total area of 11,295 km^2 and is surrounded by Senegal. It is the smallest country on mainland Africa with a population of 1,882,450. The climate is typically sub-Sahelian with one short rainy season from June to October. The country is presently divided into eight administrative divisions, including the national capital, Banjul. Prior to 2000, there were only 6 divisions in the country: Banjul, Western, North Bank, Lower River, Central River and Upper River. To retain geographic context with regard to the historical distribution of LF in The Gambia we will adhere, in this paper, with the former administrative boundaries demarcating 6 administrative divisions as shown in Fig. 1.

Study design and blood sampling surveys

This is an observational study comparing historical prevalence from studies conducted in the 1950's and 1970's (Fig. 2) with 3 cross sectional surveys conducted in 2001, 2003 and 2013 examining LF infection through the distribution of circulating filarial antigen (CFA) positive individuals, involving over 6000 children and adults in 21 districts across all administrative divisions in The Gambia.

1. In 2001, stored serum samples from people ≥12 years residing in the previously highly endemic Divisions (North Bank, Upper River and West Coast) between 1997 and 2000 were tested for CFA. The serum/plasma samples were initially collected for malaria studies and stored in the British Medical Research Council (MRC) Laboratories in Fajara, The Gambia. The MRC laboratories established in The Gambia in 1947, carried out the first LF surveys in the country in 1951 but their primary focus for research is malaria. They routinely perform malaria surveys for several projects storing serum samples which can be processed for other parasites.

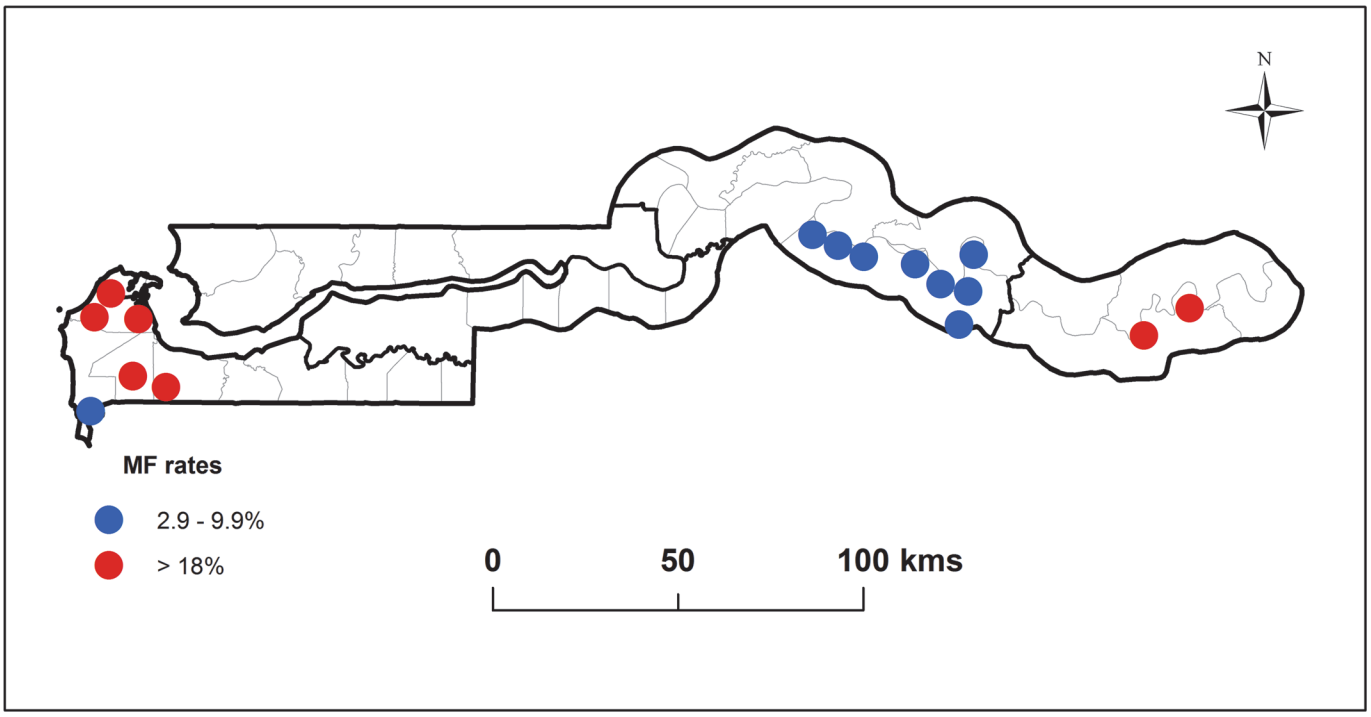

Fig 2. Map of The Gambia showing the location and microfilaria (MF rates in 15 villages surveyed in 1975 and 1976 (adapted from Reference #20) in areas of low transmission (Blue) where MF rates were less than 10% (Upper River and Western Divisions) and high transmission (Red) where MF rates where greater than 18% (Central River Division).

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2. In 2003, a national LF mapping survey was carried out by the Ministry of Health and Social Welfare (MOHSW) in 30 high risk villages in all administrative divisions across the country to determine LF distribution and endemicity, and identify implementation units eligible for MDA. High risk communities were identified based on criteria described in the WHO manual for monitoring and evaluation (M&E) of LF programs [24], including historical data on LF, presence of diseased people and ecological suitability for mosquito breeding. MDA was, however, never implemented as the 2003 mapping results were not presented to the WHO Regional (African) Programme Review Group (RPRG) for review and determination of the need for MDA. This survey was based on sampling about 100 people in each high risk village purposely selected according to WHO mapping guidelines developed in 2000 [25].
3. In 2013, a school based TAS involving 60 randomly selected schools, was carried out as described below, to verify the absence of transmission after the remarkable decline towards extinction in CFA rates revealed by the 2003 survey results when compared with historic data presented in Fig. 2[21].

Transmission assessment surveys. A transmission assessment survey (TAS) protocol, designed for post MDA surveys, was used in this study to determine incidence of LF in the absence of MDA and to verify the absence of transmission after the remarkable decline towards extinction in CFA rates revealed during the 2003 survey. Molyneux and colleagues [26] discussed disease eradication, elimination and control and the need for accurate and consistent usage and defined elimination as the reduction of incidence to zero.

The design of the TAS described in the WHO M&E manual for national elimination programmes [24, 27], is based on lot quality assurance sampling (LQAS) with the sampling

method used in the evaluation units (EU) being determined by the net primary school enrolment rate, the target population size and number of schools. Based on that, a random cluster sampling was chosen with schools as the sampling sites. A Survey Sample Builder (SSB) tool was used to determine sample size and sampling intervals. School surveys were conducted in all divisions as the net primary enrolment rates exceeded 75% (74%-78%). All children in grades 1 and 2 were eligible including a small proportion of those outside the 6–7 age range. Two evaluation units (EU) were created according to LF endemicity based on historical data as described above. The high transmission divisions of Western 1, North Bank, Lower River and Upper River were grouped into EU1 while Banjul, Western 2 and Central River Divisions were grouped into EU 2. Western 1 and Western 2 were previously part of the Western Division.

The sample sizes generated by the SSB were 30 schools clusters for each EU and 1552 children with a critical cut-off of 18 CFA positive children for the EU1 and 1556 children with a critical cut-off of 18 for EU2. Children who had not provided assent or parental custodian agreement, the severely sick and those who had lived in the community for less than two years were excluded from the TAS survey.

Immunochromatographic tests (ICT) for CFA

The rapid Immunochromatographic tests (ICT) were used to test samples collected during the three surveys described above. At the point of care, the ICT card test was performed on whole blood or serum as described in the WHO monitoring and impact assessment manual [24] and following manufacturer's instructions. The ICT cards used in 2001 and 2003 were produced by AMRAD ICT, New South Wales, Australia[28] while the cards used for the TAS surveys in 2013 were manufactured by Binax NOW Filariasis ICT card test (Alere Inc., Scarborough, ME). Both the AMRAD and Binax NOW ICTs were based on the same reagents and required the same quantify of sample volume (100 μl blood/serum) for testing. A positive [28]antigen control was used to test the validity of the ICT cards before the start of the survey in 2013.

Data entry and analysis

Data generated before 2013 was managed through a simple spread sheet that calculated percentages for comparative analysis. The TAS data was managed through a Microsoft Access based data management system specifically developed by the Centre for Neglected Tropical Diseases (CNTD) in the Liverpool School of Tropical Medicine (LSTM) to support the cluster survey. Data entry was carried out by two independent clerks using a double entry system that automatically compared the two entries to detect and reconcile any discrepancies. The TAS critical cut-off value represents the threshold of infected individuals below which transmission is expected to be no longer sustainable, even in the absence of interventions.

If the total number of positive cases is at or below the critical cut-off, the EU 'passes' the survey and it is considered that transmission will no longer be sustainable. If the total number of positive cases is above the critical cut-off transmission is still ongoing in the evaluated unit[24]. TAS sample sizes and critical cut-off values are powered so that the EU has at least a 75% chance of passing if the true antigen prevalence is half the threshold level (2% for *Culex*, *Anopheles*, and *Mansonia* vector areas, and 1% for *Aedes* vector areas). In addition, there is no more than a 5% chance of passing if the true prevalence is greater than or equal to the threshold level.

Ethical approval and consent procedures

Ethical clearance for the study was granted by The Medical Research Council (MRC) Laboratories Gambia Scientific Coordinating Committee, the LSTM Research Ethics Committee

(Research Protocol (11.89R) and the Gambian Epidemiology and Disease Control Unit (EDC), of the MOH (correspondence 23 August 2012). After approval for TAS from the Education Ministries and school authorities, the MOHSW team met with the Head Master of each school to obtain permission to conduct the survey and to schedule a date for meeting with the parents. The survey team explained the purpose of the surveys and received oral consent from teachers and parents. Non-consenting parents or non-assenting children were not included in the survey. All individuals could drop out from the study any time during the study.

Results

Historical

The results of the surveys described here are best presented in the context of the historical distribution and endemicity of LF that informed the design of the surveys carried out to determine the pattern of LF burden and distribution in the absence of MDA. Fig. 2, adapted from the results of Knight [21] shows the burden and distribution of MF in 15 villages across five of the 6 divisions involved in this study carried out in the 1970s. Villages with MF rates of 18% or higher were found only in the Upper River and Western divisions. The MF rates in study villages in the other divisions were lower than 10%.

Initial endemicity assessment: 1997–2000

The initial assessment of LF endemicity was carried out in the Upper River and Western Divisions historically known to be highly endemic and the North bank where the MRC malaria project was based. In 2001 a total of 268 stored serum samples collected between 1997 and 2000 from people ≥12 years residing in three villages (Basse, Brefet, Farafenni) in the three divisions, were tested with ICT for the presence of CFA. CFA positive individuals (n = 8) were only found in Basse village in the Upper River Division where 100 samples were tested. All 68 samples from Brefet (Western Division) and 100 from Farafenni (North Bank Division were negative for CFA. Results are shown as stars in Fig. 3. The red star indicates the location of the village where CFA positives were found in the Upper River Division.

The 8% CFA rate observed for the endemic division corresponds to between 2% and 4% MF rate [29] indicating 89–91% reduction in MF rate over 20 years in the absence of MDA in comparison to the 21% (32/148) MF rates observed in the same area in 1976 by Knight [21]. The MF rates in the Western division decreased from 15% (66/439) in 1976 to 0% (0/68) in 2000.

Nationwide mapping survey: 2003

The results of the national filariasis mapping survey conducted in 2003 are presented in Fig. 3 with circles showing the location and endemicity status of the 30 villages tested in 21 Districts across all 6 divisions. Altogether, 3113 individuals age ≥15 years were tested from the different divisions: Central River (1156), Lower River (391), North Bank (682), Upper River (423) and Western (561). CFA positive individuals were found only in 9 villages located in 5 districts outside the Central River Division where historically the MF rates had been low (Fig. 2). Among the 9 villages with CFA positives, 6 were located in the Western and Upper River Divisions where the highest MF rates were found during the 1975 and 1976 surveys (Figs 2 and 3). The remaining three villages with CFA positives were located in the North River and Lower River divisions not included in the historical surveys.

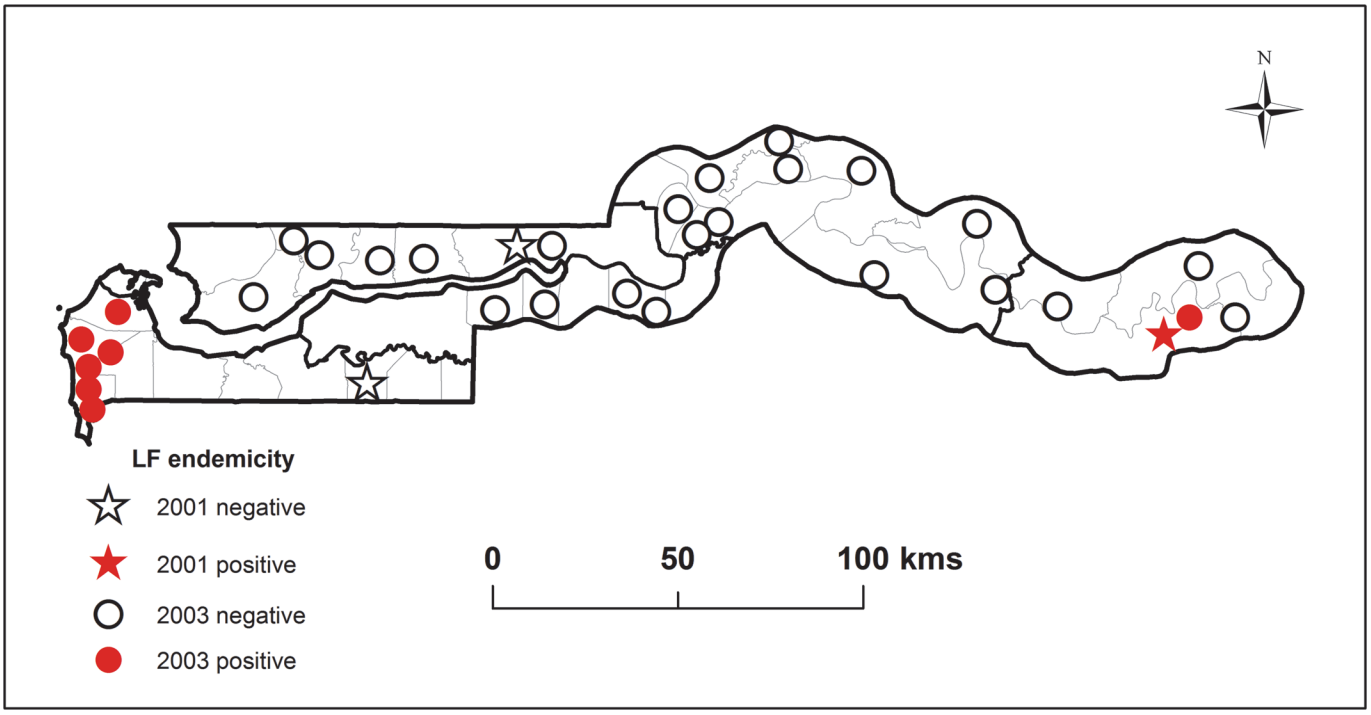

Fig 3. The lymphatic filariasis (LF) endemicity map for The Gambia showing locations of villages where at least one of the ~100 individuals tested during the mapping surveys was positive for CFA (Red dot or star) and villages where all ~100 tested individuals were found negative (White dot or star). The dots and stars represent villages surveyed during 2001 and 2003 respectively.

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Transmission assessment surveys: 2013

In 2013, TAS was conducted in 60 randomly selected schools in all Divisions in The Gambia, 30 in each of the two EUs, to assess if active transmission of *W.bancrofti* was ongoing. In total, 3180 6–7 year-old children (1509 boys and 1671 girls) were tested using ICT and none was found to be CFA positive. 1516 children were tested in EU1 and 1664 on EU2. Fig. 4 shows the location of schools tested during the TAS.

The changing pattern of LF infection rates determined by night blood and ICT surveys across the 6 administrative divisions in the Gambia between 1951 and 2013 is presented in Table 1.

Discussion

The TAS performed in this study verified the absence of transmission in all administrative divisions that were previously highly endemic for LF. The prevalence of *W.bancrofti* in The Gambia was among the highest in Africa, based on historical data [20, 21, 30–32] and reported in the first global atlas of LF compiled by Michael and Bundy in 1997 [33] and the African LF risk map of Lindsay and Thomas [30]. Village specific MF rates reported in studies carried out in the 1950s, among people 10 years or older, varied between 24.1% and 48.4% [20, 31, 32] and Hawking[4, 5] later reported that the prevalence of LF among adults during the early 1950s was about 50%. Based on our current knowledge of the relationship between MF rates and antigen prevalence such figures suggest that the majority of adults were infected with the parasite in the 1950's. Night blood surveys conducted in 15 villages in 1975 and 1976 revealed MF rates between 2.9% and 26.9% among adults ≥15 years [21]. The corresponding high prevalence of LF morbidity observed in children and adults in The Gambia in the 1970s confirmed that

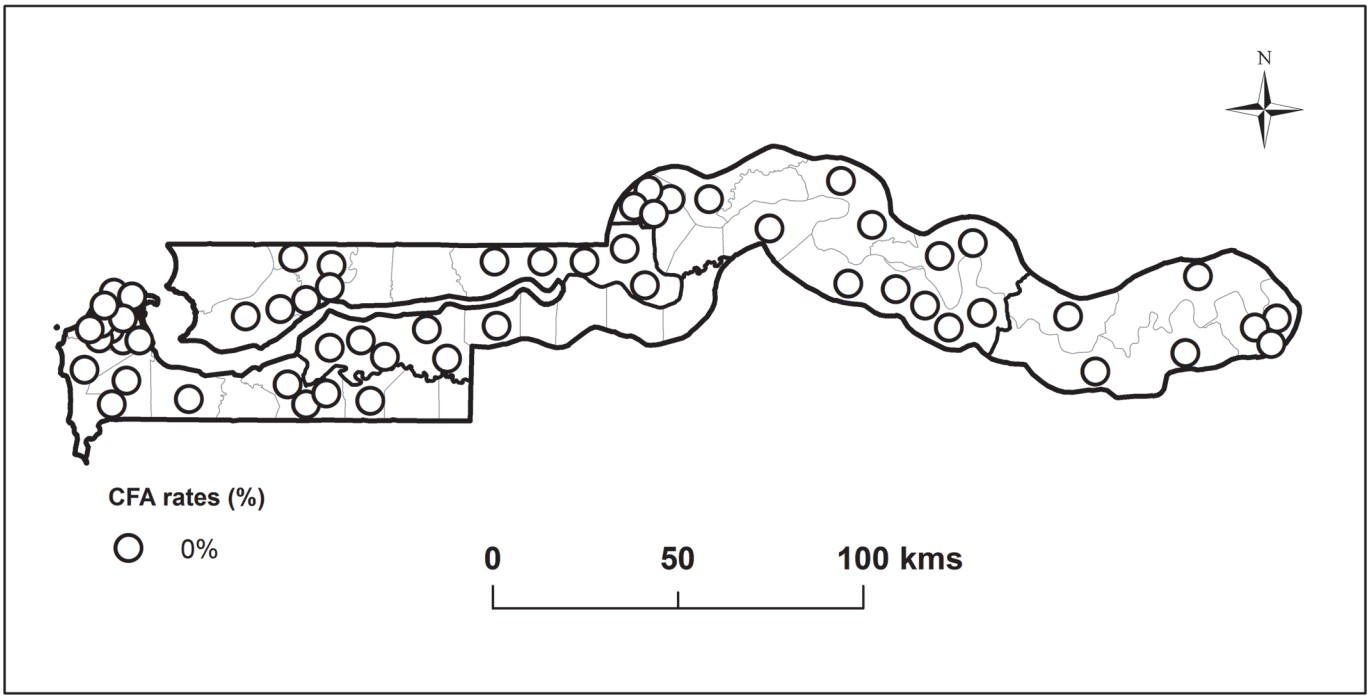

Fig 4. Map of The Gambia showing the location of 60 schools surveyed during the Transmission Assessment Surveys (TAS) carried out in 2013 and revealing no circulating filarial antigen (CFA) positive individuals in all schools indicated by the white dots.

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transmission had continued in the twenty years since the first surveys in the 1950s [21]. In adults older than 15 years, morbidity presented in the form of adenolymphangitis (13.8%–20.0%), lymphoedema (5.3%–10.0%) and hydrocele (11.7%–35.3%) [21]. Late filarial disease in the form of elephantiasis and hydrocoele was also observed in children younger than 15 years [21] suggesting early exposure and high levels of transmission.

A multi country operational research study undertaken in 11 EUs in Africa and Asia in 2011 and 2012, to test the value and practicality of the TAS tool, concluded that it was a practical and effective tool for evaluating interruption of transmission [27]. In 10 of 11 EUs surveyed during the study, the number of CFA positive cases was below the critical cut-offs of 18, leading to a cessation of MDA in these communities.

The low CFA rates ($\leq$3%) observed in the previously highly endemic divisions, during the 2003 mapping surveys, therefore implies a significant reduction in MF rates in patent infection in The Gambia between 1976 and 2003. A 9% CFA rate observed by Gass and colleagues [29] in a highly endemic area in Ghana corresponded to a 2% MF rate. Also, previous studies have shown that stored sera can remain antigen positive even after 10 years in storage [34]. In 2013, all 3180 children tested during the TAS surveys were found to be negative for CFA suggesting an interruption of the transmission of *W.bancrofti* in the absence of MDA. Results of the mapping surveys conducted in 2003 indicated that MDA for LF was required for 5 districts where CFA rates were 1% or higher [24]. No 'endemic' districts were found in the Central Division where the intensity of transmission was historically lower [17]. MDA was however not implemented by the MOHSW [1].

LF is a chronic infection perpetuated by the continuous production of microfilaria by lymphatic dwelling adult worms that can live for 4–6 years [35]. If competent mosquito vectors are present in adequate numbers, transmission will continue for as long as the MF density is

Table 1. The changing pattern of LF infection rates determined by night blood and ICT surveys across the 6 administrative divisions in The Gambia between 1951 and 2013.

Year	Administrative Division	Age group examined	Survey type and number of sampling sites	Site specific infection rate	Notes	References #
1951–1954	Western	All age groups	Night blood surveys in 4 villages	36–50%	1951 survey in area hyperendemic for malaria. No infections found in children younger than 4.5 years. 1954 survey also failed to detect mf in children <4 years.	4, 19–21,30–31
1974–1976	Western, Central, and Upper River	≥3 years	Night blood surveys in 15 villages	1.5% (≥ 3 yrs), 6.7–22.7% (≥ 5 yrs), 2.9–26.9% (≥ 15 yrs)	Night blood surveys	21
1997–2000	North Bank, Upper River and Western	≥12 years	ICT surveys on samples from 3 villages	0–8%	ICT tests performed on stored serum samples collected for a malaria project.	Current study
2003	Banjul, Central, Lower River, North Bank, Upper River and Western	≥15 years	ICT mapping surveys in 30 villages	0–3%	Mapping survey carried out in collaboration with the Ministry of Health and Social Welfare, Gambia.	Current study
2013	Banjul, Central, Lower River, North Bank, Upper River and Western	6–7 years	ICT TAS surveys in 60 schools	0%	Transmission assessment survey conducted in collaboration with the Ministry of Health and Social Welfare, Gambia	Current study

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maintained above a certain threshold. The host-parasite relationship in *W.bancrofti* transmission dynamics has been subjected to analysis using mathematical models and two infection patterns, limitation and facilitation, have emerged depending on the vector species involved [36–39]. Limitation, due to culicine transmitted filariasis, and facilitation, due to anopheline vectors have been discussed in detail [3, 40–43]. The models predict that in culicine transmitted-LF (limitation) the number of ingested microfilariae is always greater than the constant predicted by a straight line proportionality relationship so that even at low MF densities parasite uptake by mosquitoes occurs. Conversely, with anopheline-transmitted filariasis (facilitation), as in The Gambia, a lower critical threshold exists below which the number of ingested microfilariae decreases to the constant ratio. Under these conditions, transmission is not sustained and the infection will be eliminated. The critical threshold below which *Anopheles*-transmitted LF will be eliminated can be achieved either by reducing the density of microfilaria or the density of mosquitoes [44]. In The Gambia, where onchocerciasis is non endemic [45], there was never a systematic community-wide filariasis treatment that would have resulted in the reduction of *W. bancrofti* MF density [21]. The most likely explanation for our observation that transmission has been interrupted is the reduction of vector density through the widespread use of insecticide treated bednets that scaled up dramatically after 2003 and became more effective with the introduction of LLINs [12, 46] for malaria control. A Sahelian drought in neighbouring countries resulted in a significant reduction in rainfall in the Gambia that probably affected mosquito numbers and LF transmission in the late 1960s and 1970s [6, 21]. Nevertheless LF was still highly endemic in the Western and Upper River divisions during the late 1970s [6, 21].

The vectors of malaria in The Gambia are also involved in the transmission of LF with Anopheles arabiensis, *An. gambiae s.s.*, *An. melas* and *An. funestus* acting as the principal carriers of *W. bancrofti*. *Culex* mosquitoes probably play no role in the transmission of LF in The Gambia as is the case in other countries in West Africa where only *Anopheles* mosquitoes act as vectors of *W. bancrofti*. Despite the early elucidation of the biology and behaviour of *Anopheles* mosquitoes in The Gambia, there were no systematic vector control activities in the

country until insecticide treated bednets were introduced in the 1990s [21]. The report of the Liverpool School of Tropical Medicine expedition to The Gambia in 1901 recommended measures to prevent mosquito breeding in the capital, Bathurst [47]. In 1904, an ordinance covering the prevention of mosquito breeding and establishing posts for a sanitary inspector and a team of labourers to implement vector control was introduced. However, outside Bathurst no systematic attempts were made to control mosquitoes until insecticide treated bednets were introduced in the 1990s and scaled up gradually [47]. The main vectors of LF in the Gambia, are highly susceptible to insecticidal nets and indoor residual spray (IRS). The use of IRS to control malaria in the 1970s and 1980s interrupted LF transmission in Solomon Islands, parts of Papua New Guinea, Indonesia and Togo [3]. Community-wide use of long lasting insecticidal nets recently interrupted the transmission of LF in parts of Nigeria[48] and Papua New Guinea [49]. Improvements in living standards and housing characteristics probably contributed to the elimination of LF in Seychelles, Cape Verde, and Mauritius where *Culex* mosquitoes were the main vectors [50]. There is however little evidence that improvement in living standards have significantly impacted LF transmission in rural areas in Sierra Leone where LF was endemic in all districts before MDA commenced in 1998 [51].

Knight [21] who performed MF surveys in 1975 and 1976 attributed the decline in MF rates between 1950 and 1976 to a reduction in human-mosquito contact rates through decreased rainfall, improved standard of living and vector control against malaria and nuisance mosquitoes through bednets,at that time not impregnated with insecticide. Vector control in the form of IRS with DDT conducted during the malaria eradication campaign in Solomon Islands from 1974 to 1977 decreased the MF prevalence from 22% to zero without the use of anti-filarial drugs over a period of four years [17, 18].

As a result of Global Fund and PMI funding for bednet implementation, a rapid scale up in ITN coverage has occurred in many countries in Africa since 2003 when only Eritrea, Malawi, The Gambia, and Sao Tome and Principe had ITN ownership coverage greater than 20%[12]. Analysing data from suppliers of ITN to countries, National Malaria Control Program (NMCP) reports of ITNs distributed to health facilities and implementation partners and household survey data, Flaxman and co-workers[12] calculated insecticidal net ownership coverage for 44 countries in Africa between 2003 and 2008. By the middle of 2008, 8 countries including The Gambia had ITN ownership coverage of 60% or greater. There were substantial increases from 2003 in the delivery of interventions against malaria, including distribution of insecticidal nets, in The Gambia [52]. Investigating the changes in malaria indices in the country, and the causes and public-health significance of these changes, Ceesay and collaborators [13] reported that the proportions of malaria-positive slides decreased by 82% and concluded that a large proportion of the malaria burden has been alleviated in The Gambia. This remarkable reduction in malaria parasite positive individuals would confirm our views given that malaria and *W.bancrofti* have common *Anopheles* vectors, the absence of any detectable *W. bancrofti* in our sample of over 3000 children throughout the country is similarly due to the widespread use of insecticide impregnated nets and now LLINs.

Previous trials of chemoprophylaxis and insecticidal nets against malaria in The Gambia have shown reductions in all-cause morbidity and mortality greater than that directly attributable to malaria [13]. Improved treatment against malaria contributed to the reduction in malaria transmission but according to Ceesay and colleagues [13] the most significant factor was the increase of coverage of insecticidal nets because of support from the Global Fund—awarded in 2003 and implemented from 2004 onwards—for free distribution of ITNs to pregnant women and mothers of children younger than 5 years, which according to Ceesay and co-workers accounted for 55% of the population. A nationwide survey carried out to investigate the use of ITNs in rural areas of the Gambia in 1991, showed that 58% of beds had a net [46].

Bednet usage was higher in the Central Region (76%), than in the Western and Eastern Regions (both 51%)[46]. This could partly explain the difference in LF endemicity between the Central and Western divisions observed during this study.

In Kenya, one of the few African countries where the coverage of insecticidal nets exceeded 40% in 2007[12], LF prevalence continued to decrease even when MDA was interrupted for two years [53]. In Sierra Leone, where bednet usage was traditionally low [54] and scaling up of insecticide-treated nets was among the slowest in Africa [12], LF remains endemic in many parts of the country after 10 years of MDA with ivermectin for onchocerciasis and more recently for LF [45, 55]. Onchocerciasis is not endemic in The Gambia and hence mass treatment with the ivermectin has never been implemented [45]. Comprehensive reviews on the advances in knowledge of vector ecology, vector-parasite relationships, and both empirical and theoretical evidence regarding vector management to determine the role of vector control in the GPELF have concluded that MDA activities can be synergised with vector control and LF elimination will be easier to achieve [3, 7, 14, 15, 56]. Recent studies on the impact of insecticide treated nets on LF transmission in PNG [49] and Nigeria[48, 57] showed that using insecticidal nets alone in the absence of MDA interrupted the transmission of LF in highly endemic areas with MF rates over 50%.

Failure to find evidence of transmission to children of *W. bancrofti* in The Gambia in 2013 over a 6–7 year period suggests that transmission has been interrupted and the most likely cause was the extensive use of insecticide treated nets for malaria control over the last two decades built upon the causes of the decline observed [17] between the 1950 and 1970s. The growing evidence for the impact of malaria vector control activities on LF transmission was endorsed by WHO through a position statement in 2011 on integrated vector management (IVM) to control malaria and lymphatic filariasis [58]. IVM is promoted by WHO to strengthen partnerships and cross sector approaches to the control of mosquito-borne diseases like malaria and LF [15, 58]. In 2014, the Nigerian government converted these evidence based approaches into policy by launching a coordinated plan to eliminate malaria and lymphatic filariasis through the use of long lasting insecticidal nets (http://www.afro.who.int/en/nigeria/press-materials/item/6286-nigeria-launches-the-malaria-and-lymphatic-filariasis-co-implementation-guidelines.html). MDA may not be required to achieve elimination of LF in The Gambia but surveillance processes prescribed for countries during the post MDA phase, including morbidity management and disability prevention will be necessary, to acquire a non-endemic status that demands verification. The Gambia achieving non-endemic status for LF will represents huge progress in the global efforts to shrink the filariasis endemicity map and demonstrates the value of cross sector approaches in disease control. One more country will be removed from the list of LF endemic countries and 1.2 million people will be declared to be no longer at risk for the disease

Supporting Information

S1 Checklist. STROBE Checklist.
(DOC)

S1 Text. Additional survey results. Survey results for all schools that participated in the transmission assessment surveys.
(PDF)

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Author Contributions

Conceived and designed the experiments: MJB MPR DHM. Performed the experiments: MPR NKB SMS MCJ. Analyzed the data: MJB MPR LKH AGE DHM BT. Contributed reagents/materials/analysis tools: MJB MPR LKH DHM SMS MCY. Wrote the paper: MPR SMS BT NKB MCJ LKH AGE DHM MJB.

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Paper 3. Cessation of mass drug administration for Lymphatic Filariasis in Zanzibar in 2006

Rebollo, M. P[1,5,*]., K. A. Mohammed[2], B. Thomas[1], S. Ame[2], S. M. Ali[3], J. Cano[4], A. G. Escalada[5], and M. J. Bockarie[1]. 2015. 'Cessation of mass drug administration for Lymphatic Filariasis in Zanzibar in 2006: was transmission interrupted?', *PLoS Negl Trop Dis*, 9: e0003669.

Authors afilliation:

1 Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom,

2 Ministry of Health, Zanzibar, United Republic of Tanzania,

3 Public Health Laboratory-IdC, Chake-chake, Pemba, Zanzibar, Tanzania,

4 London School of Hygiene and Tropical Medicine, London, United Kingdom,

5 Rey Juan Carlos University, Madrid, Spain

* Current address: Neglected Tropical Diseases Support Center, Task Force for Global Health, Decatur, Georgia, United States of America

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Cessation of Mass Drug Administration for Lymphatic Filariasis in Zanzibar in 2006: Was Transmission Interrupted?

Maria P. Rebollo[1¤], Khalfan A. Mohammed[2], Brent Thomas[1], Shaali Ame[3], Said Mohammed Ali[2], Jorge Cano[4], Alba Gonzalez Escalada[5], Moses J. Bockarie[1]*

1 Centre for Neglected Tropical Diseases, Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom, **2** Ministry of Health, Zanzibar, United Republic of Tanzania, **3** Public Health Laboratory-IdC, Chake-chake, Pemba, Zanzibar, Tanzania, **4** London School of Hygiene and Tropical Medicine, London, United Kingdom, **5** Rey Juan Carlos University, Madrid, Spain

¤ Current address: Neglected Tropical Diseases Support Center, Task Force for Global Health, Decatur, Georgia, United States of America
* moses.bockarie@lstmed.ac.uk

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Abstract

Background

Lymphatic filariasis (LF) is targeted for elimination through annual mass drug administration (MDA) for 4–6 years. In 2006, Zanzibar stopped MDA against LF after five rounds of MDA revealed no microfilaraemic individuals during surveys at selected sentinel sites. We asked the question if LF transmission was truly interrupted in 2006 when MDA was stopped.

Methodology/Principal Findings

In line with ongoing efforts to shrink the LF map, we performed the WHO recommended transmission assessment surveys (TAS) in January 2012 to verify the absence of LF transmission on the main Zanzibar islands of Unguja and Pemba. Altogether, 3275 children were tested on both islands and 89 were found to be CFA positive; 70 in Pemba and 19 in Unguja. The distribution of schools with positive children was heterogeneous with pronounced spatial variation on both islands. Based on the calculated TAS cut-offs of 18 and 20 CFA positive children for Pemba and Unguja respectively, we demonstrated that transmission was still ongoing in Pemba where the cut-off was exceeded.

Conclusions

Our findings indicated ongoing transmission of LF on Pemba in 2012. Moreover, we presented evidence from previous studies that LF transmission was also active on Unguja shortly after stopping MDA in 2006. Based on these observations the government of Zanzibar decided to resume MDA against LF on both islands in 2013.

Author Summary

Lymphatic filariasis was highly endemic in Zanzibar when MDA commenced in 2001 to eliminate the disease. In 2006, Zanzibar, in the United Republic of Tanzania, was the first territory in Africa to complete five rounds of annual treatment using a combination of albendazole and ivermectin at 100% geographic coverage and achieving effective treatment coverage of over 65% during each round. MDA was stopped in 2006 after sentinel site surveys revealed parasite infection rates of zero in both humans and mosquito populations. In 2012, when new tools became available to verify the absence of transmission, we asked the question if transmission was truly interrupted when MDA was stopped in 2006. In January 2012, we performed the WHO recommended transmission assessment surveys (TAS) on the main islands of Unguja and Pemba to verify the absence of LF transmission in line with ongoing efforts to shrink the LF risk map. Altogether, 3275 children were tested on both islands and 89 were found to be CFA positive; 70 in Pemba and 19 in Unguja. The distribution of schools with positive children was heterogeneous with pronounced spatial variation on both islands. Based on the calculated TAS cut-offs of 18 and 20 CFA positive children for Pemba and Unguja respectively, we demonstrated that transmission was still ongoing in Pemba where the cut-off value was exceeded. We also presented evidence from previous entomological studies that LF transmission was active on Unguja shortly after stopping MDA in 2006. Based on these findings we concluded that LF transmission was still active in Zanzibar, and one million people at risk of acquiring LF, and recommended the resumption of MDA on both islands to eliminate the disease. In 2013, the government of Zanzibar decided to resume MDA with ivermectin plus albendazole on both islands.

Introduction

Lymphatic filariasis (LF) is a major cause of acute and chronic morbidity and a significant impediment to socioeconomic development in 73 countries in Africa, Southeast Asia, the Americas, and the Pacific region. The World Health Organization (WHO) estimates that in 2012 more than 1.4 billion people living in these countries were at risk of acquiring the infection [1]. LF infection occurs through intense and long-term exposure to mosquito bites from several genera of anopheline and culicine mosquitoes that are carriers of the three parasites that cause human filariasis (*Wuchereria bancrofti*, *Brugia malayi* and *B. timori* [1]. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) recommends annual mass drug administration (MDA) using albendazole in combination with either diethylcarbamazine (DEC) or ivermectin for 4–6 years as the main strategy to interrupt transmission of the disease [2]. From 2000 to 2012, more than 4.4 billion doses were used to treat over 500 million people in 56 countries, making the GPELF one of the most rapidly expanding global health programs in the history of public health [1]. Many countries implementing MDA have completed more than 5 consecutive annual treatment rounds but only China and South Korea have been verified by WHO for achieving elimination of LF [3]. In 2006, Zanzibar, in the United Republic of Tanzania, which started MDA in October 2001 [4], was the first country in Africa to complete five rounds of treatment using a combination of albendazole and ivermectin at 100% geographic coverage and achieving effective treatment coverage rate of over 65% during each round [3, 5]. A detailed description of the first round of MDA in 2001 and the subsequent four treatment rounds, including treatment coverage for each round, has been published previously [4, 5].

Parasite infection rates and intensities in the human and mosquito populations decrease after several rounds of MDA, but individuals may remain microfilaria and antigenemia-positive even after transmission has been interrupted [6]. A standard methodology called Transmission Assessment Survey (TAS) has been described by WHO to assess whether the prevalence of infection has been lowered to a level where recrudescence is unlikely to occur, even when MDA interventions have been stopped [6, 7]. After five rounds of MDA, an implementation unit (IU) is considered eligible for TAS if treatment coverage exceeds 65% on each round and the prevalence of microfilaraemia is below 1% on sentinel and spot check sites.

Treatment was stopped in Zanzibar in 2006, after five rounds of MDA and 2 sentinel and 12 spot-check site surveys in high risk urban and rural areas revealed parasite infection rates of zero in both humans and mosquitoes [3]. In line with ongoing efforts to shrink the LF map, we performed TAS in January 2012 to determine whether the successive rounds of MDA carried out between 2001 and 2006 achieved the interruption of disease transmission in the two main islands. Demonstrating the absence of active transmission of LF in Zanzibar is the first step in the verification process that could result in its reclassification as non-endemic and consequently, shrinking the LF map by yet another country.

Materials and Methods

Study area

Zanzibar is part of the United Republic of Tanzania located 35 km off the mainland Tanzania coast. It comprises two main islands, Pemba and Unguja, and a number of sparsely populated islets; the land areas of Unguja and Pemba are 1,654 km^2 and 984 km^2 respectively. About 1 million people live in Zanzibar with 65% of the inhabitants residing on Unguja. Unguja is the largest and most populated island of Zanzibar, with more than 40% of the population residing in Zanzibar town, the administrative and commercial centre of the two islands. Pemba, on the other hand, has three towns forming concentrated urban centres. Although Zanzibar is non-endemic for onchocerciasis, onchocerciasis transmission occurs in mainland Tanzania. Considering that there is free movement and settlement of the population in both the island and mainland communities, it was agreed to use ivermectin in combination with albendazole as the treatment regimen for LF [5]. In Zanzibar, the causative agent of LF is *Wuchereria bancrofti* which is transmitted by the urban mosquito, *Culex quinquefasciatus* [8, 9].

Prior to the first MDA round in 2001, parasitological and entomological surveys were carried out in two sentinel sites, Kizimkazi and Kwahani, in Unguja to collect baseline data for impact monitoring. The two sites represented the areas of highest risk of exposure to LF in rural and urban communities respectively in Zanzibar. Kizimkazi, which had a population of 3,037, is located in the dry arid stony area along the coast of the rural southern district. Pit latrines, cesspits and soakage pits, which serve as good breeding grounds for *Culex* mosquitoes, were common in Kizimkazi. Kwahani, located in the urban district of Unguja, had a population of 4,550 and featured many open drains where *Culex* mosquitoes breed. Before MDA started in 2001, the MF prevalence rates in Kizimkazi and Kwahani were 17.1% and 7.5% respectively, based on examining 500 people from each site [5]. The baseline surveys took place in September 2001 and were annually repeated before each MDA round. Finger prick blood samples (100μl) were obtained at night and examined using the counting chamber method as described in the WHO guidelines for MF surveys [7]. Treatment coverage rates over the 5 years varied from 76% (in 2001) and 83% during the subsequent 4 years with the last MDA round completed in November 2005. The prevalence of microfilaremia in the two sentinel sites dropped from 17.8% and 7.2% before treatment to 1.0% and 0.0% respectively after the fifth round [10].

Transmission assessment surveys (2012)

Transmission assessment surveys (TAS) were performed in January 2012, six years after stopping MDA, using standard operating procedures (SOP) and WHO guidelines, which have been described in detail elsewhere [6, 7]. Zanzibar met the TAS eligibility requirements of having completed at least five effective rounds of MDA in all IUs with coverage >65% over the total population and MF rates <1% in each of the sentinel sites after five MDAs. It was decided to conduct the survey in two evaluation units (EU); one per island, as this would provide a clear picture of the current LF transmission status on each island while still having less than 2 million people on each EU. The TAS design was based on the net primary school enrolment rate, the target population size and number of schools on each island, and according to the rules established for *Culex-W. bancrofti* transmission areas [7]. A Microsoft Excel computer tool, the Survey Sample Builder (SSB) [7], was used to generate random number lists and inform TAS design calculations, including sample size and sampling intervals for the two TAS evaluation units. School surveys were conducted on both islands where net primary enrolment rates exceeded 75%. Children 6–7 years old were targeted for the TAS because antigenaemia in young children would reflect recent and active transmission, while antigenaemia in older children and adults may be related to infections that occurred before MDA. All children in grades 1 and 2 were eligible including a small proportion of those outside this age range and the oldest was eight years old. The sample sizes for the two evaluation units generated by the SSB were 30 school clusters on each island, which encompassed 1556 children in Pemba and 1684 children in Unguja.

The TAS critical cut-off value represents the threshold of infected individuals below which transmission is expected to be no longer sustainable, even in the absence of MDA.

If the total number of positive cases is at or below the critical cut-off value, the EU 'passes' the survey and MDA is not consider to be required [7]. TAS sample sizes and critical cut-off values are powered so that the EU has at least a 75% chance of passing if the true antigen prevalence is half the threshold level (2% for *Culex*, *Anopheles*, *and Mansonia* vector areas, and 1% for *Aedes* vector areas). In addition, there is no more than a 5% chance of passing if the true prevalence is greater than or equal to the threshold level. The critical cut-offs generated for Pemba and Unguja were 18 and 20 respectively.

LF diagnosis

The Binax NOW Filariasis immunochromatographic card test (ICT) (Alere Inc., Scarborough, ME) was used to detect circulating filarial antigen (CFA) as described in the WHO guidelines [7]. Briefly, 100 μl of finger-prick blood was collected from each individual and then transferred to an ICT card test using a calibrated capillary tube. The test was read 10 minutes after closing the card, as instructed by manufacturers. A positive control filarial antigen was used to confirm the quality of the ICT cards. All positive controls turned out positive. The antigen contains the epitope present in circulating *Wuchereria bancrofti* antigen that is detected by the Binax Filariasis Now test. This was necessary to instil confidence in the large number of negative results expected. There was however a very minimal risk of false positives in Zanzibar which is not endemic for *Loa loa* [11]. ID number, class and test result of each child tested was recorded.

Data entry and analysis

Data analysis was conducted using SPSS and the results were mapped using ArcGIS 10.1 (ESRI, Redlands, CA). The test results from each cluster in the different EUs were collected by the MOH survey team and the Public Health Laboratory Ivo de Carneri staff on Pemba. Data

was entered using a Microsoft Access data base specifically developed by the Centre for Neglected Tropical Diseases (CNTD) in the Liverpool School of Tropical Medicine (LSTM) to support TAS. Two independent office workers entered data using a double entry system that automatically compare the two entries and detect any possible errors. Discrepancies were checked at CNTD for accuracy.

Ethical approval and consent procedures

Ethical clearance for the study was granted by the LSTM Research Ethics Committee of the Liverpool school of Tropical medicine which approved the use of oral consent (Research Protocol 11.89RS). After approval from the Ministry of Education and school authorities, the MoH team met with the head master of each school to obtain permission to conduct the survey and to schedule a date for meeting with parents. The survey team explained the purpose of the surveys and received oral consent from teachers and parents. Written consent was not required for surveys conducted by the Ministry of Health as part of the disease control activities. Non consenting parents or non-assenting children could drop out from the study at that time or any time during the study.

Results

Altogether, 3 275 children were tested on both islands and 89 were found to be CFA positive. The CFA point prevalence for Pemba and Unguja were 5.4% (70/1298) and 0.9% (19/1977) respectively. Even though the sample size required for Pemba was 1556, we decided to discontinue the tests when the number of positive children largely exceeded the critical cut-off value of 18 for the EU. Some parents did not consent to including their children in the survey but no mop up was required since the critical cut-off was largely reached. The CFA prevalence for boys (0.91%) and girls (1.0%) was almost identical on Unguja, where the whole sample size was surveyed. The distribution of schools with antigen positive children was very heterogeneous on both islands with pronounced spatial variation between and within districts as shown in the Fig. 1.

A total of 30 schools, 9 from each of the four districts on Pemba Island were surveyed and CFA positive children were detected in every school with the exception of Cheke ckeke in south Pemba, where all 296 children were negative. Among the schools with positive children the CFA prevalence rates varied from 1.17% in Mkoani in South Pemba to 14.64% in Micheweni in North Pemba. The overall CFA prevalence for Pemba was 5.4% but the district specific CFA rates for Mkoani, Wete and Micheweni were 1.0%, 7.8% and 10.3% respectively.

In Unguja, where a total of 1684 children were tested, the total found to be CFA positive (19) was just below the critical cut off value of 20. The distribution of positive schools on the island was very heterogeneous with three (Central, North B and Urban) of the 6 districts revealing no positive children. Among the 3 districts with positive school, the district specific CFA rates were 4.7% (North A), 3.2% (South) and 0.4% (West).

Discussion

Between 2001 and 2006, the Zanzibar programme for the elimination of LF carried out effective annual MDA campaigns to interrupt the transmission of the disease [5]. To effectively coordinate MDA in this predominantly Muslim country, the last Saturday of October was designated as the annual Filaria day (F-day) with a 'mop-up day' on Sunday. Ivermectin in combination with albendazole were administered by highly motivated community drug distributors known as Filarial Prevention Assistants (FPAs) who ensured a high treatment coverage ranging from 70 to 80% for all five rounds. MDA was stopped in 2006 after sentinel site surveys revealed

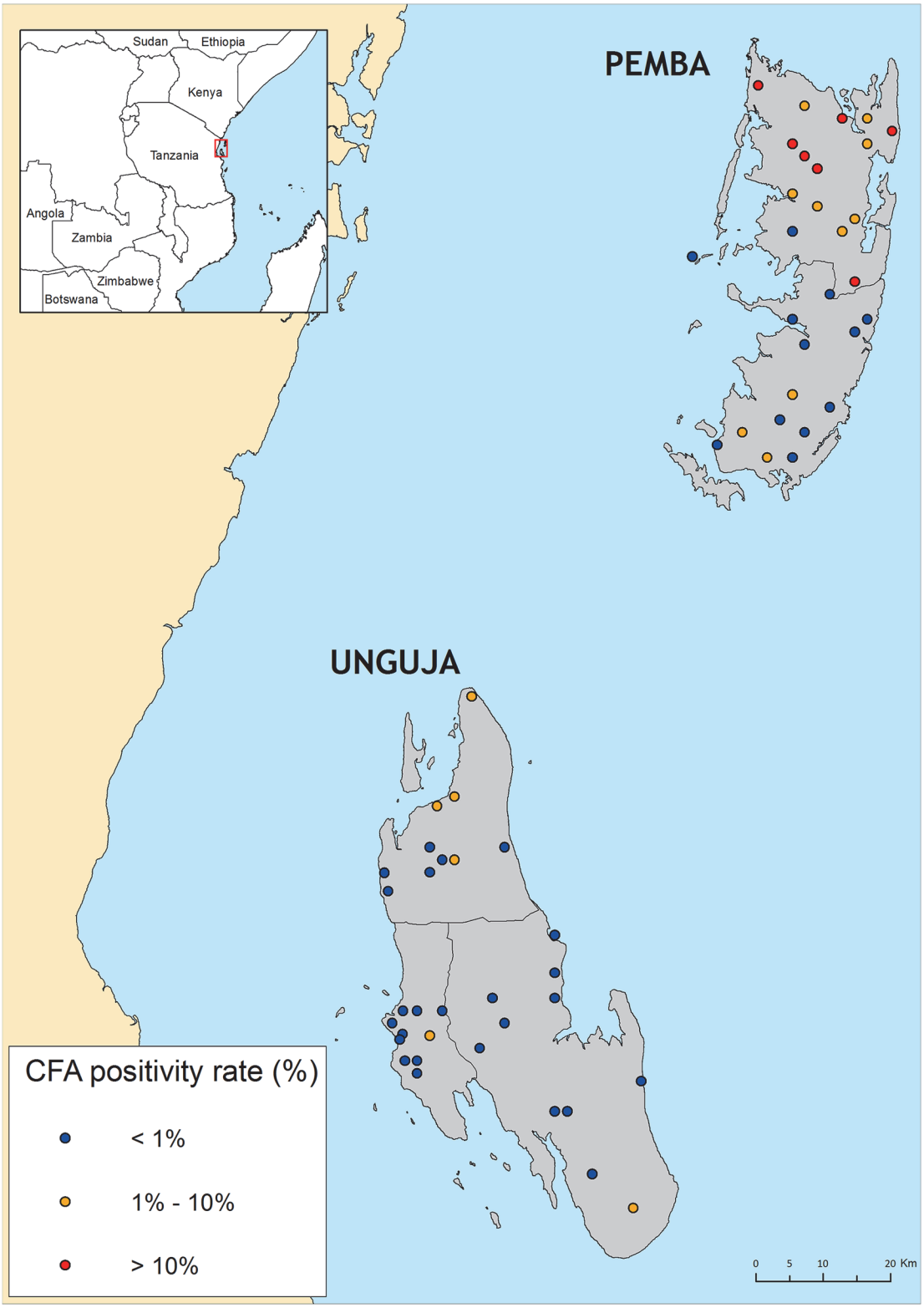

Fig 1. Spatial distribution and antigen positivity rates of schools selected for TAS surveys on Pemba and Unguja islands in Zanzibar, United Republic of Tanzania.

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parasite infection rates of zero in both humans and mosquito populations as reported by Mohammed in his 2009 PhD thesis (Lymphatic Filariasis in Zanzibar: Epidemiology, Elimination and impact) with the University of Liverpool, United Kingdom [12]. The infection rates in humans and mosquitoes were determined by night blood survey and dissections respectively. It had been demonstrated in Egypt, where *W. bancrofti* was also transmitted by *Culex* mosquitoes, that five rounds of MDA using albendazole plus DEC can interrupt the transmission of LF in a population of 2.5 million [13].

Lymphatic filariasis was endemic on both islands before MDA commenced in 2001 as described in detailed investigations carried out many years prior the initiation of MDA [5, 9, 14]. Cross-sectional clinical, parasitological and entomological surveys for LF, conducted in urban and semi urban communities on Pemba in 1990 revealed that LF endemicity and vector species composition had not changed significantly for 15 years [9]. MF prevalence rates on Pemba during a survey conducted in 1975 ranged between 11.8% and 16.2% for people aged above 1 year [9]. Clinical manifestations in the form of hydroceole and lymphoedema were also common on the island, with prevalence of 22.4% and 1.4% respectively for adults above the age of 15 years [9]. Similarly, surveys conducted on Unguja in 1975 showed that the overall prevalence of clinical signs among men aged 15 years and older was 29.6% for hydrocele and 7.9% for elephantiasis, while the MF rates varied from 7.0% to 39.0% [12].

Our TAS results showed that five rounds of MDA in Zanzibar either failed to interrupt the transmission of LF on Pemba, where the TAS cut-off of 18 was surpassed by a huge margin early in the survey, or resurgence occurred after MDA was stopped in 2006. Unfortunately, the sentinel sites selected for monitoring the impact of MDA in Zanzibar did not include communities on Pemba and therefore the intensity of LF transmission on the island in 2006 could not be verified. Studies elsewhere have demonstrated that the vector of LF in Zanzibar, the highly efficient *Culex quinquefasciatus*, can sustain transmission in areas of low density microfilaraemia, even when MF is undetectable using traditional diagnostic methods based on around 60 μl [15]. In addition, recent studies on mainland Tanzania has also demonstrated that transmission of LF can persist after seven rounds of MDA in urban areas where *Culex quinquefasciatus* are the main vectors [16]. Infective mosquitoes were found in communities in India where MF rates dropped to zero after six rounds of treatment with DEC or ivermectin suggesting that transmission can occur in the absence of detectable MF if *Culex* mosquitoes are the vectors [15, 17].

Understanding the transmission dynamics of LF by different species of mosquitoes is essential for the rational planning of control measures and impact assessment. An important determinant of transmission efficiency is the genera of vector species involved [18]. For filariasis transmission to be interrupted, vector density or microfilaria intensity needs to be lowered below a threshold that ensures no new infections occur. This threshold for parasite density has been shown by quantitative models to be higher for anopheline which interact with *W. bancrofti* in a density dependent vector-parasite relationship known as facilitation. The relationships associated with culicine mosquitoes are known as limitation and proportionality and together with facilitation they describe the quantitative relation between microfilarial uptake and yield of infective L3 larvae in the mosquito vectors [18]. Based on these vector-parasite relationships, the TAS cut-off value for *Aedes* species (1%) is lower than that for *Anopheles* and *Culex* species (2%) [7]. The basis of grouping *Culex* with *Anopheles* species in determining these TAS cut-off values is unclear but analysis of eliminations threshold for *Anopheles* and *Culex* species suggest a higher threshold for the former [18]. The persistence of transmission in *Culex* transmission zones has led to growing concerns about the effectiveness of using MDA alone to eliminate LF without the inclusion of vector control [14, 19, 20]. On the other hand,

once *Anopheles*-transmitted LF was eliminated in Solomon Island in the 1970s [20, 21], resurgence was never detected and it was declared non endemic by WHO in 2009 [22],

Vector control is effective against LF [20] but active vector control intervention did not resume in Zanzibar until after MDA for LF was stopped in 2006, when the Zanzibar Malaria Control Programme (ZMCP) started the distribution of free long lasting insecticidal nets (LLINs) targeting mainly pregnant mothers and children under the age of five years [23]. Bednet usage was initially lower in Pemba in comparison to Unguja but by 2008 every household in Zanzibar received two LLINs and, since 2006, six rounds of indoor residual spraying (IRS) have been conducted with synthetic pyrethroid lambda-cyhalothrin (ICON) resulting in over 90% coverage of all dwellings. IRS and LLINs target both endophilic (indoor resting) and endophagic (indoor feeding mosquitoes) mosquitoes including the vectors of LF on Zanzibar [20]. The combination of IRS and LLINs with other interventions resulted in a dramatic reduction of malaria prevalence in Zanzibar from 40% in 2005 to between 0.2 and 0.5% in 2011/2012 [23]. The low prevalence of LF infection in children in Unguja may be partly explained by the impact of the vector control measures as previous efforts to control LF in Unguja by vector control resulted in 65% reduction in mosquito density in houses [23]. The use of LLINs alone have resulted in the interruption of LF transmission in communities in Nigeria [24, 25] and Papua New Guinea [26]. Based on quantitative analysis of elimination thresholds for LF, the probability that the parasite will be eliminated following six rounds of MDA increases as the vector biting rates decrease [18].

The distribution of schools with antigen positive children was very heterogeneous on both islands with pronounced spatial variation between and within districts. Although Unguja barely passed the transmission interruption verification test by revealing fewer (19) CFA positive children than TAS cut-off of 20, four of the six positive schools had CFA positive rates higher than 5% and could enable transmission. Dissection of 6568 *Cx. quinquefasciatus* mosquitoes in 2006 found none to be infected with *W. bancrofti* but PCR assays on 5184 specimens collected between November 2007 and February 2008 showed a maximum likelihood infection rates of 1.13% (0.82% – 1.52%) that suggested ongoing transmission. It is therefore very likely that transmission was ongoing on the Island in 2006 when MDA was stopped [12].

The decision to stop MDA after several effective rounds of MDA should be based on statistically robust methodology. A recent multicenter evaluation to define endpoints for MDA in 11 countries concluded that TAS was superior to previous WHO guidelines used to determine when to stop MDA [6]. It was shown to be a practical and effective evaluation tool for stopping MDA although its validity for longer-term post-MDA surveillance will require the use of more sensitive tools for detection infection in humans and mosquitoes.

In conclusion, our findings in 2012 suggested that LF transmission was still active on Pemba. We also presented evidence from previous entomological studies that LF transmission was active on Unguja shortly after stopping MDA in 2006. Based on these findings including the heterogeneous distribution of CFA positive children in Unguja, and the high number of positives found compared (19) to the cut-off value (20) the government of Zanzibar decided to resume MDA with ivermectin plus albendazole on both islands in 2013. TAS will be repeated in 2015 after two rounds of treatment. However, the interpretation of the results from the TAS survey in 2015 may be confounded by the lack of treatment in Zanzibar for 7 years and pre-TAS sentinel site surveys in other evaluation units, including outside Zanzibar, may be required to determine if the criteria for TAS still holds.

Supporting Information

S1 Checklist. STROBE Checklist.
(DOC)

S1 File. Transmission assessment survey results for all participating schools in two evaluation units (EU).
(PDF)

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Author Contributions

Conceived and designed the experiments: MPR MJB AGE. Performed the experiments: MPR KAM BT SA SMA. Analyzed the data: MPR MJB JC BT. Contributed reagents/materials/analysis tools: MJB MPR SA SMA. Wrote the paper: MPR MJB AGE KAM SA SMA JC.

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Discussion

Discussion

The Sustainable Development Goals constitute a global commitment to end the epidemics of poverty related diseases, including neglected tropical diseases, by 2030 (146, 147). This will be achieved through universal health coverage, and the provision of safe and affordable medicines and vaccines for all (146, 147). WHO's proposed NTD indicator is "*the number of people needing interventions against NTDs"*. Getting this number right is the main goal of the work presented in this thesis. It will help ensure that the world's poorest and most marginalized people are prioritized at every step on the path towards SDG targets.

In 2000, the World Health Organization (WHO), in partnership with the pharmaceutical industry and other public and private partners, launched the Global Programme to Eliminate LF by 2020 (148). There was renewed commitment in 2012 through the London Declaration, to control, eliminate or eradicate by 2020 ten NTDs including Lymphatic Filariasis (130). The renewed commitment was to complete mapping and delineate affected areas that will be targeted for preventive chemotherapy.

Eliminating diseases requires a series of epidemiological and public health steps. The first one is understanding where the disease is present (the so-called "mapping' disease distribution) using sensitive and specific diagnostic tools and robust epidemiological methods, such as accurate estimates of potential distribution and control thresholds provided by modelers (138, 149). The correct diagnosis and identification of the endemic areas where transmission is active enables the cost-effective use of resources to target interventions in those areas (132).

Since the launch of the GPELF many LF endemic countries in Africa have embarked on National Programmes for Elimination of LF through annual MDA with single dose of DEC or ivermectin plus albendazole with the objective of decreasing the circulating Mf until it reaches a threshold below which infection of the vector mosquitoes is rendered impossible (150). Annual MDA although more inexpensive than most public health interventions, requires significant resources (financial cost per person per year has been estimated at 0.48 USD average while the economic cost is 4.98 USD per person treated per year) (151, 152). Therefore, despite the

engagement of partner non-profit organizations, pharmaceutical companies and WHO, Ministries of Health in Africa had been unable to reach 100% geographical coverage of MDA in all but 6 countries in Africa by 2010.

Identifying areas where active transmission is ongoing will enable targeting of the scarce resources to treat those who truly need interventions to achieve elimination. As new modelling approaches, diagnostic techniques and epidemiological assessment tools have become available; the map of LF in Africa has started to shrink. Against all odds, Africa may have always been closer to the target of elimination than expected due to the low environmental suitability of LF in many countries (3), the malaria vector control activities (153) and the overestimate of LF burden in some countries due to *Loa* antigens cross reactivity to ICT card among others (154).

The GPELF is not a static program, as new knowledge and new tools become available better and more accurate estimates of the burden caused by LF are being obtained, which in turn help re-define where resources should be focusing on to achieve elimination.

New and improved rapid diagnostic tools are central to achieving the objective of shrinking the LF map to zero endemic countries by 2020 (155). They are essential for the mapping and impact assessment processes that inform programme managers about where drugs should be targeted to initiate MDA, to determine when MDA can be stopped after transmission has been interrupted and ensure strong surveillance after completion of MDA interventions for early detection and action in the event that transmission resumes as we move towards global elimination (156). However, some of these new tests, including antigen detector, will be of limited value to inform transmission interruption because antigens from adult worms will persist in previously infected people long after transmission has been interrupted (157). In areas where LF transmission has been interrupted, antibody tests could be useful in detecting early exposure before patent infection is established. This will allow measures that can prevent recrudescence. Moreover, many molecular assays currently used for LF diagnostics, including Loop- mediated isothermal amplification (LAMP) and PCR, are laboratory based and lack some elements that can ensure quality assurance in resource poor settings (155).

When this project was started in 2013, 13 of the 73 countries in which LF was known to be endemic had not delineated all or the majority of the areas (i.e. implementation units or IU) to be targeted for mass distribution of medicines (158). In Africa, about half (48.5%) of the 464 million people exposed to LF resided in the four high burden countries of Democratic Republic of Congo (DRC) (49 million), Ethiopia (30 million), Nigeria (109 million) and Tanzania (45 million) (159). Except for Tanzania, the high burden countries in Africa had not completed LF mapping at that time in 2013. In Ethiopia, only 112 of the 817 districts in the country had so far been mapped for the disease (160). The Gambia and Gabon were still considered endemic for LF (158). It was not clear if LF transmission was active in large urban cities in West Africa. The disease was re-emerging Zanzibar in the United republic of Tanzania.

This discussion chapter is a contextualised presentation of three original research articles that were published as a result of the multi-country mapping project which have involved more studies across African LF endemic countries (150, 155, 156, 161-168). These three research works have been selected out of several papers I authored trying to answer the hypothesis presented here, because they best showcased the application of current modelling and novel epidemiological tools to identify the actual endemic areas of Lymphatic Filariasis in Africa and to produce more precise estimates of people requiring preventive chemotherapy. Targeting the actual population in need for treatment will in turn lead to accelerate the progress towards the achievement of the 2020 goals of LF elimination.

The complete mapping of LF in Ethiopia through this thesis indicated that 112 districts across the country were endemic for the disease with 11.5 million people potentially exposed to infection. The study showed that the population at risk for LF was 60% lower than the WHO estimates in 2014 (158). This result showed that coordinated mapping of multiple diseases (168) can result in significant cost savings for National NTD programmes that currently rely on disease specific mapping protocols (169).

The study was conducted as part of an integrated nationwide mapping project for LF and podoconiosis (168), a cause of non-infectious elephantiasis, and lasted three months in 2013. During that period 1315 communities were surveyed, and 130 166 individuals examined in 658 woredas across Ethiopia. The first attempt at LF mapping on a large scale in Ethiopia started in

2008 and it took two and half years to survey 11 685 individuals living in 125 villages (112 districts) of western Ethiopia (170). A detailed description of the integrated mapping of LF and podoconiosis and the lessons learnt from the exercise have been published separately (168). Mapping the overlap of multiple NTDs in endemic areas is also critical for informing strategies for interventions, morbidity management and disability prevention. This is particularly important for areas where LF may be coendemic with loiasis, malaria, onchocerciasis or podoconiosis. The Ethiopian government planning budgets for disease specific mapping of LF and podoconiosis in 658 districts were $1 212 209 and $1 211 664 respectively but the actual financial cost of our coordinated mapping of LF and podoconiosis was only $1 291 400 (168). This significant reduction in total cost for mapping both diseases was achieved through savings in the areas of team training, supply chain management and travel. The total cost of uncoordinated disease specific mapping for the two diseases was 1.9 times as high as the integrated survey approach (168).

Historically, LF has been reported as having limited distribution in the southwest of Ethiopia. The disease was first detected in a native Ethiopian in 1937 (171) but it was never reported as widely distributed outside the Gambella region where examination of 1 ml of diurnal blood in 82 adults in 1971 revealed a 24% Mf positive individuals (171). A more recent survey in Gambella in 1993 confirmed the high transmission intensity of *W. bancrofti* in this area, with an average Mf rate of 20.7% in the population surveyed at two communities adjacent to Baro river (172). Hydrocele and elephantiasis were however uncommon in the Gambella area and studies carried out in 1976 (173) ruled out the involvement of *W. bancrofti* in the occurrence of elephantiasis in the highlands, where this condition had previously been reported (174, 175). Entomological investigations in the Gambella area showed that *Anopheles gambiae s.l* (probably *An. arabiensis*) and *An. funestus* were the main vectors with no evidence for the involvement of the culicine mosquitoes despite the high biting rates (171). A total of 3228 *Mansonia* mosquitoes, secondary vectors of LF in West Africa (176), were dissected but none was found to be infected. However, the *W. bancrofti* infectivity rates in *An. gambiae s.l* (0.24%) and *An. funestus* (0.35%) were lower than what was reported for other parts of East Africa at that time, including Kenya (177), and Tanzania (178), suggesting low potential for LF transmission in the Gambella area despite the high Mf rate shown in adults. The low infectivity rates for the vectors in Ethiopia were

probably related to the low intensity of infections observed because nine of the 20 infected persons were low density microfilariae carriers with less than 6 mf/ml of blood. Only three of the 20 infected persons harboured more than 100 mf/ml (171). In his review of the significance of low density Mf (<30 mf/ml) for maintaining the transmission of LF, Southgate (179) concluded that *Anopheles* mosquitoes are poor vectors when their blood-meal source is presented by low density microfilariae carriers.

Besides the aforementioned findings, a couple of environmental-based models produced for LF across Africa, predicted low suitability for LF occurrence for over 80% of Ethiopia and moderate for narrow bands in the western and southwestern parts of the country (3, 136). Moreover, recent Bayesian geostatistical models showed Ethiopia as one of the lowest burden countries in Africa with an estimated infection rate of 2.8% for 2000 and disease distribution limited to less than 20% of the country (138, 180).

It is not clear how the WHO estimate of 30 million people at risk for LF in Ethiopia was derived. Based on the high proportion of low density microfilariae carriers in endemic areas, consistent prediction of low transmissibility in less than 20% of the landmass by models (3, 136, 180) and the relative inefficiency of the LF vectors in Ethiopia (171), it is highly unlikely that 30 million people are at risk for LF in Ethiopia. The wide distribution of malaria, which is transmitted by LF vectors, and lymphoedema associated with podoconiosis may have historically suggested a wider distribution of the disease than demonstrated by the current study. The coordinated mapping of LF and podoconiosis has shown that podoconiosis is more widespread in Ethiopia than previously estimated, but occurs in distinct geographical regions that are tied to key environmental factors (181, 182).

Mapping LF has the objective of identifying areas where transmission is active and therefore preventive chemotherapy is required. In 45 IU out of 658 surveyed during this exercise only one positive individual for circulating filarial antigen (CFA) was identified. ICT cards are not 100% sensitive and specific (157, 183), furthermore antigenemia may remain positive even after infection has been cured and individuals can move from one district to another, therefore it is arguable whether 1 positive individual for ICT can demonstrate active and sustained transmission of LF across a IU. On the other hand, due to the similarities between LF and

podoconiosis morbidity, selection of sites for mapping may have failed to identify areas where LF transmission is active.

To demonstrate that in the 45 IU reporting only 1 CFA positive out of 100 people tested (*borderline* results for a1% CFA positivitythreshold for declaring a district endemic) MDA may not be required, we eventually conducted a supplementary research using more accurate diagnostic tools (184). The supplementary study shrank the number of LF endemic woredas from 112 to 70 in Ethiopia, dramatically decreasing the estimated number of people at risk for LF transmission and requiring MDA from 30 million estimated in 2012 by WHO (158), to 11 580 010 estimated in 2016 through the first nation-wide mapping (164), to 5 893 309 in 2017 in the confirmatory survey that I also led (184). Reducing the total number of woredas requiring MDA to 70 has major resource and logistic implications for the national LF program in Ethiopia (Figure 4) . The cost required to implement 5 years of MDA, including the monitoring and evaluation requirements, is much greater than the cost of conducting the confirmatory mapping tool to confirm whether the woreda is truly endemic ($7,910) (184).

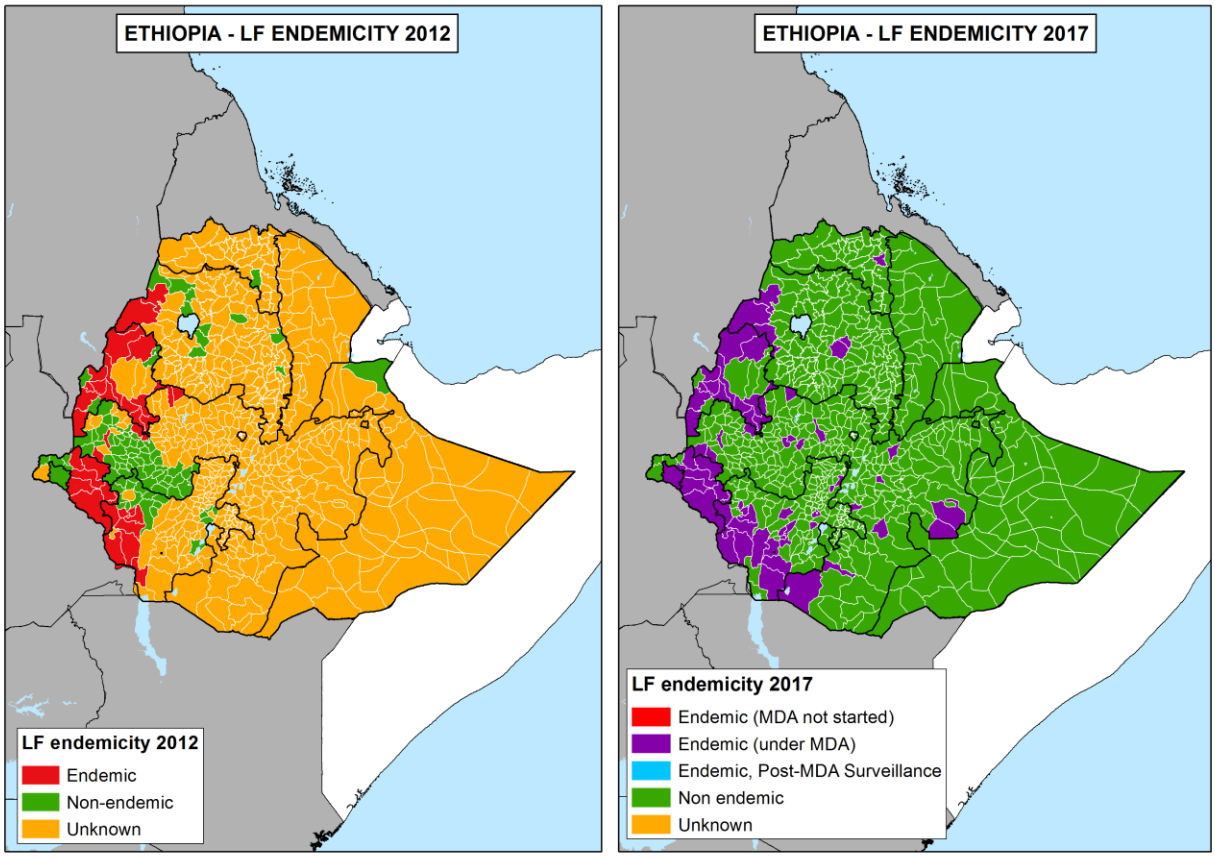

FIGURE 2. IMPACT OF THIS RESEARCH WORK ON THE LF MAP IN ETHIOPIA. 2012 (BEFORE) AND 2017 (AFTER)

Transmission assessment surveys (TAS) conducted in The Gambia verified the absence of transmission in all administrative divisions that were previously highly endemic for LF. The prevalence of *W. bancrofti* in The Gambia was among the highest in Africa, based on historic data (136, 185-188) and reported in the first global atlas of LF compiled by Michael and Bundy in 1997 (189) and the African LF risk map of Lindsay and Thomas (136). Village specific Mf rates reported in studies carried out in the 1950s, among people 10 years or older, varied between 24.1% and 48.4% (186-188). Hawking (8, 190) later confirmed a prevalence of LF among adults of 50% in the same decade. Based on our current knowledge of the relationship between Mf rates and antigen prevalence, such figures suggest that the majority of adults were infected with the LF parasite in the 1950's. Later night blood surveys conducted in 15 villages in 1975 and 1976 revealed Mf rates between 2.9 % and 26.9% among adults ≥15 years (185). The corresponding high prevalence of LF morbidity observed in children and adults in The Gambia in the 1970s confirmed that transmission had continued in the twenty years since the first surveys in the 1950s (185). In adults older than 15 years, morbidity presented in the form of adenolymphangitis (13.8% - 20.0%), lymphoedema (5.3% – 10.0%) and hydrocele (11.7% - 35.3%) (185). Late filarial disease in the form of elephantiasis and hydrocoele was also observed in children younger than 15 years(185) suggesting early exposure and high levels of transmission.

Failure to find evidence of transmission to children of *W. bancrofti* in Gambia in 2013 over a 6-7 year period suggests that transmission had been interrupted (Figure 5) and the most likely cause was the extensive use of insecticide treated nets for malaria control over the last two decades built upon the causes of the decline observed [17] between the 1950 and 1970s. The growing evidence for the impact of malaria vector control activities on LF transmission was endorsed by WHO through a position statement in 2011 on integrated vector management (IVM) to control malaria and Lymphatic Filariasis (191). IVM is promoted by WHO to strengthen partnerships and cross sector approaches to the control of mosquito-borne diseases like malaria and LF (153, 191). In 2014, the Nigerian government converted these evidence based approaches into policy by launching a coordinated plan to eliminate malaria and Lymphatic Filariasis through the use of long lasting insecticidal nets.

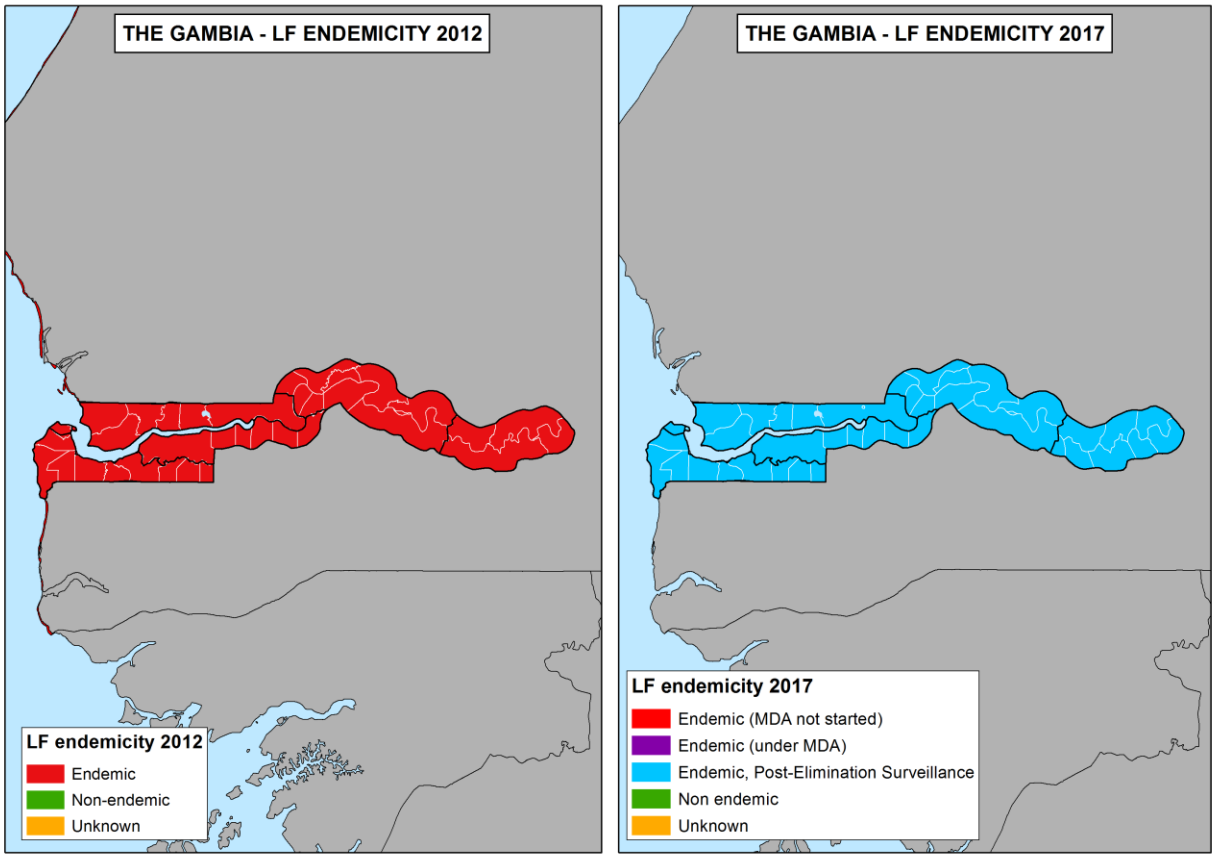

FIGURE 3.IMPACT OF THIS RESEARCH WORK ON THE LF MAP IN THE GAMBIA. 2012 (BEFORE) AND 2017 (AFTER)

The transmission assessment surveys carried in Zanzibar showed that five rounds of MDA on the island either failed to interrupt the transmission of LF on Pemba, where the TAS cut-off of 18 was surpassed by a huge margin early in the survey, or resurgence occurred after MDA was stopped in 2006. Unfortunately, the sentinel sites selected for monitoring the impact of MDA in Zanzibar did not include communities on Pemba and therefore the intensity of LF transmission on the island in 2006 could not be verified. Studies elsewhere have demonstrated that the vector of LF in Zanzibar, the highly efficient *Culex quinquefasciatus*, can sustain transmission in areas of low density Mf, even when Mf is undetectable using traditional diagnostic methods based on around 60 µl (192). In addition, recent studies on mainland Tanzania has also demonstrated that transmission of LF can persist after seven rounds of MDA in urban areas where *Culex quinquefasciatus* are the main vectors (193) . Infective mosquitoes were found in communities in India where MF rates dropped to zero after six rounds of treatment with DEC or ivermectin suggesting that transmission can occur in the absence of detectable Mf if *Culex* mosquitoes are the vectors (192, 194).

Between 2001 and 2006, the Zanzibar programme for the elimination of LF carried out effective annual MDA campaigns to interrupt the transmission of the disease (195). Ivermectin in combination with albendazole were administered by highly motivated community drug distributors known as Filarial Prevention Assistants (FPAs) who ensured a high treatment coverage ranging from 70 to 80% for all five rounds. MDA was stopped in 2006 after sentinel site surveys revealed parasite infection rates of zero in both humans and mosquito populations as reported by Mohammed in his 2009 PhD thesis (196). The infection rates in humans and mosquitoes were determined by night blood survey and dissections respectively. It had been demonstrated in Egypt, where *W. bancrofti* was also transmitted by *Culex* mosquitoes, that five rounds of MDA using albendazole plus DEC can interrupt the transmission of LF in a population of 2.5 million (197).

Lymphatic Filariasis was endemic on both islands before MDA commenced in 2001 as described in detailed investigations carried out many years prior the initiation of MDA (195, 198, 199). Cross-sectional clinical, parasitological and entomological surveys for LF, conducted in urban and semi urban communities on Pemba in 1990 revealed that LF endemicity and vector species composition had not changed significantly for 15 years (198). Mf prevalence rates on Pemba during a survey conducted in 1975 ranged between 11.8% and 16.2% for people aged above 1 year (198). Clinical manifestations in the form of hydroceole and lymphoedema were also common on the island, with prevalence of 22.4% and 1.4% respectively for adults above the age of 15 years (198). Similarly, surveys conducted on Unguja in 1975 showed that the overall prevalence of clinical signs among men aged 15 years and older was 29.6% for hydrocele and 7.9% for elephantiasis, while the Mf rates varied from 7.0% to 39.0% (196)

Vector control is effective against LF (200) but active vector control interventions did not resume in Zanzibar until after MDA for LF was stopped in 2006, when the Zanzibar Malaria Control Programme (ZMCP) started the distribution of free long lasting insecticidal nets (LLINs) targeting mainly pregnant women and children under the age of five years (201). Bednet usage was initially lower in Pemba in comparison to Unguja but by 2008 every household in Zanzibar had received two LLINs and, since 2006, six rounds of indoor residual spraying (IRS) have been conducted with synthetic pyrethroid lambda-cyhalothrin (ICON) resulting in over 90% coverage

of all dwellings. IRS and LLINs target both endophilic (indoor resting) and endophagic (indoor feeding mosquitoes) mosquitoes including the vectors of LF on Zanzibar (200). The combination of IRS and LLINs with other interventions resulted in a dramatic reduction of malaria prevalence in Zanzibar from 40% in 2005 to between 0.2 and 0.5% in 2011/2012 (201). The low prevalence of LF infection in children in Unguja may be partly explained by the impact of the vector control measures as previous efforts to control LF in Unguja by vector control resulted in 65% reduction in mosquito density in houses (201). The use of LLINs alone have resulted in the interruption of LF transmission in communities in Nigeria (202, 203) and Papua New Guinea (204). Based on quantitative analysis of elimination thresholds for LF, the probability that the parasite will be eliminated following six rounds of MDA increases as the vector biting rates decrease (205).

The surveys showed that the distribution of schools with antigen positive children was very heterogeneous on both islands with pronounced spatial variation between and within districts. Although Unguja barely passed the transmission interruption verification test by revealing fewer (19) CFA positive children than TAS cut-off of 20, four of the six positive schools had CFA positive rates higher than 5% and could enable transmission. Dissection of 6568 *Cx. quinquefasciatus* mosquitoes in 2006 found none to be infected with *W. bancrofti* but PCR assays on 5184 specimens collected between November 2007 and February 2008 showed a maximum likelihood infection rates of 1.13% (0.82% - 1.52%) that suggested ongoing transmission. It is therefore very likely that transmission was ongoing on the Island in 2006 when MDA was stopped (196).

The decision to stop MDA after several effective rounds of MDA should be based on statistically robust methodology. A recent multicenter evaluation to define endpoints for MDA in 11 countries concluded that TAS was superior to previous WHO guidelines used to determine when to stop MDA (206). It was shown to be a practical and effective evaluation tool for stopping MDA although its validity for longer-term post-MDA surveillance will require the use of more sensitive tools for detection infection in humans and mosquitoes. The TAS surveys carried out in 2012 suggested that LF transmission was still active on Pemba. Previous entomological studies suggested that LF transmission was active on Unguja shortly after stopping MDA in 2006. Based on these findings including the heterogeneous distribution of CFA positive children in Unguja,

and the high number of positives found compared (19) to the cut-off value (20) the government of Zanzibar decided to resume MDA with ivermectin plus albendazole on both islands in 2013 (Figure 6).

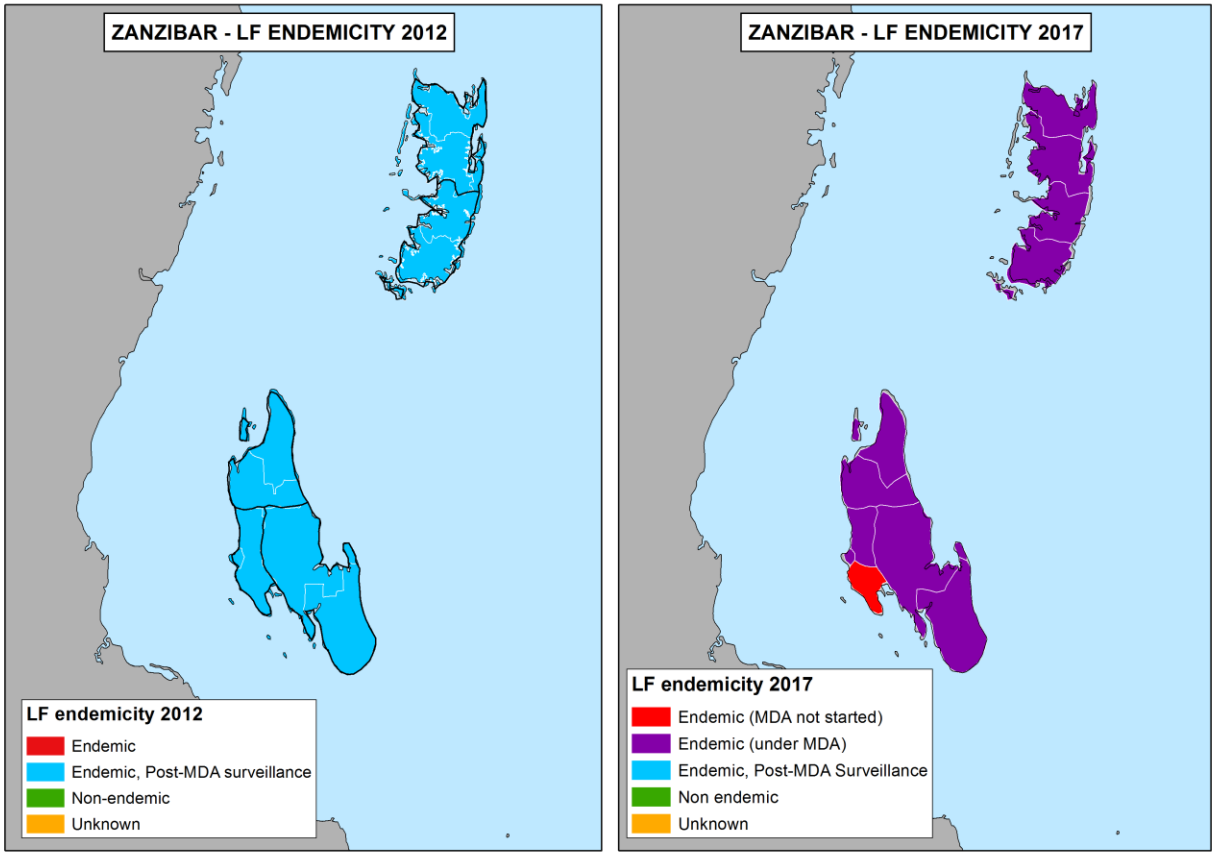

FIGURE 4. IMPACT OF THIS RESEARCH WORK ON THE ZANZIBAR MAP OF LF. 2012 (BEFORE) AND 2017 (AFTER)

It has been suggested that preventive chemotherapy may be required for more years in Africa than in India due to the higher efficacy of DEC plus albendazole (132). However LF coexists with loiasis in some implementation units in 10 countries in Africa and people infected with *Loa loa* can experience serious adverse events including progressive neurologic decline and encephalopathy following treatment with ivermectin or DEC (1, 165). In 2015 eleven countries had not started MDA for LF in Africa. Among those implementing MDA in 2015, 23 had not achieved 100% geographic coverage. Therefore 34 countries were not on track to eliminate LF as a public health problem by 2020. However, based on the recent research outputs and model predictions for alternative intervention tools and treatment strategies, fewer than the traditional 5-6 years duration of programme could be sufficient to interrupt LF in the countries left behind

(132, 149, 207). About half (23) of the 44 countries left behind are in Africa where transmission by the *Anopheles* vectors is very inefficient (161, 167). Results of ongoing research on the efficacy of the triple drug therapy of have demonstrated that treatment with ivermectin, DEC and albendazole (IDA) in combination could significantly accelerate transmission interruption in countries where onchocerciasis is not endemic(132). It is therefore not out of the question that the 2020 target for LF elimination will be met because the implementation challenges previously identified in the 2010 -2020 WHO Strategic Plan for GPELF (159) either no longer exist or have already been addressed (150, 161).

Whilst IDA may help accelerate the elimination of LF, it is important to delineate the settings where its use for MDA would be safe and appropriate (208). One major concern is the risk of severe adverse events (SAEs), which can often arise after certain drugs are given to those who are microfilaraemic. In the limited setting of the IDA pilot trial, adverse events were more common in those treated with triple therapy, which has important implications for programme safety and compliance (209). More generally, DEC cannot be used for LF campaigns in areas where onchocerciasis is present, because it induces a strong local inflammation in patients with ocular microfilariae (210). Similarly, providing ivermectin to those heavily infected (i.e. high Mf load) with the 'non-pathogenic' filarial parasite *Loa loa* has been associated with severe neurological reactions (211), precluding its use in forested areas throughout much of central Africa (212). This has led to the recommendation that twice yearly albendazole be implemented together with distribution of long-lasting insecticidal nets for control of LF in *Loa loa* co-endemic areas (213).

As a complement to this PhD project three additional topics were researched. Firstly cross reactivity of *Loa loa* antigens to LF ICT cards in Gabon have resulted in an overestimation of the population infected by LF (214). Secondly predictive risk maps for LF, onchocerciasis and *L. loa* were developed to delineate populations targetable for different MDA packages, and quantify populations at-risk of SAEs across Africa (3). Lastly lack of urban transmission of LF in West Africa (Sierra Leone and Liberia) was studied (163). It was shown that only 3.2% of the at-risk population lives in areas of high loiasis transmission, representing up to 2.4 million individuals at high risk of experiencing SAEs if treated with ivermectin. In these areas, alternative MDA

packages should be explored, including biannual albendazole monotherapy for LF (1.7 million individuals) and annual doxycycline (15 million individuals) for onchocerciasis. Studies in Gabon were unable to demonstrate presence of antibody positive individuals against Wb123 LF antigen suggesting that the previous classification of Gabon as LF endemic may be due to cross reactivity between LF and *L. loa* antigens in ICT (214). This has implications in countries that are *L. loa* endemic and may have been wrongly classified as endemic for LF. This supplementary work suggest additional methods to shrink the LF map in Africa.

Elimination of lymphatic filariasis as a public health problem by 2020 will be achieved mainly through preventive chemotherapy, based on annual MDA to entire eligible populations. However vector control may play a major role in the elimination of the disease where Anopheles mosquitoes are the main vectors and malaria vector control activities are wide spread. In 2014, after resurveying endemic districts that never started MDA for this disease, mapping surveys completed in Gambia (167) reported no evidence for lymphatic filariasis transmissions in the countries where, altogether, 2.5 million people were estimated to require preventive chemotherapy.

Similar mapping and confirmatory mapping efforts in areas where MDA has not started, guided by modelling tools and using the most up to date diagnostic and epidemiological tools could result in further shrinking the map in other high burden, *Loa loa* coendemic countries like DRC or Gabon, or where bednet coverage is high like Zambia or Equatorial Guinea.

The population at risk for LF in Africa is lower than estimated by WHO in Ethiopia (from 30 million to 5.9 million) and the Gambia (from 1.4 million to 0), while people are still at risk of LF in Zanzibar and requiring PC (from 0 million to 1.2 million) despite MDA having been stopped prematurely (Table 6).

TABLE 6. RE-ESTIMATION OF POPULATION REQUIRING TREATMENT BASED ON RESEARCH CONDUCTED FOR THIS THESIS

	Country	*Ethiopia*	*The Gambia*	*Zanzibar*
Year 2012	**Estimated Population**	68,445,633	1,412,054	1,027,674
	Endemic	30,000,000	1,412,054	1,027,674
	Non-endemic	38,445,633	-	-
Year 2018	**Estimated Population**	79,722,590	1,662,686	1,235,919
	Endemic	5,893,309	0	1,235,919
	Post elimination Surveillance	-	1,662,686	-
	Non-endemic	73,829,281	-	-

Further in the supplementary studies conducted as a complement for this thesis the suggested number of people requiring treatment for LF is reduced in the following countries: Gabón (de 1.3M a 0), Sierra Leona (Monrovia de 939 524 a 0) y Liberia (Freetown de 802 639 a 0).

In the WHO 2014 list of countries not on target to achieve 2020 goal (not starting MDA or not yet having 100% geographical coverage), 25 African countries are listed (Table 7). Among those, the Gambia, Gabon and Ethiopia appear as not having started MDA or not having reached 100% geographical coverage. The results from these PhD studies suggest that those countries were misclassified. Re-estimating the areas at risk in those countries using the tools described on this thesis may help to have a more realistic target population and identify the areas and countries that are truly behind so that efforts can be increased in those to accelerate elimination by 2020.

TABLE 7. INDICATORS OF PROGRESS BY 2014

MDA NOT STARTED	**MDA <100% GEOGRAPHICAL COVERAGE**	**MDA AT 100% GEOGRAPHICAL COVERAGE**	**POST MDA SURVEILLANCE**
Angola	Benin	Burkina Faso	Malawi
Chad	Cameroon	Comoros	Togo
Equatorial Guinea	Centro African Republic	Ghana	
Eritrea	Congo	Liberia	
Gabon	Cote d' Ivoire	Mali	
Gambia	DRC	Niger	
Sao Tome Principe	Ethiopia	Sierra Leone	
South Sudan	Guinea	Tanzania	
	Guinea Bissau		
	Kenya		
	Madagascar		
	Mozambique		
	Nigeria		
	Senegal		
	Sudan		
	Uganda		
	Zimbabwe		

Conclusions

Conclusions

- The population requiring treatment for LF in Ethiopia is at least 60% less than the estimated by WHO in 2012.

- The population at risk for LF in 112 endemic disctrics of Ethiopia is 11 580 010 instead of the estimated 30 million.

- Transmission assessment surveys conducted in The Gambia demonstrate lack on active transmission of LF across the country due to vector control for malaria.

- The Gambia has now been removed by WHO from the list of LF endemic countries and 1.2 million people declared to be no longer at risk for the disease.

- Although MDA for LF was stopped in 2006 and transmission declared interrupted, the transmission assessment surveys carried out in Zanzibar showed that five rounds of MDA on the islands failed to permanently interrupt the transmission of LF.

- As a conclusion of this research, the entire population of Zanzibar, 1 235 919 people were declared as requiring preventive chemotherapy against LF and treatment resumed in the islands.

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