Molecular Detection of *Wuchereria bancrofti* in Human Blood and Mosquitoes from Selected Endemic Areas in Egypt

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Abstract

Lymphatic filariasis is a vector-borne health problem, which have a profound impact on patient's lives and can lead to permanent disability. *Wuchereria bancrofti* (*W. bancrofti)* is the major cause of filariasis worldwide and is focally endemic in Egypt. Diagnosis of filarial infection using traditional morphologic and immunological criteria can be challenging and lead to misdiagnosis. The aim of the present cross-sectional study was the molecular detection of *W. bancrofti* in human blood and mosquitoes from selected endemic areas in Egypt. Blood samples were collected from 300 individuals residing in filariasis endemic areas, and were subjected to ELISA for the detection of *W. bancrofti* antigens and semi-nested PCR targeting *W. bancrofti* repeated DNA sequences. Mosquito pools from same endemic areas were collected, sorted, and subjected to semi-nested PCR. Additionally, a group of positive PCR products were subjected to DNA sequencing and phylogenetic analysis. Results revealed that out of the 300 collected blood samples; 7 samples were positive by ELISA (2.3%), 45 samples were positive by semi-nested PCR (15%) and 3 samples were positive by both tests (1%). All the collected mosquito pools were negative. Sequences analysis confirmed semi-nested PCR results; identifying only *W. bancrofti* species. Sequence alignment and phylogenetic analysis indicated genetically distinct clusters of *W. bancrofti* among the study population.

Key Words: *Wuchereria bancrofti,* phylogenetic analysis, semi-nested PCR, repeated DNA sequence, ELISA, blood samples, mosquitoes

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List of Abbreviations

Ab	Antibody
ADLA	Acute Dermatolymphangioadenitis
AFL	Acute Filarial Lymphangitis
Ag	Antigen
AM	Ante Meridiem
AT	Adenine - Thymine
B.	*Brugia*
bp	Base pair(s)
CFA	Circulating Filarial Antigens
Cx.	*Culex*
D.	*Dirofilaria*
DALYs	Disability-Adjusted Life Years
ddH_2O	Double Distilled Water
DEC	Diethylcarbamazine
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
ddNTPs	Dideoxynucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunsorbent Assay
FDS	Filarial Dance Sign
fg	Femtogram
FNAC	Fine Needle Aspiration Cytology
GPELF	Global Programme to Eliminate Lymphatic Filariasis
HRPO	Horseradish Peroxidase
ICT	Immunochromatographic Test
Ig	Immunoglobulin
Kb	Kilo base pairs
kDa	Kilo Dalton
L1	First -stage larvae
L2	Second-stage larvae
L3	Third-stage larvae
L4	Fourth-stage larvae
LAMP	Loop-Mediated Isothermal Amplification
LF	Lymphatic Filariasis
M.	*Mansonella*
mAb	Monoclonal Antibody
Mb	Mega base pairs

MDA	Mass Drug Administration
Mf	Microfilariae
MgCl2	Magnesium Chloride
MOHP	Ministry of Health and Population
NCBI-BLAST	National Center for Biotechnology Information – Basic Local Alignment Search Tool
NTD	Neglected Tropical Diseases
O.	*Onchocerca*
OD	Optical Density
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
pg	Picogram
PM	Post Meridiem
QBC	Quantitative Buffy Coat
q-PCR	Real Time - Polymerase Chain Reaction
rDNA	Ribosomal – DNA
spp.	Species
TBE	Tris Borate EDTA
TMB	3,3',5,5' - Tetramethylbenzidine
TPE	Tropical Pulmonary Eosinophilia
UV	Ultraviolet
W.	*Wuchereria*
WHA	World Health Assembly
WHO	World Health Organization
YLDs	Years Lived with Disability
YLLs	Years of Life Lost

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1.INTRODUCTION

1. INTRODUCTION

Lymphatic filariasis (LF) is a major vector-borne public health problem affecting more than 120 million people in over 80 endemic developing countries. *Wuchereria bancrofti (W. bancrofti)* which is responsible for 90% of cases throughout the tropics and in some subtropical areas worldwide is focally endemic in Egypt **(Melrose, 2002 & Foo *et al.*, 2011).**

The disease has been identified by World Health Organization (WHO) as the second leading cause of permanent and long term disability. In addition to medical problems, there are severe social and psychological consequences especially in those who suffer from elephantiasis or hydrocoele. LF also has a vast economic impact upon endemic communities resulting from the direct costs incurred in medical and surgical treatment, in addition to indirect costs from reduced work capacity and labour loss **(Melrose, 2002).**

To combat this disease, the WHO has launched a "Global Program to Eliminate Lymphatic Filariasis" (GPELF), aiming to eliminate LF by the year 2020 in all the endemic countries, using mass drug administration (MDA) to interrupt the disease transmission. Egypt was one of the first countries to implement a national program to eliminate LF based on WHO's strategy of repeated rounds of annual treatment in the form of albendazole with diethylcarbamazine (DEC). Subsequently, transmission was shown to be markedly reduced among villages that prior to MDA exhibited some of the highest rates of LF **(Hotez *et al.*, 2012 & Upadhyayula *et al.*, 2012).**

The exponential growth of LF elimination program has highlighted the need for sensitive tools that can be used to monitor progress towards programmatic

endpoints. As well as, to conduct surveillance, rapid and early detection of cases that can be often challenging for several reasons **(Lammie *et al.*, 2004).**

For instance, LF is characterized by a wide range of clinical presentations. One group of individuals in the endemic community shows no clinical manifestations or microfilariae (Mf). This includes individuals who have not been sufficiently exposed to be infected, individuals with prepatent infection or adult worm infection without Mf, and individuals who have cleared the infection. Another group of individuals in the endemic community shows Mf in their blood but no obvious clinical manifestations. Some of these may remain microfilaraemic and asymptomatic for years or even for the rest of their lives, while the rest may become symptomatic **(Simonsen, 2009).**

As regards the laboratory diagnosis of LF, the night time blood collection for Mf detection and the low intensity of parasites have created a problem which have led to the development of alternative methods for diagnosis. Immunological assays are reasonably sensitive and more specific than the earlier tests. The detection of specific circulating filarial antigens (CFA) are extensively used by the WHO for field diagnosis of LF, however the cost and inconsistent availability remain drawbacks to antigen detection tests **(Nuchprayoon, 2009).**

Recent studies have shown that filarial DNA could be detected in human blood and in mosquito blood meals by polymerase chain reaction (PCR) based assays, which offer the possibility of improved sensitivity and specificity. Moreover, recent advances in molecular biological technology are giving parasitologists new insights into the structure and function of the filarial genome **(Kanjanavas *et al.*, 2005 and Liu & Austin, 2013).**