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Immunological and Molecular Similarities between Human Filarial *Loa loa* and *Brugia pahangi* Antigens

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Abstract

Background: The filarial parasite *Loa loa* is restricted to Africa. This parasite impedes the WHO strategy for controlling filarial worms by worldwide mass chemotherapy. This obstacle is due to the potentially fatal side effect of *Loa loa* following treatment with ivermectin. Attempts to map the distribution of this parasite using serology encounters the issue of cross-reactivity of parasitic nematode antigens.

Aim: Analyze the extent of cross-reactivity of different antigens.

Methods: Analyse IgG subclasses reactivity to a panel of *B. pahangi* antigens by ELISA in a population exposed to *L. loa,* followed up by analysis of antigens with Western blot and deglycosylation. The *L. loa* DNA was then amplified using *Brugia pahangi* primers. The subsequent amplicons were analyzed using bioinformatic methods.

Results: The population of one village in Gabon was divided, according to the parasitological and clinical findings, into four groups: endemic controls, amicrofilaraemics, low microfilaraemics and high microfilaraemics. This study was carried out using *B. pahangi* adults, microfilariae and L3 antigens, as a surrogate for *Wuchereria bancrofti* and compared the level of IgG subclasses in these different defined groups of villagers. The study showed that the level of IgG1 was significantly higher in amicrofilaraemics compared to high and low microfilaraemics. IgG4 was high in all groups, but there was no significant increase in IgG2 and IgG3. Interestingly, the level of IgG1 was inversely correlated with microfilarial density when using the L3 antigen (Spearman's $r = -0.839$; $p < 0.0001$). Identification of the antigenic target of this response showed recognition of several molecules varying in size from 8kDa to 150 kDa. The removal of the glycosylated residues in the *B. pahangi* adult did not inhibited the reactivity of the major reacting antibodies IgG1 and IgG4 from the *L. loa*-infected population, suggesting that the reactivity is linked to the peptide backbone. Amplification of *L. loa* DNA using primers designed from the gene encoding a Brugia microfilariae 22-kDa *Brugia* gene showed an amplicon of 694-bp with similarities varying from 10% to 100% between the genes and proteins of *L. loa*, *B. pahangi*, *Brugia malayi* and *Wuchereria bancrofti.*

Conclusion: This study suggests that the map of the distribution of lymphatic filarial in *L. loa* endemic zones should be re-evaluated. However, the similarities in structural epitopes could be exploited in view of vaccine strategy designed to control *L. loa*.

Keywords: *Loa loa; Lymphatic Filaria; Antigens; Cross-Reactivity; Gene*

Introduction

Loa loa, a restricted filarial species from central and some West African countries, causes a problem to the WHO strategy on elimination of filariae in areas where *L. loa* is co-endemic with other filariae such as *Onchocerca volvulus* and *Wuchereria bancrofti*, because mass

administration of ivermectin (IVM) or diethylcarbamazine (DEC) used for this control may induce encephalitis and death after a few days [1-3]. This *Loa loa* may also involved in deep organs such as the lungs and heart or cause renal complications [4]. A recent study noted excessive mortality in individuals with heavy *L. loa* microfilariae, although it did not take into account other factors for mortality [5]. Nonetheless, *L. loa* is not mentioned on an official World Health Organization (WHO) list of neglected tropical diseases [6]. Efforts to meet the objective of control of filarial worldwide will need baseline data with which to delineate the prevalence of filarial, particularly *L. loa* in Africa due to the risk of encephalitis. Further studies are needed to design a diagnostic test which will be simple, specific and sensitive to reach this goal in the field.

To overcome this difficulty, the use of heterologous models of filarial infection may bring insight on how to control filarial. In this we utilised *Brugia pahangi,* lymphatic filarial worm originally infecting cats in Malaysia [7,8]. This species has become a model of filarial infection in *Meriones unguiculatus* [9]. The similarity between observations derived from this and human lymphatic filarial are numerous both on the molecular or immunological level [10].

The lack of a small animal model hampers similar studies on loiasis. In regions endemic for *L. loa*, efforts to map *W. bancrofti* and *L. loa* parasites encounter cross-reactivity between antigens and antibodies, resulting in insensitivity of diagnostic tests and therefore controversial conclusions. This assertion is substantiated by several other studies. Firstly, studies performs using the immunochromatographic whole blood card test (ICT) and Og4C3 enzyme linked immunosorbent assay (ELISA) show some discrepancy between ELISA and the ICT test [11] suggesting that the use of this technique to monitor the lymphatic filarial may not be accurate. Several recent studies have confirmed this assertion, even when using the new **Filariasis** *Test* Strip (FTS), the WB123 antigen test, and qPCR [12]. The study concluded that there was no *W. bancrofti* in Cameroon. Another study in DRC [13] suggests that the use of antigenemia may not be reliable in endemic zone for *L. loa*, where this may result in the overestimation of the lymphatic filarial. One implication of the use of the ICT in Cameroon concludes by suggesting that the distribution of lymphatic filarial must be reduced [14]. Beside this study, it was suggested that the association between microfilaria *L. loa* and ICT/CFA tests should be considered at both individual and community level [15] because identifying co-endemic areas will allow the identification of regions at risk of side effects [16].

All these observations are due to the cross-reactivity observe when using these tests. The cross reactivity is likely to be due to the nature of antigens. Glycoproteins are often responsible for the immunological cross-reaction [17]. A study has identified this antigen as 200 kDa in *W. bancrofti* [18]. There is also a non-protein determinant, present in all nematode parasites in many different antigen molecules like TEP 15 in phosphocholine (pc), with size between 200 and 78 kDa [19]. The wide distribution of lymphatic filariae in Africa is well accepted because of the abundance of the vector (*Aedes aegypti*, *Culex quinquefasciatus*) and some clinical signs like as hydrocele [20,21]. Currently, there is no vaccine to prevent this infection. In this situation, mass chemotherapy appears now to be the most appropriate control method of control; but this need the mapping of the distribution of filarial A good diagnostic test is a key to reach this goal.

Materials and Methods

Ambinda population

A series of surveys was organized since 1984 by the International Center for Medical research of Franceville (CIRMF) for a long-term follow-up of a community in Gabon. The village of Ambinda in southeast Gabon, 150 km north of the CIRMF, with about 170 inhabitants, was kept under observation for 5 years.

Parasite material

B. pahangi was maintained in the laboratory in cyclical passage in jirds (*Meriones unguiculatus*) and transmitted through susceptible *Aedes aegypti* by membrane feeding.

B. pahangi infective stage (L3) were obtained from *Aedes aegypti* mosquitoes 10 days after the infecting blood meal. After being immobilized at 4°C, mosquitoes were slightly crushed on a glass plate by rolling a test tube over them. The mosquitoes were then placed in a funnel containing several layers of gauze with Hank's balanced salt solution (HBSS), pH 7.4. The L3 settled at the bottom of the funnel and were collected, transferred to clean HBSS and counted.

B. pahangi adult and microfilariae were obtained from jirds infected for at least 3 months by lavage of the animal's peritoneal cavity with HBSS.

Preparation of parasite antigens

All stages of parasites used were washed three times in PBS, pH7.2, then adults and L3 were homogenized, whereas microfilariae were sonicated. Homogenization and sonication were carried out in a solution of 10 mM Tris-HCl, pH 8.3, containing protease inhibitors (2 mM PMSF, 2 mMTPCK, 2 mMTLCK). Sodium deoxycholate was added to the homogenate up to a final concentration of 1% for detergent extraction of antigen, and the mixture was left on ice for 1h. The extracts were centrifuged at 10,000 rpm for 10 minutes and the supernatant was used as the soluble antigen. The protein content was measured according to the Bradford method [22] for immunoblotting, all stages of the parasite were sonicated, or homogenized and extracted by boiling in SDS sample cocktail, containing 2% SDS and 100 mM dithiothreitol. In this case, the relative protein content of each extract was determined by titration on mini-SDS Page gels.

Enzyme linked immunosorbent assay (ELISA)

First, 100 µl of antigen at 10 µg/ml in carbonate buffer, pH 9.6, was coat, overnight at 4°C. The following day, 200 µl of diluted serum was added for an hour. Plates were washed and then incubated with 100 µl/well of anti-IgG 1, 2 (1/2000); IgG3 (1/1000) and IgG4 (1/30,000) were incubated in each well for 1 hour at room temperature. After three washes, the plates were incubated with anti-mouse IgG Fc-specific alkaline phosphatase conjugate (Sigma) for an h, followed by three further washes. In all cases, p-nitrophenyl phosphate (Sigma 104) was used as substrate, diluted in diethanolamine buffer, pH 9.8 and incubated for 6 min or 10 min in the case of the L3 antigen. The optical density (OD) was read at 410 nm (Dynatech reader) or 405 nm (Pasteur LP 200 reader).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on discontinuous gels according to Laemmeli [23], under reducing conditions and using 12.5% acrylamide. The appropriate amount was loaded in each well after boiling for 3 minutes in an SDS-PAGE sample cocktail. Gels were run overnight at 6mA/gel with cooling, or in the case of Biorad mini gels, at 200V for 1h at room temperature.

Western blotting

After SDS-PAGE under reducing conditions, the proteins on the gel were transferred onto nitrocellulose paper (NCP) by electrotransfer according to [24] The NCP was stained with Ponceau S (Sigma) to visualize the bands, cut into strips, then blocked for an hour in TBST/3% BSA, followed by three washes in TBST for 10 min each, at room temperature. Each strip was incubated overnight at 4°C with serum or plasma diluted 1/200, in TBST, 1% BSA, followed by a washing step, as before. The strips were then incubated with one of the mouse monoclonal antibodies against human IgG, IgG1, IgG2, IgG3, and IgG4 for 2 hours at room temperature at the same dilution as used for ELISA and washed as before. Subsequently, the NCP strips were incubated for 1 hour with anti-mouse IgG Fc-specific conjugated with alkaline phosphatase, diluted at 1/1000, 1/1000, 1/500, 1/400, respectively in TBST/1% BSA. This was followed by another washing step and incubation in the substrate solution containing 5-bromo-4-chloro-3-indolyl (BCIP/0.3 mg/ml) and nitro blue-tetrazolium (NBT, 0.15 mg/ml) in 1M Tris-HCL, 500 mM $MgCl_2$ buffer, for visualization of bound antibodies.

Deglycosylation of antigen with N-linked oligosaccharides

40 µg of antigen was boiled with an equal volume of 1% SDS/1.6ME for 3 minutes and incubated overnight at 37°C in three volumes of 0.55M phosphate buffer, pH 8.6, containing 100 mM of 1 - 10 phenanthrolene, 7.5% Nonidet P40 and 5 or 10 U/ml of N-glycanase (Boehringer). The reaction was stopped by adding an equal volume of the SDS-PAGE sample cocktail buffer, the sample was loaded on an SDS polyacrylamide gel after electrophoresis of the deglycosylated solution, the proteins were transferred to NCP for detection by Western blot using human sera.

Extraction of *Loa loa* genomic DNA

Two millions of purified *Loa loa* microfilaria were homogenized in 950 µl plus 50 µl of proteinase K at 1.5mg/ml and then incubated for 3 - 4h at 65°C, followed by extraction three times in a solution of phenol/chloroform (1:1). The aqueous phase of the extract was pre-

cipitated in 95% ethanol, 3M sodium acetate at −20°C. The DNA pellet was rinsed with 70% ethanol and kept in TE at 4°C until use. DNA purity was assessed by calculating the ratio between absorbance of the DNA at 260/280 nm and the concentration of DNA determined with its OD at 260X 50Xdilution factor.

Gene amplification by the polymerase chain reaction (PCR)

PCR was performed in all cases with a Gene Amp DNA amplification kit from Perkins Elmer Cetus (Norwalk, CT, USA). The reaction in general was performed by mixing 2 µl of the 10-mM solution of each mixture of all four dNTPs (dATP, dCTP, dGTP, dTTP 10 mM each); 30 ng/µl of each primer (3′; 5′ primer); 10 µl of PCR buffer; 1 - 200 ng of template DNA; and 2.5U of *Thermus aquaticus* polymerase (Taq). The volume was made up with 100 µl of sterile water and the mixture overlaid with a drop of mineral oil. The reaction tube was then put in a thermocycler for 35 cycles divided into the following steps: 95°C for 5 minutes before adding the Taq enzyme for denaturing the template, then 94°C for 1 minute for melting, 1 minute at 55°C, for annealing, 2 minutes at 72°C for extension, with a final extension at 72°C for 5 minutes. The primers, 3′TACTACTAAAGTCAATACACAACA5′ and 5′TCATTGACATGGTTGACATATTCTG, were designed from proline rich protein from *B. pahangi*, accession number X58063. The PCR products were analyzed on agarose gels.

Cloning

After PCR the amplicons were cloned with a TA cloning procedure (according to the manufacturer's instructions), the white cell colonies were picked up and grown overnight at 37°C in a shaker follow by extraction of plasmid DNA [25] Clones containing a 554- to 694-bpinsert were used for sequencing.

Agarose gel electrophoresis

We dissolved 1% or 2% agarose gel in TAE or TBE with 0.5 µg/ml ethidium bromide and poured this into a 5 X 7 cm mini gel mould. Samples were loaded after addition of sample buffer. Electrophoresis was carried out at 60V for 1h. Nucleic acids were visualized by UV light using an ultraviolet transilluminator. The size of the DNA was determined by comparison with standard markers.

Sequencing

The reactions were performed using an automated DNA sequencer (4000 Automated DNA Sequencer) with Sequi Therm Thermostable DNA polymerase and IRD41-labeled primer according to the manufacture's instruction kit. Briefly, a double-stranded DNA template was mixed with IRD41-labeled primer, 10X sequencing buffer and SequiTherm Thermostable DNA polymerase; the total was volume completed to 17 µl, 4 µl of which was distributed into four microfuge tubes labeled A, T, G and C. A drop of mineral oil was put on top of each reaction mixture tube. The four tubes were inserted into the thermocycler and the following program was applied: 95°C for 5 minutes: 95°C for 30s, 60°C for 30s and 70°C for 1 minute, for 30 cycles. The tubes were soaked at 4°C and 4 µl of sequiTherm stop solution was added to each tube, mixed thoroughly and the sample was denatured by heating at 95°C for 3 minutes before loading into the sequencing gel.

Bioinformatic analysis

The nucleotide sequence and the deduced protein were analyzed with bioinformatic software: ProtScale from Expasy for hydrophobicity scale [26] and Prosite for prediction of different domain pattern Like N-glycosylation site, myristylation, phosphorylation. Blast searches with *B. pahangi*, *B. malayi*, *Wuchereria bancrofti* genome parasite in Wormbase.org [27] were used to identify lymphatic filarial homologs of our *Loa loa* gene.

Statistical analysis

For the comparison of the difference between the median value, the Mann-Whitney U-test and the Spearman rank correlation were used to examine the relationship between groups and parameters. In each case, $p < 0.05$ was considered significant.

Results

Quantitative analysis of humoral response by ELISA

The study used *B. pahangi* adults, microfilaria, and L3 antigen to compare the level of IgG subclasses in different defined groups of villagers. This study showed that the endemic population had higher levels of IgG than the nonendemic population. The levels showed

that the endemic population had higher levels of IgG, than non-endemic population. The levels of IgG1 were significantly higher in amicrofilaremic compared to high and low microfilaremics. IgG4 was high in all groups, but there was no significant increase in IgG2 and IgG3 (Figure 1-3). Interestingly, the level of IgG1 was inversely correlated with microfilarial density when using the L3 antigen (Spearman's r = −0.839; $p < 0.0001$), but there was no relationship between microfilaria density and IgG4.

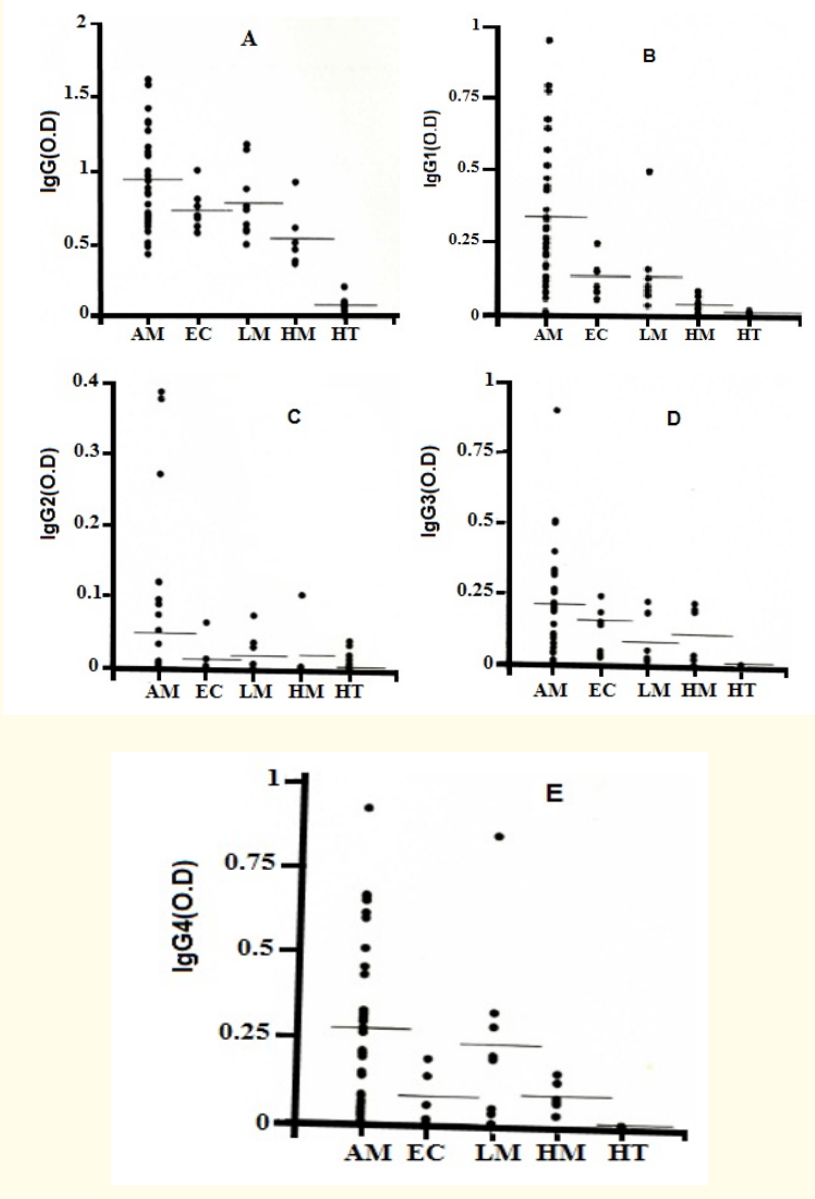
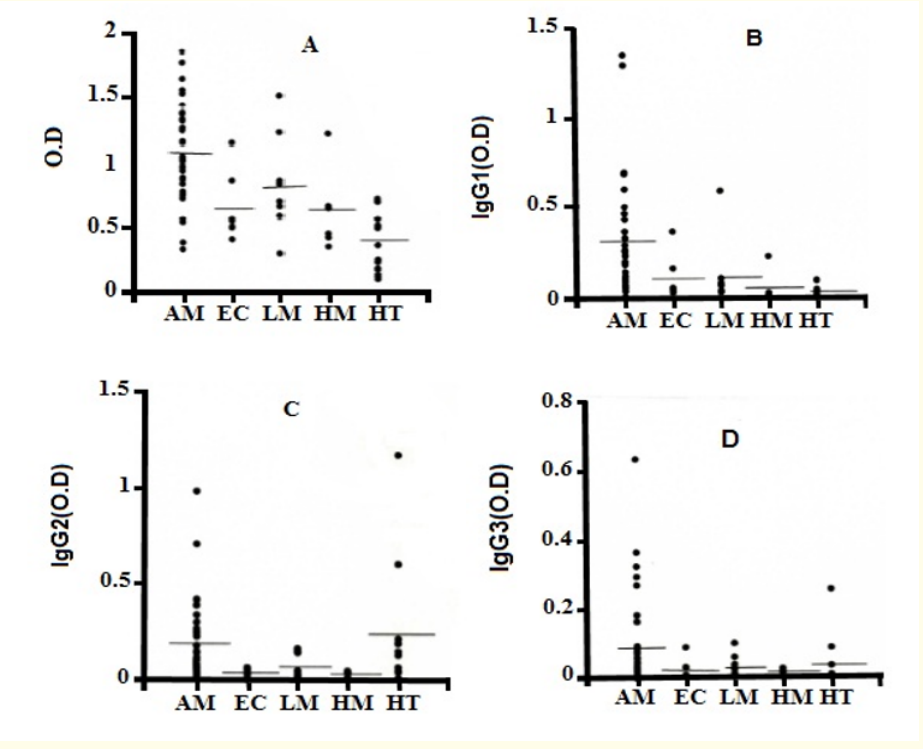


Figure 1: *The level of IgG subclasses obtained by ELISA with DOC extract of B. pahangi adults. Specific IgG (A), IgG1 (B), IgG2 (C), IgG3 (D), IgG4(E) were measured in ELISA for each individual included in the amicrofilaraemic group (AM), endemic control (EC), low microfilaraemic (LM) and high microfilaraemic (HM) from Ambinda village, or the heterologous group (HT) of non-endemic individuals without filarial infection but with other parasites. Each dot represents the level of antibody expressed as optical density (OD) and the short horizontal bar represents the mean level of the group.*



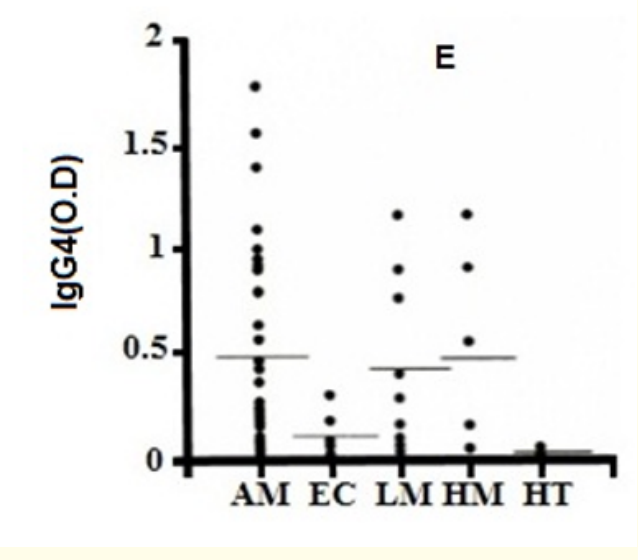
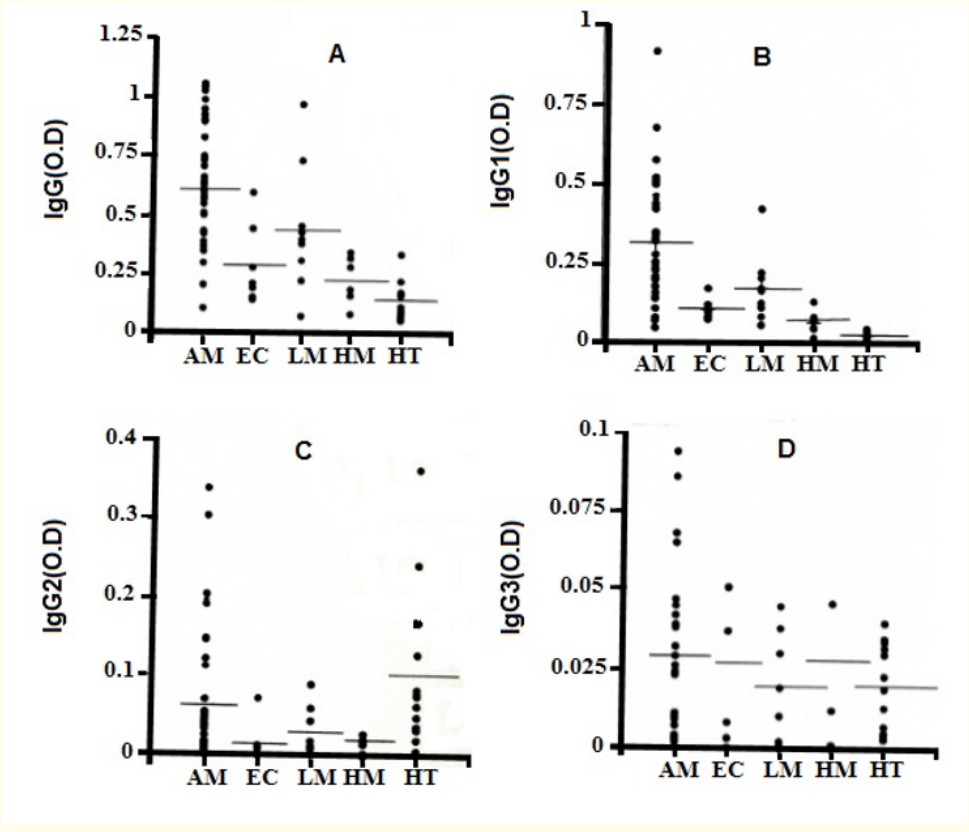


Figure 2: *The level of IgG subclasses obtained by ELISA with DOC extract of B. pahangi microfilariae. Specific IgG (A), IgG1 (B), IgG2 (C), IgG3 (D), IgG4(E) were measured in ELISA for each individual included in the amicrofilaraemic group (AM), endemic control (EC), low microfilaraemic (LM), high microfilaraemic (HM) from Ambinda village, or the heterologous group (HT) of non-endemic individuals without filarial infection but with other parasites. Each dot represents the level of antibody expressed as optical density (OD) and the short horizontal bar represents the mean level of the group.*



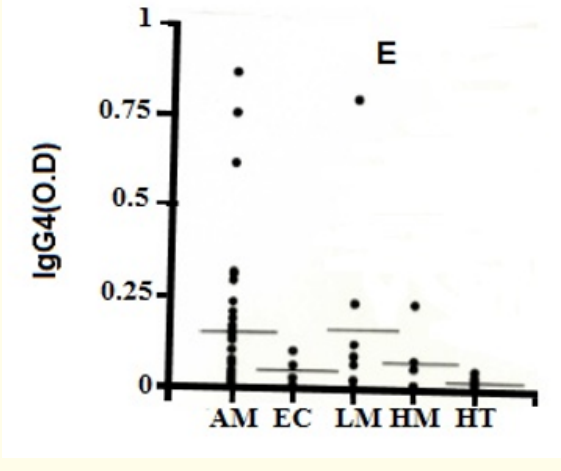


Figure 3: *The level of IgG subclasses obtained by ELISA with DOC extract of B. pahangi L3. Specific IgG (A), IgG1 (B), IgG2 (C), IgG3 (D), IgG4(E) were measured in ELISA for each individual included in the amicrofilaraemic group (AM), endemic control (EC), low microfilaraemic (LM), high microfilaraemic (HM) from Ambinda village, or the heterologous group (HT) of non-endemic individuals without filarial infection but with other parasites. Each dot represents the level of antibody expressed as optical density (OD) and the short horizontal bar represents the mean level of the group.*

Analysis of antigen recognition by isotypes using Western blot

Qualitative analysis by Western blot against antigens at all stages also showed that antigens of different molecular weights are recognized in all stages of *B. pahangi*. Several antigens were recognized by amicrofilaremics and endemic controls sera compared to a low proportion of microfilaria carriers, while a few antigens were recognized exclusively by amicrofilaremics and endemic control sera. Most isotypes of Ig recognized broad patterns. IgG1 of amicrofilaremics, endemic and low microfilaremics but not high microfilaremics recognized more antigens in *B. pahangi* while all groups had IgG4 antibodies against these same antigens. The reactivity of IgG1subclasses (Figure 4A) and IgG4 (Figure 4B) was prominent, with molecular weight varying from high to low (10 - 100 kDa), but it was notable that the amicrofilaremic group recognized more antigens than any other group.

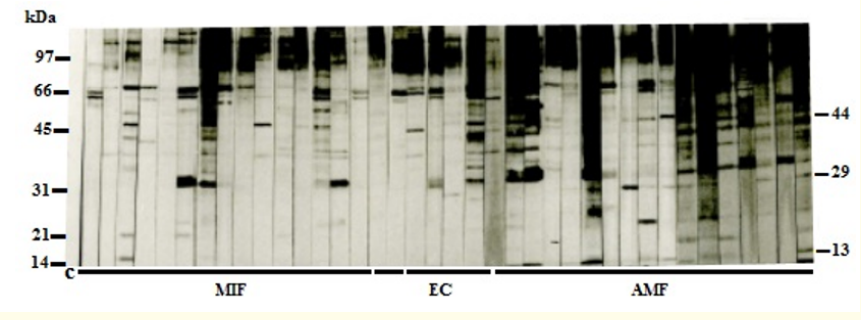


Figure 4A: *Identification of adult B. pahangi antigens by specific IgG1 antibodies.*
Western blots of adult B. pahangi antigen were prepared as described in Material and Methods. The NCP was cut into strips and each strip probed with individual human serum at a 1/200 dilution. The blot was developed with anti-human IgG1 and anti-mouse IgG alkaline phosphatase conjugate. A representative Western blot with individual sera from negative control (C), microfilariae carriers (MIF), endemic controls (EC) and amicrofilaremics (AMF). The positions of the differentially recognized antigens are marked with an arrow beside their respective molecular weights. The molecular weight standards are indicated on the left.

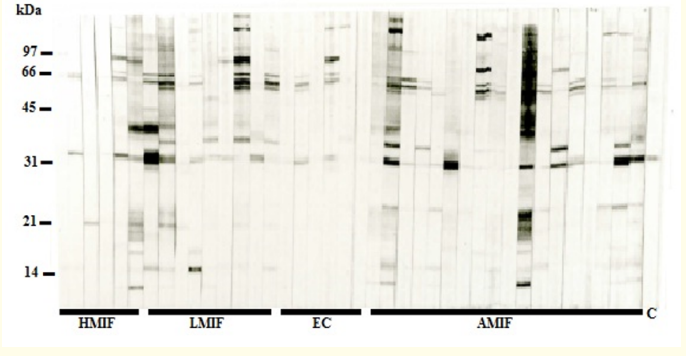


Figure 4B: *Identification of B. pahangi microfilarial antigens by specific IgG4 antibodies.*
Western blots of antigen B. pahangi microfilarial were prepared as described in material and methods. The NCP was cut into strips and each strip probed with individual human serum at a 1/200 dilution. The blot was developed with anti-human IgG1 and anti-mouse IgG alkaline phosphatase conjugate. A representative Western blot with individual sera from negative control (C), microfilariae carriers (MIF), endemic controls (EC) and amicrofilaremics (AMF). The positions of the differentially recognized antigens are marked with an arrow beside their respective molecular weights. The molecular weight standards are indicated on the left.

Molecular structure of the antigen and immunological reactivity of isotypes

We compared the reactivity of specific IgG1 (Figure 5, line 1, 2) and IgG4 (Figure 5, line 3,4) (the most immunoreactive isotypes) on both soluble antigens digested with N-glycanase and undigested *B. pahangi* antigens. It was noted that despite the shift in molecular weight of the glycosylated antigens the reactivity of IgG1 (Figure 5, line 3, 4) and IgG4 (Figure 5, line 1, 3) remained high (Figure 5, line 3,4).

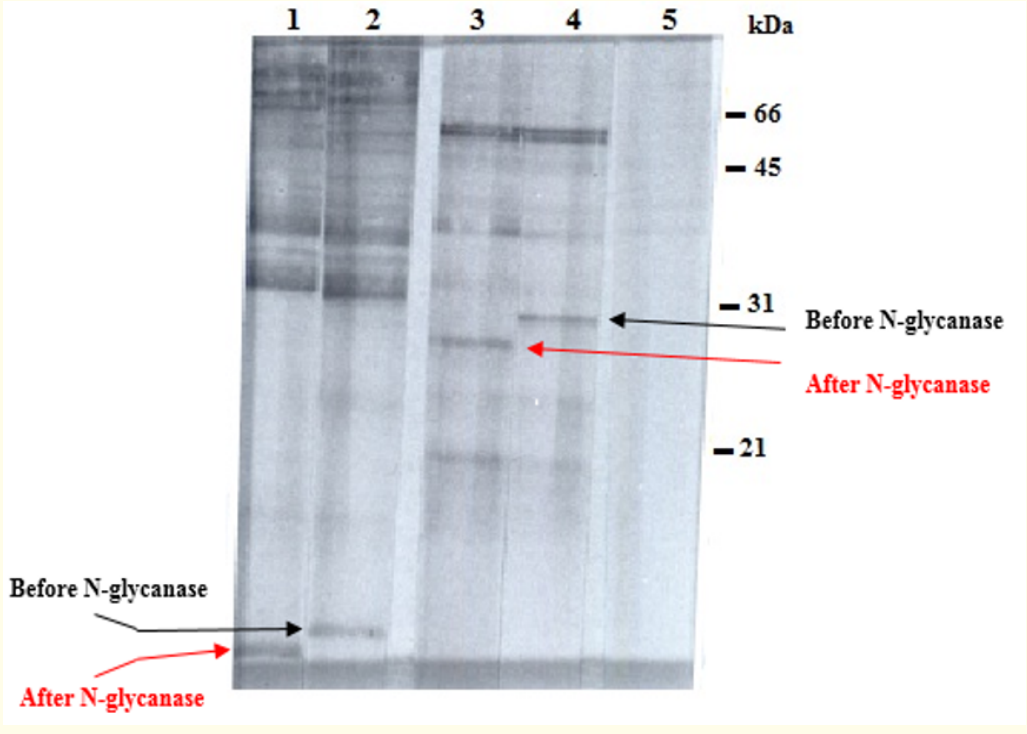


Figure 5: *Effect of deglycosylation of adult B. pahangi antigens on the reactivity of IgG1 and IgG4 subclasses by Western blot blotting. A DOC extract of B. pahangi was treated with N-glycanase (3µl of 10 U/ml) (lanes 1 and 3) or not (lanes 2 and 4) and analyzed on a 12% SDS-polyacrylamide gel. The proteins were transferred onto NCP, which was subsequently cut into strips and probed with the following antisera: human microfilaremic carrier (lanes 3 and 4), human amicrofilaremic carrier (lanes 1 and 2) and human carrier. Negative control (lane 5). The subclass reacting with the band was then detected using anti-human IgG4 (lanes 3 and 4) and anti-human IgG1 (lanes 1 and 2). Arrows indicate the shift of molecular weight of glycosylated antigens.*

Homology between *Loa loa* and *Brugia pahangi* genes

Sequencing of *L. loa* amplicons using of *B. pahangi* primers on *L. loa* genomic DNA generated an amplicon of 694-bp with two potential exons separated by a144-bp intron (Figure 6). The sequence of this amplicon blasted against the *L. loa* genome from the worm base shows 99 - 100% identity, and 47.54 and 83.33% (Table 1) with sequences from *B. pahangi*, *B. malayi*, *W. bancrofti*. When this *L. loa* amplicon was translated to protein, it generated a 17.225-kDa polypeptide with pI = 5.80, characterized by a valine rich region, leucine zipper, phosphorylation, myristylation and glycosylation site. The hydropathicity plot showed alternate regions of hydrophilic and hydrophobic region. The alignment of this polypeptide with the *L. loa* hypothetical protein *B. pahangi, B. malayi*, *W. bancrofti* showed between 47.46% and 94.55% identity with this *L. loa* amplicon (Table 2).

ATGATGATTTCAGTTATGTGTGTTGTAAGTTTATTCCCTCGTTTTACATGCTTCTATCGATAGCAAATGCGATGCATT
TGGGGCGTAATGGGCAACAGCAACCGTACTACTTCCAAAACATAAAACAGCAAGGTATGCAACCGCAAAGCATAAGA
CCACAACTTTCACGACCGCAGCCCAAGCAACTGCAATTCCTTCAACACCCAGAACCGCAACAACTGGAACAACCAGAA
CAACCGCAAAgt**atctaattaccatcatggaatattttccagaatttttacttcttacagttccctattgtgattcaaaaagtatgatcgatcaaagtt**
cgtttaaatctaagtaattttcaactaaggctaaataaaatttcaactaaatttcaacgtaaatttcaactaatttcaactttcaactaaaattcaacta
aatttctaagggcttctctgatttcagGTTTTGATAAAAAATGTGCTGGCTGCATAATAAATATAAATTGCGGAGGTACAGATT
GCATACCGACAAACTCCGTCATACACGGGAAACAACAGTATGGACGATACCGCCAACACAGACACCACCGACATTAGC
ACCGACCTGGACTTACGCAACTGCAAGACCAACGCCAAAAAGTTGCATGTGTGCATGCTATATGCCTCCGCCATGTCA
GATATGTCAACCATGTCAATGA

Figure 6: *Sequence of the amplicon derived from L. loa DNA and primers from B. pahangi. The nucleotide sequence is composed of two potentially coding sequences or exons (with upper case letters) separated with an intron (lower case letters).*
Note: The nucleotide sequence reported in this paper has been submitted to the GenBank data bank with accession number MK 341056.

Species	Name of the gene	Accession number	Length of L. loa amplicon (nucleotide)	Percentage of identity
Loa loa	Hypothetical proteinLoAG 05149	XP 003140734-1	475	99,60%
	Hypothetical proteinLoAG 14373	XP 003149918-1	149	100%
	Hypothetical proteinLoAG 15385	XP 020300957-1	191	99%
	Unnamed protein	VDN 92702-1	152	50%
Brugia pahangi	Sheath protein	CAA 57757-1	152	48,28%
	Major protein component of the microfilarial sheath	CAA 41094-1	236	47,54%
	Bm 902	CDQ 01559-1	132	56,52%
Brugia malayi	Major microfilarial sheath protein precursor	XP 001895418-1	215,14	51,72%
	MBMA-SHP-1	CDP 91850-1	222,08	51,72%
	Hypothetical protein WUBG 04310	EJW 84777-1	132	50%
Wuchereria bancrofti	Major microfilarial sheath protein	EJW 74095-1	69,4	83,33%
	Unnamed protein product	VDM 22668-1	194,32	50%

Table 1*: Homology between the amplicon of Loa loa DNA and other filarial gene.*

		Filaria species (length of amplicon/percentage identity)			
Protein	**Accession number**	**Loa loa**	**Brugia pahangi**	**Brugia malayi**	**Wuchereria bancrofti**
Hypothetical protein LOAG 05149	XP 003140734.1	23/94.55			
Hypothetical protein LOAG 14373	XP 003149918.1	12/66.67			
Unnamed protein product	VDN 92702.1		22/50		
Sheath protein	CAA 57757.1		22/48.28		
Major protein component of the microfilaria sheath	CAA 41094.1		24/47.54		
Bm 902	CDQ 01559.1			22/52.83	
Major microfilarial sheath protein precursor	XP 001895418.1			22/51.72	
BMA-SHP-1	CDP 91850.1			22/50	
Microfilarial sheath protein SHP 1a	AAA 085276.2			22/50	
Major microfilarial sheath protein precursor	XP 001895276.1			22/50	
Hypothetical protein WUBG 04310	EJW 84777.1				19/51.11
Unnamed protein product	VDM 22668.1				19/50
Sheath protein 5	EJW 76990.1				22/47.46

***Table 2**: Amino-acid homology between the Loa loa amplicon, lymphatic filarial and others L. loa protein.*

Discussion

The results reported in this paper corroborate those reported when using *L. loa* homologous antigen, from the adult worm microfilaria stage and the L3 stage [28,29]. Although it can be argued that cross-reactivity is common among parasite nematodes. Many studies have been carried out using heterologous model, but the extent to which these antigens are homologous is not known. We have demonstrated immunological cross-reactivity between *B. pahangi* antigens and *L. loa* antibodies in individuals patently exposed to *L. loa* infection after follow up in the population for several years. This suggests that this cross-reactivity is not a simple activity link to helminth family but structural activity due to the identity of antigen. This assertion is substantiated by several facts: firstly, we have shown that deglycosylation does not result in the loss of IgG reactivity despite the shift in molecular weight linked to the loss of the glycosylate portion of the molecule. In addition, the *L. loa* DNA used as template allowed the generation of a 694-bp amplicon, which showed 100% identity to *L. loa*. and different degrees similarity with many genes from different species of lymphatic filarial [30]. These results suggest that the cross-reactivity observed is not only due to carbohydrate, but also to the peptide back bone. Since the molecular structure shows that the reactivity was related to the peptide, we presume that genes of both species may have some degree of homology. This amplicon codes for a protein which is characterize by regions rich in valine without any known function, this contrast with the proline rich of the *B. pahangi* from whom the primers were design [31]. Interestingly there is also a valine-rich protein in *Mycobacterium tuberculosis* [32]. Strikingly, our previous study show that immune response against *Loa loa* may affect PPD [33]. However, it should be noted that there is a hypothetical structure called the Leucine zipper, suggesting an alpha helical conformation [34], a myristyl site responsible for acylation of protein via activation of the protein N-terminal residue transferase (NMT) [35]. The existence of the Asn-Xaa-Ser/Thr sequence is an indication of potential a N-glycosylation site [36] and cAMP, a cGMP-dependent protein kinase phosphorylation site [37].

Although not completely similar, the organization of this *L. loa* gene presents the same characteristic as the proline rich of the sheath of *B. pahangi* with two exons separated by an intron but 78bp compared to the 144bp of *L. loa*. The analysis of hydrophobicity scale show

alternate hydrophobic and hydrophilic region, suggesting a transmembrane alpha-helices structure of membrane protein. Interest to sheath is substantiated by study showing that immunoresponse against *Loa loa* microfilaria may result to clearance of these larval stage [38]. The environment of central Africa and the West African countries where *L. loa* is endemic is favorable to lymphatic filaria because of the abundance of vectors (*Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles*, etc.), shanty towns and clinical signs such as the prevalence of hydrocele [39]. Finally, its appears that, in the view of similarities between antigens molecules and their respective gene, cross-reactivity will always be present despite the use of the new rapid diagnostic test, which is claimed to be specific for *W. bancrofti* or *B. malayi.* However, the fact that there is more than 17% homology between *L. loa*, *B. malayi*, *B. pahangi* and *W. bancrofti* suggest the existence of cross-protective epitopes, which seem to be preserved among *L. loa*, *B. malayi* and *W. bancrofti*, as shown by the reactivity of the specific IgG1.

Conclusion

In conclusion, ignoring *L. loa* may lead to the failure of the WHO strategy based on mass treatment of lymphatic filariae or *O. volvulus* in areas where *L. loa* is endemic. In addition, the distribution of lymphatic filariae in areas that are co-endemic with *L. loa* need to be re-evaluated because the specific test used to detect lymphatic filariae will always cross-react with *L. loa*.

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Conflict of Interest

The authors declare they have no conflicts of interest.

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