



## Screening and evaluation of lymphatic filariasis in immigrants from endemic countries residing in a focus where it is considered eliminated in the Southern Region of Brazil: A risk of reemergence?



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### ABSTRACT

Lymphatic filariasis (LF) has been targeted by the World Health Organization for elimination by the year 2020. However, migration of infected individuals from areas where LF is endemic to areas considered non-endemic or foci for the control and elimination may jeopardize the achievement of this goal. The aim of the present study was to evaluate the occurrence of filarial infection by way of circulating filarial antigen (CFA) circulation using the point of care AD12-immunochromatography card (POC-ICT) among immigrants from Haiti residing in Chapecó, Santa Catarina, between May and October 2015. Of the 420 subjects examined, 77.4% were male, aged 19–54 years. Ten (2.38%) were POC-ICT positive. Of this total, one was not found. Two individuals were negative for Og4C3-ELISA and DNA/Wb-PCR in all biological samples, but positive for the anti-filarial antibody Bm14 and only one showed microfilaremia (1mf/mL). These findings point to the importance of the Brazilian surveillance action to reduce the possibility of reintroduction of LF in Chapecó, Santa Catarina, by infected immigrants, and to guarantee the success of the National LF Elimination Plan.

### 1. Background

Lymphatic Filariasis (LF), popularly known as elephantiasis, is a neglected disease caused by helminths of the species *Wuchereria bancrofti* (*Wb*), *Brugia malayi* and *B. timori* and transmitted by multiple genera of mosquitoes, especially *Culex quinquefasciatus* (Burkot et al., 2006; Ottesen, 2000). Currently, an estimated 1.403 billion individuals living in 55 countries are at risk of acquiring this parasite, requiring preventive chemotherapy to prevent transmission and the emergence of new cases of filarial morbidity (hydrocele and lymphedema). Sixteen years ago, the Global Program to Eliminate Lymphatic Filariasis (GPELF) was launched to prevent the spread of the infection and reduce morbidity, aiming to eliminate it by 2020. The main strategies of the GPELF are focused on two main objectives: a) the breakdown of transmission of the vector-human parasite through Mass Drug Administration (MDA) of the populations at risk of acquiring the infection and

b) prevention and relief of the suffering of the carriers with morbidity (WHO, 2013; WHO, 2015a).

In the Americas, where only *Wb* infection occurs, four countries are considered active transmission areas: Brazil, Guyana, Dominican Republic and Haiti, the latter with a higher prevalence (WHO, 2013). In Brazil, which is a signatory of the GPELF, only Recife and its metropolitan region (Olinda and Jaboatão dos Guararapes) are still an active focus of the parasitosis (NHF, 1997; PAHO, 2015). Various rounds of MDA using diethylcarbamazine citrate (DEC) alone in the metropolitan area of Recife (MAR) made it possible to reduce the prevalence of LF of 6.5% of microfilaremia in 1996–0% in 2014. Despite having reduced the prevalence of the disease to zero in the city of Recife, the MAR is still considered the main focus of LF in Brazil (Maciel et al., 1996; PAHO, 2015).

From the year 2010, thousands of immigrants from African countries (Senegal, Gambia, Ghana), the Caribbean (Dominican Republic

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and Haiti) and Asia (Bangladesh and India) all endemic for *Wb*, entered Brazil through the border states of the northern region, (Nunes et al., 2016; Ottesen et al., 1997; Santos, 2016). It is estimated that, between 2010 and 2014, about 42,000 immigrants from the Dominican Republic, Haiti and Africa entered Brazil officially or illegally in search of work, establishing themselves in the different Brazilian states (Rawlinson et al., 2014; Trezzi, 2014). Of these thousands of immigrants, approximately 11,500 (27.4%) live in the southern region of Brazil, a region called the “South Maravilha” (Duarte, 2015). About 3500 Haitians reside in several municipalities in the state of Santa Catarina (SC) and it is estimated that 1800 individuals reside in the city of Chapecó (CH), in the west of the state (Debona, 2015).

SC state was considered endemic for LF between 1951 and 1967. In these periods, control actions were carried out involving selective treatment with DEC of microfilaremic individuals, or with MDA in the resident populations of the endemic areas, making it possible to eliminate LF in the state (Schlemper et al., 2000).

The current circulating filarial antigen (CFA) for *Wb* administered through point of care AD12-immunochromatography card (POC-ICT) (Binax NOW® Filariasis, Alere Inc., Scarborough, ME), for reason of its speed and practicality, has been recommended by the World Health Organization (WHO) as a diagnostic tool to be used in the mapping of the areas to be submitted to MDA, as well as in surveillance actions (Rebollo and Bockarie, 2014). In Brazil, according to the recommendations of the Secretariat of Health Surveillance of the Ministry of Health, all positive results for *Wb* infection by POC-ICT should be confirmed using other widely known diagnostic tools (membrane filtration, CFA by Og4C3-ELISA, antibody anti Bm14-ELISA, DNA-*Wb* investigation) before treatment with DEC (SHS, 2013).

The aim of the present study was to evaluate the occurrence of filarial infection among Haitian immigrants resident in the city of CH, SC, using the CFA for *Wb* conducted by POC-ICT.

## 2. Material and methods

### 2.1. Area and population studied

CH has 183,530 inhabitants and is located in the western region of the State of Santa Catarina, southern region of Brazil, located 550 km from the capital Florianópolis (IBGE, 2015). In the period from May to October 2015, an antigenic investigation was conducted to detect LF in Haitian immigrants living in CH through the CFA survey using POC-ICT. Prior to the development of the survey, the coordinators of the present study made contact with the Representatives of the Haitian Association in CH who were fluent in the Portuguese language. This occasion provided an opportunity to communicate the objectives of the study and the importance of the participation of resident immigrants.

### 2.2. Laboratory assays

#### 2.2.1. Antigen investigation

It was carried out using the POC-ICT, a test that detects the CFA of *Wb* using the monoclonal antibody AD12, which recognizes a 200-kDa filarial antigen from either adult worms or microfilariae. The test was performed according to the manufacturer's instructions and read by trained technicians in the field after 10 min. Visualization of two lines (test and control) was interpreted as a positive result (Oliveira et al., 2014).

#### 2.2.2. Complementary laboratory assays

paired venous blood and urine samples were collected between 11:00 pm – 01:00 am from patients testing positive on the POC-ICT. 10 mL of venous blood was distributed in five mL tubes with ethylenediaminetetra-acetic acid (EDTA) to detect and quantify circulating microfilariae by filtration (QMF) (Nucleopore®) and ~ five mL to obtain serum for the assays: CFA by Og4C3-ELISA (TropBio®, Pty Ltd,

Townsville, Queensland, Australia), anti-filarial Bm-14 antibody (Filariasis CELISA, Cellabs Pty. Ltd., Brookevale, Australia tests) and DNA-*Wb*/PCR. Fifty µL of 10 mM EDTA was added to each 50 mL urine sample. The presence of DNA-*Wb*/PCR also was investigated in 100 µL of blood with EDTA and urine. Three drops of blood were distributed on two slides for study of microfilariae morphology. Blood, serum, urine and slides were stored at temperatures of 4, –20, –20 °C and room temperature, respectively, and then sent to the SRNF/CPqAM/Fiocruz-PE until the application of the standard operating procedures for each specific test. All the procedures were conducted according to the manufacturer's protocols described elsewhere (Nunes et al., 2016; Rocha et al., 2009; Weil et al., 2011; Ximenes et al., 2014).

## 3. Statistical analysis

The data were stored in a Microsoft Excel database and analyzed by descriptive statistics, the results are presented in tables that show the frequencies with which the variables under analysis occurred.

## 4. Questionnaire administration and ethics committee

Socio-demographic data and information on the use of DEC and the number of doses taken were gathered using a structured questionnaire. Those who had difficulty to understanding the questions in Portuguese were assisted by a translator. The present study was approved by the Ethics Committee of Universidade Uno Chapecó, Chapecó-SC No. CAEE. 972.980.

## 5. Results

A total of 420 individuals from Haiti were examined, 77.38% male, ranging in age from 19 to 54 years. The distribution by sex and age revealed that the majority of the individuals (69.29%) were in the young adult age range of between 20 and 34 years (Table 1). Of the individuals examined, 10 (2.38%) had positive POC-ICT, of these, 7 were male and 3 female, with a mean age of approximately 32 years.

The immigrants came from eight districts of the Republic of Haiti, all endemic for LF, the main district being Gonaives, the capital of the Artibonite Department, with 147 (35%) individuals. Of these, 7 (4.76%) were POC-ICT positive. The second most common place of origin was the capital of the Republic of Haiti, Port-au-Prince, with 83 individuals (19.76%), of whom three were POC-ICT positive.

In relation to permanence of residence in the municipality of CH, 65.23% of those surveyed stated that they had lived there for more than 2 years and 34.8% for at least 6 months, including 10 positive cases.

Less than half (43.81%) of the subjects examined reported having received at least one dose of MDA for LF, with DEC, at their place of origin. Of the POC-ICT positive immigrants, only five reported receiving some of the annual doses administered by the National Filariasis

**Table 1**

Distribution of individuals according to the results of POC-ICT surveillance, gender and age, May to October 2015, Chapecó, Santa Catarina, Brazil.

Age (years)	Male		Female		Total (%)
	Positive	Negative	Positive	Negative	
< 20	0	2	0	0	2 (0.48)
20–24	0	36	1	16	53 (12.62)
25–29	2	91	2	28	123 (29.29)
30–34	1	87	0	27	115 (27.38)
35–39	2	51	0	13	66 (15.71)
40–44	1	30	0	4	35 (8.33)
45–49	0	13	0	3	16 (3.81)
> 50	1	5	0	0	6 (1.43)
Uniformed	0	3	0	1	4 (0.95)
Total (%)	7 (1.67)	318 (75.71)	3 (0.71)	92 (21.91)	420 (100.00)

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