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BRIEF REPORT

Is there a risk of filarial infection during long-term missions in Haiti?☆

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Summary *Background:* Haiti has the highest prevalence of lymphatic filariasis (*Wuchereria bancrofti*) in the Western Hemisphere. Still, the risk of filarial infection for long-term visitors such as humanitarian aid workers or military personnel is uncertain. The presented study analyzed the exposure to *W. bancrofti* in Chilean participants of the UN Stabilization Mission in Haiti (MINUSTAH) in 2011.
Methods: Blood samples collected from 531 participants were screened for antifilarial antibodies by IgG ELISA, and, if positive, analyzed by immunofluorescence assay (IFA), IgG4 ELISA, Real-Time PCR, and circulating filarial antigen (CFA) card test.
Results: ELISA screening was positive in 10 cases. Seroconversion occurred in only two cases (0.38%) based on ELISA values determined in samples taken before and after deployment. Positive IgG ELISA values could not be confirmed by IFA and IgG4 ELISA. Real-Time PCR and CFA testing did not reveal the presence of filaria.

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Conclusions: Our data indicate that in the examined cohort of MINUSTAH participants in 2011, the risk of filarial exposure or infection was low.

1. Introduction

Haiti is one of the poorest countries in the western hemisphere and therefore still endemic for various vector-borne infections that have been controlled in neighboring countries of the Caribbean and Central America [1]. In addition, Haiti suffered a massive breakdown of the public infrastructure after the earthquake in January 2010 with a temporary downfall in control programs such as the annual mass drug administration for lymphatic filariasis (LF) [2,3]. As a consequence, there were worries about post-earthquake increases of this and other vector-borne infections that were endorsed by reports of rising malaria cases and clusters of dengue and malaria in travelers [1]. Still, due to the lack of sufficient pre- and post-earthquake surveillance data, the concrete risk for the population and for long-term visitors such as foreign aid workers is difficult to quantify [4]. The presented work studied the risk of exposure to *Wuchereria bancrofti* in Chilean soldiers participating in the United Nations Stabilization Mission in Haiti (MINUSTAH) in 2011.

2. Methods

Chile has participated since 2004 in MINUSTAH with a permanent contingent of 250—300 soldiers. In 2010, the Department of Preventive Medicine of the Chilean Armed Forces started a surveillance project to evaluate the risk of vector-borne disease including filarial infection. Chilean soldiers were stationed near Port-au-Prince and Cap Haitien at the northern coast of Haiti. In Port-au-Prince, they were part of the Engineers Company, which was responsible for road and bridge building. Activities included day- and night time exposure to swamps, rivers, and flooded roads. Almost all soldiers spent their 15 day UN leave traveling in beach resorts in the Dominican Republic. Before departure, all soldiers received an oral introduction on the risk and prevention of vector-borne infections. In Haiti measures against vector-borne infections included: personal use of DEET-based skin repellent, impregnation of occupational clothes with a permethrin-based repellent, weekly oral dose of 500 mg of chloroquine, sleeping in container-type air-conditioned facilities, and indoor residual spraying and space spraying (adulticiding) with permethrin derivates every 15 days.

To estimate the rate of exposure to *W. bancrofti*, daytime blood samples were drawn during routine medical exams 1—2 weeks before (pre-Haiti) and after (post-Haiti) deployment to Haiti. Pre- and post-Haiti serum was separated, aliquoted, and stored at −20 °C; post-Haiti EDTA-blood was stabilized by buffer AS1 (Qiagen, Germany) for PCR analysis and stored at 4 °C. Samples were shipped to the Institute of Medical Microbiology, Immunology and Parasitology in Bonn, Germany, for further testing. Serological screening was performed in all post-Haiti samples whereas pre-Haiti serum samples were analyzed only if soldiers were ELISA positive after deployment.

Antifilarial antibodies were analyzed by a commercial IgG ELISA with *Acanthocheilonema viteae* as antigen (Bordier Affinity Products, Switzerland). The cut-off was defined by the particular absorbance value of the weak positive control included in the kit. An in-house indirect immunofluorescence assay (IFA) using cryosections of adult *Brugia malayi* was applied as confirmatory test for all post-Haiti specimens with positive screening results. These samples were also tested for cross-reactivity with other helminthes like *Strongyloides stercoralis* and *Toxocara canis* (both IgG ELISA, Bordier Affinity Products), *Trichinella spiralis* and *Fasciola hepatica* (both IFA, in house assays) as well as *Echinococcus* spp. (IHA, Laboratoires Fumouze, France).

In addition, a *W. bancrofti* specific IgG4 ELISA (Filaria *Detect*™ IgG4 ELISA, InBios International, Seattle, USA) was performed with post-Haiti sera ($n = 20$) with questionable, borderline or positive results in the IgG ELISA and the available, corresponding pre-Haiti specimens ($n = 17$). Samples were tested in duplicate according to instructions of the manufacturer. The resulting OD values (450 nm) were compared with the OD values of the control samples included in the kit (negative, positive, weak positive).

From all soldiers with a positive IgG ELISA result in the post-Haiti serum, DNA was recovered from 200 μL of the corresponding stabilized EDTA-blood probe for Real-Time PCR (in-house) targeting filarial actin and *Wolbachia*. For the detection of filarial actin, a 146 b.p. fragment of the *W. bancrofti* actin (GenBank: AF184961.1) was amplified with the following protocol: 1X HotStar Taq Buffer, 4 mM $MgCl_2$, 200 μM dNTPs, 50 nM SybrGreen50, 900 nM forward (CAC TGG TGA TGG TGT TAC GC) and reverse (GAT TGC ACC ACA ATT TCA CG) primers. DNA was amplified in a Rotorgene 6000 real-time thermocycler (Corbett Research, Sydney, Australia) using the following cycling conditions: Taq polymerase activation at 95 °C for 15 min; 40 cycles of 95 °C for 10 min, 58 °C 30 s, 72 °C for 20 s. Fluorescence was acquired on the FAM channel at the end of the extension step. After cycling, a melt curve from 72 to 95C was produced to confirm the product. The products were quantified by comparing the C_t of the samples with a standard curve of a plasmid containing the *W. bancrofti* actin fragment. *W. bancrofti* Wolbachia—ftsZ gene was detected as previously described [5].

All ELISA positive samples were further tested for circulating *W. bancrofti* antigen by immunochromatographic card test (Binax NOW® Filariasis, Alere, Scarborough, USA). This rapid test was performed according to