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Short communication

## The disappearance of onchocerciasis from the Itwara focus, western Uganda after elimination of the vector Simulium neavei and 19 years of annual ivermectin treatments

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### ABSTRACT

The Itwara onchocerciasis focus is located around the Itwara forest reserve in western Uganda. In 1991. annual treatments with ivermectin started in the focus. They were supplemented in 1995 by the control of the vector Simulium neavei, which was subsequently eliminated from the focus. The impact of the two interventions on the disease was assessed in 2010 by nodule palpations, examinations of skin snips by microscopy and PCR, and Ov16 recombinant ELISA. There was no evidence of any microfilaria in 688 skin snips and only 2 (0.06%) of 3316 children examined for IgG4 were slightly above the arbitrary cut off of 40. A follow up of the same children 21 months later in 2012 confirmed that both were negative for diagnostic antigen Ov-16, skin snip microscopy and PCR. Based on the World Health Organization (WHO) elimination criteria of 2001 and the Uganda onchocerciasis certification guidelines, it was concluded that the disease has disappeared from the Itwara focus after 19 years of ivermectin treatments and the elimination of the vector around 2001. Ivermectin treatments were recommended to be halted.

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#### 1. Introduction

The Itwara onchocerciasis transmission zone (focus) is located around the Itwara forest reserve northeast of Fort Portal in Kabarole and Kyenjojo districts in western Uganda. Parasitological and clinical surveys conducted in Kigoyera parish in 1991 revealed a microfilariae (mf) skin prevalence of 88%, nodule carriers of 48% and a CMFL of 49 mf/snip in persons aged over 19 years (Fischer et al., 1993). The high endemicity in the area was later confirmed by clinical surveys carried out from 1993-1994 (Fig. 1 and Table 1) by MoH,

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GTZ, Kabarole (unpublished) and a rapid epidemiological mapping for onchocerciasis (REMO) conducted in 1994/95 (Katabarwa et al., 1999). The programme initiated mass drug administration with ivermectin in 1991, which was later replaced by the Community Directed Treatment with Ivermectin in 1997 (WHO, 1996). The recommended therapeutic coverage of at least 65% (Fig. 2) of the total population was achieved throughout all the years (Ndyomugyenyi et al., 2007).

In order to enhance the effect of the ivermectin treatment, vector control using the environmentally friendly larvicide temephos (Abate<sup>R</sup>) was initiated in 1995, which led to the elimination of the onchocerciasis vector Simulium neavei. The last biting flies in Itwara main focus located in the Itwara focus were caught in 1996. In the remaining eastern sub-foci of Siisa and Aswa the last flies were caught in 2001 (Garms et al., 2009). The study reported here was









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**Fig. 1.** Map of the Itwara onchocerciasis focus in western Uganda showing former breeding sites of the onchocerciasis vector *Simulium neavei*, study villages in 2010 (A–E), parishes where children <10 years were tested for the presence of IgG4 antibodies against the Ov-16 recombinant antigen (2010), and microfilariae prevalences (%) in villages, where clinical assessments were conducted on persons of 20 or more years in 1993–1994 about 6 months after the last ivermectin treatment (unpublished data MOH, GTZ, BNITM).

conducted in 2010 to assess whether onchocerciasis has now disappeared from the Itwara focus and ivermectin treatments can be abandoned after 19 years.

#### 2. Methods

Clinical and parasitological surveys were conducted in 2010 in five villages (Fig. 1A–E). A total of 688 persons aged 5 years and above were examined for nodules and onchocerciasis related skin lesions. Two skin biopsies were taken using a Walser punch after informed consent from study participants. The skin biopsies were placed in separate wells of microtitre plates in 150  $\mu$ l of buffered saline and incubated for 24 h. The solution was pipetted onto microscope slides and examined under a microscope (WHO, 1995). All skin snips that were microscopically examined were then subjected to PCR analysis. The biopsies were transferred to tubes containing  $500 \,\mu$ l of 10 mM EDTA and stored at 4 °C. They were processed and analyzed in the molecular laboratory of the Vector Control Division, Ministry of Health in Kampala using standard PCR procedures for detecting O-150 repeat segments (Toe et al., 1998; Unnasch & Meredith, 1996; Lindblade et al., 2007).

A serological survey was conducted in 11 parishes (Fig. 1) and the prevalence of IgG4 antibody to the recombinant antigen Ov-16 was assessed by enzyme-linked immunosorbent assay (ELISA) (Lobos et al., 1991; Lindblade et al., 2007; Cupp, 2012). From proportional samples of 3500 children (1999 aged 1–4 years, 1501 aged 5–9 years) randomly selected from all households in 100 communities, 3316 participated. Blood samples were soaked in Whatman No. 2 filter papers, allowed to dry and placed in plastic bags containing silica gel and kept at -20 °C until processed in a manner described by Lindblade in the molecular laboratory of the Vector Control Division, Kampala, Uganda (Lindblade et al., 2007).

Table 1

Villages A–E (cf. map Fig. 1) where clinical and parasitological assessments for *Onchocerca* nodules and microfilariae (microscopy, PCR) were carried out in 2010 compared with results of surveys by Ministry of Health/GTZ from November 1993 to February 1994 and Ndyomugyenyi et al. (2004).

Reference Survey (year)	ce MOH/GTZ, Kabarole 1993–1994 (year)			Ndyomugyenyi et al. (2004)			Assessments 2010		
Village (A-E)	No.	Mf carriers (%)	Nodule carriers (%)	No.	Mf. carriers (%)	Nodule carriers (%)	No.	Mf. carriers (%)	Nodule carriers (%)
Masongora (A)							146	0	0.0
Igogonya (B)	45	84	58				150	0	1.2
Busasa (C)	66	77	68				132	0	2.2
Igoma (D)	100	72*	11*	68	4.4	14.7	98	0	0.0
Mparo "A" (E)	32	66	50				162	0	0.0
**P-value								< 0.001	< 0.001

\* Fischer et al. (1993) determined a microfilaria carrier rate of 95% and a nodule carrier rate of 55% in persons aged over 19 years.

\*\* Chi-square test compared data of 1993/1994 with that of 2010.

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