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SUCCESSFUL INTERRUPTION OF THE TRANSMISSION OF *ONCHOCERCA VOLVULUS* IN MPAMBA-NKUSI FOCUS, KIBAALE DISTRICT, MID-WESTERN UGANDA

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ABSTRACT

***Background*: The Mpamba-Nkusi onchocerciasis focus is situated in the mid-western part of Uganda. It has an area of 300 km^2 and used to have *Simulium neavei* as the vector which develops in a phoretic association on freshwater crabs. Ground larviciding with temephos (Abate EC500) was initiated in June 2002. All the 330 communities in this focus have undergone annual treatment with ivermectin since 1995 and were later shifted to semi-annual treatment in 2009.**

***Objective*: To establish the impact of mass drug administration in combination with larviciding on the interruption of *O. volvulus* transmission.**

***Design*: Longitudinal study**

***Setting*: Rural areas in Mpamba-Nkusi focus, Kibaale district.**

***Subjects*: Individuals five years and above living in the focus.**

***Interventions*: Annual and semi-annual treatment with ivermectin supplemented by vector elimination were used. Epidemiological, entomological and serological assessments were conducted.**

***Results*: Freshwater crabs (n = 14,391) caught from monitoring sites (n = 41) since 2008 were negative for immature stages of *S. neavei*. The *S.neavei* population was reduced following trial and initiation of ground larviciding. No adult *S. neavei* has been caught in the focus for over five years. Parasitological examination of individuals residing in the focus revealed a microfilaria (mf) prevalence of 0.3% (95% CI 0 – 0.65%; n = 732) in 2012. Serological assays testing for *Onchocerca volvulus* antibodies conducted on 3351 children <15 years in 2009 indicated point prevalence of 0.6% , (95% CI, 0.3-0.8%) while in 2012 another survey conducted among 3,407 children, only 1/3407 (0.03%, 95% CI, 0-0.09%) individual was positive for *O. volvulus* antibodies.**

***Conclusions*: Epidemiological and entomological findings suggest that interruption of transmission has been achieved.**

INTRODUCTION

In Uganda onchocerciasis is endemic in 35 districts with estimated 1.4 million people affected and more than two million at risk of infection. It is one of the diseases targeted for elimination. In an effort to scale up national response for the disease elimination, the Government of Uganda launched onchocerciasis elimination campaign in January 2007. The policy aims to eliminate onchocerciasis through semi-annual community directed treatment with ivermectin combined with vector control and / or elimination (1, 2). The policy shift from control to elimination was a result of observations both here and in the countries of the Onchocerciasis Elimination Programme of the Americas that annual treatment alone not only failed to eliminate infections but also prolonged the period of interventions necessary in order to achieve interruption of disease transmission when compared to bi-annual treatment (3).

The Mpamba-Nkusi is one of the isolated foci earlier identified to be a candidate for elimination. It has an area of 300 km^2; the vector was *S. neavei* which develops in a phoretic association with freshwater crabs and high transmission intensities were historically found (4, 5). The focus has 330 hyper- and meso-endemic communities, of which 285 were hyperendemic for onchocerciasis before control efforts using annual distribution of ivermectin began in 1995. Ground larviciding that started in 2002 was used to supplement MDA, and this dual approach has made a big contribution in the interruption of onchocerciasis in Uganda. It was believed that this approach would be even more successful. However, it should be noted that this is only applicable in *S. neavei* areas because of its focal distribution and the slow larval development which is about four weeks (6). Semi-annual mass distribution began in 2009.

The goal of the onchocerciasis elimination programme of Uganda is to eliminate the disease in all the 17 foci in the country by 2020. The Ministry of Health, Uganda developed a number of criteria to be achieved to verify the declaration of onchocerciasis as eliminated. These include the reduction of new infections to an incidence rate of less than one new case per 1000 individuals. In foci where *S.neavei* is the vector, absence or near absence of freshwater crabs (P*otamonautes aloysiisabaudiae, P.niloticus, P.loveni*) infested with immature stages of the vector has to be observed for three years (7, 8). The objective of the present study was to establish the impact of mass drug administration in combination with larviciding in the interruption of *O. volvulus* transmission in this isolated focus.

MATERIALS AND METHODS

Study area and selection of villages for assessment: Mpamba-Nkusi focus is situated 20 km north of Kagadi Township, one of the commercial centers in Kibale district (Figure 1).The area lies 900-1500 m above sea-level. In the late 1990s, there was an improvement of the feeder road network, thus making the focus easily accessible. The main river systems that support the breeding of onchocerciasis vectors were R. Mpamba, R. Nkusi (lower), R. Nyabugando, and R. Mutungulu and their tributaries. In 1999 with the support of African Programme for Onchocerciasis Control (APOC) vector elimination was initiated in this focus and mapping activities conducted that allowed the establishment of dosing and fly catching sites (9). The focus has 330 onchocerciasis endemic communities with a total population of 194,045.

Annual and semi-annual treatment with Ivermectin: Mpamba-Nkusi onchocerciasis focus has had 20 consecutive treatment rounds of Mass Drug Administration (MDA) with ivermectin. Mass Drug Administration (MDA) with ivermectin began in 1993 with the support of Sightsavers; however, it was only from 1995 onwards that consistent and accurate treatment data became available. In 1999, a new approach of Community Directed Treatment with Ivermectin (CDTI) was introduced with the support from APOC. In this approach the communities were empowered to take responsibility of ivermectin distribution (1).

In 2009, semi-annual treatment was introduced and communities in hypo-endemic areas, formerly not under treatment, were included.

Entomological surveys

a) Collection and examinations of freshwater crabs

Mapping of vector breeding sites and their upper breeding limits began in March 1999 employing previously described procedures (2). Freshwater crabs were caught using locally designed funnel shaped basket traps baited with fresh meat. These were left in rivers for approximately one hour to overnight and later examined for crabs carrying larvae and pupae of *S. neavei* (6). Crab collection and examinations were conducted from a total of 41 sites before, during and after river treatments in rivers Nkusi, Mpamba, Nyabugando and Mutungulu including their tributaries (Figure 1).

b) Adult fly population

In the three river systems where there was evidence for vector breeding, locally recruited vector collectors

were trained. Fly collections were conducted by vector collectors twice a week at three sites of Sioni, Rulembo and Nyabugando (Figure, 1; A, B, C). Data were recorded on an hourly basis following standard fly catching procedures (10).

Ground larviciding using temephos (Abate): A total of 41 dosing points were selected in the four river systems Mpamba, Nkusi, Nyabugando and Mutungulu (Figure 1).Some trials were first carried out in June 2002 before routine larviciding commenced in rivers Mpamba, Nkusi (lower), Nyabugando in June and October 2002 using temephos (Abate, EC500) applied at a rate of 0.2-0.4 mg/L at 4 and 8-weekly intervals based on the stage of elimination. The insecticide was pre-mixed in a 15 litre knapsack sprayer and applied for a period of 30 minutes at established dosing points. The impact of the larviciding on *S. neavei* immature stages was assessed after 48 h (2, 10). Ground larviciding stopped in Nyabugando in 2004, in Mutungulu in 2005 and Mpamba system in 2009.

Parasitological survey: In 2008, five villages were selected for epidemiological assessments in the focus (Figure 1: Sioni (1.034°N, 30.813°E), Rugashali (1.0860N, 30.8200E), Burora, (1.0940N, 30.8840E), Nyabwegyereka (1.000N; 30.8040E) and Rubirizi (1.0710N, 30.6890E). Parasitological surveys were conducted in the villages of Nyabwegyereka, Sioni, Rugashali, Burora, and Rubirizi in Kibaale district. In 2012, 405 males and 327 females age 5-50 years and above were examined. Skin biopsies were taken from both the left and right iliac region using sterile lancets and surgical blades. Each biopsy was put into a single well of a microtitre plate containing physiological saline (sodium chloride, 0.9% w/v) and incubated overnight at room temperature. The solution was then pipetted onto a glass slide and examined under a low power lens of a compound microscope for the presence of *Onchocerca volvulus* microfilaria. Each slide was examined by two technicians to ensure accuracy of the results. Skin snip surveys were conducted 11 months after the last ivermectin distribution (10).

Serological survey: Serological assays testing for *Onchocerca volvulus* exposure were conducted among the population of children (5-15 years) resident in this focus for the first time in 2009 and later another survey in 2012. The children were sampled from parishes representing the overall population in the focus. Blood spots were collected in 2009 (n=3,351) and again in 2012 (n=3,407). Blood spots were collected by finger prick from each child enrolled in the study, and the dried blood spots eluted and tested for the presence of antibodies against Ov16 (11).

Ethical consideration

The procedures were reviewed and approved by the Vector Control Division Institutional Review Committee (IRC) and then Uganda National Council of Science and Technology. Community meetings were held in all selected villages within the focus to explain the research procedures, and the right of each individual to decide whether or not to participate was explained. The individuals were also informed that they would be provided with the results of the tests upon request. Before each examination, each adult who had voluntarily come to the examination point and agreed to participate were provided with a summary of the study that was prepared in a consent form and oral consent was obtained. Parents or guardians provided oral consent on behalf of all child participants.

RESULTS

Annual and semi-annual treatment with ivermectin

The mean annual treatment coverage for 14 years was consistently above 90% of the eligible population from 1995 to 2008. Bi-annual treatment was carried out from 2009 to 2012, and treatment coverage for both rounds of treatments were maintained at above 90% of the eligible population. There was gradual increase of number of people from 93,943 in 1995 to 110,924 in 2008, for bi-annual treatment from 255,285 in 2009 to 306,077 in 2012.The observed increase was due to population growth and the inclusion of hypoendemic communities.

*Crab collection and examination*s: Results of crab collections and examinations, which represent a total of 104,902 crabs, are summarised in Table 1. The highest crab infestation was 80% in 2000. Infestation rates rapidly declined after larviciding trials had been carried out in July 2002 and routine larviciding commenced in August 2002. The last positive crab was caught in January 2008.

Table 1

Numbers of freshwater crabs caught in the three rivers systems and examined for infestation with immature stages of Simulium neavei, before and after larviciding had been implemented in the Mpamba-Nkusi focus.

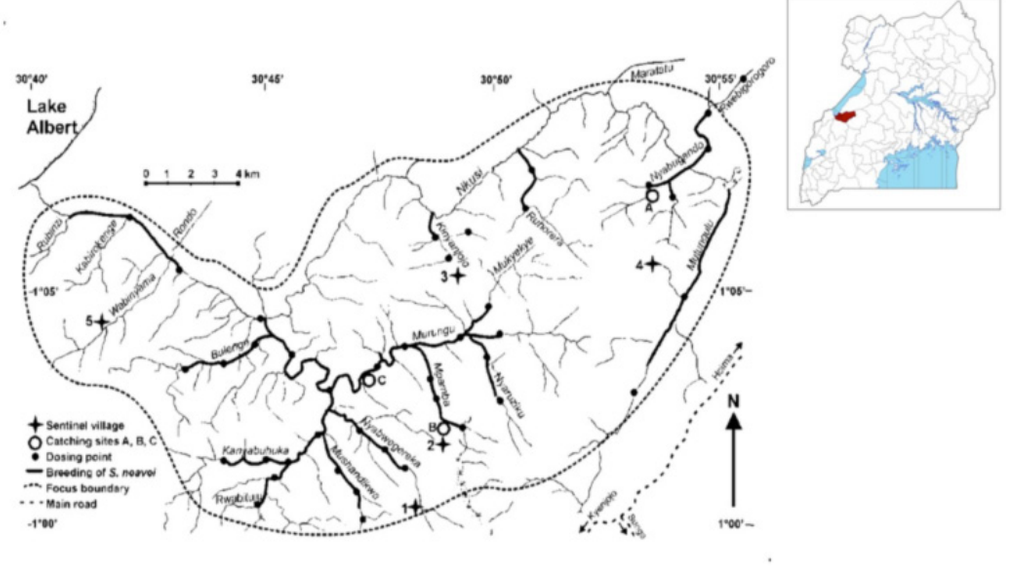
Rivers	Mpamba		Nyabugando		Mutungulu		Total	
Year	No. Crabs	% +ve	No. Crabs	% +ve	No. Crabs	% +ve		
1999	584	42.1	n.d	n.d	n.d	n.d	584	Pre-invention
2000	632	80.0	n.d	n.d	n.d	n.d	632	
2001	719	59.5	30	100	n.d	n.d	749	
*2002	5928	27.0	1262	39.0	n.d	n.d	7190	Field trial & start
§2003	10864	0.1	7989	2.9	520	25.6	19373	Larviciding & surveillance
2004	17389	0.7	3485	0.0	569	4.7	21443	
2005	10034	0.6	1032	0.0	335	0.0	11,403	
2006	10896	0.5	1271	0.0	364	0.0	12531	
2007	6393	0.06	752	0.0	57	0.0	7202	
2008	9,212	0.02	148	0.0	40	0.0	9,400	
2009	5,540	0.0	-	-	-	-	5540	
2010	4,808	0.0	594	0.0	81	0.0	5,477	
2011	2,678	0.0	-	-	-	-	2,678	
2012	696	0.0	-	-	-	-	696	
Total	86,373		16,563		1,966		104,898	

**Larviciding initiated in 2002 in Mpamba ended in 2009 while in Nyabugando river systems ended in 2004.*
§ Larviciding ended in 2005.
n.d = no data at that time because survey had not started in the systems.
% = percentage; +ve = positive; Data from 1999-2003, adopted from Lakwo, et.al., 2006.

Adult fly population: Fly densities rapidly declined at the Mpamba, Nyabugando and Mutungulu river system after larviciding commenced in June 2002. The last fly was caught in March 2008 at the Rulembo catching site (Figure 1, C), indicating that the vector had been eliminated. Surveillance activities continued for three more years and there was no sign of a re-infestation.

Figure 1

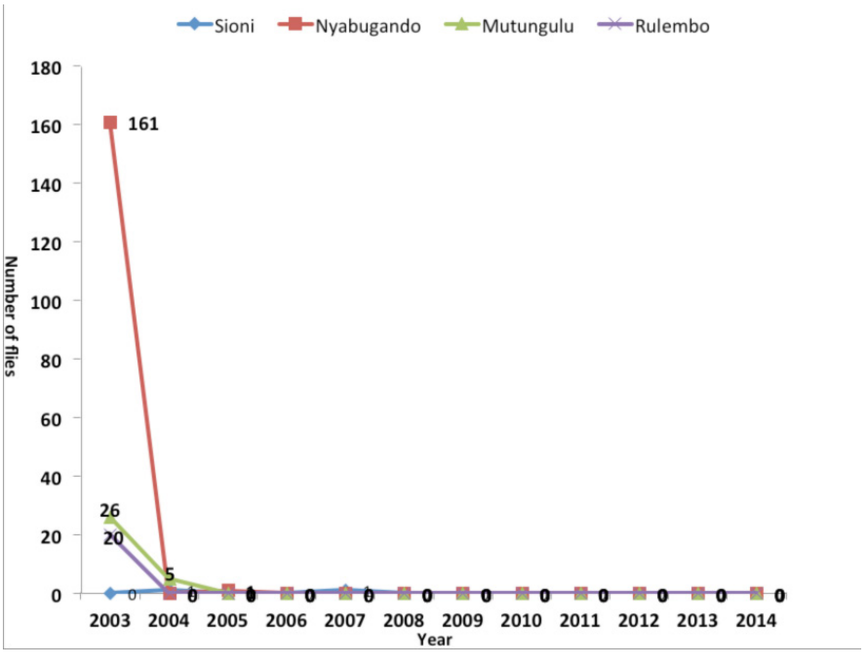
Map of Mpamba-Nkusi onchocerciasis focus in Kibale district, mid-western Uganda



When ground larviciding commenced in June 2002, there was a gradual decline of adult fly population density at Mpamba, Nyabugando and Mutungulu systems. The last fly was caught in 2008, indicating that the vector had been eliminated. Surveillance activities continued, but there was no sign of any re-infestation. (Figure 2).

Figure 2

Number ofSimulium neavei caught at four monitoring sites in Mpamba-Nkusi focus eight days per month



NB: Adult fly population density from 1999-2002 was reported by Lakwo, et al., 2006; Med. Vet. Ent. 20, 93-101; Mutungulu was one of the sites established in 2003 to monitor the Mutungulu river system.

Parasitological assessments: The results of the data collected in 2012 showing impact of ivermectin treatment and vector elimination are shown in Table 2. Pre-ivermectin treatment mf prevalence was available only for Nyabwegyereka village where mf prevalence was 92% in 1991(4). There was reduction in the prevalence of microfilaria carriers in the five villages from 92% in 1991(prior the launching of the semi-annual treatment with ivermectin in the focus) to 0.3% in 2012.

Table 2

Microfilaria carriers in Mpamba-Nkusi focus: Parasitological assessment in 2012

Village	Sex		Age group in years		Total	Mf Positive	95% CI
	Male	Female	5-19	20-50+	M+F	Number (%)	
Nyabwegyereka	72	57	20	109	129	0 (0.0)	0-0.00
Sioni	59	97	16	140	156	0 (0.0)	0-0.00
Rugashali	68	88	22	134	156	*1 (0.6)	0-0.01
Burora	67	92	27	132	159	1 (0.6)	0-0.02
Rubirizi	61	71	12	120	132	0 (0.0)	0-0.00
Total	327	405	97	635	732	2 (0.3)	0-0.01

*One mf positive case found in this village was a migrant from other onchocerciasis endemic areas..

Ov16 Serosurviellance: The results of serological survey among children are shown in Table 3. In 2009, 19 of 3,351(0.6%) children tested from 330 communities were positive for Ov16 IgG4 antibodies (point prevalence of 0.6%; 95% CI 0.3-0.8%).In a later survey in 2012 only 1 of 3407 children was positive (point prevalence of 0.03%; 95% CI 0-0.09%).

Table 3
Prevalence of IgG4 antibodies to Ov16 in the Mpamba-Nkusi focus, Mid-western Uganda

		First assessment in 2009		
Age group	Number screened	Positive IgG4	% Positive	95% CI (%)
1-4	1378	2	0.14	0-0.35
5-9	1011	4	0.40	0-0.78
10-15	962	13	1.35	0.62-2.08
Total	3351	19	0.57	0.30-0.80
		Second Assessment in 2012		
1-4	1942	0	0.0	0-0.1
5-9	1465	1	0.07	0-0.20
Total	3407	1	0.03	0-0.09

DISCUSSION

The epidemiological and entomological results strongly suggest that transmission of *O. volvulus* has been interrupted in Mpamba-Nkusi focus. No immature stages of *S. neavei* were found on more than 14,000 freshwater crabs examined after 2008 following ground larviciding.The absence of infestation in freshwater crabs with *S. neavei* immature stages following ground larviciding is strong evidence that vector elimination is a feasible option in isolated foci, where there is a realistic chance that the vector can be eliminated in a limited period of time. Several successful examples of vector elimination have been documented in Africa: elimination of *S. neavei* in a focus in Kenya in the 1950s (12) and the elimination of *S. damnosum* from the Victoria Nile, Uganda (13). Recently *S. Neavei* has been eliminated from the Itwara onchocerciasis focus in Uganda (2) and the Bioko form of *S. yahense* from the island of Bioko, Republic of Equatorial Guinea (14).Annual ivermectin treatments with coverage rates exceeding 90% of the at risk eligible population resulted in 14 complete rounds of treatment before commencement of bi-annual treatment in 2009. Coverage rates of the bi-annual treatment were also above 90% since it was initiated. Mathematical modelling by Davies (15) suggests that interruption of transmission can be achieved with an 85% coverage rate. However, for Mpamba-Nkusi focus, historic data also suggest that transmission of *O. volvulus* was very high in the 1990's, most likely attributable to lack of any control measures at the time (5). There has been extensive deforestation in the focus due to population pressure and this might have also reduced the number of breeding sites for *S. neavei*, thus contributing to reduction in transmission in some sites (6).

Work by Cupp and others in Guatemala suggested that twice yearly treatment covering all eligible persons could interrupt transmission of onchocerciasis without vector control measures (16). In this focus vector control measures were an additional strategy applied; the combination of these approaches has been shown to hasten elimination of *O. volvulus* (17). The criterion to detect the IgG4 antibodies in children only revealed 0.03%, a figure whose point prevalence and upper bound of the 95% CI are both below the 0.1% threshold value set by the WHO (7) and adopted in the Ministry of Health Uganda certification guidelines. In this focus there was only one child between the ages of 5-9 that was found positive for IgG4 antibodies. Based on the successful attainment of epidemiological, entomological and serological criteria, Mpamba-Nkusi focus was moved to Post Treatment Surveillance (PTS) period of three years before it can be declared eliminated (8).

In conclusion, with the implementation of annual mass treatment with ivermectin 14 years ago, and later introduction of ground larviciding and bi-annual treatment, interruption of *O. volvulus* has been achieved in the Mpamba-Nkusi onchocerciasis focus. According to WHO criteria (7) that were modified by Ministry of Health Uganda (8), there was no evidence of *O. volvulus* ongoing transmission in the focus. Treatment with ivermectin was recommended to be halted; however, continuous monitoring will still be required during the PTS period.

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