Short Report: Polymerase Chain Reaction Pool Screening Used To Compare Prevalence of Infective Black Flies in Two Onchocerciasis Foci in Northern Sudan

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Abstract. Onchocerciasis remains an important debilitating disease in many areas of Africa, including Sudan. The status of infection transmission in 2007 was assessed in the vectors of two disease foci in Sudan: Abu Hamed in northern Sudan, which has received at least 10 years of annual treatment and Galabat focus in eastern Sudan, where only minor, largely undocumented treatment activity has occurred. Assessment of more than 30,000 black flies for *Onchocerca volvulus* infectious stage L3 larvae by using an O-150 polymerase chain reaction protocol showed that black fly infectivity rates were 0.84 (95% confidence interval = 0.0497–1.88) per 10,000 flies for Abu Hamed and 6.9 (95% confidence interval = 1.1–16.4) infective flies per 10,000 for Galabat. These results provide entomologic evidence for suppressed *Onchocerca volvulus* transmission in the Abu Hamed focus and a moderate transmission rate of the parasite in the Galabat focus.

Onchocerciasis remains a debilitating disease in many areas of Africa and Sudan is the most northern location of the disease in the world. The World Health Organization estimated that more than two million persons are infected with *Onchocerca volvulus* in Sudan,¹ and the largest endemic focus in south-southwestern Sudan is among the most highly disease-endemic areas in the world.^{2–5} Patients in this area have severe onchocerciasis, which includes blinding disease. Two additional main disease foci in Sudan are in Abu Hamed on Nile River in northern Sudan⁶ and the Galabat area in eastern Sudan near the border with Ethiopia.⁷ These two northern foci are regions to which skin disease but not blinding disease is endemic.^{8,9} The major black fly vectors in Sudan are believed to be typical west Africa savanna species, *Simulium damnosum* s.s. and the less abundant *S. sirbarum*.¹⁰ The vector in the Abu Hamed focus is a closely related, but separate, cytospecies of the *S. damnosum* complex.¹¹ Molecular analysis of the O-150 tandem repeats molecular marker of *O. volvulus*¹² has shown that parasites in Abu Hamed also appear to have a unique molecular pattern.¹³

The Abu Hamed focus received continuous annual community-directed treatment with ivermectin (CDTI) beginning in 1998. The treatment regimen was then changed to semi-annual (every six months) treatments in 2006, and the area received 12 treatments with 53% mean coverage (range = 22–93%) by the end of 2007. In contrast, the Galabat focus only began regular annual CDTI activities in 2007 and received one treatment with 89% coverage in the same year. Parasitologic and clinical surveys conducted in Abu Hamed since 2000 have shown a considerable decrease in infection prevalence over the past 20 years. Skin snip surveys in six sentinel villages showed a major reduction in microfilarial prevalence from the 34–37% level seen in the mid 1980s⁸ to levels less than 1% in 2007 (Schewitaak T and others, unpublished data). The prevalence of persons with active clinical signs of onchocerciasis also decreased from 5% in 2006 to < 2% in 2007 in the area (Schewitaak T and others, unpublished data). For the

Galabat focus, a 63% microfilarial prevalence associated with severe onchodermatitis, commonly known as the sowda form of onchocerciasis, was reported 24 years ago.⁹

This study compared the status of parasite transmission in Abu Hamed and Galabat foci in vectors obtained during 2007–2008. The Abu Hamed focus is approximately 295 km long and 5 km wide and is located along the Nile River in the Sahara desert (Figure 1). This area received continuous CDTI with variable coverage for 10 years. The Galabat focus is located along the banks of Atbara River near the border with Ethiopia (Figure 1). This area received had minor CDTI activity in 2006; full CDTI activity began in 2007.

Two sentinel villages near vector breeding sites were selected in each focus. For the Abu Hamed focus, these were Mograt Island (19°29'N, 33°14'E, population = 932) and Nady (18°44'N, 33°39'E, population = 1,168). For the Galabat focus, they were Hilat Khatir (13°13'N, 36°1'E, population = 1,914) and Jumiza (13°25'N, 36°5'E, population = 3,799) (Figure 1). The standard human landing capture method¹⁴ was used to obtain black flies daily from the sentinel villages during March 2007–February 2008. Black flies were obtained and head pools of ≤ 100 heads were processed for *O. volvulus*-specific the O-150 polymerase chain reaction (PCR) and PCR-based enzyme-linked immunosorbent assay (ELISA) as reported.^{15–17} A sample was considered putatively positive if it had an ELISA score at or above the cutoff (i.e., the mean of 10 internal negative controls plus 3 SD). Samples positive after a second independent PCR and ELISA were reported as confirmed positive. Data were analyzed by using Poolscreen algorithm Version 2.0.¹⁸

A total of 33,818 black flies (Abu Hamed 29,401 and Galabat 4,417) were obtained during March 2007–February 2008. The monthly density of flies obtained in Abu Hamed was much higher than that obtained in Galabat (Figure 2). Within Abu Hamed, the monthly densities of flies obtained in Mograt Island were higher than those concurrently obtained in Nady (Figure 2A). Similarly, more flies were obtained in Hilat Khatir in the Galabat focus than in Jumiza (Figure 2B). The Abu Hamed focus showed major black fly activity during December–April and a peak biting activity during February–March. In contrast, major black fly activity in the Galabat focus was detected during August–December and a maximum activity during August–September (Figure 2).

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FIGURE 1. Map of Sudan showing locations of the Abu Hamed and Galabat onchocerciasis-endemic areas. Details of each focus are shown in inset maps, including the sentinel villages where black flies were obtained.

Black flies obtained in Abu Hamed were examined by PCR in 203 head pools and a maximum pool size of 100 heads (Table 1). The PCR analysis showed two confirmed positive pools with *O. volvulus* L3 larvae in 29,401 black flies. These pools were obtained in Mograt Island in March 2007 and January 2008 (Figure 2A). No positive pools were detected in black flies collected from Nady. The black fly infectivity rate in Abu Hamed was 0.84/10,000 flies (95% confidence interval [CI] = 0.0497–1.88) (Table 1). In the Galabat focus, screening of 4,417 black flies in 65 pools identified three *O. volvulus*-infective

pools. Two of these pools were obtained from Hilat Khatir in November 2007 and February 2008, and the third pool was obtained in Jumiza in October 2007 (Figure 2B). The corresponding black fly infectivity rate in Galabat was 6.9/10,000 flies (95% CI = 1.1–16.4) (Table 1).

The monthly black fly densities reported here confirm earlier observations on black fly behavior in the Abu Hamed focus⁹ and provides empirical data on annual black fly activity in the Galabat focus. Such knowledge is essential for targeted fly collections as control activities progress in these disease

FIGURE 2. Monthly density and infectivity of black flies obtained in the study area in Sudan, 2007–2008. **A**, monthly density of black flies in two sentinel villages, Mograt Island and Nady, in the Abu Hamed focus. **B**, monthly density of black flies in two sentinel villages, Hilat Khatir and Jumiza, in the Galabat focus. Cross-hatching indicates presence of a single *Onchocerca volvulus*-positive black fly heads pool in black flies obtained in the corresponding month.

TABLE 1

Summary of black fly data obtained in Abu Hamed and Galabat onchocerciasis-endemic areas in Sudan, 2007–2008 and their infectivity on the basis of polymerase chain reaction pool screen analysis

Focus	Sentinel village	No. flies	No. pools	No. positive pools	Prevalence of infective flies/10,000	95% confidence interval
Abu Hamed	Mograt	19,496	102	2	1	0.074–2.8
	Nady	9,905	101	0	0	0–1.94
	Total	29,401	203	2	0.84	0.0497–1.88
Galabat	Hilat Khatir	2,856	31	2	7.14	0.198–52.5
	Jumiza	1,561	34	1	6.47	0.25–102
	Total	4417	65	3	6.9	1.14–16.4

foci. For example, seasonal black fly collections in Abu Hamed should be carried during November–May to ensure inclusion of major fly activity. The infective black flies in Abu Hamed were generally detected during the months of high fly densities (January and March), and one of the infective pools in Galabat was detected at the end of the activity season at a time when low fly density was detected (February). This finding is supported by earlier observations of continued transmission despite apparent lack of black flies and their breeding sites in the area (Abdelnur OM, 2010, unpublished data). Presence of small pools in the Atbara River after the rainy season seem to maintain low densities of predominantly parous black flies capable of maintaining transmission, which is similar to previous observations in west Africa.¹⁹

The infected fly rate in Abu Hamed is less than the 1 in 2,000 flies (5/10,000) threshold level used by the Onchocerciasis Control Program as an indicator for interruption of disease transmission.^{20,21} This rate reflects a limit of less than 1/1,000 parous flies with a corrected level of less than 1/2,000 flies, assuming a crude parous fly rate of 50% and an infection rate of 1 infective larva per fly (Table 1).^{20,21} Thus the black fly infectivity rate in Abu Hamed suggests that transmission of the parasite might be close to the level below which the parasite population is unsustainable. In contrast, the Galabat focus showed black fly infectivity rate well above the threshold level, indicating continued moderate transmission of the disease in the area (Table 1).

The considerable difference between the prevalence of infective black flies in Abu Hamed and Galabat endemic areas was statistically masked by the minor overlap of the upper 95% CI of the Abu Hamed infective rate with the lower 95% CI limit of Galabat (Table 1). The number of black flies screened by using the O-150 PCR in Abu Hamed exceeded the minimum sample size of 10,000 flies set by the World Health Organization^{22,23} and the limit of 6,000 flies used by the Onchocerciasis Control Program.^{20,24,25} This number produced more precise estimation of the transmission level in terms of the 95% CI,^{18,26} and low number of black flies screened for the Galabat focus, < 5,000 flies, led to a much broader CI limits (Table 1).

This study is the first large-scale entomologic survey by PCR in east Africa that determined the black fly infectivity rate with *O. volvulus*. Our findings demonstrate the effect of the CDTI treatments implemented to date, and indicate that this assay can be useful for assessing future disease control activities in northern Sudan. Results for Abu Hamed show the effect of CDTI activity since 1998. These results contrast with those for Galabat, where there was virtually no treatment program before 2007. Although no baseline black fly infectivity data is available for the Abu Hamed focus, changes in black fly

infectivity levels such as those currently seen in Abu Hamed have only been reported as an impact of control intervention¹⁶ and never reported to be the result of a natural decrease in disease transmission. Thus, it is not unreasonable to suggest that the prevalence of infective black flies in the Abu Hamed and Galabat reflects CDTI activity and highlights the valuable effect of continuous ivermectin drug pressure on black fly infectivity.^{25,27}

The Abu Hamed data are consistent with recent findings of sharp decreases in parasito-clinical surveys (Schewitaak T and others, unpublished data). Taken together, these data support the suggestion that transmission can be interrupted by means of ivermectin mass treatment programs alone in some foci in Africa.^{25,27–29} However, serologic studies in children to determine recent exposure to onchocercal antigens and additional and more recent parasitologic, clinical, and entomologic data are needed to support and confirm complete interruption of the disease transmission in Abu Hamed.

In summary, PCR-based screening of black fly vectors of onchocerciasis in northern Sudan showed that only low levels of infected black flies in Abu Hamed existed before the shift to biannual treatment activities in the area. This entomologic evidence suggested low levels *O. volvulus* transmission in the Abu Hamed focus compared with a moderate transmission rate in the Galabat focus. Several years of twice per year treatment since 2007 has probably brought the parasite population in Abu Hamed to an unsustainable level. This level is paralleled in a concurrent reduction in human clinical onchocercal disease. After repeat entomologic and epidemiologic studies in the near future, it is hoped that there may be a move to cease ivermectin treatment in Abu Hamed and move to a post-treatment surveillance phase to ensure that there is no recrudescence of transmission and disease.

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