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A Serological Survey of Human Onchocerciasis in Yemen

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Abstract. Yemen is a country that has been treating severe cases of oncho-dermatitis since 1992 and is now moving to a program aimed at the elimination of the transmission of *Onchocerca volvulus*. It is important to ensure that the currently acceptable tools used in epidemiological assessment of onchocerciasis in Africa and Latin America also apply to Yemen. Five hundred and ten blood samples from three known *O. volvulus*–endemic areas, locations that have never been under a mass treatment program, were tested for the presence of antibodies against a panel of *O. volvulus*–specific antigens using enzyme-linked immunosorbent assay (Ov16) and luciferase immunoprecipitation system (OvFAR-1 and Ov-MSA1) assays. Overall, 31.4% of the samples tested were positive, with positivity increasing with age. Positivity was seen in 76.5% of those presenting with clinical onchocerciasis but importantly also in more than 28.5% of those defined as free of oncho-dermatitis; these latter individuals are likely to be serving as a source for persistent reinfection. This study supports the use of the current *O. volvulus*–specific serologic methodology in Yemen.

Onchocerciasis in Yemen was first recognized through its association with the characteristic dermatological presentation of onchocerciasis characterized by increased hyperpigmentation of the skin in localized areas of the body; indeed, this form of the disease has acquired a local Yemeni name "sowdah," the Arabic word for "dark."[1] Based on the predominance of this condition in the local endemic populations, a clinical treatment program was setup to treat those suffering from this reactive form of oncho-dermatitis.[2–4] To move the national onchocerciasis program from a disease control to an elimination program for the approximately 300,000 people likely to be exposed to this infection,[3] in keeping with the current global approach,[5,6] there is a need to ensure that the standard epidemiological indicator currently used in onchocerciasis programs (onchocerciasis serology) is applicable to Yemen. In addition, there is a need for epidemiological evidence to support a move from a successful treatment-oriented program to a mass drug program (MDA). Blood samples were collected from residents of Yemeni onchocerciasis areas in three governorates in the mountain range running parallel to the Red Sea (Figure 1) and tested for onchocerciasis.

Five hundred and ten residents were selected (298 males and 212 females) from three MDA-free wadis within the area known to be endemic for "sowdah," namely, Wadi Al Zabid, Wadi Al Ana, and Wadi Al Ghail (Figure 1). Selection of individuals living in these riverside villages was carried out on a random basis, ensuring to include those who were suffering, or had suffered, from "sowdah" and their family members. Participants were questioned about their personal histories relating to oncho-dermatitis (i.e., sowdah). Hundred and twenty-three people who had "sowdah" or had been under treatment of this condition were included, and an additional 387 individuals were, and had always been, free of clinical onchocerciasis but were exposed to the infection as they were living in endemic area also included. None of those people sampled carried onchocercal nodules, which is typical for Yemeni onchocerciasis.[3,4] Blood samples, collected by finger prick extraction, were collected on Whatman filter paper, dried, and dry-stored at –20°C for processing in the laboratory. The blood samples were extracted by standard methods and processed for the presence of onchocercal antibodies. IgG4 antibodies to Ov16 were determined by a standardized

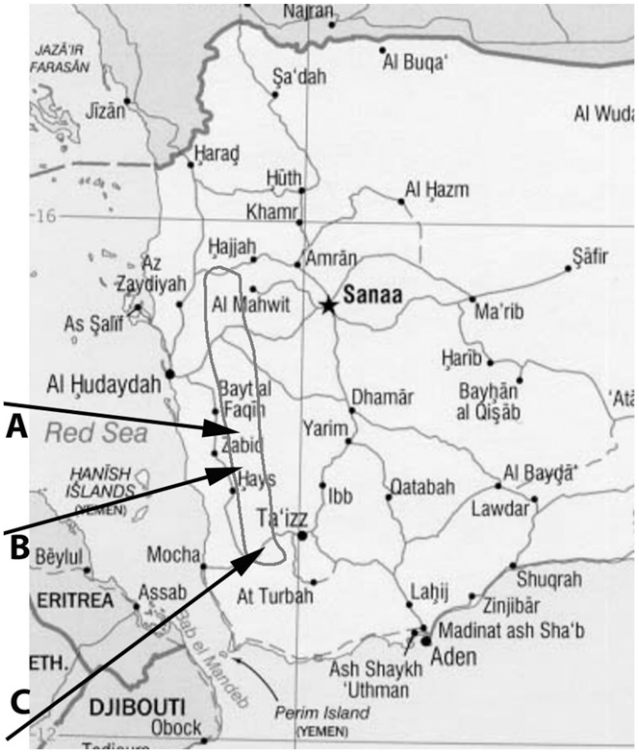

FIGURE 1. The location of the study, the three endemic sites in the Yemeni onchocerciasis area (the known endemic area indicated by the area ringed along the mountainous Red Sea border of the country). (**A**) Wadi Al Zabid, (**B**) Wadi Al Ana, and (**C**) Wadi al Ghail.

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TABLE 1

The relationship of *Onchocerca volvulus* seropositivity to the clinical presentation of onchocerciasis in 498 residents

Clinical presentation	Al Ghail		Al Ana		Al Zabid		Total	
	Ov+	Ov–	Ov+	Ov–	Ov+	Ov–	Ov+	Ov–
Presenting with oncho-dermatitis	3	1	5	6	18	1	26* (76.5%)	8
Presenting only with increased pruritus	5	15	0	0	4	2	9 (34.6%)	17
Free of either oncho-dermatitis or increased pruritus	46	136	34	105	45	72	125 (28.5%)	313

The clinical status of 12 patients in the blood survey was not recorded. Number (% of individuals in respective clinical subgroup).
* All of these were Ov antibody positive in both serological tests (see Table 3).

enzyme-linked immunosorbent assay (ELISA) performed exactly as previously described.[7] Cutoffs for positivity were based on ROC analyses. True positives were serum from skin snip positive (mf+) individuals from the Americas (Ecuador, Guatemala), as well as West (Ghana), Central (Cameroon), and East Africa (Uganda). True negatives were healthy North American donors who had traveled outside of North America. The assay was set for 99–100% specificity and the sensitivity was 80%. The skin snip biopsy technique for microfilarial assessment was not used in this study as it is currently not part of the national program protocol; indeed, "sowdah" patients are as a rule negative in this "live parasite" assay as they are actively killing the microfilariae in their dermal tissues.

The luciferase immunoprecipitation system (LIPS) assays to detect IgG antibodies to OvFAR-1 and Ov-MSA1 antigens were performed as described previously,[8] except that an input

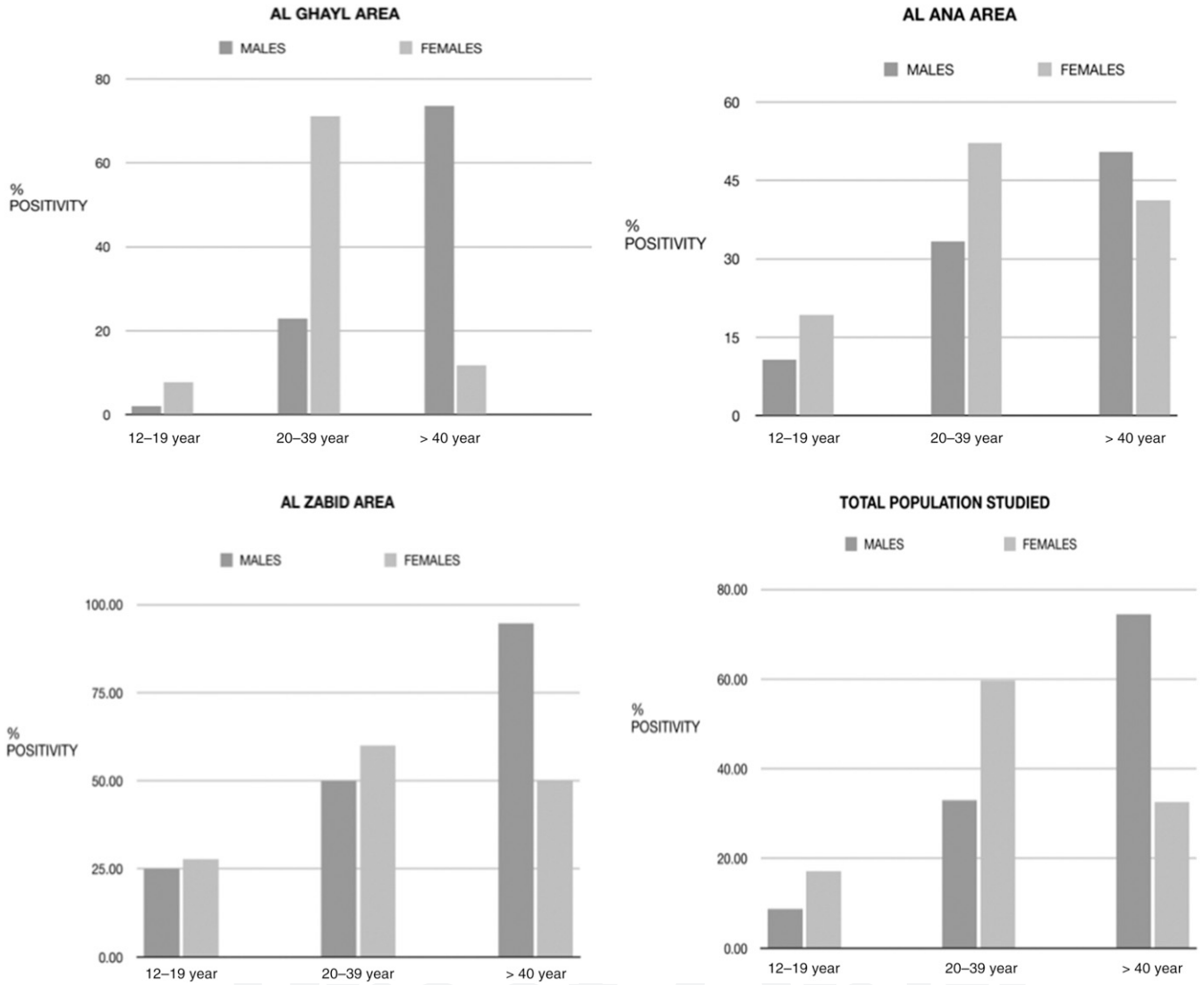

FIGURE 2. The differences in *Onchocerca volvulus* positivity between males and females resident in the three different endemic zones of onchocerciasis in Yemen.

TABLE 2

Total numbers of residents studied in each endemic area and their seropositivity for *Onchocerca volvulus*—number studied (% positivity)

Group	Wadi Al Ghail	Wadi Al Ana	Wadi Al Zabid	Total population
All residents	210 (26.2%)	151 (25.2%)	149 (45.0%)	510 (31.4%)
Males	136 (36.0%)	88 (30.7%)	74 (67.6%)	298 (42.3%)
Females	74 (9.5%)	63 (20.6%)	75 (29.3%)	212 (19.8%)
Ages 12–19 years	74 (4.1%)	54 (14.8%)	34 (26.5%)	162 (12.4%)
Ages 20–39 years	49 (36.7%)	50 (42.0%)	45 (55.6%)	144 (44.4%)
Ages > 39 years	87 (49.4%)	47 (46.8%)	70 (74.29%)	204 (75.4%)

of 1 million luminometer units (LU) of the enzyme reporter Ruc-OvFAR-1 and 300,000 LU of the Ruc-Ov-MSA1 was used. Briefly, 1 μL of patient serum was diluted 1τ10 in assay buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM, 1% Triton x-100). The plate was incubated for 5 minutes at room temperature after which 7 μL of a 30% suspension of protein A/G beads in PBS (Pierce Biotechnology, Rockford, IL) was added to the mixture in a 96-well filter HTS plate (Millipore, Bedford, MA). After 5 minutes, the filter plate containing the mixture was applied to a vacuum manifold and washed twice in assay buffer and eight times with PBS. After the final wash, all plates were processed on a Berthold LB 960 Centro microplate luminometer using a colenterazine substrate mix (Promega, Madison, WI). All data were the average of triplicates corrected for background reactivity (no serum added). The cutoffs for positivity used were based on ROC analyses as described previously.[8]

Hundred and sixty individuals, 31.4% of those tested of all those sampled, were positive to one or more of the three antigens tested, and the profile of this positive group included both individuals who had a history of oncho-dermatitis (76.5% of this clinical subgroup) and those with no history of the clinical disease (28.5% of this subgroup) (Table 1). A third (34.6%) of those who presented with increased pruritus, although not showing any other signs or symptoms of oncho-dermatitis, were positive for *Onchocerca volvulus*–specific antibodies. Both males and females were positive in this testing (Figure 2), with the prevalence of positivity increasing with age (Table 2). There were differences in age-specific prevalence that are not consistent between age groups; this may relate to differences in time of exposure to infected flies between males and females of different ages. In this society, young women tend to spend more time at the riverside (e.g., washing clothes) than older women (who tend to remain in the houses) do; in addition, men (c.f. women) often travel away from the endemic area. The testing of a larger number of people is probably needed to ensure that these differences are valid.

These results indicate first that anti-*O. volvulus* antibodies are present in residents of the onchocerciasis-endemic areas of Yemen, including antibodies directed at Ov16, the antigen target used as the global program epidemiological indicator.[6] The use of a panel of three different antigens (Ov16, OvFAR-1, and Ov-MSA1) has increased the sensitivity of the study above that if only using Ov16 (Table 3), which arguably has a sensitivity of no greater than 80%. Since its discovery in 1989, the sensitivity of Ov16 has rarely been greater than 80% in immunoassays when the specificity was tuned for > 99% against mf + Ov-infected patients. This finding is known to be related to MHC class II haplotypes, as the response to Ov16 is DR-restricted. Luciferase immunoprecipitation system constructs for Ov16 have only been recently developed and in this present study. Enzyme-linked immunosorbent assays were used in this present study as usable LIPS constructs for Ov16 have only recently been developed. In general, the sensitivity of LIPS for Ov16 antibodies has not been significantly different from that for ELISAs or for the commercially available RDT.

The substantial number of positive individuals free of any history of clinical disease, and thus not previously included in the established clinical program, are likely to have been a major source of continuing reinfection to the focus and need to be included in an MDA if elimination is to be achieved.

This study shows that the currently approved epidemiological procedures recommended in the World Health Organization elimination guidelines that involve measuring onchocercal antibodies can be applied for the elimination in Yemen.[9]

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TABLE 3

Positivity to the different antibody assays: ELISA for Ov16 and LIPS for OvFAR1/Ov-MSA1

Group	Number positive in OV16 (ELISA) only	Number positive in OvFAR1/Ov-MSA1 LIPS	Number positive for both OV16 and OvFAR1/Ov-MSA1	Positive in any test *N* (%)	Number negative in all tests *N* (%)
Wadi Al Ana *N* = 151	2	23	13	38 (25.2%)	113 (74.8%)
Wadi Al Zabid *N* = 149	2	51	14	67 (45.0%)	82 (55.0%)
Wadi Al Ghail *N* = 210	4	30	21	55 (26.2%)	155 (73.8%)
Totals	8	104	48	160 (31.4%)	350 (68.6%)

ELISA = enzyme-linked immunosorbent assay; LIPS = luciferase immunoprecipitation system.

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