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Screening for *Onchocerca volvulus* Anti-Ov16 IgG4 Response among Rural Communities Receiving Community-Directed Treatment with Ivermectin in Endemic Foci of Onchocerciasis in Yemen to Assess the Interruption of Transmission by Detecting Recent Exposure

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Contents

Executive Summary

In Yemen, onchocerciasis is endemic in certain foci near watercourses in eight governorates and is one of the most neglected diseases. Although ivermectin has been distributed to some endemic foci in the Tihama region - west of the country, there is a lack of baseline estimates of onchocerciasis and absence of monitoring for the impact of community-directed treatment with ivermectin (CDTI) as well as regular administration of the drug to affected individuals on its transmission status. Therefore, the present study is the first to determine the anti-*Ov*16 IgG4 serostatus of local communities of Hodeidah and Al-Mahwit governorates of the region as an indicator for recent exposure to and ongoing transmission of infection. Screening for anti-*Ov*16 IgG4 was conducted using the SD BIOLINE® Onchocerciasis IgG4 RDT. An overall seroprevalence rate of 18.5% (94/508) was found in four selected districts (Ad Dahi, Bani Sa'as, Al Marawi'ah and As Sukhnah), with rates of 23.7% (44/186) and 20.4% (42/206) being observed in the onchocerciasis-endemic and ivermectin-targeted districts of Ad Dahi in Hodiedah and Bani Sa'ad in Al-Mahwit, respectively. In addition, rates of 8.0% (4/50) and 6.1% (4/66) were observed in Al Marawi'ah and As Sukhnah districts of Hodeidah, which neighbor the endemic districts and were of unknown endemicity for the disease. In contrast to Ad Dahi, children of ten years or younger in Bani Sa'ad showed significantly lower anti-*Ov*16 IgG4 seropositivity than those older than ten years (9.1% *vs.* 24.5%), reflecting a possible decline in disease transmission following regular targeting of affected people with ivermectin before community-directed treatment with ivermectin (CDTI) in 2016. Moreover, age of more than ten years and residing within a large-size family were confirmed by multivariable analysis as the two independent predictors of higher exposure to infection. In conclusion, onchocerciasis transmission is still ongoing as evidenced by the higher anti-*Ov*16 IgG4 seroprevalence rate among children younger

than ten years of age in comparison to that (<0.1%) set by the World Health Organization as a serologic criterion for transmission interruption. Further large-scale studies incorporating serologic and entomologic criteria are recommended for initial mapping of *O. volvulus* in human and blackfly populations in endemic foci and their nearby areas of uncertain endemicity, determination of ivermectin distribution and coverage needed and assessment of its impact on disease transmission.

1. Background and Problem Statement

Onchocerciasis is a neglected tropical disease of the skin and eyes caused by the filarial nematode *Onchocerca volvulus* and transmitted by the bites of infected *Simulium* blackflies. It is endemic in 31 countries in sub-Saharan Africa and in some foci in Latin America and Yemen, with estimates of about 187 million people being exposed to potential transmission. [1, 2] In addition, over a million disability-adjusted life-years (DALYs) have been recently estimated to be lost due to onchocerciasis.[3] Promising strides towards the control and elimination of the disease have been made since the introduction and donation of the safe, effective microfilaricide ivermectin (Mectizan®) by Merck & Co., Inc. through Mectizan Donation Program (MDP) in the late 1980s.[4-7] Ivermectin administration at intervals has been suggested to interrupt transmission and incidence of new infections with *O. volvulus* in endemic foci in the long run.[8, 9] Concreted efforts through mass drug administration (MDA) campaigns at repeated rounds undertaken by control programs have led to the successful elimination of the disease in four countries in Latin America as certified by the World Health Organization (WHO) between 2013 and 2016; namely, Colombia, Ecuador, Mexico and Guatemala.[10]

In Yemen, the only country in Asia still afflicted with the disease, onchocerciasis mainly affects rural communities residing near the flowing streams of main seasonal watercourses (locally referred to as wadis) in western governorates.[11, 12] Clinically, onchocerciasis in Yemen is a unique form of localized, hyper-reactive onchodermatitis referred to as "sowda",[13] which is difficult to diagnosis in the laboratory by skin snip examination because of the scarcity of microfilariae.[14, 15] Although the epidemiology of onchocerciasis in the county lacks clear mapping and national burden estimates, its focal

endemicity has been documented in 33 districts of eight governorates; namely, Taiz, Ibb, Hodeidah, Dhamar, Raymah, Al-Mahwit, Sana'a and Hajjah.[12]

Ivermectin was first used for treating the clinical manifestations of sowda in Taiz in the early 1990s, and its use at three-month intervals was then recommended as a control strategy.[16] It was then distributed to patients in a few affected communities, mainly through the National Leprosy Elimination Program in Taiz and Charitable Society for Social Welfare (CSSW), a non-governmental organization contributing to ivermectin distribution to affected populations since 2000. Several campaigns have been implemented in endemic areas, particularly after approval of donating Yemen 91,000 Mectizan® treatments on a quarterly basis by the Mectizan Expert Committee of the MDP.[6] In addition to the absence of a national onchocerciasis control and elimination program in Yemen, the political crisis and war in the country since the Arab Spring revolutions in the region in 2011 have constrained the hope raised by the development of a national action plan in 2010 to eliminate the disease by 2015.[17] Moreover, no published studies on the impact of previous campaigns of CDTI) and the regular administration of the drug to affected individuals on the interruption of disease transmission in targeted areas.

Serologic markers are now widely used to determine the recent exposure to infection with *O. volvulus*, with immunoglobulin G4 (IgG4) response to the *Ov*16 antigen expressed by the third (L3) and fourth (L4) larval stages of the parasite being the most specific marker of recent infection,[18] confirming the ongoing transmission of the disease. The anti-*Ov*16 IgG4 is highly sensitive and provides evidence for recent transmission, particularly in young children. Accordingly, the negativity of anti-*Ov*16 IgG4 has been recently used to assess and confirm the interruption of disease transmission in foci

following extensive rounds of MDA or CTDI campaigns in a number of countries in Latin America and Africa.[19-23]

When tested against skin microfilaria status, a lateral flow strip RDT for detecting anti-*Ov*16 IgG4 antibodies against the parasite showed sensitivity and specificity levels of 98.0% (95% confidence interval "CI": 95.3–100.7) compared to sensitivity of 94.0% (95% CI: 89.3–98.7) and specificity of 96.0% (95% CI: 92.2–99.8) for enzyme-linked immunosorbent assay (ELISA).[24] This, in turn, makes the use of RDTs for detecting anti-*Ov*16 in sera of people in endemic settings a useful and cost-effective tool for the long-term surveillance of recent exposure to *O. volvulus* infection following MDA campaigns.[25] In 2014, SD BIOLINE® Onchocerciasis IgG4 RDT was launched as a surveillance tool for identifying exposure to *O. volvulus* by detecting anti-*Ov*16 IgG4.[26] It is noteworthy that the quality of these RDTs during field use has been successfully ensured with the use of recombinant human anti-*Ov*16 IgG4 antibody-based positive controls.[10]

2. Study Objectives

- To determine the prevalence of anti-*Ov*16 IgG4 antibodies among asymptomatic individuals in ivermectin-targeted and –non-targeted rural communities of Hodeidah and Al-Mahwit governorates.
- To determine the prevalence of anti-*Ov*16 IgG4 antibodies among symptomatic (nodule-carrier) individuals receiving ivermectin in rural communities of Hodeidah and Al-Mahwit governorates.
- To determine the prevalence and density of *O. volvulus* microfilariae among symptomatic (nodule-carrier) individuals receiving ivermectin in rural communities of Hodeidah and Al-Mahwit governorates.

3. Methodology

3.1. Study design and area

This cross-sectional study was conducted in four districts in Hodeidah and Al-Mahwit in the period from February to July 2017. Hodeidah is coastal, bordering the Red Sea and located at the coordinates of 14°48' N and 42°75' E, whereas Al-Mahwit is mountainous, bordering Hodeidah and located at the coordinates of 15°28' N and 43°32' E (Fig. 1). Both governorates are characterized by the presence of fast-flowing seasonal streams and perennial watercourses (wadis), where Wadi Surdud is the most famous one traversing the two governorates to drain to the Red Sea. Therefore, the people of rural areas residing alongside these watercourses are mainly engaged in agricultural activities.

Of the four study districts surveyed during the present study, two have been documented as being endemic for onchocerciasis; namely, Ad Dahi alongside Wadi Surdud and its tributaries in Hodeidah and Bani Sa'ad alongside Wadi Dayan and its tributaries in Al-Mahwit. The first CDTI campaign in Tihama region was implemented in both districts in 2016 (CSSW, personal communication, 2017). Moreover, ivermectin distribution campaigns targeting symptomatic patients have been conducted three times a year since 2002 in Bani Sa'ad. On the other hand, Al Marawi'ah and As Sukhnah districts of Hodeidah in the vicinity of the surveyed endemic districts, which are not listed as onchocerciasis-endemic districts (Ministry of Public Health and Population, personal communication, 2017), were included in the present study. Al Marawi'ah is traversed by

Wadi Siham and its tributaries, while As Sukhnah is traversed by Wadi Al Malih (Fig.1).

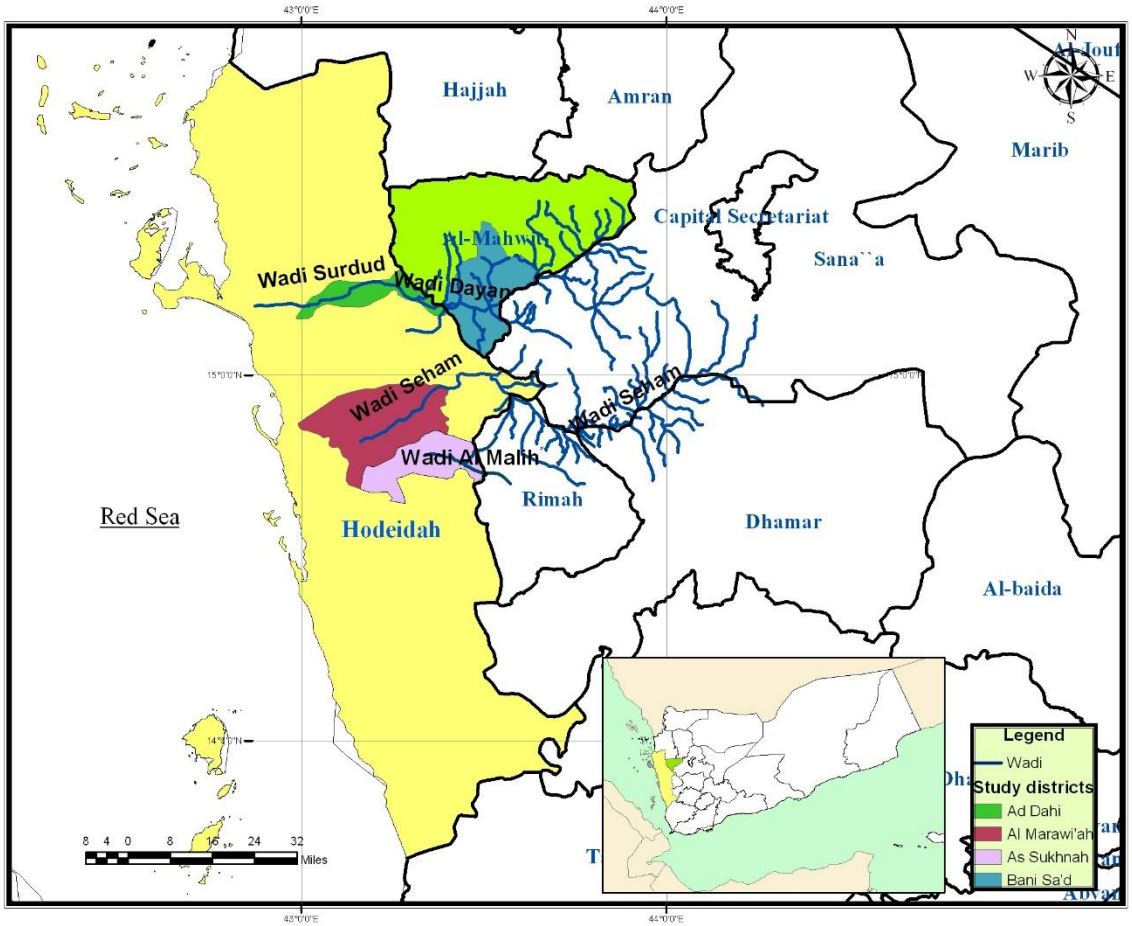

Fig. 1. Map of study areas

3.2. Sample size and sampling strategy

In accordance with the criteria set by the practical manual for the determination of sample size in health studies,[27] a minimum sample size of 384 was calculated at an expected onchocerciasis prevalence of 50.0% (due to the lack of prevalence data in the country), a confidence level of 95.0% and an accepted margin of error of 5.0%. Yet, 392 participants were recruited from the surveyed districts.

To avoid the effect that might be introduced as a result of the heterogeneity in

infection prevalence and the sparse distribution of rural communities in the study areas, multi-stage sampling was adopted to obtain the best representative sample, where endemic districts and sub-districts of the studied governorates were considered as the clusters. In the first stage, Ad Dahi and Bani Sa'ad were randomly selected from a list of endemic districts in Hodeidah and Al-Mahwit, respectively. In the second stage, two (Upper Grabeh and Lower Grabeh) and four (Al Wahaweh, Bani Ali, Gaaferat Alh and Utmah) sub-districts were randomly selected from Ad Dahi and Bani Sa'ad, respectively. Then, households were randomly selected from each sub-district, and all family members were invited to participate, ensuring the proportionality of the sample size of each sub-district to its population size. In addition, 116 participants were randomly selected from the districts of Al Marawi'ah and As Sukhnah, totaling the sample size to 508.

3.3. Data collection, blood screening for anti-*Ov*16 IgG4 and skin snip examination

Data on district of residence, gender, age, clinical signs of onchocerciasis, source of drinking water, durables of households and history of ivermectin intake were collected using a pre-designed questionnaire (Appendix A). Finger-prick blood of participants was screened for anti-*Ov*16 IgG4 using the SD BIOLINE® Onchocerciasis IgG4 RDT (Standard Diagnostics, Inc., Gyeonggi-do, Republic of Korea) according to the manufacturer's instructions. Negative and a range of low, medium and high positive controls (PATH, USA) were used to ensure the quality of each lot of RDTs at the points of testing in the field prior to blood screening. Skin snips were collected from nodule carriers and examined for detecting O. volvulus microfilariae following the standard procedure as explained previously.[28]

3.4. Data analysis

Data were analyzed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). The socio-economic status was determined using the principal component analysis (PCA) of durables owned by households. The constructed PCA-based scores of households were divided into five wealth quintiles and three socio-economic status categories, where households' residents with the lowest 40%, the middle 20% and the highest 40% of household wealth quintiles were classified as being of low, middle and high socio-economic status, respectively.[29] Prevalence of anti-*Ov*16 IgG4 and its 95% confidence interval (CI) were estimated. Associations or differences between categorical variables were tested using Pearson's chi-square test in bivariate analysis. The crude odds ratios (ORs) and the associated 95% confidence intervals of the proportion of seropositive individuals were also calculated to measure the strength of association between each independent categorical variable and the anti-*Ov*16 IgG4 seropositivity. Multivariable analysis using logistic regression was performed to determine the adjusted ORs with their associated 95% CIs so as to identify the independent predictors of anti-*Ov*16 IgG4 seropositivity. *P* values of <0.05 were considered statistically significant.

3.5. Ethical considerations

The research protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine and Health Sciences, University of Science and Technology, Sana'a, Yemen (Ref. 2016/14). Participation in the study was on a voluntary basis after explaining its purpose to the heads of households and participants, and written informed consent was obtained from the heads of households before recruiting their residents.

4. Results

Of the 508 individuals screened for anti-*Ov*16 IgG4, 56.7% (288/508) were males and 43.3% (220/508) were females. The median age of participants was 20 years (interquartile range: 11–38) with 23.2% (118/508) being children less than 11 years old. The proportion of people with history of receiving ivermectin was 42.7% (217/508). The socio-economic status of study population was classified as high (39.8; 156/508), middle (20.4%; 80/508) and low (39.8; 156/508) and 48% of study subjects are living in hut or houses composed of one room (table 1).

Table 1. Characteristics of study subjects (N = 508)

The characteristic item	*n* (%)
District of residence	
Bani Sa'ad	206 (40.6)
Ad Dahi	186 (36.6)
Al Marawi'ah	50 (9.8)
As Sukhnah	66 (13.0)
Gender	
Female	288 (56.7)
Male	220 (43.3)
Age (Years)	
≤ 10	118 (23.2)
> 10	390 (76.8)
Household's size (members)*	
≤ 5	56 (14.3)
> 5	336 (85.7)
Education status	
Secondary and above	33 (6.5)
Primary	191 (37.6)
Not educated	284 (55.9)
Occupation status	
Working	84 (16.5)
Not working	424(83.5)
Socio-economic status*	
High	156 (39.8)
Middle	80 (20.4)
Low	156 (39.8)
House structure*	
Compound of two rooms or more	204 (52)
Hut or one room	188 (48)
Source of water*	
Piped water	146 (37.2)
Others	246 (62.8)
Receiving ivermectin during the last year#	
Yes	217 (42.7)
No	291 (57.3)

*, Total number of respondents was 392; #, ivermectin has not been distributed in Al Marawi'ah and As Sukhnah districts.

4.1. Seroprevalence of anti-*Ov*16 IgG4

The overall prevalence rate of anti-*Ov*16 IgG4 was 18.5% (94/508), with a higher rate in Ad Dahi (23.7%; 95% CI: 18.0–30.0) than Bani Sa'ad (20.4%; 95% CI: 15.0–27.0), but there was no statistically significant difference (x^2 = 0.61, P = 0.435). On the other hand, lower rates of 8.0% (95% CI: 2.2–19.0) and 6.1% (95% CI: 1.7–15.0) were observed in Al Marawi'ah and As Sukhnah, respectively (Table 2).

4.2. Age-stratified seroprevalence of anti-Ov16 IgG4

In Bani Sa'ad, the prevalence of anti-*Ov*16 IgG4 among participants aged ten years or younger was significantly lower (9.1%; 95% CI: 3.0–20.0) than that among those older than ten years old (24.5%; 95%CI: 18.0–32.0) (x^2 = 5.9, P = 0.015). In contrast, no statistically significant difference (x^2 = 0.27, P = 0.61) was observed in the prevalence of anti-*Ov*16 IgG4 among the participants of the two age groups in Ad Dahi, being 20.4% (95% CI: 7.0–52.0) and 24.4% (95% CI: 19.0–32.0) for children of ten years of younger and those older than ten years, respectively. With the exception of anti-*Ov*16 IgG4 positivity in a single seven-year-old participant from Al Marawi'ah, all participants tested positive for anti-*Ov*16 IgG4 in Al Marawi'ah and As Sukhnah were older than ten years (Table 2).

4.3. Skin snip findings

Fig. 2 shows patients with suspected onchocercal dermatitis. Skin snips collected from 22 patients were negative for microfilariae.

Table 2. Age-stratified prevalence and distribution of anti-*Ov*16 IgG4 antibodies against *O. volvulus* in Hodeidah and Al-Mahwit governorates of Tihama region, Yemen (2017)

District (sub-districts)	**Prevalence of anti-*Ov*16 IgG4 stratified by age group** (years)					
	≤ 10		**> 10**		**All ages**	
	n/*N* (**%**)	95% CI	*n*/*N* (**%**)	95% CI	*n*/*N* (**%**)	95% CI
Overall prevalence	12/118 **(10.2)**	5.0–17.0	82/390 **(21.0)**	17.0–25.0	94/508 **(18.5)**	15.0–22.0
Districts endemic for onchocerciasis						
Bani Sa'ad	5/55 **(9.1)**	3.0–20.0	37/151 **(24.5)**	18.0–32.0	42/206 **(20.4)**	15.0–27.0
Al Wahaweh	1/17 (5.9)	0.2–29.0	16/54 (29.6)	18.0–44.0	17/71 (23.9)	15.0–36.0
Bani Ali	0/17 (0.0)	0.0–19.0	5/48 (10.4)	4.0–23.0	5/65 (7.7)	2.6–17.0
Gaaferat Alh	0/5 (0.0)	0.0–52.0	6/18 (33.3)	13.0–59.0	6/23 (26.1)	10.0–48.0
Utmah	4/16 (25)	7.0–52.0	10/31(32.3)	17.0–51.0	14/47 (29.8)	17.0–49.0
Ad Dahi	6/30 **(20.4)**	7.0–52.0	38/156 **(24.4)**	19.0–32.0	44/186 **(23.7)**	18.0–30.0
Upper Grabeh	1/4 (25)	0.6–81.0	15/52 (28.8)	17.0–43.0	16/56 (28.6)	17.0–42.0
Lower Grabeh	5/26 (19.2)	7.0–39.0	23/104 (22.1)	15.0–31.0	28/130 (21.5)	15.0–30.0
Districts with unknown endemicity for onchocerciasis						
Al Marawi'ah	1/5 **(20.0)**	0.5–72.0	3/45 **(6.7)**	1.4–18.0	4/50 **(8.0)**	2.2–19.0
As Sukhnah	0/28 **(0.0)**	0.0–12.0	4/38 **(10.5)**	2.9–25.0	4/66 **(6.1)**	1.7–15.0

N, Number of participants examined; *n*, number of anti-*Ov*16 IgG4-positive samples; CI, confidence interval.

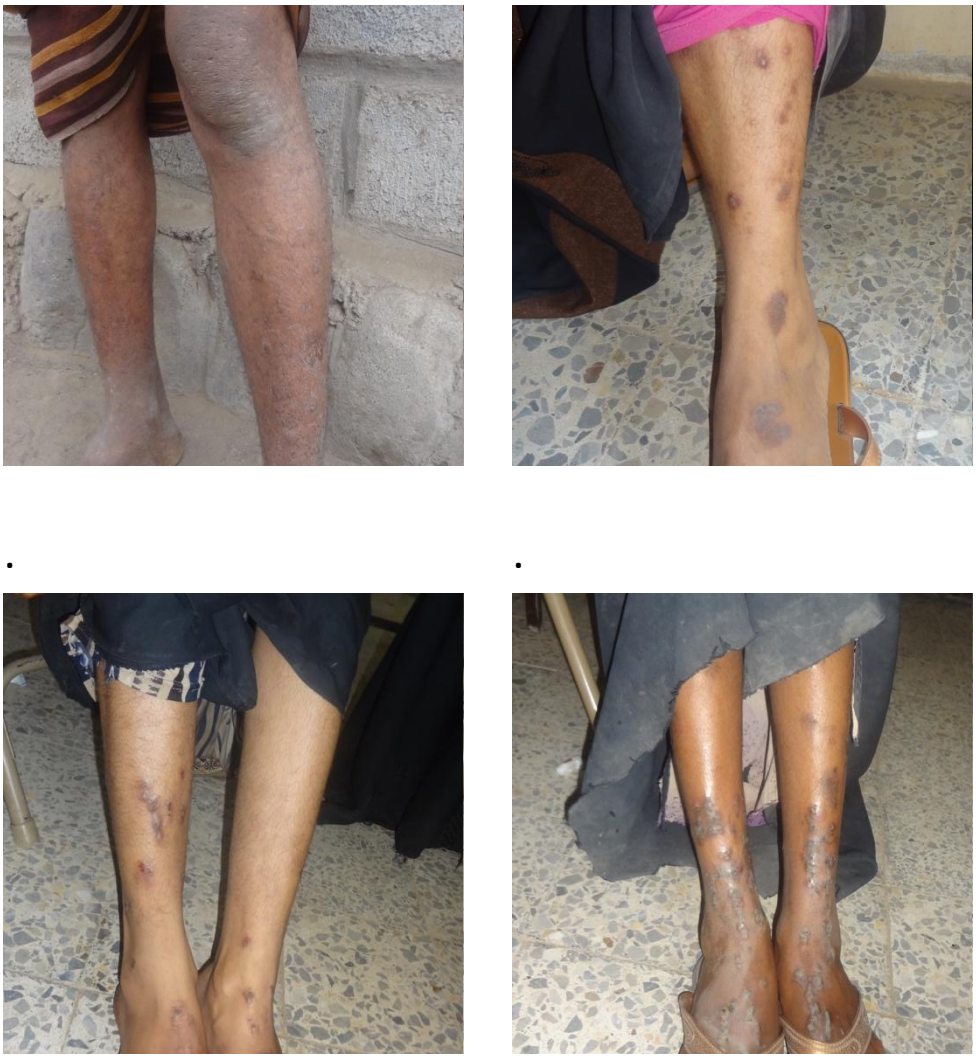

Fig. 2. Suspected cases of onchocerciasis

4.4. Factors associated with anti-*Ov*16 IgG4 seropositivity

Bivariate analysis showed that only the age of participants and their family size were significant predictors of anti-*Ov*16 IgG4 seropositivity, where those older than ten years were at about a twice higher risk of exposure to *O. volvulus* infection than those of ten years or younger (OR = 2.18; 95% CI: 1.10–4.31, *P* = 0.024). In addition, participants from large families were more than twice as likely to be exposed to infection than those from small families (OR = 2.6; 95% CI: 1.08–6.31, *P* = 0.028). However, district of residence (OR = 1.21; 95% CI: 0.75–1.95, *P* = 0.435), gender (OR =1.03; 95% CI: 0.63–1.68, *P* = 0.914), education status (OR =1.25; 95% CI: 0.77–2.03, *P* = 0.364), occupation status (OR = 0.65; 95% CI: 0.34–1.25, *P* = 0.364), socio-economic status (OR = 1.0; 95% CI: 0.58–1.73; *P* = 0.196), source of water (OR = 1.00; 95% CI: 0.61 – 1.64; *P* = 0.994), history of ivermectin intake (OR = 1.01; 95% CI: 0.74–1.57; *P* = 0.693), presence of nodules (OR = 1.17; 95% CI: 0.57 – 2.43; *P* = 0.666) or skin itching (OR = 1.10; 95% CI: 0.61–2.00; *P* = 0.734) were not found to be significantly associated with exposure to *O. volvulus* infection. On the other hand, multivariable analysis further confirmed that age of older than ten years (adjusted OR = 2.12; 95% CI: 1.07–4.23, *P* =0.032) and being a member of a large family (adjusted OR = 2.53; 95% CI: 1.04–6.14, *P* =0.040) were independent risk factors associated with exposure to infection with *O. volvulus* among residents of endemic rural areas of Hodeidah and Al-Mahwit (Table 3).

Table 3. Factors associated with anti-*Ov*16 IgG4 seropositivity among residents of onchocerciasis-endemic areas of Hodeidah and Al-Mahwit, Yemen (2017)

Variable	*N*	*n* **(%)**	**OR** (95% CI)	***P* value**
District of residence				
Bani Sa'ad	206	42 (**20.4**)	Reference	
Ad Dahi	186	44 (**23.7**)	1.21 (0.75–1.95)	0.435
Gender				
Female	239	52 (**21.8**)	Reference	
Male	153	34 (**22.2**)	1.03 (0.63–1.68)	0.914
Age (Years)				
≤ 10	85	11 (**12.9**)	Reference	
> 10	307	75 (**24.4**)	2.18 (1.10–4.31)	0.024*
Family size (members)				
≤ 5	56	6 (**10.7**)	Reference	
> 5	336	80 (**23.8**)	2.6 (1.08–6.31)	0.028*
Education status				
Educated	181	36 (**19.9**)	Reference	
Non-educated	211	50 (**23.7**)	1.25 (0.77–2.03)	0.364
Occupation status				
Working	52	15 (**28.8**)	Reference	
Not working	340	7 1 (**20.9**)	0.65 (0.34–1.25)	0.196
Socio-economic status				
High	156	32 (**20.5**)	Reference	
Middle	80	22 (**27.5**)	1.5 (0.77–2.75)	0.228
Low	156	32 (**20.5**)	1.0 (0.58–1.73)	1.00
Source of water				
Piped water	146	32 (**21.9**)	Reference	
Others	246	54 (**22.0**)	1.0 (0.61 – 1.64)	0.994
History of ivermectin intake				
Yes	217	46 (**21.2**)	Reference	
No	175	40 (**22.9**)	1.01 (0.74–1.57)	0.693
Presence of nodules				
No	347	75 (**21.6**)		
Yes	45	11 (**24.4**)	1.17 (0.57 – 2.43)	0.666
Skin itching				
No	315	68 (**21.6**)	Reference	
Yes	77	18 (**23.4**)	1.10 (0.61–2.00)	0.734

N, Number of participants examined; *n*, number of anti-*Ov*16 IgG4-positive samples; OR, Odds ratio; CI, confidence interval; * confirmed as independent risk factors using multivariable analysis.

5. Discussion

Onchocerciasis is focally endemic in eight governorates of Yemen; however, neither estimates of *O. volvulus* burden in the country nor studies on the impact of regular ivermectin campaigns or CDTI on its transmission in targeted areas are encountered. Because of the failure to achieve the goal of eliminating the disease by 2015, the WHO paid attention to its elimination from the country by 2020.[30] In Hodeidah and Al-Mahwit, control activities have been done by CSSW since 2000, mainly through the distribution of ivermectin donated by the MDP to infected people. The last activity was the MDA to endemic districts in Hodeidah and Al-Mahwit by involving local populations in CDTI campaigns in 2016. To our knowledge, the impact of campaigns in interrupting the transmission of the parasite in targeted areas has not been assessed. Therefore, the present study was the first to uncover the current transmission status of *O. volvulus* in the Tihama region of Yemen by surveillance of anti-*Ov*16 IgG4 against the parasite in the blood of residents in endemic rural areas.

The present study revealed an anti-*Ov*16 IgG4 prevalence rate of 18.5% among local populations of the four study districts in Hodeidah and Al-Mahwit, providing serologic evidence for ongoing *O. volvulus* transmission following regular ivermectin distribution to infected individuals as well as CDTI campaigns in such districts. Beyond the hope of eliminating onchocerciasis from endemic areas targeted by ivermectin, the present study is the first to uncover the transmission of the disease in districts not officially listed as endemic areas and considered as being of unknown disease endemicity. In this respect, prevalence rates of 8.0% and 6.1% were observed in the districts of Al Marawi'ah and As Sukhnah. The fact that the two districts are traversed by Wadi Siham and Wadi Al Malih, possible breeding sites for blackflies, together with the detection of anti-*Ov*16 IgG4 in a seven-year-old child in

Al Marawi'ah suggest that these areas act as potential transmission zones for onchocerciasis. This finding necessitates mapping of *O. volvulus* and its vector in all areas alongside such wadis and their tributaries before implementing MDA. The lower anti-*Ov*16 IgG4 prevalence in the latter districts compared to Bani Sa'ad and Ad Dahi could be explained by the fact that endemicity levels of onchocerciasis vary between geographic areas as a result of the interaction of several factors related to the parasite, vector, host and environmental conditions. Thus, comprehensive mapping of endemic areas is needed to geostatistically determine the level of disease endemicity and the foci of top priority for ivermectin MDA.

Although the present study revealed continuing transmission of onchocerciasis in Hodeidah and Al-Mahwit, a major issue is the lack of previously reported baseline prevalence rate of the anti-*Ov*16 IgG4 against the parasite to assess the impact of ivermectin treatment. In fact, this makes it difficult to accurately understand the extent to which ivermectin distribution had impacted the disease epidemiology. The seroprevalence rates among children of ten years or younger in the studied districts confirm continuing transmission, considering that anti-*Ov*16 IgG4 of <0.1% among under ten-year-old children is the criterion set by the WHO to confirm the interruption of disease transmission and its elimination.[30, 31] However, the significantly lower prevalence rate among children aged ten years or younger compared to those older than ten years (9.1% *vs.* 24.3%, respectively) in Bani Sa'ad raises promise regarding some degree of transmission decline. Such declining transmission could reflect the accumulative impact of the regular three-month-interval distribution of ivermectin to the affected populations in Bani Sa'ad since 2000 prior to the last CDTI campaign implemented in 2016. On the other hand, the single CDTI campaign in Ad Dahi in 2016 did not lead to significant changes in the prevalence of anti-*Ov*16 IgG4 between

children aged ten years or younger (20.4%) and those older than ten years (24.4%). In contrast to the latter district targeted by a single, recent CDTI, the early start of ivermectin distribution in Bani Sa'ad could probably maintain drug coverage for the entire reproductive lifespan of adult *O. volvulus*, which may extend between 9 and 14 years.[32] It is to be noted that ivermectin is a long-acting microfilaricidal drug that has little effect on adult worm and, therefore, controls the disease by killing microfilariae, reducing clinical manifestations and interrupting transmission by the vector but never cures the disease completely.[33] This, in turn, justifies for the rare exposure of children born by the end of MDA implementation in endemic areas to *O. volvulus* and the utility of screening such children for anti-*Ov*16 IgG4 as an indirect indicator for determining transmission interruption.[30]

In Yemen, diagnosis of sowda by the examination of skin snips is challenging due to the rare presence of microfilariae and may require repeated collection and examination of skin snips.[14, 34] This was evident in the present study, where all snips collected from nodule carriers in the surveyed districts were tested negative for the microfilariae. In fact, this could be attributed to both the poor sensitivity of skin snip microscopy for the detection of the low microfilarial load in the nodules of sowda patients due to their degeneration by the hyper-reactive immune response[35] and the possible impact of distributed ivermectin in killing microfilariae. As a rule of thumb, examination of skin snips should not be used to evaluate the impact of MDA with ivermectin on the interruption of onchocerciasis transmission or to determine the time of stopping such MDAs.[30]

Multivariable analysis showed that people older than 10 years old are at two times higher risk of exposure to *O. volvulus* infection compared to children <11 years old. It would not

seem that older age groups are more susceptible to the infection and the higher rate could be explained by the impact of ivermectin treatment on reducing the rate of infection in young children and remaining the adults who seroconverted before ivermectin treatment seropositive.[36] Identifying large family size as an independent risk factor of anti-*Ov*16 IgG4 positivity in the endemic districts may be attributed to that some members of family with large size may spend almost of their daytime outside the house. It is noteworthy that *Simulium damnosum*, the suggested vector of onchocerciasis in Yemen, bites outdoor during the daytime with two peaks of biting activity, in the morning until 09.00 and after 16.00.[37]

6. Study limitations

An important issue that has to be acknowledged is that there is no prior validation of RDTs in the study area against *Ov*16 ELISA as a reference method, and this comes in part from the unavailability of commercial ELISA kits for this purpose. Nevertheless, the quality of performance has been assured by the inclusion of anti-*Ov*16 IgG4 negative and positive controls supplied by PATH (www.path.org).

7. Conclusion

Onchocerciasis is still being transmitted in the Tihama region despite ivermectin distribution to affected individuals and the implementation of CTDI in 2016 as evidenced by the recent exposure of about a fifth of the residents being positive for anti-*Ov*16 IgG4. This could be attributed to the insufficient coverage rate with the drug and its distribution without having baseline infection rates in the targeted endemic areas and their neighboring localities, where onchocerciasis has also been found to be transmitted in two districts not

previously categorized as endemic for the disease. Despite the absence of onchocerciasis interruption, there is a decline in disease transmission in Bani Sa'ad district of Al-Mahwit as reflected by the significantly lower anti-*Ov*16 IgG4 seroprevalence among children of ten years or younger. Therefore, interruption of transmission and disease elimination is most likely in the future if good coverage with regular ivermectin MDA campaigns is achieved and their impact on disease transmission is continually monitored and evaluated.

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Appendix A

Study "Screening for *Onchocerca volvulus* anti-Ov16 IgG4 response among rural communities receiving community-directed treatment with ivermectin in endemic foci of onchocerciasis in Yemen to assess the interruption of transmission by detecting recent transmission "

Interviewer: _____ Date: _____

Start time: _____ End time: _____

GPS data: Latitude: _____ Longitude: _____Altitude:_____(m)

Governorate_____ District: _____ Uzla_____

Village name_____

1. **DEMOGRAPHIC, SOCIAL AND ECONOMIC INFORMATION**

Note: Interviewee should be head of household or adult > 18 years old

1. Name:_____
2. How many family members live in this house (including you)? #_____
3. Observe: Type of house.
   - Two floors/storey.....0
   - Compound/two rooms attached.....1
   - One room/hut.....2
   - Others, specify.....3
4. What is the source of the household's drinking water? (circle)

Piped water	0	Rainwater collection	2	Stream water	4
Well water	1	Tanker-truck	3	Spring water	5

5. Mark the number of appliances and vehicles that you have at home: ("0" if none).

Refrigerator		Stereo system or radio		Motorcycle	
TV		Electric or Gas Stove		Electric generator	
DVD		Washer		Car	
Computer		Bicycle		Agricultural tractor	

6. Is this family original in this area or came from another area? (0, original; 1, came from another area): __.
7. If came from another area, where did you come from?..... When?.....

II. HOUSEHOLD MEMBERS, CLINICAL MANIFESTATIONS AND IgG4 RDT RESULTS:

# of participant	Residents of this house	8. Sex	9. Age	10. The last Education achieved	11. Occupation (if applicable)	Taking ivermectin last year	12. Presence of Sowda	13. Sites and No. of nodules	14. Other skin signs	15. IgG4 RDT results
	Please, tell us the names of your family members, who live in your house (including you).	F=0 M=1	Years / Months ___?	0, never attend school ; 1, informal edu; 2, primary scho; 3, secondary scho; 4, university or above NA (child)=89	DK =88 NA (Student, Child)=89	No=0 Yes =1 NA = 89	No=0 Yes =1	NA=89	No=0	0=negative 1=positive
1										
2										
3										
4										
5										
6										
7										
8										

III. KNOWLEDGE, ATTITUDES, AND PRACTICES ABOUT ONCHOCERCIASIS

Note: The following sections should be answered by household heads in the endemic areas.

Knowledge of sowda

1. **Did you hear of onchocerciasis (sowda)?** (0, no; 1, yes; 2, No answer): __.

If yes, **which part of the body does it affect?** (0, false answer "parts other than the skin"; 1, correct answer "skin"; 2, No answer):___.

If the answer was correct, **what are the signs and symptoms of sowda?**

- Dermatitis:_____
- Pruritus:_____
- Skin nodules:_____
- Skin changes:_____
- Rash:_____
- Others (specify):_____

  For those reporting "itching,

  Does itching prevent you from sleeping at night? (0, no; 1, yes)

  Does itching prevent you from concentrating during daytime? (0, no; 1, yes)

2. **Had any member of your family been affected by sowda?** (0, no; 1, yes): __.

   If yes, **how many members? _____**
3. **Did sowda affect the school attendance of the infected member/s?** (0, no; 1, yes; 89,NA): __.
   If yes, is there a child (or children) not attending school at present/ this year? (0, no; yes, 1).
   If yes, how many children? _____

   For how long? _____
4. **Did sowda prevent the infected member/s from the work?** (0, no; 1, yes; 89, NA): __.
   If yes, what is the duration of work absenteeism last year? _____
5. **What is the cause of sowda?**
   - A worm:____
   - A virus:_____
   - A bacterium:_____
   - Sun scortch:_____
   - Poor personal hygiene:_____
   - Malnutrition: _____
   - Others (specify):_____
   - Do not know:_____
6. **What is its mode of transmission?**
   - Water:_____
   - Food:_____
   - Mosquito bite:_____
   - Fly (black fly) bite:_____

- Blood transfusion:_____
- Contact with infected people:_____
- Sexual intercourse:_____
- From mother to fetus:_____
- Others (specify):_____
- Do not know:_____

- If the answer was correct (black fly),

7. In which places are these flies predominant?

- Inside houses/ buidings:___.
- Along water courses:___.
- In the fields: __.
- Others (specify):_____.
- Do not know:_____.

8. **What time of the day these flies bite humans?**

- In morning hours:_____.
- In afternoon hours:_____.
- In evening hours:_____.
- During night:_____
- All the day:_____.
- Do not know:_____.

9. **Which season of the year are the flies mostly found?** (1, rainy season; 2, dry season):__.

<u>Attitudes and practices towards sowda</u>

10. Do you feel that you or your family members are at risk of sowda? (0, no; 1, yes): __.

11. Is sowda a preventable disease? (0, no; 1, yes): __.

If yes, **what are the methods of its prevention?**

- Using protective clothes:_____
- Using insect repellants:_____
- Getting vaccinated:_____
- Personal hygiene:_____
- Using drug pills:_____
- Others (specify):_____
- Do not know:_____.

12.Have you ever taken drug pills to prevent sowda delivered by healthcare workers in mass administration campaigns in the last year? (0, no; 1, yes; 3, not applicable): __.

If yes, **how many times did you take these pills?** _____

Before how long did the campaign come to your village?_____

If no, **what are the reasons for not taking the drugs?**

_____

_____

___

13. Is sowda a curable or treatable disease? (0, no; 1, yes): __.

If yes, **what are the methods you advise to treat it?**

- Herbs:_____
- Creams:_____
- Drugs:_____. If yes, what is the name of the drug?_____
- Others (specify):_____
- Do not know:_____.

14. **Do you accept that you or anyone of your male family members get married with a lady infected with sowda?** (0, no; 1, yes; I don't know, 3): __.
15. **Do you accept that you or anyone of your female family members get married with a male infected with sowda?** (0, no; 1, yes; I don't know, 3): __.

IV. Laboratory-derived data:

A. **Results of skin snips from people with sowda**
B. **Ivermectin intake by the snipped individual (0, did not take ivermectin; 1, took ivermectin):…….**
C. **Household member(s) snipped (write the number of the patient as above)**

Participant #: _____
No. of skin snip examined/ patient (mention No. and sites): _____.
Results of microscopic examination according to the sites of snips:
1. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
2. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
3. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
4. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
5. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____

Participant #: _____
No. of skin snip examined/ patient (mention No. and sites): _____.
Results of microscopic examination according to the sites of snips:
1. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
2. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
3. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
4. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
5. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____

Participant #: _____

No. of skin snip examined/ patient (mention No. and sites): _____.

Results of microscopic examination according to the sites of snips:

1. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
2. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
3. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
4. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
5. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____

Participant #: _____

No. of skin snip examined/ patient (mention No. and sites): _____.

Results of microscopic examination according to the sites of snips:

1. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
2. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
3. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
4. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____
5. **Site** (0, negative; 1, positive; if positive: microfilarial density):_____ _____

Note: If more participants were snipped in the surveyed household, add similar sections.