

RESEARCH ARTICLE

Onchocerca volvulus infection in Tihama region - west of Yemen: Continuing transmission in ivermectin-targeted endemic foci and unveiled endemicity in districts with previously unknown status

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Abstract

Background

Onchocerciasis in Yemen is one of the most neglected diseases, where baseline estimates of onchocerciasis and monitoring of the impact of ivermectin regularly administered to the affected individuals on its transmission are lacking. Therefore, this study aimed to determine the anti-*Ov*16 IgG4 seroprevalence among local communities of Hodeidah and Al-Mahwit governorates of Tihama region. The factors possibly associated with previous exposure to infection were also studied.

Methodology/Principal findings

This cross-sectional study was conducted in two ivermectin-targeted districts endemic for onchocerciasis in Hodeidah and Al-Mahwit and two untargeted districts with unknown previous endemicity in Hodeidah between February and July 2017. For 508 residents sampled by a multi-stage random approach, data were collected and blood specimens were screened for anti-*Ov*16 IgG4 using the SD BIOLINE Onchocerciasis IgG4 rapid tests. The study revealed an overall anti-*Ov*16 IgG4 rate of 18.5% (94/508) in all surveyed districts, with 10.2% (12/118) of children aged ≤10 years being seropositive. Moreover, rates of 8.0% (4/50) and 6.1% (4/66) were found in districts not officially listed as endemic for the disease. Multivariable analysis confirmed the age of more than ten years and residing within a large family as the independent predictors of exposure to infection.

Conclusions/Significance

Onchocerciasis transmission is still ongoing as supported by the higher anti-*Ov*16 IgG4 seroprevalence rate among children aged ≤10 years compared to that (<0.1%) previously

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set by the World Health Organization as a serologic criterion for transmission interruption. Further large-scale studies combining serologic and entomologic criteria are recommended for the mapping of *O. volvulus* in human and blackfly populations in endemic foci and their neighboring areas of uncertain endemicity. In addition, ivermectin distribution, coverage and impact on disease transmission need to be continually assessed.

Author summary

Onchocerciasis is endemic in certain foci in the western governorates of Yemen. Monitoring the impact of the regular ivermectin administration to affected individuals on the transmission status and providing baseline onchocerciasis estimates in endemic areas are crucial for planning effective elimination strategies. We found that the disease transmission is still ongoing in Hodeidah and Al-Mahwit governorates of Tihama region as indicated by the anti-*Ov*16 IgG4 seropositivity among children aged ≤ 10 years. In Bani Sa'ad, where affected individuals had been regularly targeted with ivermectin over the last 15 years, we found that the anti-*Ov*16 IgG4 seroprevalence rate was significantly lower among children aged ≤ 10 years (9.1%; 5/55) compared to those > 10 years (24.5%; 37/151), reflecting a possible decline in disease transmission. We also revealed onchocerciasis transmission in districts with unknown previous endemicity for the first time, with rates of 8.0% and 6.1% being found in Al Marawi'ah and As Sukhnah districts of Hodeidah. Large-scale surveys are recommended for mapping of *O. volvulus* in human and blackfly populations in endemic foci and neighboring untargeted areas of uncertain endemicity as a forward step towards the elimination of the disease from the country.

Introduction

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 approximately 187 million people being exposed to potential transmission [1, 2]. In addition, over a million disability-adjusted life years 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 microfilaricide ivermectin (Mectizan) through Mectizan Donation Program (MDP) in the late 1980s [4–7]. Ivermectin administration at intervals interrupts transmission and incidence of new infections with *O. volvulus* in endemic foci in the long run [8, 9]. Effective 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].

Yemen is the only country endemic for onchocerciasis in Asia, where the disease mainly affects the 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 diagnose in the laboratory by skin snip examination as a result of the scarcity of microfilariae [14, 15]. Although the epidemiology of onchocerciasis in the country

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].

In the early 1990s, ivermectin donated by the MDP was first distributed for treating the clinical manifestations of sowda in Wadi Al-Ghail, Taiz [16], where onchocerciasis had been reported to be endemic by Büttner et al. [11]. Its use at three-month intervals was then recommended as a control strategy, desirably through national campaigns [16]. Ivermectin has then been distributed to patients in a few affected communities, mainly through the National Leprosy Elimination Program in Taiz and the Charitable Society for Social Welfare (CSSW), a non-governmental organization (NGO) committed to Mectizan distribution to the affected populations since 2000. Several campaigns have been implemented in endemic areas following the approval of donating Yemen 91,000 Mectizan treatments on a quarterly basis by the Mectizan Expert Committee of the MDP [6].

The political crisis and war in the country since the Arab Spring revolutions in the region in 2011 have dashed the hope raised by the development of a national action plan in 2010 to eliminate the disease by 2015 [17]. The major mainstays adopted as part of the onchocerciasis elimination plan involve a combination of MDA with ivermectin to at-risk populations together with consolidating the clinic-based management of infected cases. In addition, the plan involves vector control and strengthening surveillance, including serologic and entomologic surveys (Ministry of Health and Population, personal communication, 2018). However, the current situation led to a number of challenges to the implementation of the elimination plan, including the insecurity, financial and logistic restraints besides the humanitarian priorities. In January 2016, however, the first MDA with ivermectin was implemented in Hodeidah and Al-Mahwit, targeting over 162,000 children and adults [18]. Although the disease is of focal nature and its baseline mapping in the targeted governorates is lacking, an ivermectin coverage rate of 94.8% has been reported in four targeted districts in the two governorates of Tihama region (Ministry of Health and Population, personal communication, 2018). It is worth mentioning, and to the best of our knowledge, that there are no published studies on the serostatus of onchocerciasis in the targeted areas of the country. Defining areas to be targeted by MDA with ivermectin and post-MDA surveys are key components to the success of the proposed elimination plan. This, in turn, highlights the importance of the present pilot study in providing a zoomed image to a part of the epidemiologic scene from its serologic attribute.

Serologic markers are now widely used to determine the recent exposure to infection with *O. volvulus* because of the possibility of their early detection in infection before skin snips become positive. In this regard, immunoglobulin G4 (IgG4) response to the Ov16 antigen expressed by the third (L3) and fourth (L4) larval stages of the parasite is the most specific marker of recent infection [19], confirming ongoing disease transmission. This marker is highly sensitive and provides evidence for recent transmission when detected among young children. Accordingly, the negativity of anti-Ov16 IgG4 has been recently used to confirm the interruption of disease transmission in foci following extensive rounds of MDA or community-directed treatment with ivermectin (CDTI) campaigns in a number of countries in Latin America and Africa [20–24].

When tested against skin microfilariasis status, a lateral flow rapid diagnostic test (RDT) for detecting anti-Ov16 IgG4 antibodies against the parasite showed sensitivity and specificity levels of 98.0% compared to levels of 94.0% and 96.0%, respectively, for enzyme-linked immunosorbent assay (ELISA) [25]. This, in turn, makes the use of RDTs for detecting anti-Ov16 in sera of children in endemic settings a useful and cost-effective tool for the long-term monitoring of disease transmission in the community following MDA campaigns [26, 27]. In 2014, the SD BIOLINE Onchocerciasis IgG4 RDT was launched as a surveillance tool for identifying

exposure to infection by detecting anti-Ov16 IgG4 [28]. It is noteworthy that the quality of such RDTs during field use has been successfully ensured with the use of recombinant human anti-Ov16 IgG4 antibody-based positive controls [29].

In line with the efforts to eliminate onchocerciasis from the country, there is a need to evaluate the status of its ongoing transmission after ivermectin distribution campaigns in endemic foci. Therefore, the present study aimed to determine anti-Ov16 IgG4 serostatus among rural residents in ivermectin-targeted endemic areas and neighboring untargeted areas with unknown endemicity in Hodeidah and Al-Mahwit governorates of Tihama region, west of Yemen. The factors possibly associated with previous exposure to *O. volvulus* infection were also studied.

Methods

Study design and setting

This cross-sectional study (S1 Checklist) was conducted in four districts in Hodeidah and Al-Mahwit in the period from February to July 2017. Hodeidah is located on the Red Sea at the coordinates of 14°48' N and 42°75' E, whereas Al-Mahwit is 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 into the Red Sea. It is well-known that the people of rural areas residing alongside these watercourses are mainly engaged in agricultural activities.

Of the four districts surveyed during the present study, two are 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, where breeding sites of the vector exist. The first CDTI campaign in Tihama region was implemented in both districts in 2016 (CSSW, personal communication, 2017). Moreover, ivermectin distribution campaigns targeting

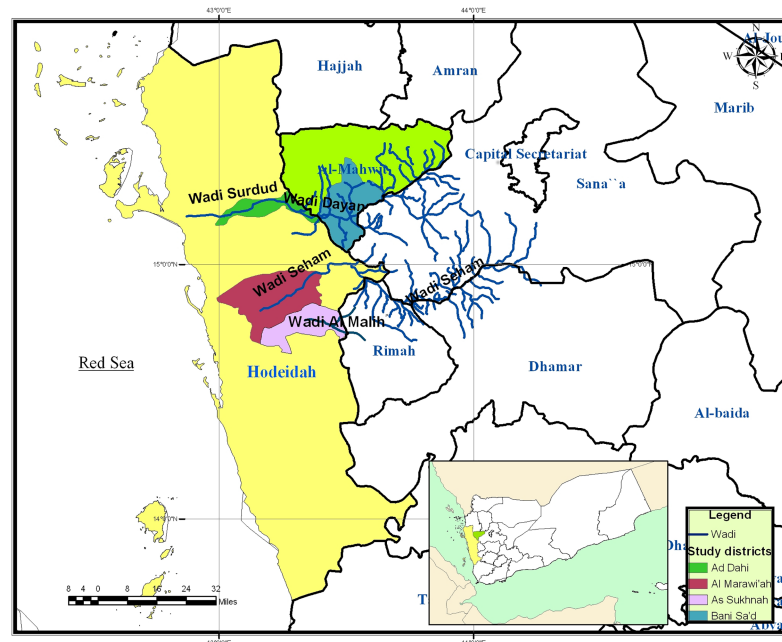


Fig 1. Map of the study area.

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symptomatic patients have been conducted three times a year since 2002 in Bani Sa'ad. Meanwhile, 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 study. Al Marawi'ah is traversed by Wadi Siham and its tributaries, while As Sukhnah is traversed by Wadi Malih, which may be potential breeding sites for the vector.

Sample size and sampling strategy

In accordance with the criteria set by the WHO for the determination of sample size in health studies [30], 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 individuals were recruited from the surveyed onchocerciasis-endemic 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. It is to be noted that cluster sampling might not always have a relationship to streams and possible breeding sites, which could yield a somewhat biased sample close to breeding sites to ensure negativity at the source of the infection. Households were then 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 to the individuals sampled from onchocerciasis-endemic districts, 116 individuals were randomly selected from Al Marawi'ah and As Sukhnah districts with unknown previous endemicity, totaling the sample size to 508.

Data collection and blood screening for anti-*Ov16* IgG4

Data on the 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. Presence of nodules and the subjective reporting of itching that usually disturbs sleeping or interferes with working capacity were recorded according to the guidelines of the WHO [31]. However, it was almost impossible to observe the nodules in body parts that are considered to be private by the participants, particularly women. Finger-prick blood was screened for anti-*Ov16* IgG4 using the SD BIOLINE Onchocerciasis IgG4 RDT (Standard Diagnostics, Inc., Gyeonggi-do, Republic of Korea) according to the manufacturer's instructions. Negative and three concentrations of positive controls (high, middle and low concentrations of anti-*Ov16* IgG4), supplied by PATH (www.path.org), were used to ensure the quality of each lot of RDTs at the points of testing in the field prior to blood screening.

Data analysis

Data were analyzed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). The socioeconomic status (SES) was determined using the principal component analysis (PCA) of durables owned by households [32]. The constructed PCA-based scores of households were divided into five wealth quintiles and three SES 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 SES, respectively [32].

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 (CIs) of the proportion of seropositive individuals were also calculated to measure the strength of association between each independent categorical variable and the anti-Ov16 IgG4 seropositivity status. 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-Ov16 IgG4 seropositivity. *P* values of < 0.05 were considered statistically significant.

Ethical statement

The study 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. In this respect, written informed consent was obtained from adults and the heads of households before recruiting their children.

Results

Of the 508 individuals screened for anti-Ov16 IgG4, the majority were females (56.7%), children aged < 11 years (23.2%), not educated (55.9%) and not working (67.5%). The median age of participants was 20 years (interquartile range: 11–38). The proportion of participants with a history of receiving ivermectin was 42.7% (217/508). Regarding the SES, individuals belonging to the high and low SES were equally distributed, representing 39.8% (156/392) of the study population each. However, 20.4% (80/392) of the participants were of the middle SES. Of the study subjects, 48.0% were living in huts or houses composed of one room (Table 1).

Seroprevalence of anti-Ov16 IgG4

The overall anti-Ov16 IgG4 seroprevalence rate was 18.5% (94/508), with a higher rate in Ad Dahi (23.7%) than Bani Sa'ad (20.4%), but there was no statistically significant difference ($\chi^2 = 0.61$, *P* = 0.435). On the other hand, lower rates of 8.0% and 6.1% were observed in Al Marawi'ah and As Sukhnah, respectively (Table 2).

Age-stratified seroprevalence of anti-Ov16 IgG4

In Bani Sa'ad, the prevalence of anti-Ov16 IgG4 among participants aged ≤ 10 years was significantly lower ($\chi^2 = 5.9$, *P* = 0.015) than among those aged > 10 years, being 9.1% and 24.5%, respectively. In contrast, no statistically significant difference ($\chi^2 = 0.27$, *P* = 0.610) was observed in the prevalence of anti-Ov16 IgG4 between the participants of the two age groups in Ad Dahi, being 20.4% and 24.4% for children aged ≤ 10 and > 10 years, respectively. With the exception of anti-Ov16 IgG4 positivity in a seven-year-old participant from Al Marawi'ah, all participants tested positive for anti-Ov16 IgG4 in Al Marawi'ah and As Sukhnah were > 10 years (Table 2).

Factors associated with anti-Ov16 IgG4 seropositivity

Bivariate analysis showed that only the age and family size were significant predictors of anti-Ov16 IgG4 seropositivity, where those aged > 10 years were at about a twice higher risk of exposure to *O. volvulus* infection than those aged ≤ 10 years (OR = 2.18; 95% CI: 1.10–4.31,

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

| Characteristic | 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 | |
| Farmer | 25 (4.9) |
| Day worker | 50 (9.8) |
| Housewife | 89 (17.5) |
| Not working | 343 (67.5) |
| SES* | |
| High | 156 (39.8) |
| Middle | 80 (20.4) |
| Low | 156 (39.8) |
| House's 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 in the year preceding the study** | |
| Yes | 217 (42.7) |
| No | 291 (57.3) |

SES, Socioeconomic status

*number of respondents was 392

**Ivermectin was not distributed in Al Marawi'ah and As Sukhnah districts.

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$P = 0.024$). In addition, participants from large families were more than twice as likely to be exposed to infection compared to those from small families (OR = 2.6; 95% CI: 1.08–6.31, $P = 0.028$). The occupations of farmers (OR = 2.93; 95% CI: 1.25–6.88, $P = 0.014$) and housewives (OR = 2.15; 95% CI: 1.16–3.96, $P = 0.015$) were significantly associated with anti-Ov16 IgG4 seropositivity. 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 = 2.69; 95% CI: 0.78–

Table 2. Age-stratified prevalence and distribution of anti-Ov16 IgG4 in Hodeidah and Al-Mahwit governorates of Tihama region, Yemen (2017).

| District (sub-districts) | Prevalence of anti-Ov16 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 |
| Ivermectin-targeted districts endemic for onchocerciasis | | | | | | |
| Bani Sa'ad | | | | | | |
| 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 |
| Total | 5/55 (9.1) | 3.0–20.0 | 37/151 (24.5) | 18.0–32.0 | 42/206 (20.4) | 15.0–27.0 |
| Ad Dahi | | | | | | |
| 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 |
| Total | 6/30 (20.4) | 7.0–52.0 | 38/156 (24.4) | 19.0–32.0 | 44/186 (23.7) | 18.0–30.0 |
| Ivermectin-untargeted districts with unknown onchocerciasis endemicity | | | | | | |
| 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 Sukhna | 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-Ov16 IgG4-positive participants; CI, confidence interval.

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9.27, $P = 0.117$), SES (OR = 1.0; 95% CI: 0.58–1.73; $P = 1.00$) 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 and/or itching (OR = 1.06; 95% CI: 0.59–1.92; $P = 0.839$) was not found to be significantly associated with anti-Ov16 IgG4 seropositivity. On the other hand, multivariable analysis further confirmed that farmers (adjusted OR = 2.67; 95% CI: 1.11–6.44, $P = 0.029$), housewives (adjusted OR = 1.92; 95% CI: 1.01–3.64, $P = 0.046$) and being a member of a large family (adjusted OR = 2.62; 95% CI: 1.07–6.45, $P = 0.063$) were the independent risk factors associated with anti-Ov16 IgG4 seropositivity among residents of endemic rural areas of Hodeidah and Al-Mahwit (Table 3).

Discussion

Onchocerciasis is focally endemic in eight governorates of Yemen. Nevertheless, 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 published in the literature. 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 [33]. In Hodeidah and Al-Mahwit, control activities have been carried out by the CSSW since 2000, mainly through the distribution of ivermectin donated by the MDP to infected individuals. The last activity was the MDA to endemic districts in Hodeidah and Al-Mahwit through involving local populations in CDTI campaigns in 2016. To the best of our knowledge, the impact of campaigns in interrupting the transmission of the parasite in targeted areas has not been assessed in Yemen.

The present study revealed an anti-Ov16 IgG4 seroprevalence rate of 18.5% among local residents of the four study districts in Hodeidah and Al-Mahwit and seropositivity among young children, providing serologic evidence for ongoing *O. volvulus* transmission following regular ivermectin distribution to infected individuals and CDTI campaigns in such districts.

Table 3. Factors associated with anti-Ov16 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 | | | | |
| Secondary and above | 29 | 3 (10.3) | Reference | |
| Primary | 152 | 33 (21.7) | 2.40 (0.69–8.44) | 0.171 |
| Non-educated | 211 | 50 (23.7) | 2.69 (0.78–9.27) | 0.117 |
| Occupation status | | | | |
| Not working | 286 | 53 (18.5) | Reference | |
| Farmer | 25 | 10 (40.0) | 2.93 (1.25–6.88) | 0.014* |
| Day worker | 20 | 3 (15.0) | 0.78 (0.22–2.74) | 0.694 |
| Housewife | 61 | 20 (32.8) | 2.15 (1.16–3.96) | 0.015* |
| SES | | | | |
| 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 and/or skin itching** | | | | |
| No | 313 | 68 (21.7) | Reference | |
| Yes | 79 | 18 (22.8) | 1.06 (0.59–1.92) | 0.839 |

N, Number of participants examined; n, number of anti-Ov16 IgG4-positive samples; OR, Odds ratio; CI, confidence interval; SES, socioeconomic status

* Confirmed as independent risk factors by multivariable analysis

** Nodules were observed with and without skin itching in 43 and two participants, respectively. The presence of skin itching only was reported by 34 participants.

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This is in contrast to the success of ivermectin MDA campaigns to interrupt onchocerciasis transmission and its elimination from a number of countries and certain endemic foci in some countries of Africa and Latin America [10, 21, 22, 34–40]. However, it could be unrealistic to compare between the successful high-coverage efforts devoted by onchocerciasis elimination programs over a long time in such countries and the low-coverage campaigns implemented by an NGO in the governorates of Tihama. Moreover, the political upheaval and wars in the country since 2011 negatively impacted the efforts of disease elimination, rescheduling the expected disease elimination from the country by 2015 [17].

The present study is the first to unveil the transmission of the infection in districts with unknown disease endemicity, where prevalence rates of 8.0% and 6.1% were observed in the districts of Al Marawi'ah and As Sukhnah. This, in turn, indicates that the disease is probably more widespread than historically and anecdotally anticipated. However, infection needs to be confirmed by a skin-snip polymerase chain reaction (PCR). In addition, serologic assessment of infection among children according to the WHO guidelines is required to assess the ongoing transmission of onchocerciasis in such new foci. PCR screening of blackfly pools for the parasite could also augment post-MDA elimination mapping in areas alongside wadis and their tributaries. The lower anti-*Ov16* 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 between 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 targeting with ivermectin MDA campaigns. Meanwhile, the high proportion (21.7%) of asymptomatic infected patients in the present study reveals that symptom-free microfilaria carriers could be a potential reservoir of infection and contribute to the ongoing transmission of the parasite in such areas, particularly with the fact that they may deny the use of ivermectin in absence of symptoms. It is worth mentioning that asymptomatic *O. volvulus* infections are not uncommon events among Yemeni patients, possibly representing a third of cases in some endemic areas [11]. In contrast, hyper-reactive sowda patients have very low-density microfilariae in the skin and usually comply with ivermectin treatment. The low rate of nodule carriers in the studied districts (11.5%; 45/392) is consistent with the published literature about the uncommon presentation of subcutaneous nodules among Yemeni patients with sowda in earlier studies [15, 41, 42]. For instance, Anderson et al. [41] reported that 14.3% (5/35) of skin snip-positive patients from the southwestern region of Yemen were nodule carriers. In addition, Büttner and Racz [42] reported that only 15.0% (16/104) of patients with onchocerciasis in Taiz were nodule carriers, with nodules being mostly observed over the calf or thigh.

The lack of baseline anti-*Ov16* IgG4 seroprevalence rates makes it difficult to accurately understand the extent to which ivermectin distribution had impacted the disease epidemiology. However, seropositivity rate among children ≤ 10 years in the studied districts confirms recent exposure and continuing transmission. It is noteworthy that anti-*Ov16* IgG4 of $< 0.1\%$ among children < 10 years old is the criterion set by the WHO to confirm the interruption of disease transmission and its elimination [33, 43].

In the present study, the significantly lower rate among children aged ≤ 10 years compared to those > 10 years (9.1% vs. 24.3%, respectively) in Bani Sa'ad raises promise regarding a partial impact of the ivermectin on onchocerciasis transmission. This could reflect the accumulative impact of the regular three-month-interval distribution of ivermectin to the affected individuals in Bani Sa'ad since 2000 prior to the last campaign in 2016. On the other hand, the single campaign in Ad Dahi in 2016 did not lead to significant changes in the prevalence of anti-*Ov16* IgG4 between children aged ≤ 10 years (20.4%) and those > 10 years (24.4%). The early start and repeated distribution of ivermectin in Bani Sa'ad could probably maintain drug coverage for the entire reproductive life span of *O. volvulus* adult worms, which may extend between 9 and 14 years [44]. It is to be noted that ivermectin is a long-acting microfilaricidal drug that has a little effect on the adult worms and, therefore, controls the disease by killing microfilariae, reducing clinical manifestations and interrupting transmission by the vector but does not cure the disease completely [45]. This, in turn, justifies for the rare exposure to *O. volvulus* among children born by the end of MDA implementation in endemic areas and the

utility of screening such children for anti-*Ov16* IgG4 as an indirect indicator for determining transmission interruption [33].

Mathematical modeling demonstrates the utility of anti-*Ov16* IgG4 seropositivity among children aged <10 years as a marker for the post-MDA interruption of onchocerciasis transmission [46]. Yearly MDAs significantly reduce human infection rates and, hence, reduce seroconversion rates in newborns and young children proportionately to the MDA duration and coverage, but adults who seroconverted before the start of MDA remain seropositive [46]. Moreover, a lower force of infection (FOI) is usually associated with younger age groups [47], where FOI is an epidemiologic measure of the rate of infection acquisition by susceptible individuals and is usually used in mathematical modeling to compare the rate of disease transmission between two different groups [48].

Continuing transmission of *O. volvulus* after MDA with ivermectin has been suggested in three Senegalese districts after more than ten years of 45–90% coverage, where the anti-*Ov16* IgG4 was prevalent among 6.9% of the population and 2.5% of five- to nine-year-old children [49]. On the other hand, the interruption of onchocerciasis transmission following years of MDA has been evidenced, among other criteria, by seronegativity or <0.1% seroprevalence of anti-*Ov16* IgG4 among children aged ≤10 years in certain foci of endemic countries, including Guatemala [33, 34], Uganda [36], Mexico [37], Sudan [21, 38], Ecuador [22], Nigeria [39] and Equatorial Guinea [40].

In Yemen, skin snip examination for sowda is challenging due to the rare presence of microfilariae that may require repeated collection and examination of skin snips [14, 41]. This was evident in the present study, where all skin snips from nodule carriers were negative for microfilariae. This is 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 [42] and the possible impact of distributed ivermectin in killing microfilariae. As a rule of thumb, however, 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 MDA campaigns [33].

In the present study, individuals aged >10 years are at a twice higher risk of exposure to *O. volvulus* infection compared to those aged ≤10 years. Repeated risk of contact with the vector could explain the higher exposure by increasing age. Moreover, the impact of ivermectin on reducing the infection exposure rate among young children and seroconversion of adults before the start of ivermectin administration could not be ruled out [46]. Although large family size was an independent risk factor of anti-*Ov16* IgG4 positivity in the endemic districts, the reason behind this association is not clear but it could be attributed to the fact that more family members are engaged in agricultural activities and spend most daytime outside houses compared to small families with younger members. The significantly higher seropositivity rates among farmers and housewives compared to those not working could be explained by the increased exposure of these population categories to the bites of blackflies. In such rural communities, farmers and housewives are usually engaged in agricultural activities in the farms along the breeding sites of the vectors for long periods during the daytime. In addition, housewives are mainly responsible for bringing water from seasonal watercourses to their homes, usually several times a day based on the amount of the water needed by their household members. It is noteworthy that the vector of *O. volvulus* in Yemen belongs to *Simulium damnosum* complex, which is a distinctive subspecies referred to as *S. rasyani* and bites outdoors during the daytime with two peaks of biting activity, in the morning until 09.00 and after 16.00 [50, 51]. It should be stressed that there is a need for a detailed study of the factors affecting the extent of human contact with blackfly populations and the intensity of exposure to infection, including the distance of human dwellings from probable breeding sites.

Although the present study is limited by adopting only *Ov16* serology as a criterion, its primary aim was to provide baseline seroprevalence of anti-*Ov16* IgG4 in endemic areas of the country because of the lack of published studies in this regard. Moreover, skin snip microscopy is not suitable for the evaluation of transmission status and is absolutely insensitive for the detection of microfilariae in case of sowda. On the other hand, it was rather difficult to meet the entomologic criterion set by the WHO [33], which recommends a minimum sample size of 6000 blackflies to determine the prevalence of infective flies by PCR. In fact, this is in accordance with the recommendations of the WHO Guideline Development Group [33], which declared that resources, cost, feasibility and acceptability should be considered when choosing the tests to be used to demonstrate the interruption of onchocerciasis transmission.

It has to be acknowledged is that there is no prior validation of RDTs in the study area against *Ov16* ELISA as a reference method, and this comes in part from the unavailability of commercial ELISA kits for this purpose. Nevertheless, the quality of RDT performance has been assured by the inclusion of serial dilutions of anti-*Ov16* IgG4 positive and negative controls supplied by PATH (www.path.org). Moreover, the feasibility of integrating the use of anti-*Ov16* IgG4 RDTs into onchocerciasis surveillance activities instead of the use of traditional skin snip microscopy has been recently demonstrated from Senegal [52]. Another issue to be considered is that the numbers of investigated cases are few when allocated over the four districts of the study. However, this could be justified by the fact the total sample size was statistically calculated to address the study objective.

In conclusion, onchocerciasis is still being transmitted in the Tihama region of Yemen despite ivermectin distribution to the affected individuals and the implementation of CDTI in 2016. This is supported by the recent exposure of children aged ≤ 10 years in the region to the parasite, who were positive for anti-*Ov16* 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. Moreover, onchocerciasis transmission has also been found in two districts not previously categorized as endemic for the disease and had never been targeted by ivermectin. Therefore, there is a need to establish valid baseline data for onchocerciasis mapping in endemic or potentially endemic areas before being targeted by ivermectin MDAs, with continuous monitoring with respect to elimination mapping. 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-*Ov16* IgG4 seroprevalence among children aged ≤ 10 years. Therefore, interruption of disease transmission and its elimination is most likely a future task if good coverage with regular ivermectin MDA campaigns is achieved and its impact on disease transmission is continually monitored and evaluated.

Supporting information

S1 Checklist. STROBE checklist.
(DOC)

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