OV16 antibody seropositivity reveals persistent onchocerciasis transmission and cross-border risk in Magwi County, South Sudan: a population-based survey

Ezbon WApary[1], Yak Yak[2], Benson Ojara[3], Wilson Ladu[2], David Oguttu[4], Ian Hennessee[5], Pita Jane[6], Johan Willems[3], Thok Chol[2], Kamulete Horasio[3], Okot William[2], Amanya Jacob[2]

Author Affiliation:

1. University of Juba, Juba, South Sudan
2. Ministry of Health, Juba, South Sudan
3. Christian Blind Mission, Juba, South Sudan
4. Ministry of Health, Kampala, Uganda
5. The Carter Center, Juba, South Sudan
6. World Health Organization, Juba, South Sudan

Correspondence:
Ezbon WApary
aulyaezbon@gmail.com

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ABSTRACT

Introduction: Onchocerciasis, or river blindness, is the second leading infectious cause of blindness after trachoma. It is a parasitic disease caused by *Onchocerca volvulus*, a filarial worm transmitted by black flies. In 34 countries, 249.5 million people are at risk, of which 14.6 million are infected, including 1.15 million people who are visually impaired or have gone blind. Onchocerciasis remains endemic in many parts of South Sudan. Magwi is an onchocerciasis-endemic county in South Sudan bordering northern Uganda districts where onchocerciasis transmission has been suppressed. The study was conducted in Magwi County to determine the prevalence of onchocerciasis by testing for *Onchocerca volvulus-16* (OV16) antibodies to estimate the risk of cross-border reintroduction of onchocerciasis into northern Uganda.

Method: The study adapted a community-based cross-sectional serological survey in Magwi County of South Sudan. The study recruited children aged 6-9 years. The study used a stratified cluster sampling approach, informed by data on population distribution and the proximity of communities to rivers infested with blackflies. Blood samples were collected using filter paper as dried blood spots. ELISA testing was done in the Uganda Onchocerciasis Reference Laboratory to confirm OV16 antibodies. Analysis software was Stata v17. The ethical standards were adhered to.

Results: 3,290 children participated in the study. 1,746 (53.1%) were males, and 6-7 years were 1,723 (52.4%). The overall OV16 seropositivity was 0.9%. Among the children, those aged 8-9 years had the highest seropositivity prevalence of 17 (1.1%). Across the clusters, transmission varied statistically ($\chi^2 = 15.23$, $p < 0.001$), with a small effect size (Cramer's V = 0.068). Whereas most clusters had no positive cases, the highest seroprevalence was in Garamu (9.6%) which followed by Malakia Central (7.6%), Olikwi (4.2%), and Kamuli (2.7%).

Conclusion: Persistent onchocerciasis transmission in Magwi County poses a risk of cross-border transmission into Northern Uganda.

Key words: OV16 antibody seropositivity, onchocerciasis transmission, cross-border, population-based survey, Magwi County, South Sudan

Introduction

Onchocerciasis, or river blindness, is the second leading infectious cause of blindness after trachoma. It is a parasitic disease caused by *Onchocerca volvulus*, a filarial worm transmitted by blackfly bites. In 34 countries, 249.5 million people are at risk of which 14.6 million are infected, 1.15 million people are visually impaired and/or have gone blind.[1] Twenty-six African countries shouldered 96% of all global cases.[2]

In South Sudan, Rapid Epidemiological Mapping of Onchocerciasis identified 48 endemic counties out of total 80 counties.[3] These areas are inhabited by communities classified as priority areas requiring Community-Directed Treatment with Ivermectin (CDTI), areas not requiring treatment, and possible endemic areas that need further investigation. The main endemic foci are in Western Bahr El-Ghazal, Western Equatoria, Central Equatoria, and Lakes states. In Unity State, the prevalence was <20%. Despite the country shifting focus from control to elimination, Mass Drug Administration (MDA) with Ivermectin was carried out in 2022 in all the 48 identified endemic counties for onchocerciasis, of which 34 were treated for onchocerciasis with >5 effective rounds of MDA (≥80% coverage) and lymphatic filariasis/Oncho-LF (co-endemic), while 14 were treated for onchocerciasis only.[3]

Onchocerciasis MDA has been implemented since 2005. The aim is to achieve≥80% ivermectin coverage. The target set for 2027 on onchocerciasis is that 30% of counties should have suppressed transmission. The percentage of counties with suppressed transmission in 2023 was 0% and was used as the baseline; in 2025, it was 20% as a midterm.[3] Magwi County was selected for an onchocerciasis prevalence survey due to its proximity to northern Uganda districts where the transmission is suppressed. Additionally, population movements, farming near rivers, and environmental disruption maintain exposure to blackflies.

The *Onchocerca volvulus*-16 (OV16) antibody, specifically the human IgG4 subclass, is a serological biomarker used to detect exposure to *Onchocerca volvulus.* Monitoring this antibody is a critical component of elimination programs as it serves as an early marker of infection, often detectable months before the parasite's larvae (microfilariae) appear in the skin.[4]

The study was conducted in Magwi County to monitor the prevalence of onchocerciasis by testing for OV16 antibody to estimate the risk of cross-border reintroduction of onchocerciasis into northern Uganda.

Method

The study adapted a community-based cross-sectional serological survey design utilising a stratified cluster sampling technique.[5] The survey covered 32 clusters in Magwi County (Figure 1), selected based on proximity to Simulium breeding sites. The breeding sites were identified by walking along the rivers, locating fast-flowing sections with suitable submerged or trailing vegetation.

The survey included children aged 6-9 years, as they serve as a core indicator of recent transmission; resided in these clusters for at least 5 years; had guardians' or parents' consent; and assented. The exclusion criteria were individuals with temporary or recent (<5 years) residence in endemic areas; individuals with bleeding disorders or medical contraindications to finger-prick sampling.

The study engaged with community leaders and sensitisation on the survey objectives was carried out. Initial meetings were with community leaders and local government. Verbal and written sensitisation was done to inform communities about the survey. The study recruited children from house-to-house who met the inclusion criteria, recorded their demographic data, pricked their fingers to collect blood samples on filter paper, safely discarded sharps into safety boxes, and disposed daily other biohazardous waste in the nearest health facility's incinerators. Anti-OV16 ELISA testing was done in the Uganda Onchocerciasis Reference Laboratory.

The survey teams were trained on the study protocol with emphasis on community entry, sensitisation technique, ethical guidelines, tools for data collection, DBS sampling procedures, and sample handling. Indoor and outdoor practicums were conducted prior to the survey's commencement to ensure the quality of the survey team members.

Ethical consideration

The ethical standards were adhered to, with reference number RERB/A/75/2025 obtained from the Research and Ethical Review Board, Ministry of Health, South Sudan. Written consent was obtained from the responsible party, with assent from minors (children). As the names were de-identified, the participant codes and sample codes were the same for each participant.

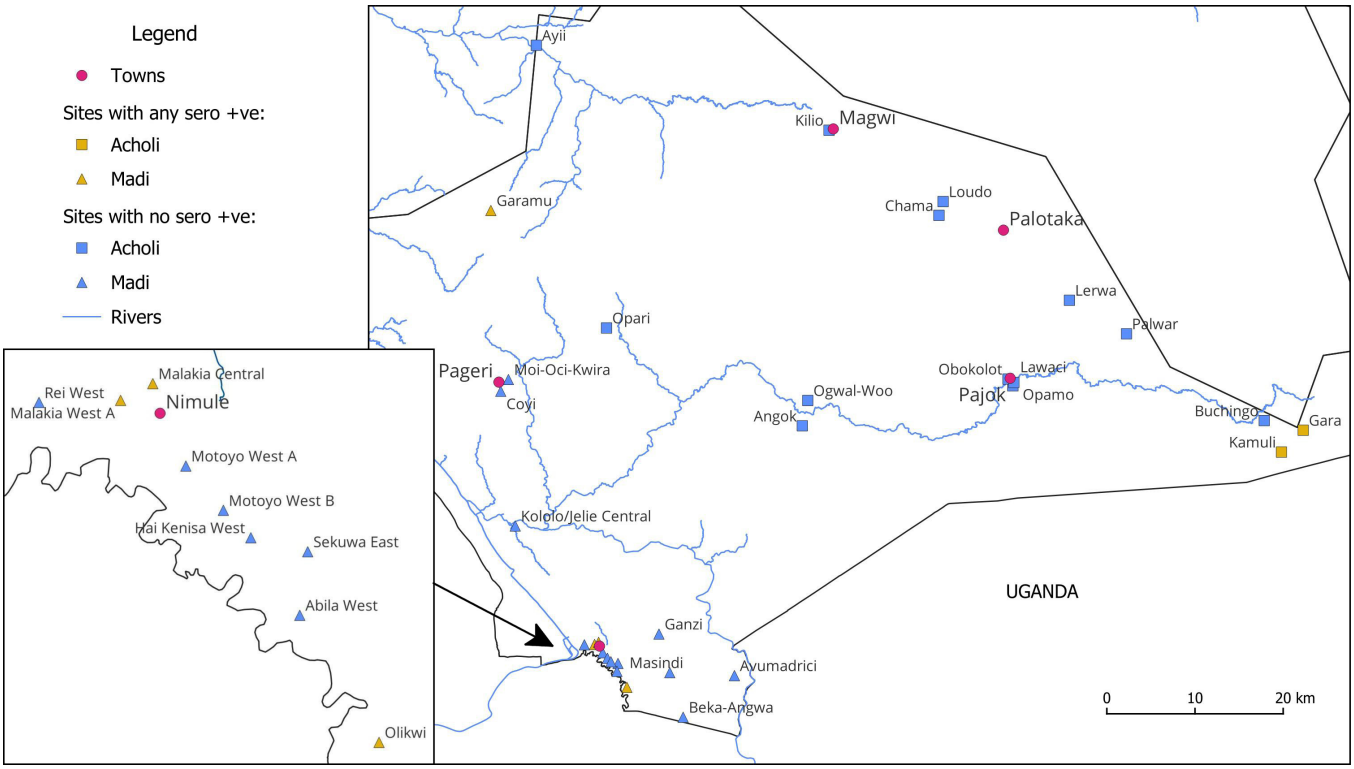

Figure 1. Location of sites within Magwi county, Eastern Equatoria State. Background data from the Humanitarian Data Exchange (https://data.humdata.org).

Data analysis

Field data were gathered using ESPEN Collect installed on mobile devices. Data collected included coded informed consent and demographic characteristics, and were submitted daily into the ESPEN-hosted server. The data were exported into Stata v17 software for Analysis. Descriptive statistics summarised participant characteristics, including age, sex, and cluster distribution. Seroprevalence of OV16 antibodies was calculated as proportions with corresponding percentages. Bivariate analysis was conducted to assess associations between OV16 seropositivity and categorical variables (age group, sex, and geographic clusters) using the chi-squared (χ^2) test. Effect sizes were estimated using Cramer's V to assess the strength of associations. Cluster-level seroprevalence was analysed to identify spatial heterogeneity and potential transmission hotspots. Statistical significance was set at $p<0.05$. Results were presented in tables and interpreted in the context of onchocerciasis elimination thresholds.

Results

Of the 3,290 children who participated in the study, males comprised 53.1%, 6-year-olds comprised 33.3%, and 42.1% were from Nimule Payam. The overall OV16 seropositivity was 0.9%. Among the children, those aged 8-9 years had the highest seroprevalence of 1.1%. Age and sex showed no statistical significance, indicating even exposure to the transmission. The effect size (Cammer's V) further confirms the absence of a strong relationship (Table 1).

Children residing in the Madi Corridor clusters had a higher seropositivity of 1.5% compared to 0.02% in the Acholi Corridor (Table 1). The highest seroprevalence was in Garamu (9.6%), followed by Malakia Central (7.6%), Olikwi (4.2%), and Kamuli (2.7%) (Table 2).

Discussion

Analysis of the ELISA findings revealed that 0.9%of the children aged 6-9 years tested positive for OV16 antibodies. This low seroprevalence aligns with results from similar surveys in hypo-endemic settings, indicating limited ongoing transmission of *Onchocerca volvulus* in the county, or, rather, in South Sudan.[6,7] The 0.9%

Table 1. Seropositivity of onchocerciasis in children by age, sex and location

	ELISA Test Results			Chi-squared	Cramer's V
	- ve n (%)	+ ve n (%)	Total n (%)	(p-value)	
Age in years				0.99 (0.319)	0.017
6-7	1710 (99.2)	13 (0.8)	1723 (52.4)		
8-9	1550 (98.9)	17 (1.1)	1567 (47.6)		
Sex				0.01 (0.977)	0.001
Male	1730 (99.1)	16 (0.9)	1746 (53.1)		
Female	1530 (99.1)	14 (0.9)	1544 (46.9)		
Clusters				15.23 (<.001)	0.068
Acholi Corridor	1601 (99.8))	4 (0.2)	1605 (48.8)		
Madi Corridor	1659 (98.5)	26 (1.5)	1685 (51.2)		
Total	3260 (99.1)	30 (0.9)	3290 (100)		

OV16 seropositivity exceeds the 0.1% WHO threshold for stopping MDA with ivermectin and indicates ongoing transmission in Magwi County.[5] Therefore, CDTI should continue in Magwi County and, where appropriate, be intensified, especially in identified hotspots, to prevent cross-border transmission into northern Uganda.

Children aged nine years exhibited the highest seropositivity compared to those aged 6-8 years. Despite this trend, the results showed no statistically significant differences, implying that age may not be a strong predictor of exposure in this population.[8]

Even though overall seropositivity is minimal, the detection of seropositive cases among these age groups reflects residual pockets of transmission. In East African foci, studies have revealed that low seropositivity can continue post-ivermectin MDA, necessitating targeted follow-up interventions.[9,10] The OV16 ELISA remains a sensitive and specific tool for detecting antibodies in these children, providing vital data to inform onchocerciasis elimination efforts.[11,12]

Seropositivity was homogeneously low across these age groups, extending from 0.4% to 1.4%. Similarly, the effects of age have been weak or absent in some endemic settings when analysis inquiry is restricted to a limited age group, whereas broader age ranges in adult populations, such as livestock or humans, show more often strong age gradients in seropositivity.[13,14]

Male and female children had similar seroprevalence (0.9%) with no statistically detectable association between ELISA status and sex. This contrasts with many occupationally mediated or sexually transmitted infections, where men often show higher seroprevalence or clustering,[13,15,16] and with brucellosis in cattle in South Sudan, where females had substantially greater likelihood of seropositivity than males.[14] The absence of variation suggests that exposure is likely transmitted through shared household or environmental factors rather than through sex-related behaviours.

Clusters were observed as strong determinants of transmission homogeneity (e.g. Garamu, Malakia Central, Malakia West A, Olikwi) showed seroprevalence between 3.1% and 9.6%. The higher seropositivity in the Madi Corridor may indicate favourable localised ecological factors for vector breeding or transmission. This is analogous to riverside villages in Cameroon, Nigeria, and Ethiopia, where higher biting and infection are documented.[17–20] This pattern is consistent with focal "hotspots" of transmission embedded in a largely low-risk landscape, as seen for HIV, brucellosis, and other infections where particular communities or locations account for a disproportionate share of ongoing transmission.[13–16] In such settings, spatially targeted interventions in high-prevalence clusters may be more efficient than uniform county-wide measures.

Several limitations were considered. OV-16 antibodies indicate exposure; however, they may persist after treatment or infection. Entomological investigations were not concurrently conducted. This limits direct inference about the intensity of the transmission. Mobility of population data may also affect interpretation. Despite these limitations, the significance of seroprevalence strongly indicates that transmission of onchocerciasis has not yet been interrupted.

Conclusion

Persistent transmission of onchocerciasis in Magwi

Table 2 Seropositivity of onchocerciasis in children by clusters within Magwi county

	ELISA Test Results		
	- ve n (%)	+ ve n (%)	Total n (%)
Acholi Corridor Clusters			
Angok	110 (100)	0 (0)	110 (3.3)
Ayii	109 (100)	0 (0)	109 (3.3)
Buchingo	111 (100)	0 (0)	111 (3.4)
Chama	110 (100)	0 (0)	110 (3.3)
Gara	109 (99.1)	1 (0.9)	110 (3.3)
Kamuli	107 (97.3)	3 (2.7)	110 (3.3)
Kilio	110 (100)	0 (0)	110 (3.3)
Lawaci	112 (100)	0 (0)	112 (3.4)
Lerwa	111 (100)	0 (0)	111 (3.4)
Loudo	110 (100)	0 (0)	110 (3.3)
Obokolot	110 (100)	0 (0)	110 (3.3)
Ogwal-Woo	110 (100)	0 (0)	110 (3.3)
Opamo	110 (100)	0 (0)	110 (3.3)
Opari	62 (100)	0 (0)	62 (1.9)
Palwar	110 (100)	0 (0)	110 (3.3)
Madi Corridor Clusters			
Abila West	123 (100)	0 (0)	123 (3.7)
Avumadrici	42 (100)	0 (0)	42 (1.3)
Beka-Angwa	64 (100)	0 (0)	64 (1.9)
Coyi	33 (100)	0 (0)	33 (1)
Ganzi	52 (100)	0 (0)	52 (1.6)
Garamu	75 (90.4)	8 (9.6)	83 (2.5)
Hai Kenisa West	138 (100)	0 (0)	138 (4.2)
Kololo/Jelie Central	99 (100)	0 (0)	99 (3)
Malakia Central	109 (92.4)	9 (7.6)	118 (3.6)
Malakia West A	125 (96.9)	4 (3.1)	129 (3.9)
Masindi	56 (100)	0 (0)	56 (1.7)
Moi-Oci-Kwira	34 (100)	0 (0)	34 (1)
Motoyo West A	148 (100)	0 (0)	148 (4.5)
Motoyo West B	149 (100)	0 (0)	149 (4.5)
Olikwi	113 (95.8)	5 (4.2)	118 (3.6)
Rei West	150 (100)	0 (0)	150 (4.6)
Sekuwa East	149 (100)	0 (0)	149 (4.5)
Total	3260 (99.1)	30 (0.9)	3290 (100)

County, South Sudan, poses a risk of cross-border transmission into Northern Uganda districts. The study underscores the importance of shifting programmatic interventions from broad, uniform approaches toward more specific, spatially updated strategies that address focal transmission, thereby consolidating gains accomplished and preventing resurgence in high-risk clusters.

Conflict of interest: None

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