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RESEARCH ARTICLE

REVISED Rapid diagnostic testing for onchocerciasis in Maridi (South Sudan) before and after improving elimination strategies: a repeated cross-sectional survey

[version 2; peer review: 2 approved, 1 approved with reservations]

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Abstract

Background

Maridi County is an onchocerciasis-endemic area in South Sudan. Annual community-directed treatment with ivermectin (CDTi) was instituted in Maridi since the early 2000s, but with low coverage. In 2021, the CDTi programme was strengthened to a six-monthly programme. Additionally, the community-based vector control strategy "Slash and Clear" has been implemented since 2019 at the Maridi Dam, the only blackfly breeding site in the area. This study assessed the effect of these reinforced onchocerciasis elimination interventions on the *Onchocerca volvulus* seroprevalence among young children, an indicator of ongoing transmission.

Methods

Baseline and follow-up serosurveys were conducted in Maridi in 2019 (prior to strengthening onchocerciasis elimination efforts) and 2023, respectively. During both surveys, children aged three to nine years were recruited from five study sites situated at different distances from the Maridi Dam. Ov16 antibodies were detected via rapid diagnostic tests (RDTs) using whole blood obtained by finger-pricking the participants. Baseline and follow-up Ov16 prevalence rates were calculated and compared.

Open Peer Review

Approval Status ? ✓ ✓

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Results

In 2019, the Ov16 seroprevalence among children aged three to nine years was 24.5% compared to 30.6% in 2023 (p=0.22). Both surveys found a particularly high Ov16 seroprevalence in the study site closest to the Maridi Dam (35.0% in 2019 and 44.0% in 2023, p=0.52). The Ov16 seroprevalence had a non-significant decreasing trend in the three-year-old children, from 12.5% (3/24) in 2019 to 8.8% (3/34) in 2023 (p=0.65).

Conclusion

The persistent Ov16 RDT seropositivity among three-year-old children in 2023 indicates ongoing *O. volvulus* transmission. Therefore, further strengthening of the onchocerciasis elimination programme is required. The study highlights the utility of RDTs in monitoring onchocerciasis transmission in highly endemic settings.

Keywords
Onchocerciasis, Onchocerca volvulus, antibodies, transmission, testing, seroprevalence, children, ivermectin, vector control

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REVISED **Amendments from Version 1**

As a result of the comments of the reviewers, we have revised our manuscript. We have chosen for a more streamlined way to present numbers and percentages throughout the document. Resulting in a changed lay-out in Table 1 and Table 2. Additionally, we included specifics on the WHO recommendations regarding *O. volvulus* diagnosis using Ov16 based rapid diagnostic tests and Enzyme-linked immunosorbent Assay (ELISA). More information was added on the study site and Onchocerciasis control interventions performed in South Sudan. Including past studies on epilepsy and Onchocerciasis prevalence and the prevalence in relation to the Maridi Dam. Furthermore, we added background information on the occurrence of skin disease in the area.
Finally, we refined our discussion by referring to a modelling study presenting an initial rise in prevalence after vector control. Providing an additional potential explanation on the increased seroprevalence in the three to nine years old age category in Maridi, South Sudan.

Any further responses from the reviewers can be found at the end of the article

Introduction

Onchocerciasis is a neglected tropical disease primarily found in Africa, caused by the filarial nematode *Onchocerca volvulus* and transmitted through bites of infectious blackflies (*Simulium* spp.)[1]. Onchocerciasis is known to cause dermatitis and blindness ("river blindness") and is associated with a clinical spectrum of seizures and disorders grouped under the termonchocerciasis-associated epilepsy (OAE)[2]. In areas where there is high ongoing *O. volvulus* transmission, children are at risk of developing OAE, with seizure onset typically occurring between the ages of three to 18 years[2]. Elimination of onchocerciasis depends mainly on community-directed treatment with ivermectin (CDTi) in at-risk communities, which can be supplemented with activities to eradicate the blackflies[3].

Onchocerciasis was first reported in Sudan in 1947 by Kirk *et al.* Ivermectin distribution started in South Sudan in 1996 through the Southern Sudan Onchocerciasis Control Programme, but annual CDTi was only formally initiated in 2005[4,5]. Moreover, there have been several years of low coverage treatment due to insecurity, and community drug distributors' high attrition rate, which, in combination with high onchocerciasis transmission potential, has allowed the disease to remain endemic most of the country[4–6]. Although CDTi was interrupted in 2020 because of the COVID-19 pandemic, it has been re-introduced biannually (six-monthly) since 2021.

Currently, the World Health Organisation (WHO) recommends using serodiagnostic tools based on the detection of antibodies specific to a 16KDa species-specific antigen of *O. volvulus* (Ov16), utilising either a rapid diagnostic test or an Enzyme-Linked Immunosorbent Assay (ELISA) platform[7]. The antigen Ov16 serves as an early marker of onchocerciasis infection, so seroconversion to Ov16 can be used to detect pre-patent infection, which is not possible with skin snips[8]. Since its discovery in 1991, the use of Ov16 has emerged as the gold standard for onchocerciasis serology, with sensitivities in the 80–89% range and specificity values in the 97–99% range depending on the assay used and the sample collection assessed[9,10].

In 2018, a house-to-house survey in Maridi County, an onchocerciasis-endemic area in Western Equatoria State, South Sudan, showed a very high epilepsy prevalence of 43.8 per 1000 individuals[11]. Notably, in Kazana 2, the site closest to the major blackfly breeding site in the area (Maridi Dam), an epilepsy prevalence of 119.0 per 1000 individuals was noted[11]. In December 2019, onchocerciasis transmission was assessed in Maridi in three sites at different distances from the Maridi Dam via an Ov16 serosurvey in children three to nine years of age. The overall seroprevalence of the children was 24.5%[12]. As these findings indicated high onchocerciasis transmission, a community-based vector control strategy, "Slash and Clear", has been implemented at least once a year at the Maridi Dam since December 2019 (Figure 1)[13]. This strategy consists of clearing the trailing vegetation to eliminate the blackfly breeding site and reduce biting density in the nearby communities.

In February 2023, a follow-up *O. volvulus* serosurvey was conducted among children aged three to nine years in Maridi to evaluate possible changes in transmission patterns. This paper presented the effect of strengthening the onchocerciasis elimination interventions as demonstrated by seroprevalence in children aged three to nine years using the Ov16 SD BIOLINE rapid diagnostic test (RDT).

Methods

Ethical standards

Ethical approval for this study was obtained from the University of Antwerp's ethics committee (Ref: BUN B300201940004) and from the ethics committee of the Ministry of Health of South Sudan (MOH/RERB 56/2022). All participants were



Figure 1. Slash-and-Clear at the Maridi Dam, South Sudan.

recruited only after informed parental consent was granted (and assent for children aged seven years and above). The research procedures were in compliance with the declaration Helsinki, and all personal data were treated confidentially.

Study setting
South Sudan is known to have multiple endemic hotspots of onchocerciasis, including Maridi County in the Western Equatoria State (Figure 2).

Maridi County is home to over 115,000 individuals[14] and is crossed by the Maridi River, upon which a dam was built in the 1950s. The dam spillway was identified as the sole blackfly breeding site in Maridi[12], driving onchocerciasis transmission in the neighbouring villages. This study was conducted between 2019 (baseline) and 2023 (follow-up) in five villages, of which Kazana 1, Kazana 2, and Hai-Matara are situated in the proximity of the dam (high-transmission zone; HTZ) and Hai-Tarawa and Hai-Gabat are located further away from the dam (low-transmission zone; LTZ) (Figure 3).

Study procedures
A cross-sectional study was conducted before and after strengthening the control interventions against onchocerciasis. The procedures of the baseline survey (2019) have been described in detail previously[12]. Several weeks before the follow-up survey in 2023, the research team contacted the community leaders to inform them about the study and obtain their consent and collaboration. Five local healthcare workers were trained to perform the Ov16 SD BIOLINE RDT and administer a questionnaire to the parents of the children. One day before participant recruitment, community mobilisers notified villagers about the study and of the site(s) chosen in each village (schools, churches, etc.) to test the eligible children (three to nine years old). On the day of the study in each study site, the mobilisers used megaphones, and healthcare workers went from house to house to gather more participants. All children for whom informed consent was provided by a parent/guardian were enrolled in the study (assent was obtained from children aged seven to nine years).



Figure 2. Location of Maridi County in Western-Equatorian State, South Sudan (produced in Scribble Maps).

A short questionnaire was administered to the parent/guardian and the child to collect relevant information about the child's history (age, sex, village of residence, previous ivermectin intake, itching/skin lesions, epilepsy). Thereafter, all the participating children were finger-pricked with a single-use, retractable lancet of 2.00 millimetres (Ergo lance). Whole blood from each finger prick was used for Ov16 rapid diagnostic testing (RDT) (SD Bioline, Inc, Gyeonggi-do, South Korea). The Ov16 RDT test was read after 30 minutes and performed in the field following the manufacturers' instructions.

Data analysis
Study data was checked daily and uploaded to the REDCap secure online platform. The final datasets were exported from REDCap, cleaned in Excel spreadsheets, then transferred to the R software version 4.2.2 for analysis. Continuous variables were summarised as median with interquartile range (IQR), while categorical variables were expressed as percentages. The findings of the baseline (2019)[12] and follow-up (2023) surveys were compared using the Chi-squared test (or Fisher's exact test when appropriate), and 95% confidence intervals (CIs) were produced using the Wilson score with continuity correction.

Results
Ov16 seroprevalence in Maridi 2023
During the 2023 *O. volvulus* seroprevalence study in Maridi, 248 children aged three to nine years were recruited. The median age was six years (IQR: 4–8), and 114 were males (46.0%). Ninety-three of the participating children (37.5%) had some form of dermatitis, as evidenced by itching and/or visible skin lesions. Furthermore, four children (1.6%) were identified as having epilepsy.

The overall Ov16 RDT seroprevalence was 30.7% (76/248). The seroprevalence was significantly different across villages ($p<0.001$), with Kazana 1 and Kazana 2 being the most affected and Hai-Gabat and Hai-Tarawa less affected (Figure 4).

The Ov16 RDT seroprevalence was similar for males and females ($p=0.98$) and did not vary significantly across the younger (30.7% seropositivity among children aged <7 years) and older (30.5% seropositivity among aged ≥7 years) age-groups ($p=0.78$) (Table 1). However, the 3-year-olds, had lower Ov16 seropositivity: 8.8% (3/34) versus 34.1% (73/214) in the older children; $p<0.001$.

Children with dermatitis had a higher Ov16 RDT seroprevalence (43.5%, 40/92) compared to their peers without dermatitis (22.6%, 35/155); $p< 0.001$. Of the four children with epilepsy, none tested positive for Ov16 antibodies.

Ov16 seroprevalence in Maridi over time
Comparing baseline and follow-up Ov16 RDT results, the overall Ov16 seroprevalence increased non-significantly from

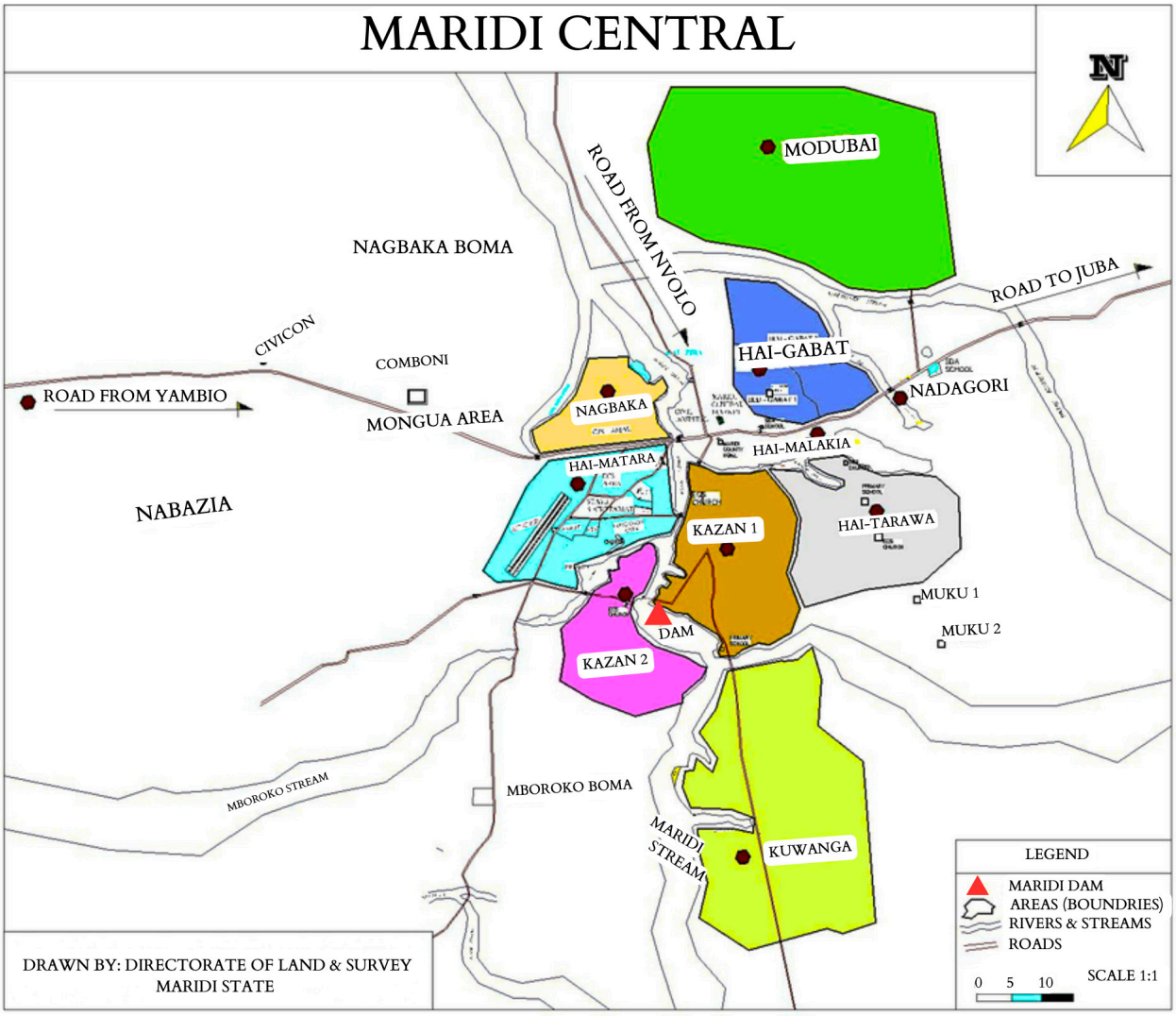


Figure 3. The Maridi central area with the location of high- (Kazana 1 and 2 and Hai-Matara sites) and low-onchocerciasis transmission zones (Hai-Gabat and Hai-Tarawa sites) and the Maridi Dam (adapted from Colebunders *et al.*[11]).

24.3% in 2019 to 30.6% in 2023 (p=0.22) (Figure 5). The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, was measured at 8% in 2023 and was thus lower than the level of 12.5% measured among three-year-olds in 2019. However, the difference was not significant (p=0.68). Conversely, the Ov16 seropositivity rose non-significantly from 26.7% in 2019 to 34.1% in 2023 among children aged four years and above (p=0.16). Transmission zone-specific Ov16 seroprevalence did not significantly change across surveys (HTZ: p= 0.70; LTZ: p= 0.18; Table 2).

Discussion

In 2023, an Ov16 seroprevalence of 30.7% was found among children aged three to nine years in Maridi, representing a non-significant increase from the 24.3% seroprevalence documented in 2019[12] (p=0.98). As the elimination measures were only introduced three years ago, children between the ages of four and nine years in the 2023 cohort had already been highly exposed to onchocerciasis before strengthening the control interventions. An increase in the seroprevalence in the age group of three to nine years old children was to be expected, particularly because of the very high pre-intervention endemicity[15]. Indeed, some of the children might harbour prepatent infection before the start of the intervention, which may break out in the course of the intervention. A force-of-infection model for onchocerciasis has shown that upon interrupting *O. volvulus* transmission by vector control, the microfilarial prevalence of the zero to four and five to nine year old age groups continues to rise during the first five years, after which it starts falling[16].

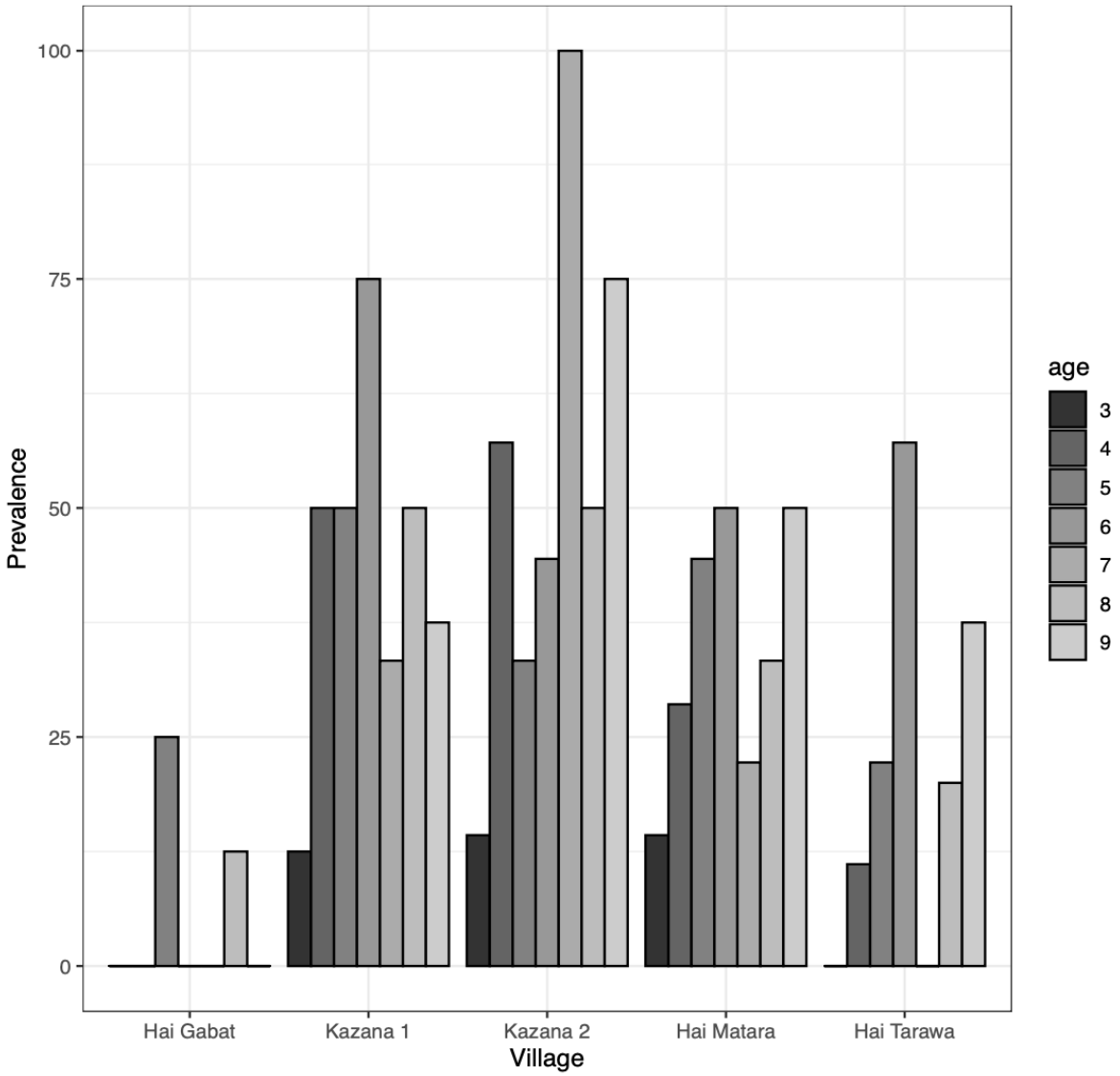


Figure 4. Ov16 rapid diagnostic test seroprevalence in 2023 per study site and age (years old).

The fact that the Ov16 seroprevalence among the three-year-olds did not decrease significantly may also be related to the small sample size, short follow-up duration since the strengthening of the control interventions and the lack of CDTi in 2021. Indeed, mathematical modelling has suggested that a one-year CDTi interruption may significantly impact onchocerciasis elimination prospects in settings such as Maridi, with short CDTi histories and high onchocerciasis endemicity[17,18]. The one-year CDTi interruption in 2020 and the consistently low CDTi coverage rates achieved in Maridi (40.8% in 2017[11] and 56.6% in 2021[19]) explain why some three-year-old children became infected with *O. volvulus*. Achieving a CDTi coverage of at least 80% of the total population is strongly recommended for the successful elimination of onchocerciasis transmission, particularly in areas with a high prevalence of the disease[20].

The high Ov16 seroprevalence among young children in Maridi reveals that the onchocerciasis elimination measures must be further strengthened to protect children from developing onchocerciasis-associated morbidities. Over one-third (37.5%) of the children had some form of dermatitis, as evidenced by itching and/or visible skin lesions. A significantly higher proportion of children with dermatitis tested Ov16 RDT positive (43.5%) compared to their peers without dermatitis (22.6%, $p<0.001$), suggesting the dermatitis was likely caused by an active *O. volvulus* infection in a large proportion of children. In resource-constrained settings such as many parts of sub-Saharan Africa, when children present with dermatitis and itching, scabies should be considered as a possible diagnosis. However, the absence of characteristic scabies lesions was noted in the children included in the study. Additionally, since scabies is treatable with ivermectin, its

Table 1. **Ov16 rapid diagnostic test seroprevalence in 2023 by age group and sex across included villages.**

	Ov16 RDT seroprevalence %(Ratio, 95%CI)					Comparisons	
Village	**Hai-Gabat**	**Kazana 1**	**Kazana 2**	**Hai-Matara**	**Hai-Tarawa**	**Overall**	***p*-value***
Age-groups							
3–6 years	3.4 (1/29, 0.2-19.6)	46.7 (14/30, 28.8-65.4)	43.8 (14/32, 26.0-60.6)	35.5 (11/31, 19.8-54.6)	7/30 (23.3, 10.6-42.7)	30.7 (47/153, 23.7-38.8)	0.98
7–9 years	4.8 (1/21, 0.2-25.9)	8/20 (40.0, 20.0-63.6)	66.7 (8/12, 38.9-89.6)	33.3 (7/21, 15.5-57.0)	4/20 (20.0, 0.7-44.3)	30.5 (29/95, 21.7-40.9)	
Sex							
Male	3.6 (1/28, 0.2-20.2)	8/16 (50.0, 28.0-72.0)	55.0 (11/20, 32.0-76.2)	38.9 (7/18, 18.3-64.0)	6/31 (19.4, 8.1-38.1)	29.2 (33/113, 21.2-38.6)	0.78
Female	4.5 (1/22, 0.2-24.9)	14/34 (42.2, 25.2-59.2)	46.6 (12/26, 27.1-66.3)	32.4 (11/34, 18.0-50.6)	5/19 (26.3, 10.1-51.4)	31.9 (43/135, 24.3-40.5)	
Overall	30.6 (76/248, 25.1-36.9)						

* All p-values were obtained with a Chi-square test/Fisher's exact test. CI – confidence interval.

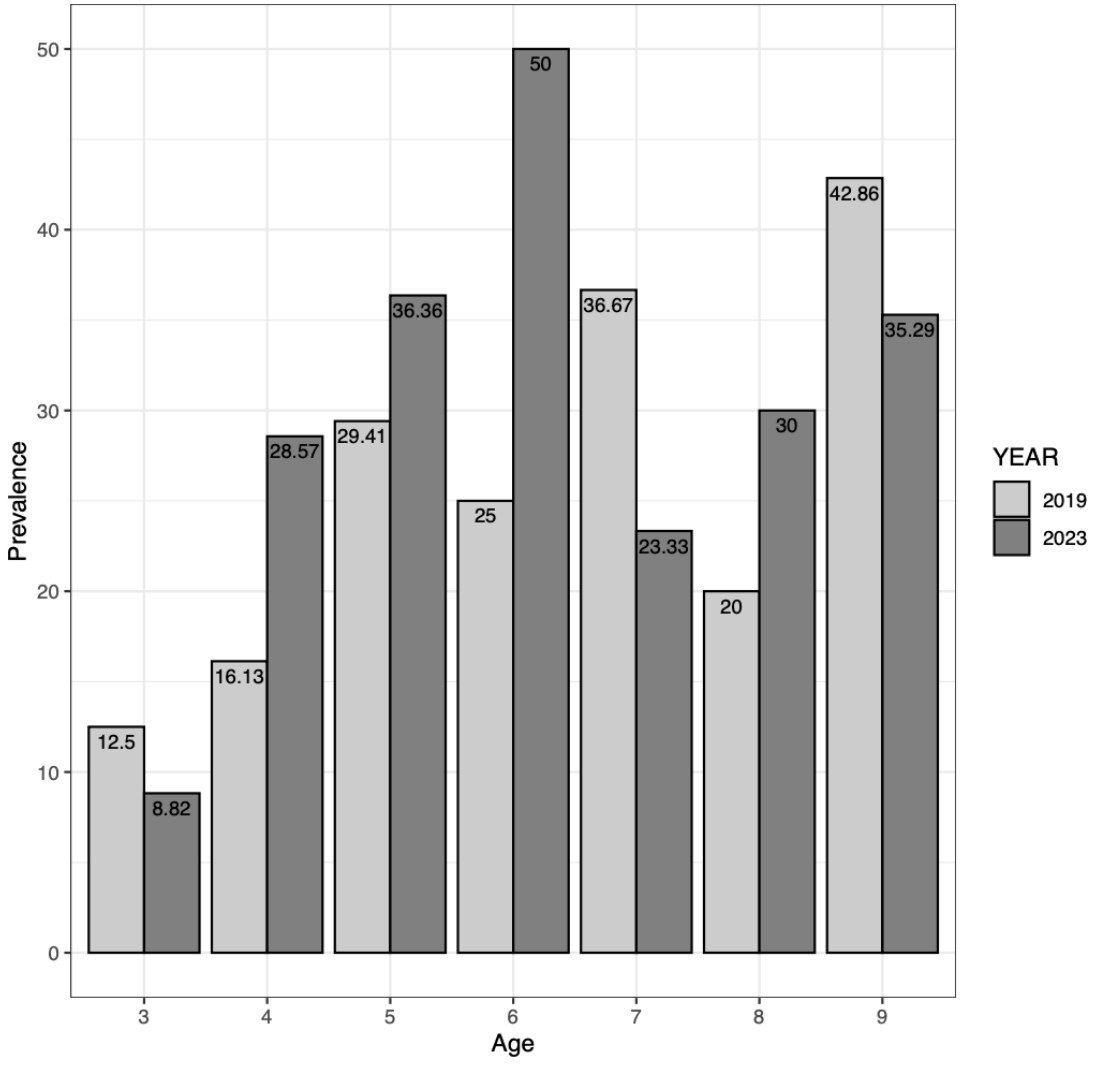


Figure 5. **Ov16 rapid diagnostic test seroprevalence (%) by age and year surveyed (2019 and 2023).**

Table 2. Ov16 rapid diagnostic test seroprevalence among three- to nine-year-olds per survey (2019/2023) and village.

	2019 seroprevalence %(Ratio, 95%CI)	2023 seroprevalence %(Ratio, 95%CI)	*p*-value*
Village			
Hai-Gabat	0.0 (0/24, 0.0-17.2)	4.2 (2/48, 0.7-14.9)	N.A.
Kazana 1	35.0 (14/40, 21.1-51.7)	44.0 (22/50, 30.3-58.7)	0.52
Kazana 2	50.0 (12/24, 31.4-68.6)	50.0 (23/46, 36.1-63.9)	1.00
Hai-Matara	14.8 (4/27, 4.9-34.6)	34.6 (18/52, 22.3-49.2)	N.A.
Hai-Tarawa	17.2 (5/29, 6.5-36.5)	22.0 (11/50, 12.0-36.3)	0.83
Transmission zones			
Low-transmission zone	9.4 (5/53, 3.5-21.4)	13.0 (13/100, 7.4-21.6)	0.70
High-transmission zone	33.0 (30/91, 23.7-43.7)	42.6 (63/148, 34.6-51.0)	0.18
Overall	24.3 (35/144, 17.7-32.3)	30.7 (76/248, 25.1-36.9)	0.22

* All p-values were obtained with a Chi-square test/Fisher's exact test. N.A.: not applicable due to a too-rare event; CI – confidence interval.

prevalence typically decreases in regions where mass ivermectin distribution is implemented[21].

This study demonstrates the feasibility of using rapid diagnostic tests for evaluating onchocerciasis transmission in highly endemic settings. Until recently, field Ov16 RDTs were overlooked in monitoring onchocerciasis transmission in endemic foci since the gold standard technique recommended by the World Health Organization (WHO) was Ov16 enzyme-linked immunosorbent assay (ELISA)[22]. However, in practice, there are logistical challenges to performing Ov16 ELISA (need for a cold chain, specialised equipment, and capacity), particularly in remote sites like Maridi. Meanwhile, studies in Cameroon[23] and the Democratic Republic of Congo[24] have found that Ov16 RDT done in the field was helpful in investigating onchocerciasis transmission patterns. Therefore, it is worth considering the widespread use of RDTs as a point-of-care approach for monitoring onchocerciasis in resource-limited sites.

Our study had some limitations. A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested, and a longer follow-up is needed to investigate effect of strengthening an onchocerciasis elimination programme. Moreover, in the absence of skin snips for microfilariae quantification and blackfly infection rates, it is difficult to obtain precise estimates of the intensity of *O. volvulus* transmission in Maridi. Further research may be required to better understand the Ov16 antibodies dynamics during and after *O. volvulus* infection. Strengthening the onchocerciasis elimination program certainly decreased the *O. volvulus* transmission in Maridi. This was suggested by the results of a recent longitudinal population-based study that showed that the incidence of onchocerciasis-associated epilepsy, including nodding syndrome, considerably decreased in Maridi between 2018 and 2022[25].

In conclusion, using Ov16 RDT, we were were able to demonstrate that Maridi remains a hotspot for *O. volvulus* transmission even after the implementation of elimination measures. These measures should be continued and strengthened even further, including increasing the CDTi coverage to protect children from developing OAE and to move towards the eliminationgoals of WHO in 2030.

Data availability

EUDAT. Rapid diagnostic testing for onchocerciasis in Maridi (South Sudan) before and after improving elimination strategies. DOI: 10.23728/b2share.e98101581be64e6188836d73a19d0243[26].

This project contains the following data:

- Baseline and follow-up serosurveys were conducted in Maridi in 2019 (prior to strengthening onchocerciasis elimination efforts) and 2023, respectively. During both surveys, children aged three to nine years were recruited from five study sites situated at different distances from the Maridi Dam. Ov16 antibodies were detected via rapid diagnostic tests (RDTs) using whole blood obtained by finger-pricking the participants. Baseline and follow-up Ov16 prevalence rates were calculated and compared.

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Author contributions

Conceptualisation by AH, SRJ and RC; data curation by AH and SRJ; formal analysis by AH and JNSF; funding acquisition by RC; investigation by SRJ and AH; methodology by SRJ, RC and AH; project administration by SRJ; resources by RC, JR; software by AH; supervision by RC and JNSF; validation by RC; visualisation by AH; writing – original draft by AH, JNSF, L-JA and RC; and writing – review & editing by AH, SRJ, L-JA, RC, and JNSF.

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References

1. Brattig NW, Cheke RA, Garms R: **Onchocerciasis (river blindness) – more than a century of research and control.** *Acta Trop.* 2021; **218**: 105677.
PubMed Abstract | Publisher Full Text

2. Colebunders R, Njamnshi AK, Menon S, *et al.*: ***Onchocerca volvulus* and epilepsy: A comprehensive review using the Bradford Hill criteria for causation.** *PLoS Negl Trop Dis.* 2021; **15**(1): e0008965.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Boussinesq M, Fobi G, Kuesel AC: **Alternative treatment strategies to accelerate the elimination of onchocerciasis.** *Int Health.* 2018; **10**(suppl_1): i40–i48.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Rumunu J, Brooker S, Hopkins A, *et al.*: **Southern Sudan: an opportunity for NTD control and elimination?** *Trends Parasitol.* 2009; **25**(7): 301–7.
PubMed Abstract | Publisher Full Text | Free Full Text

5. WHO: **Rapid assessment of loiasis and onchocerciasis in Equatoria region of Southern Sudan: 4th to 24th 2005**.
Reference Source

6. Noma M, Zoure HGM, Tekle AH, *et al.*: **The geographic distribution of onchocerciasis in the 20 participating countries of the African Programme for Onchocerciasis Control: (1) priority areas for ivermectin treatment.** *Parasit Vectors.* 2014; **7**: 325.
PubMed Abstract | Publisher Full Text | Free Full Text

7. WHO: **Onchocerciasis: diagnostic target product profile to support preventive chemotherapy**. 2021.
Reference Source

8. Lobos E, Weiss N, Karam M, *et al.*: **An immunogenic *Onchocerca volvulus* antigen: a specific and early marker of infection.** *Science.* 1991; **251**(5001): 1603–5.
PubMed Abstract | Publisher Full Text

9. Cama VA, McDonald C, Arcury-Quandt A, *et al.*: **Evaluation of an OV-16 IgG4 Enzyme-Linked Immunosorbent Assay in Humans and Its Application to Determine the Dynamics of Antibody Responses in a Non-Human Primate Model of *Onchocerca volvulus* Infection.** *Am J Trop Med Hyg.* 2018; **99**(4): 1041–8.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Golden A, Steel C, Yokobe L, *et al.*: **Extended result reading window in lateral flow tests detecting exposure to *Onchocerca volvulus*: a new technology to improve epidemiological surveillance tools.** *PLoS One.* 2013; **8**(7): e69231.
PubMed Abstract | Publisher Full Text | Free Full Text

11. Colebunders R, Carter JY, Olore PC, *et al.*: **High prevalence of onchocerciasis-associated epilepsy in villages in Maridi County, Republic of South Sudan: A community-based survey.** *Seizure.* 2018; **63**: 93–101.
PubMed Abstract | Publisher Full Text | Free Full Text

12. Lakwo TL, Raimon S, Tionga M, *et al.*: **The Role of the Maridi Dam in Causing an Onchocerciasis-Associated Epilepsy Epidemic in Maridi, South Sudan: An Epidemiological, Sociological, and Entomological Study.** *Pathogens.* 2020; **9**(4): 315.
PubMed Abstract | Publisher Full Text | Free Full Text

13. Raimon S, Lakwo TL, Sebit WJ, *et al.*: **"Slash and Clear", a Community-Based Vector Control Method to Reduce Onchocerciasis Transmission by *Simulium sirbanum* in Maridi, South Sudan: A Prospective Study.** *Pathogens.* 2021; **10**(10): 1329.
PubMed Abstract | Publisher Full Text | Free Full Text

14. **South Sudan - County Population Estimates - 2015-2020**. Humanitarian Data Exchange. accessed 11 Mar 2023.
Reference Source

15. Lont YL, Coffeng LE, de Vlas SJ, *et al.*: **Modelling Anti-Ov16 IgG4 Antibody Prevalence as an Indicator for Evaluation and Decision Making in Onchocerciasis Elimination Programmes.** *PLoS Negl Trop Dis.* 2017; **11**(1): e0005314.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Remme J, Ba O, Dadzie KY, *et al.*: **A force-of-infection model for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area.** *Bull World Health Organ.* 1986; **64**(5): 667–81.
PubMed Abstract | Free Full Text

17. Nana-Djeunga HC, Djune-Yemeli L, Domche A, *et al.*: **High infection rates for onchocerciasis and soil-transmitted helminthiasis in children under five not receiving preventive chemotherapy: a bottleneck to elimination.** *Infect Dis Poverty.* 2022; **11**(1): 47.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Hamley JID, Blok DJ, Walker M, *et al.*: **What does the COVID-19 pandemic mean for the next decade of onchocerciasis control and elimination?** *Trans R Soc Trop Med Hyg.* 2021; **115**(3): 269–80.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Jada SR, Amaral LJ, Lakwo T, *et al.*: **Effect of Onchocerciasis Elimination Measures on the Incidence of Epilepsy in Maridi, South Sudan: A Three-Year Prospective Study.** *The Lancet.* Preprint.
Publisher Full Text

20. **Conceptual and operational framework of onchocerciasis elimination with ivermectin treatment**. African Programme for Onchocerciasis Control. 2010.
Reference Source

21. Lake SJ, Kaldor JM, Hardy M, *et al.*: **Mass Drug Administration for the Control of Scabies: A Systematic Review and Meta-analysis.** *Clin Infect Dis.* 2022; **75**(6): 959–67.
PubMed Abstract | Publisher Full Text | Free Full Text

22. WHO: **Onchocerciasis guidelines for stopping mass drug administration and verifying elimination of human onchocerciasis**. In: Organisation WH, editor. 2016; 44.
Reference Source

23. Ekanya R, Beng AA, Anim MA, *et al.*: **Concordance between Ov16 Rapid Diagnostic Test(RDT) and Ov16 Enzyme-Linked Immunosorbent Assay (ELISA) for the Diagnosis of Onchocerciasis in Areas of Contrasting Endemicity in Cameroon.** *Parasite Epidemiol Control.* 2023; **21**: e00290.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Lenaerts E, Mandro M, Mukendi D, *et al.*: **High prevalence of epilepsy in onchocerciasis endemic health areas in Democratic Republic of the Congo.** *Infect Dis Poverty.* 2018; **7**(1): 68.
PubMed Abstract | Publisher Full Text | Free Full Text

25. Jada SR, Amaral LJ, Lakwo T, *et al.*: **Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study.** *Lancet Glob Health.* 2023; **11**(8): e1260–e8.
PubMed Abstract | Publisher Full Text

26. Hadermann A, Jada SR, Amaral LJ, *et al.*: **Rapid diagnostic testing for onchocerciasis in Maridi (South Sudan) before and after improving elimination strategies.** [Data set]. 2023.
http://www.doi.org/10.23728/B2SHARE.E98101581BE64E6188836D73A19D0243

Open Peer Review

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Version 2

Reviewer Report 22 April 2024

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✓ **Yankum Dadzie**
Emory University, Atlanta, Georgia, USA

The paper reads well and the discussion has been improved significantly. As it has been implied, the impact of the additional intervention measures which need to be strengthened will be apparent in a few more years.

However, the discussion on the use and attributes of Ov 16 RDT as a diagnostic tool includes a statement that: 'until recently, the RDT tool has been overlooked in the monitoring of onchocerciasis transmission in endemic foci since WHO recommended the use of ELISA as in reference 22 of this paper'. This statement, however, is misleading in the sense that the WHO Guidelines which the reference 22 refers, discusses only the modalities required to demonstrate that interruption of onchocerciasis transmission has occurred and therefore mass drug administration (MDA) can be stopped and surveillance installed. The Guideline's recommendation selected a trialed serological tool over skin snip for this specific activity on the basis of diagnostics characteristics and at a time when RDT had only just been available. In research recommendations, the Guidelines even advocated for validation of RDT as a possible replacement of the standard test, being ELISA, recognizing the potential of RDT even at the time it had not been widely trialed. The Guidelines were also very clear that tools for monitoring onchocerciasis intervention up to the point of demonstrating interruption of transmission, did not need to be restricted to Ov 16 ELISA.

In the meantime, the WHO's Onchocerciasis Technical Advisory Subgroup, since 2017, has been annually reviewing, among other things, the performance characteristics of two available Ov 16 ELISAs and RDT and provided clear recommendations on their uses based on technical capabilities. In addition, onchocerciasis diagnostic target product profile has been developed to guide development of the appropriate diagnostic tool required for use in onchocerciasis elimination process, as found in Biamonte *et al.* (2022[1]). This suggests that none of the available diagnostic tools is really very satisfactory. Finally, from informal conversations with African Programme Managers of onchocerciasis, who have not published their results in peer reviewed journals, all confirm that they use RDT for all situations in onchocerciasis intervention because of its simplicity in use, which suggest that this judgement might be based on a narrower view of

events.

It therefore appears evident that the statement is not accurate, judges the WHO unfairly and does not quite fit this place in the discussion. I think it should be deleted whilst the good attributes of the RDT remain standing.

References

1. Biamonte MA, Cantey PT, Coulibaly YI, Gass KM, et al.: Onchocerciasis: Target product profiles of in vitro diagnostics to support onchocerciasis elimination mapping and mass drug administration stopping decisions.*PLoS Negl Trop Dis*. 2022; **16** (8): e0010682 PubMed Abstract | Publisher Full Text

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Onchocerciasis epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 19 April 2024

https://doi.org/10.21956/openreseurope.18760.r39102

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?

Norbert W Brattig
Department Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

I thank the authors for clarifying and correcting the manuscript according to the comments and critique of the reviewers. There are still some points in the revision which should be addressed - please see the attached file linked here for more information.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology, microbiology, immunology, diagnostics, pathogen-host-interaction

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 13 April 2024

https://doi.org/10.21956/openreseurope.18760.r39103

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Kiswendsida Thierry Guiguemde
Joseph Ki-Zerbo University, Ouagadougou, Burkina Faso

The authors worked on the importance of using rapid tests for the monitoring and control of onchocerciasis.
The revised version of the manuscript has taken into account our comments and suggestions. This allows a good understanding of the survey procedures and the analysis of the data obtained.
We have no further comments

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 19 January 2024

https://doi.org/10.21956/openreseurope.17376.r36629

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Yankum Dadzie
[1] Emory University, Atlanta, Georgia, USA
[2] Emory University, Atlanta, Georgia, USA

This is an interesting paper reporting on the effect of using a new onchocerciasis diagnostic tool to follow up an onchocerciasis intervention in an uncontrolled area, comparable to an onchocerciasis naïve area with a high endemicity. Every observation is thus intriguing whilst the interpretation of what is being observed needs to be done cautiously, particularly as regards the application of the new diagnostic tool.

This study is well set out and appears well conducted. However, the paper must address two

areas/points which constitute a setback for the paper's strength. The first point is the use of some words or phrases in the paper which are not scientifically appropriate and give the wrong information on the situation in question. The second is about some points in the discussion of the results which portray an opinion which do not seem to be backed by evidence.

The following concern comments on the first point with the use of inappropriate words or phrases:
Introduction: Line 7-10, replace 'Prevention' with 'Elimination' and continue with the sentence and in the 10th line substitute 'control' for 'eradicate'.
The last sentence of the introduction is confusing as what it states is not was what was done. I suggest the following formulation: "This paper presented the effect of strengthening the onchocerciasis elimination interventions as demonstrated by seroprevalence in children aged 3-9 years using the Ov16 SD BIOLINE rapid diagnostic test (RDT)".

Results:
Under the subtitle 'Ov16 seroprevalence in Maridi over time', the following sentence in lines 3-5, 'The seroprevalence among the three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, non-significantly decreased from 12.5% in 2019 to 8.8% in 2023 (p=0.68)', needs to be reformulated. The three-year-olds born after implementation of more robust onchocerciasis elimination measures could be found only in 2023 and could therefore not have contributed to the RDT seroprevalence in 2019 to enable showing a reduced level in 2023. As the measurements are cross-sectional values the sentence should read correctly thus: The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi measured in 2023 8% and was thus lower than the level of 12.5% measured among three-year-olds in 2019 but the difference was not significant, (p=0.68).

My main concern is with the "discussion" and it is specifically about the interpretation of the results from the study. The first paragraph of the discussion points out the increase, albeit insignificant, of the seroprevalence in the 4–9-year-olds following enhanced onchocerciasis measures and interprets the 'increased seroprevalence as 'an indication of intensification of transmission'. Such an interpretation, "increase in transmission" cannot be correct as the enhanced intervention measures clearly must have had increased effect entomologically as well as epidemiologically, through twice a year CDTi even if the coverage was not optimal yet, on the transmission. The entomological impact of "slash and clear" has been reported in the ref. no 5. The publication, "A force-of-infection model for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area by Remme, J *et al.* in the Bulletin of the World Health Organization, 64: 667-681 (1986) on which the onchocerciasis mathematical model is based, shows that at the onset of intervention of vector control, which interrupts transmission almost immediately, the mf prevalence of age groups in years 0-4, 5-9, continues to rise during the first five years after which it starts falling. It is only in the adults that there is a fall of mf prevalence which is seen in the first three years. A similar effect is found with CDTi where it is even more accentuated the higher the pre-intervention endemicity level of the community. It may be recalled that impact assessment of CDTi in APOC countries did not occur until after five years of CDTI when impact would be expected. The notion was based on this observation. Recently, Lont *et al.* have shown that there are basic similarities in the effect of application of mf prevalence and seroprevalence in their publication (Lont *et al.*, 2017[1]). The increase in the seroprevalence recorded in the age group of 3-9 years old children is normal and it

is to be expected in that age group particularly when dealing with communities with very high pre-intervention endemicity. It may be noted that some of the young might harbour prepatent infection before the start of the intervention and which may break out in the course of the intervention as Lont *et al.* discuss in their paper.

As regards to the above considerations, certain statements in the discussion will have to be reviewed and I would like to point them out:
1st paragraph, line 3-5 "These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population". The sentence may be deleted as what has been found is to be expected and importantly does not suggest an absence of impact. The continuation is correct as it takes a few years before interruption of transmission can occur showing also the absence of new infections or new seroconversions as applicable to CDTi.

Line 10, "may indicate an intensification of *O. volvulus transmission"* may be deleted for the same reason as above.

Paragraph 2 is well discussed. However, I would like to point out a striking mitigating factor which has either been downplayed or overseen. It is true that no CDTi was carried out in 2020 because of Covid-19 with the negative implications for which Hamley's good work has been quoted to highlight the effect on this study. However, a good look at the first-year results following the first slash and clear activity as read from the paper by Raimon *et al.* suggests that Maridi focus possibly did not do as badly as it has been described in this paper. A proper analysis of the events could very well indicate that there could have been more reduction in transmission in the year 2020 effected by the slash and clear than what could have been achieved by annual CDTI alone before the slash and clear was introduced.

In the penultimate line of the paragraph, kindly substitute 'eliminate blackflies' for 'eradicate blackflies'.

Paragraph 5:
I wonder whether after the foregoing discussions it is still worth retaining this statement, particularly the second half of the statement. "A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested before and after interventions to evaluate the short-term effect of strengthening an onchocerciasis elimination programme".

References

1. Lont YL, Coffeng LE, de Vlas SJ, Golden A, et al.: Modelling Anti-Ov16 IgG4 Antibody Prevalence as an Indicator for Evaluation and Decision Making in Onchocerciasis Elimination Programmes. *PLoS Negl Trop Dis*. 2017; **11** (1): e0005314 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Onchocerciasis epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Feb 2024

Amber Hadermann

Response to the reviewer: Rapid diagnostic testing for onchocerciasis in Maridi (South Sudan) before and after improving elimination strategies: a repeated cross-sectional survey
Reviewer

1 : **Norbert Brattig** The study represents a local investigation on the success of onchocerciasis control endeavour in South Sudan. The authors analysed the antibody response in children to the diagnostic Onchocerca protein Ov16 in 2023 compared to 2019. The reported data indicate that despite the annual treatment with ivermectin (CDTI) and the vector control strategy “Slash and Clear” the Ov16 response did not decrease but showed insignificant increase in 4-9-year-old children (about 27% to 34%) but an insignificant decrease in 3-year-old children (about 12% to 9%). The cross-sectional survey indicates the ongoing Onchocerca transmission demanding strengthening of the onchocerciasis elimination programme.
The work is clearly and accurately presented. The authors cited current references except the indications noted below. The study design is appropriate, the authors exhibit sufficient details of the analysis – except the constraint below – including the statistics and applied methods which in part should be better introduced. The article merits indexing for scientists involved in onchocerciasis control. The source data are available. The conclusions drawn adequately are supported by the results.
However, the title appears not to represent the documented data as their conclusion and should be improved. Thus, not the applied diagnostic test pertains the core statement of the article, furthermore the notification “*improving elimination strategies*” rather indicates a success in onchocerciasis control. The title more preferably should read: “*Continuance of onchocerciasis transmission to children in a highly endemic area in South Sudan after ivermectin treatment and vector control*” or “*Continuance of onchocerciasis transmission to children in the*

highly endemic Maridi county (South Sudan) after ivermectin treatment and vector control strategy requiring strengthening of onchocerciasis elimination programmes"

Response: ***We prefer to keep our title. Indeed, the aim of this paper was to report on the use of the OV16 rapid test to evaluate onchocerciasis elimination programs. In Maridi, because of the high prevalence of epilepsy and the high Ov16 seroprevalence among children, the onchocerciasis elimination program was already strengthened by switching to six monthly distribution of ivermectin and the implementation of the "Slash& Clear" programme. We now clarify this in the new introduction of the paper. This programme most likely decreased (but not yet interrupted) onchocerciasis transmission because we observed a decrease in the incidence of onchocerciasis-associated epilepsy, including nodding syndrome. These findings were recently published by Jada et al. Jada SR, Amaral LJ, Lakwo T, Carter JY, Rovarini J, Bol YY, Logora MY, Hadermann A, Hopkins A, Fodjo JNS, Colebunders R. Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study. Lancet Glob Health. 2023 Aug;11(8):e1260-e1268. We now also mention this in the discussion of the paper and include this additional reference. We agree that the programme needs further strengthening as 8.8% of the 3-year-old children were still Ov16 positive. Our work also suggests Ov16 rapid testi**ng in 3-year olds as a potential tool to monitor changes in transmission over time.*

Objections:

1. The authors should not state "*Maridi Dam, the only blackfly breeding site in the area*" since this statement cannot be accurate, there surely are other breeding sites.

Response: ***The rivers in the Maridi central area were extensively investigated for the presence of breeding sites by Lakwo et al. (2020), and the Maridi dam was found to be the single black fly breeding site in the area. The reference of the paper by Lakwo et al. is included in the reference list. We agree this is a unique situation for an onchocerciasis endemic area. Our population-based studies showed that the Maridi Dam explains the reason why an "epilepsy epidemic" started in Maridi. Nevertheless, we now state "Maridi Dam, the major blackfly breeding site in the area".***

1. The authors stated that the "a*nnual CDTi was introduced in South Sudan in the early 2000s",* however, they in addition should refer to the detection and occurrence of onchocerciasis in Sudan. Thus, onchocerciasis had been reported in 1947 by Kirk, in 1985 by Williams et al..

Response*: We added the sentence: « Onchocerciasis was first reported in Sudan in 1947 by Kirk et al.» We also included the reference: Kirk R, Morgan HV, Haseeb MA, Satti MH. Onchocerciasis in the Sudan Republic. Ann Trop Med Parasitol. 1959;53(1):97-102.*

1. The authors stated the application of Ov16 rapid diagnostic tests by citing an earlier study of their research group, however, they should explain to the readers the significance and specificity of this *O. volvulus* antigen. Thus, *O. volvulus*-specific antibodies demonstrated in 1987 by Sisley et al., Ov16 antibodies by Higazi et al. in 2013 and 2016; Lobos et al. documented first the high specificity of Ov16 in 1991, later Richards et al. documented 2018 the specificity of the operational performance of *O. volvulus* Ov16 ELISA serological assay, followed by Weil et al. in 2000.

Response*: We have now added more information about the Ov16 test and included in the introduction: "Currently, the World Health Organisation (WHO) recommends using serodiagnostic tools based on the detection of antibodies specific to a 16KDa species-specific antigen of O. volvulus (Ov16), utilising either a rapid diagnostic test or an Enzyme-*

Linked Immunosorbent Assay (ELISA) platform. The antigen Ov16 serves as an early marker of onchocerciasis infection, so seroconversion to Ov16 can be used to detect pre-patent infection, which is not possible with skin snips. Since its discovery in 1991, the use of Ov16 has emerged as the gold standard for onchocerciasis serology, with sensitivities in the 80–89% range and specificity values in the 97–99% range depending on the assay used and the sample collection assessed. We also added the following references: World Health Organisation. Onchocerciasis: diagnostic target product profile to support preventive chemotherapy, 2021 https://www.who.int/publications/i/item/9789240024496 Lobos E, Altmann M, Mengod G, Weiss N, Rudin W, Karam M. Mol Biochem Parasitol. Identification of an Onchocerca volvulus cDNA encoding a low-molecular-weight antigen uniquely recognized by onchocerciasis patient sera. 1990 Feb;39(1):135-45. Cama VA, McDonald C, Arcury-Quandt A, Eberhard M, Jenks MH, Smith J, et al. Evaluation of an OV-16 IgG4 Enzyme-Linked Immunosorbent Assay in Humans and Its Application to Determine the Dynamics of Antibody Responses in a Non-Human Primate Model of Onchocerca volvulus Infection. Am J Trop Med Hyg. 2018;99(4):1041-8. Golden A, Steel C, Yokobe L, Jackson E, Barney R, Kubofcik J, et al. Extended result reading window in lateral flow tests detecting exposure to Onchocerca volvulus: a new technology to improve epidemiological surveillance tools. PLoS One. 2013;8(7):e69231.

1. The note of the recommendation of the Ov16 diagnostic test by the WHO in the text should include the respective reference (WHO, 2016[1]).

Response: reference added.

1. The author correctly accentuated the limitation of the presented study by (i) the small sample size (n=248), (ii) the short follow-up duration, and (iii) the impact of the interrupted ivermectin treatment by Covid-19 pandemic. Thus, this study needs follow-up survey.

Response: Yes, we are planning a follow-up survey.

Reviewer 2: Kiswendsida Thierry Guiguemde The authors worked on the importance of using rapid tests for the monitoring and control of onchocerciasis. Which is very interesting. However, a few comments could help improve the manuscript.

Methods:

Study procedures:

1. There were community mobilizers to spread the information. It would be good to specify who injected the children for the test and whether they were trained beforehand.

Response: We now included in the study procedure "Five local health care workers were trained to perform the Ov16 SD BIOLINE RDT and administer a questionnaire to the parents of the children."

Results:

Ov16 seroprevalence in Maridi 2023

1. "During the 2023 *O. volvulus* seroprevalence study in Maridi, 248 children aged three to nine years were recruited. The median age was six years (IQR: 4-8), and 114 (46.0%) were males. Ninety-three (37.5%) of the participating children had so To make reading easier, it is good to harmonize the way you write the numbers. Either write in letters or write in numbers as for the 248 children. It must be me form of dermatitis, as evidenced by itching

and/or visible skin lesions. Furthermore, four (1.6%) children were identified as having epilepsy2. “applied throughout the entire manuscript. Put the percentage after the number and the specimen. Example: Ninety-three of the participating children (37.5%) instead of "Ninety-three (37.5%) of the participating children". four children (1.6%) were identified instead of four (1.6%) children were identified.

Response: ***We have written the text to fit the following standards: “Spell out numbers one through nine, except in the case of units of measure or time. For these, and for values of 10 and higher, use Arabic numerals. Always spell out numbers at the beginning of a sentence if the sentence cannot be rearranged to avoid starting with a number.” - Springer. Therefore, we have chosen to keep our way of writing numbers the same throughout this document. However, we have adapted the placement of percentages as suggested.***

2. The overall Ov16 RDT seroprevalence was 76/248 (30.7%). The overall Ov16 RDT seroprevalence was 76/248 (30.7%).
The overall Ov16 RDT seroprevalence is in percentage, putting 30.7% (76/248). Correct everywhere in the text the way of writing.

Response: ***We have adapted the text to fit the suggested standards.***

Discussion:
1. A significantly higher proportion of children with dermatitis tested Ov16 RDT positive (43.5%) compared to their peers without dermatitis (22.6%, p<0.001), suggesting the dermatitis was likely caused by an active *O. volvulus* infection in a large proportion of children.
Other causes of dermatitis in children in the study area must be clarified and discussed.

Response: ***We now include in the discussion: “In resource-constrained settings, when African children present with dermatitis and itching, scabies should be considered as a possible diagnosis. However, the absence of characteristic scabies lesions was noted in the children included in the study. Additionally, since scabies is treatable with ivermectin, its prevalence typically decreases in regions where mass ivermectin distribution occurs.”***

2. More elements of discussion should be provided. Studies have shown limitations in the detection of antibodies against the OV-16 antigen. This is after discrepancies in the results with skin snip method according to WHO standards for the detection of microfilariae in the blood. It is important to specify this and give possible reasons. Carrying out the skin snip method in children over 5 with dermatitis could provide more precision on the cause of dermatitis.

Response: ***Indeed, the addition of skin snips would have added more information on active disease in the children. However, skin snip testing is increasingly not very well accepted by local populations because it is an invasive procedure. The use of rapid diagnostic tests is not to diagnose an O. volvulus infection on an individual level but to identify whether transmission is ongoing or not. Furthermore, in the age of ivermectin, the use of skin snip microscopy is becoming redundant. The number of microfilariae in the skin after ivermectin use is low and often below the detection limit. Finally, the fact that Ov16***

antibodies can be detected even before the production of microfilariae make it a much better proxy for recent onchocerciasis transmission as it can identify pre-patent infections in recently infected children.

Reviewer 3 : Yankum Dadzie This is an interesting paper reporting on the effect of using a new onchocerciasis diagnostic tool to follow up an onchocerciasis intervention in an uncontrolled area, comparable to an onchocerciasis naïve area with a high endemicity. Every observation is thus intriguing whilst the interpretation of what is being observed needs to be done cautiously, particularly as regards the application of the new diagnostic tool.

This study is well set out and appears well conducted. However, the paper must address two areas/points which constitute a setback for the paper's strength. The first point is the use of some words or phrases in the paper which are not scientifically appropriate and give the wrong information on the situation in question. The second is about some points in the discussion of the results which portray an opinion which do not seem to be backed by evidence.

1. The following concern comments on the first point with the use of inappropriate words or phrases:
   Introduction: Line 7-10, replace 'Prevention' with 'Elimination' and continue with the sentence and in the 10th line substitute 'control' for 'eradicate'.
   The last sentence of the introduction is confusing as what it states is not was what was done. I suggest the following formulation: "This paper presented the effect of strengthening the onchocerciasis elimination interventions as demonstrated by seroprevalence in children aged 3-9 years using the Ov16 SD BIOLINE rapid diagnostic test (RDT)".

Response: ***Suggested alterations done.***

1. Results:
   Under the subtitle 'Ov16 seroprevalence in Maridi over time', the following sentence in lines 3-5, 'The seroprevalence among the three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, non-significantly decreased from 12.5% in 2019 to 8.8% in 2023 (p=0.68)', needs to be reformulated. The three-year-olds born after implementation of more robust onchocerciasis elimination measures could be found only in 2023 and could therefore not have contributed to the RDT seroprevalence in 2019 to enable showing a reduced level in 2023. As the measurements are cross-sectional values the sentence should read correctly thus: The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi measured in 2023 8% and was thus lower than the level of 12.5% measured among three-year-olds in 2019 but the difference was not significant, (p=0.68).

Response: ***Text altered. We now state" The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, was measured at 8% in 2023 and was thus lower than the level of 12.5% measured among three-year-olds in 2019. However, the difference was not significant (p=0.68)."***

1. My main concern is with the “discussion” and it is specifically about the interpretation of the results from the study. The first paragraph of the discussion points out the increase, albeit insignificant, of the seroprevalence in the 4–9-year-olds following enhanced onchocerciasis measures and interprets the ‘increased seroprevalence as 'an indication of intensification of transmission’. Such an interpretation, “increase in transmission” cannot be correct as the enhanced intervention measures clearly must have had increased effect entomologically as well as epidemiologically, through twice a year CDTi even if the coverage was not optimal yet, on the transmission. The entomological impact of “slash and clear” has been reported in the ref. no 5. The publication, “A force-of-infection model for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area by Remme, J *et al.* in the Bulletin of the World Health Organization, 64: 667-681 (1986) on which the onchocerciasis mathematical model is based, shows that at the onset of intervention of vector control, which interrupts transmission almost immediately, the mf prevalence of age groups in years 0-4, 5-9, continues to rise during the first five years after which it starts falling. It is only in the adults that there is a fall of mf prevalence which is seen in the first three years. A similar effect is found with CDTi where it is even more accentuated the higher the pre-intervention endemicity level of the community. It may be recalled that impact assessment of CDTi in APOC countries did not occur until after five years of CDTI when impact would be expected. The notion was based on this observation. Recently, Lont *et al.* have shown that there are basic similarities in the effect of application of mf prevalence and seroprevalence in their publication (Lont *et al.*, 2017[1]). The increase in the seroprevalence recorded in the age group of 3-9 years old children is normal and it is to be expected in that age group particularly when dealing with communities with very high pre-intervention endemicity. It may be noted that some of the young might harbour prepatent infection before the start of the intervention and which may break out in the course of the intervention as Lont *et al.* discuss in their paper.

As regards to the above considerations, certain statements in the discussion will have to be reviewed and I would like to point them out:

1st paragraph, line 3-5 “These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population”. The sentence may be deleted as what has been found is to be expected and importantly does not suggest an absence of impact. The continuation is correct as it takes a few years before interruption of transmission can occur showing also the absence of new infections or new seroconversions as applicable to CDTi. *Response: We thank you for these important comments. We eliminated the sentence “These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population”. We now included in the discussion “An increase in the seroprevalence in the age group of 3-9 years old children was to be expected, particularly because of the very high pre-intervention endemicity. Indeed some of the children might harbour prepatent infection before the start of the intervention which may break out in the course of the intervention. A force-of-infection model for onchocerciasis has shown that even during interruption of O. volvulus transmission by vector control, the microfilarial prevalence of the 0-4 and 5-9 year old age groups continues to rise during the first five years after which it starts falling.” And we added the following two references Lont YL, Coffeng LE, de Vlas SJ, Golden A, de Los Santos T, Domingo GJ, Stolk WA. PLoS Negl Trop Dis. 2017 Jan 23;11(1):e0005314. doi: 10.1371/journal.pntd.0005314. eCollection 2017 Remme J, Ba O, Dadzie KY, Karam M. A force-of-*

infectionmodel for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area. Bull World Health Organ. 1986;64(5):667-81

1. Line 10, "may indicate an intensification of *O. volvulus transmission"* may be deleted for the same reason as above.

Response*: We deleted the the text mentioning the ' intensification of *O. volvulus transmission"

1. Paragraph 2 is well discussed. However, I would like to point out a striking mitigating factor which has either been downplayed or overseen. It is true that no CDTi was carried out in 2020 because of Covid-19 with the negative implications for which Hamley's good work has been quoted to highlight the effect on this study. However, a good look at the first-year results following the first slash and clear activity as read from the paper by Raimon *et al.* suggests that Maridi focus possibly did not do as badly as it has been described in this paper. A proper analysis of the events could very well indicate that there could have been more reduction in transmission in the year 2020 effected by the slash and clear than what could have been achieved by annual CDTI alone before the slash and clear was introduced.

***Response*: We agree that the strengthening of the onchocerciasis elimination program decreased the O. volvulus transmission in the area. This was strongly suggested by the results of a longitudinal population-based study that showed that the incidence of onchocerciasis-associated epilepsy, including nodding syndrome, decreased in Maridi. This paper has now been published and is included among the references. Jada SR, Amaral LJ, Lakwo T, Carter JY, Rovarini J, Bol YY, Logora MY, Hadermann A, Hopkins A, Fodjo JNS, Colebunders R. Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study. Lancet Glob Health. 2023 Aug;11(8):e1260-e1268. doi: 10.1016/S2214-109X(23)00248-6.**

1. In the penultimate line of the paragraph, kindly substitute 'eliminate blackflies' for 'eradicate blackflies'.

Response: Text altered.

1. Paragraph 5:
   I wonder whether after the foregoing discussions it is still worth retaining this statement, particularly the second half of the statement. "A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested before and after interventions to evaluate the short-term effect of strengthening an onchocerciasis elimination programme".

Response: We now state "A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested and a longer follow-up is needed to investigate the effect of strengthening an onchocerciasis elimination programme."

Competing Interests: No competing interests were disclosed.

Reviewer Report 19 January 2024

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Kiswendsida Thierry Guiguemde
[1] Joseph Ki-Zerbo University, Ouagadougou, Burkina Faso
[2] Joseph Ki-Zerbo University, Ouagadougou, Burkina Faso

The authors worked on the importance of using rapid tests for the monitoring and control of onchocerciasis. Which is very interesting. However, a few comments could help improve the manuscript.

Methods:
Study procedures:
1. There were community mobilizers to spread the information. It would be good to specify who injected the children for the test and whether they were trained beforehand.

Results:
Ov16 seroprevalence in Maridi 2023
1. “During the 2023 O. volvulus seroprevalence study in Maridi, 248 children aged three to nine years were recruited. The median age was six years (IQR: 4-8), and 114 (46.0%) were males. Ninety-three (37.5%) of the participating children had some form of dermatitis, as evidenced by itching and/or visible skin lesions. Furthermore, four (1.6%) children were identified as having epilepsy2. “
- To make reading easier, it is good to harmonize the way you write the numbers. Either write in letters or write in numbers as for the 248 children. It must be applied throughout the entire manuscript.
- Put the percentage after the number and the specimen. Example: Ninety-three of the participating children (37.5%) instead of "Ninety-three (37.5%) of the participating children". four children (1.6%) were identified instead of four (1.6%) children were identified.

2. The overall Ov16 RDT seroprevalence was 76/248 (30.7%). The overall Ov16 RDT seroprevalence was 76/248 (30.7%).
The overall Ov16 RDT seroprevalence is in percentage, putting 30.7% (76/248). Correct everywhere in the text the way of writing.

Discussion:
1. A significantly higher proportion of children with dermatitis tested Ov16 RDT positive (43.5%) compared to their peers without dermatitis (22.6%, $p<0.001$), suggesting the dermatitis was likely caused by an active *O. volvulus* infection in a large proportion of children.
Other causes of dermatitis in children in the study area must be clarified and discussed.

2. More elements of discussion should be provided. Studies have shown limitations in the detection of antibodies against the OV-16 antigen. This is after discrepancies in the results with skin snip method according to WHO standards for the detection of microfilariae in the blood. It is important to specify this and give possible reasons. Carrying out the skin snip method in children over 5 with dermatitis could provide more precision on the cause of dermatitis.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Feb 2024

Amber Hadermann

Response to the reviewer: Rapid diagnostic testing for onchocerciasis in Maridi (South Sudan) before and after improving elimination strategies: a repeated cross-sectional survey
Reviewer

1 : **Norbert Brattig** The study represents a local investigation on the success of onchocerciasis control endeavour in South Sudan. The authors analysed the antibody response in children to the diagnostic Onchocerca protein Ov16 in 2023 compared to 2019. The reported data indicate that despite the annual treatment with ivermectin (CDTI) and the vector control strategy “Slash and Clear” the Ov16 response did not decrease but showed insignificant increase in 4-9-year-old children (about 27% to 34%) but an insignificant decrease in 3-year-old children (about 12% to 9%). The cross-sectional survey indicates the ongoing Onchocerca transmission demanding strengthening of the onchocerciasis elimination programme.
The work is clearly and accurately presented. The authors cited current references except the indications noted below. The study design is appropriate, the authors exhibit sufficient details of the analysis – except the constraint below – including the statistics and applied methods which in part should be better introduced. The article merits indexing for scientists

involved in onchocerciasis control. The source data are available. The conclusions drawn adequately are supported by the results.
However, the title appears not to represent the documented data as their conclusion and should be improved. Thus, not the applied diagnostic test pertains the core statement of the article, furthermore the notification "*improving elimination strategies*" rather indicates a success in onchocerciasis control. The title more preferably should read: "*Continuance of onchocerciasis transmission to children in a highly endemic area in South Sudan after ivermectin treatment and vector control*" or "*Continuance of onchocerciasis transmission to children in the highly endemic Maridi county (South Sudan) after ivermectin treatment and vector control strategy requiring strengthening of onchocerciasis elimination programmes*"

Response:* We prefer to keep our title. Indeed, the aim of this paper was to report on the use of the OV16 rapid test to evaluate onchocerciasis elimination programs. In Maridi, because of the high prevalence of epilepsy and the high Ov16 seroprevalence among children, the onchocerciasis elimination program was already strengthened by switching to six monthly distribution of ivermectin and the implementation of the "Slash& Clear" programme. We now clarify this in the new introduction of the paper. This programme most likely decreased (but not yet interrupted) onchocerciasis transmission because we observed a decrease in the incidence of onchocerciasis-associated epilepsy, including nodding syndrome. These findings were recently published by Jada et al. Jada SR, Amaral LJ, Lakwo T, Carter JY, Rovarini J, Bol YY, Logora MY, Hadermann A, Hopkins A, Fodjo JNS, Colebunders R. *Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study.* Lancet Glob Health. 2023 Aug;11(8):e1260-e1268. We now also mention this in the discussion of the paper and include this additional reference. We agree that the programme needs further strengthening as 8.8% of the 3-year-old children were still Ov16 positive. Our work also suggests Ov16 rapid testing in 3-year olds as a potential tool to monitor changes in transmission over time.

Objections:

1. The authors should not state "*Maridi Dam, the only blackfly breeding site in the area*" since this statement cannot be accurate, there surely are other breeding sites.

***Response:* The rivers in the Maridi central area were extensively investigated for the presence of breeding sites by Lakwo et al. (2020), and the Maridi dam was found to be the single black fly breeding site in the area. The reference of the paper by Lakwo et al. is included in the reference list. We agree this is a unique situation for an onchocerciasis endemic area. Our population-based studies showed that the Maridi Dam explains the reason why an "epilepsy epidemic" started in Maridi. Nevertheless, we now state "Maridi Dam, the major blackfly breeding site in the area".**

1. The authors stated that the "a*nnual CDTi was introduced in South Sudan in the early 2000s",* however, they in addition should refer to the detection and occurrence of onchocerciasis in Sudan. Thus, onchocerciasis had been reported in 1947 by Kirk, in 1985 by Williams et al..

***Response*: We added the sentence: « Onchocerciasis was first reported in Sudan in 1947 by Kirk et al.» We also included the reference: Kirk R, Morgan HV, Haseeb MA, Satti MH. Onchocerciasis in the Sudan Republic. Ann Trop Med Parasitol. 1959;53(1):97-102.**

1. The authors stated the application of Ov16 rapid diagnostic tests by citing an earlier study of their research group, however, they should explain to the readers the significance and specificity of this *O. volvulus* antigen. Thus, *O. volvulus*-specific

antibodies demonstrated in 1987 by Sisley et al., Ov16 antibodies by Higazi et al. in 2013 and 2016; Lobos et al. documented first the high specificity of Ov16 in 1991, later Richards et al. documented 2018 the specificity of the operational performance of *O. volvulus* Ov16 ELISA serological assay, followed by Weil et al. in 2000.

Response*: We have now added more information about the Ov16 test and included in the introduction: "Currently, the World Health Organisation (WHO) recommends using serodiagnostic tools based on the detection of antibodies specific to a 16KDa species-specific antigen of O. volvulus (Ov16), utilising either a rapid diagnostic test or an Enzyme-Linked Immunosorbent Assay (ELISA) platform. The antigen Ov16 serves as an early marker of onchocerciasis infection, so seroconversion to Ov16 can be used to detect pre-patent infection, which is not possible with skin snips. Since its discovery in 1991, the use of Ov16 has emerged as the gold standard for onchocerciasis serology, with sensitivities in the 80–89% range and specificity values in the 97–99% range depending on the assay used and the sample collection assessed. We also added the following references: World Health Organisation. Onchocerciasis: diagnostic target product profile to support preventive chemotherapy, 2021 https://www.who.int/publications/i/item/9789240024496 Lobos E, Altmann M, Mengod G, Weiss N, Rudin W, Karam M. Mol Biochem Parasitol. Identification of an Onchocerca volvulus cDNA encoding a low-molecular-weight antigen uniquely recognized by onchocerciasis patient sera. 1990 Feb;39(1):135-45. Cama VA, McDonald C, Arcury-Quandt A, Eberhard M, Jenks MH, Smith J, et al. Evaluation of an OV-16 IgG4 Enzyme-Linked Immunosorbent Assay in Humans and Its Application to Determine the Dynamics of Antibody Responses in a Non-Human Primate Model of Onchocerca volvulus Infection. Am J Trop Med Hyg. 2018;99(4):1041-8. Golden A, Steel C, Yokobe L, Jackson E, Barney R, Kubofcik J, et al. Extended result reading window in lateral flow tests detecting exposure to Onchocerca volvulus: a new technology to improve epidemiological surveillance tools. PLoS One. 2013;8(7):e69231.

1. The note of the recommendation of the Ov16 diagnostic test by the WHO in the text should include the respective reference (WHO, 2016[1]).

Response: reference added.

1. The author correctly accentuated the limitation of the presented study by (i) the small sample size (n=248), (ii) the short follow-up duration, and (iii) the impact of the interrupted ivermectin treatment by Covid-19 pandemic. Thus, this study needs follow-up survey.

***Response:* Yes, we are planning a follow-up survey.**

Reviewer 2: Kiswendsida Thierry Guiguemde The authors worked on the importance of using rapid tests for the monitoring and control of onchocerciasis. Which is very interesting. However, a few comments could help improve the manuscript.

Methods:

Study procedures:

1. There were community mobilizers to spread the information. It would be good to specify who injected the children for the test and whether they were trained beforehand.

***Response:* We now included in the study procedure "Five local health care workers were trained to perform the Ov16 SD BIOLINE RDT and administer a questionnaire to the parents of the children."**

Results:
Ov16 seroprevalence in Maridi 2023
1. “During the 2023 *O. volvulus* seroprevalence study in Maridi, 248 children aged three to nine years were recruited. The median age was six years (IQR: 4-8), and 114 (46.0%) were males. Ninety-three (37.5%) of the participating children had so To make reading easier, it is good to harmonize the way you write the numbers. Either write in letters or write in numbers as for the 248 children. It must be me form of dermatitis, as evidenced by itching and/or visible skin lesions. Furthermore, four (1.6%) children were identified as having epilepsy2. “applied throughout the entire manuscript. Put the percentage after the number and the specimen. Example: Ninety-three of the participating children (37.5%) instead of "Ninety-three (37.5%) of the participating children". four children (1.6%) were identified instead of four (1.6%) children were identified.

Response: ***We have written the text to fit the following standards: “Spell out numbers one through nine, except in the case of units of measure or time. For these, and for values of 10 and higher, use Arabic numerals. Always spell out numbers at the beginning of a sentence if the sentence cannot be rearranged to avoid starting with a number.” - Springer. Therefore, we have chosen to keep our way of writing numbers the same throughout this document. However, we have adapted the placement of percentages as suggested.***

2. The overall Ov16 RDT seroprevalence was 76/248 (30.7%). The overall Ov16 RDT seroprevalence was 76/248 (30.7%).
The overall Ov16 RDT seroprevalence is in percentage, putting 30.7% (76/248). Correct everywhere in the text the way of writing.

Response: ***We have adapted the text to fit the suggested standards.***

Discussion:
1. A significantly higher proportion of children with dermatitis tested Ov16 RDT positive (43.5%) compared to their peers without dermatitis (22.6%, $p<0.001$), suggesting the dermatitis was likely caused by an active *O. volvulus* infection in a large proportion of children.
Other causes of dermatitis in children in the study area must be clarified and discussed.

Response: ***We now include in the discussion: “In resource-constrained settings, when African children present with dermatitis and itching, scabies should be considered as a possible diagnosis. However, the absence of characteristic scabies lesions was noted in the children included in the study. Additionally, since scabies is treatable with ivermectin, its prevalence typically decreases in regions where mass ivermectin distribution occurs.”***

2. More elements of discussion should be provided. Studies have shown limitations in the detection of antibodies against the OV-16 antigen. This is after discrepancies in the results with skin snip method according to WHO standards for the detection of microfilariae in the blood. It is important to specify this and give possible reasons. Carrying out the skin snip method in children over 5 with dermatitis could provide more precision on the cause of dermatitis.

Response: ***Indeed, the addition of skin snips would have added more information on active disease in the children. However, skin snip testing is increasingly not very well accepted by local populations because it is an invasive procedure. The use of rapid diagnostic tests is not to diagnose an O. volvulus infection on an individual level but to identify whether transmission is ongoing or not. Furthermore, in the age of ivermectin, the use of skin snip microscopy is becoming redundant. The number of microfilariae in the skin after ivermectin use is low and often below the detection limit. Finally, the fact that Ov16 antibodies can be detected even before the production of microfilariae make it a much better proxy for recent onchocerciasis transmission as it can identify pre-patent infections in recently infected children.***

Reviewer 3 : Yankum Dadzie This is an interesting paper reporting on the effect of using a new onchocerciasis diagnostic tool to follow up an onchocerciasis intervention in an uncontrolled area, comparable to an onchocerciasis naïve area with a high endemicity. Every observation is thus intriguing whilst the interpretation of what is being observed needs to be done cautiously, particularly as regards the application of the new diagnostic tool.

This study is well set out and appears well conducted. However, the paper must address two areas/points which constitute a setback for the paper's strength. The first point is the use of some words or phrases in the paper which are not scientifically appropriate and give the wrong information on the situation in question. The second is about some points in the discussion of the results which portray an opinion which do not seem to be backed by evidence.

1. The following concern comments on the first point with the use of inappropriate words or phrases:
   Introduction: Line 7-10, replace 'Prevention' with 'Elimination' and continue with the sentence and in the 10th line substitute 'control' for 'eradicate'.
   The last sentence of the introduction is confusing as what it states is not was what was done. I suggest the following formulation: "This paper presented the effect of strengthening the onchocerciasis elimination interventions as demonstrated by seroprevalence in children aged 3-9 years using the Ov16 SD BIOLINE rapid diagnostic test (RDT)".

Response: ***Suggested alterations done.***

1. Results:
   Under the subtitle 'Ov16 seroprevalence in Maridi over time', the following sentence in lines 3-5, 'The seroprevalence among the three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, non-significantly decreased from 12.5% in 2019 to 8.8% in 2023 (p=0.68)', needs to be reformulated. The three-year-olds born after implementation of more robust onchocerciasis elimination measures could be found only in 2023 and could therefore not have contributed to the RDT seroprevalence in 2019 to enable showing a reduced level in 2023. As the measurements are cross-sectional values the sentence should read correctly thus: The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination

measures like vector control and bi-annual CDTi measured in 2023 8% and was thus lower than the level of 12.5% measured among three-year-olds in 2019 but the difference was not significant, (p=0.68).

Response: ***Text altered. We now state" The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, was measured at 8% in 2023 and was thus lower than the level of 12.5% measured among three-year-olds in 2019. However, the difference was not significant (p=0.68)."***

1. My main concern is with the "discussion" and it is specifically about the interpretation of the results from the study. The first paragraph of the discussion points out the increase, albeit insignificant, of the seroprevalence in the 4–9-year-olds following enhanced onchocerciasis measures and interprets the 'increased seroprevalence as 'an indication of intensification of transmission'. Such an interpretation, "increase in transmission" cannot be correct as the enhanced intervention measures clearly must have had increased effect entomologically as well as epidemiologically, through twice a year CDTi even if the coverage was not optimal yet, on the transmission. The entomological impact of "slash and clear" has been reported in the ref. no 5. The publication, "A force-of-infection model for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area by Remme, J *et al.* in the Bulletin of the World Health Organization, 64: 667-681 (1986) on which the onchocerciasis mathematical model is based, shows that at the onset of intervention of vector control, which interrupts transmission almost immediately, the mf prevalence of age groups in years 0-4, 5-9, continues to rise during the first five years after which it starts falling. It is only in the adults that there is a fall of mf prevalence which is seen in the first three years. A similar effect is found with CDTi where it is even more accentuated the higher the pre-intervention endemicity level of the community. It may be recalled that impact assessment of CDTi in APOC countries did not occur until after five years of CDTI when impact would be expected. The notion was based on this observation. Recently, Lont *et al.* have shown that there are basic similarities in the effect of application of mf prevalence and seroprevalence in their publication (Lont *et al.*, 2017[1]). The increase in the seroprevalence recorded in the age group of 3-9 years old children is normal and it is to be expected in that age group particularly when dealing with communities with very high pre-intervention endemicity. It may be noted that some of the young might harbour prepatent infection before the start of the intervention and which may break out in the course of the intervention as Lont *et al.* discuss in their paper.

As regards to the above considerations, certain statements in the discussion will have to be reviewed and I would like to point them out:

1st paragraph, line 3-5 "These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population". The sentence may be deleted as what has been found is to be expected and importantly does not suggest an absence of impact. The continuation is correct as it takes a few years before interruption of transmission can occur showing also the absence of new infections or new seroconversions as applicable to CDTi. *Response: We thank you for these important comments. We eliminated the sentence "These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population". We now included in the discussion "An increase in the seroprevalence in the age group of 3-9 years old children was to be expected, particularly*

because of the very high pre-intervention endemicity. Indeed some of the children might harbour prepatent infection before the start of the intervention which may break out in the course of the intervention. A force-of-infection model for onchocerciasis has shown that even during interruption of O. volvulus transmission by vector control, the microfilarial prevalence of the 0-4 and 5-9 year old age groups continues to rise during the first five years after which it starts falling." And we added the following two references Lont YL, Coffeng LE, de Vlas SJ, Golden A, de Los Santos T, Domingo GJ, Stolk WA. PLoS Negl Trop Dis. 2017 Jan 23;11(1):e0005314. doi: 10.1371/journal.pntd.0005314. eCollection 2017 Remme J, Ba O, Dadzie KY, Karam M. A force-of-infectionmodel for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area. Bull World Health Organ. 1986;64(5):667-81

1. Line 10, "may indicate an intensification of *O. volvulus transmission"* may be deleted for the same reason as above.

Response*: We deleted the the text mentioning the '** **intensification of *O. volvulus transmission"

1. Paragraph 2 is well discussed. However, I would like to point out a striking mitigating factor which has either been downplayed or overseen. It is true that no CDTi was carried out in 2020 because of Covid-19 with the negative implications for which Hamley's good work has been quoted to highlight the effect on this study. However, a good look at the first-year results following the first slash and clear activity as read from the paper by Raimon *et al.* suggests that Maridi focus possibly did not do as badly as it has been described in this paper. A proper analysis of the events could very well indicate that there could have been more reduction in transmission in the year 2020 effected by the slash and clear than what could have been achieved by annual CDTI alone before the slash and clear was introduced.

***Response*: We agree that the strengthening of the onchocerciasis elimination program decreased the O. volvulus transmission in the area. This was strongly suggested by the results of a longitudinal population-based study that showed that the incidence of onchocerciasis-associated epilepsy, including nodding syndrome, decreased in Maridi. This paper has now been published and is included among the references. Jada SR, Amaral LJ, Lakwo T, Carter JY, Rovarini J, Bol YY, Logora MY, Hadermann A, Hopkins A, Fodjo JNS, Colebunders R. Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study. Lancet Glob Health. 2023 Aug;11(8):e1260-e1268. doi: 10.1016/S2214-109X(23)00248-6.**

1. In the penultimate line of the paragraph, kindly substitute 'eliminate blackflies' for 'eradicate blackflies'.

Response: Text altered.

1. Paragraph 5:
   I wonder whether after the foregoing discussions it is still worth retaining this statement, particularly the second half of the statement. "A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested before and after interventions to evaluate the short-term effect of strengthening an onchocerciasis elimination programme".

Response: We now state "A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested and a longer follow-up is needed to investigate the effect of strengthening an onchocerciasis elimination programme."

Competing Interests: No competing interests were disclosed.

Reviewer Report 19 January 2024

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Norbert W Brattig
[1] Department Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2] Department Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

The study represents a local investigation on the success of onchocerciasis control endeavour in South Sudan. The authors analysed the antibody response in children to the diagnostic *Onchocerca* protein Ov16 in 2023 compared to 2019. The reported data indicate that despite the annual treatment with ivermectin (CDTI) and the vector control strategy "Slash and Clear" the Ov16 response did not decrease but showed insignificant increase in 4-9-year-old children (about 27% to 34%) but an insignificant decrease in 3-year-old children (about 12% to 9%). The cross-sectional survey indicates the ongoing *Onchocerca* transmission demanding strengthening of the onchocerciasis elimination programme.

The work is clearly and accurately presented. The authors cited current references except the indications noted below. The study design is appropriate, the authors exhibit sufficient details of the analysis – except the constraint below – including the statistics and applied methods which in part should be better introduced. The article merits indexing for scientists involved in onchocerciasis control. The source data are available. The conclusions drawn adequately are supported by the results.

However, the title appears not to represent the documented data as their conclusion and should be improved. Thus, not the applied diagnostic test pertains the core statement of the article, furthermore the notification "*improving elimination strategies*" rather indicates a success in onchocerciasis control. The title more preferably should read: "*Continuance of onchocerciasis transmission to children in a highly endemic area in South Sudan after ivermectin treatment and vector control*" or "*Continuance of onchocerciasis transmission to children in the highly endemic Maridi county (South Sudan) after ivermectin treatment and vector control strategy requiring strengthening of onchocerciasis elimination programmes*"

Objections:

- The authors should not state "*Maridi Dam, the only blackfly breeding site in the area*" since this statement cannot be accurate, there surely are other breeding sites.

- The authors stated that the "a*nnual CDTi was introduced in South Sudan in the early 2000s",* however, they in addition should refer to the detection and occurrence of onchocerciasis in Sudan. Thus, onchocerciasis had been reported in 1947 by Kirk, in 1985 by Williams et al..

- The authors stated the application of Ov16 rapid diagnostic tests by citing an earlier study of their research group, however, they should explain to the readers the significance and specificity of this *O. volvulus* antigen. Thus, *O. volvulus*-specific antibodies demonstrated in 1987 by Sisley et al., Ov16 antibodies by Higazi et al. in 2013 and 2016; Lobos et al. documented first the high specificity of Ov16 in 1991, later Richards et al. documented 2018 the specificity of the operational performance of *O. volvulus* Ov16 ELISA serological assay, followed by Weil et al. in 2000.

- The note of the recommendation of the Ov16 diagnostic test by the WHO in the text should include the respective reference (WHO, 2016[1])**.**

- The author correctly accentuated the limitation of the presented study by (i) the small sample size (n=248), (ii) the short follow-up duration, and (iii) the impact of the interrupted ivermectin treatment by Covid-19 pandemic. Thus, this study needs follow-up survey.

In summary, this article should be indexed after minor revision:
Decision: approved with minor reservation.

References

1. Guidelines for Stopping Mass Drug Administration and Verifying Elimination of Human Onchocerciasis: Criteria and Procedures. 2016. PubMed Abstract

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology, microbiology, immunology, diagnostics, pathogen-host-interaction

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Feb 2024

Amber Hadermann

Response to the reviewer: Rapid diagnostic testing for onchocerciasis in Maridi (South Sudan) before and after improving elimination strategies: a repeated cross-sectional survey Reviewer

1 : **Norbert Brattig** The study represents a local investigation on the success of onchocerciasis control endeavour in South Sudan. The authors analysed the antibody response in children to the diagnostic Onchocerca protein Ov16 in 2023 compared to 2019. The reported data indicate that despite the annual treatment with ivermectin (CDTI) and the vector control strategy "Slash and Clear" the Ov16 response did not decrease but showed insignificant increase in 4-9-year-old children (about 27% to 34%) but an insignificant decrease in 3-year-old children (about 12% to 9%). The cross-sectional survey indicates the ongoing Onchocerca transmission demanding strengthening of the onchocerciasis elimination programme.
The work is clearly and accurately presented. The authors cited current references except the indications noted below. The study design is appropriate, the authors exhibit sufficient details of the analysis – except the constraint below – including the statistics and applied methods which in part should be better introduced. The article merits indexing for scientists involved in onchocerciasis control. The source data are available. The conclusions drawn adequately are supported by the results.
However, the title appears not to represent the documented data as their conclusion and should be improved. Thus, not the applied diagnostic test pertains the core statement of the article, furthermore the notification "*improving elimination strategies*" rather indicates a success in onchocerciasis control. The title more preferably should read: "*Continuance of onchocerciasis transmission to children in a highly endemic area in South Sudan after ivermectin treatment and vector control*" or "*Continuance of onchocerciasis transmission to children in the highly endemic Maridi county (South Sudan) after ivermectin treatment and vector control strategy requiring strengthening of onchocerciasis elimination programmes*"
Response: ***We prefer to keep our title. Indeed, the aim of this paper was to report on the use of the OV16 rapid test to evaluate onchocerciasis elimination programs. In Maridi, because of the high prevalence of epilepsy and the high Ov16 seroprevalence among children, the onchocerciasis elimination program was already strengthened by switching to six monthly distribution of ivermectin and the implementation of the "Slash& Clear" programme. We now clarify this in the new introduction of the paper. This programme most likely decreased (but not yet interrupted) onchocerciasis transmission because we observed a decrease in the incidence of onchocerciasis-associated epilepsy, including nodding syndrome. These findings were recently published by Jada et al. Jada SR, Amaral LJ,***

***Lakwo T, Carter JY, Rovarini J, Bol YY, Logora MY, Hadermann A, Hopkins A, Fodjo JNS, Colebunders R. Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study. Lancet Glob Health. 2023 Aug;11(8):e1260-e1268. We now also mention this in the discussion of the paper and include this additional reference. We agree that the programme needs further strengthening as 8.8% of the 3-year-old children were still Ov16 positive. Our work also suggests Ov16 rapid testi**ng in 3-year olds as a potential tool to monitor changes in transmission over time.*

Objections:

1. The authors should not state "*Maridi Dam, the only blackfly breeding site in the area*" since this statement cannot be accurate, there surely are other breeding sites.

***Response:** The rivers in the Maridi central area were extensively investigated for the presence of breeding sites by Lakwo et al. (2020), and the Maridi dam was found to be the single black fly breeding site in the area. The reference of the paper by Lakwo et al. is included in the reference list. We agree this is a unique situation for an onchocerciasis endemic area. Our population-based studies showed that the Maridi Dam explains the reason why an "epilepsy epidemic" started in Maridi. Nevertheless, we now state "Maridi Dam, the major blackfly breeding site in the area".*

1. The authors stated that the "a*nnual CDTi was introduced in South Sudan in the early 2000s",* however, they in addition should refer to the detection and occurrence of onchocerciasis in Sudan. Thus, onchocerciasis had been reported in 1947 by Kirk, in 1985 by Williams et al..

***Response**: We added the sentence: « Onchocerciasis was first reported in Sudan in 1947 by Kirk et al.» We also included the reference: Kirk R, Morgan HV, Haseeb MA, Satti MH. Onchocerciasis in the Sudan Republic. Ann Trop Med Parasitol. 1959;53(1):97-102.*

1. The authors stated the application of Ov16 rapid diagnostic tests by citing an earlier study of their research group, however, they should explain to the readers the significance and specificity of this *O. volvulus* antigen. Thus, *O. volvulus*-specific antibodies demonstrated in 1987 by Sisley et al., Ov16 antibodies by Higazi et al. in 2013 and 2016; Lobos et al. documented first the high specificity of Ov16 in 1991, later Richards et al. documented 2018 the specificity of the operational performance of *O. volvulus* Ov16 ELISA serological assay, followed by Weil et al. in 2000.

***Response**: We have now added more information about the Ov16 test and included in the introduction: "Currently, the World Health Organisation (WHO) recommends using serodiagnostic tools based on the detection of antibodies specific to a 16KDa species-specific antigen of O. volvulus (Ov16), utilising either a rapid diagnostic test or an Enzyme-Linked Immunosorbent Assay (ELISA) platform. The antigen Ov16 serves as an early marker of onchocerciasis infection, so seroconversion to Ov16 can be used to detect pre-patent infection, which is not possible with skin snips. Since its discovery in 1991, the use of Ov16 has emerged as the gold standard for onchocerciasis serology, with sensitivities in the 80–89% range and specificity values in the 97–99% range depending on the assay used and the sample collection assessed. We also added the following references: World Health Organisation. Onchocerciasis: diagnostic target product profile to support preventive chemotherapy, 2021 https://www.who.int/publications/i/item/9789240024496 Lobos E, Altmann M, Mengod G, Weiss N, Rudin W, Karam M. Mol Biochem Parasitol. Identification of an Onchocerca volvulus cDNA encoding a low-molecular-weight antigen uniquely recognized by onchocerciasis patient sera. 1990 Feb;39(1):135-45. Cama VA, McDonald C,*

Arcury-Quandt A, Eberhard M, Jenks MH, Smith J, et al. Evaluation of an OV-16 IgG4 Enzyme-Linked Immunosorbent Assay in Humans and Its Application to Determine the Dynamics of Antibody Responses in a Non-Human Primate Model of Onchocerca volvulus Infection. Am J Trop Med Hyg. 2018;99(4):1041-8. Golden A, Steel C, Yokobe L, Jackson E, Barney R, Kubofcik J, et al. Extended result reading window in lateral flow tests detecting exposure to Onchocerca volvulus: a new technology to improve epidemiological surveillance tools. PLoS One. 2013;8(7):e69231.

1. The note of the recommendation of the Ov16 diagnostic test by the WHO in the text should include the respective reference (WHO, 2016[1]**).**

Response: reference added.

1. The author correctly accentuated the limitation of the presented study by (i) the small sample size (n=248), (ii) the short follow-up duration, and (iii) the impact of the interrupted ivermectin treatment by Covid-19 pandemic. Thus, this study needs follow-up survey.

Response: Yes, we are planning a follow-up survey.

Reviewer 2: Kiswendsida Thierry Guiguemde The authors worked on the importance of using rapid tests for the monitoring and control of onchocerciasis. Which is very interesting. However, a few comments could help improve the manuscript.

Methods:
Study procedures:
1. There were community mobilizers to spread the information. It would be good to specify who injected the children for the test and whether they were trained beforehand.

Response: We now included in the study procedure "Five local health care workers were trained to perform the Ov16 SD BIOLINE RDT and administer a questionnaire to the parents of the children."

Results:
Ov16 seroprevalence in Maridi 2023
1. "During the 2023 *O. volvulus* seroprevalence study in Maridi, 248 children aged three to nine years were recruited. The median age was six years (IQR: 4-8), and 114 (46.0%) were males. Ninety-three (37.5%) of the participating children had so To make reading easier, it is good to harmonize the way you write the numbers. Either write in letters or write in numbers as for the 248 children. It must be me form of dermatitis, as evidenced by itching and/or visible skin lesions. Furthermore, four (1.6%) children were identified as having epilepsy2. "applied throughout the entire manuscript. Put the percentage after the number and the specimen. Example: Ninety-three of the participating children (37.5%) instead of "Ninety-three (37.5%) of the participating children". four children (1.6%) were identified instead of four (1.6%) children were identified.

Response: We have written the text to fit the following standards: "Spell out numbers one through nine, except in the case of units of measure or time. For these, and for values of 10 and higher, use Arabic numerals. Always spell out numbers at the beginning of a sentence if the sentence cannot be rearranged to avoid starting with a number." - Springer. Therefore, we have chosen to keep our way of writing numbers the same throughout this

document. However, we have adapted the placement of percentages as suggested.

2. The overall Ov16 RDT seroprevalence was 76/248 (30.7%). The overall Ov16 RDT seroprevalence was 76/248 (30.7%).
The overall Ov16 RDT seroprevalence is in percentage, putting 30.7% (76/248). Correct everywhere in the text the way of writing.

Response: ***We have adapted the text to fit the suggested standards.***

Discussion:
1. A significantly higher proportion of children with dermatitis tested Ov16 RDT positive (43.5%) compared to their peers without dermatitis (22.6%, p<0.001), suggesting the dermatitis was likely caused by an active *O. volvulus* infection in a large proportion of children.
Other causes of dermatitis in children in the study area must be clarified and discussed.

Response: ***We now include in the discussion: "In resource-constrained settings, when African children present with dermatitis and itching, scabies should be considered as a possible diagnosis. However, the absence of characteristic scabies lesions was noted in the children included in the study. Additionally, since scabies is treatable with ivermectin, its prevalence typically decreases in regions where mass ivermectin distribution occurs."***

2. More elements of discussion should be provided. Studies have shown limitations in the detection of antibodies against the OV-16 antigen. This is after discrepancies in the results with skin snip method according to WHO standards for the detection of microfilariae in the blood. It is important to specify this and give possible reasons. Carrying out the skin snip method in children over 5 with dermatitis could provide more precision on the cause of dermatitis.

Response: ***Indeed, the addition of skin snips would have added more information on active disease in the children. However, skin snip testing is increasingly not very well accepted by local populations because it is an invasive procedure. The use of rapid diagnostic tests is not to diagnose an O. volvulus infection on an individual level but to identify whether transmission is ongoing or not. Furthermore, in the age of ivermectin, the use of skin snip microscopy is becoming redundant. The number of microfilariae in the skin after ivermectin use is low and often below the detection limit. Finally, the fact that Ov16 antibodies can be detected even before the production of microfilariae make it a much better proxy for recent onchocerciasis transmission as it can identify pre-patent infections in recently infected children.***

Reviewer 3 : Yankum Dadzie This is an interesting paper reporting on the effect of using a new onchocerciasis diagnostic tool to follow up an onchocerciasis intervention in an uncontrolled area, comparable to an onchocerciasis naïve area with a high endemicity. Every observation is thus intriguing whilst the interpretation of what is being observed needs to be done cautiously, particularly as regards the application of the new diagnostic tool.

This study is well set out and appears well conducted. However, the paper must address two areas/points which constitute a setback for the paper's strength. The first point is the use of some words or phrases in the paper which are not scientifically appropriate and give the wrong information on the situation in question. The second is about some points in the discussion of the results which portray an opinion which do not seem to be backed by evidence.

1. The following concern comments on the first point with the use of inappropriate words or phrases:
   Introduction: Line 7-10, replace 'Prevention' with 'Elimination' and continue with the sentence and in the 10th line substitute 'control' for 'eradicate'.
   The last sentence of the introduction is confusing as what it states is not was what was done. I suggest the following formulation: "This paper presented the effect of strengthening the onchocerciasis elimination interventions as demonstrated by seroprevalence in children aged 3-9 years using the Ov16 SD BIOLINE rapid diagnostic test (RDT)".

***Response:* Suggested alterations done.**

1. Results:
   Under the subtitle 'Ov16 seroprevalence in Maridi over time', the following sentence in lines 3-5, 'The seroprevalence among the three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, non-significantly decreased from 12.5% in 2019 to 8.8% in 2023 (p=0.68)', needs to be reformulated. The three-year-olds born after implementation of more robust onchocerciasis elimination measures could be found only in 2023 and could therefore not have contributed to the RDT seroprevalence in 2019 to enable showing a reduced level in 2023. As the measurements are cross-sectional values the sentence should read correctly thus: The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi measured in 2023 8% and was thus lower than the level of 12.5% measured among three-year-olds in 2019 but the difference was not significant, (p=0.68).

***Response:* Text altered. We now state" The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, was measured at 8% in 2023 and was thus lower than the level of 12.5% measured among three-year-olds in 2019. However, the difference was not significant (p=0.68)."**

1. My main concern is with the "discussion" and it is specifically about the interpretation of the results from the study. The first paragraph of the discussion points out the increase, albeit insignificant, of the seroprevalence in the 4–9-year-olds following enhanced onchocerciasis measures and interprets the 'increased seroprevalence as 'an indication of intensification of transmission'. Such an interpretation, "increase in transmission" cannot be correct as the enhanced intervention measures clearly must have had increased effect entomologically as well as epidemiologically, through twice a year CDTi even if the coverage was not optimal yet, on the transmission. The entomological impact of "slash and clear" has been reported in the ref. no 5. The publication, "A force-of-infection model for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta

River basin area by Remme, J *et al.* in the Bulletin of the World Health Organization, 64: 667-681 (1986) on which the onchocerciasis mathematical model is based, shows that at the onset of intervention of vector control, which interrupts transmission almost immediately, the mf prevalence of age groups in years 0-4, 5-9, continues to rise during the first five years after which it starts falling. It is only in the adults that there is a fall of mf prevalence which is seen in the first three years. A similar effect is found with CDTi where it is even more accentuated the higher the pre-intervention endemicity level of the community. It may be recalled that impact assessment of CDTi in APOC countries did not occur until after five years of CDTI when impact would be expected. The notion was based on this observation. Recently, Lont *et al.* have shown that there are basic similarities in the effect of application of mf prevalence and seroprevalence in their publication (Lont *et al.*, 2017[1]). The increase in the seroprevalence recorded in the age group of 3-9 years old children is normal and it is to be expected in that age group particularly when dealing with communities with very high pre-intervention endemicity. It may be noted that some of the young might harbour prepatent infection before the start of the intervention and which may break out in the course of the intervention as Lont *et al.* discuss in their paper.

As regards to the above considerations, certain statements in the discussion will have to be reviewed and I would like to point them out:

1st paragraph, line 3-5 "These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population". The sentence may be deleted as what has been found is to be expected and importantly does not suggest an absence of impact. The continuation is correct as it takes a few years before interruption of transmission can occur showing also the absence of new infections or new seroconversions as applicable to CDTi. *Response: We thank you for these important comments. We eliminated the sentence "These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population". We now included in the discussion "An increase in the seroprevalence in the age group of 3-9 years old children was to be expected, particularly because of the very high pre-intervention endemicity. Indeed some of the children might harbour prepatent infection before the start of the intervention which may break out in the course of the intervention. A force-of-infection model for onchocerciasis has shown that even during interruption of O. volvulus transmission by vector control, the microfilarial prevalence of the 0-4 and 5-9 year old age groups continues to rise during the first five years after which it starts falling." And we added the following two references Lont YL, Coffeng LE, de Vlas SJ, Golden A, de Los Santos T, Domingo GJ, Stolk WA. PLoS Negl Trop Dis. 2017 Jan 23;11(1):e0005314. doi: 10.1371/journal.pntd.0005314. eCollection 2017 Remme J, Ba O, Dadzie KY, Karam M. A force-of-infectionmodel for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area. Bull World Health Organ. 1986;64(5):667-81*

1. Line 10, "may indicate an intensification of *O. volvulus transmission"* may be deleted for the same reason as above.

Response: We deleted the the text mentioning the '* intensification of *O. volvulus transmission"

1. Paragraph 2 is well discussed. However, I would like to point out a striking mitigating factor which has either been downplayed or overseen. It is true that no CDTi was carried out in 2020 because of Covid-19 with the negative implications for which Hamley's good work has been quoted to highlight the effect on this study. However, a

good look at the first-year results following the first slash and clear activity as read from the paper by Raimon *et al.* suggests that Maridi focus possibly did not do as badly as it has been described in this paper. A proper analysis of the events could very well indicate that there could have been more reduction in transmission in the year 2020 effected by the slash and clear than what could have been achieved by annual CDTI alone before the slash and clear was introduced.

***Response*: We agree that the strengthening of the onchocerciasis elimination program decreased the O. volvulus transmission in the area. This was strongly suggested by the results of a longitudinal population-based study that showed that the incidence of onchocerciasis-associated epilepsy, including nodding syndrome, decreased in Maridi. This paper has now been published and is included among the references. Jada SR, Amaral LJ, Lakwo T, Carter JY, Rovarini J, Bol YY, Logora MY, Hadermann A, Hopkins A, Fodjo JNS, Colebunders R. Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study. Lancet Glob Health. 2023 Aug;11(8):e1260-e1268. doi: 10.1016/S2214-109X(23)00248-6.**

1. In the penultimate line of the paragraph, kindly substitute 'eliminate blackflies' for 'eradicate blackflies'.

Response: Text altered.

1. Paragraph 5:
   I wonder whether after the foregoing discussions it is still worth retaining this statement, particularly the second half of the statement. "A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested before and after interventions to evaluate the short-term effect of strengthening an onchocerciasis elimination programme".

Response: We now state "A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested and a longer follow-up is needed to investigate the effect of strengthening an onchocerciasis elimination programme."

Competing Interests: No competing interests were disclosed.

Comments on this article

Version 1

Author Response 19 Feb 2024

Amber Hadermann

Response to the reviewer: Rapid diagnostic testing for onchocerciasis in Maridi (South Sudan) before and after improving elimination strategies: a repeated cross-sectional survey Reviewer

1 : **Norbert Brattig** The study represents a local investigation on the success of onchocerciasis control endeavour in South Sudan. The authors analysed the antibody response in children to the diagnostic Onchocerca protein Ov16 in 2023 compared to 2019. The reported data indicate that

despite the annual treatment with ivermectin (CDTI) and the vector control strategy "Slash and Clear" the Ov16 response did not decrease but showed insignificant increase in 4-9-year-old children (about 27% to 34%) but an insignificant decrease in 3-year-old children (about 12% to 9%). The cross-sectional survey indicates the ongoing Onchocerca transmission demanding strengthening of the onchocerciasis elimination programme.
The work is clearly and accurately presented. The authors cited current references except the indications noted below. The study design is appropriate, the authors exhibit sufficient details of the analysis – except the constraint below – including the statistics and applied methods which in part should be better introduced. The article merits indexing for scientists involved in onchocerciasis control. The source data are available. The conclusions drawn adequately are supported by the results.
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Response: ***We prefer to keep our title. Indeed, the aim of this paper was to report on the use of the OV16 rapid test to evaluate onchocerciasis elimination programs. In Maridi, because of the high prevalence of epilepsy and the high Ov16 seroprevalence among children, the onchocerciasis elimination program was already strengthened by switching to six monthly distribution of ivermectin and the implementation of the "Slash& Clear" programme. We now clarify this in the new introduction of the paper. This programme most likely decreased (but not yet interrupted) onchocerciasis transmission because we observed a decrease in the incidence of onchocerciasis-associated epilepsy, including nodding syndrome. These findings were recently published by Jada et al. Jada SR, Amaral LJ, Lakwo T, Carter JY, Rovarini J, Bol YY, Logora MY, Hadermann A, Hopkins A, Fodjo JNS, Colebunders R. Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study. Lancet Glob Health. 2023 Aug;11(8):e1260-e1268. We now also mention this in the discussion of the paper and include this additional reference. We agree that the programme needs further strengthening as 8.8% of the 3-year-old children were still Ov16 positive. Our work also suggests Ov16 rapid testi**ng in 3-year olds as a potential tool to monitor changes in transmission over time.*
Objections:

1. The authors should not state "*Maridi Dam, the only blackfly breeding site in the area*" since this statement cannot be accurate, there surely are other breeding sites.

Response: ***The rivers in the Maridi central area were extensively investigated for the presence of breeding sites by Lakwo et al. (2020), and the Maridi dam was found to be the single black fly breeding site in the area. The reference of the paper by Lakwo et al. is included in the reference list. We agree this is a unique situation for an onchocerciasis endemic area. Our population-based studies showed that the Maridi Dam explains the reason why an "epilepsy epidemic" started in Maridi. Nevertheless, we now state "Maridi Dam, the major blackfly breeding site in the area".***

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***Response*: We added the sentence: « Onchocerciasis was first reported in Sudan in 1947 by Kirk et al.» We also included the reference: Kirk R, Morgan HV, Haseeb MA, Satti MH. Onchocerciasis in the Sudan Republic. Ann Trop Med Parasitol. 1959;53(1):97-102.**

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***Response*: We have now added more information about the Ov16 test and included in the introduction: "Currently, the World Health Organisation (WHO) recommends using serodiagnostic tools based on the detection of antibodies specific to a 16KDa species-specific antigen of O. volvulus (Ov16), utilising either a rapid diagnostic test or an Enzyme-Linked Immunosorbent Assay (ELISA) platform. The antigen Ov16 serves as an early marker of onchocerciasis infection, so seroconversion to Ov16 can be used to detect pre-patent infection, which is not possible with skin snips. Since its discovery in 1991, the use of Ov16 has emerged as the gold standard for onchocerciasis serology, with sensitivities in the 80–89% range and specificity values in the 97–99% range depending on the assay used and the sample collection assessed. We also added the following references: World Health Organisation. Onchocerciasis: diagnostic target product profile to support preventive chemotherapy, 2021 https://www.who.int/publications/i/item/9789240024496 Lobos E, Altmann M, Mengod G, Weiss N, Rudin W, Karam M. Mol Biochem Parasitol. Identification of an Onchocerca volvulus cDNA encoding a low-molecular-weight antigen uniquely recognized by onchocerciasis patient sera. 1990 Feb;39(1):135-45. Cama VA, McDonald C, Arcury-Quandt A, Eberhard M, Jenks MH, Smith J, et al. Evaluation of an OV-16 IgG4 Enzyme-Linked Immunosorbent Assay in Humans and Its Application to Determine the Dynamics of Antibody Responses in a Non-Human Primate Model of Onchocerca volvulus Infection. Am J Trop Med Hyg. 2018;99(4):1041-8. Golden A, Steel C, Yokobe L, Jackson E, Barney R, Kubofcik J, et al. Extended result reading window in lateral flow tests detecting exposure to Onchocerca volvulus: a new technology to improve epidemiological surveillance tools. PLoS One. 2013;8(7):e69231.**

1. The note of the recommendation of the Ov16 diagnostic test by the WHO in the text should include the respective reference (WHO, 2016[1]**).**

Response: reference added.

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Ov16 seroprevalence in Maridi 2023
1. "During the 2023 *O. volvulus* seroprevalence study in Maridi, 248 children aged three to nine years were recruited. The median age was six years (IQR: 4-8), and 114 (46.0%) were males. Ninety-three (37.5%) of the participating children had so To make reading easier, it is good to harmonize the way you write the numbers. Either write in letters or write in numbers as for the 248 children. It must be me form of dermatitis, as evidenced by itching and/or visible skin lesions. Furthermore, four (1.6%) children were identified as having epilepsy2. "applied throughout the entire manuscript. Put the percentage after the number and the specimen. Example: Ninety-three of the participating children (37.5%) instead of "Ninety-three (37.5%) of the participating children". four children (1.6%) were identified instead of four (1.6%) children were identified.

Response: ***We have written the text to fit the following standards: "Spell out numbers one through nine, except in the case of units of measure or time. For these, and for values of 10 and higher, use Arabic numerals. Always spell out numbers at the beginning of a sentence if the sentence cannot be rearranged to avoid starting with a number." - Springer. Therefore, we have chosen to keep our way of writing numbers the same throughout this document. However, we have adapted the placement of percentages as suggested.***

2. The overall Ov16 RDT seroprevalence was 76/248 (30.7%). The overall Ov16 RDT seroprevalence was 76/248 (30.7%).
The overall Ov16 RDT seroprevalence is in percentage, putting 30.7% (76/248). Correct everywhere in the text the way of writing.

Response: ***We have adapted the text to fit the suggested standards.***

Discussion:
1. A significantly higher proportion of children with dermatitis tested Ov16 RDT positive (43.5%) compared to their peers without dermatitis (22.6%, $p<0.001$), suggesting the dermatitis was likely caused by an active *O. volvulus* infection in a large proportion of children.
Other causes of dermatitis in children in the study area must be clarified and discussed.

Response: ***We now include in the discussion: "In resource-constrained settings, when African children present with dermatitis and itching, scabies should be considered as a possible diagnosis. However, the absence of characteristic scabies lesions was noted in the children included in the study. Additionally, since scabies is treatable with ivermectin, its prevalence typically decreases in regions where mass ivermectin distribution occurs."***

2. More elements of discussion should be provided. Studies have shown limitations in the detection of antibodies against the OV-16 antigen. This is after discrepancies in the results with skin snip method according to WHO standards for the detection of microfilariae in the blood. It is important to specify this and give possible reasons. Carrying out the skin snip method in children over 5 with dermatitis could provide more precision on the cause of dermatitis.

Response: ***Indeed, the addition of skin snips would have added more information on active disease in the children. However, skin snip testing is increasingly not very well accepted by local populations because it is an invasive procedure. The use of rapid diagnostic tests is not to diagnose an O. volvulus infection on an individual level but to identify whether transmission is ongoing or not. Furthermore, in the age of ivermectin, the use of skin snip microscopy is becoming redundant. The number of microfilariae in the skin after ivermectin use is low and often below the detection limit. Finally, the fact that Ov16 antibodies can be detected even before the production of microfilariae make it a much better proxy for recent onchocerciasis transmission as it can identify pre-patent infections in recently infected children.***

Reviewer 3 : Yankum Dadzie This is an interesting paper reporting on the effect of using a new onchocerciasis diagnostic tool to follow up an onchocerciasis intervention in an uncontrolled area, comparable to an onchocerciasis naïve area with a high endemicity. Every observation is thus intriguing whilst the interpretation of what is being observed needs to be done cautiously, particularly as regards the application of the new diagnostic tool.

This study is well set out and appears well conducted. However, the paper must address two areas/points which constitute a setback for the paper's strength. The first point is the use of some words or phrases in the paper which are not scientifically appropriate and give the wrong information on the situation in question. The second is about some points in the discussion of the results which portray an opinion which do not seem to be backed by evidence.

1. The following concern comments on the first point with the use of inappropriate words or phrases:
   Introduction: Line 7-10, replace 'Prevention' with 'Elimination' and continue with the sentence and in the 10^{th} line substitute 'control' for 'eradicate'.
   The last sentence of the introduction is confusing as what it states is not was what was done. I suggest the following formulation: "This paper presented the effect of strengthening the onchocerciasis elimination interventions as demonstrated by seroprevalence in children aged 3-9 years using the Ov16 SD BIOLINE rapid diagnostic test (RDT)".

Response: ***Suggested alterations done.***

1. Results:
   Under the subtitle 'Ov16 seroprevalence in Maridi over time', the following sentence in lines 3-5, 'The seroprevalence among the three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, non-significantly decreased from 12.5% in 2019 to 8.8% in 2023 (p=0.68)', needs to be reformulated. The three-year-olds born after implementation of more robust onchocerciasis elimination measures could be found only in 2023 and could therefore not have contributed to the RDT seroprevalence in 2019 to enable showing a reduced level in 2023. As the measurements are cross-sectional values the sentence should read correctly thus: The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi measured in 2023 8% and was thus lower than the level of 12.5% measured among three-year-olds in 2019 but the difference was not significant, (p=0.68).

Response: ***Text altered. We now state" The seroprevalence among three-year-olds, born after implementation of more robust onchocerciasis elimination measures like vector control and bi-annual CDTi, was measured at 8% in 2023 and was thus lower than the level of 12.5% measured among three-year-olds in 2019. However, the difference was not significant (p=0.68)."***

1. My main concern is with the "discussion" and it is specifically about the interpretation of the results from the study. The first paragraph of the discussion points out the increase, albeit insignificant, of the seroprevalence in the 4–9-year-olds following enhanced onchocerciasis measures and interprets the 'increased seroprevalence as 'an indication of intensification of transmission'. Such an interpretation, "increase in transmission" cannot be correct as the enhanced intervention measures clearly must have had increased effect entomologically as well as epidemiologically, through twice a year CDTi even if the coverage was not optimal yet, on the transmission. The entomological impact of "slash and clear" has been reported in the ref. no 5. The publication, "A force-of-infection model for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area by Remme, J *et al.* in the Bulletin of the World Health Organization, 64: 667-681 (1986) on which the onchocerciasis mathematical model is based, shows that at the onset of intervention of vector control, which interrupts transmission almost immediately, the mf prevalence of age groups in years 0-4, 5-9, continues to rise during the first five years after which it starts falling. It is only in the adults that there is a fall of mf prevalence which is seen in the first three years. A similar effect is found with CDTi where it is even more accentuated the higher the pre-intervention endemicity level of the community. It may be recalled that impact assessment of CDTi in APOC countries did not occur until after five years of CDTI when impact would be expected. The notion was based on this observation. Recently, Lont *et al.* have shown that there are basic similarities in the effect of application of mf prevalence and seroprevalence in their publication (Lont *et al.*, 2017[1]). The increase in the seroprevalence recorded in the age group of 3-9 years old children is normal and it is to be expected in that age group particularly when dealing with communities with very high pre-intervention endemicity. It may be noted that some of the young might harbour prepatent infection before the start of the intervention and which may break out in the course of the intervention as Lont *et al.* discuss in their paper.

As regards to the above considerations, certain statements in the discussion will have to be reviewed and I would like to point them out:
1st paragraph, line 3-5 "These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population". The sentence may be deleted as what has been found is to be expected and importantly does not suggest an absence of impact. The continuation is correct as it takes a few years before interruption of transmission can occur showing also the absence of new infections or new seroconversions as applicable to CDTi.
Response: We thank you for these important comments. We eliminated the sentence "These results suggest that the elimination measures did not significantly impact the seroprevalence of the study population". We now included in the discussion "An increase in the seroprevalence in the age group of 3-9 years old children was to be expected, particularly because of the very high pre-intervention endemicity. Indeed some of the children might harbour prepatent infection before the start of the intervention which may break out in the course of the intervention. A force-of-infection model for onchocerciasis has shown that even during interruption of O. volvulus transmission by vector control, the microfilarial prevalence of the 0-4 and 5-9 year old age groups continues to rise during the first five years after which it starts falling." And we added the following two references Lont YL, Coffeng LE, de Vlas SJ, Golden A, de Los Santos T, Domingo GJ, Stolk WA. PLoS Negl Trop Dis. 2017 Jan 23;11(1):e0005314. doi: 10.1371/journal.pntd.0005314. eCollection 2017 Remme J, Ba O, Dadzie KY, Karam M. A force-of-infectionmodel for onchocerciasis and its applications in the epidemiological evaluation of the Onchocerciasis Control Programme in the Volta River basin area. Bull World Health Organ. 1986;64(5):667-81

1. Line 10, "may indicate an intensification of *O. volvulus transmission*" may be deleted for the same reason as above.

Response: We deleted the the text mentioning the '* intensification of *O. volvulus transmission"

1. Paragraph 2 is well discussed. However, I would like to point out a striking mitigating factor which has either been downplayed or overseen. It is true that no CDTi was carried out in 2020 because of Covid-19 with the negative implications for which Hamley's good work has been quoted to highlight the effect on this study. However, a good look at the first-year results following the first slash and clear activity as read from the paper by Raimon *et al.* suggests that Maridi focus possibly did not do as badly as it has been described in this paper. A proper analysis of the events could very well indicate that there could have been more reduction in transmission in the year 2020 effected by the slash and clear than what could have been achieved by annual CDTI alone before the slash and clear was introduced.

Response: We agree that the strengthening of the onchocerciasis elimination program decreased the O. volvulus transmission in the area. This was strongly suggested by the results of a longitudinal population-based study that showed that the incidence of onchocerciasis-associated epilepsy, including nodding syndrome, decreased in Maridi. This paper has now been published and is included among the references. Jada SR, Amaral LJ, Lakwo T, Carter JY, Rovarini J, Bol YY, Logora MY, Hadermann A, Hopkins A, Fodjo JNS, Colebunders R. Effect of onchocerciasis elimination measures on the incidence of epilepsy in Maridi, South Sudan: a 3-year longitudinal, prospective, population-based study. Lancet Glob Health. 2023 Aug;11(8):e1260-e1268. doi: 10.1016/S2214-109X(23)00248-6.

1. In the penultimate line of the paragraph, kindly substitute ‘eliminate blackflies’ for ‘eradicate blackflies’.

Response: Text altered.

1. Paragraph 5:
   I wonder whether after the foregoing discussions it is still worth retaining this statement, particularly the second half of the statement. “A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested before and after interventions to evaluate the short-term effect of strengthening an onchocerciasis elimination programme”.

Response: We now state “A sufficiently large sample size of 3-year-old children needs to be Ov16 RDT tested and a longer follow-up is needed to investigate the effect of strengthening an onchocerciasis elimination programme.”

Competing Interests: No competing interests were disclosed.