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Ways to mitigate the public health problem caused by onchocerciasis-associated epilepsy

Thesis submitted for the degree of Doctor of Medical Sciences at the University of Antwerp to be defended by

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Cover picture – Onchocerciasis Larvae at Itimbi river, Ituri Area, Democratic Republic of Congo. Photo credit : Anne Laudisoit

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To my family and relatives, this work is dedicated to you. A special thanks to my dad Jean Ntamuturano, my mum Josephine Nyiramahane, my uncle Jean Dusingizimana, and my aunt Patricie Nyirabageni who supported me in great and small things. This work is also dedicated to my partner Janvière Hirwa Basenga who was always there to encourage me. I also dedicate this dissertation to my friends who supported me throughout the process.

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List of acronyms

APOC	African Programme for Onchocerciasis Control
CDDs	Community-Directed Distributors
CDTI	Community-Directed Treatment with Ivermectin
CMFL	Community Microfilaria Load
DRC	Democratic Republic of Congo
ESPEN	Expanded Special Programme for Elimination of Neglected Tropical Diseases
ILAE	International League Against Epilepsy
LMIC	Low- and Middle-Income Countries
MDA	Mass Drug Administration
Mf	Microfilariae
NS	Nodding Syndrome
OAE	Onchocerciasis-Associated Epilepsy
OCP	Onchocerciasis Control Programme in West Africa
Ov16	Anti-*Onchocerca volvulus* IgG4 antibodies
PWE	Person(s) With Epilepsy
RDT	Rapid Diagnostic Test
REMO	Rapid Epidemiological Mapping of Onchocerciasis
WHO	World Health Organization

Summary

Samenvatting

Summary

Epilepsy is a chronic condition characterized by repeated unprovoked seizures, currently affecting over 50 million individuals globally with more than 80% of those residing in developing countries. *Onchocerca volvulus* (*O. volvulus*) is the parasite that causes onchocerciasis (river blindness), well known for causing skin and eye disease. Currently, over 20.9 million people are *O. volvulus*-infected worldwide, of which 14.6 million have skin disease and 1.15 million suffer from vision loss. Epidemiological evidence, mainly recently generated by the ERC funded Nodding syndrome aetiology (NSETHIO)-project, suggests that an *O. volvulus* infection potentially could trigger epilepsy, so-called onchocerciasis-associated epilepsy (OAE). OAE is an important public health problem in onchocerciasis endemic areas with high past or ongoing *O. volvulus* transmission. With this thesis we aim to identify ways to mitigate the impact of OAE in onchocerciasis endemic areas.

The studies presented in this thesis were carried out as part of the NSETHIO project. In a NSETHIO case control study conducted in an ivermectin-naïve population living in an onchocerciasis endemic region in Ituri, Democratic Republic of the Congo, the *O. volvulus* Ov16 IgG4 antibodies seropositivity and the presence of skin microfilarial density in persons with epilepsy (PWE) were nearly twice as many compared to the controls.

In a first study, we assessed the *O. volvulus* Ov16 IgG4 antibody seroprevalence among PWE in four onchocerciasis-endemic regions in Africa. In these regions, the Ov16 antibody seroprevalence among the PWE ranged from 35.2 to 59.7% and increased with age until the age of 39 years, after which it decreased considerably. This suggests that in onchocerciasis-endemic regions, epilepsy in the younger age groups is often associated with recent exposure to blackflies that transmit *O. volvulus* infection, while in older persons the epilepsy is not related to recent *O. volvulus* exposure.

In a second study, we investigated why in onchocerciasis endemic regions, despite many years of community-directed treatment with ivermectin (CDTI), still a high prevalence of OAE is observed. More specifically, we investigated the ivermectin treatment response in *O. volvulus*-infected PWE. We did not observe evidence for an

overall sub-optimal ivermectin treatment response. However, a higher pre-ivermectin microfilarial (mf) density was associated with an increased probability of a positive skin snip after ivermectin treatment. Furthermore, a higher number of ivermectin treatment rounds was associated with a higher probability of having a detectable post-treatment mf density. Our findings suggest that ongoing onchocerciasis transmission in endemic sites may be a consequence of a poor performance of local onchocerciasis elimination program and not of a sub-optimal ivermectin treatment response.

In a third study, we assessed the potential effect of ivermectin on the frequency of epileptic seizures in *O. volvulus*-infected PWE. Mediation analysis showed that ivermectin reduced the frequency of seizures of *O. volvulus*-infected PWE through reduction in skin mf density, but also potentially through a direct anti-seizure effect. This reduction was most pronounced in *O. volvulus*-infected PWE with a high seizure frequency before ivermectin intake.

In a fourth study, we investigated the use of Ov16 IgG4 rapid diagnostic testing (RDT) among 6–10-year-old children (a proxy for ongoing *O. volvulus* transmission) to monitor the performance of onchocerciasis elimination programs. Using data collected during door-to-door epilepsy surveys in 18 onchocerciasis-endemic foci in 8 countries, we found that Ov16 RDT seroprevalence among 6-10-year-old children, epilepsy prevalence and incidence, and ivermectin therapeutic coverage data convey useful information to identify areas where onchocerciasis-elimination programs need to be strengthened or introduced.

Finally, we evaluated the societal and economic impact of a community-based epilepsy treatment program (CBETP) implemented in an onchocerciasis endemic area in the Logo health zone, Ituri province, the Democratic Republic of Congo. The CBETP induced a positive change in perceptions and attitudes towards PWE. The decentralization of epilepsy treatment at the community level empowered the local health care workers and enriched the knowledge to manage epileptic seizure attacks in the community. PWE and their family experienced less epilepsy related stigma and consulted less frequently traditional healers. The latter showed a growing willingness to collaborate with local health professionals in the management of PWE. The CBETP by providing anti-seizure medication reduced the direct and indirect costs incurred by the family because of epilepsy.

Conclusion

Our studies confirm the recent and overwhelming epidemiological evidence for the association between onchocerciasis and epilepsy. The current high prevalence of OAE seems to be the consequence of sub-optimal performance of onchocerciasis elimination programs and not of a sub-optimal ivermectin treatment response. Ineffective onchocerciasis elimination programs can be identified by an Ov16 RDT seroprevalence study among 6–10-year-old children. Onchocerciasis elimination programs should prioritize efforts towards onchocerciasis-endemic regions with a high epilepsy prevalence and incidence.

Samenvatting

Epilepsie is een chronische aandoening gekenmerkt door herhaalde niet-uitgelokte aanvallen. Epilepsie treft momenteel wereldwijd meer dan 50 miljoen personen, rond 80% leven in ontwikkelingslanden. *Onchocerca volvulus* (*O. volvulus*) is de parasiet die onchocerciasis (rivierblindheid) veroorzaakt, en waarvan bekend is dat ze huid- en oogaandoeningen veroorzaakt. Momenteel zijn meer dan 20,9 miljoen mensen wereldwijd besmet met *O. volvulus*, van wie 14,6 miljoen een huidziekte hebben en 1,15 miljoen verlies van gezichtsvermogen. Epidemiologisch bewijs, voornamelijk recent gegenereerd door het door het ERC gefinancierde Nodding Syndrome etiology (NSETHIO)-project, suggereert dat een infectie met *O. volvulus* epilepsie zou kunnen veroorzaken (onchocerciasis-geassocieerde epilepsie; OAE). OAE is een belangrijk volksgezondheidsprobleem in onchocerciasis endemische gebieden met een hoge transmissie van *O. volvulus*. Met dit proefschrift willen we manieren identificeren om de impact van OAE in onchocerciasis endemische gebieden te verminderen.

De studies die in dit proefschrift worden gepresenteerd, zijn uitgevoerd als onderdeel van het NSETHIO-project. In een NSETHIO case-control studie uitgevoerd bij een ivermectine-naïeve populatie in een onchocerciasis endemisch gebied in Ituri, Democratische Republiek Congo, bleek dat ongeveer twee keer zoveel personen met epilepsie (PWE) *O. volvulus* Ov16 IgG4-antilichamen vertoonden en microfilarial in huidknipsels in vergelijking met controles.

In een eerste studie bepaalden we de seroprevalentie van *O. volvulus* Ov16 IgG4-antilichamen bij personen met epilepsie (PWE) in vier onchocerciasis-endemische regio’s in Afrika. De Ov16 seroprevalentie varieerde van 35.2% tot 59.7% en nam toe met de leeftijd tot de leeftijd van 39 jaar, waarna deze aanzienlijk daalde. Dit suggereert dat in onchocerciasis-endemische regio's epilepsie in de jongere leeftijdsgroepen vaak geassocieerd is met blootstelling aan *O. volvulus*, terwijl bij oudere personen epilepsie niet gerelateerd is aan blootstelling hieraan.

In een tweede studie onderzochten we waarom in onchocerciasis endemische gebieden, ondanks vele jaren van gemeenschapsgerichte behandeling met ivermectine (CDTI), nog steeds een hoge prevalentie van OAE wordt waargenomen.

In het bijzonder onderzochten we respons op behandeling met ivermectine bij met *O. volvulus* geïnfecteerde PWE. We hebben geen bewijs voor een veralgemeend suboptimale respons op de behandeling met ivermectine. Een hogere pre-ivermectine microfilaria (mf) dichtheid was echter geassocieerd met een verhoogde kans op een positieve huidsnip na behandeling met ivermectine. Bovendien was een groter aantal ivermectine behandelingsrondes geassocieerd met een detecteerbare mf in de huid na de behandeling. Onze bevindingen suggereren dat aanhoudende overdracht van onchocerciasis op endemische plaatsen een gevolg is van een lage CDTI-dekking en niet van een suboptimale respons op de ivermectine behandeling.

In een derde studie onderzochten we het effect van ivermectine op de frequentie van epileptische aanvallen bij met *O. volvulus* geïnfecteerde PWE onderzocht. Mediatie-analyse toonde aan dat ivermectine de frequentie van aanvallen bij met *O. volvulus* geïnfecteerde PWE verminderde door een vermindering van de mf-dichtheid in de huid, maar mogelijks ook door een direct anti-epileptisch effect. Deze vermindering was het meest uitgesproken bij met *O. volvulus* geïnfecteerde PWE met een hoge baseline frequentie van epileptische aanvallen.

In een vierde studie onderzochten we het gebruik van Ov16 IgG4 snelle diagnostische testen (RDT) bij 6-10-jarige kinderen (als proxy voor voortdurende *O. volvulus*-overdracht) om de prestaties van onchocerciasis-eliminatieprogramma's te beoordelen. Met behulp van gegevens verzameld tijdens epilepsie-onderzoeken in 18 onchocerciasis-endemische foci in 8 landen, ontdekten we dat Ov16 RDT-seroprevalentie bij 6-10-jarige kinderen, epilepsie prevalentie en incidentie, en gegevens over de dekkingsgraad van de behandeling met ivermectine nuttige informatie is om gebieden te identificeren waar de onchocerciasis-eliminatie-programma's moeten worden versterkt of ingevoerd.

Tenslotte evalueerden we het effect van een community-based epilepsie behandelingsprogramma (CBETP) geïmplementeerd in een onchocerciasis endemisch gebied in de Logo gezondheidszone, in Ituri in de Democratische Republiek Congo. Dit CBETP veroorzaakte een positieve verandering in de perceptie en houding ten opzichte van PWE. De decentralisatie van epilepsiebehandeling op gemeenschapsniveau sterkte de lokale gezondheidswerkers en verrijkte de kennis om aanvallen te beheersen in de gemeenschap. PWE en hun familie ervoeren minder

epilepsie gerelateerde stigmatisering en raadpleegden minder vaak traditionele genezers. Deze laatsten toonde een groeiende bereidheid om samen te werken met gezondheidswerkers bij de begeleiding van PWE. Door het verstrekken van anti-epileptische medicatie verminderde de directe en indirecte epilepsie kosten voor de familie wegens epilepsie.

Conclusie

Onze studies bevestigen het recent en overweldigend epidemiologische bewijs voor de associatie tussen onchocerciasis en epilepsie. De huidige hoge prevalentie van OAE lijkt het gevolg te zijn van ineffectieve onchocerciasis-eliminatieprogramma's en niet van een suboptimale respons op de ivermectine behandeling. Ineffectieve onchocerciasis eliminatieprogramma’s kunnen worden identificeerd bepaald aan de hand van een Ov16 RDT-seroprevalentieonderzoek bij 6-10-jarige kinderen. Onchocerciasis eliminatieprogramma’s moeten vooral versterkt worden in onchocerciasis-endemische regio's met een hoge prevalentie en incidentie van epilepsie.

CHAPTER 1.

General Introduction

Chapter 1.I. Background

I. 1. Onchocerciasis

Onchocerciasis known as river blindness is a parasitic disease frequently occurs in tropical areas. Onchocerciasis is caused by the filarial nematode *Onchocerca volvulus* (*O. volvulus*) and transmitted by blackflies (*Simuliidae*) through the repeated bites [1]. Blackflies breed along fast-flowing rivers and streams. Individuals residing in a blackflies breeding area accumulate the worms through the repeated life-long exposures to blackfly bites [1]. *O. volvulus* infection is known to cause dermal and ocular disease (onchocerciasis) induced by an acute inflammatory response to dead or dying worms in subcutaneous tissue or cornea [2].

I.1.1 Life cycle of Onchocerciasis

***O. volvulus* life cycle within the blackfly:** During a blood meal, the blackfly ingests the microfilariae (mf) from an *O. volvulus*-infected person. These ingested mf develop in the salivary glands of the blackfly into third stage larvae (L3) (Figure 1). A female blackfly with L3 larvae is infectious.

***O. volvulus* life cycle within humans:** *O. volvulus* infection occurs when the infectious female blackfly bites a health person and drops *O. volvulus* (L3) into the skin. The L3 larvae dropped by infectious blackfly remain in the dermis and subcutaneous tissue and mature into adult male/female worms after 6 to 12 months [1]. The female fertile adult worms are capable to daily produce 1000-3000 mf that migrate through the skin, eyes and other human organs [3]. After several years the adult worms in subcutaneous tissue start to form the onchocercoma (nodules) which are located on bony prominences and easily palpable [4]. Mf can stay in the human host up to 2 years, but the adult female worms typically live from 2-15 years in human hosts and continue to produce mf during their entire life span [1].

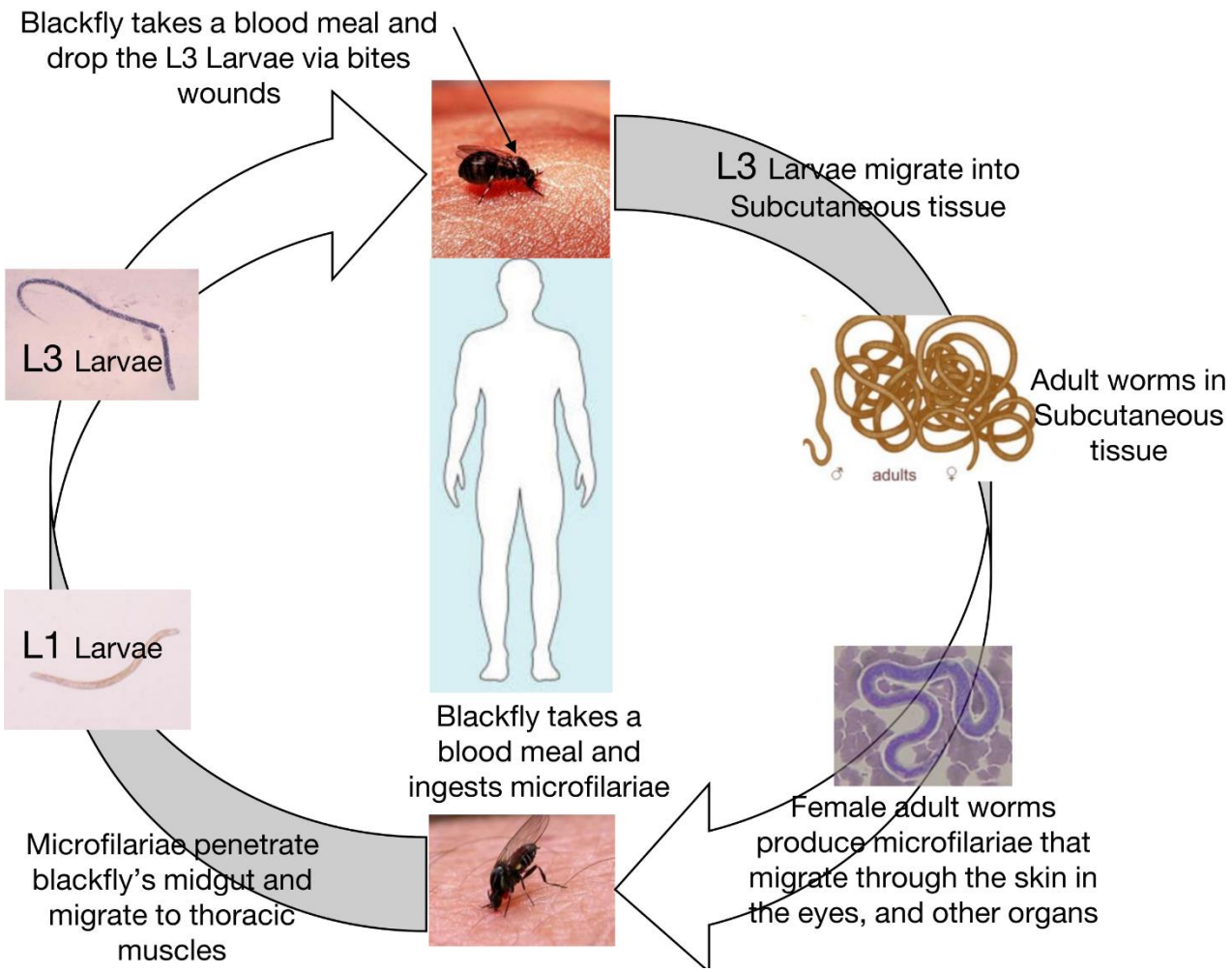

Figure 1. *Life cycle of O. volvulus infection adapted from a figure created for the Centre for Disease Control and Prevention [5].*

I. 2. Epidemiology of Onchocerciasis

Of 34 countries in which onchocerciasis is still present, 31 are located in Sub-Saharan Africa (Figure 2) [6]. Onchocerciasis is also present in some foci in Latin America (Brazil and Venezuela) and Yemen. Since 2012, four countries in South and Central America (Colombia in 2013, Ecuador in 2014, Mexico in 2015, and Guatemala 2016) have been certified to be free of onchocerciasis after successfully controlling and implementing elimination activities for decades [6].

In 2017, at least 20.9 million people were *O. volvulus*-infected worldwide, of which 14.6 million had skin disease and 1.15 million had vision loss [7]. According to the recent data of WHO, in 2020 over 240 million people worldwide were estimated to be at risk

of contracting onchocerciasis and therefore potentially requiring chemotherapy against *O. volvulus* infection [6]. Of these, only 112.6 million (46.6%) received treatment against onchocerciasis in 2020 [8]. In 2020, during the (start of the) SARS-CoV-2 (or COVID-19) pandemic the number of individuals who received treatment for onchocerciasis declined by 26.9% as compared to 2019. A survey conducted by the WHO to quantify the impact of COVID-19 on neglected tropical diseases (NTDs) in May-July 2020 and January-March 2021 revealed that the interventions targeting NTDs were disrupted in 19% of the 109 participating countries due to the interruption of community-based activities notably mass treatment with ivermectin and vector control [9].

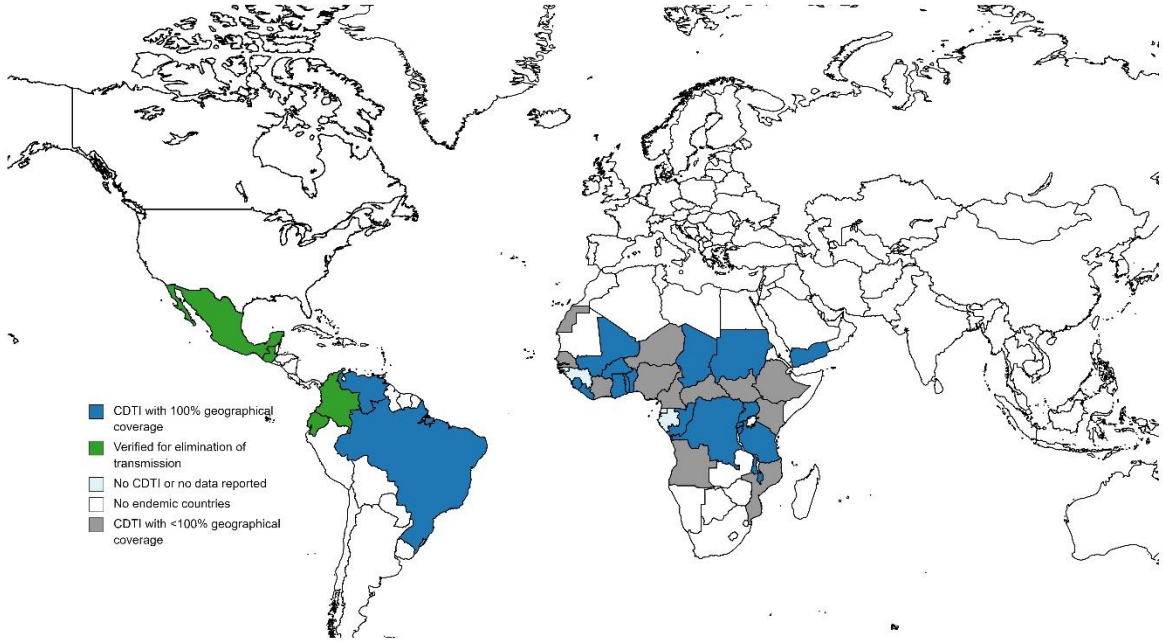

Figure 2. *Worldwide distribution of onchocerciasis and status of community-based treatment with ivermectin in 2019 [6]*

Onchocerciasis and its related morbidities constitute a public health problem and a barrier to socio-economic development in the affected areas. In 2017, it was estimated that 92% of 1.34 million corresponding to 1.24 million years lived with disabilities (YLDs) for onchocerciasis were caused by onchocercal skin disease and 96,100 YLDs were caused by onchocercal eye disease [7]. The mitigation of onchocerciasis-associated morbidity and optimal onchocerciasis elimination would lead to substantial health and economic benefits in the endemic countries. Predictive models revealed

that an optimal onchocerciases strategy in onchocerciasis endemic countries would prevent 4.3 to 5.6 million of disability-adjusted life years (DALYs) until 2045 [10].

I. 3. Onchocerciasis clinical manifestations

I. 3. 1. Skin and Eye disease

Onchocerciasis is well reputed to cause the skin and eye diseases. Skin and ocular lesions are induced by an inflammatory reaction to the death or dying of mf [2]. The most common and early skin symptom of onchocerciasis is onchocercal dermatitis [1]. Onchocercal dermatitis is characterised by severe itching, alterations in skin pigment, and scratching [11].

The subcutaneous nodules (onchocercoma) are the consequence of adult worms encapsulated in subcutaneous tissue [12]. The onchocercoma are formed because of a response of the human host to an organism continuously producing foreign proteins [11].

Ocular symptoms appear at a later stage of *O. volvulus* infection when the mf invade the cornea. The dead or dying mf in the cornea provocate corneal inflammation which results in visual impairment and river blindness [1]. Since the ivermectin era, the blindness caused by onchocerciasis has significantly decreased. According to the data collected by the Onchocerciasis Control Programme in West Africa (OCP) during 1971–2001, the percentage of people with blindness caused by onchocerciasis was reduced from 29.7% of the total of *O. volvulus*-infected persons to 19.6% after the introduction of ivermectin treatment (during 1988-2001) [14].

However, blindness caused by onchocerciasis is still prevalent in areas where onchocerciasis elimination programs are poorly implemented. In a recent study conducted in Mvolo in South Sudan, an area with high ongoing *O. volvulus* transmission and low coverage of ivermectin, 445 (2.8%) of 15,699 study participants were blind or had a blurred vision in at least one eye [15]. In a study conducted in an *O. volvulus* endemic area, in Northern District of Ghana where mass community-directed treatment with ivermectin (CDTI) had been ongoing for more than twenty years, about 4.4% of the *O. volvulus*-infected persons were blind [13].

I. 3. 2. Neurological manifestation of onchocerciasis

A study conducted in Cameroon among children aged 6–16 years suggested that *O. volvulus* infection may alter the cognitive function of children exposed to *O. volvulus* infection [16]. In Nigeria *O. volvulus* infection was linked to poor school performance and a higher drop-out rate among school-aged children [17].

Recent epidemiological evidence suggests that onchocerciasis is linked with epilepsy, so-called onchocerciasis-associated epilepsy (OAE) [18]. The link between *O. volvulus* infection and epilepsy was suspected since in 1938 in an onchocerciasis endemic area in Mexico by Guillaume Casis Sacre [19]. In Cameroon, it has been shown that the risk to develop epilepsy is determined by the level of *O. volvulus* infection in early childhood [20].

An operational definition of OAE term was first proposed in 2018 to be able to estimate the burden of epilepsy potentially associated with onchocerciasis in the epidemiological studies conducted mainly in onchocerciasis foci [21]. The initial proposed definition was later refined in 2019 where OAE is suspected in a person meeting all six criteria listed below [22].

Criteria for OAE :

1. History of ≤ 2 unprovoked seizures at least 24 hours apart.
2. No severe disease that can cause epilepsy during the 5 years preceding seizure onset : perinatal brain insult, head trauma, previous central nervous system infection or any other disease
3. Age onset of epilepsy between 3 and 18 years.
4. Normal psychomotor development before the onset of epileptic seizures
5. Living in blackflies biting regions at least 3 years
6. Living in a village with a high frequent occurrence of clusters of persons with epilepsy in the same family

OAE can also present with different clinical presentations including nodding syndrome and Nakalanga syndrome.

I. 3. 3. Nodding syndrome

Initially, documented in Mahenge village, Tanzania in 1970 [23], Nodding Syndrome (NS) is a form of epilepsy characterized by repeated head nodding attacks. NS has now been reported in other onchocerciasis endemic areas in Uganda, South Sudan and Democratic Republic of Congo (DRC) [24], Cameroon [25], Liberia [26] and the Central African Republic [27].

Nodding syndrome is mainly reported in the remote villages with high ongoing *O. volvulus* transmission [28] and mostly occurs in children within the same family [24]. A study in Uganda where NS was endemic in the past showed that 33.6% of the children with NS had at least one sibling with another form of epilepsy [29]. The clustering of NS in the same family was also documented in the village of Mvolo, Western Equatoria State in South Sudan where nine (40.9%) of 22 families participated in the study had at least two children with nodding syndrome or another form of epilepsy [24].

I. 3. 4. Nakalanga syndrome

Initially described in Uganda in 1962 [30], Nakalanga Syndrome was later reported in South Sudan, Burundi, Ethiopia, and Cameroun [31]. It mainly affects healthy children aged between 3-10 years that were previously developing normally [30, 31]. Nakalanga is characterized by retarded growth, poor muscle development, delayed sexual development, postural deformities, epilepsy and mental impairment [31].

I. 4. Onchocerciasis Diagnosis

I. 4. 1. Clinical Evaluation

Onchocerciasis can be suspected by observing onchodermatitis and the presence of onchocerciasis nodules which can be detected by palpation. However, the detection of onchocerciasis nodules by palpation requires qualified personnel [32].

Onchocerciasis can also be examined with a slit lamp to visualise *O. volvulus* nematodes in the anterior chamber [32]. Similarly, ocular examination with a split lamp to detect mf in the eye requires special expertise and expensive equipment.

I. 4. 2. Detection of *O. volvulus* by skin snip microscopy

O. volvulus active infection is diagnosed microscopically by detecting mf in skin snips (small superficial skin biopsies) taken from the left and right iliac crests [33]. Microscopic evaluation of skin biopsies has been used by the most onchocerciasis control programmes in Africa [34] for diagnosis and monitoring of *O. volvulus* active infection [35]. The skin snip method is performed using a sclerocorneal biopsy punch by removing about 2 mg of skin tissue. The tissue is then incubated in normal saline for 24 hours at room temperature to allow the larvae to emerge. After the incubation period, the mf larvae can be identified microscopically. The presence of *O. volvulus* mf in any of the skin snips confirms the diagnosis.

The performance of skin snip microscopy is limited in a setting with a low intensity of *O. volvulus* infection and in a pre-patent stage of infection [36]. Studies showed that, sensitivity of skin snip microscopy among individuals with low microfiladermia (mean between left and right mf count below 2 mf per skin snip) decreased to 40.4% [36]. Because of its low sensitivity, currently microscopic skin snip testing, is often replaced by DNA-based skin snip testing [36-38]. Therefore, the microscopic skin snips test method is not useful to determine when to stop ivermectin mass drug distribution.

I. 4. 3. Detection of *O. volvulus* infection by polymerase chain reaction (PCR) of skin snips

O. volvulus infection is also diagnosed by PCR to detect *O. volvulus*-DNA in skin biopsies. DNA-based methods have been developed mainly to detect *O. volvulus* infection when the intensity of *O. volvulus* infection is very low [34, 36-38]. Because of its high sensitivity as compared to standard microscopic skin snip test [39-41], the *O. volvulus*-specific PCR test is recommended to confirm the interruption of *O. volvulus* transmission [34].

I. 4. 4. Serological test to detect exposure to *O. volvulus* by Ov16 IgG4 antibodies

The *O. volvulus* serological test detects the presence of IgG4 antibodies to the Ov16 antigen by Ov16 enzyme-linked immunosorbent assay (Ov16 ELISA) [42] or by Ov16

rapid diagnostic test (Ov16 RDT) [43]. The accuracy of serological tests depends on the level of onchocerciasis endemicity in the area [44]. In the highly onchocerciasis endemic areas, the serological test was found to be more accurate (sensitivity : 70-90%) and (specificity : 80-100%) [38, 43]. Currently, the Ov16 ELISA test in children under 11 years is recommended by WHO for the monitoring ongoing *O. volvulus* transmission and for deciding the interruption of transmission in the area [45].

I. 4. 5. Diethylcarbamazine (DEC) Patch Test

Initially, DEC has been used to treat onchocerciasis [46], but because of its side effects among *O. volvulus*-infected persons, its use was stopped in 1980 [47]. A DEC patch test can be used to detect mf in skin of *O. volvulus*-infected persons via a localized skin reaction. This reaction is caused by mf killed upon skin exposure to DEC [33, 48]. The DEC patch test results are only available after 24-48 hours [37]. In low endemicity setting, the DEC patch test is less sensitive. Therefore this test is inadequate to prove the success of an onchocerciasis elimination program and is currently only used in research settings [32].

I. 5. Onchocerciasis control and elimination

To eliminate onchocerciasis, WHO recommends at least annual large-scale mass drug distribution of ivermectin administrated under direct observation (CDTI) or the combination of CDTI and vector control [45].

I. 5. 1. Vector control

Initially implemented in 1975 [49], *simulium spp.* vector control was the main strategy to control onchocerciasis until anti-parasitic medications against onchocerciasis became available in 1987 [50]. The large scale s*imulium spp.* vector control initially implemented in 11 countries located in West Africa by onchocerciasis control programme (OCP) and was originally based on aerial larviciding against blackfly larvae over fast-flowing rivers and streams [51]. The vector control in OCP area, successfully prevented approximately 600,000 cases of blindness and interrupted the transmission of *O. volvulus* in West Africa [52]. The strategy of controlling *O. volvulus* infection by killing blackflies larvae experienced technical challenges including insecticide resistance and blackfly migration across the borders [53] and it was also

very costly. Following the approval ivermectin treatment in 1987, OCP coupled the blackfly control strategy with ivermectin treatment. The combination of vector control and ivermectin treatment led to the elimination of onchocerciasis as public health problem in West Africa [52].

I. 5. 2. Onchocerciasis control with chemotherapy

I. 5. 2. 1 Ivermectin Treatment

Onchocerciasis is effectively treated by ivermectin treatment (brand name Mectizan®). The use of ivermectin treatment in human was first approved in 1987 [54, 55]. Since its approval, over 2.7 billion treatments were administered for control and elimination of onchocerciasis [56, 57].

Given orally (150 µg/kg), ivermectin rapidly kills mf, relieves intense skin itching and halts the progression towards blindness [54, 58]. A single dose of ivermectin treatment reduces microfiladermia by half after 24 hours, by 85% after 72 hours, by 94% after 1 week, and by 98-99% after 1-2 months [58].

Ivermectin does not kill adult worms but suppresses the production of mf by adult female worms for a few months following treatment [59, 60]. Adult worms can live for 15 years in the human body and continue to produce mf until they die naturally [51, 61]. Ivermectin treatment must be taken at least yearly for 16-18 years to stop onchocerciasis transmission [62, 63] but may need to be continued longer in high endemic areas with high blackfly biting rates [64].

I. 5. 2. 2 Moxidectin treatment

Recently, a new anti-parasitic drugs moxidectin was approved to treat human *O. volvulus* infection [65]. Phase II and III clinical trials demonstrated a more prolonged microfilaricidal and embryostatic effect of moxidectin as compared to ivermectin treatment [66]. Moxidectin treatment leads to a sustained low skin mf load, with no detectable mf within the 6 months following treatment intake [66]. Therefore, moxidectin is a promising, effective and safe agent that potentially will accelerate onchocerciasis elimination.

I. 5. 2. 3 Other drugs targeting *Wolbachia*

Wolbachia is an endosymbiont of the *O. volvulus* parasite. This bacteria is essential for the reproduction and the long-term viability of the adult worms [67]. *Wolbachia*-derived molecules released by death or dying worms induce an inflammatory response in the skin and eye [68]. This inflammatory response in the cornea leads to river blindness.

Recently, anti-parasitic drugs with anti-*Wolbachia* effect have been of interest. Doxycycline is an effective and safe anti-*Wolbachia* therapy [69]. A proof-of-concept clinical trial using doxycycline in humans demonstrated the sustained microfilaricidal and macrofilaricidal effects of 6-weeks doxycycline for up to two years [70]. The use of anti-*Wolbachia* therapy in onchocerciasis elimination programmes is limited by the fact that it requires two weeks treatment and for safety reasons doxycycline should not be given to children under eight years [70]. Studies are ongoing to investigate the safety of new anti-*Wolbachia* agents with a shorter course of treatment [71].

I. 6. The ivermectin treatment use in onchocerciasis control

Building on the OCP success, the African Programme for Onchocerciasis Control (APOC) was launched in 1995 with the main mission to control onchocerciasis as a public health problem in 20 African countries not covered by OCP [72]. APOC implemented a CDTI as core strategy where the affected communities took the responsibilities for the distribution, supervision and monitoring of ivermectin treatment [73]. Twenty-years of APOC activities resulted in the decline of *O. volvulus* infection prevalence and river blindness in all the covered countries [74]. Between 1996-2015, APOC saved 17.4 million disability-adjusted life-years (DALYs) [72].

In 2009, APOC together with WHO started debating the possibility to shift from control to elimination of onchocerciasis in Africa. The debate was stimulated by the success of the onchocerciasis elimination efforts in the Americas [75] where onchocerciasis was eliminated in 11 of the 13 endemic foci. Similarly, empirical evidence in some onchocerciasis foci in Senegal, Mali [62], Uganda [76] and Sudan [77], showed that long-term CDTI with high coverage can interrupt onchocerciasis transmission.

However, in Mali and Senegal the evidence for the interruption of onchocerciasis was based on parasitological test results (skin snips microscopic and a DEC patch test results). According to WHO guidelines, parasitological tests should only be used to monitor progress during the treatment phase of onchocerciasis elimination but not to verify interruption of transmission [45].

After the discussion and revision of data generated by APOC, in 2009 the paradigm was shifted from control to elimination of onchocerciasis in all endemic countries [6]. It was considered that the long-term implementation of CDTI with >80% coverage would lead to an elimination of onchocerciasis-related morbidity and mortality [78]. Annual or bi-annual CDTI already interrupted onchocerciasis transmission in 11 of 15 foci in four countries in South America and in some foci in Mali, Senegal, Sudan and Uganda [6]. WHO expect the interruption of onchocerciasis transmission by 2030 in 8 additional countries [6].

In 2015, following the closure of APOC operations, WHO established a new structure to tackle the impact of neglected tropical diseases (NTDs). The Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN) was launched mainly to provide technical support for country's activities focusing on the elimination of five NTDs including onchocerciasis, trachoma, lymphatic filariasis, soil transmitted helminths and schistosomiasis [79]. Since the establishment of ESPEN, 69.2-212.7 million of the 600 million people in need of preventive chemotherapy were treated with a coverage between 54.8% and 71.4% [80]. In 2019, the number of people treated for *O. volvulus* infection in Africa increased with 15% from 132 million in 2016 to 152 million in 2019 [81]. In 2020, WHO developed the new road map for NTDs 2021–2030 with global targets to prevent, control, eliminate or eradicate 20 diseases including *O. volvulus* infection [82].

I. 7. Onchocerciasis elimination phases in the endemic countries

The elimination of onchocerciasis using CDTI is carried out in three phases [45] (Figure 3). The first phase (phase 1) consists of the stop of active transmission using annual or bi-annual CDTI. This phase lasts at least 12-15 years with a minimum CDTI coverage of 80% of the eligible population during each round. Although the first phase

lasts more than 10 years, modelling studies suggest that with a higher CDTI coverage, elimination will occur in a shorter period of time [83].

Post-treatment surveillance phase (PTS) starts after CDTI phase which typically lasts 3-5 years. During this phase the country onchocerciasis elimination program conduct rigorous research to ascertain the interruption of *O. volvulus* transmission. At the end of the PTS phase, the post-elimination surveillance phase (PES) starts and aims to provide additional evidence for the permanent interruption of *O. volvulus* transmission.

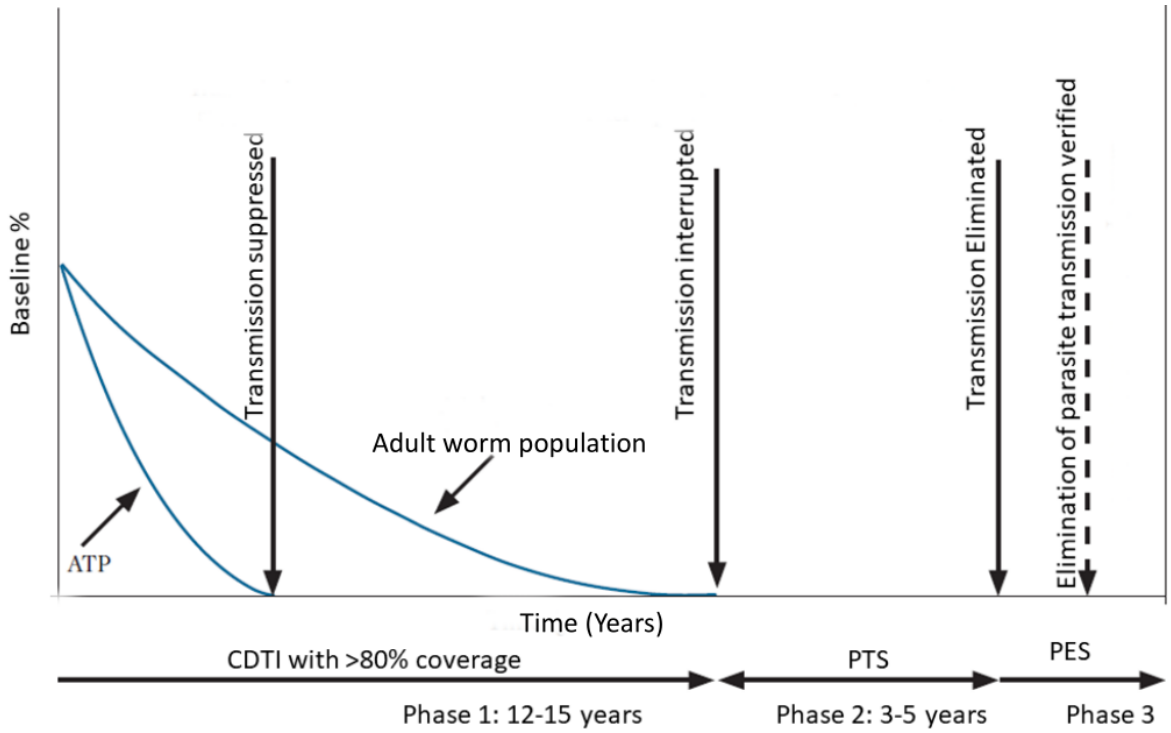

Figure 3. *Phases of elimination of human onchocerciasis, initially developed by WHO [45].*

ATP: Annual transmission potential; PTS: Post-treatment surveillance; PES: Post-elimination surveillance

I. 8. Public health problem caused by onchocerciasis-associated epilepsy

In studies performed in onchocerciasis meso- and hyper-endemic villages in the DRC, Cameroon, Uganda, Tanzania, and South Sudan, an epilepsy prevalence of 2−8% was documented, which is considerably higher than the median prevalence of 1.4% in

African non-endemic regions [18]. Overall 200,000 to 400,000 persons in Africa are estimated to have OAE [84]. OAE is associated with severe co-morbidities and high premature mortality; it is accompanied by severe psycho-socio-economic consequences, including stigma and loss of employment; and it brings a high-cost burden [18]. These psycho-socio-economic burdens affect both children and their families and their communities. Most OAE-affected children present with intellectual disabilities, and they may develop other psychiatric disabilities. Many communities with the highest prevalence of OAE, believe that epilepsy-affected people are possessed by evil spirits [85]. Parents of children with epilepsy often consult traditional healers with many not being aware that evidence-based medical treatment may improve the health condition of the affected children when diagnosed and treated early on. For many children, OAE-related changes become irreversible.

I. 9. The prevention of OAE

Recent evidence suggest that CDTI prevents OAE including NS and Nakalanga Syndrome [18]. In 2017, in the Kitgum and Pader villages of Northern Uganda, the cumulative incidence of NS decreased from 490 to 43 per 100,000 persons per year in eight years after implementing a bi-annual CDTI and ground larviciding of blackfly-infested rivers [86]. Similarly, in Western Uganda OAE occurrence stopped when onchocerciasis was eliminated [87]. In Cameroon, a drastic decrease in epilepsy incidence (773.5 vs 98.0 per 100,000 persons per year) was noted after 13 years of onchocerciasis control using CDTI in onchocerciasis endemic villages along the Mbam and Sanaga river valleys [88]. Despite the strong evidence that CDTI prevents OAE [18], in many onchocerciasis-endemic locations in Africa where CDTI coverage has been sub-optimal or interrupted, there is still high prevalence of onchocerciasis associated morbidity including OAE.

I. 10. Pathogenesis of OAE

Until now, pathophysiological mechanism by which *O. volvulus* infection trigger epilepsy needs to be elucidated. Since 1932, research to identify the mechanism by which *O. volvulus* infection causes neurological conditions has been conducted, but the results are inconsistent. Hissette postulated that mf could invade the central nerves system (CNS) [89]. In 1956 Mazotti [90] and in 1976 Duke et al. [91] detected mf in

cerebrospinal fluid (CSF). However, since the CDTI era no *O. volvulus* or its DNA has been detected in the CNS. For instance, in a recent study performed in *O. volvulus*-infected persons with epilepsy (PWE) in South Sudan, no *O. volvulus* mf nor *O. volvulus* and *Wolbachia* DNA were detected in CSF of persons with OAE [92]. Also in a post-mortem study of OAE cases in Uganda, no *O. volvulus* mf nor *O. volvulus* and *Wolbachia* DNA were detected [93]. This suggests that the invasion of the CNS by mf is unlikely. It may be that in the studies by Mazotti and Duke, because of a very high microfilarial load, mf from the skin entered the CSF during the cerebrospinal tap.

It was also hypothesised that OAE is another neurogenerative disease caused by pathogenic tau protein deposits in the brain which induce neuronal dysfunction. The recent findings on this hypothesis are contradictory. The post-mortem study performed in 2018 on five fatal cases of OAE in Northern Uganda confirmed that NS is tauopathy [94]. In contrast, a recent study conducted on nine post-mortems of people who died with NS or other forms of OAE revealed that OAE is less likely caused by tau protein deposits in the brain [93].

It was suggested that OAE is an autoimmune disorder induced by Leiomodin-1 antibodies cross-reacting with *O. volvulus* proteins [95]. In a recent study no difference in Leiomodin-1 antibodies levels were observed in persons with OAE compared to controls [96]. However, it remains possible that OAE is an autoimmune disease as a consequence of *O. volvulus* infection and induced neuro-toxic antibodies that are still to be discovered.

Chapter 1.II. Thesis outline

II. 1 Study framework

This PhD research was carried out as part of the NSETHIO project, an ERC funded project that started in October 2015 to perform epidemiological, entomological and laboratory studies to identify the cause of nodding syndrome. Until now, NSETHIO generated epidemiological evidence for the association between onchocerciasis and epilepsy. NSETHIO studies also demonstrated that by strengthening onchocerciasis elimination programs OAE including Nodding Syndrome (NS) can be eliminated. The current PhD work is focusing on interventions that could make onchocerciasis elimination programs more effective to prevent OAE and to improve the quality of life of persons with epilepsy (PWE) in onchocerciasis-endemic regions.

II. 2 Rationale

Despite the strong epidemiological evidence concerning the association between onchocerciasis and epilepsy [97], this is still not accepted by all scientists. One of the reasons is that the pathophysiological mechanism of OAE still needs to be elucidated. Moreover, it was considered that annual CDTI, because it will eliminate onchocerciasis, it will also eliminate onchocerciasis-associated morbidities including OAE. However, recent studies conducted by NSETHIO project showed that in several meso- and high onchocerciasis endemic areas, despite many years of CDTI, a high incidence and prevalence of epilepsy was still observed and that persons of epilepsy were often not treated. Therefore, there is a need to evaluate interventions to prevent the epilepsy in onchocerciasis-endemic regions, to reduce the epilepsy treatment gap in these regions and to mitigate the public health problem caused by OAE.

II. 3 Overall objectives

The overall objective of this PhD work is to better understand the association between onchocerciasis and epilepsy, and to investigate ways to mitigate the public health problem caused by OAE.

II. 4 Specific objectives

1. Assess the prevalence of *O. volvulus* antibodies in persons with epilepsy in onchocerciasis-endemic areas

2. Investigate whether there is a sub-optimal ivermectin treatment response in onchocerciasis-endemic regions with a high prevalence of OAE

3. Assess the effect of ivermectin treatment on the frequency of seizures in onchocerciasis-infected persons with epilepsy

4. To investigate how the anti-Ov16 immunoglobulin G4 (IgG4) antibodies seroprevalence among 6–10-year-old children as a proxy for ongoing *O. volvulus* transmission, together with epilepsy prevalence and ivermectin treatment coverage data, can be used to monitor the performance of the onchocerciasis-elimination programs

5. Evaluate the benefits of a community-based epilepsy treatment program in the Logo health zone, Ituri, Democratic Republic of Congo (DRC) on the quality of life of persons with epilepsy, epilepsy related stigma and cost for the family to care for a persons with epilepsy

II. 5 Chapter's overview

Chapter 1. General introduction

The **Chapter 1** covers the general introduction, the rational and objectives of the thesis. An overview of onchocerciasis, control and elimination of onchocerciasis, the recent burden of onchocerciasis and the onchocerciasis-associated epilepsy are discussed in this chapter.

Chapter 2. The seroprevalence of *O. volvulus* antibodies in PWE in onchocerciasis-endemic areas

In this chapter, we described the Ov16 antibody seroprevalence among the PWE from four onchocerciasis-endemic regions in Mahenge area (Tanzania), Kitgum and Pader districts (Uganda), the Mbam and Sanaga river valleys (Cameroon), and the Logo health zone regions (DRC).

Chapter 3. Ivermectin treatment response in onchocerciasis-endemic region with a high prevalence of epilepsy.

Chapter 3I. Ivermectin treatment response in onchocerciasis-endemic region with a high prevalence of onchocerciasis-associated epilepsy.

In **Chapter 3I,** we investigated the ivermectin treatment response among the *O. volvulus* infected PWE. We also assessed factors associated with higher mf density in 3-5 months after ivermectin treatment in *O. volvulus* infected PWE in three onchocerciasis-endemic areas (Maridi, South Sudan; Aketi, DRC; and Mahenge, Tanzania) with high epilepsy prevalence.

Chapter 3II. The ivermectin treatment on epileptic seizures in onchocerciasis-infected persons with epilepsy (PWE)

In **Chapter 3II,** we presented the results of an assessment of the ivermectin treatment effect on seizure frequency in *O. volvulus* PWE with and without anti-seizure medication in three onchocerciasis-endemic areas (Maridi, South Sudan; Aketi, DRC; and Mahenge, Tanzania).

Chapter 4. Surveillance for onchocerciasis-associated epilepsy and Ov16 IgG4 antibodies testing of children 6–10 years old should be used to identify areas where onchocerciasis elimination programs need strengthening

In **Chapter 4**, we presented the assessment of an association between ongoing *O. volvulus* transmission in the area and prevalence and incidence of epilepsy in the same area. Between 2015 and 2021, door-to-door surveys were conducted in onchocerciasis-endemic villages in Cameroon, the DRC, Nigeria, South Sudan, and Tanzania to determine epilepsy prevalence and incidence of epilepsy and ivermectin therapeutic coverage. During these surveys the degree of onchocerciasis transmission in the participating villages was assessed by testing children 6–10 years old for onchocerciasis antibodies using the Ov16 IgG4 RDT (Standard Diagnostics, Inc., Giheung-gu, Yongin-si, Gyeonggi-do, Korea). In this chapter we summarised the door-to-door data and assessed association between ongoing transmission in the area and prevalence and incidence of epilepsy in the same area.

Chapter 5. The management of epilepsy in remote onchocerciasis-endemic setting in the Logo health zone, in Ituri in the DRC.

This chapter presents the results of a qualitative assessment of an impact of a community-based epilepsy treatment program (CBETP) initiated in the Logo health zone on the community's perceptions and attitudes regarding epilepsy and the epilepsy related cost.

Chapter 6. Discussion and future perspectives

In Chapter 6, we discussed the findings of the five research papers of this thesis and implications to the onchocerciasis-associated epilepsy.

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CHAPTER 2.

The seroprevalence of *O. volvulus* antibodies in person with epilepsy in onchocerciasis-endemic regions

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Abstract

There is growing epidemiological evidence that onchocerciasis may induce epilepsy. High prevalence of onchocerciasis has been reported in onchocerciasis-meso and hyper-endemic regions. We aimed to determine the Ov16 antibody seroprevalence in persons with epilepsy (PWE) in four onchocerciasis-endemic regions.

PWE were identified during studies in Mahenge area (Tanzania), Kitgum and Pader districts (Uganda), the Mbam and Sanaga river valleys (Cameroon), and the Logo health zone (Democratic Republic of Congo). Exposure to *Onchocerca volvulus* was assessed by testing PWE for Ov16 IgG4 antibodies using a rapid diagnostic test.

The Ov16 seroprevalence among PWE in the four onchocerciasis-endemic study sites ranged from 35.2 to 59.7%. Ov16 seroprevalence increased with age until the age of 39 years, after which it decreased drastically. Our study suggests that, in onchocerciasis-endemic regions, epilepsy in young people is often associated with onchocerciasis, while epilepsy in older persons seems unrelated to *O. volvulus* exposure.

Keywords: epilepsy; onchocerciasis; Ov16 IgG4; ivermectin; age

1. Background

Onchocerciasis elimination programs using community directed treatment with ivermectin (CDTI) have significantly reduced *Onchocerca volvulus* (*O. volvulus*) transmission and onchocerciasis associated blindness (river blindness) in many African countries [1]. Nevertheless, despite many years of CDTI, there is still active onchocerciasis transmission in many areas [2]. The explanation for this could be the absence or sub-optimal performance of onchocerciasis elimination programs with low ivermectin uptake in certain areas.

Currently, there is very strong epidemiological evidence that onchocerciasis is able to induce epilepsy [3-5], including the nodding syndrome in previously healthy children 3-18 years old [6]. This type of epilepsy is called onchocerciasis-associated epilepsy (OAE) [7]. OAE is a major public health problem in onchocerciasis-endemic areas, where onchocerciasis elimination programs are working sub-optimally or where such programs still need to be implemented [4, 8] but disappear when onchocerciasis is eliminated from the area [5, 9]. In recent years, we conducted many epidemiological studies to investigate the association between onchocerciasis and epilepsy in areas with high ongoing or past onchocerciasis transmission [10]. During these studies, persons with epilepsy (PWE) were tested for the presence of *O. volvulus* Ov16 antibodies. In this paper, we describe the Ov16 seroprevalence in PWE, considering their age, gender, and past ivermectin use.

2. Methods

Ethical approval was obtained from the Ethics committee of the School of Public Health in Kinshasa, (Logo: January 2017, ESP/CE/006/2017), the ethical committee of the National Institute for Medical Research, Tanzania (NIMR/HQ/R.8a/Vol.IX/2278) the Ethics committee of the Makerere University and St. Mary's Hospital Lacor, Uganda (LHIREC 001/02/2017), the National ethical committee for the public health research, Cameroon (No: 2017/02/875/CE/CNERSH/SP), and the Ethics Committee of the Antwerp University Hospital (May 24, 2017, B300201733011). Informed consent was obtained from all study participants.

Most PWE were identified during door-to-door epilepsy prevalence surveys in onchocerciasis-endemic areas with different histories of CDTI implementation. In Mahenge, Ulanga district, Tanzania [11, 12], CDTI had been implemented for more than 20 years, Kitgum and Pader districts, Uganda [5], CDTI had been implemented since 2009, and in Cameroon, in Bilomo (Mbam valley), mass drug administration of ivermectin was implemented in 1998 and around mid-1990s in Kelleng (Sanaga valley) [13]. Other PWE were enrolled during the screening of PWE for participation in a clinical trial to investigate the effect of ivermectin on the frequency of seizures in persons with *O. volvulus* infection in the Logo health zone, Ituri province, Democratic Republic of Congo (DRC) [14]. In the Logo health zone, CDTI had never been implemented [15].

Recruitment of PWE was done following a two-step approach: screening using a validated questionnaire [16], followed by epilepsy case confirmation by a neurologist or clinician trained in epilepsy. This questionnaire was translated and adapted to make the questions more understandable by the local population [17]. The socio-demographic information of PWE was obtained, as well as their ivermectin use during the last CDTI round. Consented participants were finger-pricked, and a few drops of blood was collected for the detection of Ov16 antibodies using a rapid diagnostic test (SD Bioline Onchocerciasis IgG4 rapid test, Abbott Standard Diagnostics, Inc., Yongin, Republic of Korea). The testing procedures were aseptic and followed the manufacturer's instructions.

3. Statistical Analysis

The collected data and Ov16 test results (positive or negative) were entered into electronic spreadsheets and prepared for analysis. Continuous variables were summarized using median and interquartile range (IQR), while proportions were expressed as counts and percentages. The Cochran-Armitage trend test [18] was used to assess Ov16 seroprevalence trends across age groups. A logistic regression model was used to assess the association between age and Ov16 seroprevalence

adjusted for gender and study sites and past ivermectin exposure. The model goodness-of-fit was assessed using deviance and the Pearson chi-square test. Data were analysed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 4.0.2, and a two-sided 5% significance level was used.

4. Results

Overall, Ov16 serologic data of 760 PWE were analysed. The Ov16 seroprevalence among PWE in the four onchocerciasis-endemic study sites ranged from 35.2 to 59.7% (Table 1).

Table 1. Characteristics of persons with epilepsy in the four study sites.

Characteristics	Logo, DRC (n = 391)	Mbam and Sanaga Valley, Cameroon (n = 77)	Mahenge, Tanzania (n = 187)	Kitgum and Pader, Uganda (n = 105)
Age in years: median (IQR)	20.0 (14.0–29.0)	26.0 (21.0–31.0)	24.0 (18.0–34.5)	20.0 (16.0–24.0)
Male gender: *n* (%)	205 (52.4)	34 (44.2)	85 (47.2)	56 (53.3)
Past ivermectin use: *n* (%)	0	51 (66.2)	89 (47.6)	82 (78.1)
OV16 positive: *n* (%)	149 (38.1)	46 (59.7)	102 (54.5)	37 (35.2)

IQR : Interquartile range, DRC : Democratic Republic of Congo

Ov16 seroprevalence increased with increasing age in the younger age-groups but progressively decreased among older PWE (Cochran-Armitage trend test statistics of −4.75 and a two-sided *p*-value of <0.001) (Table 2).

Table 2. Age-specific Ov16 seroprevalence.

Age group	*Ov16 Seroprevalence, n (%)*
10 years or younger (*n* = 79)	7 (8.9)
11–20 years (*n* = 253)	103 (40.7)
21–30 years (*n* = 235)	128 (54.5)
31–40 years (*n* = 93)	39 (51.6)
41–50 years (*n* = 39)	17 (53.8)
51 years or older (*n* = 47)	15 (46.8)

The multivariable analysis revealed a significant association between age and Ov16 serostatus (Table 3). Sex did not influence this association (Table S1, supplementary material). Among younger participants (up to 39 years), increasing age was associated with increasing odds for Ov16 seropositivity; conversely, in the older PWE (above 39 years), the probability of Ov16 seropositivity decreased with increasing age (Figure 1).

Table 3. Predictors of a positive Ov16 RDT test among persons with epilepsy in four onchocerciasis-endemic areas.

Parameter	***Coeff***	***95% CI***		***P-value***
Age (years)	0.1256	0.078	0.1733	<0.0001
Age*age	−0.0016	−0.0023	−0.0009	<0.0001
Female gender	0.0994	−0.2225	0.4213	0.5451
Ivermectin intake vs no intake during last CDTI round	−0.3471	−0.9851	0.2908	0.2862
Country site (CMR vs DRC)	0.384	−0.2788	1.0468	0.2562
Country site (TZD vs DRC)	0.3682	−0.3455	1.0819	0.312
Country site (UG vs DRC)	−0.3582	−1.086	0.3696	0.3347

Estimated coefficient from logistic regression (Coeff); Wald confidence interval (CI); Mbam and Sanaga valley, Cameroon (CMR); Logo, Democratic Republic of Congo (DRC); Mahenge, Tanzania (TZD); Kitgum and Pader, Uganda (UG)

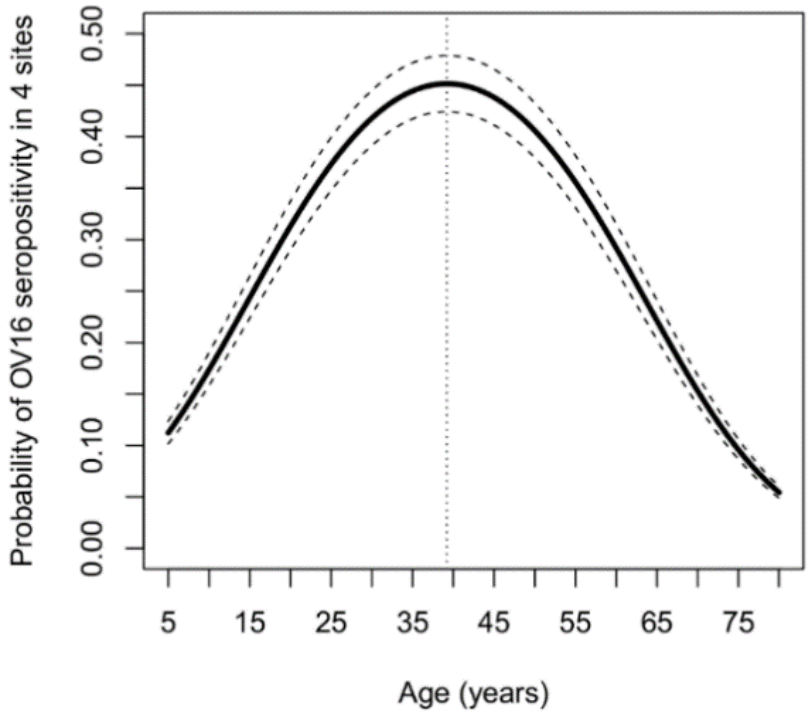

Figure 1*. The probability Ov16 seropositivity as a function of age, considering gender and ivermectin history in four onchocerciasis-endemic areas. Solid black lines represent the point estimates and dashed lines indicate pointwise 95% confidence bands based on the delta method.*

5. Discussion

The increase of Ov16 seroprevalence with increasing age up to the age of 39 years was expected among the enrolled PWE, as the same trend was also observed in the general population in onchocerciasis-endemic regions [19-21]. However, the decrease in Ov16 seroprevalence in the PWE of 39 years and above was unexpected. Few population-based studies have investigated the Ov16 antibody response in the older age groups and the results are not consistent. In a study conducted in an onchocerciasis-endemic area in Yemen, where ivermectin was never distributed, the Ov16 seroprevalence continued to rise with age in men but decreased in women after the age of 39 years [21]. Differences according to the sex may be due to the fact that men and women get exposed to infected blackflies at different ages in their life because of different socio-economic activities, such as farming. Indeed, both younger and older men in Yemen are likely to work daily in the fields where they can be bitten by blackfly vectors, but young women tend to spend more time at the river side (for example, to wash clothes) than older women, who tend to remain in the houses, away from infective blackfly bites [21].

In a study conducted in Togo after decades of *Simulium damnosum* vector control and mass drug administration of ivermectin, the mean Ov16-specific IgG4 reactivity was often low during the first 2 decades of life; from 16 years onwards, an enhanced responsiveness was observed and from 20 years and older, the mean participants' serologic IgG4 responses to Ov16 continued to rise steadily until the fifth decade [20]. In another study in Togo, the Ov16 prevalence increased up to the age of 41 and then remained at nearly the same level [19]. A previous study in the Logo health zone (DRC) found that the proportion of positive Ov16 test results among the general population increased with increasing age, up to the age of 39 years. After 39 years, there was a slight decrease but the seroprevalence remained high [22]. In that same study, Ov16 tests were positive in 18 (58.1%) of 31 participants who had previously exposed to ivermectin and in 260 (29.5%) of 881 participants who were ivermectin-naïve. Twenty-five (46.3%) of the 54 persons with epilepsy on whom Ov16 tests were performed were positive, compared to 253 (29.5%) of 858 subjects without epilepsy ($p = 0.014$) [22], suggesting that onchocerciasis was more prevalent among PWE.

The drastic decrease of the Ov16 seroprevalence among PWE in our study after the age of 39 years is in contrast with the observed Ov16 seroprevalence among the general population in the DRC [22], Yemen [21], and Togo [20]. The most likely explanation for this observation is that the epilepsy beyond the age of 39 years in our study population was unrelated to onchocerciasis. Most of the younger PWE in the study areas met the criteria of onchocerciasis associated epilepsy (OAE) [11, 13, 23, 24]. OAE typically develops in onchocerciasis-exposed children between the ages of 8 and 18 and has a high mortality, with very few cases reaching the age of 30 years [25]. It is therefore plausible that the older PWE with negative Ov16 test results in our study did not develop OAE during their childhood/adolescence, but rather experienced epilepsy onset during the later decades of their lives. Frequent causes of epilepsy at older age include cerebrovascular accidents, head trauma, cerebral infections, or tumours [26, 27] and those conditions are unrelated to onchocerciasis.

Moreover, in all study sites, except the Logo health zone (DRC), older PWE had been exposed to many years of ivermectin and this may have decreased the prevalence of Ov16 seropositivity [28]. In addition, PWE generally avoid coming close to the rivers because of the risk of drowning during seizure attack and therefore are less exposed to infectious blackflies. As a consequence, after many years of non-exposure, Ov16 antibodies may have disappeared [28]. The level *O. volvulus* microfilaria load in children was shown to be a predictor for developing OAE later in life [3, 4]; however, the pathophysiological mechanism on how the *O. volvulus* parasite causes the seizures remains unknown. No parasites or *O. volvulus* DNA and no *Wolbachia* DNA (an *O. volvulus* endosymbiont) were found in cerebrospinal fluid [29] or in brain tissue of persons who had died of OAE [30]. An auto-immune mechanism induced by neurotoxic *O. volvulus* cross-reacting antibodies was proposed [31], but this hypothesis still needs to be confirmed.

Our findings should be interpreted in light of several limitations, including the fact that the data on Ov16 seroprevalence among the general population in our study sites was available only for the Logo health zone (DRC). Another limitation is that no other laboratory test or brain imaging investigations were done to identify the epilepsy aetiology in our study participants. Moreover, the presence of *O. volvulus* antibodies

was only assessed using an Ov16 rapid diagnostic test, which is less sensitive compared to Ov16 ELISA [32].

In conclusion, our study confirms that Ov16 seroprevalence is high among PWE, particularly in the younger age groups. Our findings suggest that, in onchocerciasis-endemic areas, epilepsy in young people may be associated with exposure to *O. volvulus* and that, in older PWE, additional investigations need to be performed to identify risk factors for epilepsy other than onchocerciasis.

Supplementary Materials

Table S1: Predictors of an Ov16 RDT seropositivity among persons with epilepsy in four onchocerciasis-endemic areas by gender.

Parameter	***Male***				***Female***			
	Coeff	**95% CI**		**P-value**	**Estimates**	**95% CI**		**P-value**
Age (years)	0.1349	0.0699	0.1999	<.0001	0.1116	0.0392	0.1841	0.0025
Age*age	-0.0018	-0.0028	-0.0009	0.0002	-0.0013	-0.0024	-0.0003	0.0154
Female gender	-0.4673	-1.4557	0.5211	0.3541	-0.2762	-1.1284	0.576	0.5253
Ivermectin intake vs no intake during last CDTI round	0.2752	-0.6932	1.2436	0.5775	0.4728	-0.4477	1.3933	0.3141
Country site (CMR vs DRC)	0.3438	-0.7194	1.407	0.5262	0.3566	-0.6201	1.3333	0.4742
Country site (TZD vs DRC)	-0.5616	-1.7042	0.5809	0.3353	-0.2275	-1.1805	0.7256	0.6399

Estimated coefficient from logistic regression (Coeff); Wald confidence interval (CI); Mbam and Sanaga valley, Cameroon (CMR); Logo, Democratic Republic of Congo (DRC); Mahenge, Tanzania (TZD); Kitgum and Pader, Uganda (UG)

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CHAPTER 3

Ivermectin treatment response in onchocerciasis-endemic regions with a high prevalence of epilepsy

I. Ivermectin treatment response in onchocerciasis-endemic region with a high prevalence of onchocerciasis-associated epilepsy

II. The ivermectin treatment effect on epileptic seizures in onchocerciasis-infected persons with epilepsy

Ivermectin treatment response in onchocerciasis-endemic region with a high prevalence of onchocerciasis-associated epilepsy.

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Abstract

Despite a long history of community-directed treatment with ivermectin (CDTI), a high ongoing *Onchocerca volvulus* transmission is observed in certain onchocerciasis-endemic regions in Africa with a high prevalence of epilepsy. We investigated factors associated with higher microfilarial (mf) density after ivermectin treatment. Skin snips were obtained from *O. volvulus*-infected persons with epilepsy before, and 3 to 5 months after ivermectin treatment. Participants were enrolled from 4 study sites : Maridi (South Sudan); Logo and Aketi (Democratic Republic of Congo); and Mahenge (Tanzania).

Of the 329 participants, 105 (31.9%) had a post-treatment mf density >20% of the pre-treatment value. The percentage reduction in the geometric mean mf density ranged from 69.0% (5 months after treatment) to 89.4% (3 months after treatment). A higher pre-treatment mf density was associated with increased probability of a positive skin snip after ivermectin treatment (p = 0.016). For participants with persistent microfiladermia during follow-up, a higher number of previous CDTI rounds increased the odds of having a post-treatment mf density >20% of the pre-treatment value (p = 0.006).

In conclusion, the high onchocerciasis transmission in the study sites may be due to initially high infection intensity in some individuals. Whether the decreasing effect of ivermectin with increasing years of CDTI results from sub-optimal response mechanisms warrants further research.

Keywords: Ivermectin; sub-optimal response; onchocerciasis; *Onchocerca volvulus*; epilepsy

1. Introduction

With an estimated 20.9 million people infected worldwide, of whom over 99% live in sub-Saharan Africa [1], *Onchocerca volvulus (O. volvulus)* infections are an important global health problem. *O. volvulus* is a filarial nematode causing onchocerciasis and is transmitted by blackflies (*Simuliidae*). Clinical manifestations associated with onchocerciasis include skin and eye disease, nodding syndrome, and other forms of epilepsy (hereafter referred to as onchocerciasis-associated epilepsy (OAE)) [2]. Adult female worms reside in subcutaneous nodules and produce larvae, called microfilariae (mf), that migrate under the skin.

Ivermectin, a broad-spectrum anti-helminthic drug, rapidly kills mf. A single dose of ivermectin leads to a decline in skin mf density of approximately 99% within the first two months [3]. Unfortunately, ivermectin does not kill the adult worms, it only temporarily represses mf production by the adult female worms and therefore mf production resumes at a slow rate after approximately 3 to 6 months [4, 5]. Nonetheless, mf loads are expected to remain below 20% of the pre-ivermectin levels for up to 10 months following a single dose of ivermectin [3]. Therefore, many onchocerciasis-endemic areas have adopted annual or bi-annual community-directed treatment with ivermectin (CDTI) as a strategy to decrease onchocerciasis transmission [6].

Onchocerciasis elimination programs using CDTI have significantly reduced *O. volvulus* transmission and onchocerciasis-related blindness in many African countries [6]. Nevertheless, despite a long history of CDTI, there is still active onchocerciasis transmission in many endemic areas in Ghana [7], Cameroon [8], Democratic Republic of Congo (DRC) [9], and Tanzania [10]. Moreover, such meso- and hyperendemic settings have also been noted to harbour a high burden of OAE [9-12]. In Ghana and Cameroon, an *O. volvulus* phenotype with sub-optimal ivermectin response has been documented [4, 13] which may contribute, at least partly, to the persistence of *O. volvulus* transmission. To determine host factors associated with the high onchocerciasis prevalence and transmission observed in OAE hotspots, we conducted a study assessing individual mf density before and after ivermectin administration.

2. Materials and Methods

2.1. Study participants and study sites

We performed the study among persons with epilepsy (PWE) with the intention to investigate the effect of ivermectin on microfilarial (mf) density but also to evaluate whether ivermectin could decrease the frequency of seizures in PWE infected with *O. volvulus.* Study participants were PWE that were identified during door-to-door surveys in four onchocerciasis-endemic study sites. The four study sites were the Aketi health zone, Bas Uélé province and the Logo health zone, Ituri province in the Democratic Republic of Congo (DRC), Mahenge, Ulanga district in Tanzania and Maridi in South Sudan. In Maridi, Aketi and Mahenge participants were enrolled only for a short follow up study, while in the Logo health zone, the 132 participants were enrolled in a randomized clinical trial to evaluate the potential added value of ivermectin in decreasing the frequency of seizures in PWE with *O. volvulus* infection treated with phenobarbital [14]. Right and left skin snips were obtained from each participant before ivermectin treatment intake. Participants with detectable mf in their skin snips were asked to have the skin snip testing repeated 3 to 5 months after ivermectin treatment. Results of the effect of ivermectin on the frequency of seizures were published elsewhere [15]. All study sites had a different history of CDTI and a different schedule of skin snip testing, as depicted in Table 1.

Table 1. Years of community-directed treatment with ivermectin (CDTI) and timing of skin snip testing per study site.

Characteristic	South Sudan	DRC		Tanzania
	Maridi	Logo	Aketi	Mahenge
Years of CDTI	1	0	14	21
Number of months since last CDTI	12	NA	12	5
Number of months between pre-treatment skin snip (followed by ivermectin administration) and post-treatment skin snip	5	4	3	3

NA = not applicable because of no previous CDTI round

2.2. Aketi health zone, DRC

The Aketi health zone is an onchocerciasis hyper-endemic area in Bas-Uélé province in the DRC where CDTI was initiated 14 years ago [16]. Despite this long history of

CDTI, an epilepsy prevalence of 5.7% was reported in Aketi rural town during a door-to-door household survey in 2017. In addition, a seroprevalence of Ov16 IgG4 antibodies against *O. volvulus* of 64.5% was reported in children aged 7-10 years old, suggesting a high ongoing onchocerciasis transmission [9]. In January 2018, skin snips were obtained from selected PWE from Wela, Makoko, and Aketi rural town. Eighty-one *O. volvulus*-infected PWE [17] were asked to participate in a follow up study and to be skin snipped again three months after ivermectin intake. Post-ivermectin skin snips could be obtained from 74 of 81 PWE.

2.3. Logo health zone, DRC

In October 2017, a proof-of-concept randomized clinical trial investigating the effect of ivermectin on seizure frequency in *O. volvulus* infected PWE was initiated in the Logo health zone, an onchocerciasis-endemic area with a high epilepsy prevalence (4.6%) where ivermectin had never been distributed before [12]. In February 2018, PWE were asked to participate in a randomized clinical trial to evaluate the effect of ivermectin on the frequency of seizures in *O. volvulus* infected PWE [14]. In 392 PWE, skin snips were obtained and 143 (36.5%) of them had detectable mf [17]. These 143 skin snips positive PWE were asked to participate in a follow up study and to be skin snip tested again four months after ivermectin intake. In 136 (95%) of them, pre-and post-ivermectin skin snip results were obtained.

2.4. Maridi, south sudan

In May 2018, a door-to-door household survey in eight villages in an onchocerciasis-endemic area in Maridi county showed an overall epilepsy prevalence of 4.4%, reaching up to 11.9% in the Kazana II village, which is located closest to the Maridi dam, a blackfly breeding site [11]. CDTI had been interrupted for at least ten years in this region and was re-initiated in 2017. During a CDTI round in December 2018, PWE identified in the initial survey were asked to participate in a study to determine the prevalence of *O. volvulus* infection among PWE. Of the 318 PWE who agreed to participate, 270 (84.9%) had detectable mf in their skin snips pre-ivermectin [18]. These 270 skin snips positive PWE were asked to participate in a follow up skin snip study 5 months after ivermectin intake. In 102 (38%) of them, pre- and post-ivermectin skin snip results were obtained.

2.5. Mahenge, Tanzania

Located in the Ulanga district, Mahenge is a known onchocerciasis-endemic focus. The Tanzanian National Onchocerciasis Control Programme initiated annual CDTI since 1997 and bi-annual CDTI in 2019. In January 2017, we conducted an epilepsy prevalence survey in two rural villages in the Mahenge area (Mdindo, Msogezi), and documented a high prevalence of epilepsy (3.5%) among the general population in these villages as well as a high prevalence of Ov16 IgG4 antibodies in children 6–10 years (42.5%) [10]. PWE identified during the initial survey were asked to participate in a study to determine the prevalence of *O. volvulus* infection among PWE. Of the 42 PWE who agreed to participate, 22 (52.4%) presented mf in skin snips (Bhwana D., personal communication). In April 2019, these 22 PWE with positive skin snip were asked to participate in a follow up study which entailed collecting a second skin snip 3 months after ivermectin intake. In 17 (77%) of them, pre- and post-ivermectin skin snip results were obtained.

2.6. Participant data

Upon enrolment in the study, we obtained the following data from all study participants: age, gender, weight, height, and town or village of residence. In addition, we obtained from the health authorities the exact number of previous rounds of CDTI in each study site (Table 1).

2.7. Skin snip testing

Skin snips were obtained from each posterior iliac crest of eligible participants using a Holt-type punch. Snips were immediately placed in two wells of a microtiter plate containing 3 drops of normal saline solution and incubated for 24 hours at room temperature to allow mf to emerge into the fluid. After the incubation period, mf in the solution was examined microscopically under a x40 magnification and counted by a trained technician. Mf densities were expressed as arithmetic means between mf count at right skin snips and mf count at left skin snips. One punch was used per subject and punches were sterilized between subjects using steam under pressure (autoclave).

2.8. Ivermectin treatment

Ivermectin was given under direct observation at approximately 150 µg per kg of body weight or using a height equivalent, as prescribed by the World Health Organization guidelines [19]. Our primary indicator of ivermectin treatment efficacy was the reduction in skin mf densities in *O. volvulus*-infected PWE measured 3–5 months after ivermectin treatment, both at the individual level and per study site. We also investigated host-factors associated with a persistent microfiladermia in the post-ivermectin skin snip samples.

2.9. Data processing

The mf density of participants before and after ivermectin treatment were entered into electronic spreadsheets. Given that mf density was recorded as mf per skin snip, and that mf density per skin snip in a small body surface area (BSA) (mostly for children) is likely to be higher than in a large BSA, we calculated participants' BSA using Boyd's formula:

$$BSA\ (in\ m^2) = \ 0.0003207 * W^{(0.7285 - 0.0188 \log(W))} * H^{0.3},$$

where W = weight of the participant (in kg) and H = height of the participant (in m). We then used the individual BSA to normalize individual *O. volvulus* skin mf density [20]. A BSA of 1.73 is mostly used in medical physiology to normalize a number of physiological variables [21]. The absolute value of individual pre-ivermectin skin snip mf density was therefore normalized as: pre- or post-ivermectin skin mf density*individual relative BSA (rBSA) with rBSA equal to BSA/1.73.

2.10. Statistical Analysis

Baseline characteristics of all participants within each study site were summarized using the absolute and relative frequency for categorical variables, and median (with interquartile range, IQR) for continuous variables. Mf density before and after ivermectin for each study site were summarized with geometric mean (GM) calculated as the *n*th root of the product of individual mf density (to which 1 was added so as to include post-ivermectin negative skin snips), with *n* equal to number of PWE per study site. The percentage reduction in GM mf density per study site was calculated as the difference between pre- and post-ivermectin GM mf density divided by pre-ivermectin

GM mf density multiplied by 100. The distribution of post-ivermectin *O. volvulus* mf density was skewed to the right and a large proportion of *O. volvulus* mf density values was zero. Therefore, post-ivermectin *O. volvulus* mf density data were modelled using Poisson and negative binomial hurdle models and zero-inflated Poisson and negative binomial models [22]. Akaike's Information Criteria (AIC) was used to select the 'best' model among all candidate models [23]. Parameter estimation in the different models was done using maximum likelihood estimation. In a hurdle model, the analysis consists of a separate logistic regression model and a truncated Poisson (or negative binomial) regression model. The logistic regression model is used to assess the effect of predictors on the probability of having a positive skin snip after ivermectin treatment, whereas the truncated Poisson (or negative binomial) regression approach studies the effects of covariates on post-ivermectin *O. volvulus* mf densities conditional on having a positive skin snip after ivermectin treatment. All possible two-way interactions between baseline characteristics were tested to be included in the final model using the likelihood ratio test. Data were analysed using SAS 9.4 (SAS Institute Inc.) and two-sided tests at a significance level of 5%.

3. Results

A total of 329 *O. volvulus*-infected PWE from four sites participated in the study (Table 2). All the study sites were comparable in terms of gender and age, except for Mahenge where participants appeared to be older (Kruskal-Wallis chi-square test = 41.8, and degree of freedom (df) = 3, $p < 0.001$)

Table 2. Characteristics of study participants in the four study sites.

Participant Characteristic	South Sudan	DRC		Tanzania
	Maridi (n = 102)	**Logo (n = 136)**	**Aketi (n = 74)**	**Mahenge (n = 17)**
Age (years), Median (IQR)	16.5 (14.0–18.0)	22.0 (17.0–30.5)	17.0 (15.0–20.0)	35.0 (25.0–45.0)
Male gender: *n* (%)	53 (50)	69 (49)	40 (49)	11 (46)
Weight (kg), Median (IQR)	38.0 (30.0–48.0)	45.0 (38.5–50.0)	37.5 (43.5–54.5)	45.0 (40.0–51.0)
Height (cm), Median (IQR)	158.0 (154.5–162.0)	154.0 (147.0–160.0)	154.0 (138.0–156.0)	154.0 (146.0–160.0)
Pre-ivermectin mf density per skin snip, Median (IQR)	22.7 (12.0–44.5)	28.0 (7–93.0)	9.4 (3.0–52.5)	2 (2.0–20.6)
Pre-ivermectin mf density, GM (SE)	15.0 (1.1)	24.7 (3.2)	12.9 (2.1)	5.7 (1.6)

n : number; mf: microfilariae; IQR = Interquartile range; GM: Geometric mean; SE: Geometric mean standard error

3.1. Effects of ivermectin treatment on the participants mf density

Of the 329 PWE, 105 (31.9%) experienced a <80% decrease in mf density in their post-treatment skin snips (implying that the post-treatment mf density was >20% of the pre-treatment value). The proportion of PWE with <80% decrease in mf density increased with the number of months before the second skin snip was obtained (Mantel–Haenszel chi-square: 48.8, df =1, $p < 0.001$), being highest in Maridi (second skin snips after 5 months) and lowest in Aketi (second skin snips after 3 months). At the community level, reduction in geometric mean mf density was highest in the Aketi study site (89% reduction) followed by Logo (86% reduction) (Table 3).

Table 3. Post-ivermectin parasitological data of study participants

Parasitological variables	Maridi (n = 102)	Logo (n = 136)	Aketi (n = 74)	Mahenge (n = 17)
Months post-ivermectin intake: *n*	5	4	3	3
Positive skin snip: *n* (%)	82 (80)	66 (48)	18 (24)	9 (53)
<80% decrease in individual mf density: *n* (%)	52 (51)	38 (28)	8 (11)	7 (41)
Overall Mf per skin snip, Median (IQR)	4.5 (1.0–17.5)	0.0 (0.0–9.0)	0.0 (0.0–0.5)	0.5 (0.0–1.0)
Mf per skin snip of PWE with post-ivermectin positive skin snip; Median (IQR)	5.0 (1.5–19.0)	10.5 (2.5–38.0)	2.0 (0.5–5.5)	1 (0.5–1.0)
Post-ivermectin GM mf density per skin snip, GM (SE)	4.7 (0.6)	3.4 (0.5)	1.4 (0.2)	1.6 (0.3)
Overall percentage reduction in GM mf density per skin snip (%)	69	86	89	72

n: number; mf: microfilariae density, IQR = Interquartile range; GM: Geometric mean; SE: Geometric mean standard error

3.2. Host factors associated with post-ivermectin mf density values given a post-ivermectin positive skin snip test

To investigate host factors associated with post-ivermectin *O. volvulus* mf density, we described the average post-ivermectin mf density value, conditional on having a positive skin snip after ivermectin, while adjusting for gender, age, height, number of previous CDTI rounds in the site, duration since last ivermectin intake, and mf density before ivermectin treatment. The negative binomial hurdle model, for which results are presented in Table 4, outperformed other hurdle and zero-inflated models in terms of AIC (Table A1). A higher pre-ivermectin *O. volvulus* mf density was associated with an increased probability of having detectable mf in the follow-up skin snip (Table 4). The effect of pre-ivermectin *O. volvulus* mf density did not change when BSA was included as an offset in the model.

Table 4. Negative binomial hurdles model to assess host factors associated with post-ivermectin *O. volvulus* microfilarial density.

	Post-ivermectin O. volvulus Mf Density				Probability of Positive Skin Snip after Ivermectin Treatment			
Factors	**Coeff**	**95% CI**		**P-value**	**Coeff**	**95% CI**		**P-value**
Female gender	−0.312	−0.946	0.323	0.336	−2.518	−4.913	−0.124	0.039
Male gender (reference)								
Age (years)	0.012	−0.041	0.064	0.661	−0.391	−0.656	−0.125	0.004
Age*age (years)	−0.001	−0.003	0.001	0.349	0.012	0.003	0.020	0.007
Height in cm	−0.007	−0.055	0.041	0.773	0.091	−0.010	0.192	0.076
Number of previous CDTI rounds in site	−0.216	−0.515	0.083	0.157	−0.591	−1.252	0.069	0.079
>1 year since last ivermectin dose	0.455	−0.476	1.386	0.338	−2.020	−4.137	0.097	0.062
<1 year since last ivermectin dose (reference)								
Follow-up skin snip at month 3	2.734	−1.823	7.290	0.240				
Follow-up skin snip at month 4	0.533	−0.384	1.450	0.255				
Follow-up skin snip at month 5 (reference)								
Pre-ivermectin microfilarial density[#]	0.337	0.064	0.611	0.016	1.187	0.161	2.213	0.023

*#: Log-transformed; Coeff = Negative binomial hurdle model coefficients, CI = Confidence interval; Age*age = Quadratic effect of age*

In Figure 1, we graphically display the quadratic effect of age on the probability of a positive skin snip after ivermectin treatment. More specifically, a plot of age-dependent odds ratio for a positive follow-up skin snip reveals that 38 years is the transition age before which the probability of a positive skin snip after ivermectin treatment decreases with increasing age, whereas the reverse is true for individuals aged 38 years or more (Figure 1).

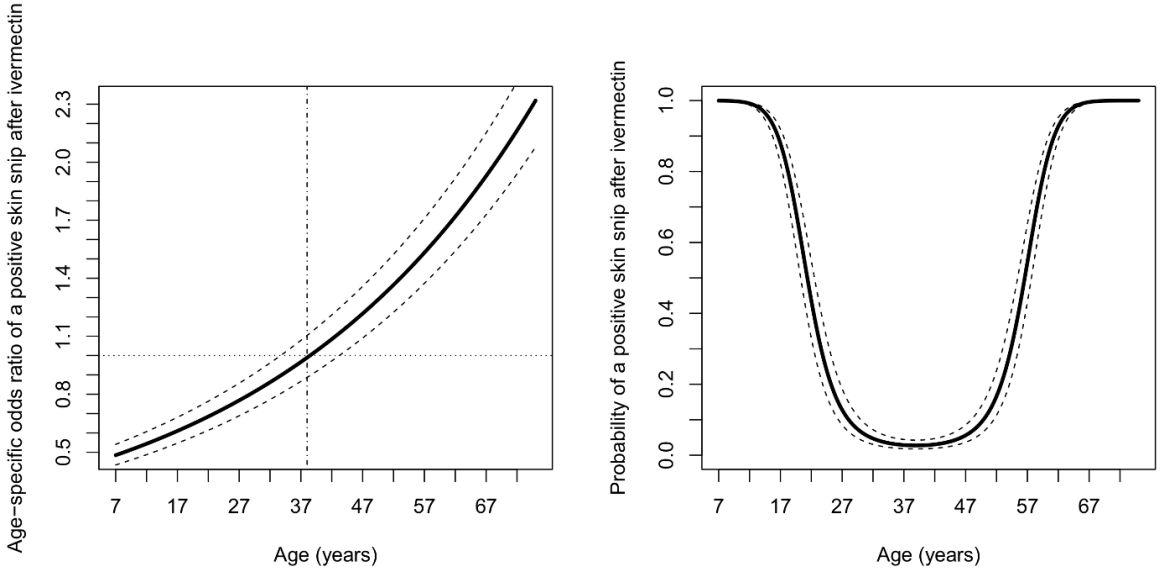

Figure 1. *The age-dependent odds ratio of having a positive skin snip after ivermectin for a unit increase in age (left panel). Probability of having a positive skin snip after ivermectin treatment as function of age for female persons of height 180 cm with more than a year since last CDTI round, with 175 mf per skin snip before ivermectin and living in a community where CDTI has been implemented for at least 10 years (right panel). Solid black lines represent the point estimates and dashed lines indicate pointwise 95% confidence bands based on the delta method*

A higher number of previous CDTI rounds in the study site and a longer time lapse between ivermectin intake and the follow-up skin snip increased the odds for <80% individual skin mf reduction (Table 5).

Table 5. Logistic regression to assess the factors associated with *O. volvulus* mf density reduction of <80% following ivermectin treatment in the four study sites.

Factors	Coeff	95% CI		P-value
Female gender	−0.058	−0.568	0.453	0.825
Male gender (reference)				
Age (years)	−0.097	−0.192	−0.002	0.044
Age*age (years)	0.001	0.001	0.003	0.045
Height in cm	−0.019	−0.048	0.010	0.207
Number of previous CDTI rounds in site	0.282	0.080	0.484	0.006
Follow-up skin snip at month 3	−6.943	−10.064	−3.822	<0.001
Follow-up skin snip at month 4	−1.101	−1.850	−0.351	0.004
Follow-up skin snip at month 5 (reference)				
Pre-ivermectin microfilarial density #	0.202	0.012	0.392	0.037

*#: Log-transformed; Coeff = Logistic regression model coefficients, CI = Confidence interval; Age*age = Quadratic effect of age*

4. Discussion

In this study, we investigated treatment response to ivermectin in 329 *O. volvulus*-infected PWE living in four onchocerciasis-endemic areas in sub-Saharan Africa. The post-ivermectin mf density was >20% of the pre-treatment value in 105 (31.9%) participants, suggesting that their response to ivermectin warrants further investigation [7]. We observed the highest GM mf reduction of 89.4% three months after ivermectin treatment, as compared to 69.0% GM mf reduction five months post-treatment. Previous studies reported up to 98% reduction of GM mf density, 14 to 90 days after a single dose of ivermectin, with a recuperation of adult worm fertility starting around the third month resulting in a reduction in GM mf density of about 84%, four months post-ivermectin [3].

In the three study sites where the second skin snip was obtained within four months, the percentage of PWE with positive skin snips after ivermectin was lower when compared with a study in Ghana, where 70% of participants had detectable mf four months post-ivermectin [7]. This was not the case with participants from Maridi (South Sudan) whose skin snips were obtained 5 months after treatment and up to 80.4% of participants still presented with microfiladermia. The proportion of participants with positive skin snips post-treatment was higher in our study than observed in moxidectin-treated participants during a comparative trial with ivermectin in the Logo health zone.

In this trial, only 8% of the participants who received moxidectin still had detectable mf six months after treatment [24]. In addition to being more potent in reducing mf density, the microfilaricidal effects of moxidectin also last longer than ivermectin [25]. Therefore, annual moxidectin or bi-annual ivermectin treatment should be considered for mass treatment in hyperendemic settings as it results in a long-lasting suppression of mf compared to ivermectin once a year.

In our study, we observed that a high pre-ivermectin mf density was significantly associated with a lower mf reduction in the follow-up skin snip. This was previously reported by Pion et al. [26] and in a meta-analysis by Churcher et al. [27], possibly because of the presence of a higher adult worm burden and/or higher numbers of newly patent adult worms in individuals with higher mf density. Therefore, we assume that almost all the post-treatment positive skin snips in our study can be explained by skin mf repopulation, which is in line with our observation that post-ivermectin skin snip positivity increased with increasing time between ivermectin treatment and follow-up skin snipping.

We found that in younger persons, the post-ivermectin *O. volvulus* mf densities initially decreased with an increase in age as previously documented by Pion et al. [26]. A potential explanation is that skin mf repopulation may be more rapid in younger individuals because the adult worms are still maturing (lifespan of about 9–11 years) and rapidly releasing huge numbers of mf in the early phase of the infection [26, 28]. In the older participants (>38 years) however, post-ivermectin *O. volvulus* mf densities increased as they got older (Figure 1). A possible explanation for the reversal in trends observed after 38 years could be the absence of adjustments for the number of nodules in our multivariate model [26, 28, 29].

An increasing number of previous CDTI rounds was associated with higher probability of achieving <80% mf reduction after ivermectin treatment (Table 5). This finding concurs with previous reports from Cameroon [26, 28, 29] where the embryostatic effect of ivermectin was reduced among individuals who had been treated several times with ivermectin in the past, compared to ivermectin-naïve individuals. In contrast, a longer history of CDTI tended to decrease the chances of having a positive skin snip post-ivermectin, although this trend was not statistically significant (p = 0.079; Table

4). This could be due to a very low pre-treatment mf density among participants who had been receiving ivermectin for several years prior to our study.

Our study has several limitations. Firstly, our study sites were very different in many aspects, including the time between ivermectin treatment and follow-up skin snipping, and the number of previous CDTI rounds. Secondly, we only evaluated mf densities at two time points and given that we did not collect adult female worms at either of the four sites, we were not able to assess the fecundity and mf density dynamics immediately after ivermectin use, which are needed to confirm sub-optimal treatment response. Moreover, the number of palpable nodules, as a proxy for the total number of adult worms, was not assessed in our study participants. Finally, PWE who take anti-epileptic drugs may experience decreased ivermectin drug levels. It is well known that phenobarbital can influence the *p*-glycoprotein (MDR1) transporter, which plays an important role in the elimination of ivermectin. Unfortunately, ivermectin plasma concentrations were not measured in this study.

In conclusion, our study shows that ivermectin effectively reduces mf density in our study participants, similar to what was observed in other onchocerciasis-endemic areas. The fact that almost one third of our participants still had >20% of their pre-treatment mf density, as well as the high ongoing *O. volvulus* transmission observed at our study sites are most likely related to elevated mf densities at baseline as a result of ineffective or inexistent onchocerciasis elimination programs. While we clearly demonstrate that post-ivermectin parasitic load depends on pre-treatment infection intensity, it is unclear whether some study participants exhibit a sub-optimal response to ivermectin. Resistance to the ivermectin treatment has been reported in several parasites in veterinary studies [30-32]. Given the rapid rise and spread of ivermectin resistance in the veterinary field, studies should also investigate the possibility of ivermectin resistance in human medicine. In the light of the apparent decreasing effect of ivermectin as the number years of CDTI increases, studies need to investigate the number/fecundity of adult female worms that may be contributing to skin repopulation, and whether a sub-optimal response genotype is present in these areas as was the case in Ghana [13].

Appendix

Table A1. Model comparison information's with relative rankings.

Model	−2 LL	AIC	Pearson Chi-Square	Ranking
Poisson	4907.6	8732.6	8566.8	5
Negative Binomial	4270.8	1467.6	536.4	3
Poisson Hurdle	5115.7	5157.7	1320.2	4
ZINB	1432.4	1472.4	465.7	2
ZIP	6303.1	5961.7	2200.8	6
Negative Binomial Hurdle	1408.8	1448.8	343.7	1

LL: Log-likelihood; AIC: Akaike information criterion.

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The effect of ivermectin treatment on epileptic seizures in onchocerciasis-infected persons with epilepsy

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Abstract

A clinical trial performed in the Democratic Republic of Congo (DRC), among persons with epilepsy (PWE) infected with *Onchocerca volvulus* treated with anti-seizure medication suggested that ivermectin reduces the seizure frequency. We assessed the effect of ivermectin treatment on seizure frequency in PWE with and without anti-seizure medication in three onchocerciasis-endemic areas (Maridi, South Sudan; Aketi, DRC; and Mahenge, Tanzania).

Pre- and 3–5 months post-ivermectin microfilariae densities in skin snips and seizure frequency were assessed. After ivermectin, the median (IQR) percentage reduction in seizure frequency in the study sites ranged from 73.4% (26.0–90.0) to 100% (50.0–100.0). A negative binomial mixed model showed that ivermectin significantly reduced the seizure frequency, with a larger decrease in PWE with a high baseline seizure frequency. Mediation analysis showed that ivermectin reduced the seizure frequencies indirectly through reduction in microfilariae densities but also that ivermectin may have a direct anti-seizure effect.

However, given the short half-life of ivermectin and the fact that ivermectin does not penetrate the healthy brain, such a direct anti-seizure effect is unlikely. A randomized controlled trial assessing the ivermectin effect in people infected with *O. volvulus* who are also PWE on a stable anti-seizure regimen may be needed to clarify the causal relationship between ivermectin and seizure frequency.

Keywords: epilepsy; ivermectin; onchocerciasis; seizures

1. Introduction

The filarial nematode *Onchocerca volvulus* (*O. volvulus*) is known to cause skin disease, and ocular problems (river blindness). There is growing evidence that this parasite directly or indirectly can cause nodding syndrome as well as other forms of epilepsy (so-called onchocerciasis-associated epilepsy or OAE) [1].

Ivermectin is currently the drug of choice to treat human onchocerciasis [2]. Although ivermectin does not kill the adult filarial worm, it decreases its fertility, kills the microfilariae, and has an excellent safety profile with adverse events occurring only as a consequence of an immunological response against the killed microfilariae [3]. These effects termed as “Mazzotti reactions” are usually mild and disappear within days without further treatment [4, 5].

Annual or bi-annual community-directed treatment with ivermectin (CDTI) is the cornerstone of onchocerciasis elimination programs [6]. One dose of ivermectin of 150 to 200 µg/kg body weight eliminates microfilariae very rapidly [7]. A mathematical model predicted that microfilariadermia would be reduced by half after 24 hours, by 85% after 72 hours, and by 94% one week after the intake of ivermectin [7]. If microfilarial density plays a role in causing OAE, it is expected that a reduction in microfilarial density following treatment with ivermectin may also be able to decrease the frequency of seizures.

Until recently there was only anecdotal evidence suggesting that ivermectin use in onchocerciasis-endemic regions reduces the frequency of seizures. In 1992, a study in Kabarole reported a decrease in either frequency or severity of seizures after a single dose of ivermectin at 150 µg/kg in 34 out of 91 persons with epilepsy (PWE) [8]. After being treated with ivermectin, 13 (14%) individuals experienced no seizures for 3.7 months on average; seizures were unchanged in 51 (56%) and worsened in 6 (7%) [8]. A positive correlation between seizure frequency and microfilarial density was reported previously in the Aketi health zone, Bas Uélé province and the Logo health zone in Ituri province, onchocerciasis-endemic regions in the Democratic Republic of Congo (DRC) [9]. A proof of concept randomised trial to investigate whether ivermectin had an added value in reducing the frequency of seizures in persons with

onchocerciasis-associated epilepsy (OAE) also treated with anti-epileptic drugs, showed a borderline association between ivermectin treatment and seizure-freedom at 4 months of the trial [10]. Upon prolonging the follow-up period of this initial trial to one year while providing additional doses of ivermectin to some participants, seizure freedom during the last four months of follow-up was more likely among those treated with ivermectin twice (OR: 5.09, 95% CI: 1.38–19.75) and thrice (OR: 2.47, 95% CI: 0.94–6.77) than in those treated once [11].

Recently, we investigated the ivermectin response on *O. volvulus* microfilarial densities in persons with epilepsy (PWE) in four onchocerciasis-endemic areas in Africa, with and without a history of CDTI. A higher pre-ivermectin microfilarial density was associated with increased probability of a positive skin snip after ivermectin treatment and among the participants with persistent microfiladermia during follow-up, a higher number of previous community-based treatment rounds with ivermectin increased the probability of having a post-treatment microfilarial density >20% of the pre-treatment value [12]. In the present paper, we study the effect of ivermectin on the frequency of seizures in people infected with *O. volvulus* who are also PWE whereby some individuals were on anti-epileptic treatment and others were not.

2. Materials and Methods

2.1. Study setting and participants

We carried out three pre–post-observational studies of PWE with an *O. volvulus* infection treated with ivermectin in three different sites: one in the DRC, one in Tanzania and another in South Sudan. These are all rural settings with a high prevalence of epilepsy and onchocerciasis.

2.1.1. Aketi health zone, DRC

In 2015, a high prevalence of epilepsy was documented in Wela (6.5%) and Makoko (7.8%), two villages in the Aketi health zone, an onchocerciasis hyper-endemic area in the Bas-Uélé province in the DRC [13]. CDTI had been initiated in this health zone for 14 years. In 2017, an epilepsy prevalence of 5.7% was also observed in Aketi rural

town together with a 64.5% seroprevalence of Ov16 antibodies in children aged 7-10 years old, suggesting high ongoing onchocerciasis transmission [14]. PWE were selected in the villages of Wela, Makoko and Aketi rural town. All individuals received a single dose of ivermectin and skin snip testing was performed before ivermectin treatment and repeated after three months.

2.1.2. Maridi, South Sudan

In May 2018, during a door-to-door household survey in 8 villages in an onchocerciasis-endemic area in Maridi County, an overall epilepsy prevalence of 4.4% was documented with the highest epilepsy prevalence of 11.9% in a village close to the Maridi dam, a blackfly breeding site [15]. CDTI had been interrupted for many years and was re-initiated in 2017. In December 2018, PWE identified during the survey in May were asked to participate in this study. After an initial skin snip, the participants received a single dose of ivermectin and skin snip testing was repeated after five months.

2.1.3. Mahenge, Ulanga District, Tanzania

The National Onchocerciasis Control Programme has conducted CDTI in Mahenge since 1997. In January 2017, an epilepsy prevalence survey was conducted in two rural villages in the Mahenge area (Mdindo, Msogezi), and documented a high prevalence of epilepsy of 3.5% together with evidence of ongoing *O. volvulus* transmission (42.6% children 6–10 years old presented with OV16 antibodies) [16]. PWE identified during this survey were asked to participate in the study. A first skin snip was obtained, after which all participants received a single dose of ivermectin, and skin snip testing was repeated after three months.

2.2. Skin snip testing

Skin snips were obtained from each posterior iliac crest of eligible participants using a Holt-type punch. Snips were immediately placed in two wells of a microtitre plate containing 3 drops of normal saline solution and incubated for 24 hours at room

temperature to allow microfilariae to emerge into the fluid. After the incubation period, mf in the solution were examined microscopically under a ×40 magnification and counted by a trained technician. Microfilarial (mf) densities of positive samples were expressed as the arithmetic mean from both right and left skin snips (mean microfilariae density per skin snip). One punch was used per subject and punches were sterilized between subjects using steam under pressure (autoclave). Only PWE with positive skin snips at baseline were included in this study.

2.3. Ivermectin treatment

All ivermectin tablets (3mg) were taken orally once under direct observation according to participant height, as recommended by the World Health Organization [17, 18].

2.4. Medical history and clinical examination

PWE and their caretakers were questioned about seizure frequency per day, per week, and per month during the month preceding ivermectin intake. Information was also obtained regarding the use of anti-seizure drugs, and past ivermectin intake. Three to five months after ivermectin treatment, the seizure frequency assessment was repeated.

2.5. Statistical Analysis

As the primary objective was to evaluate the effect of ivermectin treatment on seizure frequency, assessed at two time points (before ivermectin intake and 3–5 months after ivermectin treatment depending on the study site under consideration), this analysis included PWE with at least one seizure in the month preceding ivermectin intake. Continuous variables were summarized using the median and interquartile range (IQR). Categorical data were reported using absolute and relative proportions. The percentage reduction in frequency of seizures was calculated as the difference between pre- and post-ivermectin values divided by the pre-ivermectin values multiplied by 100. Pre- and post-ivermectin seizure frequencies were compared using the Wilcoxon signed-rank test. We assumed that the seizure frequency conditional on

covariates follows a negative binomial distribution. This distribution is an extension of the Poisson distribution commonly used to model count data. We used a negative binomial mixed model to obtain marginal estimates of the effect of ivermectin on the frequency of seizures while adjusting for relevant individual- and community-level covariates. The negative binomial mixed model included individual-specific random effects to accommodate association in the data as a result of repeated measurements at the same individual. The covariates included gender, age, study site, anti-seizure drug use, previous ivermectin use, and baseline mf density per skin snip. Ivermectin treatment was represented by time period (pre- and post-ivermectin). All possible two-way interactions between covariates were checked to be included in the final model, and inclusion into the model was determined based on the likelihood ratio test.

We conducted a mediation analysis to determine the effect of ivermectin itself (direct effect) and the effect of ivermectin through the reduction in mf density (indirect effect) on seizure frequency. Assuming that all confounders are controlled in the model and there are no time-varying confounders with respect to ivermectin and microfilarial density, we used the mediation analysis method for longitudinal data proposed by Bind et al., [19] available in the statistical software program SAS. We estimated the asymptotic sampling variances of the direct and indirect effects of ivermectin on seizure frequency using bootstrapping procedures. To preserve the correlation between pre- and post-ivermectin seizures and mf density, we resampled the longitudinal data with replacement. Unstandardized direct and indirect effects were computed for each of 500 bootstrapped samples, and bootstrap-based 95% percentile confidence intervals were computed. Data were analysed using R version 4.0.2, and SAS 9.4 (SAS Institute Inc, Cary, NC, USA).

2.6. Ethical approval and consent to participate

Ethical approvals were obtained from the ethics committees of the University of Antwerp (Belgium), the ethical committee of the School of Public Health, Kinshasa (DRC), the Ministry of Health of the Republic of South Sudan, and the National Institute of Medical Research, Tanzania. The purpose and procedures of the study were explained to all participants in their local languages. Participants were free to abstain

from participation in the study or to withdraw consent to participate at any time. No direct benefits for participation in the study were provided. All participants were asked to sign an informed consent form and only consenting individuals were enrolled. Minors >12 years and <18 years signed an assent form, while parents or legal guardians consented for younger participants. All individual data were encoded and treated confidentially.

3. Results

3.1. Baseline characteristics of participants

A total of 215 PWE from three study sites participated: Maridi, South Sudan (*n* = 105; 48.8%), Aketi, DRC (*n* = 87; 40.5%), and Mahenge, Tanzania (*n* = 23; 10.7%). About half of the study participants (48.8%) were males. Of these, 35 (16%) PWE had less than one seizure per month during the month preceding ivermectin intake. Generalized tonic-clonic seizures were the most frequent type of seizures in the three study sites. The median age was 17.0 years (IQR: 15.0–21.0) (Table 1).

Table 1. Baseline characteristics of people infected with onchocerciasis who are also persons with epilepsy (PWE).

Characteristic	Maridi (*n* = 105)	Aketi (*n* = 87)	Mahenge (*n* = 23)	All participants (*n* = 215)
Age (years): median (IQR)	17.0 (14.0–18.0)	17.0 (15.0–20.0)	33.0 (27.3–42.0)	17.0 (15.0–21.0)
Gender: Male, *n* (%)	53 (50.5)	40 (49.4)	9 (39.1)	102 (48.8)
Weight (kg): median (IQR)	37.0 (30.0–48.0)	39.0 (30.0–43.0)	45.0 (40.5–49. 5)	40.0 (31.0–48.0)
History of anti-seizure drug use at enrolment, *n* (%)	97 (93.3)	55 (63.2)	21 (95.5)	173 (81.2)
Most frequent types of seizure				
Generalized tonic-clonic seizures: *n* (%)	58 (55.2)	74 (85.1)	17 (73.9)	149 (69.3)
Atonic seizures (drop attacks): *n* (%)	1 (0.9)	0 (0.0)	1 (4.3)	2 (0.9)
Absences: *n* (%)	3 (2.9)	4 (4.6)	3 (13.0)	10 (4.6)
Nodding seizures: *n* (%)	25 (23.8)	7 (8.0)	2 (8.7)	34 (15.8)
Generalized tonic-clonic with nodding seizures: *n* (%)	18 (17.1)	2 (2.3)	0 (0.0)	20 (9.3)
Ivermectin use in 2018, *n* (%)	41 (39.0)	81 (93.1)	18 (78.3)	140 (65.1)

IQR = Interquartile range

3.2. Pre- and post-ivermectin microfilarial density and seizure frequency

In the Aketi health zone, three months after ivermectin intake, 77.8% (63/81) of PWE were cleared of microfilariae and had negative skin snips. The median (IQR) % reduction in seizure frequency three months after ivermectin intake was 100% (50.0–100.0) (Table 2).

Table 2. Ivermectin response in 87 people infected with onchocerciasis who are also PWE in Aketi, DRC.

Variables	Before Ivermectin Intake	3 months after Ivermectin inTake	*P*-value
Positive skin snip for mf: *n* (%)	87 (100)	18 (22.2)	<0.001[a]
mf density: median (IQR)	12.0 (4.0–63.0)	0.0 (0.0–0.0)	<0.001[b]
Seizures per month: median (IQR)	1.0 (0.5–2.0)	1.0 (0.0–2.0)	<0.001[b]
Number of PWE with <1 seizure per month: *n* (%)	10 (11.5)	55 (63.3)	<0.001[a]
% reduction in frequency of seizures: median (IQR)	NA	100 (50.0-100)	NA

[a]p-values based on McNemar Test; [b]p-values based on Wilcoxon signed-rank test; mf = microfilarial density

In Maridi, five months after ivermectin intake, 21.0% (22/105) of PWE were free from microfilariae in skin snips. Median seizure frequency reduced significantly between baseline and follow up (Table 3).

Table 3. Ivermectin response in people infected with onchocerciasis who are also PWE in Maridi, South Sudan.

Variables	Before ivermectin intake	5 months after ivermectin intake	P-value
Positive skin snip for mf: *n* (%)	105 (100)	83/105 (79.0%)	<0.001[a]
mf density: median (IQR)	52.0 (29.0–84.0)	3.0 (1.0–10.0)	<0.001[b]
Seizures per month: median (IQR)	12.0 (2.0–90.0)	8.0 (2.0–20.0)	<0.001[b]
Number of PWE with <1 seizure per month: *n* (%)	8 (7.6)	45 (42.9)	NA
% reduction in frequency of the seizures: median (IQR)	NA	73.4 (26.0–90.0)	NA

[a] *P-values based on McNemar Test;* [b] *P values based on Wilcoxon signed ranked test; mf= microfilarial density*

In Mahenge, three months after ivermectin intake, 52.2% (12/23) of PWE were free from microfilariae in skin snips. Median mf density reduced significantly after ivermectin intake. The median % reduction in seizure frequency was 100% (85.8–100.0) (Table 4).

Table 4. Ivermectin response in people infected with onchocerciasis who are also PWE in Mahenge, Tanzania.

Parameters	Before ivermectin intake	3 months after ivermectin intake	P-value
Positive skin snip for mf: *n* (%)	23 (100)	11/23 (47.8)	0.002[a]
mf density: median (IQR)	3.0 (1.0–5.0)	0.0 (0.0–1.0)	<0.001[b]
Seizures per month: median (IQR)	2.0 (1.0–2.0)	0.0 (0.0–1.0)	0.093[b]
Number of PWE with <1 seizure per month: *n* (%)	2 (8.7)	12 (52.2)	<0.001[a]
% reduction in frequency of seizures: median (IQR)	NA	100 (85.8–100.0)	NA

[a] p-values based on McNemar Test; [b] p-values based on Wilcoxon signed-rank test; mf= microfilarial density.

Combining the results of the three study sites, the median (IQR) seizure frequency per month was 6.0 (2.0–60.0) before ivermectin treatment and 3.0 (1.0–16.0) after ivermectin treatment. There was a significant difference between median frequency of seizure before and after ivermectin treatment (sign test statistic = 62, p-value ≤ 0.001).

According to the negative binomial mixed effect model, the expected seizure frequency significantly decreased after ivermectin treatment with a larger decrease in PWE with higher baseline seizure frequencies (Table 5 and Figure 1). In the left panel of Figure 1, we present the relative difference in seizure frequency as a function of the baseline seizure frequency (i.e. the ratio of seizure frequency after vs. before ivermectin treatment). The relative difference decreases monotonically with increasing baseline seizure frequency. The number of seizures after ivermectin treatment as a function of the baseline seizure frequency is depicted in the right panel of Figure 1. More specifically, for persons with ten seizures at baseline, 60% of the seizures, or six seizures, are on average observed after ivermectin treatment.

Table 5. Predictors of seizure frequency using a negative binomial mixed model.

Effect	Estimate	95% CI		*P*-value
Intercept	2.322	0.736	3.908	0.004
Age (years)	−0.048	−0.107	0.012	0.114
Gender (male vs female)	−0.309	−0.833	0.215	0.245
Site (Mahenge vs Aketi)	−0.610	−2.207	0.987	0.452
Site (Maridi vs Aketi)	1.646	0.908	2.385	<0.001
Baseline mf/skin snip	0.002	−0.003	0.006	0.471
After ivermectin treatment (vs before)	−0.338	−0.592	−0.084	0.009
Seizure before ivermectin* After ivermectin treatment (vs before)	−0.014	−0.019	−0.008	<0.001
Previous ivermectin (used vs not used)	−0.371	−1.009	0.268	0.253
Anti-seizure drug use with ivermectin	0.144	−0.436	0.724	0.624
$Var\{b0\}$ (se)	2.129 (0.325)			

mf = microfilarial density; $Var\{b0\}$*: Variance of random intercepts;* se *: standard error; *: interaction* effect.

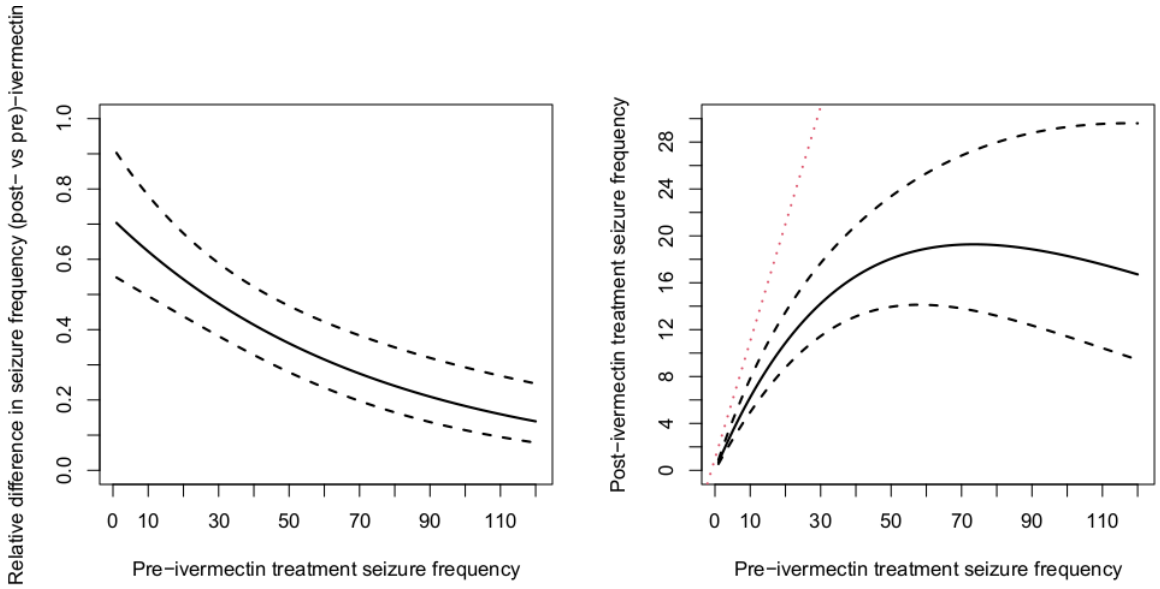


Figure 1. *The relative difference in post- vs pre-ivermectin seizure frequency as a function of pre-ivermectin seizure frequency. The solid black line represents the estimated relative difference and dashed lines indicate pointwise 95% confidence bands computed based on estimated variance-covariance matrix of the parameter (left panel). The right panel shows the expected seizure frequency rate after ivermectin treatment as a function of the baseline seizure frequency (solid black line); 95% confidence limits are displayed using dashed lines. Red dotted line displays no effect of ivermectin treatment (i.e frequency of seizure before ivermectin treatment = frequency of seizure after ivermectin treatment)*

After adjusting for age, gender, history of ivermectin, past exposure to anti-seizure drug and study site, both the direct and indirect ivermectin effect on the frequency of seizures was significant (Table 6). A schematic representation of the decomposition of the ivermectin effect in a direct and indirect effect is presented in Figure S1 of the Supplementary Material.

Table 6. Causal mediation effects of microfilarial density.

Estimates	Estimate	95% CI	
Indirect effect	−0.014	−0.020	−0.006
Direct effect	−0.715	−0.955	−0.465
Total effect	−0.730	−0.970	−0.470
Percentage mediated	1.867	1.363	2.087

CI: 95% bootstrap confidence interval

4. Discussion

This study assessed the effect of ivermectin on the frequency of seizures among PWE living in onchocerciasis-endemic areas. Our findings demonstrate that ivermectin treatment intake reduces the frequency of seizures in people infected with *O. volvulus* who are also PWE, three to five months after treatment. PWE with a higher pre-ivermectin seizure frequency experienced a larger reduction in post-ivermectin seizure frequency.

PWE in Maridi presented higher post-ivermectin seizure frequency as compared to those from Aketi. This could be due to the higher pre-ivermectin mf density among PWE in Maridi, which was probably due to the prolonged CDTI interruption in South Sudan. High mf densities have been shown to be associated with more severe forms of OAE, with more seizures and disabilities [20]. In a recent randomised trial among people infected with *O. volvulus* who are also PWE, seizure freedom was more often observed in the last 4 months of a one year trial in those who received two or three doses of ivermectin compared to only one dose [11]. This difference was explained by a higher mf load during this period in the one dose treatment arm. The probability of being seizure-free was found to be positively associated with the absence of mf; OR = 2.618 (95% CI: 1.136–6.289). A problem with this trial was that at enrolment all PWE were not on a regular anti-seizure treatment and that during the trial, anti-seizure drug doses frequently had to be adapted, complicating the interpretation of the trial results.

In our three-country study, because of the lack of a control group and the problem with potential confounders, it was difficult to investigate the relationship between the effect of ivermectin on mf load and frequency of seizures. However, using mediation analysis, both the direct and indirect effects of ivermectin led to a significant reduction in post-ivermectin treatment seizure frequency. This suggests that next to the reduction in mf density and the effect thereof on the seizure frequency, ivermectin treatment itself may have an additional beneficial effect in lowering the frequency of seizures. An anti-convulsive effect of ivermectin is unlikely because, at the therapeutic dose, ivermectin is unable to cross the human blood–brain barrier [21]. Moreover, given the short half-life of ivermectin (about 56.50 ± 7.01 hours) [22], it is difficult to

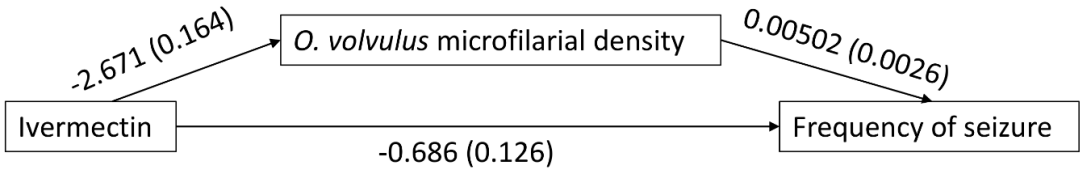
explain how one dose of ivermectin would have a direct anti-seizure effect during 3-5 months.

An anti-convulsive effect of ivermectin was suggested in a small non-randomised trial conducted in Spain among individuals with refractory epilepsy [22]. However, in the latter study, ivermectin was given three or seven times a week at a dose of 10mg per day along with anti-seizure drugs, and some patients had brain lesions which possibly compromised the integrity of the blood–brain barrier. It is unclear how ivermectin may have had a direct anti-seizure effect in our study. In the informed consent of the study, we mentioned that one of the study objectives was to determine whether ivermectin is able to decrease the frequency of seizures. Therefore, it is possible that the observed decrease in seizures was partially caused by a placebo effect. On the other hand, the relation between seizure frequency and mf density, prior to and after ivermectin treatment, is not fully captured by the model, thereby inducing a direct effect of ivermectin to explain the entire extent of the reduction in seizure frequency. Quantification of seizure frequency and mf density based on a single measurement before and after ivermectin treatment potentially introduces additional variability, masking the relation between frequency of seizures and mf density, at least partly.

A strength of our study is that similar results were obtained in three different study sites by three different teams of investigators. However, our results have to be interpreted in light of some limitations. The information on seizure frequency and anti-seizure drug use was obtained by interviewing PWE/family members, and therefore, we cannot rule out recall bias. We did not obtain detailed information about anti-seizure drug use (including treatment adherence) prior to the ivermectin intake and during the follow-up period. Mf densities were only measured per skin snip and not per mg of skin, rendering standardization and comparison across sites difficult. Moreover, our analysis included only *O. volvulus* infected PWE who came back for a second skin snip test. The lack of information on frequency of seizures of PWE who did not came back for a follow up could have influenced the study results. Indeed, some of them may not have come back because they did not observe any improvement in frequency of seizures thereby potentially biasing the estimated ivermectin effect on seizure frequency. Finally, no additional laboratory studies, nor neuro-imaging studies were performed to determine the aetiology of the epilepsy.

In conclusion, PWE with *O. volvulus* infection were found to have fewer seizures 3–5 months after receiving ivermectin, suggesting that ivermectin is effective in reducing seizures. Our finding that ivermectin is able to reduce the frequency of seizures through the reduction in mf density confirms the results of a recently performed clinical trial that investigated the effect of ivermectin on the frequency of seizures. However, our study suggests that the decrease in seizure frequency following ivermectin treatment cannot solely be ascribed to a reduction in mf density. Given the short half-life of ivermectin and the fact that ivermectin does not penetrate the brain, a direct anti-seizure effect of ivermectin is unlikely. A randomized controlled trial evaluating the effect of ivermectin in people infected with *O. volvulus* who are also PWE on a stable anti-seizure regimen may be needed to clarify the causal relationship between ivermectin use and the frequency of seizures.

Supplementary Materials: Figure S1: Mediation pathways.



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CHAPTER 4.

Surveillance for onchocerciasis-associated epilepsy and Ov16 IgG4 testing of children 6–10 years old should be used to identify areas where onchocerciasis elimination programs need strengthening

Surveillance for onchocerciasis-associated epilepsy and Ov16 IgG4 testing of children 6–10 years old should be used to identify areas where onchocerciasis elimination programs need strengthening

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Abstract

To eliminate onchocerciasis-associated morbidity, it is important to identify areas where there is still high ongoing *Onchocerca volvulus* (*O. volvulus*) transmission. Between 2015 and 2021, door-to-door surveys were conducted in onchocerciasis-endemic villages in Cameroon, the Democratic Republic of Congo (DRC), Nigeria, South Sudan, and Tanzania to determine epilepsy prevalence and incidence, type of epilepsy and ivermectin therapeutic coverage. Moreover, children aged between six and 10 years were tested for anti-*Onchocerca* antibodies using the Ov16 IgG4 rapid diagnostic test (RDT). A mixed-effect binary logistic regression analysis was used to assess significantly associated variables of Ov16 antibody seroprevalence.

A high prevalence and incidence of epilepsy was found to be associated with a high Ov16 antibody seroprevalence among 6–10-year-old children, except in the Logo health zone, DRC. The low Ov16 antibody seroprevalence among young children in the Logo health zone, despite a high prevalence of epilepsy, may be explained by a recent decrease in *O. volvulus* transmission because of a decline in the *Simulium* vector population as a result of deforestation. In the Central African Republic, a new focus of *O. volvulus* transmission was detected based on the high Ov16 IgG4 seropositivity among children and the detecting of nodding syndrome cases, a phenotypic form of onchocerciasis-associated epilepsy (OAE).

In conclusion, Ov16 IgG4 RDT testing of 6–10-year-old children is a cheap and rapid method to determine the level of ongoing *O. volvulus* transmission and to assess, together with surveillance for OAE, the performance of onchocerciasis elimination programs.

Keywords: onchocerciasis; onchocerciasis-associated epilepsy; epilepsy prevalence; incidence; ivermectin; OV16 antibodies; Africa

1. Introduction

Onchocerciasis, commonly known as river blindness, is caused by the filarial worm *Onchocerca volvulus* (*O. volvulus*) [1]. It is estimated that 99% of the 20.9 million *O. volvulus* infected individuals live in 31 African countries [2]. Over 70% (14.6 million) of the *O. volvulus* infected individuals are considered to have onchocerciasis-induced skin disease and 5.5% (1.15 million) to have vision loss [3]. Moreover, accumulating evidence suggest that *O. volvulus* infection is also able to trigger epilepsy in a manner that is dependent on the microfilarial (mf) load in the skin [4-6], so-called onchocerciasis-associated epilepsy (OAE) [7].

Onchocerciasis-elimination programs rely on community-directed treatment with ivermectin (CDTI) and vector control [3]. Using CDTI, the African Programme for Onchocerciasis Control (APOC) has successfully eliminated onchocerciasis as a public health problem in several African countries [3, 8]. However, in some onchocerciasis-endemic areas in Africa there is still high ongoing *O. volvulus* transmission and a high prevalence of onchocerciasis-associated morbidity including OAE due to low CDTI coverage and in some areas resulting from CDTI interruptions during the periods of insecurity [7, 9, 10].

Several new promising drugs for the treatment of onchocerciasis are being tested in clinical trials [11, 12], of which moxidectin was shown to reduce and maintain low skin microfilarial density for longer than ivermectin [13]. Macrofilaricides, currently only in an early phase of development, will be needed to drastically reduce the elimination time of onchocerciasis [11, 12]. However, today none of these new drugs are available for mass drug administration programmes.

The interruption of *O. volvulus* transmission is evaluated by screening pooled blackflies using the O-150 PCR technique targeting parasite-specific markers and by dissecting the heads and thorax of blackflies to determine the level of infective *O. volvulus* larvae (L3 stage) under a binocular microscope [14]. Moreover, the prevalence of anti-Ov16 immunoglobulin G4 (IgG4) antibodies in children aged <10 years, determined by an Ov16 ELISA test, is also used to assess *O. volvulus* transmission interruption [14]. This method has been used by the South American

onchocerciasis elimination programme to document the elimination of onchocerciasis in several Latin American countries [15-19], and also in some African countries such as Senegal [20] and Uganda [21]. However the threshold required to determine when it is safe to stop CDTI and to declare interruption of transmission is still under debate [22]. According to World Health Organization (WHO) guidelines, 2000 children under 10 years of age have to be tested for Ov16 antibodies, and a seroprevalence below 0.1% is required to assume a sufficient reduction of *O. volvulus* transmission such that CDTI can be stopped [14]. A modelling study suggested that the Ov16 antibody prevalence in children aged 5–14 years would perform better in predicting elimination and that a threshold value for this age group of 2.0% and even higher threshold values would be safe to use in lower-endemic areas [23].

While it is important to know when a CDTI program can be stopped, it is also important to identify CDTI programs that are working sub-optimally in order to strengthen them. To do so, CDTI coverage is assessed, and skin snip testing has been used to monitor community microfilarial loads. There are, however, problems with both methods: CDTI coverage data reported by the community-directed distributors of ivermectin are often not very reliable [24]. Independent surveys, as recommended by the WHO, provide more reliable results but are relatively costly. Skin snip testing is also problematic because it requires punches that are difficult to obtain and that are quite expensive. It also requires an experienced lab technician and a good microscope to read the skin snips and differentiate *O. volvulus* microfilariae from other filarial larvae, with results only made available the next day. Moreover, as it is an invasive and slightly painful procedure, therefore populations are increasingly reluctant to be skin snip tested. Therefore, we propose to use the Ov16 IgG4 rapid diagnostic testing (Ov16 RDT) of children 6–10 years old as an easier alternative way to determine the degree of ongoing onchocerciasis transmission. In different onchocerciasis-endemic foci in sub-Saharan Africa, we investigated how the Ov16 RDT seroprevalence among 6–10-year-old children as a proxy for ongoing *O. volvulus* transmission, together with epilepsy prevalence and ivermectin coverage data, can be used to evaluate the performance of the onchocerciasis-elimination programs.

2. Materials and Methods

2.1. Epilepsy surveys in different onchocerciasis-endemic foci

Between 2015 and 2021, door-to-door epilepsy surveys were conducted in onchocerciasis-endemic villages across Central African countries: Cameroon (Sanaga valley in the Littoral region: Kelleng [25], and Mbam valley in the Littoral region: Bilomo, Bayomen, Nyamongo, and Ngongol [25, 26]); the Central African Republic (CAR) [27, 28]; West African countries: Nigeria (Imo River Basin) [29]; Central and East African countries including the Democratic Republic of Congo (DRC) in Aketi, Bas Uéle [30], and Logo, Ituri [31, 32]; Tanzania (Mahenge) [33]; South Sudan, Maridi [9], Mvolo [10] and Mundri, West County [34]. In total, eighteen study sites in eight different onchocerciasis foci were included in the study (Figure 1).

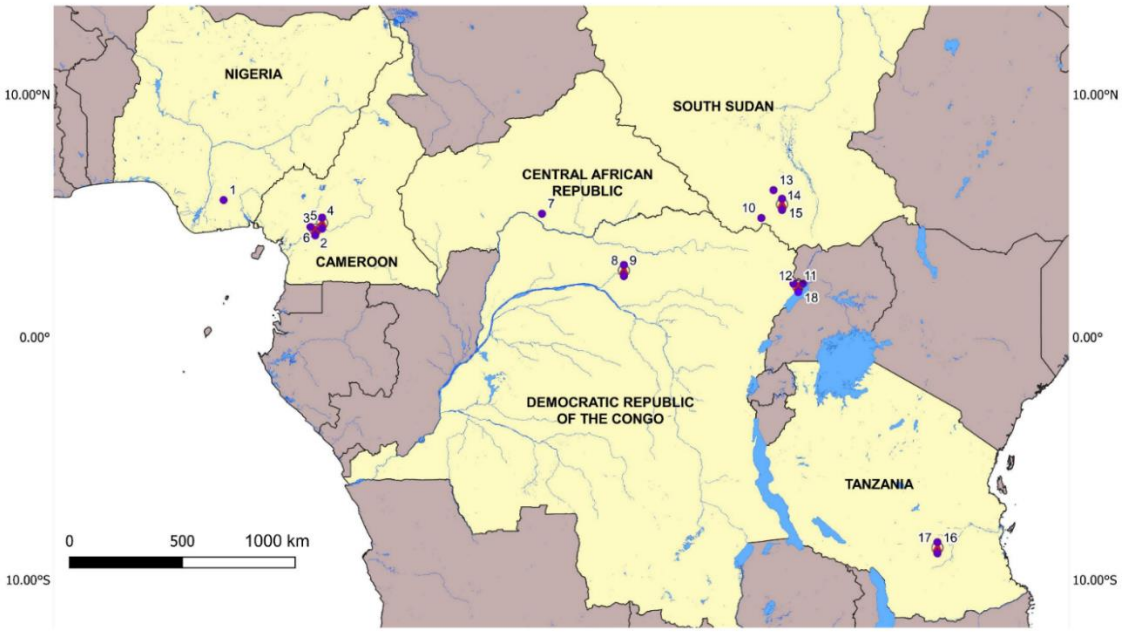

Figure 1. Map with the localisation of the villages included in the study; (1) Imo River Valley (Imo State); (2) Kelleng (Littoral Region); (3) Bayomen (Mbam River Valley); (4) Nyamongo (Mbam River Valley); (5) Bilomo (Centre region); (6) Ngongol (Mbam River Valley); (7) Kodjo (Landja Mboko District); (8) Makoko (Bas Uélé Province); (9) Wela (Bas Uélé Province); (10) Maridi (Western Equatoria state); (11) Kuda valley (Ituri Province); (12) Draju (Ituri Province); (13) Mvolo (Western Equatoria state); (14) Mundri center Payam (Western Equatoria state); (15) Amadi Payam (Western Equatoria state); (16) Mahenge Sub-urban villages (Ulanga district); (17) Mahenge Rural villages (Ulanga district); (18) Kuda valley (Ituri Province).

Two steps were used to identify people suspected to have epilepsy. In the first step, trained research assistants accompanied by village volunteers (usually community health workers) carried out house-to-house visits in the study villages. After they obtained informed consent, household members were interviewed using a validated questionnaire containing five epilepsy screening questions [35]. This questionnaire was translated into the local languages of each study site. If the answer to one of the five questions was positive, the person was suspected to have epilepsy. Additionally during the survey, household members were asked whether they had taken ivermectin during the most recent CDTI round.

In a second step, all suspected epilepsy cases were seen by a clinical officer or medical doctor trained to diagnose epilepsy, and/or a neurologist. These clinicians took a detailed medical history of the suspected epilepsy cases and performed a complete clinical examination, and a targeted neurological evaluation to confirm or reject the diagnosis of epilepsy using a structured pre-tested questionnaire.

Epilepsy was defined as recommended by the International League Against Epilepsy (ILAE): the occurrence of at least two unprovoked seizures with a minimum of 24 h between the two episodes [36].

Onchocerciasis-associated epilepsy (OAE) was defined previously [7], using published criteria which included: residence in the study village for at least three years, the high prevalence of epilepsy in the village, the onset of epilepsy between the age of three and 18 years, normal psychomotor development prior to the onset of seizures, and no obvious cause for epilepsy obtainable from the medical history. As potential “obvious causes for epilepsy”, we considered a history of perinatal trauma (including prolonged labour and birth by emergency caesarean section), severe measles, severe malaria, encephalitis or meningitis, or head injury with loss of consciousness within the two years preceding the onset of seizures.

2.2. Assessment of the level of onchocerciasis transmission

We assessed the degree of onchocerciasis transmission in the participating villages by testing children 6-10 years old for onchocerciasis antibodies using the Ov16 IgG4 RDT (Standard Diagnostics, Inc., Giheung-gu, Yongin-si, Gyeonggi-do, Korea). Six

and 10-year-old children were only tested at certain study sites but seven- to nine-year-old children were tested at all study sites. After informed consent was obtained from the parents of the children, all procedures were followed as per the manufacturer's instructions, and Ov16 RDT results were noted for each participant. Parents of the children were also asked whether their children had taken ivermectin during the most recent CDTI. In four study sites (Aketi and Logo health zones in the DRC; Mahenge in Tanzania; and Maridi in South Sudan), microfilarial loads in skin snips of persons with epilepsy were measured before the ivermectin intake.

2.3. Data Analysis

Categorical variables were summarized as absolute frequencies and percentages. Epilepsy incidence and prevalence, Ov16 seropositivity among children 6–10 years old, and ivermectin coverage among the different onchocerciasis foci were calculated per study site. Epilepsy incidence was estimated retrospectively by summing up all the confirmed cases of epilepsy that reported an onset of seizure within the last five years (i.e., duration of epilepsy between zero and five years) divided by the total number of individuals involved in the house-to-house survey and dividing by five for yearly incidence. Ivermectin coverage in the population was calculated as the number of the survey participants that took ivermectin over the total number of individuals involved in the survey. The epilepsy prevalence, incidence and OV16 seropositivity with its corresponding exact Clopper-Pearson confidence intervals were visually presented.

A generalized linear mixed model (GLMM) using a logit link was fitted to assess factors associated with Ov16 seropositivity (binary response) among the children residing in onchocerciasis areas with the study site being considered as the random effect to account for the correlation that can occur among children residing in the same study site. We first fitted the model to assess an association between Ov16 seropositivity and the children's characteristics such as age and gender of the children and ivermectin intake during the last CDTI round. Secondarily, we fitted the model to investigate whether Ov16 seropositivity of 6–10-year-old children could be used to assess the performance of an onchocerciasis elimination program at the population level. At the population level, the fixed variables included ivermectin coverage and epilepsy prevalence in the study site community. The GLMM results were reported as

adjusted odds ratios with 95% confidence intervals (CIs). A two-sided 5% significance level was used. Data were analysed using SAS software version 9.4, (SAS Institute, Inc., Cary, NC, USA) and R software version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Summary statistics related to the epilepsy surveys and the Ov16 serosurveys are presented in Tables 1 and 2, respectively. In total, 47,935 individuals from eight different onchocerciasis foci participated in the epilepsy door-to-door surveys and Ov16 RDT were performed in 1821 children aged 6–10 years. Of these tested children, 907 (49.4%) were boys, and 1059 (58.2%) had taken ivermectin during the last distribution round.

Table 1. Prevalence and incidence of epilepsy, the proportion of epilepsy individuals meeting the OAE criteria, skin snip positivity of persons with epilepsy and ivermectin coverage at each study site.

Study Site, (Study Years)	Prevalence	Incidence [B]	Epilepsy Meeting OAE Criteria (%)	Positive Skin Snip [D]	GMF+ (SD) [D]	Ivermectin Coverage
Nigeria						
Umuoparaodu and Umuezeala, Imo river valley (2018) [A] [29]	4/843 (0.50%)	23.7	3/4 (75%)	0/4 (0%)		672/843 (79.7%)
Landja Mboko, Central African Republic [C]						
Kodjo (2021) [27]	55/6175 (0.9%)		NS reported			0/6175 (0%)
Sanaga river valley, Cameroon [E]						
Kelleng (2018) [25]	16/204 (7.8%)	98.0	93.8%			141/204 (69.2%)
Bilomo (2017) [25]	61/1321 (4.6%)	227.1	98.2%			847/1321 (64.1%)
Mbam river valley, Cameroon						
Nyamongo (2017) [26]	42/1151 (3.7%)	173.8	92.3%			
Bayomen (2017) [26]	15/582 (2.6%)	68.7	93.3%			
Ngongol (2017) [26]	24/553 (4.3%)	144.4	95.7%			
Bas Uélé, DRC						
Aketi town (2017) [30]	125/2180 (5.7%)		75.8%	18/74 (24%)	12.9 (2.1)	1219/2180 (55.9%)
Wela (2014–2016) [32]	39/570 (6.8%)	596.5				298/570 (52.3%)
Makoko (2014–2016) [32]	31/367 (8.4%)	817.4				217/367 (59.1%)
Ituri, DRC [F]						
Draju (Logo health zone) (2016) [G] [31]	64/1389 (4.6%)	719.9	94.0%	66/136 (48%)	24.7 (3.2)	0/1339 (0%)
Western Equatoria state, South Sudan						
Maridi (2018) [9, 37]	736/17,652 (4.4%)	321.8	85.2%	82/102 (80%)	15.0 (1.1)	7209/17,652 (40.8%)
Mvolo (2020) [10]	798/15,699 (5.1%)	191.1	78.4%			9859/13,780 (71.5%)
Mundri West County [H]						
Amadi Payam (2021) [34]	14/317 (4.5%)	126.2	76.6%			155/317 (48.9%)
Mundri Centre Payam (2021) [34]	43/1400 (3.1%)	14.3	80.5%			775/1400 (55.4%)
Lui town Payam (2021) [34]	26/626 (4.1%)	31.9	84.0%			231/626 (37.0%)
Mahenge, Tanzania [I]						
Sub-urban villages (2017) [33]	39/2618 (1.4%)	120.1				2039/2618 (77.9%)
Rural villages (2017) [33]	88/2499 (3.5%)	91.7	77.9%	22/42 (52.4%)	5.7 (1.6)	2028/2499 (81.6%)

[A]: Three persons with epilepsy meeting the OAE criteria were not born in the study village and had not received ivermectin prior to seizure onset. [B]: Per 100,000 person per year. OAE: Onchocerciasis-associated epilepsy. [C]: OAE criteria were not systematically assessed but only nodding syndrome cases were reported [28]. NS: Nodding syndrome. GMF+: Geometric mean of microfilarial load of two skin snips obtained per individuals. SD: standard error of the geometric mean. [D]: Skin snip performed in four study sites to determine microfilarial loads in persons with epilepsy reported in Dusabimana et al. [37]. [E]: Proportion of persons with epilepsy meeting OAE criteria were calculated based on data collected by Siewe Fodjo et al. [38]. [F]: Persons with epilepsy meeting OAE criteria were calculated and reported by Mandro et al. [39]. [G]: Incidence in Draju (Logo health zone) was calculated based on people with epilepsy who reported an onset of seizures within the last 12 months preceding the survey (i.e., epilepsy duration ≤ one year) divided by the total number of people who completed the questionnaire during house-to-house surveys. [H]: Jada et al., [34]. [I]: Persons with epilepsy meeting OAE criteria were calculated based on data collected by Bhwana et al. [40].

Table 2. Ov16 RDT prevalence among children 6–10 years old and ivermectin coverage among 7–9-year-old children at each study site.

Study Site (Study Years)	Ov16 RDT Seroprevalence in the children					Ivermectin Coverage 7–9 Years
	6 years	7 years	8 years	9 years	10 years	
Nigeria						
Umuoparaodu and Umuezeala, Imo river valley (2018) [29]	0/5 (0.0%)	0/9 (0.0%)	0/5 (0.0%)	0/17 (0.0%)	0/14 (0.0%)	21/31 (67.7%)
Landja Mboko, Central African Republic						
Kodjo (2021) [27]	2/20 (10.0%)	2/5 (40.0%)	4/12 (33.3%)	2/13 (15.4%)		0/30 (0.0%)
Sanaga river valley, Cameroon						
Kelleng (2018) [25]		3/6 (50%)	4/7 (57.1%)	2/3 (66.7%)	4/9 (44.4%)	15/16 (93.7%)
Bilomo (2017) [25]		31/52 (53.1%)	14/40 (41.7%)	10/20 (47.8%)	13/33 (39.4%)	43/112 (38.4%)
Mbam river valley, Cameroon						
Nyamongo (2017) [26]		17/32 (44.4%)	5/12 (45.5%)	11/23 (31.3%)	13/33 (42.9%)	40/67 (59.7%)
Bayomen (2017) [26]		25/39 (64.1%)	17/29 (58.6%)	13/23 (56.5%)	12/29 (41.4%)	43/91 (47.3%)
Ngongol (2017) [26]		16/36 (44.4%)	5/11 (45.4%)	5/16 (31.2%)	9/21 (42.8%)	36/63 (57.1%)
Bas Uélé, DRC						
Wela (2014–2016) [30, 32]		46/60 (76.6%)	33/43 (76.7%)	18/21 (85.7%)	25/28 (89.3%)	96/124 (77.4%)
Makoko (2015–2016) [30, 32]		19/43 (44%)	18/35 (51%)	6/17 (35%)	17/35 (48.6%)	90/95 (94.7%)
Ituri, DRC						
Draju (Logo health zone) (2016) [31, 32]	0/51 (0%)	4/39 (10.3%)	3/41 (7.3%)	3/36 (8.3%)	2/25 (8%)	0/116 (0%)
Kuda valley (Logo health zone) (2018)	0/4 (0.0%)	0/13 (0.0%)	0/6 (0.0%)	1/11 (9.1%)	0/11 (0.0%)	0/60 (0%)
Kuda valley (Nyarambe health zone) (2021)		0/49 (0.0%)	0/19 (0.0%)	0/26 (0.0%)		85/94 (90.4%)
Equatoria State, South Sudan						
Maridi (2016) [9, 37]	6/24 (25%)	11/30 (36.6%)	2/10 (20%)	3/8 (37.5%)		34/48 (70.8%)
Mvolo (2020) [10]	7/22 (31.8%)	7/15 (46.6%)	4/20 (20%)	7/18 (38.8%)		22/53 (41.5%)
Mundri West County [A]						
Amadi Payam (2021) [34]	2/7 (28.5%)	1/11 (9.1%)	4/14 (28.6%)	6/12 (50.0%)		16/37 (43.2%)
Mundri Centre Payam (2021) [34]	1/18 (5.5%)	0/26 (0.0%)	1/23 (4.3%)	0/16 (0.0%)		18/65 (27.7%)
Mahenge, Tanzania						
Sub-urban villages (2018) [33]	0/26 (0.0%)	1/42 (2.4%)	3/65 (4.6%)	1/48 (2.1%)	5/91 (5.5%)	111/155 (71.6%)
Rural villages (2018) [33]	2/16 (12.5%)	19/52 (36.5%)	11/37 (29.7%)	26/54 (48.1%)	41/99 (41.4%)	106/143 (74.1%)

[A]: Jada et al., [34].

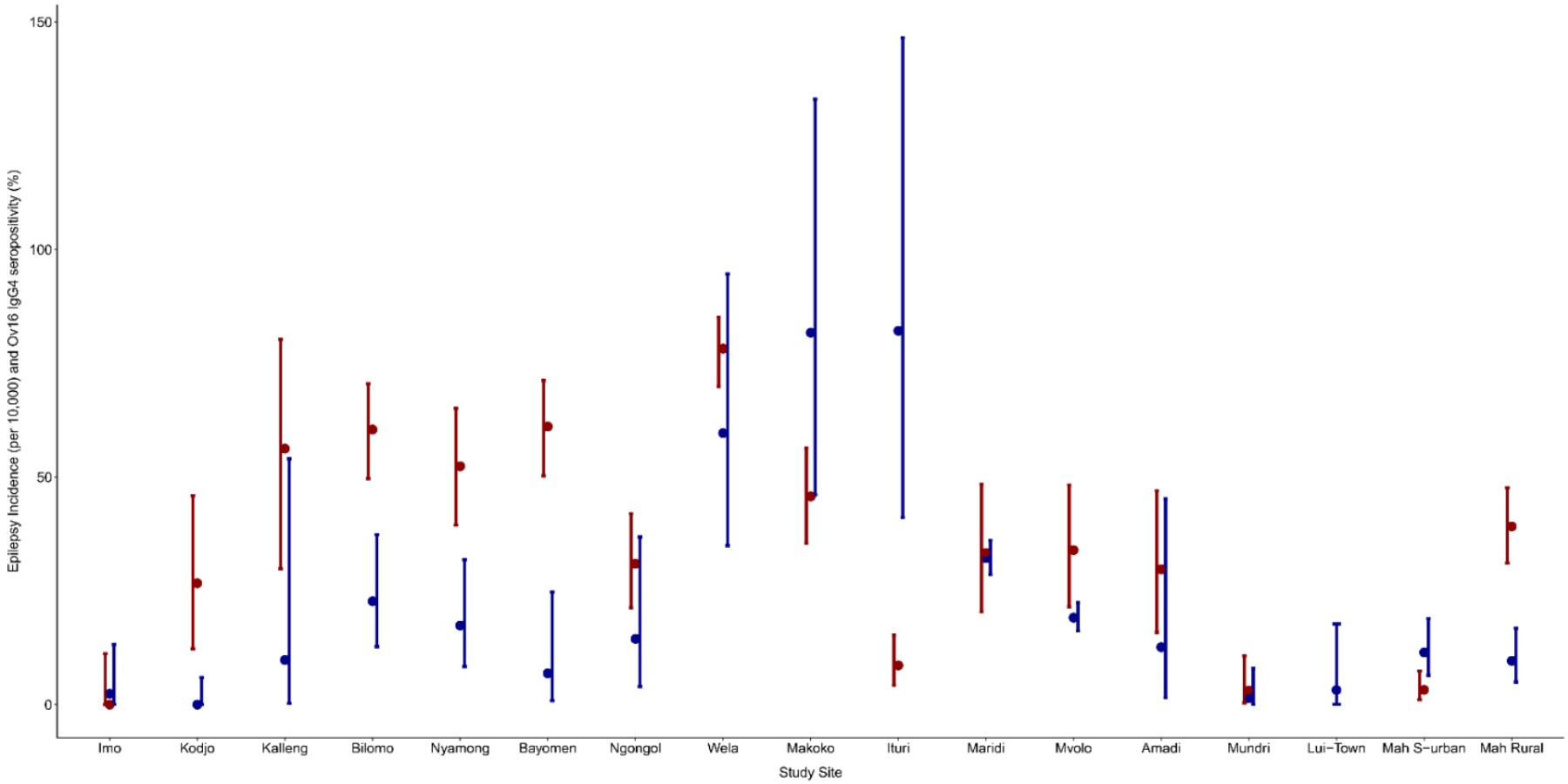

Figure 2. *Ov16 IgG4 seropositivity (%) in children aged 7–9 years (red dots) with 95% exact Clopper-Pearson confidence interval and incidence of epilepsy (per 10,000) per study site (blue dots) with 95% exact Clopper-Pearson confidence interval in the study site.*

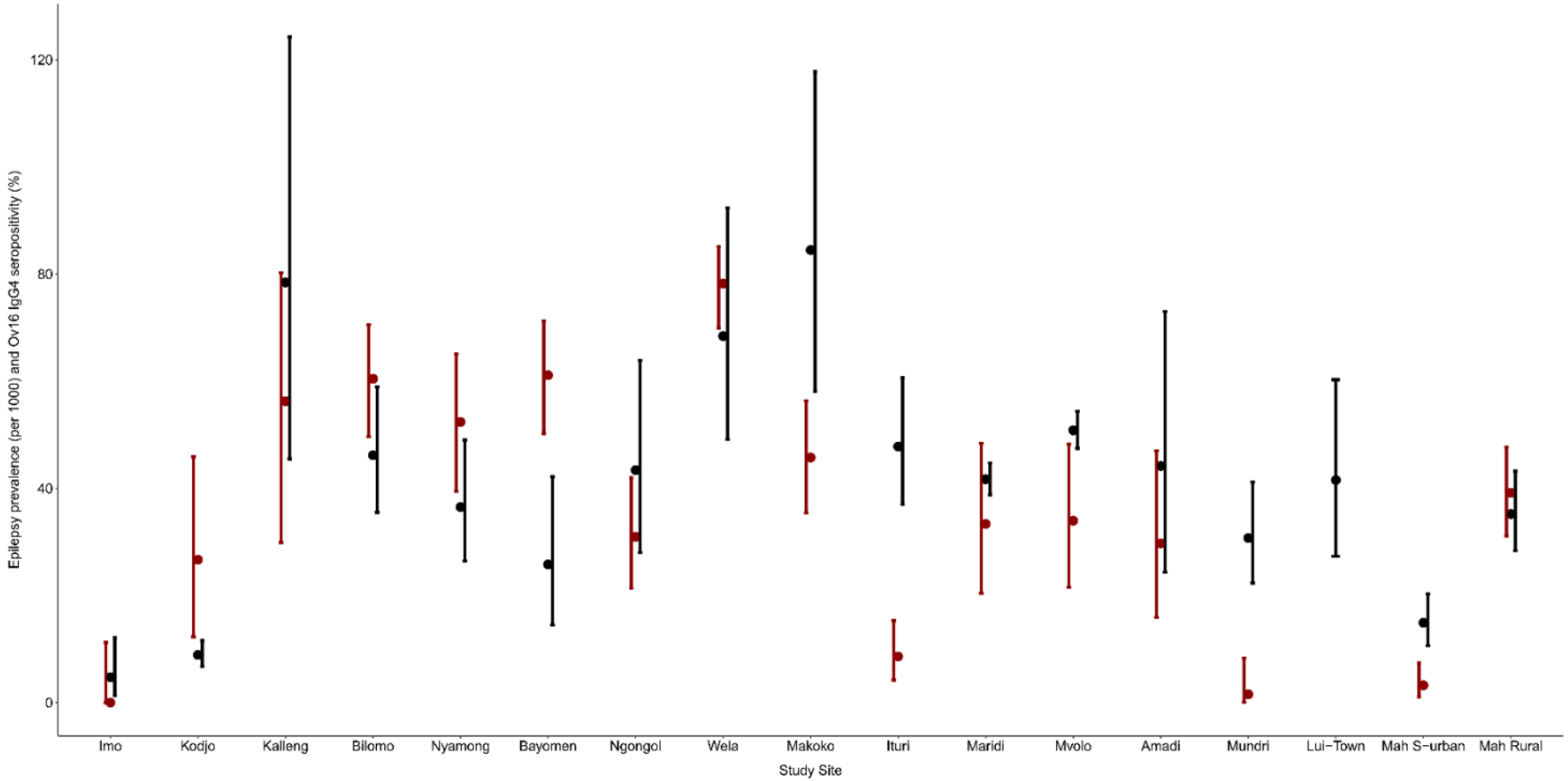

Figure 3. *Ov16 IgG4 seropositivity (%) in children aged from 7–9 years (red dots) with 95% Clopper-Pearson (Exact) confidence interval and prevalence of epilepsy (per 1000) per study site (black dots) with 95% Clopper-Pearson confidence interval in the study site*.

In all the villages, at least 75% of the epilepsy cases met the OAE criteria. However, in the Imo River Valley in Nigeria, the three persons with epilepsy meeting the OAE criteria were immigrants and had developed their first seizures in another onchocerciasis-endemic area in Nigeria. In most study sites, a high Ov16 seropositivity in children was observed in villages with high epilepsy prevalence (Figures 2 and 3). A high epilepsy prevalence in the village was associated with a high Ov16 seropositivity (Odds Ratio (OR): 1.288, 95% CI: 1.194–1.390, $p < 0.001$). In contrast, a high ivermectin coverage in the village was associated with a low Ov16 seropositivity among children residing in that village (OR: 0.961, 95% CI: 0.951–0.972, $p < 0.001$) (Table 3 and Figure 4).

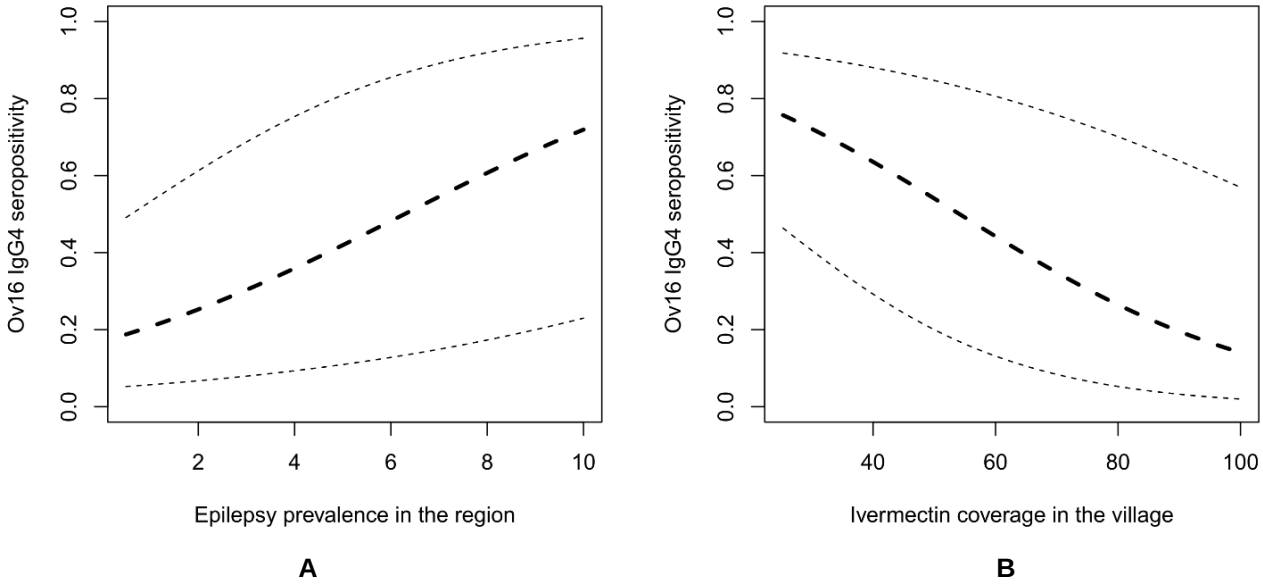

Figure 4. *The probability of Ov16 seropositivity estimated from a generalized linear mixed model with village (excluding Ituri because of recent decrease in vector population and excluding Kodjo in the Central African Republic because no state-of-the-art epilepsy survey was done) considered as random effect plotted as the function of epilepsy prevalence in the study sites (**A**) and ivermectin coverage in population residing in the study site (**B**). Black solid lines represent the estimated probability of Ov16 seropositivity with pointwise 95% Wald-type confidence bands.*

Table 3. Generalized linear mixed model to assess an association between Ov16 IgG4 seropositivity (as a proxy for ongoing O. volvulus transmission in the participated community) and the ivermectin coverage and epilepsy prevalence considering the study village as random effect.

Effect	Estimated OR	95% CI		*P*-value
Intercept	2.777	1.414	5.453	0.003
Ivermectin coverage in the village (in %)	0.961	0.951	0.972	<0.001
Epilepsy prevalence in village (in %)	1.288	1.194	1.390	<0.001
$Var(b0)$ (se) [A]	0.055 (0.170)			

$Var(b0)$: Variance of random intercept. se: Standard error. [A]: Ituri, DRC and Kodjo, RCA were excluded in the analysis. OR: Estimated odds ratio

The Ov16 seropositivity of six-year-old children was significantly lower compared to that of the 10-year-old children; however, no difference in Ov16 seropositivity was observed when comparing 7-, 8- and 9-year-old children with 10-year-old children (Table 4).

Table 4. Generalized linear mixed model to assess the variables associated with Ov16 IgG4 seropositivity among children 6–10 years of age, considering the study village as random effect

Variables	Estimated OR	95% CI		*P*-value
Intercept	0.439	0.201	0.959	0.040
Male gender	1.036	0.827	1.299	0.756
Female gender (reference)				
Age (6 years)	0.466	0.259	0.839	0.011
Age (7 years)	1.127	0.816	1.558	0.466
Age (8 years)	0.878	0.621	1.242	0.463
Age (9 years)	1.160	0.816	1.651	0.408
Age (10 years) (reference)				
Children ever used ivermectin	0.954	0.730	1.248	0.733
Children never used ivermectin (reference)				
$Var(b0)$ (se) [A]	1.551 (0.631)			

$Var(b0)$: Variance of random intercept. se: Standard error. [A]: Ituri, DRC and Kodjo, RCA were excluded in the analysis. OR: Estimated odds ratios

4. Discussion

In onchocerciasis-endemic foci with a high epilepsy prevalence and incidence, we observed a high Ov16 seropositivity among children less than 11 years old, except in villages from the Logo health zone in Ituri, DRC where Ov16 seroprevalence was only 6.3%. In all onchocerciasis-endemic foci with a high Ov16 seroprevalence among young children, more than 75% (ranging from 75.0% to 94%) of all persons with epilepsy in the village met the criteria of OAE. In addition, a high prevalence and incidence of epilepsy was observed in areas of low ivermectin coverage, or where ivermectin was never distributed, such as in the Logo health zone. These data suggest that high ongoing *O. volvulus* transmission is associated with a high prevalence and incidence of epilepsy.

The high epilepsy prevalence and incidence in villages in the Logo health zone, despite low Ov16 seroprevalence among young children (a proxy for ongoing *O. volvulus* transmission), is most likely the result of high *O. volvulus* transmission in the past and a recent decrease in transmission. Indeed, during a randomized clinical trial comparing the efficacy of moxidectin with ivermectin in 2009 in the Logo health zone, a high number of *O. volvulus* infected individuals with a high microfilarial load was observed [13]. However, except for one dose of ivermectin or moxidectin that was administered to the individuals who participated in this clinical trial, ivermectin and moxidectin were never distributed in this health zone. Despite a REMO assessment that had documented a prevalence of onchocerciasis nodules in Draju and certain other villages in the area, the rest of the Logo health zone had been considered to be an onchocerciasis hypo-endemic region and therefore had not been included in the CDTI program [31]. The fact that the epilepsy incidence was still high in 2016 may be explained by the fact that ivermectin-naïve children, who were already infected several years earlier, still harboured high microfilarial loads, putting them at risk of developing epilepsy even with the declining *O. volvulus* transmission. We can exclude a problem of quality of the Ov16 RDT, because at the same time the 6–10-year-old children tested negative, two persons with epilepsy meeting the OAE criteria from the same area tested Ov16 RDT positive.

In Draju, a mountainous area located in the Logo health zone, a number of 6–10-year-old children were still Ov16 seropositive in 2016. In 2018 in the Goma and Jabi villages

(which are closer to the Kuda river in the Kuda valley of the Logo health zone), only one of the Ov16 RDT tested children was positive. The explanation of the recent decline in *O. volvulus* transmission in the Logo health zone could be that the abundance of the blackfly vector of *O. volvulus* in the area recently declined possibly due to deforestation. According to the local population of the Kakoi-Koda onchocerciasis focus, they started slash-and-burn agriculture and commercial farming around 1990 which enormously increased the deforestation in this area [41] . They sometimes had to stop logging the forest because of the intensity of the blackfly bites. Local elders reported that around 1987, in the early years of the settlement of houses near the forests, the nuisance caused by the blackflies was terrible and it was sometimes necessary to flee and stay at home or to move up the hills to escape the bites [42]. However, in 2017, people mentioned that they were still being bitten by blackflies around the Kakoi river at lower altitude and during the cold season or in rainy and foggy weather [42]. A similar level of deforestation (up to 6.8% between 2000 and 2020) has also been noted in many other parts of the DRC [43, 44]. Another explanation of the low *O. volvulus* transmission in the Logo health zone in DRC could be the restrictions of movements to crop fields far from houses due to the conflicts and insecurity that have increased since 2017 [45].

In a recent investigation, only two types of blackflies in the Logo area, *Simulium vorax* and *Simulium dentulosum,* were found to be infested with *O. volvulus* [46] . Of these two types of blackflies, only *S. dentulosum* was found to be infective (presence of *O. volvulus* L3 larvae in the head). *Simulium neavei* was found breeding in some rivers outside the Kakoi-Koda onchocerciasis focus. Therefore, it is possible that *S. neavei* was the main (or the only) vector in the past but recently became rare as a result of the removal of tree cover, as a result of land use changes, and because the crabs they used as substrates also became rare. In 2021 in the Goro, Jupagassa, Jupafoyo, Jupupedero and Jupumvuga villages of the Kuda valley in the Nyarambe health zone, a part of the Kakoi-Koda onchocerciasis focus where CDTI was implemented, all 7–9-year-old children also tested Ov16 RDT negative. However, 90.4% of these children had taken ivermectin, but this should not have completely erased their *O. volvulus* immune response if they had been exposed.

The highest Ov16 seropositivity among 7–10-year-old children as well as the highest epilepsy prevalence and incidence was observed in Wela and Makoko (Bas Uéle province, DRC). In these villages, CDTI had been implemented for 14 years but geographic coverage had been very low [30]. The high onchocerciasis endemicity in these villages was also shown by a REMO assessment: 43 (86%) of 50 adult men examined in Wela and 21 (70%) of 30 examined in Makoko presented onchocercal nodules [30]. While performing surveys on OAE in 2015 in the Salambongo area (Tshopo province) [32, 47], *S. naevei* larvae were identified in the Aketi area on crabs in the Mobi and the Onane river [42].

A high Ov16 seropositivity among 7–10-year-old children and a high epilepsy prevalence and incidence were observed in villages in the Mbam and Sanaga valleys in Cameroon, despite many years of CDTI [25]. However, in this onchocerciasis focus, CDTI coverage has been sub-optimal. The percentage of infective blackflies in the area was found to be relatively low (0.10–0.36%), but in certain villages extremely high densities of biting blackflies were documented [48]. Despite a high Ov16 seropositivity in the children, a relatively low epilepsy prevalence (2.6%) was observed in Bayomen. This lower epilepsy prevalence in Bayomen could be explained by the high number of recent immigrants from other parts of Cameroon. Indeed, a stratified analysis including only indigenous households found a crude epilepsy prevalence of 6.7% (14/208) in Bayomen, 6.5% (15/232) in Ngongol and 5.5% (50/905) in Nyamongo [26]. The high Ov16 seropositivity in children in Bayomen concurs with the results of an entomological study that observed an even higher *O. volvulus* annual transmission based on blackfly parity and infection rates and transmission potentials in Bayomen (from dissection data) compared to Nyamongo [48].

In South Sudan, a high prevalence and incidence of epilepsy was observed in villages with high ongoing onchocerciasis transmission and with a history of many years of interrupted CDTI [9, 10]. In Maridi, the highest Ov16 seropositivity and epilepsy prevalence was observed close to the Maridi dam, the only blackfly breeding site in the area where very high blackfly biting rates were observed [49]. In Mundri center Payam, the Ov16 seroprevalence was low, contrasting with a relatively high epilepsy prevalence. This is explained by the fact that the site where the children were tested

was located in a more urban area further away from the river, while the epilepsy survey also included communities very close to the river.

In Tanzania, a high Ov16 seropositivity among 6–10-year-old children and a high prevalence and incidence of epilepsy were observed in the rural but not in the suburban villages of the Mahenge area [33]. *O. volvulus* transmission by *Simulium damnosum* s.l. was found to have continued in Mahenge despite 19 years of annual CDTI [50]. In 2016, the percentage of *S. damnosum* s.l. carrying infective L3 stage parasites was found to be 0.57% (95% CI 0.43–0.74%) [50], similar to infective rates reported in the 1960s [51]. In 2019, the geometric mean microfilarial density among persons with epilepsy prior to the intake of ivermectin was lowest in the Mahenge villages compared with geometric mean microfilarial densities in Maridi, Aketi and the Logo health zone [37], suggesting a lower transmission in Mahenge compared to the other sites.

In Nigeria, a 0% Ov16 seropositivity and a low epilepsy prevalence were observed after more than 20 years of CDTI in the Imo River Valley, with optimal coverage rates recorded during annual and then biannual CDTI rounds [29].

In Landja Mboko in the Central African Republic, an area located about 9 km from the capital city of Bangui where ivermectin was never distributed, a total of 6175 individuals were screened for epilepsy in 799 households [28]. In this study, 55 of the 75 epilepsy suspected cases examined by a neurologist were confirmed to have epilepsy, corresponding to an epilepsy prevalence of 0.89%. In addition to the 55 persons with epilepsy, five (9.1%) were classified as presenting nodding syndrome [28]. Ov16 RDT testing was performed in four settlements within the selected area at the four sites (Belespoir, Landja 1 and 2, Mangapou 2 and Kodjo), but a high Ov16 IgG4 seropositivity among 7–9-year-old children was observed only in Kodjo. Compared to other villages, Kodjo is situated only 200 m away from the Oubangui river, which most likely constitutes a suitable breeding ground for blackflies. When taken together, the high Ov16 seropositivity among 7–9-year-old children and the presence of nodding syndrome suggests that there may be a high prevalence of OAE in this area. However, because of insecurity in the area, an exhaustive house-to-house survey to assess the epilepsy prevalence was not appropriately conducted. A more in-depth investigation of the onchocerciasis and epilepsy situation in the Landja Mboko

area is urgently needed to evaluate whether CDTI should be implemented in the area to prevent children from developing OAE, and to contribute to the global elimination effort.

Ov16 IgG4 seropositivity of the six-year-old children was lower compared to that among 7–10-year-old children. The reason for this lower Ov16 seropositivity among the very young children most likely resides in their different degrees of exposure to blackfly bites, as this was found to increase with age; moreover, it takes many months for exposed children to build an immune response with detectable levels of antibodies [23]. Therefore, lower titers of Ov16 IgG4 antibodies may be detected in younger children.

Several limitations of our study need to be mentioned. To qualitatively determine the presence of onchocerciasis antibodies, only the Ov16 IgG4 RDT was used and not the Ov16 IgG4 ELISA (the gold standard technique for Ov16 IgG4 antibody detection, which is more sensitive than Ov16 IgG4 RDT) [52]. Moreover, no laboratory studies nor imaging investigations were performed to identify the causes of epilepsy. Given the cross-sectional study design, the incidence of epilepsy could only be estimated retrospectively by interview, and the information obtained could have been influenced by recall bias and the deaths of some of the individuals with epilepsy prior to the survey. We performed skin snips to determine microfilarial loads in persons with epilepsy in only four study sites; no community microfilarial load calculations were done, but we succeeded in carrying out REMO assessments in two study sites. Finally, entomological studies were carried out in only a few study sites; as a consequence we do not have data on the number of infected blackflies and biting rates in each community to determine the intensity and degree of exposure to the infected vectors per study site.

5. Conclusions

Epilepsy incidence and prevalence, ivermectin coverage, and Ov16 RDT testing among 6–10-year-old children constitutes three important parameters to evaluate the performance of onchocerciasis-elimination programs and/or to identify sites where potentially such a program needs to be introduced. The Ov16 RDT, because of its low

sensitivity [52], cannot be used to decide whether a CDTI program can be stopped, but could be used to rapidly assess the performance of a CDTI program in onchocerciasis-endemic areas with a high prevalence of epilepsy where no laboratory is available for performing ELISA testing and where ivermectin coverage data are not reliable. Epilepsy prevalence and incidence may also be used to estimate the performance of an onchocerciasis-elimination program. However, one needs to consider non-onchocerciasis related causes of epilepsy and the degree of in and out migration in the area. Moreover, to assess the performance of onchocerciasis-elimination programs, other parameters such as ecological and entomological parameters also need to be considered. Our data confirm the association between high ongoing or past *O. volvulus* transmission and epilepsy prevalence. Finally, the surveys performed in the DRC (Aketi), Cameroon, and Tanzania show that many years of annual CDTI with insufficient coverage cannot interrupt onchocerciasis transmission, possibly predisposing the affected communities to a high prevalence of OAE.

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Informed Consent Statement: During the house-to-house surveys, signed or thumb-printed informed consent was obtained from family members, parents or caregivers of children, and from adolescents (aged 12–18 years). Informed consent was also obtained from parents of the children who participated in the OV16 prevalence studies.

Data Availability Statement: The datasets with de-identified patients' data generated during the current study are available from the corresponding authors on reasonable request.

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CHAPTER 5

Community perceptions and attitudes regarding epilepsy and disease cost after implementation of a community-based epilepsy treatment program in onchocerciasis-endemic communities in the Democratic Republic of Congo

Community perceptions and attitudes regarding epilepsy and disease cost after implementation of a community-based epilepsy treatment program in onchocerciasis-endemic communities in the Democratic Republic of Congo

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Abstract

Background : In October 2017, a community-based epilepsy treatment program (CBETP) was initiated in the Logo health zone (Ituri province, Democratic Republic of Congo), consisting mainly of community epilepsy education, provision of free anti-epileptic drugs, and monthly follow-up of persons with epilepsy (PWE). Prior to the implementation of the CBETP, qualitative research had revealed several misconceptions about the cause of epilepsy, major epilepsy-related stigma and high economic cost for families of PWE mainly because of costly treatment by traditional healers. One year after the implementation of the CBETP, we assessed the perceived effect of this program on the community's perceptions and attitudes regarding epilepsy and effect on epilepsy-related costs.

Methods : Focus group discussions (FGD) and semi-structured in-depth interviews (SSI) were conducted with different target groups. Additionally, the cost associated with epilepsy was evaluated using questionnaires administered to 74 PWE and/or their families.

Results : Nine FGDs and 16 SSIs were conducted. There was a notable shift in perceptions and attitudes, as most community members no longer believed that epilepsy is contagious, while acknowledging that this condition can be treated in local health centres. PWE and their family experienced less epilepsy related stigma and consulted less frequently traditional healers; the latter showed a growing willingness to collaborate with health professionals in the management of PWE. The direct and indirect cost for families caring for a PWE decreased by 95.2% and 95.7%, respectively.

Conclusion: The main perceived benefits of the CBETP were the decrease of misconceptions about epilepsy and epilepsy-related stigma. Families with PWE understood the benefit of seeking health care from trained health professionals rather than traditional healers. The direct and indirect cost for families to take care of a PWE reduced considerably after the program. However, the cost-effectiveness and long-term sustainability of this approach remains to be assessed.

Key words: Epilepsy, community-based, anti-epileptic, stigma, misconceptions, sustainability

1. Background

Of the estimated 50 million people worldwide living with epilepsy, more than 80% reside in low and middle-income countries (LMICs) [1]. Despite being one of the most cost-effective neurological disorders to treat, epilepsy remains a major concern in low-income countries. If diagnosed properly and treated with proper anti-epileptic drugs (AEDs), up to seven in ten persons with epilepsy (PWE) could have their seizures fully controlled [2]. Although there are cheap AEDs on the market, about three quarters of PWE in LMICs are still not receiving appropriate treatment [1], mainly because of long distance to health facilities, lack of trained health care providers, cost of AEDs, cultural believe in traditional medicine or unavailability of AEDs [3]. Bridging the treatment gap and fully controlling the seizures in PWE is still a big challenge in LMICs. A meta-analysis of 12 studies reported that 70% of PWE in LMICs do not get the appropriate AEDs [4].

Epilepsy poses an important economic burden for the health system as well as on the PWE and their family because it is associated with increased healthcare needs, premature death and lost work productivity [5]. We previously showed that the cost of taking care of a PWE in the Logo health zone in Ituri province in the Democratic Republic of Congo (DRC), constituted almost half of the household income [6]; 68.2% of the direct epilepsy related cost was spent only on traditional medicine. Other costs included cost of outpatient care, AEDs cost, and expenses for transport particularly from remote areas to healthcare facilities. A study in Burundi revealed that healthcare expenses among PWE were 6 times higher compared to the rest of the population, and that families with PWE had on average 5 times more disrupted days than other families [7].

In October 2017, a clinical trial was initiated in the Logo health zone to investigate whether ivermectin is able to decrease the frequency of seizures [8]. During that project, we also aimed at establishing a community-based epilepsy treatment program (CBETP) to treat all PWE in the area [9]. Before setting up this program, formative research was conducted to investigate the community's knowledge, perception and attitudes regarding epilepsy. Qualitative findings revealed several misconceptions

about epilepsy, including the belief that epilepsy is a family-related disease, that it is contagious, transmitted by saliva or by contact with a person during seizures, or caused by evil spirits [10]. In the latter study, the traditional healers were found to play an important role in spreading these misconceptions. The study also reported barriers to access AEDs, the lack of trained healthcare workers to diagnose and treat epilepsy, the high cost for medical treatment and lack of drugs in the village health centres. The affected villages strongly advocated that a CBETP be set-up to alleviate the sufferings of PWE [10]. Therefore, in December 2018, one years after setting-up a CBETP, we re-evaluated the community's perceptions and attitudes about epilepsy and experience with the program. Moreover, we re-evaluated the cost for a family to take care for a PWE among the participants in trial.

2. Methodology

2.1 Study sites

This study was conducted in Draju, Kanga and Thedeja health areas in the Logo health zone, an onchocerciasis-endemic area with a high epilepsy prevalence of 4.6% and where ivermectin for onchocerciasis control had never been distributed [11]. The Logo health zone has only one reference hospital with five doctors, and 26 health centres headed by nurses [6]. The zone is located not far from the Ugandan border, therefore Ugandan shillings (USH) is the currency commonly used in the area. The majority of residents in this zone are from the Alur ethnic group (around 98%) and speak the Alur language which is the Dhu-Alur. The most practiced religion is Catholicism (80%) with a growing popularity of traditional religions such as "Mungu lonycon" or "Karwo" who believe that God is able to solve all problems in response to the prayers and whose leaders are preaching against all modern practices (modern medicines).

2.2 The community-based epilepsy treatment program

Prior to the start of the CBETP, local authorities, study participants, nurses, community health workers known as relais communautaires (RECO) and teachers at the local schools were contacted to introduce the epilepsy treatment program and were asked to participate. Consenting individuals were trained by two neurologists. RECO were trained to screen persons suspected to have epilepsy using a pre-tested validated

questionnaire with five questions [9]. All persons suspected to have epilepsy were referred to the trained local health care workers who took a detailed medical history and performed a clinical examination to confirm the diagnosis of epilepsy. All persons with confirmed epilepsy were offered free AED treatment. First line AED treatment was phenobarbital. *O. volvulus* infected PWE were asked to participate in a randomised clinical trial to evaluate the effect of ivermectin on the frequency of seizures [12]. Ivermectin was administered orally and directly observed by the healthcare worker, while weekly home visits were conducted by the RECO to ensure that AEDs were taken daily as prescribed.

2.3 Community perceptions, attitudes and experience about epilepsy and the CBETP

Focus group discussions (FGD) were carried out among PWE and their families, RECO, teachers and residents without any PWE in their household (Table 1). Semi-structured in-depth interviews (SSIs) were performed with PWEs and their families, nurses at the local health centres and local known traditional healers. The interviews were conducted in the presence of a principal investigator (MM), supported by one or two moderators (moderators were trained healthcare professionals who were native of the study area and fluent in the local languages: Alur and Swahili). All interviewees were informed of the interviewer's background and qualifications, and the motivations for undertaking the research project. The interview topic guides used for the FGDs, and SSI are presented in supplemental material 1.

Table 1. Characteristics of the participants in the focus group discussions (FGDs) and semi-structured interviews (SSIs).

Categories	Inclusion criteria
RECO, teachers and Community members not related to the PWE	- RECO (community health workers) or teachers in the study village - Served at least one year as a RECO or teacher - Participated in the introduction of the current CBETP - Resident in the study villages with no direct relationship with the PWE
PWE/PWE's family members	- Having epilepsy or living in the same household with a PWE - Resident of the study village - Participating in the current CBETP
Traditional healers	- Resident of the health zone - Known in the community as traditional healer
Nurses	- Being a nurse from the health zone - Having participated in the CBETP

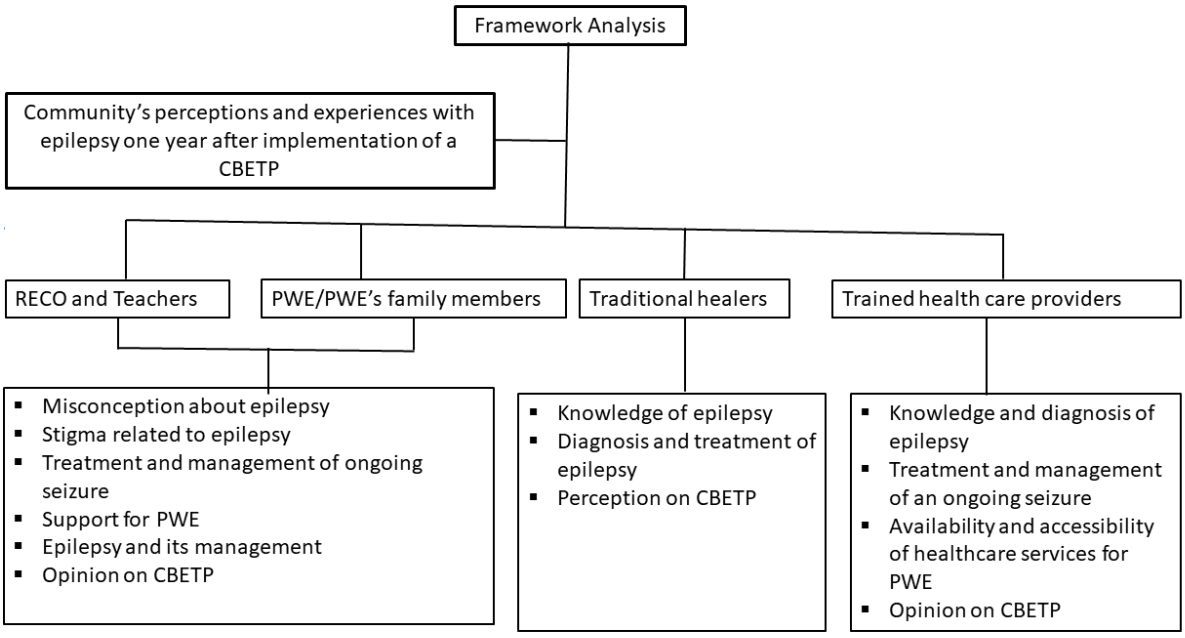
PWE: person with epilepsy; RECO: relais communautaires ; CBETP: Community-based epilepsy treatment program.

Purposive sampling was used to ensure that interviewees were diverse in demographics, clinical expertise, and relationship with PWE. The FGDs and SSIs participants inclusion criteria are presented in Table 1. In each participating village, FGDs were conducted separately with RECO and teachers, PWE and their families and traditional healers. Participants were contacted one day before the interview to identify a suitable time frame to meet with the research team. At the end of each FGD and SSI, the moderator summarized the discussions in French to enable the principal investigator (M.M) to ask additional questions. Both FGDs and SSIs were audio recorded.

2.4 Qualitative analysis

FGDs and SSIs were transcribed by trained staff and translated verbatim from the local languages to French by a professional local translator. All the interviewees were assigned pseudonyms during transcribing. The analysis started at the end of all planned interviews. Initially, A.D and H.D independently read and highlighted the transcripts to first familiarize themselves with the data and then to identify emergent themes and understand and report positive and/or negative changes from the baseline data (Figure 1) (see Dolo et al., [10]). The codes were reviewed and confirmed by M.M, J.N.S.F, R.C and S.C. Transcripts were then uploaded to NVivo 12 (QSR International, Pty, Doncaster, Victoria, Australia), which was followed by second level, line-by-line coding of text by A.D and H.D. A framework analysis as described by Srivastava and Thomson [13] was used. The SSIs and FGDs transcripts were

processed and analysed according to the five key stages of framework qualitative data analysis (familiarization, identifying a thematic framework, indexing, charting, mapping and interpretation) [14], and reported in accordance with standards for reporting qualitative research (SRQR) [15]. Transcript analysis was completed in French to ensure fidelity and consistency of findings. During the process of data analysis, A.D and H.D frequently communicated with M.M who was involved in data collection and was in closer contact with local team to discuss emerging codes and categories, as well as the interpretation of key texts. Quotes included in this article were translated from French to English by A.D and confirmed by H.D (authors who are bilingual) and were verified for cultural accuracy by M.M who participated in fieldwork.



RECO: (Rélais Communautaire); CBETP: Community-based epilepsy treatment program; PWE: Persons with epilepsy

Figure 1 *Research question with the framework analysis themes*

2.5 Cost related to epilepsy

Some PWE and their family members who participated in a clinical trial to evaluate the added value of ivermectin on the frequency of seizures in *O. volvulus*-infected PWE [8, 16] were asked to participate in a study to evaluate the epilepsy related cost for the family. The direct and indirect cost due to epilepsy was assessed by interviewing PWE and their family using a questionnaire that was used prior to the implementation of the

CBETP [6]. Direct costs due to epilepsy included medical consultations and hospitalization cost, transportation cost to the health centre/hospital and the cost of traditional medicine. All expenses in USH were changed to United States Dollars (USD) using the following conversion factor: 1 USD = 3600 USH. Indirect cost due to epilepsy consisted of time (in days) lost by the PWE and/or the caretaker due to seizures, converted to monetary value by assuming that a day's wage is worth 1/365th of the annual Gross Domestic Product (GDP) per capita in the DRC [17]. More specifically, the number of workdays lost was multiplied with GDP per capita per day. Considering GDP of DRC in 2019 of 48.994 billion USD and a total population of 86,791,000 [18], the GDP per capita is equal to 564.5 USD and GDP per capita per day is equivalent to 1.56 USD. SAS 9.4, (SAS Institute Inc.) was used in the analysis.

2.6 Ethical Considerations

The study protocol was approved by the ethics committee of the Antwerp University Hospital/University of Antwerp (May 2017, B300201733011) and of the School of Public Health of the University of Kinshasa (January 2017, ESP/CE/006/2017). All participants provided a signed or verbal informed consent to participate in the study.

3. Results

3.1 Participants' characteristics

Overall, 9 FGDs (3-9 persons per FGD) and 14 SSIs were conducted, for a total of 67 study participants. The following study procedures were conducted at each participating health centre: three FGDs (at least one for teachers, one for PWE or/and their families, and one for RECO and community members not affected by epilepsy) and four SSIs (one with a nurse or head of nurse, two with PWE, and one or two with traditional healers) (Table 2). Moreover, 74 PWE participated in the epilepsy cost follow-up study.

Table 2. Participants in the focus group discussions (FGDs) and semi-structured interviews (SSIs) per health centre

Draju health centre (n=22)	Kanga health centre (n=22)	Thedeja health centre (n=23)
Teachers (n=5, FGD)	Teachers (n=6, FGD)	Teachers (n=5, FGD)
PWE or/and their families (n=3, FGD)	PWE or/and their families (n=6, FGD)	PWE or/and their families (n=8, FGD)
RECO and community members not related with PWE (n=9, FGD)	RECO (n=5, FGD)	RECO (n=6, FGD)
PWE (n=2, SSI)	PWE (n=2, SSI)	PWE (n=2, SSI)
Traditional healer (n=2, SSI)	Traditional healer (n=2, SSI)	Traditional healer (n=1, SSI)
Nurse (n=1, SSI)	Nurse (n=1, SSI)	Nurse (n=1, SSI)

PWE: person with epilepsy; RECO: relais communautaires; FGD: Focus Group Discussion; SSI: Semi-Structured In-depth Interviews

3.2 Qualitative research findings

Five main themes emerged from the analysis of FGDs and SSIs data; the findings for each target group are summarized in Table 3.

3.2.1 Epilepsy-related misconceptions

False beliefs related to epilepsy partially disappeared in the Logo community. Most people do not believe anymore that epilepsy is contagious or is a “hereditary disease”.

‘*Following the sensitization, PWE and their family and the communities where they live changed their mentality. PWE now participate in various activities, they participate in church activities, children who have epilepsy go to play with other children....*’(P1, male nurse at local health centre, SSI)

‘…because of the information from the current project, the communities have understood that epilepsy is a disease that is not contagious and not caused by evil spirits, rather it is a brain disturbance which can be treated by appropriate anti-epileptic medication.’ (P10, male RECO, FGD)

Although the negative attitudes towards the PWE and the community mentality have positively changed, a large proportion of the affected communities remained uninformed, resulting in the false belief that epilepsy may be contagious.

‘*Those who don't have it (people without epilepsy) in their families misjudge us as evil, and they say that we are the ones who bring and cause it (epilepsy), and we too are left with it, and nobody likes suffering because everyone likes happiness…*’ (P2, male PWE, SSI)

Table 3. Summary of the main findings by themes and by target group

Themes	PWE and their family	Traditional healers	RECO/teachers	Nurses
Epilepsy related misconceptions	Less misconceptions	Persistence of misconceptions	Less misconceptions	
Stigma related to epilepsy	Less epilepsy related stigma	Persistence of stigma	Less epilepsy related stigma	
Traditional practices for diagnosing and treating epilepsy	The health care system is preferred as first point of care	Need for dialogue and cooperation with health professional to eliminate epilepsy	Positive change to choose the health system as first point of care	
Perception and effect of the epilepsy treatment program	Very satisfied with the program and the care received Improved health status		Very satisfied with the program and the care offered	Very satisfied with the program Gained self-confidence in treating PWE
Experience and prospect of epilepsy treatment program	Concerns about the program sustainability		Concerns about the program sustainability	Concerns about the program sustainability

PWE: person with epilepsy; RECO: relais communautaires

3.2.2 Epilepsy-related stigma

One year following the implementation of the CBETP, both epilepsy knowledge and epilepsy attitudes improved and there was a notable reduction of epilepsy-related stigma in the affected communities.

'*In my village, they (PWEs) are no more stigmatized like before, they are working normally, the community is no longer seeing them as people who can infect them....*' (P3, male teacher at local primary school, FGD)

The PWE still experience divorce which might be the result of concealing epilepsy status before marriage and once it is discovered resulted in divorce.

'*Once my family in-law was informed of my health status (that I have epilepsy), this created something else… I am married and this disease (epilepsy) found me in the marriage and once epilepsy was detected, I was forced to leave the marriage and until now I live with my parents and suffering from epilepsy alone...*' (P4, female PWE, SSI)

3.2.3 Traditional healers' perspectives

The influence of the traditional healers in the community was still present.

'There is still a huge influence of traditional healers in the villages. PWE's families are disrupted with the beliefs that epilepsy is caused by evil spirits, witchcraft, maledictions, sorceries and so on, mostly spread in the community by the traditional healers' (P5, male RECO, FGD).

Some traditional healers still believe that the cause of epilepsy is the evil spirit.

'*…epilepsy is caused by the evil spirit and the convulsion'* (P6, female traditional healer, SSI)

'*We are capable to detect epilepsy with our powerful spirit that we have, that powerful spirit shows us the PWE and tells us what plant to administer*' (P7, male traditional healer, SSI)

'Our powerful mind sends us to take the right plant to treat the evil spirit in the people (PWE). The same mind guides the way we administer this plant. There are medicines to chew and others to drink' (P6, female traditional healer, SSI).

The RECO proposed a collaboration between the trained heath professional and the traditional healers to cope with the epilepsy.

'As the traditional healers mostly spread wrong beliefs about the causes and treatment of epilepsy, we need to work together to cope with this disease (epilepsy). All we need to do is to ask them if a PWE visit them for care, they have to refer that PWE to the nearest health centre' (P5, male RECO, FGD).

The traditional healers showed a growing willingness to collaborate with trained health professionals and local health care workers (RECO) in coping with epilepsy.

'*The collaboration with trained health professionals is possible, we can work together to serve our people to eliminate this bad evil. By the way, during the era of MOBUTU (former president of DRC from 1965 to 1997), we were given documents to work together with trained health professionals, but nothing has happened*' (P8, male traditional healer, SSI).

'*I would say, when patients (PWE) come to us (traditional healers), we can re-direct them to the nearest health centre*' (P9, male traditional healer, SSI).

'*The best thing would be, when the patient come to our home for epilepsy and we can write their names and give the names to the head of health centre in our area'* (P6, female traditional healers, SSI).

3.2.4 Perception of the community-based epilepsy treatment program

PWE enrolled in treatment program and their families expressed their level of satisfaction.

'*These people (PWE) are satisfied because they had not had enough medication before. In the past, there was always a shortage of anti-epileptic drugs in the stock and in the pharmacy…. But now on, the medication is available, PWE and their families are satisfied'* (P10, female RECO, FGD).

'*We had a child in our school from the village which is not far from here (village of Djupamamba), so this child was suffering a lot from the epilepsy and his father embarrassed himself and did everything to get his child back to normal, unfortunately it did not work out, when this program arrived here at Thedeja health area, this child*

was enrolled to the program, after this care he feels very well. His father is satisfied because the life of his child returned to normal (P11, male teacher at local school, FGD).

Families of PWE reported a remarkable improvement of the health status of the PWE enrolled in the program.

'…my big sister was often rude. Since the start of the treatment in this program there has been a remarkable change, she now smiles with people, it is like a miracle in our family. Before the program, she often had a seizure almost every day but now she has not experienced any seizure attack over a long period' (P12, female PWE's family member, FGD)

'In the past, I usually used the traditional medicines, these medicines have already disappointed me. From the beginning of my illness (epilepsy), I have used all kinds of traditional medicine with no success, I changed many times the traditional healers without any success, and I have even changed my religion to find the solution of my illness, and no success, since the start of this project, almost a year I had only 2 seizures, I would say the treatment from the program has helped me considerably, it relieved my suffering; no more seizure disruption as in the past' (P13, male PWE, SSI).

PWE and their families gained crucial experience in managing PWE during seizure attacks.

'*In case of seizure attack, we firstly have to take him (PWE) straight to the nearest health centre. In the past, we feared them (PWE), but with the knowledge we gained from the training during the program we should not be afraid of them (PWEs)*' (P14, PWE's family member, SSI).

Nurses and doctors at health centre reported increased work satisfaction as they observed day to day improvement of their patients.

'*Because of the services and AEDs that PWE are receiving, we have observed an enormous improvement in their health status, these positive improvements of the PWE give a good feeling to the nurses that their work has an impact to the society*' (P15, female nurse at local health centre, SSI)

PWE and their family changed their attitudes towards the local health care services. Health care providers experienced a meaningful collaboration with PWE and their families. Participants advocated that the epilepsy problem in the area could end if the CBETP continues.

'Nowadays, there is a good collaboration between the nurses at the local health centre and PWE and their families, because they are happy and they are satisfied with the services they get here at the health centre and they experience the amelioration of their health status (P16, male nurse at local health centre, FGD).

This project has made PWE and their families, health professionals and the RECOs to work together as one and everyone feel satisfied because we are all in the same community, in the same family no one is happy when other is feeling the pain (P17, male RECO, FDG).

3.2.5 Experience and prospects of the epilepsy treatment program

PWE and their families reported a positive feeling concerning the way there are treated in the community. One year after starting the treatment program, some PWE returned to their normal activities (mainly farming) and students returned to school, and ladies have married.

'*I observed the patients (PWE) that I was following who were inactive, now they play football very well, some could not cultivate or trade, but now they cultivate and trade'* (P10, female RECO, FGD)

'*… among my patients, there is one who returned to school and another who was always bothered with seizures, after starting the program, it has been already 10 months without any seizure attack*' (P17, male RECO, FGD).

'*…One of my patients did not even walk to the health centre - he always needs a motorbike to come to the health centre, but now he comes to the health centre himself by feet, he does his own laundry, takes a bath himself'* (P15, female nurse at local health centre, SSI)

I also (health care provider) have a patient now started to do business, another who got re-married (P18, male nurse at local health centre, SSI).

The practice to first seek care with traditional healers has changed.

'Nowadays, we receive few patients (PWE), because a lot of people do not accept the traditional care, they qualify it as wrong treatment, more people go to seek care in the hospitals. This could be the reason why my patients are no more looking for care at my house…' (P8, male traditional healer, SSI)

Nurses and RECO were concerned about the sustainability of the program. They felt ending the program will lead to poor adherence with AED treatment, thereby increasing the frequency and severity of seizures.

Our main concern is to abandon our patients in the coming days, we would like to help these people so that they continue and finish their treatment, because if we abandon them like that, their life will not be like others, they will suffer with stigmatization, and discrimination in the society as they were before, (P15, female nurse at local health centre, SSI).

We would need the time to inform them (PWE) that the AEDs is no more free…, to reassure that they will continue to get the AEDs for small amount and the medication will always be available every day' (P19, male RECO at local health centre, FGD).

3.3 Cost related to epilepsy

A total of 74 PWE and their family completed the questionnaire to assess the direct and indirect epilepsy cost for the family one year after the implementation of the CBETP. Of these, 40 (54%) were female, and 71 (96%) were taking AEDs regularly. The median age was 22.5 years (IQR: 18-31), and the median duration of epilepsy among the PWE was 8.5 years (IQR: 3-17).

The weighted mean direct cost per month due to epilepsy was reduced from 10.5 USD/per month to 0.5 USD per month after one year of CBETP. PWE spent about 38% of the total cost on transportation from or to the health centre. Within a year, the expenses on traditional medicine had reduced from 68% to 5% of the direct epilepsy cost (Table 4).

Table 4. Impact of community-based epilepsy treatment program on direct epilepsy-related cost for the family

Direct cost related to :	Before implementation of the CBETP				One year after the implementation of the CBETP			
	Number of PWE spending on this item	Average cost (SD) (USD)	Weighted average cost (USD)	Weighted average cost (%)	Number of PWE spending on this item	Average cost	Weighted average cost (USD)	Weighted average cost (%)
Anti-epileptic drugs	163/258	3.4 (5.6)	2.1	20	0	0	0	0
Medical consultation	48/258	3.0 (5.2)	0.6	6	5/74	1.5 (2.4)	0.10	20
Hospitalization	11/258	9.1 (11.6)	0.4	4	1/74	14.3	0.19	38
Transportation	30/258	2.8 (5.0)	0.3	3	6/74	2.4 (1.8)	0.19	38
Traditional medicine	126/258	14.6 (24.5)	7.1	68	2/74	0.9 (0.8)	0.02	5
Total direct cost per month			10.5	100			0.5	100

CBETP: community-based epilepsy treatment program; USD: United states dollar, SD : Standard deviation of the mean

On average PWE lost 7.1 days of work per month while the caretaker lost 3.7 days of work per month before the CBETP. The average weighted indirect cost due to epilepsy for PWE was reduced from 11.1 USD/month to 1.09 USD after 1 year. Indirect cost incurred by the caretaker/family members of PWE also reduced from 5.8 USD to 0.6 USD per month (Table 5).

Table 5. Estimated impact of the community-based epilepsy treatment program on indirect epilepsy related cost for the family.

	Before the implementation of the CBETP					One year after the implementation of the CBETP				
Indirect cost related to:	Number of persons concerned	Average number of workdays lost (SD) (days/month)	Total workdays lost due to epilepsy (days/month)	Average cost (USD)	Weighted average cost (USD)	Number of persons concerned	Average number of workdays lost (SD) (days/month)	Total workdays/month lost	Average cost (USD)	Weighted average cost (USD)
PWE	199/258	7.1 (8.3)	1824	8.9	6.8	23/74	0.7 (1.4)	51	0.9	0.3
Family of PWE	138/244	3.7 (5.8)	965	4.6	2.6	9/70	0.4 (1.0)	21	0.5	0.1
Total			2789.9		9.4			72		0.4

CBETP : community-based epilepsy treatment program; PWE: persons with epilepsy; USD : United states dollar

4. Discussion

Our study shows that the CBTEP had a very positive impact in the Logo community. One year after the introduction of the program, epilepsy misconceptions had positively changed, and most people did not believe anymore that PWE are infectious as was previously reported [10]. A similar effect was observed in a comparable program in Tanzania [19]. The CBTEP changed the health seeking behaviour in the study area. PWE now prefer to first seek care at a health centre instead of consulting traditional healers.

However, despite epilepsy sensitization efforts, traditional healers in the Logo health zone still believe that epilepsy is contagious and is caused by evil spirits, and witchcraft, as was also observed in several other sub-Saharan Africa studies [20-25]. Because of the influence of traditional healers and past cultural beliefs [10], some individuals in the Logo community still prefer to consult traditional healers and the churches alongside trained health care providers.

The program contributed to the reduction of epilepsy-related stigma and increased adherence to AEDs as was reported in other studies [16, 26, 27]. Traditional healers in the zone experienced an important reduction of PWE people seeking their services. A similar experience was observed in Kenya, where misconception about epilepsy, cultural treatment, and negative stereotypes positively changed after a community-based educational intervention [27].

Consistent to other studies, traditional healers in the Logo health zone expressed the willingness to collaborate with trained health professionals in coping with epilepsy [25, 28, 29]. Given the strong influence of traditional healers and the persistence of epilepsy related stigma, such collaboration should be considered. Similar initiatives have been tried in the past whereby an agreement was signed, even though there was “little or no follow up” (traditional healer said). In Tanzania, traditional healers are recognized as pillars of seizure management because of their position as custodians of Tanzanian culture and their ability to counsel PWE with very severe or mild seizures [24]. In South-Africa it has been attempted to establish a collaboration with traditional healers for epilepsy management, but the idea was not approved by South-African health policy makers [25, 30].

PWE and their families who participated in our study were very satisfied with the CBETP and reported a considerable improvement in their quality of life thanks to the program. A similar experience was reported in Brazil [31], where after healthcare workers received training in seizure management, the majority of PWE reported health status improvement. Health care providers expressed increased satisfaction in their work as they observed day-to-day PWE's life improvement.

Our results demonstrated that a community-based intervention aimed to increase the access to AEDs at little or no cost will have a prominent effect on the overall economic cost of epilepsy and will improve the quality of life of PWE in high endemic setting. Following the implementation of a CBETP, epilepsy-related cost for the family reduced by 95%. Our findings concur with previous findings in similar settings where providing epilepsy care at the community level reduced the cost related to epilepsy by 80% to 90% of the initial cost [32, 33].

The results of our study need to be interpreted in the light of some limitations. The full impact of the CBETP cannot be evaluated using only a qualitative study. Moreover, the program included only the PWE and their families who participated in the trial to evaluate the effect of ivermectin on the frequency of seizures [8]. Persons not included in the trial who were followed up less strictly may have had different and less positive experiences. The community-based intervention covered only one health zone; therefore, traditional healers from other neighbouring health zones may still be actively spreading false knowledge about epilepsy. The reported epilepsy costs are only estimations of the real-life scenario, because we did not quantify intangible costs (cost due to psychosocial pain and suffering), as well as the costs related to lost schooling or employment opportunities because of seizures. Some FGDs had few participants, and they cannot be considered representative of all RECO, PWE, healthcare workers, traditional healer or community members in the health zone. Moreover, during FGDs, certain participants may not express themselves freely and they may influence each other. In addition, it is possible that the presence of a moderator in the group discussion may have affected the views and opinions of the participants.

A lesson learned from this study is that we missed the opportunity to conduct a full evaluation of the community-based epilepsy treatment program including an estimation of its cost benefit. To do so we should have developed a protocol and data

collection tools before the start of the programme. Data should have been collected at baseline and prospectively during implementation of the programme. Quality of life and epilepsy related stigma should have been evaluated using validated questionnaires. Moreover, information about epilepsy related costs, not only for the family but also concerning the implementation of the programme should have been collected.

In conclusion, our study shows the benefit of the CBETP in the Logo health zone. The main perceived benefits were the positive changes related to the misconceptions about epilepsy and epilepsy related stigma. Families with PWE understood the benefit of seeking health care with trained health professionals rather than with traditional healers. The direct and indirect cost for families to take care of a PWE reduced considerably after the program. Further quantitative research with larger and unbiased samples is needed to assess the generalizability of our findings. Moreover, the cost-effectiveness and long-term sustainability of this approach remains to be investigated.

Competing interests

The authors declare no conflict of interest. The study sponsors had no role in the design, execution, interpretation, or writing of the study.

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CRediT authorship contribution statement

A. Dusabimana: Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization. M.N. Mandro: Validation, Investigation, Data curation, Writing - review & editing, Visualization, Project administration. J.N. Siewe Fodjo: Methodology, Validation, Investigation, Data curation, Writing - review & editing, Visualization. H. Dolo: Methodology, Software, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization. S. Coenen: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing - review & editing, Visualization, Supervision. R. Colebunders: Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Chapter 6.

Discussion, Implications and future perspectives

6. I. Discussion

With an estimated 200,000−400,000 persons affected [1], onchocerciasis-associated epilepsy (OAE) is a public health problem that need urgent action. Until now, there is still reluctance by some scientists and public health decision makers to accept the existence of OAE and its public health impact. International recognition of the link between onchocerciasis and epilepsy on the one hand and that OAE is a preventable condition on the other hand is essential in order to implement appropriate interventions to mitigate the public health problem caused by OAE.

What have we learned from our studies that could be used to mitigate the public health problem caused by OAE?

1. Our studies provide additional evidence for an association between onchocerciasis and epilepsy
    a) High prevalence of *O. volvulus* antibodies in people with epilepsy (PWE) of the age under 39 years in *O. volvulus* endemic areas with no or sub-optimal onchocerciasis control programs (Chapter 2).
    b) Ivermectin treatment may reduce the frequency of seizures among *O. volvulus*-infected PWE via its microfilaricidal effect (Chapter 3II). The interpretation of the results of our study was not straightforward because the persons with OAE included in our study were also treated with anti-seizure medication. However, this study clearly demonstrated that ivermectin use certainly is not the only cause of the epilepsy observed in onchocerciasis-endemic areas.
    c) High incidence of epilepsy in onchocerciasis-endemic regions associated with high Ov16 IgG4 seroprevalence in children 6-10 years old (an indicator of ongoing *O. volvulus* transmission) (Chapter 4).
2. We learned that ivermectin treatment, even in onchocerciasis-endemic areas with many years of community-directed treatment with ivermectin (CDTI), is still highly effective in killing microfilaria (Chapter 3I). Therefore, most likely the high prevalence and incidence of OAE in onchocerciasis-endemic areas is mainly caused by onchocerciasis elimination programmes that perform sub-optimally because of low ivermectin population coverage.

3. We observed a large epilepsy treatment gap in onchocerciasis-endemic areas. However, in Ituri, a community-based epilepsy treatment programme was able to reduce epilepsy related stigma, increase the knowledge of epilepsy and reduce the cost related to epilepsy for the family (Chapter 5).

What can we do to mitigate the problems caused by OAE?

1. CDTI coverage needs to be increased and if possible annual CDTI should be replaced by bi-annual distribution to accelerate the onchocerciasis elimination. Onchocerciasis-endemic areas with a high incidence of epilepsy need to follow the example of northern Uganda where after implementing bi-annual CDTI and larviciding blackfly-infested rivers in 2012, no new cases of NS have been reported and the prevalence of other forms of epilepsy also decreased [2].

A bi-annual CDTI may be too expensive for countries with limited resources such as South Sudan. Therefore, it should be considered to increase the coverage of annual CDTI, but also to add an extra distribution of ivermectin to children 5-15 years during “school health days” six months after CDTI. Those children if infected with an *O. volvulus* infection with a high microfilarial load are at risk of developing OAE [3, 4]. If these children are treated twice a year with ivermectin they will have no microfilaria during the entire year and therefore not at risk for developing OAE. This school based ivermectin distribution is a low-cost intervention that could even be done in areas co-endemic for schistosomiasis together with the distribution of praziquantel.

Although CDTI revolutionised the control of onchocerciasis, the CDTI campaigns confront with some problems that may hinder its optimal effectiveness. A problem of CDTI campaigns is that they are organised only during a few days by a limited number of community-directed distributors (CDDs). This is problematic in rural villages where households are often located far from each other. Moreover, in most onchocerciasis areas there is no possibility to distribute ivermectin from a stock that was not used after the CDTI campaign. As consequence, the pregnant women who are advised not to take ivermectin are unable to obtain ivermectin after delivery and after the first week of breast feeding as recommended by WHO [5].

2. OAE awareness campaigns need to be implemented in onchocerciasis-endemic regions with high onchocerciasis transmission and high OAE prevalence. Indeed, explaining to the local population and health care workers that the bites by infectious blackflies are the consequence of the high prevalence of epilepsy and that this form of epilepsy can be prevented by the intake of ivermectin will stimulate the affected communities to distribute and take the ivermectin and this will increase CDTI coverage. This clarification may also decrease epilepsy-related stigma because people will understand that epilepsy is not caused by bad spirits, that families are not cursed and that persons with epilepsy are not contagious. Not only the people affected with OAE, but also their parents and their communities will benefit from such an awareness campaign.

3. The efficacy of onchocerciasis-elimination programs needs to be monitored. This could be done by monitoring ivermectin coverage. However, the ivermectin coverage data provided by CDDs are often not reliable. Therefore, OAE prevalence and incidence data using the OAE case definition [6] and Ov16 IgG4 RDT test results of 6–10-year-old children are useful additional indicators to monitor the performance of onchocerciasis-elimination programs.

4. Primary health services, mental health programs and neglected tropical disease (NTD) programs need to collaborate and share information. A high incidence of epilepsy with >75% of epilepsy cases met OAE criteria in a certain onchocerciasis-endemic area suggest that onchocerciasis-elimination efforts need to be strengthened. On the other hand, if CDTI coverage in an area is low or CDTI has been interrupted, this indicates that a high incidence of OAE is to be expected and that additional anti-seizure medication may be need.

5. OAE should be recognised as a manifestation of onchocerciasis and be included in the calculation of the burden of disease caused by onchocerciasis. If this is done, this will increase the burden of disease caused by onchocerciasis considerably and therefore will help to mobilise extra resources for onchocerciasis elimination and for OAE treatment.

6. More advocacy is needed to reduce the epilepsy treatment gap. The creation of epilepsy peer support groups could be a way to increase the support for PWE and their families. Currently, in onchocerciasis-endemic areas many children drop out of school or are not sent to school [7]. Treating children with anti-seizure medication early after the start of their first seizures would allow many children with epilepsy to attend school.

6. II. Future research

1. Studies are still needed to evaluate interventions that could increase the efficacy of onchocerciasis elimination programs particularly in the areas with high incidence of OAE. The reasons for low CDTI coverage need to be investigated in each onchocerciasis-endemic areas where there are indicators suggesting that the program is performing sub-optimally. This should be done using qualitative and quantitative mixed methods. Based on the results of these studies, innovative interventions need to be implemented and evaluated to increase CDTI coverage.
2. Until now ivermectin treatment is not administered to the children below the age of five because of lack of evidence on the safety of ivermectin treatment in this younger age group [5]. However, because OAE, including NS has been reported in children aged 3 and 4 years, clinical trials to investigate the safety and the efficacy of ivermectin treatment in children below the age of 5 are needed.
3. Research is needed to determine how to optimally organise OAE awareness campaigns and to decrease epilepsy-related stigma in onchocerciasis-endemic areas.
4. State-of-the-art studies are still needed to accurately estimate the burden of OAE, and the disability associated with OAE.
5. The pathogenesis of OAE still needs to be elucidated. A recent case-control study in Uganda suggested that pre-term birth could be a risk factor for children to develop NS when they acquire an *O. volvulus* infection [8]. The explanation for this may be that the pre-term birth was caused by an *O.volvulus* infection in the pregnant women [9]. Such an infection may modify the immune response of the mother toward the parasite causing “parasitic tolerance” [10]. This parasitic tolerance is transmitted to the offspring and may lead to a high microfilarial load in children exposed to *O. volvulus* infectious blackflies. This high microfilarial load is a known risk factor for developing OAE. To investigate this hypothesis further, cohort studies of *O. volvulus*-infected and non-infected pregnant women with long-term follow-up of their children should be set up. In addition, a clinical trial to determine the safety and benefit of ivermectin

treatment on *O. volvulus*-infected pregnant women should be considered. In such studies serial blood samples will need to be collected in the children to detect biomarkers associated with the development of OAE. Co-factors including genetic factors most likely play a role and should be investigated in large case-control studies.

6. Research is needed to investigate how to reduce the epilepsy treatment gap. Currently it is considered that sodium valproate is the best anti-seizure medication for persons with NS [11]. However, this has never been proven in a randomised clinical trial. Moreover, sodium valproate is too expensive for countries such as South Sudan and it is teratogenic [12] and therefore not indicated in women of childbearing age. Clinical trials are needed to determine the most cost-effective treatment strategy for OAE. A much cheaper anti-seizure medication such as phenobarbital could be considered before switching to a more expensive drug in case seizures are insufficiently controlled. The possibility to increase access to anti-seizure medication and improve the quality of life of PWE in onchocerciasis-endemic areas by creating epilepsy peer support groups should also be investigated.

6. III. General conclusion

Our studies confirm the recent overwhelming epidemiological evidence for the association between onchocerciasis and epilepsy. However, the “association” between onchocerciasis and epilepsy does not mean that the *O. volvulus* parasite causes the epilepsy. To identify a causal relation, additional studies, including more research concerning the biology of the *O. volvulus* parasite, need to be done. Indeed, it may be possible that it is not the *O. volvulus* parasite itself that triggers the epilepsy, but an unknown pathogen that is part of the microbiome of the worm that induces the epilepsy. However, we can not wait until we have a complete understanding of the pathogenesis of OAE to implement interventions that have been shown to be successful in eliminating OAE. The main intervention that is able to rapidly stop the incidence of OAE is the distribution of ivermectin twice a year to children. For those who already suffer from OAE more advocacy is needed to decrease the epilepsy treatment gap, but the quality of life of PWE and their families in onchocerciasis-endemic areas could already be improved by information campaigns about how children acquire OAE and how this condition can be treated.

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