Onchocerciasis-associated epilepsy in the Democratic Republic of Congo: Clinical description and relationship with microfilarial density

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Abstract

Background: High epilepsy prevalence and incidence were observed in onchocerciasis-endemic villages in the Democratic Republic of Congo (DRC). We sought to investigate the clinical characteristics of onchocerciasis-associated epilepsy (OAE), and the relationship with microfilarial density.

Methods: In October 2017, ivermectin-naive persons with epilepsy (PWE) were recruited from onchocerciasis-endemic areas in the Logo health zone in the DRC. Additional PWE were enrolled in the Aketi health zone, where ivermectin had been distributed annually for 14 years. Past medical history, clinical characteristics and skin snips for *Onchocerca volvulus* detection were obtained from participants. Bivariate and multivariable analyses were used to investigate associations with microfilarial density.

Results: Of the 420 PWE in the Logo health zone, 392 were skin snipped (36.5% positive). Generalized motor seizures were most frequent (392 PWE, 93.3%), and nodding seizures were reported in 32 (7.6%) participants. Twelve PWE (3.1%) presented Nakalanga features. More skin snip-positive participants reported a family history of epilepsy (p=0.027). Eighty-one onchocerciasis-infected PWE were recruited in the Aketi health zone. Positive correlations between seizure frequency and microfilarial density were observed in Logo (Spearman-rho=0.181; p=0.0003) and Aketi (Spearman-rho=0.228; p=0.046). In the multivariable analysis which adjusted for age, gender and previous anti-epileptic drug use, factors associated with high seizure frequency included: high microfilarial density (RR=1.004, 95% CI: 1.002–1.007; p<0.001), history of nodding seizures (RR=3.852, 95% CI: 2.926–5.082; p<0.001) and shorter duration of epilepsy (RR=0.948, 95% CI: 0.928–0.968; p<0.001). In Aketi, previous ivermectin use was associated with reduced seizures (RR=0.69, 95% CI: 0.58–0.83; p<0.001).

Conclusion: In onchocerciasis-endemic regions in the DRC, a wide spectrum of seizures was observed. Nodding seizures, Nakalanga features, and a positive association between microfilarial density and seizures suggest a high OAE prevalence in the study villages, requiring a double management strategy: treatment with anti-epileptic drugs and stronger onchocerciasis elimination programs.

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47 ## Author summary

48 Several epidemiological surveys suggest that onchocerciasis (a disease resulting from an infection with the
49 parasite *Onchocerca volvulus*) is a cause of epilepsy. We conducted a study to describe the clinical
50 characteristics of persons with epilepsy (PWE) living in onchocerciasis-endemic villages in the Democratic
51 Republic of Congo. Our study revealed that the frequency of seizures increased with increasing number of
52 *O. volvulus* microfilariae detected in skin snips of participants. A wide spectrum of seizures was observed,
53 including generalized tonic-clonic seizures, absence seizures, and focal seizures. Growth retardation and
54 household clustering of PWE were common. Specific clinical presentations such as nodding seizures and
55 Nakalanga features were encountered. These results suggest a high prevalence of onchocerciasis-associated
56 epilepsy (OAE) in the study villages.

Introduction

As early as the 1930s, onchocerciasis was already suspected to cause seizures [1]. A meta-analysis has reported a 0.4% increase in epilepsy prevalence, for every 10% increase in onchocerciasis prevalence [2]. Today, there is increasing evidence that onchocerciasis is a risk factor for epilepsy [3–6] and that proper onchocerciasis elimination strategies can reduce the incidence of onchocerciasis-associated epilepsy (OAE) [7]. However, the physiopathology explaining how *Onchocerca volvulus* (the parasite responsible for the clinical manifestations of onchocerciasis) may cause seizures remains unclear.

Recent studies in the Democratic Republic of Congo (DRC) have revealed a high epilepsy prevalence in hyper-endemic onchocerciasis foci, particularly where control measures are sub-optimal and transmission is ongoing [8–11]. Although specific phenotypic features of OAE such as the nodding and Nakalanga syndromes have already been reported in the DRC [9], the full clinical spectrum of OAE in the DRC remains unknown. In a bid to further elucidate the association between epilepsy and onchocerciasis, a randomized clinical trial evaluating the effect of ivermectin on the frequency of seizures in PWE living in the Logo health zone was initiated in October 2017 [12] (Trial Registration Number NCT03052998; available at: www.clinicaltrials.gov). During the recruitment phase of this trial, all consenting PWE were examined and skin snipped to assess eligibility criteria. This paper describes the clinical features observed in ivermectin-naïve PWE encountered during the trial. Additional data to investigate the relationship between seizures and infection with *O. volvulus* were obtained from the Aketi health zone, another hyper-endemic onchocerciasis focus in the DRC with high epilepsy prevalence [10].

Methods

Study design

We carried out a cross-sectional, descriptive study of persons with epilepsy in the Democratic Republic of Congo.

Study sites

The study was carried out in two health zones in the DRC, namely Logo (in the Ituri province) and Aketi (in the Bas-Uélé province). In the Logo health zone, five onchocerciasis-endemic health areas where community directed treatment with ivermectin (CDTI) had never been implemented were selected: Draju, Kanga, Tedheja, Ulyeko and Wala. In the Aketi health zone, the study sites had already benefited from 14 years of CDTI and included Wela, Makoko, and Aketi rural town. The ecology and setting was similar in all study sites; these were essentially rural communities, with several fast-flowing rivers providing suitable breeding grounds for the blackflies (*Simulium spp*), vectors of *O. volvulus*. The main economic activity of the residents was farming.

Study procedures

In the Logo Health zone

This study was conducted within the scheme of a wide program launched in October 2017, aiming to treat all the PWE in the health zone, including a clinical trial investigating the effect of ivermectin on seizures [12]. Prior to the start of the study, local authorities were contacted and the study was explained to them in detail. After obtaining their collaboration, we proceeded to recruit participants using a community-based approach. Massive sensitization was carried out in the target villages, inviting persons known to have epilepsy to spontaneously report to the mobile clinics set up by the research team at the health centers. Additional potential participants were referred to the clinic by community health workers who had been trained by the research team to screen persons suspected to have epilepsy in their respective villages.

All persons suspected to have epilepsy who reported to the mobile clinics were briefed on the study objectives and procedures in the local language (*Alur),* and informed consent was provided by the participant and/or the caretaker. Upon confirmation of the epilepsy diagnosis, PWE were further interviewed and examined by a neurologist (DM) or a medical doctor trained in epilepsy (JNSF, MM, AA, RC). Participants' weight was measured using a weighing scale, and their heights obtained with a stadiometer. Information was collected on seizure semiology, seizure frequency, past medical history,

antiepileptic treatment history and family history of epilepsy. Cognitive and behavioural symptoms were grossly assessed by investigating if the participant was coherent in speech, obedient to orders or displayed any unexplained aggressive attitudes and/or wandering episodes.

Two approaches were used to assess growth retardation among our participants. For PWE below 20 years, the World Health Organization (WHO) height-for-age Z-scores were used, and any participant whose height was found below -2Z was considered to be growth retarded [13]. For PWE aged 20 years and above, the mean height of an adult residing in the DRC was retrieved from literature as being 157.4 ± 7.56 cm (only women's height was available) [14]. We therefore adopted 157.4 – 7.6 = 149.8 cm, as the cut-off height under which adult participants were considered to be growth retarded.

Onchocerciasis was diagnosed in two ways. Participants were initially tested for Ov16 antibodies using rapid diagnostic tests (Ov16 RDT, Standard diagnostics, Inc., Yongin-si, Gyeonggi-do, Korea). Thereafter, two skin snip samples were collected from each participant for the microscopic detection of *O. volvulus* microfilariae (mf). All relevant clinical and laboratory information was collected on paper and later entered in computers using the REDCap platform (https://www.project-redcap.org/), a secure web-based electronic database. The collected data was extracted and analyzed.

In the Aketi Health Zone

In January 2018, our research team recruited PWE in Wela, Makoko and Aketi rural town just before the yearly distribution of ivermectin. Community health workers and local health personnel referred suspected cases of epilepsy to a physician (FT) for confirmation. Skin snips were collected from confirmed PWE and examined for mf. The sociodemographic information, history of previous ivermectin and anti-epileptic drug use as well as seizure frequencies were obtained from participants with positive skin snips. A detailed clinical examination was not done for PWE in Aketi, because the main research objective in this health zone was to evaluate seizure frequency and mf density among PWE prior to ivermectin treatment, and to determine their response to the treatment. All collected data was entered in Microsoft Excel 2016 spreadsheets.

Epilepsy diagnosis and seizure classification

PWE were diagnosed in a two-step approach. Firstly, suspected cases were identified by administering a 5-item validated questionnaire [15]. Any individual who answered affirmatively to at least one question was further clerked and examined by a neurologist or a physician with training in epilepsy. Epilepsy diagnosis was confirmed according to the 2014 International League Against Epilepsy (ILAE) operational definition: two or more unprovoked seizures with at least 24 hours separating the two events [16]. All reported seizures were classified following the ILAE 2017 nomenclature [17], and the evaluation of the seizure frequency included all diagnosed seizure types. Previously suggested criteria were applied to identify OAE [7].

Detection of *Onchocerca volvulus* microfilariae

Skin snips were taken from the left and right iliac crests of participants using a sterile Holtz corneo-scleral punch (2mm) to investigate infection with *O. volvulus*. The collected skin snips were incubated for 24 hours in isotonic saline in a flat-bottomed microtiter plate. The mf that emerged were counted using an inverted microscope, and the average count for both skin snips from each participant was calculated. Mf densities were expressed as mf/skin snip. The same experienced laboratory technician (GA) examined the skin snips from all study sites.

Data analysis

Data was analysed in Rstudio version 1.1.456. A Shapiro-Wilk test was done and showed that the continuous variables were not normally distributed. Therefore, continuous variables were expressed as median and interquartile range (IQR) and compared across groups (*O. volvulus*-infected vs uninfected) using the Wilcoxon rank sum test. As for categorical data, they were expressed as proportions and compared using the Chi-square test. The Spearman rho was used to test for correlations. A generalized linear model fitted with a Poisson distribution was used to investigate the factors associated with seizure frequency in the study participants. Selected variables including age, sex, burn scars, and mf density [4], as well as covariates with a p-value less than 0.2 during bivariate analysis with seizure frequency [18], were included in the final model. Any p-value less than 0.05 was considered to be statistically significant.

Ethical considerations

Ethical approval for the study was obtained from the ethical committee of the School of Public Health of the University of Kinshasa in the DRC (Approval number: ESP/CE/013/2018) and the ethical committee of the University of Antwerp (Registration number: B300201733350). All PWE willingly participated in the study and provided signed/thumb-printed informed consents. The identity and information of participants was kept confidential. In collaboration with the non-governmental organizations Malteser international and VZW Aketi, decentralized community-based programs were implemented to provide anti-epileptic drugs to PWE in the study sites.

Results

PWE in the Logo Health Zone

A total of 420 PWE in the Logo health zone were enrolled in the study between October 2017 and July 2018. Their ages ranged from 1 – 72 years. Skin snip data was available for 392 (93.3%) of participants; of these, 143 (36.5%) were positive. The median mf density was 0.0 (IQR: 0.0 – 9.6 mf/skin snip), range 0.0 – 384.5 mf/skin snip. More than 90% of participants had not gone beyond primary education (Table 1).

Table 1. Sociodemographic characteristics of PWE in the Logo Health Zone

	All PWE N = 420	**Skin snip negative n = 249**	**Skin snip positive n = 143**	**P-value**
Median age in years (IQR)	19.0 (14.0 – 29.0)	18.0 (13.0 – 29 .0)	23.0 (18.0 – 31.0)	< 0.001
Gender				0.776
Number of males (%)	218 (51.9%)	129 (51.8%)	72 (50.3%)	
Number of females (%)	202 (48.1%)	120 (48.2%)	71 (49.7%)	
Level of education*				0.263
None (%)	155 (37.5%)	85 (35.0%)	49 (34.5%)	
Primary (%)	218 (52.8%)	129 (53.1%)	84 (59.2%)	
Secondary (%)	39 (9.4%)	28 (11.5%)	9 (6.34%)	
University (%)	1 (0.2%)	1 (0.41%)	0 (0%)	

**7 missing values*
IQR: Interquartile range

Epilepsy duration ranged from 0 – 53 years, with a median of 7 years (IQR: 3 – 14). In 51 (12.3%) participants, the duration of epilepsy was ≤ 1 year (new cases of epilepsy). The median age for epilepsy onset was 11 years (IQR: 6.3 – 16.0), with 308 (73.3%) experiencing the first epileptic seizure between 3 – 18 years (Fig 1).

Fig 1. Ages of participants at seizure onset

Generalized motor seizures were reported in 392 (93.3%) PWE, and included 388 (92.1%) with generalized tonic-clonic seizures, 2 (0.5%) generalized myoclonic seizures, 2 (0.5%) generalized atonic seizures ("drop attacks"), and 1 (0.2%) generalized tonic seizures. Nodding seizures were reported in 32 (7.6%) participants. One hundred and sixty-five (39.3%) PWE experienced more than one seizure type. Table 2 summarizes the clinical presentations of participants in the Logo health zone, stratified by skin snip status; the denominators may vary for the different parameters because PWE with missing data were excluded.

Table 2: Clinical presentations of PWE in the Logo health zone

	All PWE N = 420	**Skin snip negative n = 249**	**Skin snip positive n = 143**	**P-value**
Anthropometric characteristics				
Growth retardation (%)	122/386 (31.6%)	71/216 (32.9%)	41/142 (28.9%)	0.425
Seizure characteristics				
Seizure frequency per month (IQR)	2.0 (0.5 – 3.0)	2.0 (0.3 – 3.0)	2.0 (1.0 – 4.0)	<0.001
Age at seizure onset in years (IQR)*	11.0 (6.3 – 6.0)	10.0 (6.0 – 15.2)	13.0 (9.0 – 17.0)	0.001
Epilepsy duration in years (IQR)*	7.0 (3.0 – 14.0)	7.0 (4.0 – 12.6)	10.0 (3.0 – 16.8)	0.052
Generalized motor seizures (%)	392/420 (93.3%)	227/248 (91.5%)	138/143 (96.5%)	0.057
Absence seizures (%)	168/420 (40.0%)	101/248 (40.7%)	62/143 (43.4%)	0.603
Nodding seizures (%)	32/420 (7.6%)	16/248 (6.5%)	14/142 (9.9%)	0.223
Focal motor seizures, conserved awareness (%)	8/386 (2.1%)	3/216 (1.4%)	5/142 (3.5%)	0.189
Focal motor seizures, reduced awareness (%)	34/386 (8.8%)	17/216 (7.9%)	16/142 (11.3%)	0.278
Focal to bilateral tonic-clonic seizures (%)	22/359 (6.1%)	13/217 (6.0%)	9/142 (6.3%)	0.908
Focal non-motor seizures, mainly visual hallucinations (%)	74/349 (21.2%)	47/224 (21.0%)	27/125 (21.6%)	0.896

Unclassified seizures (%)	1/358 (0.3%)	1/216 (0.5%)	0/142 (0%)	NA
Clinical and laboratory findings				
Itching (%)	141/414 (34.1%)	83/245 (33.9%)	57/142 (40.1%)	0.222
Palpable nodules (%)	24/406 (5.9%)	8/236 (3.4%)	14/143 (9.8%)	0.010
Burn scars (%)	98/417 (23.5%)	60/249 (24.1%)	38/142 (26.8%)	0.554
Cognitive impairment (%)	143/415 (34.5%)	87/245 (35.5%)	48/143 (33.6%)	0.705
Abnormal behaviour (%)	47/120 (39.2%)	27/69 (39.1%)	18/47 (38.3%)	0.931
Spinal/thoracic deformity	5/385 (13.0%)	2/216 (0.9%)	3/142 (2.1%)	0.341
Nakalanga features**	12/386 (3.1%)	7/216 (3.2%)	5/142 (3.5%)	0.877
Positive Ov16 rapid test result	127/362 (35.1%)	49/211 (23.2%)	76/123 (61.8%)	< 0.001
OAE criteria met [7]	284/420 (67.6%)	165/249 (66.3%)	110/143 (76.9%)	0.027

**2 missing data*
***Growth retardation, delayed sexual development, cognitive impairment, and/or deformities* [19]
IQR: Interquartile range; OAE: Onchocerciasis-associated epilepsy; NA: Not available

Among the 284 PWE (67.6%) who met the OAE diagnostic criteria, 110/275 (40.0%) and 99/150 (39.8%) were positive for skin snips and Ov16 rapid tests, respectively. Only 258 of these OAE participants had complete data for both Ov16 and skin snip results, and 147 (57.0%) of them were positive for at least one onchocerciasis test. Seizure frequency was higher among PWE who met the OAE criteria: 2.0 seizures/month (IQR: 0.7 – 4.0), compared to 1.5 seizures/month (IQR: 0.5 – 2.0) in PWE who did not meet the criteria; p = 0.006 (Wilcoxon rank sum test). Moreover, a higher mf density was observed among the PWE who fulfilled the OAE criteria: 0.0 mf/skin snip (IQR: 0.0 – 16.0) vs 0.0 mf/skin snip (IQR: 0.0 – 1.5); p = 0.02.

When considering all PWE in the Logo health zone, PWE who were positive for at least one onchocerciasis laboratory test (Ov16 test or skin snip) were older (median age: 23.0 years, IQR: 18.0 – 31.0 vs 17.0 years, IQR: 12.0 – 26.8; p < 0.001); had a higher seizure frequency (2 seizures/month, IQR: 0.8 – 4.0 vs 2 seizures/month, IQR: 0.3 – 2.0; p = 0.003); more absence seizures (42.8% vs 27.8%, p = 0.003); more itching (41.5% vs 30.2%, p = 0.028); more traumatic wounds/burns (29.5% vs 19.1%; p = 0.024); and more

family history of epilepsy (41.2% vs 30.2%; p = 0.032) when compared with PWE who were negative for both tests.

Nodding seizures were reported in 32 (7.6%) PWE. When compared with PWE without a history of nodding seizures, PWE with nodding seizures were younger (median ages: 16.0 years (IQR: 13.0 – 19.0) vs 20.0 years (IQR: 14.2 – 29.0); p = 0.01), had a higher seizure frequency (3.0 seizures/month (IQR: 2.0 – 16.2) vs (2.0 seizures/month (IQR: 0.5 – 3.0); p < 0.001), experienced absence seizures more frequently (75.0% vs 37.1%; p < 0.001), were more often cognitively impaired (71.9% vs 31.2%; p < 0.001), and had a higher prevalence of delayed secondary sexual development (11.1% vs 2.5%; p = 0.01). Age at seizure onset was not significantly different among participants who reported nodding seizures (age at onset: 9.5 years; IQR: 6.0 – 12.0) compared to those who did not (age at onset: 11.0 years; IQR: 7.0 – 17.0); p = 0.09.

Twelve PWE presented with Nakalanga features (Table 3), of which 4 (33.3%) were males. Their ages ranged from 16 – 30 years. In all the 11 PWE with Nakalanga features for whom the age of epilepsy onset was known, the first seizure occurred between 3 and 12 years; in one individual, the age at seizure onset was not known but a febrile seizure was reported at the age of one year. Seizure types included generalized tonic-clonic seizures in 11/12 (91.7%), absence seizures in 9/12 (75.0%), and nodding seizures in 3/12 (25.0%). Seizure frequency ranged from 0.2 – 90.0 per month, and 11 of these PWE experienced two or more seizure types. Two thirds (8/12) of PWE presenting Nakalanga features were positive for at least one onchocerciasis test.

Table 3: Clinical features and onchocerciasis diagnosis in PWE with the Nakalanga features

Case	Socio-demography		Anthropometry			Seizure history				Other clinical manifestations			OAE	Onchocerciasis diagnosis		
	Sex	Age	Height (cm)	Height-for-age Z-score[1]	Summary	Age at onset	Seizure types	Frequency (monthly)	Epileptic siblings	Cognitive impairment	Sexual development	Deformity	Criteria met[2]	Number of nodules	Mf density[3]	Ov16
1	Female	16 years	145	-2.6	Moderate stunting	4 years	Generalized tonic clonic; Absence; focal sensory	12	0	No	Mature breast No pubic hair	None	Yes	0	0	+
2	Male	22 years	140	ND	Below the mean adult height*	8 years	Generalized tonic clonic	90	2	Yes	No pubic hair	Lordosis; facial dysmorphia	Yes	0	155.5	+
3	Female	18 years	143	-3.0	Severe stunting	NA	Generalized tonic clonic; Absence; focal sensory	3	1	Yes	Mature breast No pubic hair	None	No	2	159.5	-
4	Male	18 years	144	-4.3	Severe stunting	12 years	Nodding; Absence	3	0	Yes	No pubic hair	None	Yes	0	0	+
5	Female	30 years	136	ND	Below the mean adult height*	5 years	Generalized tonic clonic; Absence; Nodding; focal sensory	0.2	0	Yes	Mature breast Pubic hair present	None	Yes	0	0	-
6	Male	29 years	137	ND	Below the mean adult height*	12 years	Generalized tonic clonic	3	0	Yes	No pubic hair	Kyphosis, facial dysmorphia	Yes	0	0	-
7	Female	19 years	136	-4.2	Severe stunting	5 years	Generalized tonic clonic; Absence	3	2	No	Immature breast No pubic hair	None	Yes	0	27.5	NA
8	Female	19 years	152	-1.7	Low height, not stunted	7 years	Generalized tonic clonic; Absence	5	0	Yes	Mature breast Pubic hair present	None	Yes	0	0	-
9	Female	19 years	142	-3.2	Severe stunting	8 years	Generalized tonic clonic; Absence; Nodding; focal seizure + impaired awareness	15	0	Yes	Mature breast No pubic hair	Kyphosis	Yes	0	0.5	-
10	Male	24 years	155	ND	Below the mean adult height*	8 years	Generalized tonic clonic; Absence; focal sensory	15	0	Yes	No pubic hair	Thoracic deformity	Yes	1	126.5	NA
11	Female	19 years	150	-2.1	Moderate stunting	3 years	Generalized tonic clonic; Absence	16	2	Yes	Mature breast Pubic hair present	None	Yes	0	0	+
12	Female	27 years	145	ND	Below the mean adult height*	5 years	Generalized tonic clonic	2	0	Yes	Mature breast Pubic hair not examined	None	Yes	0	0	-

[1]*For participants aged 5 – 19 years, based on the World Health Organization growth curves* [13]
[2]*Based on previously published criteria* [7]
[3]*Number of microfilariae per skin snip*
**Mean height of a female adult in the Democratic Republic of Congo: 157.4 cm* [14]
Mf: microfilaria; NA: Not available; ND: Not done; OAE: Onchocerciasis-associated epilepsy

Table 4 summarizes the past history of PWE in the Logo health zone. Overall, 136 probable neurological events during childhood were reported, of which 62 (45.6% of the events) were seizures with fever. Of the 288 PWE who reported ever taking anti-epileptic drugs (AED), the molecules used included: phenytoin (91 PWE, 31.6%), phenobarbital (13 PWE, 4.5%) and carbamazepine (1 PWE, 0.3%). The remaining participants could not recall the name of the AED used. Compared to their counterparts with no other PWE among their first degree relatives, participants with a family history of epilepsy had more positive skin snips (44.1% vs 32.9%; p = 0.027) and higher mf densities (0 mf/skin snip, IQR: 0 – 29 vs 0 mf/skin snip, IQR: 0 – 3; p = 0.007).

Table 4. Past history of PWE in the Logo Health Zone

	All PWE N = 420	**Skin snip negative n = 249**	**Skin snip positive n = 143**	**P-value**
Head trauma with loss of consciousness	6/413 (1.5%)	6/246 (2.4%)	0/139 (0%)	NA
Probable perinatal asphyxia*	20/380 (5.3%)	11/233 (4.7%)	5/132 (3.8%)	0.687
Meningitis/encephalitis	4/412 (1.0%)	4/246 (1.6%)	0/138 (0%)	NA
Malaria	38/384 (9.9%)	27/245 (11.0%)	11/139 (7.9%)	0.389
Measles	6/350 (1.7%)	5/212 (2.4%)	1/138 (0.7%)	0.234
Seizure with fever in childhood	62/380 (16.3%)	38/234 (16.2%)	16/120 (13.3%)	0.473
Ever used anti-epileptic drugs	288/418 (68.9%)	171/248 (69.0%)	94/143 (65.7%)	0.502
Ever used traditional medicine	167/385 (43.4%)	99/215 (46.0%)	59/142 (41.5%)	0.403
Family history of epilepsy**	151/420 (36.0%)	82/249 (32.9%)	63/143 (44.1%)	0.027

**Difficult labor and/or birth by emergency caesarean section*
***Epilepsy in a first degree relative, either parent or sibling*
NA: Not available

Different seizure triggers were identified, including food, cold weather, and storms (Fig 2). Eight of the nine PWE (88.9%) who reported food as a trigger were experiencing nodding seizures.

Fig 2. Seizure triggers among PWE in the Logo Health Zone

234 Bivariate analysis showed a positive correlation between seizure frequency and mf density among PWE in
235 the Logo health zone: Spearman rho = 0.181; p = 0.0003 (Fig 3A). The multivariable analysis revealed that
236 female gender, age, mf density, history of nodding seizures, behavioural abnormalities, deformities, growth
237 retardation and reduced autonomy were associated with the high seizure frequency in the study participants
238 (Table 5). In contrast, epilepsy duration, absence seizures, burn scars, previous AED use and cognitive
239 impairment had a negative association with seizure frequency.

240 **Table 5.** Multivariable analysis for factors associated with seizure frequency in the Logo Health Zone

Covariate	Risk Ratio	95% confidence interval	P-value
Female gender	2.163	1.708 – 2.747	< 0.001
Age	1.043	1.032 – 1.054	< 0.001
Duration of epilepsy	0.948	0.928 – 0.968	< 0.001
Mf density	1.004	1.002 – 1.007	< 0.001
Nodding seizures	3.852	2.926 – 5.082	< 0.001
Absence seizures	0.723	0.557 – 0.936	0.01
Head trauma	0.310	0.005 – 2.081	1
Previous AED use	0.579	0.432 – 0.774	< 0.001
Cognitive impairment	0.046	0.027 – 0.077	< 0.001
Abnormal behaviour	1.867	1.425 – 2.441	< 0.001
Reduced autonomy	2.749	1.688 – 4.738	< 0.001
Burn scars	0.620	0.460 – 0.831	0.001
History of malaria	1.078	0.787 – 1.466	1
Growth retardation	2.678	1.919 – 3.758	< 0.001
Spinal/thoracic deformity	2.001	1.121 – 3.412	0.01

Mf: Microfilariae; AED: Anti-epileptic drug

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243 ## PWE in the Aketi Health Zone

244 All 81 PWE recruited in the Aketi health zone had positive skin snips. Their median age was 17 years (IQR:
245 15 – 20) and the median mf density was 10.5 mf/skin snip (IQR = 3.5 – 53.0). Forty-one (50.6%) were
246 males. The median seizure frequency was 1.0 seizure/month (IQR: 0.5 – 2.0). CDTI coverage among the

247 participants in the year prior to the study was 50/81 (61.7%). Bivariate analyses showed a positive
248 correlation between seizure frequency and mf density, albeit a borderline significance (Spearman rho:
249 0.228, p = 0.046; Fig 3B). After adjusting for age, sex, previous AED and ivermectin use, the seizure
250 frequency of participants was still associated with mf density (RR = 1.0065, 95% confidence interval:
251 1.0057 – 1.0073; p < 0.001). Of note, previous ivermectin use was associated with reduced seizure
252 frequency (RR = 0.69, 95% confidence interval: 0.58 – 0.83; p < 0.001).

253 **Fig 3.** Correlation between frequency of seizures and microfilarial density among PWE

254

255 When comparing the intensity of *O. volvulus* infection among PWE in the Logo and Aketi health zones, we
256 found that the median mf densities from skin snip-positive participants in both sites differed significantly
257 (Logo: 27.5 mf/skin snip vs Aketi: 10.5 mf/skin snip; p = 0.01).

258

259 Discussion

260 This is the first paper describing the clinical characteristics of epilepsy and its relationship with microfilarial
261 density in onchocerciasis-endemic areas in the DRC. A wide spectrum of seizures was observed, with more
262 than one third of participants reporting at least two seizure types. The most frequently reported seizures
263 were generalized motor seizures (93.3%), followed by absences (40.0%). Nodding seizures and Nakalanga
264 features were reported, suggesting a high prevalence of OAE in these communities as previously observed
265 in Ituri (DRC) [9], in the Mbam valley (Cameroon) [20], Mahenge (Tanzania) [21], and Maridi (South
266 Sudan) [22]. Moreover, two thirds of participants in the Logo health zone met the OAE criteria. A positive
267 correlation between the frequency of seizures and mf density supports recent findings from a cohort study
268 in Cameroon which showed that the risk to develop epilepsy increases with increasing intensity of
269 childhood infection with *O. volvulus* [5]. In that cohort study, the population attributable fraction of epilepsy
270 associated with onchocerciasis was estimated at 91.7% [5], and PWE in the investigated villages had similar
271 clinical manifestations as observed in our study [23].

By meticulously taking the history of our study participants, we were able to identify 32 PWE who reported experiencing nodding seizures. They all met the criteria of the consensual case definition of probable nodding syndrome [24]. Persons with a history of nodding seizures in our study were younger, more often cognitively impaired and had more food-triggered seizures; all these clinical aspects align with the nodding syndrome definition [24]. In addition, the description of the 12 PWE with Nakalanga features presented in Table 3 closely matched previous reports from other African countries [19]. The fact that these phenotypic presentations have only been reported in onchocerciasis-endemic settings till date strongly suggests the role of *O. volvulus* in triggering these conditions.

An unexpected finding from our multivariable model was the negative association between seizure frequency and cognitive impairment. This could be related to the natural history of the nodding syndrome, as it usually begins at a younger age with frequent, debilitating nodding seizures, which generally evolve to less frequent generalized convulsive seizures [25]. This is confirmed by the reduction in seizure frequency observed with increasing duration of epilepsy. It is therefore conceivable that some PWE were already cognitively impaired because of past nodding seizures, though presenting with fewer generalized convulsions at the time of the study. Persons with cognitive impairment and high seizure frequency are also likely to have a reduced life expectancy and therefore were not very prevalent in the study population. A similar explanation may hold true for participants with burn scars.

In our study, head trauma was not associated with seizure frequency in contrast with previous findings from Ethiopia [18]. Other factors associated with high seizure frequency such as behavioral symptoms and reduced autonomy are probably co-morbid conditions resulting from recurrent seizures. The fact that the female gender was associated with two times more seizures compared to the male gender in our study warrants further research. Absence seizures may have been underestimated, explaining their negative association with the overall seizure frequency.

Growth retardation was a very frequent trait among PWE in the Logo health zone, irrespective of skin snip status. However, our multivariable model showed that growth retardation and mf density are each associated with increased seizure frequency. This complements previous suspicions from a recent study in

298 the DRC, that both epilepsy and retarded growth may be related to *O. volvulus* exposure [26]. Although
299 stunting is a common feature in persons with OAE including nodding syndrome [7,23,26], other factors
300 such as undernutrition and poverty observed among PWE may contribute to their abnormal growth, as
301 reported in an Ethiopian study [27]. However, given that we did not investigate the feeding habits of our
302 participants, our study is unable to confirm this.
303 Participants with a family history of epilepsy had a higher prevalence and intensity of *O. volvulus* infection.
304 This suggests a greater exposure to onchocerciasis and explains the clustering of PWE in such households,
305 which is a characteristic feature of OAE [7]. This is in line with previous reports of villages and families
306 who are closer to blackfly breeding sites having more PWE [3,11,20,22].
307 Only skin snip-positive PWE were recruited in the Aketi health zone. However, their mf densities were
308 much lower compared to skin-snip positive PWE in the Logo health zone. This is most probably due to 14
309 rounds of CDTI that had already taken place in Aketi, while the participants from Logo were ivermectin-
310 naïve. Up to 61.7% of PWE in Aketi had received ivermectin in the year preceding the study, much higher
311 than the 25% CDTI coverage observed among PWE in onchocerciasis-endemic areas in Cameroon [23]; $p < 0.001$. The belief that ivermectin may help to reduce epilepsy is frequent in the Aketi health zone [10]
313 and this probably motivates PWE to adhere to CDTI.
314 While this was not the purpose of the study, we noted some discrepancies in the onchocerciasis diagnosis
315 using skin snips (reference technique in our study) and Ov16 rapid tests (Table 2); the rapid tests yielded
316 23.2% of false positives. Rapid tests may therefore not be optimal for diagnosing ongoing *O. volvulus*
317 infection, although they do provide information about exposure to the parasite. They however remain key
318 and convenient for field use when assessing onchocerciasis transmission by testing children aged 10 years
319 and below, as was the case in Cameroon [20], Nigeria [28], DRC [10] and Tanzania [21].

320 Limitations of the study

321 Our study has several limitations. Laboratory and imaging investigations to exclude other possible causes
322 of epilepsy such as neurocysticercosis were not performed. However, previous studies had suggested that

T. solium infection is not prevalent in the Logo Health zone [4] and in the Bas-Uélé province [29]. In addition, the high proportion of PWE meeting the OAE criteria makes it unlikely for another infectious pathology to be the main reason behind the high epilepsy prevalence. Another limitation is the fact that seizure information and past history of participants were obtained by questioning the PWE and caretakers, and could be subject to recall bias. Absence seizures and some focal seizures which are more subtle may have been under-reported as a consequence.

In conclusion, PWE in onchocerciasis-endemic villages in the Logo Health zone presented with wide clinical spectrum including generalized seizures, nodding seizures, Nakalanga features and other OAE characteristics. Mf density was significantly and positively associated with seizure frequency in both Logo and Aketi. It is expedient that onchocerciasis control measures be strengthened to prevent new OAE cases, while providing comprehensive care to confirmed PWE using appropriate AED and cognitive rehabilitation services. The possible added value of anti-filarial drugs in the treatment of OAE including nodding syndrome is currently being investigated [12,30].

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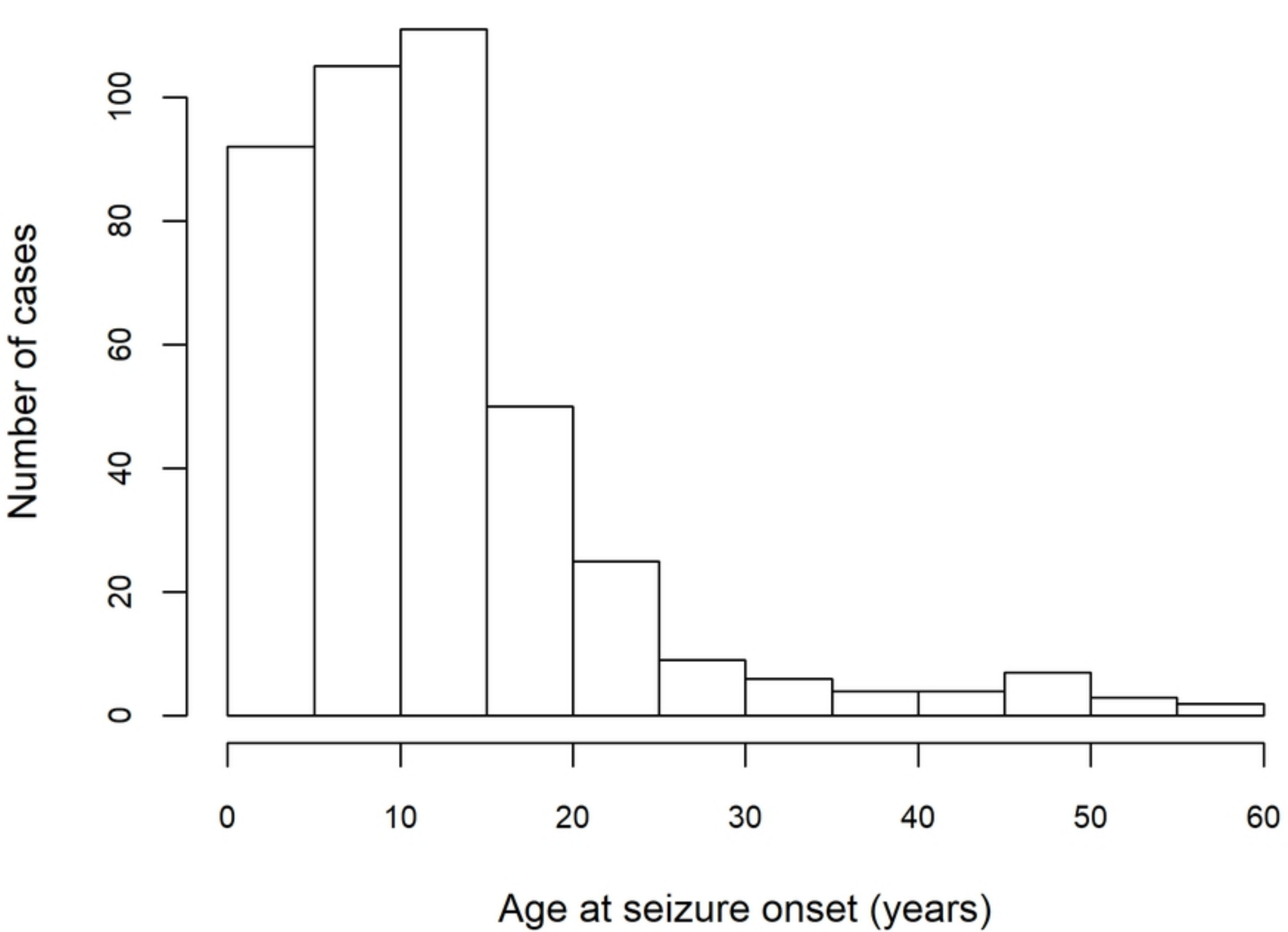
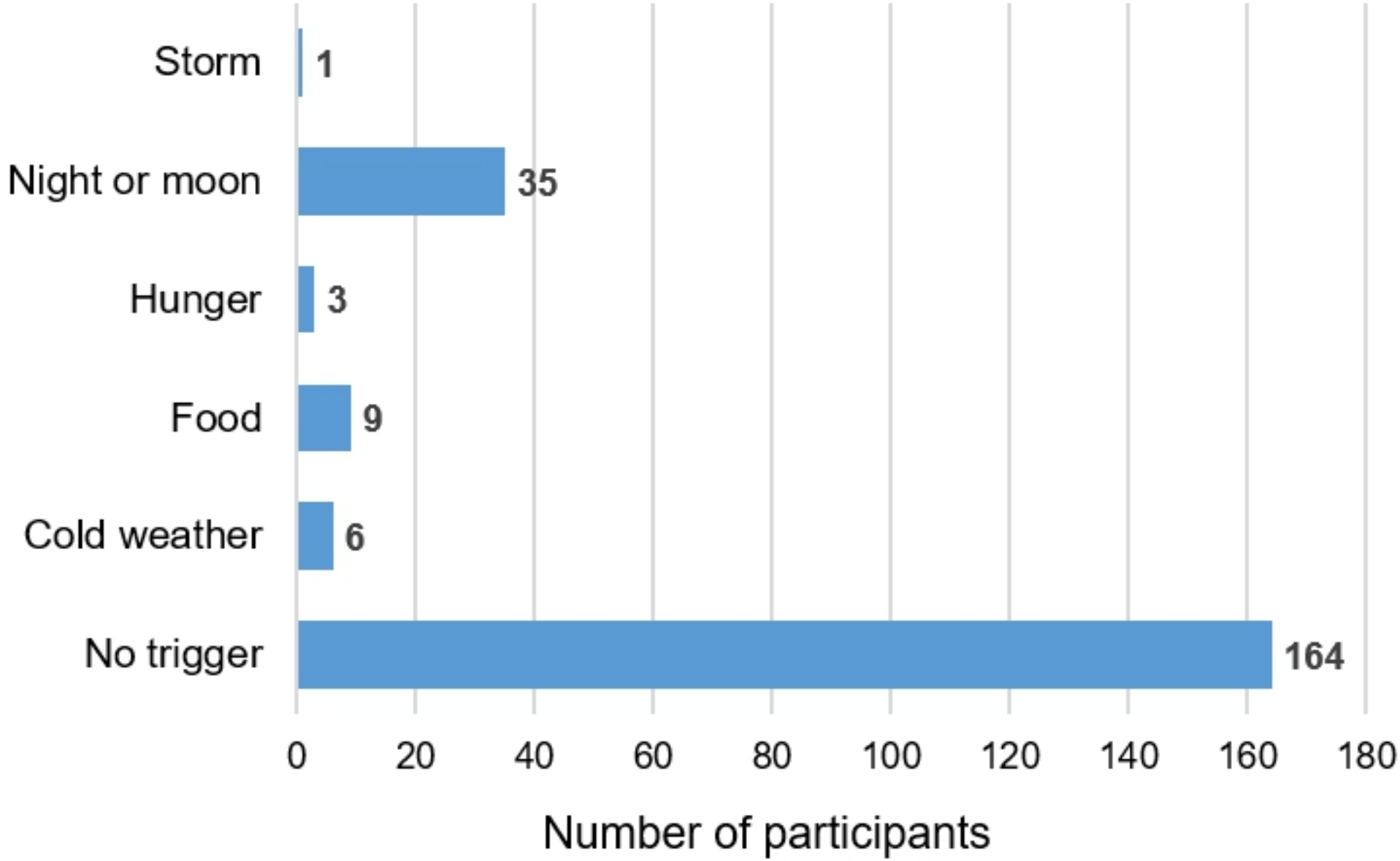
Supporting information

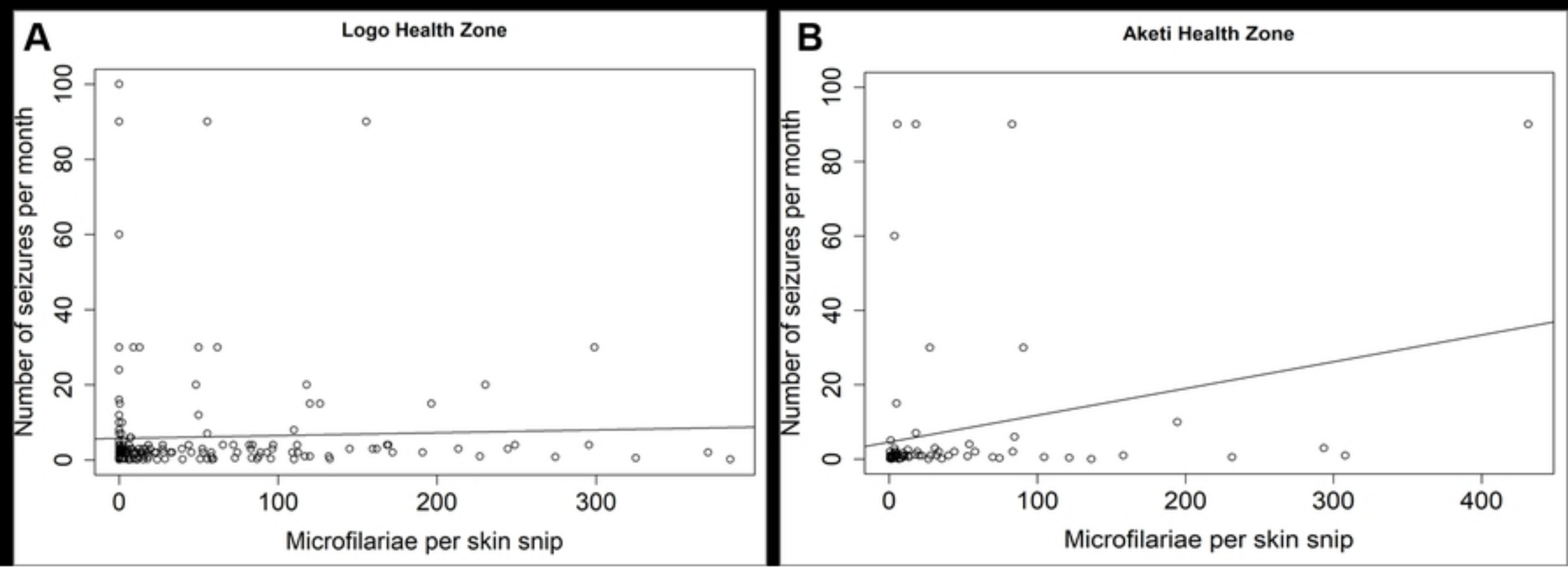
S1 File. STROBE checklist.


Number of cases
0
20
40
60
80
100
0
10
20
30
40
50
60
Age at seizure onset (years)

Figure


Storm
1
Night or moon
35
Hunger
3
Food
9
Cold weather
6
No trigger
164
0
20
40
60
80
100
120
140
160
180
Number of participants

Figure


A
Logo Health Zone
Number of seizures per month
0
20
40
60
80
100
0
100
200
300
Microfilariae per skin snip
B
Aketi Health Zone
Number of seizures per month
0
20
40
60
80
100
0
100
200
300
400
Microfilariae per skin snip

Figure