The temporal relationship between onchocerciasis and epilepsy: a population-based cohort study

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Summary

Background Many studies have suggested that onchocerciasis might be associated with epilepsy. Therefore, we did a cohort study to assess the incidence of epilepsy relative to *Onchocerca volvulus* skin microfilarial density (MFD) measured during childhood and to assess the possibility of a temporal relationship.

Methods During onchocerciasis surveys undertaken in 25 villages in Cameroon during 1991–93, we measured MFD in individuals aged 5 years or older. In 2017, we revisited seven of these villages. With a standardised five-item questionnaire, we collected information on the occurrence of epilepsy in 856 individuals who were aged 5–10 years in 1991–93, and had MFD determined during the original surveys. We did multivariable analyses to assess the overall incidence and incidence ratios taking into account age, sex, individual MFD in 1991–93, and onchocerciasis endemicity level in the village.

Findings In 2017, we obtained data on the history of epilepsy for 85% (729 of 856) of individuals. Among these individuals, we classified 60 as being suspected cases of epilepsy. The overall incidence of epilepsy was 350 per 100 000 person-years (95% CI 270–450). The adjusted incidence ratio for developing epilepsy was 7·07 (95% CI 0·98–51·26; p=0·0530) in individuals with initial MFD of one to five microfilariae per skin snip (mf per snip), 11·26 (2·73–46·43) in individuals with six to 20 mf per snip, 12·90 (4·40–37·83) in individuals with 21–50 mf per snip, 20·00 (3·71–108·00) in individuals with 51–100 mf per snip, 22·58 (3·21–158·56) in individuals with 101–200 mf per snip, and 28·50 (95% CI 3·84–211·27; p=0·0010) in individuals with more than 200 mf per snip, compared with that of individuals without detectable densities of skin microfilariae.

Interpretation Individual *O volvulus* MFD in childhood was associated with the risk of either seizures or epilepsy in an onchocerciasis focus in Cameroon. This temporal relationship suggests a potential causal link between onchocerciasis and epilepsy.

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Introduction

There are between 50 and 70 million people with epilepsy worldwide,[1] with 80% living in low-income and middle-income countries.[1,2] Prevalences are estimated to be 9·4 per 1000 people in sub-Saharan Africa and 59·7 per 1000 people in central Africa.[3] Similarly, although findings from a meta-analysis showed that the global median incidence of epilepsy is 50·4 per 100 000 person-years,[4] values recorded in sub-Saharan Africa range between 64 and 187 per 100 000 person-years.[3]

The high prevalences and incidences recorded in sub-Saharan Africa are mainly due to perinatal brain damage and the presence of many pathogens that can induce severe neurological complications. The possibility that *Onchocerca volvulus*, the filarial worm causing onchocerciasis, can induce epilepsy was first raised in the 1930s, in Mexico.[5] Since then, findings from most subsequent studies,[6–8] as well as meta-analyses,[9–11] have shown a significant association between onchocerciasis and epilepsy prevalence, even when adjusted for other risk factors and infections.[12,13] In 2017, the term onchocerciasis-associated epilepsy was proposed[14] to describe different forms of epilepsy occurring in onchocerciasis foci, including nodding syndrome.

Because these studies could not establish a causal relationship between *O volvulus* and epilepsy, and the parasite was never found in the brain and only rarely in the cerebrospinal fluid (CSF), this association has remained controversial. To bring new insights into the discussion and, especially, to bring evidence of a temporal relationship, we did a cohort study to assess whether the level of infection with *O volvulus* measured in 1991–93, in a population of children aged 5–10 years, was associated with an increased risk of developing epilepsy later in life.

Methods

Initial parasitological surveys

Between 1991 and 1993, we undertook parasitological surveys with WHO's recommended methods in 25 villages of the Mbam valley (Centre Region, Cameroon) to measure the levels of infection with *O volvulus* among children aged 5 years or older (appendix, p 1). After registration of full name, sex, age, and history of antifilarial treatment, we took two skin snips from each

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See Online for appendix

Research in context

Evidence before this study

Onchocerciasis, a filarial infection caused by *Onchocerca volvulus* and transmitted by blackflies (*Simulium* spp), is primarily endemic in Africa. We reviewed articles published in English or French before February 5, 2018, identified through a PubMed search with the terms "onchocerciasis", "filariasis", "*Onchocerca volvulus*", "epilepsy", "central Africa", "seizures", "nodding syndrome", "lymphatic system", and "central nervous system". We also identified relevant articles through searches in Google Scholar, theses, and reports. Several studies, including meta-analyses, suggested an association between onchocerciasis and epilepsy, at the community and individual levels. We found that all previous studies were cross-sectional, and that reports of parasite presence in the cerebrospinal fluid were rare. Consequently, the question of a causal relationship between *O volvulus* infection and epilepsy is still unresolved. Moreover, to our knowledge, no cohort study has been done to assess the temporal relationship between *O volvulus* exposure and the development of epileptic seizures.

Added value of this study

Our study was done in seven villages of central Cameroon and included 856 individuals whose intensity of infection with *O volvulus* (measured in the number of microfilariae per skin snip) had been measured in 1991–93, when they were 5–10 years old. In 2017, we assessed the appearance of epileptic seizures since the initial parasitological survey, with use of a five-item questionnaire. Our cohort study showed that early childhood infection with *O volvulus* is associated with an increased risk of developing either seizures (provoked or unprovoked) or epilepsy, with a strong dose–response relationship, in which the extent of the risk increased with the intensity of infection with *O volvulus*.

Implications of all the available evidence

Our study adds substantial support to the hypothesis of a causal relationship between *O volvulus* infection and epilepsy, and confirmation in other endemic sites is needed. Further studies are needed to elucidate the possible direct and indirect effects of the parasite on the CNS underlying this relationship; if the indirect effects are due to the presence of microfilariae in the brain, studies need to access the role of live parasites (associated with provoked seizures) and dead parasites (associated with unprovoked seizures). Our study should motivate the increase of mass treatment to reduce the prevalence and intensity of *O volvulus* infection and the inclusion of children younger than 5 years in the mass treatment programmes, therefore helping to possibly decrease the high burden of epilepsy in Africa.

participant with a 2 mm Holth-type corneoscleral punch, and left the snips in saline at room temperature for 24 h. We counted the emerged microfilariae under a microscope and calculated each individual's microfilarial density (MFD, microfilariae per snip [mf per snip]) as the arithmetic mean of the counts in each snip. Community microfilarial load (CMFL), defined as the Williams geometric mean of MFD in individuals aged 20 years or older, was calculated for each village.

Selection of villages and individuals for the 2017 survey

Seven of the 25 communities surveyed in 1991–93 (table 1) were selected to be revisited in 2017, on the basis of the high proportion of the population included in the 1991–93 survey and including villages with a wide range of CMFL (18·8–114·5 mf per snip). Mass treatments with ivermectin were organised in February, 1994, 1995, and 1996, in all seven villages, except Yambassa and Nyamongo, as part of a clinical trial. Therapeutic coverages during these mass treatments were low (about 30%), with a weak effect on the intensity of transmission of *O volvulus*. From 1998 onwards, all villages in the study area benefited from annual ivermectin mass treatment, from the community-directed strategy developed by the African Programme for Onchocerciasis Control. No vector control activity was ever done in the study area.

3280 individuals aged 5 years or older were examined during 1991–93, in the seven selected villages. Initial observations showed that people with epilepsy usually have their first epileptic seizure between the ages of 10 and 15 years.[15] Therefore, we approached all individuals aged 5–10 years during the baseline surveys for participation in our study. We excluded eight children who had already presented a seizure at the time of the baseline surveys and two others who had taken ivermectin before the initial survey, resulting in 856 adults that we sought to contact for our 2017 survey.

Evaluation and definition of epilepsy

In July, 2017, we identified, with the help of informants, the place of residence in the village of the 856 selected individuals or of their family members. With the assistance of local health workers, we then visited the selected individuals or family members at home and introduced ourselves, our study, and its objectives, and asked them whether they would agree to participate in the study. After informed consent was obtained, if the selected 1991–93 survey participant was not home, we asked the family members whether the person was still alive. If the individual had died, we recorded the year and the potential cause of death. This information was used to estimate the duration of follow-up for each individual. Afterwards, we administered a validated and standardised five-item questionnaire,[16] developed by the Institut d'Épidémiologie Neurologique et de Neurologie Tropicale (Limoges, France), to the selected individuals or their family members (if the individual was absent or dead) to identify suspected cases

	Children in baseline study (% of total)	Individuals with information collected in 2017 (% of total)	Individuals with or without suspected development of epilepsy*			p value†	Proportion of individuals with suspected development of epilepsy among those who could be traced (per 1000)
			Without epilepsy	With epilepsy	Missing data‡		
Total	856	729	669 (78%)	60 (7%)	127 (15%)	..	82·3
Age	..	..	..	..	..	0·0402	
5 years	126 (15%)	103 (14%)	90 (71%)	13 (10%)	23 (18%)	..	126·2
6 years	176 (21%)	148 (20%)	138 (78%)	10 (6%)	28 (16%)	..	67·6
7 years	171 (20%)	148 (20%)	134 (78%)	14 (8%)	23 (13%)	..	94·6
8 years	122 (14%)	95 (13%)	87 (71%)	8 (7%)	27 (22%)	..	84·2
9 years	122 (14%)	110 (15%)	103 (84%)	7 (6%)	12 (10%)	..	63·6
10 years	139 (16%)	125 (17%)	117 (84%)	8 (6%)	14 (10%)	..	64·0
Sex	..	..	..	..	..	0·219	
Male	434 (51%)	376 (52%)	340 (78%)	36 (8%)	58 (13%)	..	95·7
Female	422 (49%)	353 (48%)	329 (78%)	24 (6%)	69 (16%)	..	68·0
CMFL§ (mf per snip)	..	..	..	..	..	0·0308	
<38	268 (31%)	221 (30%)	208 (78%)	13 (5%)	47 (18%)		58·8
38–58	289 (34%)	254 (35%)	240 (83%)	14 (5%)	35 (12%)	..	55·1
>58	299 (35%)	254 (35%)	221 (74%)	33 (11%)	45 (15%)	..	129·9
Skin snip	..	..	..	..	..	0·0308	
Negative	205 (24%)	165 (23%)	164 (80%)	1 (<1%)	40 (20%)	..	6·1
Positive	651 (76%)	564 (77%)	505 (78%)	59 (9%)	87 (13%)	..	104·6
MFD (mf per snip)	..	..	..	..	..	0·499	
0	205 (24%)	165 (23%)	164 (80%)	1 (<1%)	40 (20%)	..	6·1
1–5	132 (15%)	114 (16%)	109 (83%)	5 (4%)	18 (14%)	..	43·9
6–20	141 (16%)	125 (17%)	116 (82%)	9 (6%)	16 (11%)	..	72·0
21–50	123 (14%)	106 (15%)	97 (79%)	9 (7%)	17 (14%)	..	84·9
51–100	96 (11%)	82 (11%)	71 (74%)	11 (11%)	14 (15%)	..	134·1
101–200	61 (7%)	52 (7%)	44 (72%)	8 (13%)	9 (15%)	..	153·8
>200	98 (11%)	85 (12%)	68 (69%)	17 (17%)	13 (13%)	..	200·0
Village	..	..	..	..	..	0·0146	
Bitang	268 (31%)	221 (30%)	208 (78%)	13 (5%)	47 (18%)	..	58·8
Yambassa	259 (30%)	229 (31%)	220 (85%)	9 (3%)	30 (12%)	..	39·3
Nyamongo	30 (4%)	25 (3%)	20 (67%)	5 (17%)	5 (17%)	..	200·0
Kiboum	73 (9%)	65 (9%)	57 (78%)	8 (11%)	8 (11%)	..	123·1
Ngongol	39 (5%)	30 (4%)	26 (67%)	4 (10%)	9 (23%)	..	133·3
Yebekolo	142 (17%)	127 (17%)	110 (78%)	17 (12%)	15 (11%)	..	133·9
Biatsotta	45 (5%)	32 (4%)	28 (62%)	4 (9%)	13 (29%)	..	125·0

The number of individuals suspected to have developed epilepsy after the initial parasitological survey was assessed according to answers to the five-item questionnaire by the individual or the family. CMFL=community microfilarial load. Mf per snip=microfilariae per skin snip. MFD=individual microfilarial density. *Data are n (% of total in each category at baseline). †p value for comparison between available and missing data. ‡Individual or family not met in 2017. §CMFL in mf per snip: Bitang 18·8 mf per snip, Yambassa 38·0 mf per snip, Nyamongo 45·8 mf per snip, Kiboum 58·5 mf per snip, Ngongol: 63·7 mf per snip, Yebekolo 100·7 mf per snip, Biatsotta 114·5 mf per snip.

Table 1: **Study population**

of epilepsy, defined as individuals for whom the answer to at least one of the five questions was positive (appendix, p 2). The interviewers had no information on the individuals' MFD measured during the initial parasitological survey. The study protocol and the information to be provided to study participants or their families were approved by the ethical committee of the University of Antwerp (registration number B300201731362) and the Cameroon National Ethics Committee for Research in Human Health (Registration number 2017/02/875/CE/CNERSH/SP).

Variables

The dependent variable was the suspicion of epilepsy on the basis of the answers to the questionnaire, and

the independent variables were the sex, age (5, 6, 7, 8, 9, or 10 years), MFD (seven categories: zero, one to five, six to 20, 21–50, 51–100, 100–200, and >200 mf per snip), and intensity of infection in the individual's village of residence recorded during the initial survey (1991–93). The intensity of infection was defined by the CMFL and organised into three categories, each including a similar number of selected individuals: fewer than 38, 38–58, and more than 58 mf per snip (appendix, p 2).

Statistical analysis

The primary analysis included only individuals for whom the answers to the five-item questionnaire could be collected from the individuals themselves or their family members. This primary analysis was complemented by sensitivity analyses that included data from individuals without answers to the questionnaire. We did three main sensitivity analyses. In the first, all individuals for whom no information could be collected in 2017 (individuals unknown or family members not met, considered as lost to follow-up) were considered as not having developed epilepsy. In the second analysis, all these individuals were considered as being suspected cases of epilepsy. In the third sensitivity analysis, we checked the influence of the missing data (for the individuals who were lost to follow-up) by assuming that our missing data depends only on observed values and not on unobserved values—the missing-at-random model—and by use of a multiple imputation method. To take into account these two methods, we applied the multivariate imputation by chained equations using the appropriate package of the R software (version 3.2.3), with 50 iterations. We used age, sex, individual MFD, villages, and CMFL as variables. This method allowed us to simulate the epileptic status for the individuals lost to follow-up and, once the imputation was done, we did the same multivariable Poisson model. Lastly, an additional sensitivity analysis was done with the use alone of the response to the fifth question of the questionnaire ("Did someone tell the subject that he/she had epilepsy or that he / she already had epileptic fits?"; specificity 94·8%);[16] and including only individuals for whom the answers to the five-item questionnaire were collected.

Survival data and incidences

Data related to vital status information were censored at the date of visit in 2017. For individuals who were unknown to the informants in 2017, data were censored at half-time of the follow-up period. Data concerning individuals who died between the initial survey and 2017 were censored at the date of death if it was known or at half-time of the follow-up period if it was unknown. Incidences were estimated by dividing the number of suspected cases of epilepsy by the total number of person-years of follow-up.

Assessment of individual risk factors associated with suspected epilepsy (incidence ratios)

We first did a procedure to check the statistical hypotheses and to compare models (appendix, p 3). Finally, we did a multivariable Poisson model, including the four independent variables previously mentioned, and a cluster option on the villages to obtain better confidence interval estimates. This allowed us to estimate the predicted incidences, using the margin function, and a population-attributable fraction (PAF; appendix, p 3) to estimate the proportion of suspected cases of epilepsy that would have been prevented if all the individuals had a MFD equal to zero at baseline. This was estimated using the Punaf function, with unconditional option for the variance. All analyses were done with Stata (version 14.0).

Role of the funding source

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study, and had final responsibility for the decision to submit for publication.

Results

Table 1 shows characteristics of children in the baseline study. The proportion of children with skin microfilariae in 1991–93 was 76% (651 of 856), with a median MFD of 29·0 mf per snip (7·5–96·5; appendix, p 4). In 2017, the questionnaire was completed for 729 (85%) of 856 individuals who had been surveyed in 1991–93. The proportion of missing data was 15% (127 of 856). Missing data were less frequent for individuals who were 9 and 10 years old at the time of the initial surveys (p=0·040), and were not associated with sex, initial MFD category, or the CMFL of the village of residence (table 1). Among the 729 individuals for whom information could be collected in 2017, 564 (77%) presented with microfilariae in 1991–93, with a median MFD of 28·5 mf per snip (IQR 7·8–95·5).

In 2017, 60 of 729 individuals were identified as suspected cases of epilepsy; therefore, the overall proportion of individuals suspected to have developed epileptic seizures was 82·3 per 1000. This proportion increased with increasing baseline MFD, from 6·1 per 1000 in individuals without skin microfilariae in 1991–93, to 200·0 per 1000 in 2017 in those with initial MFD greater than 200 mf per snip.

The mean follow-up period for the 729 individuals with answers to the questionnaire was 23·7 years (SD 0·13), and the number of person-years of follow-up was 17275. The overall incidence of suspected cases of epilepsy was 350 per 100000 person-years. The incidence tended to decrease as age at the time of the initial survey increased. The incidence did not differ significantly between sexes, but was higher for individuals living in villages with high onchocerciasis endemicity levels (table 2). The most striking result was the

	PY	SCE	Incidence (per 100 000 PY, 95% CI)	p value*
Total	17 275	60	350 (270–450)	NA
Age	..	..	..	0·108
5 years	2440	13	530 (310–920)	..
6 years	3556	10	280 (150–510)	..
7 years	3497	14	400 (240–680)	..
8 years	2185	8	370 (180–730)	..
9 years	2618	7	270 (130–560)	..
10 years	2979	8	270 (130–540)	..
Sex	..	..	..	0·102
Male	8855	36	410 (290–560)	..
Female	8420	24	290 (190–430)	..
CMFL (mf per snip)	..	..	..	0·0014
<38	5180	13	250 (150–430)	
38–58	6210	14	230 (120–380)	..
>58	5885	33	560 (400–790)	..
Skin snip	..	..	..	0·0001
Negative	4006	1	20 (0–180)	
Positive	13 269	59	440 (340–570)	..
MFD (mf per snip)	..	..	..	<0·0001
0	4006	1	20 (0–180)	..
1–5	2737	5	180 (80–440)	..
6–20	2938	9	310 (160–590)	..
21–50	2471	9	360 (190–700)	..
51–100	1908	11	580 (320–1004)	..
101–200	1253	8	640 (320–1280)	..
>200	1961	17	870 (540–1390)	..
Villages	..	..	..	<0·0001
Bitang	5180	13	250 (150–430)	..
Yambassa	5597	9	160 (80–310)	..
Nyamongo	613	5	820 (340–1960)	..
Kiboum	1469	8	540 (270–1090)	..
Ngongol	723	4	550 (210–1470)	..
Yebekolo	2890	17	590 (370–950)	..
Biatsotta	804	4	500 (190–1330)	..

PY=person-years. SCE=suspected cases of epilepsy. NA=not applicable. CMFL=community microfilarial load. Mf per snip=microfilariae per skin snip. MFD=individual microfilarial density. *p values were calculated within each variable and assessed with log-rank test for sex, skin snip positivity, and village variables, and with trend's modified log-rank test for the variables age, CMFL, and skin snip in seven categories.

Table 2: **Incidences of epilepsy**

	Incidence ratio (95% CI)	p value
Age*		
5 years	2·28 (0·72–7·22)	0·163
6 years	1·28 (0·58–2·80)	0·537
7 years	1·58 (0·57–4·40)	0·377
8 years	1·47 (0·48–4·55)	0·501
9 years	1·03 (0·36–3·00)	0·953
Sex (male)†	1·10 (0·70–1·72)	0·688
CMFL (mf per snip)‡		
38–58	0·85 (0·38–1·89)	0·687
>58	1·32 (1·04–1·69)	0·0251
MFD (mf per snip)§		
1–5	7·07 (0·98–51·26)	0·0530
6–20	11·26 (2·73–46·43)	0·0008
21–50	12·90 (4·40–37·83)	<0·0001
51–100	20·00 (3·71–108·00)	0·0005
101–200	22·58 (3·21–158·56)	0·0017
>200	28·50 (3·84–211·27)	0·0010

CMFL=community microfilarial load. Mf per snip=microfilariae per skin snip. MFD=individual microfilarial density. *p values are of each category versus age 10 years. †p value is male versus female. ‡p values are of each category versus CMFL of less than 38 mf per snip. §p values are of each category versus MFD of 0 mf per snip.

Table 3: **Multivariable model for incidence of epilepsy**

gradual increase of incidence of suspected cases of epilepsy with the increase in baseline MFD (table 2).

The risk of being identified as a suspected case of epilepsy in 2017 was higher, but not significantly so, for individuals who were 5 years old during the initial surveys than for 10-year-olds. Sex was not associated with a higher risk of developing epilepsy (table 3). Individuals who lived in villages with the highest CMFL were at higher risk of being a suspected case of epilepsy in 2017 than were those who lived in villages with a lower CMFL, and the risk increased gradually with the increase in individual baseline MFD (table 3). Sensitivity analyses showed the same trends as in the primary analysis, although the effect of MFD was not significant in scenario 2, in which all 127 individuals who were lost to follow-up were considered as being suspected cases of epilepsy (table 4). Additionally, analyses restricted to question five of the questionnaire, to increase our specificity, were similar to the analyses that used all five items of the questionnaire (appendix, p 5).

The risk of an individual being a suspected case of epilepsy increased rapidly with the increase in baseline MFD, when the density was lower than 50 mf per snip, and increased more slowly (but linearly) for higher values of MFD (figure 1). A closer look at the risk for the 26 individuals with MFD higher than 400 mf per snip suggested that the risk continued to increase at such high infection intensities, with the slope being even more marked than for lower MFD (appendix, pp 6–7). In fact, six of 13 individuals who had baseline MFD higher than 500 mf per snip had developed epilepsy by 2017 (one of one child aged 5 years, two of three aged 7 years, two of three aged 8 years, and one of one aged 10 years; p=0·112). An interaction between baseline MFD and the CFML seemed to exist, but was not taken into account in the analyses (figure 2; appendix, p 7).

Lastly, the contribution of infection with *O volvulus* to epilepsy was very high (PAF 91·7%, 95% CI 56·7–98·4; p=0·0021). Consequently, the proportion of suspected cases of epilepsy in the study villages would have been reduced by 91·7% if none of the individuals had had detectable densities of skin microfilariae at the age of 5–10 years.

	Scenario 1		Scenario 2		Scenario 3	
	IR (95% CI)	p value	IR (95% CI)	p value	IR (95% CI)	p value
Age*						
5 years	2·28 (0·72–7·22)	0·163	1·87 (1·39–2·51)	<0·0001	1·85 (0·55–6·23)	0·323
6 years	1·28 (0·59–2·80)	0·537	1·43 (1·23–1·66)	<0·0001	1·13 (0·41–3·16)	0·809
7 years	1·58 (0·57–4·40)	0·377	1·38 (0·98–1·94)	0·069	1·24 (0·35–4·40)	0·737
8 years	1·47 (0·48–4·55)	0·501	1·82 (1·11–2·97)	0·0166	1·35 (0·35–5·26)	0·666
9 years	1·03 (0·36–3·00)	0·953	1·00 (0·56–1·73)	0·959	0·79 (0·25–2·54)	0·696
Sex† (male)	1·10 (0·70–1·72)	0·688	0·91 (0·80–1·04)	0·177	1·14 (0·75–1·73)	0·538
CMFL‡ (mf per snip)						
38–58	0·85 (0·38–1·89)	0·687	0·72 (0·52–0·99)	0·0429	0·76 (0·34–1·68)	0·498
>58	1·32 (1·04–1·69)	0·0251	1·02 (0·74–1·40)	0·908	1·29 (1·02–1·64)	0·0332
MFD§ (mf per snip)						
1–5	7·07 (0·98–51·26)	0·0530	0·87 (0·56–1·34)	0·516	3·36 (0·53–21·31)	0·198
6–20	11·26 (2·73–46·43)	0·0008	0·89 (0·89–1·36)	0·604	7·30 (1·93–27·70)	0·0034
21–50	12·90 (4·40–37·83)	<0·0001	1·04 (0·82–1·33)	0·730	8·08 (4·46–14·62)	<0·0001
51–100	20·00 (3·71–108·00)	0·0005	1·31 (0·81–2·13)	0·277	3·19 (1·77–21·59)	0·0043
101–200	22·58 (3·21–158·56)	0·0017	1·21 (0·66–2·63)	0·426	13·90 (9·90–19·53)	<0·0001
>200	28·50 (3·84–211·27)	0·0010	1·54 (0·89–2·69)	0·124	18·10 (7·02–46·71)	<0·0001
Number of individuals at baseline	856	..	856	..	856	..
Number of suspected cases of epilepsy	60	..	187	..	75	..
Incidence (per 1000 person-years)	3·1	..	9·7	..	3·9	..
Population attributable fraction	91·7%	..	5·9%	..	87·4%	..

Scenario 1: all lost to follow-up individuals (LTFU) are considered non-epileptic. Scenario 2: all LTFU individuals are considered epileptic. Scenario 3: LTFU individuals were handled using a missing-at-random model and multiple imputations. IR=incidence ratio. CMFL=community microfilarial load. Mf per snip=microfilariae per skin snip. MFD=individual microfilarial density. *p values are of each category versus age 10 years. †p value is male versus female. ‡p values are of each category versus CMFL of less than 38 mf per snip. §p values are of each category versus MFD of 0 mf per snip.

Table 4: **Sensitivity analysis and missing data assessment for the individual analysis**

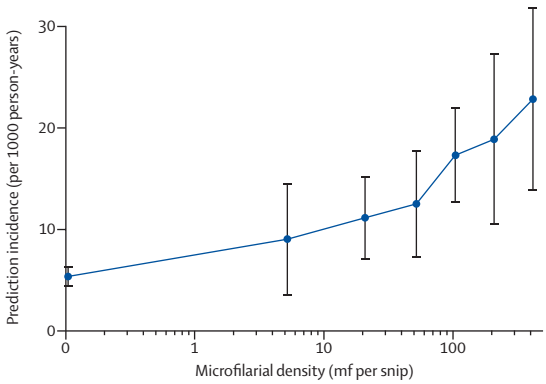

Figure 1: **Predicted incidences of epilepsy according to individual microfilarial densities**
Vertical lines indicate the 95% CI for each point of measure from the final model. X axis is presented in a logarithm scale. Mf per snip=microfilariae per skin snip.

Discussion

To our knowledge, this is the first cohort study investigating the association between onchocerciasis infection at a young age and epilepsy later in life. The patients identified as suspected cases of epilepsy using the five-item questionnaire included individuals with epilepsy and with provoked seizures. However, an evaluation of the questionnaire in neighbouring villages where all individuals identified as suspected cases of epilepsy were subsequently examined by a neurologist showed that 88% of these individuals had epilepsy (data not shown). Given this high positive predictive value, as well as the results of our sensitivity analyses, we believe that this study provides strong evidence that the level of individual infection with *O volvulus* during childhood is associated with an increased risk of developing epilepsy later in life. This temporal relationship substantiates results obtained from cross-sectional studies showing that the prevalence of onchocerciasis is significantly associated with that of epilepsy at the community level, and that the frequency and intensity of infection are higher in people with epilepsy than in sex-matched and age-matched controls. Even if the 95% CIs of the incidences for the different MFD categories are fairly wide, our results indicate that the association between baseline MFD and the risk of epilepsy is strong, with high incidence ratio, and that the dose–response relationship is also strong. Our findings are also consistent with observations suggesting that the prevalence of epilepsy in onchocerciasis foci decreases after distributions of ivermectin, a drug that reduces rapidly and for several months the MFD of *O volvulus*;[17,18] furthermore, mass treatment with ivermectin was associated with a decrease in the incidence of epilepsy in

neighbouring villages (authors' unpublished data). These arguments support a causal relationship between the intensity of infection with *O volvulus* in childhood and the subsequent occurrence of epilepsy. Further similar studies, including neurological confirmation and classification according to the clinical definitions of the International League Against Epilepsy,[19] are needed in other endemic areas to substantiate these results.

The biological plausibility of onchocerciasis-associated epilepsy is yet to be demonstrated. The physiopathogenic mechanisms are not known, but several hypotheses can be proposed. Although most studies did not find any microfilariae in the CSF,[20] epilepsy might result from a direct effect of microfilarial penetration into the CNS, and at least three entry routes are possible. First, although microfilariae are mainly found in the skin, they have been repeatedly found in the blood (appendix, p 8), and associations between the frequency and density of microfilariae in skin and in blood have been reported, both at individual and community levels.[21,22] Therefore, parasite entry into the CNS through the blood circulation is plausible. Microfilariae circulating in the arteries of the subarachnoid space could pass to the arteries penetrating into the brain parenchyma and then either stay there without crossing the blood–brain barrier or cross it to reach the perivascular space; these microfilariae could also secondarily cross the glia limitans and reach the brain parenchyma, where the presence of blood-dwelling *Loa loa* microfilariae has been registered (appendix p 8). Alternatively, *O volvulus* blood microfilariae could cross the blood–CSF barrier at the level of the choroid plexuses, circulate in the CSF of the subarachnoid space, and ultimately reach the brain parenchyma through the perivascular space. A second possible entry route to the CNS is the lymphatic system, which might be the usual place where the microfilariae live in the dermis.[23] *O volvulus* microfilariae have been found in the lymph nodes[24] and in the lymphatic vessels of onchocercal nodules.[25] Microfilariae could penetrate into the CSF through the lymphatic system of the olfactory mucosa of the cribriform plate, in the ethmoid bone (appendix p 8), or through the lymphatic system of the dura mater,[26] and reach the CSF or the vessels of the brain parenchyma. Finally, microfilariae could penetrate into the brain through the optic nerve, where they have been repeatedly found.[27,28]

In addition to the direct effects of microfilariae in the brain parenchyma, indirect mechanisms could also explain the association between onchocerciasis and epilepsy. Autoimmune mechanisms involving molecular mimicry between *O volvulus* antigens and host antigens (leiomodin-1) have been proposed to explain the development of nodding syndrome,[29] a specific type of epilepsy that is particularly frequent in onchocerciasis foci of east Africa, but which also exists in the region of this study (personal observations). However, this hypothesis still needs to be substantiated, and the strong dose–response relationship found during this study supports a direct mechanism in which the intensity of infection with *O volvulus* has the main role.

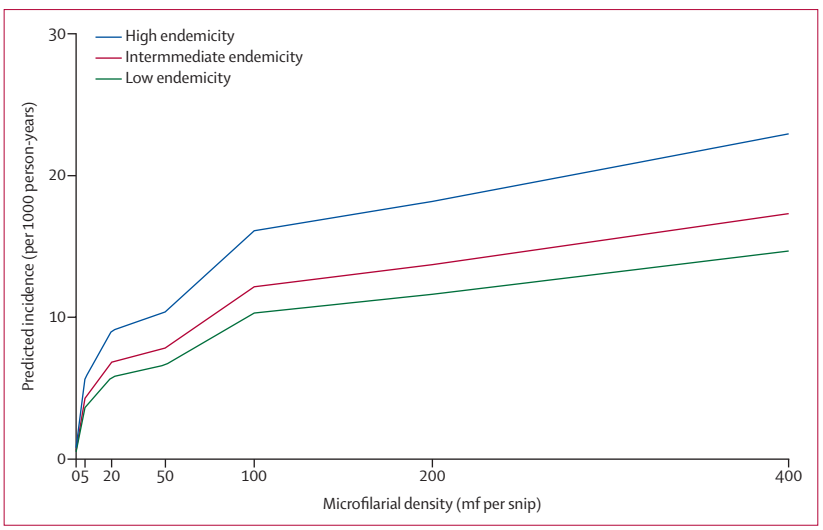

Figure 2: **Predicted incidences of epilepsy according to both individual microfilarial densities and the endemicity level in the village of residence**
Mf per snip=microfilariae per skin snip.

Many studies have shown that *O volvulus* microfilariae can invade blood and urine (appendix p 8) and CSF after antifilarial treatment with diethylcarbamazine[23,30,31] or ivermectin, or spontaneously. *O volvulus* microfilariae and adult worms secrete enzymes necessary for their migration to the host tissues,[32] and these enzymes might help the microfilariae to cross the blood–brain barrier. Should seizures be due to the presence of microfilariae in the brain, it would be important to assess whether seizures are due to live or moribund parasites (causing provoked seizures) or due to dead parasites (causing unprovoked seizures). Genetic factors might explain why some children with a high MFD develop epilepsy, whereas others do not. General concomitant viral, bacterial, or parasitic infections, some of which are particularly frequent in children, as well as immunological aspects related to individual genetic factors, might facilitate the pathogenic processes. Alternatively, we cannot exclude the possibility that high *O volvulus* MFD increases the pathogenicity of other epileptogenic infectious agents.

Our analyses suggest that the duration of follow-up did not influence the risk of an individual being identified as a suspected case of epilepsy (appendix, p 3). This result suggests that either first seizure episodes are distributed at random across follow-up, regardless of the individual's age, or that the probability of first seizure occurs within a narrow age range for most individuals. The observation that most of the first epileptic episodes in the study area occurred in individuals aged 10–15 years supports the second hypothesis. Therefore, the occurrence of epilepsy would not be caused by cumulative high levels of infection with *O volvulus*, but by a high MFD associated with an acute event during childhood.

Because suspected cases of epilepsy could not be confirmed by detailed neurological investigations, the definition of suspected case of epilepsy used could be a limitation of our study. An evaluation of the five-item questionnaire suggested that it would have 95·1% sensitivity and 65·6% specificity (appendix, p 2). However, it seems unlikely that the proportion of false positive suspected cases of epilepsy would differ between the MFD groups. Furthermore, the number of suspected cases of epilepsy in the MFD group with 0 mf per snip (only one of 165) reassured us that there was little, if any, bias related to performance of the questionnaire. Other possible causes of epilepsy were not included in our analyses, but the strength of the relationship between MFD and suspected cases of epilepsy, the dose–effect response, and the fact that this association was previously not influenced by other classic risk factors,[12] seems to support this decision.

In conclusion, our cohort study brings new arguments supporting a causal relationship between *O volvulus* microfilariae and the subsequent development of epilepsy. Although autoimmune mechanisms are possible, the dose–response relationship that we identified in our study supports the hypothesis of a direct effect of the parasite. However, we cannot exclude the possibility of a concomitant direct and indirect effect of the parasite. After many years of distribution of ivermectin, *O volvulus* infection levels have dropped in most of the onchocerciasis foci, associated with a sharp decrease in the incidence of epilepsy, further supporting the conclusion of a causal relationship between *O volvulus* infection and epilepsy (appendix, p 8). *O volvulus* transmission is still continuing at high levels in some areas, requiring intensified efforts to reduce the prevalence of infection and individual microfilariae loads. In such areas, the results of our study could be used to sensitise populations to take ivermectin. Given that our study showed that infection at the age of 5 years already constitutes a risk of developing epilepsy, studies to support regulatory registration of ivermectin for treatment of children younger than 5 years should be encouraged.

Contributors

CBC and MB conceived and designed the study. MB collected the baseline information (1991–93). CBC, HCN-D, AKN, CGL-N, CB, A-CZ-KB, JK, RC, and MB did the survey in 2017. CBC developed, performed, and interpreted the statistical analyses and wrote the first version of the manuscript. All authors critically reviewed and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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References

1 Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. *Epilepsia* 2010; **51:** 883–90.

2 Newton CR, García HH. Epilepsy in poor regions of the world. *Lancet* 2012; **380:** 1193–201.

3 Ba-Diop A, Marin B, Druet-Cabanac M, Ngoungou EB, Newton CR, Preux P-M. Epidemiology, causes, and treatment of epilepsy in sub-Saharan Africa. *Lancet Neurol* 2014; **13:** 1029–44.

4 Ngugi AK, Kariuki SM, Bottomley C, Kleinschmidt I. Incidence of epilepsy: a systematic review and meta-analysis. *Neurology* 2011; **77:** 1005–12.

5 Casis Sacre G. El síndrome epiléptico y sus relaciones con la oncocercosis. *Bol Salub Hig (Mexico)* 1938; **1:** 11–31.

6 Kaiser C, Kipp W, Asaba G, Mugisa C, Kabagambe G. The prevalence of epilepsy follows the distribution of onchocerciasis in a west Ugandan focus. *Bull World Health Organ* 1996; **74:** 361.

7 Boussinesq M, Pion SDS, Demanga-Ngangue, Kamgno J. Relationship between onchocerciasis and epilepsy: a matched case-control study in the Mbam Valley, Republic of Cameroon. *Trans R Soc Trop Med Hyg* 2002; **96:** 537–41.

8 Dozie INS, Onwuliri COE, Nwoke BEB, et al. Onchocerciasis and epilepsy in parts of the Imo river basin, Nigeria: a preliminary report. *Public Health* 2006; **120:** 448–50.

9 Druet-Cabanac M, Boussinesq M, Dongmo L, Farnarier G, Bouteille B, Preux PM. Review of epidemiological studies searching for a relationship between onchocerciasis and epilepsy. *Neuroepidemiology* 2004; **23:** 144–49.

10 Pion SDS, Kaiser C, Boutros-Toni F, et al. Epilepsy in onchocerciasis endemic areas: systematic review and meta-analysis of population-based surveys. *PLoS Negl Trop Dis* 2009; **3:** e461.

11 Kaiser C, Pion SDS, Boussinesq M. Case-control studies on the relationship between onchocerciasis and epilepsy: systematic review and meta-analysis. *PLoS Negl Trop Dis* 2013; **7:** e2147.

12 Kamuyu G, Bottomley C, Mageto J, et al. Exposure to multiple parasites is associated with the prevalence of active convulsive epilepsy in sub-Saharan Africa. *PLoS Negl Trop Dis* 2014; **8:** e2908.

13 Ngugi AK, Bottomley C, Kleinschmidt I, et al. Prevalence of active convulsive epilepsy in sub-Saharan Africa and associated risk factors: cross-sectional and case-control studies. *Lancet Neurol* 2013; **12:** 253–63.

14 Colebunders R, Njamnshi AK, van Oijen M, et al. Onchocerciasis-associated epilepsy: from recent epidemiological and clinicalfindings to policy implications. *Epilepsia Open* 2017; **2:** 145–52.

15 Kamgno J, Pion SDS, Boussinesq M. Demographic impact of epilepsy in Africa: results of a 10-year cohort study in a rural area of Cameroon. *Epilepsia* 2003; **44:** 956–63.

16 Diagana M, Preux PM, Tuillas M, Ould Hamady A, Druet-Cabanac M. Dépistage de l'épilepsie en zones tropicales: validation d'un questionnaire en Mauritanie. *Bull Soc Pathol Exot* 2006; **99:** 103–07.

17 Kipp W, Burnham G, Kamugisha J. Improvement in seizures after ivermectin. *Lancet* 1992; **340:** 789–90.

18 Levick B, Laudisoit A, Tepage F, et al. High prevalence of epilepsy in onchocerciasis endemic regions in the Democratic Republic of the Congo. *PLoS Negl Trop Dis* 2017; **11:** e0005732.

19 Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: practical clinical definition of epilepsy. *Epilepsia* 2014; **55:** 475–82.

20 König R, Nassri A, Meindl M, et al. The role of *Onchocerca volvulus* in the development of epilepsy in a rural area of Tanzania. *Parasitology* 2010; **137:** 1559–68.

21 Fain A, Vandepitte J, Wery M. Microfilarémie par *Onchocerca volvulus*. *Ann Soc Belg Med Trop* 1974; **54:** 121–27.

22 Fuglsang H, Anderson J. Microfilariae of *Onchocerca volvulus* in blood and urine before, during, and after treatment with diethylcarbamazine. *J Helminthol* 1974; **48:** 93–97.

23 Duke BO, Vincelette J, Moore PJ. Microfilariae in the cerebrospinal fluid, and neurological complications, during treatment of onchocerciasis with diethylcarbamazine. *Tropenmed Parasitol* 1976; **27:** 123–32.

24 Connor DH, Morrison DE, Kerdel-Vegas F, et al. Onchocercal dermatitis, lymphadenitis, and elephantiasis in the ubangi territory. *Human Pathology* 1970; **1:** 553–79.

25 Attout T, Hoerauf A, Dénécé G, et al. Lymphatic vascularisation and involvement of lyve-1+ macrophages in the human onchocerca nodule. *PLoS One* 2009; **4:** e8234.

26 Louveau A, Smirnov I, Keyes TJ, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature* 2015; **523:** 337–41.

27 Rodger FC. The pathogenesis and pathology of ocular onchocerciasis. *Am J Ophthalmol* 1960; **49:** 104–35.

28 Paul EV, Zimmerman LE. Some observations on the ocular pathology of onchocerciasis. *Human Pathology* 1970; **1:** 581–94.

29 Johnson TP, Tyagi R, Lee PR, et al. Nodding syndrome may be an autoimmune reaction to the parasitic worm *Onchocerca volvulus*. *Sci Transl Med* 2017; **9:** eaaf6953.

30 Mazzotti L. Presencia de microfilarias de *Onchocerca volvulus* en el liquido cefalorraquideo de enfermos tratados con hetrazan. *Rev Inst Salubr Enferm Trop* 1959; **19:** 1–5.

31 Bureau JP, Nozais JP, Botreau-Roussel Y. Lethal coma in the course of notezine treatment and with the presence of microfilaria of *Onchocerca volvulus* in the cerebrospinal fluid. *Nouv Presse Med* 1976; **5:** 2807 (in French).

32 Haffner A, Guilavogui AZ, Tischendorf FW, Brattig NW. *Onchocerca volvulus*: microfilariae secrete elastinolytic and males nonelastinolytic matrix-degrading serine and metalloproteases. *Exp Parasitol* 1998; **90:** 26–33.