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BRIEF REPORT

A Second Population-Based Cohort Study in Cameroon Confirms the Temporal Relationship Between Onchocerciasis and Epilepsy

Cédric B. Chesnais,[1] Charlotte Bizet,[1,2] Jérémy T. Campillo,[1] Wepnyu Y. Njamnshi,[3,4] Jean Bopda,[2] Philippe Nwane,[2] Sébastien D. Pion,[1] Alfred K. Njamnshi,[3,4] and Michel Boussinesq[1]

[1]UMI 233, Institut de Recherche pour le Développement (IRD), Université Montpellier, INSERM Unité 1175, Montpellier, France, [2]Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Yaoundé, Cameroon, [3]Neurology Department, Central Hospital/Faculty of Medicine & Biomedical Sciences, The University of Yaoundé I, (FMBS-UYI), Yaoundé, Cameroon, and [4]Brain Research Africa Initiative, BRAIN, Geneva, Switzerland/Yaoundé, Cameroon

To confirm our earlier evidence of a temporal and dose–response relationship between onchocerciasis and epilepsy, we conducted another cohort study in a different setting in Cameroon. Individuals whose *Onchocerca volvulus* microfilarial density (*Ov*-MFD) was measured in 1992–1994 when they were children were revisited in 2019 to determine if they acquired epilepsy. With reference to individuals with no microfilariae in 1992–1994, the relative risks of acquiring epilepsy were 0.96, 2.76, 3.67, and 11.87 in subjects with initial *Ov*-MFD of 1–7, 8–70, 71–200, and > 200 microfilariae per skin snip, respectively. This study further demonstrates reproducibility using the Bradford Hill's criteria for causality.

Keywords. onchocerciasis; epilepsy; causal relationship; Africa; cohort.

Eighty percent of the 50–70 million people with epilepsy (PWE) worldwide are found in low- and middle-income countries [1, 2]. This over-representation of PWE in the general population is particularly patent in Central Africa, where the prevalence is estimated at 59.7 per 1000 people [3]. Furthermore, a meta-analysis showed that the median incidence rate of epilepsy worldwide is 50.4 per 100 000 persons-years, whereas values recorded in Sub-Saharan Africa range between 64 and 187 per 100 000 person-years [3].

A possible association between onchocerciasis and epilepsy was originally suggested in 1938 in Mexico [4]. Several studies and meta-analyses subsequently demonstrated a significant relationship between the 2 diseases [5, 6], even after adjusting for other risk factors and infections [7, 8]. Consequently, the concept of onchocerciasis-associated epilepsy (OAE) was proposed, and in 2015, 381 000 people were estimated to have OAE [9].

A longitudinal study conducted in 2017 in the Mbam valley onchocerciasis focus of Cameroon demonstrated that the incidence of epilepsy was positively correlated with the intensity of *Onchocerca volvulus* infection at a young age (5–10 years old) [10]. This first cohort study provided evidence for 2 of the main Bradford Hill criteria, supporting causality between onchocerciasis and epilepsy (temporality and biological gradient) but had to be replicated in another setting to provide evidence for another criterion, namely consistency (reproducibility). In the present study, we used a design similar to that used in the Mbam valley to evaluate whether the level of infection with *O. volvulus* measured in 1992–1994 in children aged 5–15 years living in the Lékié Division (Center Region, Cameroon) was associated with an increased risk of subsequently developing epilepsy.

METHODS

Initial Parasitological Surveys and Selection of Subjects for the 2019 Survey

Between 1992 and 1994, parasitological surveys were conducted in 18 villages of the Lékié Division to measure the levels of *O. volvulus* infection in individuals aged ≥5 years (Table 1; Supplementary Figure 1). Two skin snips were collected from each volunteer using a Holth-type corneoscleral punch and incubated in saline at room temperature for 24 hours. Emerged microfilariae (mf) were counted using a microscope, and the individuals' microfilarial density (MFD), expressed as mf per snip, were calculated using the arithmetic mean of the counts. Community microfilarial load (CMFL), defined as the Williams geometric mean of the MFD in subjects aged ≥20 years, was calculated for each village. In addition, standardized thick blood smears were prepared between 10:00 and 16:00 to measure the participants' *Loa loa* and *Mansonella perstans* MFD (mf/mL). All 18 communities surveyed in 1992–1994 were re-visited in 2019. Since the average age of first seizure in subjects with OAE is between 10 and 14 years old [11, 12], we sought information for all those 1258 individuals who were 5–15 years old during the baseline surveys.

Evaluation and Definition of Epilepsy

In November 2019, we investigated, with the help of key informants (village authorities, long-standing residents, health workers), whether the selected subjects were still alive and which ones had developed epilepsy. Once identified, we visited, with

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Correspondence: Michel Boussinesq, MD, PhD, UMI 233—TransVIHMI, IRD, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France (michel.boussinesq@ird.fr).

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Table 1. Data by Village

Health District	Village	Subjects Targeted	Subjects Retrieved in 2019	No. (%) SR With *O. volvulus* mf[a]	CMFL[a]	*Loa loa* prevalence[a]	No. (%) of SR with *L. loa* mf[a]	*Mansonella perstans* prevalence[a]	No. (%) SR with *M. perstans*[a]	PY	No. SCE	IR (per 100 000 PY)	95% CI	CDTI Start Year
Ebebda	Djouniat	40	37	34 (91.9)	32.4	13.1	1 (2.7)	30.3	2 (5.4)	972.2	1	102.9	14.5–730.2	1999
Ebebda	Eyene	85	73	56 (76.7)	61.8	10.3	1 (1.4)	17.4	2 (2.7)	1981.2	0	0.0	N.A.	1999
Ebebda	Mbenega	70	50	47 (94.0)	53.1	4.0	1 (2.0)	8.0	3 (6.0)	1208.7	10	827.3	445.2–1537.6	1999
Ebebda	Nega Lendong	155	111	90 (81.1)	29.3	21.1	8 (7.2)	10.2	5 (4.5)	2873.6	7	243.6	116.1–511.0	1999
Elig Mfomo	Elig Mfomo	31	23	11 (47.8)	2.5	31.2	4 (17.4)	32.6	2 (8.7)	573.9	2	348.5	57.2–1393.4	1999
Evodoula	Nkolakok	42	35	26 (74.3)	17.6	27.3	2 (5.7)	28.1	2 (5.7)	864.0	1	115.7	16.3–821.6	1994
Evodoula	Nkolassa	50	26	24 (92.3)	53.8	15.7	2 (7.7)	38.6	6 (23.1)	612.3	4	653.3	245.2–1740.7	1994
Evodoula	Nkolmeyos I	35	27	24 (88.9)	118.4	16.7	1 (3.7)	40.5	5 (18.5)	663.9	1	150.6	21.2–1069.3	1994
Monatélé	Nkongmessa	42	23	21 (91.3)	81.3	9.6	1 (4.4)	4.1	0	449.1	10	2226.5	1198.0–4138.1	1994
Obala	Nkolfep	82	51	4 (7.8)	0.3	31.9	7 (13.7)	3.5	0	1259.6	1	79.4	11.2–563.6	1999
Okola	Ayos	81	73	35 (48.0)	5.7	24.9	11 (15.1)	31.4	6 (8.2)	1847.8	0	0.0	N.A.	1999
Sa'a	Lebamzip I	46	27	4 (14.8)	1.3	33.1	2 (7.4)	3.1	0	729.7	0	0.0	N.A.	1999
Sa'a	Nkolbogo I	70	65	26 (40.0)	3.8	25.8	5 (7.7)	28.1	10 (15.4)	1735.5	0	0.0	N.A.	1999
Sa'a	Nkolbogo II	63	58	38 (65.5)	22.8	26.1	8 (13.8)	54.5	29 (50.0)	1541.5	2	129.7	32.4–518.8	1999
Sa'a	Nkolebassimbi	73	53	40 (75.5)	24.8	13.4	1 (1.9)	11.4	2 (3.8)	1419.1	1	70.5	9.9–500.3	1999
Sa'a	Nkolntsa	104	44	13 (29.6)	2.3	28.5	6 (13.6)	8.0	0	908.0	10	1101.3	592.6–2046.9	1999
Sa'a	Nkolossang	62	47	10 (21.3)	3.3	26.4	5 (10.6)	18.2	3 (6.4)	1206.0	0	0.0	N.A.	1999
Sa'a	Ntsan Mendouga	127	99	52 (52.5)	10.8	18.2	6 (6.1)	13.8	3 (3.0)	2441.3	3	112.9	39.6–381.0	1999

Abbreviations: CDTI, start year of community-directed treatment with ivermectin; CMFL, *O. volvulus* community microfilarial load (in mf/snip) in the village during the baseline parasitological survey; IR, incidence rate (number of cases per PY); Ov, *Onchocerca volvulus*; PY, person-years; SCE, suspected cases of epilepsy (number); SR, Subjects Retrieved.

[a]Assessed in 1992–1994.

the assistance of local health workers or local authorities, the household of each selected individual or that of their relatives. If the targeted person was not at home, we asked his/her relatives whether he/she was still alive. If the subject had died, the year of death was recorded. For all individuals, a standardized 5-item questionnaire [13] was used to identify "suspected cases of epilepsy" (SCE). SCE was identified when a positive answer was given to at least 1 of the 5 questions. Interviewers had no information on the individuals' MFD measured during the initial parasitological survey. When no information could be obtained from the families, we used the responses from the key informants to define the SCE status. This study was approved by the Cameroon National Ethics Committee for Research in Human Health (registration number 2018/12/1123/CE/CNERSH/SP).

Statistical Analyses

The variable of interest was SCE status. Independent variables were gender, age during the initial survey (5, 6–7, 8–9, 10–11 [reference group], 12–13, or 14–15 years), MFD (0 [reference group], 1–7, 8–70, 71–200, and > 200 mf/snip), the presence of blood *L. loa* mf (negative vs positive), the presence of *M. perstans* mf, CMFL measured in 1992–1994 in the subject's village of residence (<4, 4–19, 20–29, and ≥30 mf/snip), and the start year of community-directed treatment with ivermectin (CDTI) for onchocerciasis control in the health district (HD; 1994 or 1999). HDs are health administrative units in charge of the implementation of health programs.

Data (individual duration of follow-up) concerning individuals who were not identified as SCE and who died between the initial survey and 2019 were censored by the year of death if it was known, or at half-time of the follow-up period if it was not known. Data concerning the SCE were censored at half-time of the follow-up period if the patient was alive or at half-time of the period between baseline and the year of death if declared dead. Incidence rates (IRs) were estimated by dividing the number of SCE by the total number of person-years of follow-up. Then, in order to assess individual risk factors associated with SCE (incidence rate ratios [IRRs]), we performed a multivariate Poisson regression model including all the independent variables mentioned above.

All possible and relevant interactions as well as random effects on the HD were assessed using likelihood ratio tests. All analyses were performed with Stata (version 14.0).

RESULTS

Population Interviewed and Incidence Rates

In 2019, information on SCE could be obtained for 922 of the 1258 targeted subjects (73.3%). The mean follow-up period for these 922 subjects was 25.2 years, and the number of person-years of follow-up was 23 287. Fifty-three SCE were identified, including 45 through questionnaires applied directly to the person or his/her relatives, and 8 were identified through questionnaires applied to key informants. The overall IR of epilepsy was 53/23 287 (227.6 per 100 000 person-years). The IR increased gradually with the initial MFD, with values ranging from 117.8 per 100 000 persons-years for individuals without skin mf to 952.8 per 100 000 persons-years for those with an MFD >200 mf/snip ($P < .0001$) (Table 2).

Individual Risk Factors Associated With SCE

The risk of being identified as an SCE in 2019 was higher for those who were 5 years old during the initial surveys than for the 10-year-olds (adjusted IRR [aIRR], 3.60; $P < .0001$). There was no difference between genders or between CMFL categories (after adjustment for individual MFD). The risk increased gradually with the individual MFD: aIRRs for individuals with 1–7, 8–70, 71–200, and >200 mf/snip were 0.96 ($P = .937$), 2.76 ($P = .017$), 3.67 ($P < .001$), and 11.87 ($P < .001$), respectively. No significant association was found between SCE status and presence of *L. loa* or *M. perstans* microfilaremia, or start year of CDTI. No interactions between the covariates were found. Inclusion of a random effect at the HD level ($P = .090$) did not affect the strength and significance of the effect of the MFD on SCE status.

DISCUSSION

This study confirms the results obtained in the neighbouring Mbam division [10]: a temporal relationship between onchocerciasis and epilepsy and a dose–effect relationship with a risk of developing epilepsy increasing gradually with the MFD during childhood. This study also confirms the absence of any gender effect but an increased risk for the younger children, all other parameters being equal. Lowering the minimal age of inclusion in CDTI (presently 5 years) should be considered.

Considering that all villages in a given HD benefitted from similar CDTI-related activities, the random effect was evaluated at the HD level (not the village level). As this random effect was close to significance, the risk of developing epilepsy could vary slightly between the HDs.

Unexpectedly, the start year of CDTI was not associated with SCE incidence. However, one should consider that the HDs where CDTI started in 1994 had the highest onchocerciasis endemicity levels. Although there is a possible lack of statistical power, one may consider that the first years of CDTI in these HDs had little impact on the intensity of transmission and/or that children were less treated than adults during the first years of CDTI implementation. As individual history of treatment is lacking, it is impossible to support one hypothesis or the other.

As in the previous study [10], our current study presents these possible biases: (i) the possible misclassification of SCE for individuals with a history of provoked seizures, (ii) the absence of control for other possible risk factors (eg, cysticercosis), (iii)

Table 2. Population Study, Follow-up Data, Incidence Rates, and Incidence Rate Ratios

		No. Examined in Baseline Study (% of Total)	No. With Information Collected in 2017 (% of Total)	SCE, No.	PY	IR (per 100 000 PY) (95% CI)	*P*[a]	Model Without Random-Effect		Model With a Random-Effect at HD Level	
								aIRR[b] (95% CI)	*P*	aIRR[c] (95% CI)	*P*
Total		1258	922	53	23 287.4	227.6 (173.9–297.9)					
Age, y	5	95 (7.6)	59 (6.4)	7	1445.8	484.1 (230.8–1015.6)	.380	3.60 (2.19–5.92)	<.0001	3.39 (1.25–9.14)	.016
	6–7	273 (21.7)	182 (19.7)	6	4673.4	128.4 (57.7–285.8)		0.83 (0.37–1.85)	.645	0.74 (0.26–2.09)	.572
	8–9	222 (17.7)	169 (18.3)	8	4285.5	186.7 (93.4–373.3)		1.03 (0.35–3.31)	.956	1.07 (0.42–2.74)	.889
	10–11	273 (21.7)	212 (23.0)	10	5410.4	184.8 (99.4–343.5)		Ref			
	12–13	235 (18.7)	182 (19.7)	12	4556.2	263.4 (149.6–463.8)		1.06 (0.54–2.07)	.868	0.99 (0.42–2.36)	.991
	14–15	160 (12.7)	118 (12.8)	10	2916.0	342.9 (184.5–637.4)		1.19 (0.436–3.86)	.775	1.17 (0.47–2.90)	.735
Sex	Female	637 (50.6)	449 (48.7)	25	11 216.6	222.9 (150.6–329.9)	.892	Ref		Ref	
	Male	621 (49.4)	473 (51.3)	28	12 070.8	232.0 (160.2–336.0)		0.75 (0.33–1.73)	.505	0.78 (0.44–1.38)	.388
CMFL, mf/snip	<4	395 (31.4)	257 (27.9)	13	6412.7	202.7 (117.7–349.1)	.004	Ref		Ref	
	4–19	250 (19.9)	207 (22.4)	4	5153.1	77.6 (29.1–206.8)		0.24 (0.04–1.36)	.106	0.29 (0.08–1.02)	.054
	20–29	291 (23.1)	222 (24.1)	10	5834.1	171.4 (92.2–318.6)		0.32 (0.07–1.47)	.143	0.20 (0.10–0.93)	.036
	≥30	322 (25.6)	236 (25.6)	26	5887.5	441.6 (300.7–648.6)		0.51 (0.09–2.86)	.447	0.49 (0.15–1.66)	.251
Ov in skin snip	Negative	524 (41.7)	367 (39.8)	11	9341.8	117.8 (65.2–212.6)	.004				
	Positive	734 (58.43)	555 (60.2)	42	13 945.6	301.2 (222.6–407.5)					
MFD, mf/snip	0	524 (41.7)	367 (39.8)	11	9341.8	117.8 (65.2–212.6)	<.001	Ref		Ref	
	1–7	241 (19.2)	182 (19.7)	4	4638.1	86.2 (32.4–229.8)		0.96 (0.38–2.43)	.937	0.93 (0.28–3.07)	.909
	8–70	245 (19.5)	178 (19.3)	10	4558.8	219.4 (118.0–407.7)		2.76 (1.20–6.35)	.017	2.66 (0.98–7.19)	.054
	71–200	129 (10.3)	100 (10.9)	7	2544.8	275.1 (131.1–577.0)		3.67 (1.86–7.21)	<.001	3.29 (1.01–10.71)	.048
	>200	119 (9.5)	95 (10.3)	21	2203.9	952.8 (621.3–1461.4)		11.87 (5.56–25.33)	<.001	11.60 (3.89–34.61)	<.0001
Loa loa, mf/mL	Negative	1077 (85.6)	796 (86.3)	47	20 046.7	234.5 (176.2–312.0)	.484	Ref		Ref	
	Positive	99 (7.9)	72 (7.8)	2	1848.7	108.2 (27.1–432.6)		0.46 (0.14–1.55)	.211	0.46 (0.11–1.96)	.292
	Missing	82 (6.5)	54 (5.9)	4	1391.9	287.4 (107.9–765.7)		1.46 (0.75–2.83)	.265	1.52 (0.48–4.77)	.476
Mansonella perstans, mf/mL	Negative	1074 (85.4)	788 (85.5)	43	19 857.7	216.5 (160.6–292.0)	.679	Ref		Ref	
	Positive	102 (8.1)	80 (8.7)	6	2037.7	294.4 (132.3–655.4)		0.93 (0.31–2.75)	.896	1.15 (0.45–2.97)	.770
	Missing	82 (6.5)	54 (5.9)	4	1391.9	287.4 (107.9–765.7)		N.A.		N.A.	
CDTI	1994	169 (13.4)	111 (12.0)	16	5589.4	617.9 (378.6–1008.6)	<.001	Ref		Ref	
	1999	1089 (86.6)	811 (98.0)	37	20 698.0	178.8 (129.5–246.7)		0.63 (0.11–3.80)	.617	0.49 (0.13–1.75)	.270

Abbreviations: aIRR, adjusted incidence rate ratio; CDTI, start year of community-directed treatment with ivermectin; CI, confidence interval; CMFL, community microfilarial load (in mf/snip); HD, health district; IR, incidence rate (number of cases per PY); MFD, individual microfilarial density (in mf/snip); Ov, *Onchocerca volvulus* mf; PY, persons-years; SCE, suspected cases of epilepsy (number).

[a] *P* values were calculated within each variable and assessed using the log-rank test for sex, skin snip positivity, *Loa loa* microfilariae positivity, *Mansonella perstans* positivity, CDTI, and the trends modified log-rank test for age, CMFL, and skin snip in 5 categories of variables.

[b] Multivariate logistic model with a cluster-robust standard errors to account for possible intra-community clustering.

[c] Multivariate logistic model with a random effect at the HD level.

and the absence of confirmation of epilepsy by detailed neurological examination of the SCE.

Nevertheless, this study provides significant new findings, investigating for the first time the possible role of *L. loa* and *M. perstans* in inducing epilepsy and demonstrating that it was not the case.

In conclusion, this study supports the previous findings and consequently adds the reproducibility principle to the Bradford Hill's criteria [14], supporting the causal nature of the relationship between *O. volvulus* infection and epilepsy.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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