PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Evolution of epilepsy prevalence and incidence in a Tanzanian area endemic for onchocerciasis, and the potential impact of community-directed treatment with ivermectin: a cross sectional study and comparison over 28 years
AUTHORS	Greter, Helena; Mbando, Bruno; Makunde, Williams; Mnacho, Mohamed; Matuja, William; Kakorozya, Advocatus; Suykerbuyk, Patrick; Colebunders, Robert

VERSION 1 – REVIEW

REVIEWER	Christoph Kaiser Pediatric Practice Balzenbergstr. 73 76530 Baden-Baden Germany
REVIEW RETURNED	12-May-2017

GENERAL COMMENTS	MAJOR COMMENT In the present form, the study proposal is suggesting to provide evidence of an effect of community directed treatment with ivermectin (CDTi) on reducing epilepsy prevalence and incidence in a defined population with high endemicity of onchocerciasis. This would require accurate information from the examined village populations about: a) The pre- and post-treatment data of epilepsy prevalence and incidence. This is sufficiently done with the baseline data available from the study of Rwiza et al (Epilepsia, 1992) and the planned repetition of an epilepsy survey 28 years later which is considered of yielding appropriate data for comparison (For details see comments on epilepsy methods). b) Proof of the actual implementation of the intervention (CDTi) and its extent/ intensity in the village population. This could be derived from i) pre- and ii) post-treatment endemicity data for onchocerciasis and iii) data of CDTi treatment coverage and the number of years of annual CDTi administration at village level. In this respect, the study protocol is appropriate to only assess post-treatment onchocerciasis endemicity at village level, but no sufficient information is provided on points b)i) and b)ii). (For details see minor comment N° 4). c) Assessment of relevant confounding factors. This would require the significance of possible alternative etiologic factors (other than onchocerciasis) in the village populationor the individual patients wth epilepsy. These factors in particular comprise traumatic causes at birth or from injuries and infections of the CNS (cerebral malaria,

	neurocystocercosis, meningitis, encephalitis). Although the authors mention the significance of confounding factors, the study protocol does not contain sufficient information on how these shall be examined and valued. Despite the mentioned limitations of the study protocol, the question of a possible reduction of epilepsy burden following CDTi in onchocerciasis endemic areas is of great interest. The authors are encouraged to carry out a study on this issue, by use of available earlier and present ecological information on epilepsy and onchocerciasis in the Mahenge focus, and combining this with a thorough update of the present situation. This would allow for a statement to what extent the study results are compatible with the hypothesis that there is an effect of CDTi of reducing epilepsy prevalence and incidence in onchocerciasis infested areas. It will however be difficult to provide sufficient evidence to fully demonstrate the assumed effect with the observations obtained in the study. ---------- MINOR COMMENTS 1. TITLE The title gives the impression that the planned study should be appropriate to conclude on the possible effect of CDTi on epilepsy frequency measures, but the expected results will probably not allow for this (see MAJOR COMMENTS, point b) and c). It is suggested to use a more cautious title (such as: "Evolution of epilepsy prevalence and incidence over 28 years in an Tanzanian area endemic for onchocerciasis, and the possible influence of CDTi"). 2.INTRODUCTION: page 4, line 79 - 88 (In Tanzania ... South Sudan.) Instead of refering to the article of Aall-Jilek of 1965 (see reference 9 of the manuscript) who described patients with head nodding seizures in Tanzania but did not propose epilepsy in these patients as a distinct syndrome, it would be more appropriate to cite the article of Winkler et al (Epilepsia 2008, 2008-2015). Nodding syndrome has also been confirmed in western Uganda (Kaiser et al. Am J Trop Med & Hyg 2015, 198-202) in an area non-contingent with those mentionned by the authors. 3. INTRODUCTION: page 7, line 167 - 170 (An epidemiological ... Cameroon.) Prior to the publication of Boussinesq et al. (TRSTM&H, 2002, 537-541), an epidemiological relationship between epilepsy and onchocerciasis was reported by Ovuga et al. (East Afr Med J 1992, 554-556) and Kipp et al (Lancet 1994, 183-184). 4. INTRODUCTION: page 8, line 190 - page 9, line 198 (In Tanzania ...estimated at 20.3%). In this paragraph, the authors present some general information on the assumed endemicity of onchocerciasis in the study area, Mahenge. Refering to Zoure et al. (Parasites & Vectors, 2014, 326, reference 25 of the manuscript), the authors mention a figure of 69% for onchocerciasis prevalence in the Mahenge area, not specifying if with this the prevalence of skin microlfilaria (mf) or that of palpable nodules is meant. In the publication of Zoure et al. (reference 25 of the submitted manuscript), which is constructing a map about the

	geographic distribution in the 20 African APOC countries, the mentioned figure of a prevalence of onchocerciasis in Mahenge (69%) cannot be found. A publication of 1990 from Tanzania (Mwaiko et al. Centr Afr J Med 1990, 94-96, not cited by the authors) is reporting a percentage of 58.6% positive for skin mf in a sample of 482 inhabitants of the Mahenge area. In the publication of Tekle AH et al. (Infect Dis Poverty 2016, 66; cited in the submitted manuscript as reference 28, although not in the specific context of onchocerciasis prevalence in Mahenge) the "maximum PRE-CONTROL prevalence of NODULES" in the villages examined in Mahenge with the REMO surveys in 1998 by the African Program for Onchocerciasis Control (APOC) was reported at 78.7%, and the maximum POST-CONTROL village prevalence of SKIN MF was found at 21.9% in 2009, after at least 7 years CDTi with a coverage of >60%. This is evidence of a sharp decline of the overall onchocerciasis endemicity in Mahenge which can be attributed to CDTi. However, the above mentioned summary figures of a high PRE-CONTROL onchocerciasis prevalence in Mahenge do not give sufficient evidence that PRE-CONTROL onchocerciasis prevalence was in the same range in the study villages assigned to the survey planned by H. Greter et al. (Mdindo, Vigoi, Misegezi and Matumbala village). Even in highly endemic areas, onchocerciasis prevalence varies widely depending on the local transmission pattern, the vector species and the proximity of a village to vector breeding sites. The efficacy of CDTi may also vary substantially between different villages depending on the compliance of the particular village community. For this reason, the conclusion that a reduced epilepsy prevalence and incidence in the study villages should be evidence of the effect of CDTi in the specific study villages is not warranted only on the ground of the mentioned summary data. This would need to be validated by immediate PRE-CONTROL data of the study villages. When the original files of the APOC database on the REMO surveys in Mahenge in 1998 were screened for the village names of the intended survey (Mdindo, Vigoi, Misegezi and Matumbala village) these names could not be traced (M. Boussinesq, personal comunication; consultation with permission of BMJ editorial office). Possibly, the actual PRE-CONTROL onchocerciasis prevalence in the study villages could be better estimated by use of a map of Mahenge indicating the local river system, the geographic GPS location of the study villages (Mdindo, Vigoi, Misegezi and Matumbala village) and the GPS locations of the villages of the 1998 REMO survey which could be retrieved from the APOC database. 5. METHODS AND ANALYSIS; Definition of epilepsy: page 14, line 314 - 326 The current ILAE definition of epilepsy which is intended for use in the study protocol of H. Greter et al. (Fisher et al. Epilepsia 2014, 475-482) is differing from that used by Rwiza et al. (Epilepsia 1992, 1051-1056) mainly because the revised definition in part considers patients with single seizures as cases of epilepsy. Formerly, two seizures were required as mandatory for the definition of epilepsy. As the authors mention, the use of the revised 2014 ILAE defintion is expected to result in epilepsy prevalence data (and even more incidence data) which will substantially deviate from results which would be obtained if the earlier definition was used. This will impede the comparability of baseline data of Rwiza et al. with those of the planned replicating survey. It is therefore suggested that in the planned study prevalence and

	incidence data are assessed and reported with both epilepsy definitions, the revised 2014-ILAE definition AND the earlier definition used by Rwiza et al. (Epilepsia 1992, 1051-1056). This would allow to compare the results of the updating survey with the baseline data of 1989, and also with data of possible future epilepsy studies relying on the effective 2014-ILAE definition.

REVIEWER	Dziedzom K. de Souza Noguchi Memorial Institute for Medical Research, University of Ghana
REVIEW RETURNED	13-Sep-2017

GENERAL COMMENTS	This study is a necessary and relevant work, given that treatment with ivermectin may help lower the incidence of epilepsy. However, the methodological issues are not clearly defined, thus reducing the importance of the work. Also, I suggest a thorough editing of the text for typos. MAJOR COMMENTS Abstract: 1. I am not sure if the section on ethics and dissemination is needed in the abstract. This should be removed. 2. "It will contribute to a better understanding of the linkage between the onset of epilepsy and NS in particular, onchocerciasis and CDTi. Comparing the epidemiological data on epilepsy from pre-CDTi and 20 years after its introduction will allow identifying a potential protective effect of ivermection on the onset of epilepsy" should be moved under the section on methods and analysis. The introduction is lengthy and can be significantly summarized. Kindly present a figure summarizing the study design. Also kindly use the STROBE checklist. Sample Size: Please present the formula for calculating the sample size. Table 4: How were the 7007 and 745 determined? These do not add up. Kindly double check these figures. Section under data management and analysis: There is the need to describe the statistics that will undertaken. I can see several scenarios here: For example; a. No Ivermectin treatment ------ Epilepsy b. Ivermectin treatment ---------- Epilepsy c. No Ivermectin treatment ------ No Epilepsy d. Ivermectin treatment ---------- No Epilepsy How will these various outcomes be taken into consideration in the final analysis? Would there be any models to assess the impact of the interventions? Statistics is not my strong point, but I could think of odds and risk ratios, protective effects of ivermectin against NS/epilepsy, etc... There is the need to put more thoughts into this. Children under 5 years are usually not treated with ivermectin. It would be useful to discuss the implications of this on the observations of epilepsy in the population. Also kindly factor this into the study design. For example, if more cases are observed in children 5 years and below, what would this mean for the study.

	The study design should also consider examining relationships between the number of ivermectin treatment taken by study participants and the outcome on NS/epilepsy. It would be useful to see/attach the questionnaire (or the 5 questions referred to in line 297), that will be used in identifying cases. Figure 1: I think a better figure caption could be presented. The current description is not adequate. MINOR COMMENTS: Line 32. "Suspected cases are invited for to neurological examination" I suggest to change this to "Suspected cases will be invited for neurological examination" Line 40. "and 20 years after its introduction". Is it 20 years or 27 years? The first bullet under the section on Strengths and limitations (line 48) indicates 27 years. Line 49. "Answers key questions" I suggest changing this to "The study answers key questions" Line 57. Correct "limited" to "limit" Line 73. delete "those" Line 74. Replace "also" with "other" Line 83 - 84. Revise sentence " ... and stunted growth in formerly normally developing children..." Line 87: suggest you insert the word "then" after since (Since then, an NS epidemic) Line 94: Correct first word to "anti-epileptic" Line 98: Evil spirits? Please correct. Line 130: "...years, until all the adult worms die". please revise sentence. Line 137: suggest changing "limitated" to "limited" Lines 219-221: The sentence is not clear. Please revise. Line 239: it would be useful to define the coverage being referred to. Is it geographic or therapeutic coverage? The coverage should also be addressed throughout the text. Line 241: "serological survey" Clearly state the serological method to be used. Line 257. "...subsistence agriculture, livestock breeding, and also mining is practiced" I suggest changing this to "...subsistence agriculture and livestock breeding. Mining is practiced" Line 258: "presented" delete.

	Line 265: Define LMICs Line 271: "... or such". Suggest replacing with "etc..." Line 282: change "optain" to "obtain" Line 286: 6'600. Please change to 6,600. Also, is this the sample size for the study or the population of the village. Please clarify this. The 6,600 does not add up to what is in Table 3. Please check. Line 293: Correct "workerw" Line 295 & 300: "The interview team..." Line 301; Correct "accompagnied" to "accompanied" Line 307: Suggest you delete medical doctor Line 330: "I part one". "In part one"? Line 342: "746". it is 745 in Table 4. Line 349: "all data collection forms are developed..." I suggest you change this to " all data collection forms will be developed..." Line 351: "Coordinator is assigned..." Please change to "coordinator will be assigned" Line 369: "optainded" change to "obtained"? Line 379: "withness" Correct to "witness" Line 413: "unreveal" Correct to "unravel" Line 414: "... an evidence base for to treatment..." Delete "for"

REVIEWER	Hugues Nana Djeunga Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Cameroon
REVIEW RETURNED	21-Sep-2017

GENERAL COMMENTS	The manuscript submitted by Greter and colleagues is a protocol whose main objective is to assess the impact of mass ivermectin administration against onchocerciasis on prevalence and incidence of epilepsy in Tanzania. This study is of high interest in its field since it provide guidelines while evaluating the association between onchocerciasis and epilepsy. The techniques or approaches presented in this protocol are clear and sufficiently detailed to allow implementation or replication. However, the authors need to address many points to improve the quality of their manuscript, and to make it smooth to readers. Point #1 The title should be slightly modify to reflect the objectives and the orientation of the paper; the authors should consider “impact” rather than “effect”.

	. Point #2 The paper need to be reorganized since many good ideas are developed but sometimes in wrong order; as a consequence, they seem to repeat many information. We would suggest to authors: o to choose either to structure their introduction (using titles and sub-titles) or to organize ideas into paragraphs; o to organize the first part of their introduction (before "Onchocerciasis, a treatable … elimination") in three paragraphs as follow: (i) definition and distribution of epilepsy, (ii) burden and stigma of epilepsy; (iii) etiology and hypothesis. So, they need to bring back line number (LN) 78-84 ("Epilepsy manifest … with the disease") to LN 74 (after "fully understood"; Move the sentence "Many of these … anti-parasitic treatment" (LN77-78) to LN 92 after "from epilepsy", at the end of page 4. The LN 93-102 should be move to LN 74 (page 4), after "living in LMICs". o the information on the situation of onchocerciasis and epilepsy in the study area (Page 8, LN 190, to page 9, LN 213) should be organized, reshaped and given in the section "Study site and population"; please consider "Study site and population" rather than "Study site and study population". o the objectives and specific objectives need to be reshaped and the justification section should be deleted; the authors should organize their introduction in such a way that the problem is well presented; o the method section also need to be reorganized; please consider the following organization: 1. Study area and population 2. Study design 1.1. Sample size 2.1. Data collection procedures (including epilepsy and onchocerciasis) 3. Data analysis 4. Ethical considerations (including Data storage and handling). Point #3 The authors declared that adverse events associated with ivermectin are "close to nil"; this is not true and the authors can refer to the paper "Ivermectine" by Boussinesq in 2005 to revise this. Rather than "close to nil", the authors should list the main and use the term "moderate" … Point #4 There is a hypothesis for sample size calculation (page 12, LN 279); why assuming whereas 2017 figures are given in the table 2 (Page 10) by the authors themselves. Point #5 Study design: Rather than nodule palpation to have a rough estimate of the prevalence, the authors should choose to use skin snipping. Indeed, nodule palpation was recommended as a rapid strategy to delineate areas needing mass treatments; however, the objective here is not to have just an idea of the situation of onchocerciasis but to have more precise data; doing skin snips will provide also information on the intensity of infection and community microfilarial load (CMFL) that can be helpful to further assess the relationship between epilepsy and onchocerciasis, in a context of mass ivermectin administrations

	Point #6 The statistical analysis is appropriate but may need some further details. Point #7 The authors declared that they will evaluate the impact of CDTI (please consider CDTI rather than CDTi throughout the text) on incidence of epilepsy but they didn't detailed how they will achieve this goal. They only focussed on prevalence. Point #8 In their discussion, the authors evoked potential factors that may explain the persistence of onchocerciasis post-MDA (page 17, LN 402-403); the authors should also simply consider that the disease can persist as a consequence of high level of transmission (activity of flies), and baseline data are therefore needed. Point #9 The authors may need to go through the manuscript and polish the language since there are numerous typos. Here are some examples: o Page 4, LN 70: consider "are reported" rather than "is reported" o Delete "also" (Page 4, LN 74) and add "as well" after "…trigger epilepsy" (Page 4, LN 75) o Page 4, LN 85: consider "in onchocerciasis" rather than "from onchocerciasis" o Page 4, LN 87: consider "A NS" rather than "Since, an NS" o Page 4, LN 90: delete "South Sudan" after "Mvolo" o Page 8, LN 174: consider "is still" rather than "still is" o Page 13, LN 293: consider "workers" rather than "workerw" o Page 18, LN 414: consider "…evidence base to …" rather than ""…evidence base for to …" o … There are so many typos and it would have been better to rewrite the manuscript.

VERSION 1 – AUTHOR RESPONSE

Editorial Requirements:

- Please revise your title to include the study design. This is the preferred format for the journal.

We have now adjusted the title to include the study design. Additionally, the title has been modified in response to Reviewer #1 feedback (see revised manuscript, clean version, lines 1 -3).

- Please revise the Strengths and Limitations section (after the abstract) to focus on the methodological strengths and limitations of your study.

We have now revised the 'Strength and Limitations' section and put the focus on the methodological strengths and limitations of our study (see revised manuscript, clean version, lines 51 – 70)

- Please ensure the manuscript is correctly formatted as per our guidelines for protocol articles: http://bmjopen.bmj.com/pages/authors/

We have now formatted our manuscript according to the guidelines of the journal. If any issue remains, please do not hesitate to contact us.

- Please include an Ethics and Dissemination section in the main text of the manuscript.

We now included an Ethics section in the main text (see revised manuscript, clean version, lines 355-372).

The dissemination strategy is presented in the Outlook section (see revised manuscript, clean version, lines 413– 415).
- Please include the dates for the study in the main text of the manuscript.
We have now included the study dates and duration in the main text (see revised manuscript, clean version, lines 208-209).

Reviewer #1
Reviewer Name: Christoph Kaiser
Institution and Country: Pediatric Practice, Balzenbergstr. 73 76530 Baden-Baden, Germany
Please state any competing interests: None declared

MAJOR COMMENT
In the present form, the study proposal is suggesting to provide evidence of an effect of community directed treatment with ivermectin (CDTi) on reducing epilepsy prevalence and incidence in a defined population with high endemicity of onchocerciasis.
This would require accurate information from the examined village populations about:
a) The pre- and post-treatment data of epilepsy prevalence and incidence. This is sufficiently done with the baseline data available from the study of Rwiza et al (Epilepsia, 1992) and the planned repetition of an epilepsy survey 28 years later which is considered of yielding appropriate data for comparison (For details see comments on epilepsy methods).
b) Proof of the actual implementation of the intervention (CDTi) and its extent/ intensity in the village population. This could be derived from i) pre- and ii) post-treatment endemicity data for onchocerciasis and iii) data of CDTi treatment coverage and the number of years of annual CDTi administration at village level. In this respect, the study protocol is appropriate to only assess post-treatment onchocerciasis endemicity at village level, but no sufficient information is provided on points b)i) and b)ii). (For details see minor comment N° 4).
We have now included detailed epidemiological data on pre -treatment and 12 years post-treatment endemicity, and also provide de according references (see revised manuscript, clean version, lines 228-233, references 47 and 33).

c) Assessment of relevant confounding factors. This would require the significance of possible alternative etiologic factors (other than onchocerciasis) in the village population or the individual patients with epilepsy. These factors in particular comprise traumatic causes at birth or from injuries and infections of the CNS (cerebral malaria, neurocystocercosis, meningitis, encephalitis). Although the authors mention the significance of confounding factors, the study protocol does not contain sufficient information on how these shall be examined and valued.
We thank reviewer #1 for raising this important point. In the method section we now mention" During interview questions will be asked to identify possible etiologic factors (other than onchocerciasis) such as traumatic causes at birth or head injuries and infections of the CNS (cerebral malaria, neurocysticercosis, meningitis, encephalitis)" (see revised manuscript, clean version, lines 254 – 256)
.
Despite the mentioned limitations of the study protocol, the question of a possible reduction of epilepsy burden following CDTi in onchocerciasis endemic areas is of great interest. The authors are encouraged to carry out a study on this issue, by use of available earlier and present ecological information on epilepsy and onchocerciasis in the Mahenge focus, and combining this with a thorough update of the present situation. This would allow for a statement to what extent the study results are compatible with the hypothesis that there is an effect of CDTi of reducing epilepsy prevalence and incidence in onchocerciasis infested areas. It will however be difficult to provide sufficient evidence to fully demonstrate the assumed effect with the observations obtained in the study.
We thank Reviewer #1 for acknowledging the importance of our planned research. In view of the limitation mentioned, and also initiated by Reviewer #1 and Reviewer #2, we have now adapted the title accordingly and replaced the term 'effect' by 'impact' which seems more appropriate to the

expected outcome of our study. Moreover in the discussion we now added a statement on the option of combining the CDTI with larviciding rivers, in case our study reveals an insufficient reduction of onchocerciasis reached by annual CDTI alone (see revised manuscript, clean version, lines 396-397)

MINOR COMMENTS

1. TITLE

The title gives the impression that the planned study should be appropriate to conclude on the possible effect of CDTi on epilepsy frequency measures, but the expected results will probably not allow for this (see MAJOR COMMENTS, point b) and c). It is suggested to use a more cautious title (such as: "Evolution of epilepsy prevalence and incidence over 28 years in an Tanzanian area endemic for onchocerciasis, and the possible influence of CDTi").

We thank Reviewer #1 for this most useful comment. We have now adjusted our title taking Reviewer #1 consideration into account. In addition, the title now also contains the study design. (see revised manuscript, clean version, lines 1-3)

2.INTRODUCTION: page 4, line 79 - 88 (In Tanzania ... South Sudan.)

Instead of refering to the article of Aall-Jilek of 1965 (see reference 9 of the manuscript) who described patients with head nodding seizures in Tanzania but did not propose epilepsy in these patients as a distinct syndrome, it would be more appropriate to cite the article of Winkler et al (Epilepsia 2008, 2008-2015). Nodding syndrome has also been confirmed in western Uganda (Kaiser et al. Am J Trop Med & Hyg 2015, 198-202) in an area non-contingent with those mentionned by the authors.

Indeed, we thank Reviewer #1 for this observation. We have now replaced reference 9 as suggested (see revised manuscript reference 11), and added the reference reporting NS from western Uganda (see revised manuscript reference 12).

3. INTRODUCTION: page 7, line 167 - 170 (An epidemiological ... Cameroon.)

Prior to the publication of Boussinesq et al. (TRSTM&H, 2002, 537-541), an epidemiological relationship between epilepsy and onchocerciasis was reported by Ovuga et al. (East Afr Med J 1992, 554-556) and Kipp et al (Lancet 1994, 183-184).

Again, we thank Reviewer #1 for pointing out these important references. We have now adapted the manuscript (see revised manuscript, clean version, lines 166-167) and included the two references suggested (see revised manuscript reference 22 and reference 23).

4. INTRODUCTION: page 8, line 190 - page 9, line 198 (In Tanzania ...estimated at 20.3%).

In this paragraph, the authors present some general information on the assumed endemicity of onchocerciasis in the study area, Mahenge. Refering to Zoure et al. (Parasites & Vectors, 2014, 326, reference 25 of the manuscript), the authors mention a figure of 69% for onchocerciasis prevalence in the Mahenge area, not specifying if with this the prevalence of skin microlfilaria (mf) or that of palpable nodules is meant. In the publication of Zoure et al. (reference 25 of the submitted manuscript), which is constructing a map about the geographic distribution in the 20 African APOC countries, the mentioned figure of a prevalence of onchocerciasis in Mahenge (69%) cannot be found. A publication of 1990 from Tanzania (Mwaiko et al. Centr Afr J Med 1990, 94-96, not cited by the authors) is reporting a percentage of 58.6% positive for skin mf in a sample of 482 inhabitants of the Mahenge area. In the publication of Tekle AH et al. (Infect Dis Poverty 2016, 66; cited in the submitted manuscript as reference 28, although not in the specific context of onchocerciasis prevalence in Mahenge) the "maximum PRE-CONTROL prevalence of NODULES" in the villages examined in Mahenge with the REMO surveys in 1998 by the African Program for Onchocerciasis Control (APOC) was reported at 78.7%, and the maximum POST-CONTROL village prevalence of SKIN MF was found at 21.9% in 2009, after at least 7 years CDTi with a coverage of >60%. This is

evidence of a sharp decline of the overall onchocerciasis endemicity in Mahenge which can be attributed to CDTi.
We thank Reviewer #1 for pointing this out and have now adjusted the manuscript with the numbers and references kindly recommended by the Reviewer #1 (see revised manuscript lines 229-233, references 33 and 48). The sub-section was moved to the 'Methods' section as recommended by Reviewer #3.

However, the above mentioned summary figures of a high PRE-CONTROL onchocerciasis prevalence in Mahenge do not give sufficient evidence that PRE-CONTROL onchocerciasis prevalence was in the same range in the study villages assigned to the survey planned by H. Greter et al. (Mdindo, Vigoi, Misegezi and Matumbala village). Even in highly endemic areas, onchocerciasis prevalence varies widely depending on the local transmission pattern, the vector species and the proximity of a village to vector breeding sites. The efficacy of CDTi may also vary substantially between different villages depending on the compliance of the particular village community. For this reason, the conclusion that a reduced epilepsy prevalence and incidence in the study villages should be evidence of the effect of CDTi in the specific study villages is not warranted only on the ground of the mentioned summary data. This would need to be validated by immediate PRE-CONTROL data of the study villages. When the original files of the APOC database on the REMO surveys in Mahenge in 1998 were screened for the village names of the intended survey (Mdindo, Vigoi, Misegezi and Matumbala village) these names could not be traced (M. Boussinesq, personal comunication; consultation with permission of BMJ editorial office). Possibly, the actual PRE-CONTROL onchocerciasis prevalence in the study villages could be better estimated by use of a map of Mahenge indicating the local river system, the geographic GPS location of the study villages (Mdindo, Vigoi, Misegezi and Matumbala village) and the GPS locations of the villages of the 1998 REMO survey which could be retrieved from the APOC database.
We are truly thankful to Reviewer #1 for this in-depth search for more precise pre-control onchocerciasis prevalence data from the villages selected for this study. Indeed, we cannot find pre-control prevalence data at village level. Yet, village selection was done due to two major criteria: 1) a high level of epilepsy prevalence in the Rwiza study; and 2) proximity to fast flowing rivers. Criteria 2 was defined as such as to take into account the lack of village-specific onchocerciasis prevalence. The proximity to fast flowing rivers and therewith to blackfly breeding sites underlines the potential for onchocerciasis transmission in the village. We are therefore confident that the villages selected for this proposed study are exposed to onchocerciasis and that the population might have had an elevated level of onchocerciasis prevalence in the pre-control period. An indicator for onchocerciasis transmission in the study villages are results presented from an entomological study by W. Häusermann from the 1960 who found a considerable number of blackflies collected from rivers in the study area contained infective L3 stage parasites in their heads. We now include this in the text (see revised manuscript, clean version, lines 319-322, reference 56)

5. METHODS AND ANALYSIS; Definition of epilepsy: page 14, line 314 - 326
The current ILAE definition of epilepsy which is intended for use in the study protocol of H. Greter et al. (Fisher et al. Epilepsia 2014, 475-482) is differing from that used by Rwiza et al. (Epilepsia 1992, 1051-1056) mainly because the revised definition in part considers patients with single seizures as cases of epilepsy. Formerly, two seizures were required as mandatory for the definition of epilepsy. As the authors mention, the use of the revised 2014 ILAE defintion is expected to result in epilepsy prevalence data (and even more incidence data) which will substantially deviate from results which would be obtained if the earlier definition was used. This will impede the comparability of baseline data of Rwiza et al. with those of the planned replicating survey.
It is therefore suggested that in the planned study prevalence and incidence data are assessed and reported with both epilepsy definitions, the revised 2014-ILAE definition AND the earlier definition used by Rwiza et al. (Epilepsia 1992, 1051-1056). This would allow to compare the results of the

updating survey with the baseline data of 1989, and also with data of possible future epilepsy studies relying on the effective 2014-ILAE definition.
This is a very valuable comment of Reviewer #1. We now mention that “For comparison, the ILAE epilepsy case definition valid in 1989 will be applied, which means that only cases with more than one seizure will be included in the comparison data analysis. Results obtained by applying the current ILAE definition will be analysed and presented seperately.” (see revised manuscript, clean version, lines 344-347)

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Reviewer #2
Reviewer Name: Dziedzom K. de Souza
Institution and Country: Noguchi Memorial Institute for Medical Research, University of Ghana
Please state any competing interests: None declared

This study is a necessary and relevant work, given that treatment with ivermectin may help lower the incidence of epilepsy. However, the methodological issues are not clearly defined, thus reducing the importance of the work. Also, I suggest a thorough editing of the text for typos.

MAJOR COMMENTS
Abstract:
1. I am not sure if the section on ethics and dissemination is needed in the abstract. This should be removed.
The BMJ Open guidelines for protocol papers demand a section on ‘ethics and dissemination’ in the abstract.

2. "It will contribute to a better understanding of the linkage between the onset of epilepsy and NS in particular, onchocerciasis and CDTi. Comparing the epidemiological data on epilepsy from pre-CDTi and 20 years after its introduction will allow identifying a potential protective effect of ivermection on the onset of epilepsy" should be moved under the section on methods and analysis.
We thank Reviewer #2 for pointing this out. We have now moved these two sentences from ‘Ethics and dissemination’ section to the ‘introduction’ section. Indeed both ideas represent the main objectives of the study and are therefore best placed in the ‘introduction’ section of the abstract (see revised manuscript, clean version, lines 25-27)

The introduction is lengthy and can be significantly summarized.
We fully agree with Reviewer #2. Yet, as epilepsy caused by onchocerciasis remains a controversial issue we feel the importance to provide the reader with sufficient background information about the topic and the reason why we have written the protocol. Nevertheless, we have re-organized the introduction and shortened the text considerably (see revised manuscript, clean version, ‘Introduction’).

Kindly present a figure summarizing the study design. Also kindly use the STROBE checklist.
We feel that the study design is now, thanks to the most useful comments of three Reviewers, well presented and described in the re-organised methodology section and have therefore decided to not further lengthen the manuscript with a second figure. The STROBE checklist is a most useful tool for reporting results of epidemiological studies. Here, we present a study protocol. The STROBE checklist will be completed with the publication of the study results.

Sample Size: Please present the formula for calculating the sample size.
The sample size calculation was done using OpenEpi: http://www.openepi.com/Menu/OE_Menu.htm

Table 4: How were the 7007 and 745 determined? These do not add up. Kindly double check these figures.
All numbers in all tables have been double checked. The number 7007, now in Table 2, refers to the population in the 5 most affected villages in 1989 . In table 4, the number 745 has been corrected, 722 is the number of children aged 7-10 years old we expect to be able to test for OV16 antibodies (80% of the estimated number of 900 children) (see revised manuscript, clean version, Table 4).

Section under data management and analysis: There is the need to describe the statistics that will undertaken. I can see several scenarios here: For example;
a. No Ivermectin treatment ------ Epilepsy
b. Ivermectin treatment ---------- Epilepsy
c. No Ivermectin treatment ------ No Epilepsy
d. Ivermectin treatment ---------- No Epilepsy
How will these various outcomes be taken into consideration in the final analysis? Would there be any models to assess the impact of the interventions? Statistics is not my strong point, but I could think of odds and risk ratios, protective effects of ivermectin against NS/epilepsy, etc... There is the need to put more thoughts into this.
Ivermectin treatment coverage, epilepsy prevalence and incidence and OV16 positivity rate among children 7-10 years old will be compared among villages
We follow the input from Reviewer #3 and we have now further developed the data analysis section (see revised manuscript, clean version, lines 339-354)

Children under 5 years are usually not treated with ivermectin. It would be useful to discuss the implications of this on the observations of epilepsy in the population. Also kindly factor this into the study design. For example, if more cases are observed in children 5 years and below, what would this mean for the study.
Epilepsy in children below the age of 5 is generally related to problems during or post delivery , or may have a genetic aetiology. The onset of epilepsy triggered by onchocerciasis generally is between the age of 5-20 years.
We now mention in the methodology that in every person with epilepsy we will determine the onset of the seizures and investigate possible causes of epilepsy such as: birth trauma, head injury, meningo-encephalitis, cerebral malaria, etc. (see revised manuscript, clean version, lines 253-256)

The study design should also consider examining relationships between the number of ivermectin treatment taken by study participants and the outcome on NS/epilepsy.
This would indeed be an additional desirable result of this study. Yet, since ivermectin intake is not documented at individual level during CDTI, the ivermectin intake can only be assessed by recall. It is a well-known fact that recall bias is increasing with regard to questions that go more than one year in the past. To obtain reliable results, we limit the data collection on the individual ivermectin intake to the last CDTI round.

It would be useful to see/attach the questionnaire (or the 5 questions referred to in line 297), that will be used in identifying cases.
We know provide the 5 question questionnaire in the annex (see revised manuscript, clean version, lines 609-638).

Figure 1: I think a better figure caption could be presented. The current description is not adequate.
We fully agree with Reviewer #3 and have now shortened and sharpened the figure caption by deleting the second sentence. The caption is now to-the-point (see revised Figure 1).

MINOR COMMENTS:

Line 32. "Suspected cases are invited for to neurological examination" I suggest to change this to "Suspected cases will be invited for neurological examination"
Manuscript adjusted as recommended (see revised manuscript, clean version, line 34)
Line 40. "and 20 years after its introduction". Is it 20 years or 27 years? The first bullet under the section on Strengths and limitations (line 48) indicates 27 years.
The epilepsy survey by Rwiza et al was performed 27 (28) years ago. CDTI was introduced 20 years ago. Manuscript adjusted as recommended (see revised manuscript, clean version, lines 27-29)
Line 49. "Answers key questions" I suggest changing this to "The study answers key questions"
Manuscript adjusted as recommended (see revised manuscript, clean version, line 50)
Line 57. Correct "limited" to "limit"
Manuscript adjusted as recommended (see revised manuscript, clean version, line 56)
Line 73. delete "those"
Done
Line 74. Replace "also" with "other"
Manuscript adjusted as recommended (see revised manuscript, clean version, line 76)
Line 83 - 84. Revise sentence " ... and stunted growth in formerly normally developing children..."

Line 87: suggest you insert the word "then" after since (Since then, an NS epidemic)
Solved (see revised manuscript, clean version, line 85)
Line 94: Correct first word to "anti-epileptic"
Manuscript adjusted as recommended (see revised manuscript, clean version, line 92)
Line 98: Evil spirits? Please correct.
Manuscript adjusted as recommended (see revised manuscript, clean version, line 94)
Line 130: "...years, until all the adult worms die". please revise sentence.
Manuscript adjusted as recommended (see revised manuscript, clean version, lines 125-126)
Line 137: suggest changing "limitated" to "limited"
Manuscript adjusted as recommended (see revised manuscript, clean version, line 132)
Lines 219-221: The sentence is not clear. Please revise.

Line 239: it would be useful to define the coverage being referred to. Is it geographic or therapeutic coverage? The coverage should also be addressed throughout the text.
It should be treatment coverage. We now mention this in the text.
Line 241: "serological survey" Clearly state the serological method to be used.
Manuscript adjusted as recommended (see revised manuscript, clean version, lines 321-323)
Line 257. "...subsistence agriculture, livestock breeding, and also mining is practiced" I suggest changing this to "...subsistence agriculture and livestock breeding. Mining is practiced"
This sentence has been deleted.
Line 258: "presented" delete.
Solved
Line 265: Define LMICs
The abbreviation is introduced in the Introduction, line 72.
Line 271: "... or such". Suggest replacing with "etc..."
Solved by deleting.
Line 282: change "optain" to "obtain"
Solved
Line 286: 6'600. Please change to 6,600.
Also, is this the sample size for the study or the population of the village. Please clarify this.
The 6,600 does not add up to what is in Table 3. Please check.
We thank Reviewer #2 for this observation. It leads us to re-calculate the table and revealed an error. We apologize for this and have now corrected all numbers in the manuscript and all tables.
Line 293: Correct "workerw"
Solved

Line 295 & 300: "The interview team..."
Solved
Line 301; Correct "accompagnied" to "accompanied"
Solved
Line 307: Suggest you delete medical doctor
Solved
Line 330: "I part one". "In part one"?
Solved
Line 342: "746". it is 745 in Table 4.
We thank Reviewer #2 for this observation. It leads us to re-calculate the table and revealed an error. We have now corrected all numbers (see revised manuscript, clean version, Tables)
Line 349: "all data collection forms are developed..." I suggest you change this to " all data collection forms will be developed..."
Solved
Line 351: "Coordinator is assigned..." Please change to "coordinator will be assigned"
Solved. 'Will be' now used throughout the manuscript, where approbriate
Line 369: "optainded" change to "obtained"?
Solved
Line 379: "withness" Correct to "witness"
Solved
Line 413: "unreveal" Correct to "unravel"
Solved
Line 414: "... an evidence base for to treatment..." Delete "for"
Solved
****

Reviewer #3
Reviewer Name: Hugues Nana Djeunga
Institution and Country: Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Cameroon
Please state any competing interests: None declared

The manuscript submitted by Greter and colleagues is a protocol whose main objective is to assess the impact of mass ivermectin administration against onchocerciasis on prevalence and incidence of epilepsy in Tanzania. This study is of high interest in its field since it provide guidelines while evaluating the association between onchocerciasis and epilepsy. The techniques or approaches presented in this protocol are clear and sufficiently detailed to allow implementation or replication.
We thank Reviewer #3 for this positive appraisal of our planned research and the presented study protocol.
However, the authors need to address many points to improve the quality of their manuscript, and to make it smooth to readers.
Thanks to the most useful comments of 3 reviewers we had the opportunity to sharpen our manuscript and feel that it has now further gained in precision.

Point #1
The title should be slightly modify to reflect the objectives and the orientation of the paper; the authors should consider "impact" rather than "effect".
We thank the Reviewer #3 for this feedback. We fully agree and have integrated this input in the title. The title was further adjusted following a most useful feedback from Reviewer #1. We now feel that thanks to the two Reviewers inputs the title has gained precision and is now to-the-point. (see revised manuscript, clean version, lines 1-3)

Point #2

The paper need to be reorganized since many good ideas are developed but sometimes in wrong order; as a consequence, they seem to repeat many information.

We agree with this comment and have now reorganised the structure of the paper, as well as eliminated repetitions in different sections.

We would suggest to authors:

o to choose either to structure their introduction (using titles and sub-titles) or to organize ideas into paragraphs;

o to organize the first part of their introduction (before "Onchocerciasis, a treatable … elimination") in three paragraphs as follow: (i) definition and distribution of epilepsy, (ii) burden and stigma of epilepsy; (iii) etiology and hypothesis. So, they need to bring back line number (LN) 78-84 ("Epilepsy manifest … with the disease") to LN 74 (after "fully understood"; Move the sentence "Many of these … anti-parasitic treatment" (LN77-78) to LN 92 after "from epilepsy", at the end of page 4. The LN 93-102 should be move to LN 74 (page 4), after "living in LMICs".

Thanks to the feedback from all three Reviewers, we reorganised the introduction and structured it – including also the suggestions from Reviewer #3 - and also shortened it considerably. It has now gained in precision (see revised manuscript, clean version, Introduction).

o the information on the situation of onchocerciasis and epilepsy in the study area (Page 8, LN 190, to page 9, LN 213) should be organized, reshaped and given in the section "Study site and population"; please consider "Study site and population" rather than "Study site and study population".

We followed this most useful recommendation of Reviewer #3 and moved the information onchocerciasis and epilepsy to the methods section. For further improving the structure, we have devided the section in 'Study site' and 'Study population? (see revised manuscript, clean version, lines 210-238)

o the objectives and specific objectives need to be reshaped and the justification section should be deleted; the authors should organize their introduction in such a way that the problem is well presented;

We rewrote the objectives and reorganised the methods section. We deleted the justification section as recommended by Reviewer #3. As described above, the introduction has also been sharpened.

o the method section also need to be reorganized; please consider the following organization:

1. Study area and population
2. Study design

1.1. Sample size

2.1. Data collection procedures (including epilepsy and onchocerciasis)

3. Data analysis
4. Ethical considerations (including Data storage and handling).

We now introduced the method section with the following sentence "The study consists of two parts: (I) a study to determine the prevalence and incidence of epilepsy and (II) a study to determine ivermectin coverage, onchocerciasis prevalence and level of transmission." (see revised manuscript, clean version, lines 206-209).

Also, we followed Reviewer #3 in the proposed re-structuring of the methods section (see revised manuscript, clean version, Methods and analysis)

Point #3

The authors declared that adverse events associated with ivermectin are "close to nil"; this is not true and the authors can refer to the paper "Ivermectine" by Boussinesq in 2005 to revise this. Rather than "close to nil", the authors should list the main and use the term "moderate" …

We thank the Reviewer #3 to point out this important detail. While carefully restructuring the introduction, we decided to delete the comment regarding adverse events in the here mentioned sentence. In lines 144-147 the complications associated with Loa loa co-infection are well described

and a relevant reference by Boussinesq et al. is cited (see revised manuscript, clean version, reference 34)

Point #4
There is a hypothesis for sample size calculation (page 12, LN 279); why assuming whereas 2017 figures are given in the table 2 (Page 10) by the authors themselves.
In line 262 onwards, we assume two factors: 1) to observe a reduction in epilepsy prevalence by 33.3%, and 2) that 80% of the village population will participate. The hypotheses are not related to the population figures given in table 3, but to calculate the sample size with a sufficient power.

Point #5
Study design: Rather than nodule palpation to have a rough estimate of the prevalence, the authors should choose to use skin snipping. Indeed, nodule palpation was recommended as a rapid strategy to delineate areas needing mass treatments; however, the objective here is not to have just an idea of the situation of onchocerciasis but to have more precise data; doing skin snips will provide also information on the intensity of infection and community microfilarial load (CMFL) that can be helpful to further assess the relationship between epilepsy and onchocerciasis, in a context of mass ivermectin administrations
We plan a REMO study which will allow to compare 2017 data with REMO data obtained in the past by the national onchocerciasis control program in Mahenge. To evaluate the level of on-going onchocerciasis transmission we have chosen on OV16 testing in children < 10 years of age, as described in the methods section lines 322-327. These two indicators will provide an accurate picture of the current onchocerciasis situation in the study villages.

Point #6
The statistical analysis is appropriate but may need some further details.
We have now included the analyses of the ivermectin treatment coverage (see revised manuscript, clean version, lines 348-350).

Point #7
The authors declared that they will evaluate the impact of CDTI (please consider CDTI rather than CDTi throughout the text) on incidence of epilepsy but they didn't detailed how they will achieve this goal. They only focussed on prevalence.
We now use CDTI instead of CDTi throughout the text (changes not marked in the revised manuscript). The effect of CDTI on onchocerciasis transmission will be evaluated using the OV16 testing in children < 10 years old. A potential effect of CDTI on the onset of onchocerciasis associated epilepsy will be addressed by the incidence data. We hypothesise to find a lower incidence than in the 1989 survey (see revised manuscript, clean version, Table 1).

Point #8
In their discussion, the authors evoked potential factors that may explain the persistence of onchocerciasis post-MDA (page 17, LN 402-403); the authors should also simply consider that the disease can persist as a consequence of high level of transmission (activity of flies), and baseline data are therefore needed.
Indeed onchocerciasis transmission can persist despite CDTI in case of high activity of the flies. Thanks to the very useful comments of Reviewer #3 and Reviewer #1, who also raised the topic, we have now adjusted the discussion and included a statement on the option of combining the CDTI with larviciding rivers, in case our study reveals an insufficient reduction of onchocerciasis reached by annual CDTI alone (see revised manuscript, clean version, lines 397-398).

Point #9

The authors may need to go through the manuscript and polish the language since there are numerous typos. Here are some examples:
o Page 4, LN 70: consider “are reported” rather than “is reported”
Solved
o Delete “also” (Page 4, LN 74) and add “as well” after “…trigger epilepsy” (Page 4, LN 75)
Solved
o Page 4, LN 85: consider “in onchocerciasis” rather than “from onchocerciasis”
Solved)
o Page 4, LN 87: consider “A NS” rather than “Since, an NS”
Solved)
o Page 4, LN 90: delete “South Sudan” after “Mvolo”
Solved
o Page 8, LN 174: consider “is still” rather than “still is”
Solved
o Page 13, LN 293: consider “workers” rather than “workerw”
Solved
o Page 18, LN 414: consider “…evidence base to …” rather than ““…evidence base for to …”
Solved

There are so many typos and it would have been better to rewrite the manuscript.
We hope we have now identified and corrected all the typos in the manuscript.

VERSION 2 – REVIEW

REVIEWER	Dziedzom de Souza Noguchi Memorial Institute for Medical Research, Ghana
REVIEW RETURNED	27-Nov-2017

GENERAL COMMENTS	There is a considerable improvement in the current version of the paper. However, there are a couple of issues which would need to be strengthened. - The data analysis section can still be improved. I think at this stage the authors have an idea of the type of data that will be obtained in the study and can describe the statistical method that will be used. For example, the authors state "Prevalence and incidence will be compared between villages and to the 1989 data". What type of statistical method will be used (t-test, chi square, non-parametric)? How would statistical significant be determined, if any? - The discussion could be strengthened by the various assumptions and arguments presented in responding to the reviewers' comments. I believe these will make it easier for the readers to understand the concept of the study and the approach adopted. - Please check the typos in the manuscripts

REVIEWER	Christoph Kaiser Pediatrician, Pediatric Practice, Baden-Baden, Germany
REVIEW RETURNED	04-Dec-2017

GENERAL COMMENTS	General Comments

	Thank you for the intense work on the manuscript in response to the reviewers` comments on the first version. In my view, progress has been made in making the MS more concise, and more cautious in its statements. The most interesting point of this proposal is the longitudinal approach by which evidence supporting (or refuting) the supposed connection between onchocerciasis and epilepsy is searched for. This concept is based on the assumption that changes over time in measurements presenting each of the two phenomena can be related to each other. Earlier measures are available from the area, but have so far not been taken at the same time, in the same population, and with identical methodology (with the exception of some data presented by Winkler et al., (Epilepsia 2008: 2008-15), and König et al. (Parasitology 2010: 1559-68); see also below: specific comment, VIII. § 4,(„Onchocerciasis and CDTI … prevalence of 8.3%.[33]“)). Although the disparity of data formats of the earlier studies is largely preventing a statistical analysis of possible interactions between onchocerciasis and epilepsy, the present proposal is of major interest because of the general scarcity of information about the issue. Yet, more information is needed for the proper evaluation of the impact of onchocerciasis control on epilepsy morbidity in endemic areas, in particular the impact of CDTI and vector control. For a comprehensive presentation of the information about onchocerciasis, epilepsy and nodding syndrome in the Mahenge area, I suggest to add in the proposal a table listing all available publications on this subject. A number of references were already included in my review of the first version of the manuscript, and the authors have found the most interesting article of Häusermann (Acta Tropica 1969:29-69). Possibly, more pertinent information could be retrieved with a systematic literature search. For a better understanding of the spatial conditions of Mahenge, it would also be helpful to include a map of the study area, representing the river system, altitudes and the geographical positioning of earlier surveys, and of the planned survey. Specific Comments INTRODUCTION

	I. § 1, line 13-14 („The seizures … 18 years [14]“) The authors cite Kaiser et al (Epilepsia 2009: 2325-6) as a reference for characterization of the typical head nodding seizures found in NS. However, this reference is a letter to the editor in response to the pivotal article of Winkler et al (Epilepsia 2008: 2008-15), and it does not contain original clinical information and no information on seizure semiology. It is therefore not suitable as a reference at this point. I would suggest to rather refer directly to Winkler et al. (Epilepsia 2008: 2008-15) or to Dowell et al. (Emerg If Dis 2013: 1374-84). II. § 1, line 16-18 („Since its … African countries[16 17]“) The term of „Nodding Syndrome“ or „Head Nodding Syndrome“ was not conceptualized and formulated prior to the publication of the article of Winkler et al. (Epilepsia 2008: 2008-15). Therefore the statement that „Nodding Syndrome was described in 1960“ is not justified, even if, in retrospect, the cases described by Jilek-Aall with seizures characterized by head nodding movements were probably affected by the disease later named „Nodding Syndrome“. I suggest to use a wording such as: „In the 1960, Aall-Jillek was the first to describe an unusual form of epileptic seizures characterized by nodding movements of the head …“. This would be correct and at the same time would recognize the achievements of Louise Aall-Jillek. The statement of the submitted MS of Greter et al. that „until the mid-1990 NS was a rare condition in African countries“ is unclear. As mentioned, Nodding Syndrome was not defined as a medical entity until 2008. The authors do not provide data or pertinent references to support this statement. III. § 1, line 19-23 („The weight … from epilepsy.[19]“) The authors refer to an article of Colebunders et al (BMC Res Notes 2016: 182), reporting a 2-days-visit in a South Sudanese village where “Thirteen (59%) households had at least one child with NS or another form of epilepsy“. Because in the reference only the combined number of NS AND other forms of epilepsy is reported, it is likely that in fact only a proportion of these 13 households had a child with NS and this could be less than 50%. Thus, strictly speaking, the statement in the submitted manuscript is not warranted. Furthermore, the term „epilepsy of the NS type“ is not well defined. Although features determining epilepsy types (focal/ generalized/ combined/ unknown brain pathology) are also constituents for epilepsy syndromes, epilepsy types and epilepsy syndromes are not the same (Scheffer I et al, Epilepsia 2017: 512-521; Berg AT et al, Epilepsia 2010: 676-685). In my view, the use of arbitrary terms and expressions should be avoided. Perhaps, the

	verbatim quotation of the above mentioned statement of Colebunders et al (BMC Res Notes 2016: 182) would be correct and most appropriate at this place. IV. § 3, line 5-6 (Study results … this association.[8 25]) Here, I suggest to introduce the term OAE (onchocerciasis associated epilepsy) which is taken up later in the article and is the major theme of the planned study. The term of OAE describing an epidemiological phenomenon was used first by Kaiser et al (PLoS NTD 2013: e2147). V. § 6, line 9-13 (Considering … exceed 100`000. [1 7])  The issue of the disease burden attributable to OAE is a highly interesting issue. In my view, the number of people affected by OAE is probably higher than the number of 100`000 mentioned in the submitted manuscript of Greter et al. which is based on an ALL-AGES excess prevalence of OAE. When looked at more closely, the extremely high age-specific prevalence rate in areas of high onchocerciasis endemicity at age 10-19 years (Kaiser et al. Bull WHO 1996, 361-367; Colebunders et al. PLoS NTD 2016, e0004478) would exceed an expected baseline from other regions of 0.5 – 1.0 by a factor of 5x / up to 10x, the decreasing prevalence in older age groups is probably due to the high mortality from epilepsy in these areas (Kamgno et al. Epilepsia 2003: 956-63; Kaiser et al. Trans Roy Soc Trop Med & Hyg 2007, 48-55), and the (all-ages) incidence would by about seven times higher (Kaiser et al Epilepsy Res. 1998: 247-51). Both estimates, that suggested by Greter et al. as well as my own, are rather „back-of-the-envelope“ calculations. The magnitude of the disease burden of OAE should be analyzed more exactly. METHODS AND ANALYSIS VI. § 3, line 1-2 (In the 1960, … first NS cases) see Minor Comment II. INTRODUCTION, § 1, line 16-18 („Since its … African countries[16 17]“) VII. § 4, („Onchocerciasis and CDTI … prevalence of 8.3%.[33]“) In this paragraph, the author make a literature based appraisal of onchocerciasis endemicity in Mahenge. It might be useful to include here the reference of Häusermann et al. (reference 56) and give more details of this study because it is not easily retrieved by the

	reader. Data on onchocerciasis prevalence are also available from surveys carried out in Mahenge in 2005 (König R et al. Parasitology 2010: 1559-68; König R et al. Proceedings of the Austrian Society of Tropical Medicine meeting 2006; Schmutzhard E et al. Proceedings of the AAN meeting 2008), summarized by Kaiser et al. (PLoS NTD 2013: e2147). In these surveys, onchocerciasis infection was found with skin snip or skin PCR in 35 of 104 adult healthy controls. These investigations were done immediately in the catchment area of the Mahenge Epilepsy Clinic, hence many of these household controls may have originated from the villages planned to be studied in the protocol of Greter et al. VIII. § 7, Epilepsy and NS prevalence / incidence study, Study design („The study … community leaders“) IX. § 9, Epilepsy and NS prevalence / incidence study („The community … potential clustering“). Perhaps this paragraph is more appropriately shifted to the sub-heading „study design“ below the paragraph: „The study is designed … or community leaders.[50]“ It is recommended to translate and re-translate the 5-items questionnaire and to pilot-test the translated questionnaire in a small sample. This would improve the validity of the screening tool to detect convulsive seizures, because its validation in Mauritanean patients cannot be readily transfered to the setting of Mahenge. With regard to the detection of cases with Nodding Syndrome, it is unclear to what extent this can be attained with the 5-items questionnaire. A recent anthropological investigation on Nodding Syndrome in Mahenge found that there is no clarity about the terms of „Nodding Syndrome“ and two locally applied terms („amesinzia kichwa“ and „kifafa cha kusinzia“) amongst local health workers and some community members (Van Bemmel K & Van der Weegen K. Anthropol Med 2017, Epub ahead of print). I want to suggest to use the planned introductory key persons interviews to explore the community concept and the possible terms used for head nodding seizures/ Nodding Syndrome in the study villages. The appropriate local term for NS or an accepted paraphrase could then be added to the 5-item screening tool. This would enhance the sensitivity of NS case finding in the study. I recommend to report the number of persons detected with the door-to-door survey as possibly affected by epilepsy as „the number of persons with suspected epilepsy“. This would be in line with the ILAE guidelines (Thurman J et al. Epilepsia 2011; Suppl 7: 2-26). Accordingly, the number of persons detected as possibly affected by NS should be clearly reported as „the number of persons with suspected NS“. This would follow the system of the consented case

	definition for NS (Dowell et al. Emerg Infect Dis 2013: 1374-73). X. § 10, Case verification and validation (All suspected epilepsy … epilepsy treatment history) I recommend to specify the name of the neurologist and her/his experience in epileptology and specifically Nodding Syndrome. The time devoted for the diagnostic interview and examination should be specified. If a self-constructed questionnaire is used, this should be added as supplementary information. I want to encourage the authors to use the standardized questionnaire developped by the „Institut d`Épidemiologie Neurologique et de Neurologie Tropicale de Limoges“ which they cite as reference 52 (Preux PM. Bull Soc Pathol Exot 2000: 276-8). This questionnaire contains more than 150 questions and can probably not directly applied in the local language. Additional questions and standardized observations should be added for the verification of possible/ confirmed NS. Therefore, the setting of the interview situation should be specified (e. g. Description of the professional and cultural background of the translater; Pre-testing of the questionnaire). The results of verification/confirmation process should be reported for persons with confirmed epilepsy AND for those with <u>non-confirmed</u> epilepsy. As a suitable format for presentation, I would like to suggest the presentation taken in our earlier epilepsy study in a western Ugandan population (Kaiser et al. Bull WHO 1996:361-367; see Table 3), giving details of diagnosis in non-confirmed epilepsy patients. This should also be done for persons with suspected, probable and confirmed Nodding Syndrome, according to the consented case definition for NS (Dowell et al. Emerg Infect Dis 2013: 1374-73). The numbers of those patients in whom epilepsy AND Nodding Syndrome will be confirmed (designated „Head Nodding plus“ by Winkler et al. (Epilepsia 2008: 2008-15) should also be reported. XI. § 11 Definitions („A case of epilepsy“) The definition of epilepsy proposed by the authors is not in full agreement with the definition they refer to (Fisher RS et al. Epilepsia: 475-482). In this, presently valid, definition Fisher et al. add a third criterion to those two criteria considered in the submitted manuscript of Greter et al., namely: „One provoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk … „. This additional criterion which relies mostly on information from ancilliary technical studies is not practical in the setting of rural Africa. Therefore, as explained in the review of the first version of the manuscript and agreed by the authors, a correct and approved reference on the former definition should be cited here

	(e.g. the classical study of Hauser WA et al. (Epilepsia 1991), or Gastaut H., Dictionary of Epilepsy, WHO, Geneva 1980), together with the baseline article of Rwiza et al. (Epilepsia 1992:1051-56) in Mahenge. The discrepancies between the former definition and the current definition, and the rational for choosing the former, could be explained here or in the discussion. XII. § 13 Definitions („A case suspected NS") I recommend to add a definition for a „probable case" and a „confirmed" case of NS according to the consented case definition for NS (Dowell et al. Emerg Infect Dis 2013: 1374-73). (Along with the specification of the procedures allowing to classify a probable and a confirmed NS case in the planned study. See also Minor Comment XI). XIII § 15 Onchocerciasis prevalence study („This study … in their heads.[56]) The procedure for assessment of onchcerciasis endemicity is appropriate, although the REMO methodology which relies on nodule palpation may be of limited accuracy in areas with a low prevalence. The authors make no attempt to estimate the pre-control endemicity of onchocerciasis more exactly, indicating that there is no data on village level. This is probably true with regard of the specific villages of Mdindo, Vigoi and Misegezi where the present study is planned. Nevertheless, the excellent study of W Häusermann (Acta Tropica 1969: 29-69) cited by the authors, besides entomological data and a description of the local river system, also demonstrated onchocerciasis prevalence rates ranging from 73% to 100% skin snip positives in adults aged >30 years in three villages of the Mselezi valley located immediatly South of Mahenge town. As mentioned, it would be of interest to include in the proposal a map with the exact location of the study villages of the planned survey in relation to the river system and to the villages studied by W Häusermann in 1969. This would help to have a better idea about the pre-CDTI situation in the villages of Mdindo, Vigoi and Misegezi.

REVIEWER	Hugues Nana Djeunga Centre for Research on Filariasis and other Tropical Diseases (CRFilMT)
REVIEW RETURNED	12-Dec-2017

GENERAL COMMENTS	Dear Editor, The revised version of the manuscript submitted by Greter and colleagues was significantly improved and all but one of my comments and revisions have been appropriately addressed. Indeed, I still have a concern regarding this protocol. It was recently

	demonstrated (although still unpublished) that the association between onchocerciasis and epilepsy is mostly driven by the intensity of onchocerciasis infection, rather than just the fact to harbour the parasite or not (measure by the pevalence and/or incidence in this study). The findings would likely be more robust if the intensity of infection was also captured. If this is not done, I would like the authors discussing this important point as a limitation of the study. Lastly, there are still some typos that I have corrected in the clean pdf version of the revised manuscript uploaded. - The reviewer provide a marked copy with additional comments. Please contact the publisher for full details

VERSION 2 – AUTHOR RESPONSE

Editorial Requirements:
Please revise the Strengths and Limitations section (after the abstract) to focus on the methodological strengths and limitations of your study rather than summarizing the results.

- We have rewritten the 'Strengths and Limitations' section (see revised manuscript, lines 46-62)

****

Reviewer #1
Reviewer Name: Christoph Kaiser
Institution and Country: Pediatric Practice, Balzenbergstr. 73 76530 Baden-Baden, Germany
Please state any competing interests: None declared

The most interesting point of this proposal is the longitudinal approach by which evidence supporting (or refuting) the supposed connection between onchocerciasis and epilepsy is searched for. This concept is based on the assumption that changes over time in measurements presenting each of the two phenomena can be related to each other.
Earlier measures are available from the area, but have so far not been taken at the same time, in the same population, and with identical methodology (with the exception of some data presented by Winkler et al., (Epilepsia 2008: 2008-15), and König et al.(Parasitology 2010: 1559-68); see also below: specific comment, VIII. § 4,(„Onchocerciasis and CDTI… prevalence of 8.3%.33“)). Although the disparity of data formats of the earlier studies is largely preventing a statistical analysis of possible interactions between onchocerciasis and epilepsy, the present proposal is of major interest because of the general scarcity of information about the issue. Yet, more information is needed for the proper evaluation of the impact of onchocerciasis control on epilepsy morbidity in endemic areas, in particular the impact of CDTI and vector control.

- Indeed it will not be easy to compare pre-CDTI data with the data we are going to collect. Previous studies of Winkler et al and König et al were clinic based.
Only the study by Rwiza et al was a population based study, and therefore the main information source to compare to.

For a comprehensive presentation of the information about onchocerciasis, epilepsy and nodding syndrome in the Mahenge area, I suggest to add in the proposal a table listing all available publications on this subject. A number of references were already included in my review of the first version of the manuscript, and the authors have found the most interesting article of Häusermann (Acta Tropica 1969:29-69). Possibly, more pertinent information could be retrieved with a systematic

literature search. For a better understanding of the spatial conditions of Mahenge, it would also be helpful to include a map of the study area, representing the river system, altitudes and the geographical positioning of earlier surveys, and of the planned survey.

- We agree, and the most important papers concerning epilepsy in the Mahenge area relevant for this protocol are cited in the reference list, including those kindly pointed out by Reviewer #1 in the first round of revision. Also we feel that a literature revue on epilepsy in Mahenge is of importance, the present paper is already quite long we do think an extensive table citing all published papers on the topic would exceed this format. Yet, such a literature review could stand as a publication alone, specifically of interest to the concerning research community and the Tanzanian public health authorities.
A detailed map providing geographical information on previous and the present study, as well as detailed river and village locations is planned to be published together with the results of this study, as mentioned in the manuscript in lines 297-300.

The authors cite Kaiser et al (Epilepsia 2009: 2325-6) as a reference for characterization of the typical head nodding seizures found in NS. However, this reference is a letter to the editor in response to the pivotal article of Winkler et al (Epilepsia 2008: 2008-15), and it does not contain original clinical information and no information on seizure semiology. It is therefore not suitable as a reference at this point. I would suggest to rather refer directly to Winkler et al. (Epilepsia 2008: 2008-15) or to Dowellet al. (Emerg If Dis 2013: 1374-84).

- We are grateful to Reviewer #1 to point this out, and have now replaced the reference as suggested by the reference of Winkler et al. (Epilepsia 2008: 2008-15).

II. § 1, line 16-18 („Since its … African countries16 17“)
The term of „Nodding Syndrome“ or „Head Nodding Syndrome“ was not conceptualized and formulated prior to the publication of the article of Winkler et al. (Epilepsia 2008: 2008-15). Therefore the statement that „Nodding Syndrome was described in 1960“ is not justified, even if, in retrospect, the cases described by Jilek-Aall with seizures characterized by head nodding movements were probably affected by the disease later named „Nodding Syndrome“. I suggest to use a wording such as: In the 1960, Aall-Jillek was the first to describe an unusual form of epileptic seizures characterized by nodding movements of the head …“. This would be correct and at the same time would recognize the achievements of Louise Aall-Jillek.

- We accept the comment and have adjusted the manuscript as follows: The suggested sentence is now included in the revised manuscript, lines 82-83, including the relevant references by Aall-Jillek. We also include a statement on the term nodding syndrome in line 85.

The statement of the submitted MS of Greter et al. that „until the mid-1990 NS was a rare condition in African countries“ is unclear. As mentioned, Nodding Syndrome was not defined as a medical entity until 2008. The authors do not provide data or pertinent references to support this statement.

- We agree and omitted this statement.

III. § 1, line 19-23 („The weight … from epilepsy.19“) The authors refer to an article of Colebunders et al (BMC Res Notes 2016: 182), reporting a 2-days visit in a South Sudanese village where “Thirteen (59%) households had at least one child with NS or another form of epilepsy“. Because in the reference only the combined number of NS AND other forms of epilepsy is reported, it is likely that in fact only a proportion of these 13 households had a child with NS and this could be less than 50%. Thus, strictly speaking, the statement in the submitted manuscript is not warranted. Furthermore, the term „epilepsy of the NS type“ is not well defined. Although features determining epilepsy types (focal/

generalized/ combined/ unknown brain pathology) are also constituents for epilepsy syndromes, epilepsy types and epilepsy syndromes are not the same (Scheffer I et al, Epilepsia 2017: 512-521; Berg AT et al, Epilepsia 2010: 676-685). In my view, the use of arbitrary terms and expressions should be avoided. Perhaps, the verbatim quotation of the above mentioned statement of Colebunders et al (BMC Res Notes 2016: 182) would be correct and most appropriate at this place.

- Indeed in the South Sudanese village not all 13 households had a child with NS. We now state that "The weight of the public health burden caused by epilepsy in onchocerciasis endemic regions can be illustrated by the situation in the West Equatorial State in South Sudan, where in the village of Mvolo, over 50% of the families had at least one child with epilepsy, resulting in one in six children of the village suffering from epilepsy. » (see revised manuscript, lines 92-96).

IV. § 3, line 5-6 (Study results … this association.8 25) Here, I suggest to introduce the term OAE (onchocerciasis associated epilepsy) which is taken up later
in the article and is the major theme of the planned study. The term of OAE describing an epidemiological phenomenon was used first by Kaiser et al (PLoS NTD 2013: e2147).

- We now include "To describe this epidemiological phenomenon the term onchocerciasis associated epilepsy (OAE) was proposed by Kaiser and colleagues" and refer to the publication of Kaiser et al (PLoS NTD 2013: e2147). (see revised manuscript, lines 109-111).

V. § 6, line 9-13 (Considering … exceed 100`000. 1 7)
The issue of the disease burden attributable to OAE is a highly interesting issue. In my view, the number of people affected by OAE is probably higher than the number of 100`000 mentioned in the submitted manuscript of Greter et al. which is based on an ALL-AGES excess prevalence of OAE. When looked at more closely, the extremely high age-specific prevalence rate in areas of high onchocerciasis endemicity at age 10-19 years (Kaiser et al. Bull WHO 1996, 361-367; Colebunders et al. PLoS NTD 2016, e0004478) would exceed an expected baseline from other regions of 0.5 – 1.0 by a factor of 5x / up to 10x, the decreasing prevalence in older age groups is probably due to the high mortality from epilepsy in these areas (Kamgno et al. Epilepsia 2003: 956-63; Kaiser et al. Trans Roy Soc Trop Med & Hyg 2007, 48-55), and the (all-ages) incidence would by about seven times higher (Kaiser et al Epilepsy Res. 1998: 247-51). Both estimates, that suggested by Greter et al. as well as my own, are rather „back-of-the-envelope" calculations. The magnitude of the disease burden of OAE should be analyzed more exactly.

- We agree that the burden of disease caused by OAE is probably greater > 100.000.
We are preparing a paper on this topic with mathematical modellers and burden of disease experts. They estimated the number of persons with OAE between 136 thousand (95%CI: 63-447 thousand) (this is if OAE only occurs in countries where epilepsy prevalence studies were done in oncho areas) and 454 thousand (95%CI: 232-1,361 thousand). We assume it is about 400 000 (this is considering OAE occurs in all meso hyper endemic oncho areas).
We now mention cases of epilepsy attributed to onchocerciasis most likely exceeds 100'000" (see revised manuscript, lines 182-183).

VI. § 3, line 1-2 (In the 1960, … first NS cases)
see Minor Comment II. INTRODUCTION, § 1, line 16-18 („Since its … African countries16 17")

- Solved (see above).

VII. § 4, („Onchocerciasis and CDTI … prevalence of 8.3%.33")

In this paragraph, the author make a literature based appraisal of onchocerciasis endemicity in Mahenge. It might be useful to include here the reference of Häusermann et al. (reference 56) and give more details of this study because it is not easily retrieved by the reader.

- We now provide more information about the study of Häusermann et al. by including the skin snip positivity rates in the three villages (see revised manuscript, lines 336-338).

Data on onchocerciasis prevalence are also available from surveys carried out in Mahenge in 2005 (König R et al. Parasitology 2010: 1559-68; König R et al. Proceedings of the Austrian Society of Tropical Medicine meeting 2006; Schmutzhard E et al. Proceedings of the AAN meeting 2008), summarized by Kaiser et al. (PLoS NTD 2013: e2147). In these surveys, onchocerciasis infection was found with skin snip or skin PCR in 35 of 104 adult healthy controls. These investigations were done immediately in the catchment area of the Mahenge Epilepsy Clinic, hence many of these household controls may have originated from the villages planned to be studied in the protocol of Greter et al.

- Indeed, the studies mentioned here by Reviewer #1 were performed post introduction of CDTI, yet these were, to our knowledge, not population based studies. This is the main difference to the here proposed study

VIII. § 7, Epilepsy and NS prevalence / incidence study, Study design („The study … community leaders“)
IX. § 9, Epilepsy and NS prevalence / incidence study („The community … potential clustering“). Perhaps this paragraph is more appropriately shifted to the sub-heading „study design“ below the paragraph: „The study is designed … or community leaders.50“

- We feel that the structure of the methods section has considerably improved thanks to the comments received from the reviewers in the first round and are convinced that it now follows a logical flow. Therefore no paragraph switch as done.

It is recommended to translate and retranslate the 5-items questionnaire and to pilot-test the translated questionnaire in a small sample. This would improve the validity of the screening tool to detect convulsive seizures, because its validation in Mauritanean patients cannot be readily transfered to the setting of Mahenge.

- The here described procedure is common good practice in epidemiological studies and is described in the present manuscript by stating that the questionnaire is pre-tested and validated. Yet, to make this clear we have added a description of the process (see revised manuscript, lines 293-294).

With regard to the detection of cases with Nodding Syndrome, it is unclear to what extent this can be attained with the 5- items questionnaire. A recent anthropological investigation on Nodding Syndrome in Mahenge found that there is no clarity about the terms of „Nodding Syndrome“ and two locally applied terms („amesinzia kichwa“ and „kifafa cha kusinzia“) amongst local health workers and some community members (Van Bemmel K & Van der Weegen K. Anthropol Med 2017, Epub ahead of print). I want to suggest to use the planned introductory key persons interviews to explore the community concept and the possible terms used for head nodding seizures/ Nodding Syndrome in the study villages. The appropriate local term for NS or an accepted paraphrase could then be added to the 5-item screening tool. This would enhance the sensitivity of NS case finding in the study.

- We fully agree with Reviewer #1's comment. As we describe in our manuscript in lines 294-295, we will use the pre-identified, adequate terms to allow for a clear identification of the symptoms. We also mention example terms in the manuscript. During the pilot test, these terms will be verified.

I recommend to report the number of persons detected with the door-to-door survey as possibly affected by epilepsy as „the number of persons with suspected epilepsy“. This would be in line with the ILAE guidelines (Thurman J et al. Epilepsia 2011; Suppl 7: 2-26). Accordingly, the number of persons detected as possibly affected by NS should be clearly reported as „the number of persons with suspected NS“. This would follow the system of the consented case definition for NS (Dowell et al. Emerg Infect Dis 2013: 1374-73).

- We now use the term persons with suspected epilepsy /NS throughout the manuscript.

X. § 10, Case verification and validation (All suspected epilepsy … epilepsy treatment history) I recommend to specify the name of the neurologist and her/his experience in epileptology and specifically Nodding Syndrome. The time devoted for the diagnostic interview and examination should be specified. If a self-constructed questionnaire is used, this should be added as supplementary information. I want to encourage the authors to use the standardized questionnaire developped by the „Institut d`Épidemiologie Neurologique et de Neurologie Tropicale de Limoges“ which they cite as reference 52 (Preux PM. Bull Soc Pathol Exot 2000: 276-8). This questionnaire contains more than 150 questions and can probably not directly applied in the local language. Additional questions and standardized observations should be added for the verification of possible/ confirmed NS. Therefore, the setting of the interview situation should be specified (e. g. Description of the professional and cultural background of the translater; Pre-testing of the questionnaire).

- The neurologists involved in the study are Prof Matuja a very experience neurologist who has been several times investigating epilepsy in the Mahenge area and who was involved in the initial survey in 1989 and Mohamed Mnacho, neurologist at the Department of neurology of Muhimbili University of Health Sciences, Dar es Salaam. Both are members of the investigator team since the early planning stage of this study, and both are also among the authors of this protocol manuscript. Both neurologists are native Tanzanian citizens and no translation is needed for the clinical examination and associated interviews.

The results of verification/confirmation process should be reported for persons with confirmed epilepsy AND for those with non-confirmed epilepsy. As a suitable format for presentation, I would like to suggest the presentation taken in our earlier epilepsy study in a western Ugandan population (Kaiser et al. Bull WHO 1996:361-367; see Table 3), giving details of diagnosis in non-confirmed epilepsy patients.

- Indeed we will provide details about the diagnosis of non-confirmed epilepsy patients in the publication of the results of this study. Yet, as this is an epidemiological study, clinical examination does not include EEG and results will be presented as identified within the frame of the given conditions.

This should also be done for persons with suspected, probable and confirmed Nodding Syndrome, according to the consented case definition for NS (Dowell et al. Emerg Infect Dis 2013: 1374-73). The numbers of those patients in whom epilepsy AND Nodding Syndrome will be confirmed (designated „Head Nodding plus“ by Winkler et al. (Epilepsia 2008: 2008-15) should also be reported.

- As mentioned before, we are not planning to videotape the nodding episodes or perform EEG. The objective of the study is not to describe in detail all clinical aspects of OAE but to investigate whether there is an effect of ivermectin on the incidence and prevalence of epilepsy.

XI. § 11 Definitions („A case of epilepsy“)
The definition of epilepsy proposed by the authors is not in full agreement with the definition they refer to (Fisher RS et al. Epilepsia: 475-482). In this, presently valid, definition Fisher et al. add a third

criterion to those two criteria considered in the submitted manuscript of Greter et al., namely: „One provoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk… „. This additional criterion which relies mostly on information from ancilliary technical studies is not practical in the setting of rural Africa. Therefore, as explained in the review of the first version of the manuscript and agreed by the authors, a correct and approved reference on the former definition should be cited here (e.g. the classical study of Hauser WA et al. (Epilepsia 1991), or Gastaut H., Dictionary of Epilepsy, WHO, Geneva 1980), together with the baseline article of Rwiza et al.(Epilepsia 1992:1051-56) in Mahenge. The discrepancies between the former definition and the current definition, and the rational for choosing the former, could be explained here or in the discussion.

- We agree and will use the definition omitting the third criterion. We now explain that we use the same definition as Rwiza, added the suggested references, and omitted the reference by Fisher et al. (see revised manuscript, lines 311-325, Ref. 52, and lines 323-324).

XII. § 13 Definitions („A case suspected NS“)
I recommend to add a definition for a „probable case“ and a „confirmed“ case of NS according to the consented case definition for NS (Dowell et al. Emerg Infect Dis 2013: 1374-73). (Along with the specification of the procedures allowing to classify a probable and a confirmed NS case in the planned study. See also Minor Comment XI).

- This would go beyond the here presented epidemiological study, as mentioned above we will not perform the clinical examinations necessary for rigorous NS case confirmation (see also comment above).

XIII § 15 Onchocerciasis prevalence study („This study … in their heads.56)
The procedure for assessment of onchcerciasis endemicity is appropriate, although the REMO methodology which relies on nodule palpation may be of limited accuracy in areas with a low prevalence. The authors make no attempt to estimate the pre-control endemicity of onchocerciasis more exactly, indicating that there is no data on village level. This is probably true with regard of the specific villages of Mdindo, Vigoi and Misegezi where the present study is planned. Nevertheless, the excellent study of W Häusermann (Acta Tropica 1969: 29-69) cited by the authors, besides entomological data and a description of the local river system, also demonstrated onchocerciasis prevalence rates ranging from 73% to 100% skin snip positives in adults aged >30 years in three villages of the Mselezi valley located immediatly South of Mahenge town.

- We fully agree with this comment and have now adapted the manuscript accordingly (see revised manuscript, lines 336-338)

As mentioned, it would be of interest to include in the proposal a map with the exact location of the study villages of the planned survey in relation to the river system and to the villages studied by W Häusermann in 1969. This would help to have a better idea about the pre-CDTI situation in the villages of Mdindo, Vigoi and Misegezi.

- Please see comment above. (response to the second comment of Reviewer #1)

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Reviewer #2
Reviewer Name: Dziedzom K. de Souza
Institution and Country: Noguchi Memorial Institute for Medical Research, University of Ghana
Please state any competing interests: None declared

There is a considerable improvement in the current version of the paper. However, there are a couple of issues which would need to be strengthened.
The data analysis section can still be improved. I think at this stage the authors have an idea of the type of data that will be obtained in the study and can describe the statistical method that will be used. For example, the authors state "Prevalence and incidence will be compared between villages and to the 1989 data". What type of statistical method will be used (t-test, chi square, non-parametric)? How would statistical significant be determined, if any?

- We have now further developed the analysis section of our manuscript and included the information on the statistical significance level and the test methods used in the text (see revised manuscript, lines 364-366 and 367-368).

The discussion could be strengthened by the various assumptions and arguments presented in responding to the reviewers' comments. I believe these will make it easier for the readers to understand the concept of the study and the approach adopted.

- We have rewritten several parts of the discussion in view of Reviewer #2 valuable comment. We also more clearly specify the limitations of our study design.

Please check the typos in the manuscripts

- We have once more carefully checked our manuscript for any typos. Additionally, we are grateful for the careful proof reading provided by Reviewer #3.
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Reviewer #3
Reviewer Name: Hugues Nana Djeunga
Institution and Country: Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Cameroon
Please state any competing interests: None declared

The revised version of the manuscript submitted by Greter and colleagues was significantly improved and all but one of my comments and revisions have been appropriately addressed. Indeed, I still have a concern regarding this protocol. It was recently demonstrated (although still unpublished) that the association between onchocerciasis and epilepsy is mostly driven by the intensity of onchocerciasis infection, rather than just the fact to harbour the parasite or not (measure by the pevalence and/or incidence in this study). The findings would likely be more robust if the intensity of infection was also captured. If this is not done, I would like the authors discussing this important point as a limitation of the study.

- Indeed, we are grateful to Reviewer #3 for pointing out this important aspect. We have now included this aspect in the limitation statement in the discussion (see revised manuscript, lines 421-423).

Lastly, there are still some typos that I have corrected in the clean pdf version of the revised manuscript uploaded

- We thank Reviewer#3 very much indeed for the careful proof reading of our manuscript and have now integrated all corrections as suggested.

VERSION 3 – REVIEW

REVIEWER	Christoph Kaiser

	Pediatrician, Pediatric Practice, Baden-Baden, Germany
REVIEW RETURNED	30-Dec-2017

GENERAL COMMENTS	I want to thank the authors for carefully and patiently responding to my numerous, at time lengthy, comments. In my view, the study protocol is now at a stage to yield useful results which hopefully will contribute to a better understanding of OAE/ NS in Mahenge, and to more effective control measures. One final comment on the Definition of Epilepsy: This issue is of extreme importance to Neurology/ Epileptology as it is touching the core of the discipline. Any imprecision could affect acceptance of the study results in the neurological scientific community. The confusion about the Definition of Epilepsy arose from the revision proposed by Fisher RS et al in 2005 and adopted by ILAE (Epilepsia 2005; 46: 470-472), correctly stating that a single seizure would be sufficient to diagnose epilepsy in many individual patients and thus to define epilepsy. This was not unanimously accepted in the epileptological world and provoked many controversial debates. Therefore, the Definition of Epilepsy again underwent some modification and the (today valid !) guidelines of 2011 differentiate between a "conceptual" definition, corresponding to that introduced in 2005 (see Fisher RS et al. above), and an "operational definition" for use in epidemiological studies (Thurman DJ et al, Epilepsia 2011; 52 Suppl 7: 2-26, cited in the submitted manuscript of H Greter et al.). Fortunately, the "operational definition" is largely coinciding with the "good old" earlier definition which had also been used be Rwiza et al. in their baseline study in Mahenge (Epilepsia 1992: 1051-1056). With this background, I want to suggest the following formulation for the Definition of Epilepsy in the submitted manuscript of H Greter et al.: "A case of epilepsy will be defined as a patient who had at least two times nonfebrile seizures unrelated to any acute metabolic disorder or to withdrawal of alcohol or drugs, with a minimal time difference of 24h between the two events. This is in accordance with the current guidelines of the International League Against Epilepsy (ILAE) for an operational definition of epilepsy (ref. 49) and with the definition used by Rwiza et al in their baseline study performed in 1989 (ref. 43, ref. 52)." I am looking forward to soon read the results of the proposed study.

REVIEWER	Dziedzom de Souza Noguchi Memorial Institute for Medical Research, University of Ghana
REVIEW RETURNED	15-Jan-2018

GENERAL COMMENTS	This revised version of the manuscript is a much improved version. There are a couple of typos and the authors should have a careful read through the manuscript to ensure all mistakes are addressed.

REVIEWER	Hugues Nana Djeunga Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Cameroon
REVIEW RETURNED	27-Jan-2018

GENERAL COMMENTS	The authors thoroughly addressed my previous comments. However, there are still many typos remaining that I have revised to the attention of the authors (please see the attachment). - The reviewer provide a marked copy with additional comments. Please contact the publisher for full details

VERSION 3 – AUTHOR RESPONSE

Reviewer #1
Reviewer Name: Christoph Kaiser
Institution and Country: Pediatric Practice, Balzenbergstr. 73 76530 Baden-Baden, Germany
Please state any competing interests: None declared

I want to thank the authors for carefully and patiently responding to my numerous, at time lengthy, comments. In my view, the study protocol is now at a stage to yield useful results which hopefully will contribute to a better understanding of OAE/ NS in Mahenge, and to more effective control measures. Indeed, it is us who would like to express our sincere thanks to Reviewer #1 for a rigorous and most useful review process. The comments and inputs received contributed strongly to sharpen our study protocol.

One final comment on the Definition of Epilepsy: This issue is of extreme importance to Neurology/ Epileptology as it is touching the core of the discipline. Any imprecision could affect acceptance of the study results in the neurological scientific community. The confusion about the Definition of Epilepsy arose from the revision proposed by Fisher RS et al in 2005 and adopted by ILAE (Epilepsia 2005; 46: 470-472), correctly stating that a single seizure would be sufficient to diagnose epilepsy in many individual patients and thus to define epilepsy. This was not unanimously accepted in the epileptological world and provoked many controversial debates. Therefore, the Definition of Epilepsy again underwent some modification and the (today valid !) guidelines of 2011 differentiate between a "conceptual" definition, corresponding to that introduced in 2005 (see Fisher RS et al. above), and an "operational definition" for use in epidemiological studies (Thurman DJ et al, Epilepsia 2011; 52 Suppl 7: 2-26, cited in the submitted manuscript of H Greter et al.). Fortunately, the "operational definition" is largely coinciding with the "good old" earlier definition which had also been used be Rwiza et al. in their baseline study in Mahenge (Epilepsia 1992: 1051-1056). With this background, I want to suggest the following formulation for the Definition of Epilepsy in the submitted manuscript of H Greter et al.:

"A case of epilepsy will be defined as a patient who had at least two times nonfebrile seizures unrelated to any acute metabolic disorder or to withdrawal of alcohol or drugs, with a minimal time difference of 24h between the two events. This is in accordance with the current guidelines of the International League Against Epilepsy (ILAE) for an operational definition of epilepsy (ref. 49) and with the definition used by Rwiza et al in their baseline study performed in 1989 (ref. 43, ref. 52)."
- We fully agree with Reviewer #1 that the definition is critical and we have integrated the text as suggested.

I am looking forward to soon read the results of the proposed study.
- We thank Reviewer #1 for this encouragement.

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Reviewer: 2
Reviewer Name: Dziedzom de Souza
Institution and Country: Noguchi Memorial Institute for Medical Research, University of Ghana
Please state any competing interests: None declared

This revised version of the manuscript is a much improved version. There are a couple of typos and the authors should have a careful read through the manuscript to ensure all mistakes are addressed.
- We thank Reviewer #2 for the appreciation of our revised manuscript. Thanks to his and Reviewer #3 careful proofreading, we hope that now all the typos have been eliminated.

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Reviewer: 3
Reviewer Name: Hugues Nana Djeunga
Institution and Country: Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Cameroon
Please state any competing interests: None declared

The authors thoroughly addressed my previous comments. However, there are still many typos remaining that I have revised to the attention of the authors (please see the attachment)
- We are grateful to Reviewer #3 for accepting our excuses for the remaining typos in the R2 version of our manuscript and hope that now all the typos have been eliminated.