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The effect of community-directed treatment with ivermectin on the prevalence and incidence of epilepsy in an onchocerciasis endemic area of Tanzania: a study protocol

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ABSTRACT

Introduction

Worldwide, there are an estimated 50 million people affected by epilepsy. Its etiology is manifold, and parasitic infections play an important role, specifically onchocerciasis. In Tanzania, Uganda and South Sudan a distinctive form of epilepsy has been described as nodding syndrome (NS), affecting mainly children and causing nodding seizures, mental retardation and debilitating physical development. NS is only described from onchocerciasis endemic areas.

Onchocerciasis is treatable with ivermectin. Control programs using community directed treatment with ivermectin (CDTi) are implemented in endemic countries. This study is designed to test whether the implementation of CDTi decreases the incidence of epilepsy and NS in onchocerciasis endemic regions.

Methods and analysis

The study will be conducted in the Mahenge highlands in Tanzania. Study site selection is based on an in-depth study on epilepsy in that area dating from 1989. CDTi was introduced in 1997. By a door-to-door approach, the population will be screened using a validated questionnaire to identify suspected epilepsy cases. Suspected cases are invited for to neurological examination for case verification. Additonally, ivermectin use will be assessed at household level. Data will be analysed in comparison to the 1989 data to reveal pre- and post-CDTi implementation prevalence and incidence of epilepsy.

Ethics and dissemination

This study has obtained ethical approval from the relevant ethics committees in the countries. 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. Resullts and lessons learned will be published in peer-

reviewed journals, presented at scientific conferences and to the health authorities in Tanzania, and are of relevance for other regions where onchocerciasis associated epilepsy occurs.

Strengths and limitations of this study:

- This study will be carried out in an onchocerciasis endemic area of Tanzania, where nodding syndrome (NS) has been described for the first time and will allow comparison of large scale population based data on epilepsy epidemiology pre- and 27 years post-CDTi.
- Answers key questions of the impact of community directed treatment with ivermectin (CDTi) targeting onchocerciasis and its potential preventive effect on epilepsy, and specifically nodding syndrome.
- Bridging expertise on infectious diseases (onchocerciasis control) and chronic diseases (epilepsy) through the close collaboration of experts in both fields.
- Pre-CDTi epilepsy surveys in Tanzania dates from 1989 and adjusting for potential confounding factors other than CDTi that may influence epilepsy incidence and prevalence in the area need to be carefully assessed.
- Slightly differing methodology might limited the potential to compare data, and focus on villages with high epilepsy burden in the past may lead to an overestimation of the effect of CDTi.

Key words

Epilepsy

Nodding disease

Onchocericasis

Ivermectin

Tanzania

INTRODUCTION

Epilepsy is a chronic disease estimated to affect 50 million people worldwide according to World Health Organisation (WHO) [1]. In general, higher prevalence and incidence is reported from those populations living in low and middle income countries (LMICs) when compared to industrialized countries [2, 3]. In fact, more than 85% of the global burden of epilepsy occurs in those 49% of the global population living in LMICs [4, 5]. The etiology of epilepsy is very diverse and not yet fully understood. Besides birth trauma and head injury involving the brain, also infections can trigger epilepsy. Specifically parasitic infections are in the focus and those most described as associated with the onset of epilepsy are cerebral malaria, neurocysticercosis, echinococcosis, and onchocerciasis [6, 7]. Many of these epilepsy cases could be prevented through timely anti-parasitic treatment. Epilepsy manifests in a variety of seizure types and degrees of intensity [8]. In Tanzania, Uganda and South Sudan, a distinct form of epilepsy has been described as nodding syndrome (NS) [9, 10]. NS is a debilitating epileptic encephalopathy mainly affecting children at the ages of 3 and 18 years [11]. The seizures are characterized by a brief loss of muscle-tone in the neck, leading to repetitive head-nodding [12]. In contrast to other forms of epilepsy, cognitive decline, retardation in cognitive development, and stunted growth in formerly normally developing children are also associated with the disease [13]. So far, NS is solely described from onchocerciasis endemic areas [10]. Since its first description from Tanzania in the 1960s [9] until the mid-1990s, NS was a rare condition with single cases reported predominantly in African countries [14]. Since, an NS epidemic has been observed in the past two decades in northern Uganda [15] and in neighboring South Sudan [13]. The weight of the public health burden caused by NS can be illustrated by the situation in the West Equatorial State in South Sudan, where in the village of Mvolo in South Sudan, over 50% of the families had at least one child affected by epilepsy of the NS type, resulting in one in six children of the village suffering from epilepsy [16].

Health systems services in remote rural regions in Africa are rarely capable to provide continous anti-epiletic treatment to those patients in need [5]. Further more, most health care workers lack training to diagnose and treat persons with epilepsy adequately. The costs associated to seeking professional care by a trained doctor or a neurologist in a major city can often not be afforded by epileptic patients and their families from rural areas. In many societies in Africa, and in addition to the clinical burden, epilepsy is perceived as a possession by evil spirists and hence bears a stigma that puts the whole family of a diseased individual at risk for social isolation [17]. Moreover, the family's economy is negatively impacted by the disease, since an epileptic family member needs specific care and supervision, detaining care takers from their subsistence duties [18].

Many hypotheses on the etiology of NS, and epilepsy in general, have been raised but currently, the causing mechanism is still not fully understood [15]. Several studies have shown epidemiological association of epilepsy with parasitic infections, for NS particularly onchocerciasis [7, 19, 20]. NS is only known to occur in some onchocerciasis endemic areas, and case-control studies have demonstrated a statistically significant higher prevalence of onchocerciasis in individuals with NS than in controls [13, 15]. It is, however, unclear how onchocerciasis might cause NS [20]. Although the eye and the optical nerve are affected when onchocerciasis causes blindness, microfilariae and adult *Onchocerca volvulus* (OV) worms are not generally considered able to invade the central nervous system. Recent research hypothesises that an immunological cross-reaction of onchocerciasis-specific antibodies may provoke a neurotoxic reaction and trigger NS [21].

Onchocerciasis, a treatable neglected tropical disease facing obstacles on the road to elimination

Onchocerciasis is a parasitic disease caused by an infection with the the worm *Onchocerca volvulus* whose filarial larvae are transmitted by blackflies (*Simulidae* spp.). In the final host,

humans, the adult female worms encapsulate in the subcutaneous tissue forming visually detectable nodules. Each female worm releases up to one thousand microfilariae per day. These microfilariae provoke itching, dermatitis and – if left untreated – blindness, which led to the disease also being called river blindness. Blackflies get infected with microfilariae when biting infected humans in proximity to fast flowing rivers, the breeding site of the blackflies. After passing through several larval stages, infected blackflies spread the parasite by biting other people. Onchocerciasis is treatable with ivermectin, a drug with high efficacy and close to nil adverse effects [22]. The drug has a twofold mechanism of action: (i) it kills the microfilariae and (ii) it inhibits their release by the adult female worm for up to two years after a single dose treatment [23]. Hence, ivermectin has a strong impact on reducing transmission. However, ivermectin is not lethal to adult worms and infected persons have to take it annually for up to 15 years, until all the adult worms die [24]. Onchocerciasis is a priority disease scheduled for elimination by 2025 by the WHO [1]. Today, 99% of the globally 37 million people infested live in Africa [1]. In 1995, the African Program for Onchocerciasis Control (APOC) was initiated for the implementation of the onchocerciasis control programme based on community directed treatment with ivermectin (CDTi). APOC was coordinating this activities in endemic areas of 22 African countries [25]. Since May 2016, CDTi control programmes are integrated in the WHO Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN) [26]. CDTi overcomes the limitated performance of weak health systems in rural areas by using an active, strategic involvement of the community [27]. To reach the entire population, ivermectin distribution is organized by trained volunteers in each village, resulting in a large geographical coverage. CDTi, in certain regions combined with the control of the blackfly by larviciding of breeding sites (i.e. fast flowing rivers), resulted in a measurable impact reflected by a remarkable transmission reduction in the past 20 years [28]. But success and effectiveness of these targeted interventions lack comprehensiveness. Certain onchocerciasis endemic regions remain undersupplied or unreachable for CDTi due to political instability, insecurity or armed conflicts [29]. As a

consequence in the war-affected regions of South Sudan and northern Uganda, onchocerciasis control measures were stopped or implementation started only recently. Moreover, misconceptions and the fear of adverse effects result in suboptimal therapeutic coverage and reduce the effectiveness of control programs [30]. Adding to the complexity of onchocerciasis control, in regions where *Loa loa* and onchocerciasis are co-endemic, ivermectin may cause severe adverse effects (encephalopathy, coma or death) in *Loa loa* infected individuals with high microfilariae load [31, 32]. Thus, CDTi implementation in such regions requires additional precautions [33]. This is the case in certain regions of Cameroon and the DRC [34]. Compliance to CDTi programs can also decrease over the years since less direct positive effects can be observed when onchocerciasis prevalence drops, and healthy feeling people may not appreciate the importance of continuing repeated drug treatment [35].

The potential of CDTi to reduce the incidence of epilepsy in onchocerciasis endemic regions

The endemicity of onchocerciasis and the prevalence and incidence of epilepsy are influenced by a multitude of factors (Figure 1). These factors are of uncontrollable nature (climate, environment, ecology), and of controllable nature (prevention and intervention programs, access to health care and treatment). The controllable factors can be addressed. i.e. onchocerciasis cases can be cured and new onchocerciasis associated epilepsy (OAE) cases prevented.

<<<<<<Figure 1 near here>>>>>>

An epidemiological association between epilepsy and onchocerciasis was first documented by Boussinesq *et al.* (2002) in a case-control study performed in 1991-92 in the Mbam valley in Cameroon [20]. This study demonstrated a significantly higher microfilarial load in persons with epilepsy than in controls. During the past 25 years, study results from further onchocerciasis-

endemic African countries underline this association [19, 36]. Case-control studies in northern Uganda and South Sudan produced similar results, showing a higher prevalence of onchocerciasis in NS cases compared to non-epileptic controls [13, 15]. Both areas were - and South Sudan still is - affected by armed conflicts. In 2012, after the conflict ended in northern Uganda, the NS epidemic was investigated. Following on, epilepsy treatment was provided [37], biannual CDTi was implemented and larviciding of major rivers was carried out. Since 2013 the NS epidemic in northern Uganda has reportedly been halted, although the situation has not yet been empirically assessed [38]. In contrast in South Sudan, where CDTi was stopped because of insecurity, new cases of NS continue to appear [16]. Based on the observations from northern Uganda, it has been suggested that ivermectin may reduce the incidence of NS and other forms of epilepsy in onchocerciasis endemic areas [39]. Considering that 30% of the globally 37 million individuals infected with onchocerciasis do not have access to effective treatment [1], and that 1% of those develop epilepsy (equivalent to the estimated excess prevalence of epilepsy over non-onchocerciasis areas) [7], the number of excess cases of epilepsy attributed to onchocerciasis could exceed 100'000. It is hypothesized that with annual administration of ivermectin these cases are preventable.

Recent observations in the DRC, and northern Uganda suggest that optimal coverage of ivermectin as an onchocerciasis control intervention, may stop the incidence of NS and other forms of OAE [39].

In Tanzania, Morogoro region is among the five regions where onchocerciais is endemic. The region presents four foci: Uluguru mountain, Ulanga/Mahenge, Kilosa and the Nguru mountain focus [40]. The Ulanga district, specifically the Mahenge area, was known for its high endemicity of onchocerciasis since the early last century [41]. Mass drug administration (MDA) using CDTi on annual bases as recommended by WHO for elimination of onchocerciasis was introduced in the Mahenge area in 1997 [42]. Before CDTi was implemented, onchocerciasis prevalence in the area was estimated at 69% [25]. During the last rapid epidemiological mapping of onchocerciasis

(REMO) in 10 villages during 2008/2009, onchocerciasis prevalence was estimated at 20.3% [43].

Epilepsy in the Mahenge area, Tanzania

In the 1960s, Aall-Jilnek was the first to report a high burden of epilepsy in the Mahenge area, and also described the first NS cases [9]. In 1989, Rwiza et al. carried out a population based survey to determine prevalence and incidence of epilepsy in the Ulanga district and found an annual incidence of 73.3 new cases in 100 000. The prevalence was 10.2 cases in 1000, and varied between villages from 5.1 to 37.1 cases in 1000. Those villages with the highest prevalence were located in the Mahenge highlands (Table 1) [44].

Table 1. Population size and prevalence of epilepsy in those villages of the Ulanga district, Tanzania, showing the highest prevalence in 1989, as reported by Rwiza et al. in 1992

Name of Village	Population size	Number of Epilepsy cases	Prevalence per 1000 people
Sali$^{\delta}$	1282	14	10.9
Mdindo$^{\delta}$	539	20	37.1
Vigoi$^{\delta}$	1822	23	12.6
Lupiro	1697	17	10
Misegezi	1667	18	10.8
Total	**7007**	**92**	**13.13**

$^{\delta}$Located in Mahenge highlands

Numberless rivers and streams have their source in the Mahenge highlands, and these provide breeding sites for the blackfly, the intermediate host of *Onchocerca volvulus.*

Goal

This study aims at investigating the effect of CDTi for the treatment and prevention of onchocerciasis on the incidence of epilepsy and NS. Bearing the association of onchocerciasis

and epilepsy in mind, it is expected that, since the onchocerciasis interventions have been launched, the prevalence of epilepsy has diminished and the incidence has decreased accordingly. If CDTi for onchocerciasis control has an effect on the onset of epilepsy and NS, it is expected to observe an age shift in epilepsy cases when compared to the study from 1989 by Rwiza et al. (1992) [44].

Objectives

The main objective of this study is to identify the potential effect of long-term onchocerciasis control measures using CDTi on the prevalence and incidence of epilepsy and NS in selected villages in the Mahenge area of the Ulanga district in Tanzania. Based on the epidemiological data available from 1989 by Rwiza et al., a comparison of prevalence and incidence from 2017 in different age groups will allow testing for an association of epilepsy with onchocerciasis interventions, specifically annual CDTi (Table 2).

Table 2. The pre- and post-CDTi epilepsy study periods and estimated epilepsy prevalence and incidence in the Mahenge area, Ulanga district in Tanzania.

Mahenge area, Ulanga district, Tanzania		
Year of epilepsy study data collection	1989 [44]	2017
Status of CDTi at study year	Pre-CDTi	Post-CDTi (ongoing since 1997)
Estimated epilepsy prevalence	20.2/1000	14/1000*
Estimated epilepsy incidence	146/100'000	81.7/100'000**

*Median epilepsy prevalence [4] and **incidence in LMICs [3]

Specific objectives

1. To determine the prevalence of all forms of epilepsy in selected villages of the Mahenge area and compare the related data from 2017 to the 1989 data.

2. To determine the incidence rate of annual new onset cases of all forms of epilepsy in the Mahenge area and compare the related data from 2017 to the 1989 data.

3. To assess the ivermectin coverage and the onchocerciasis prevalence with a REMO assessment in selected villages of the Mahenge area.

4. To determine the level of ongoing onchocerciasis transmission by a serological survey among children in selected villages of the Mahenge area.

5. To investigate the potential difference in clinical appearance of epilepsy in patients with negative onchocerciasis serology to patients with positive onchocerciasis serology.

Justification

The initial population based study from 1989 established the baseline prevalence of all forms of epilepsy in the Ulanga district, Tanzania. With this 2017 population based study we aim to further evaluate the prevalence and the incidence rate of new epilepsy cases, including a complete case ascertainment of all forms of epilepsy encountered, and compare the data to the 1989 data. The study includes an assessment of the ivermectin coverage in the study villages, and a concurrent REMO assessment will provide the onchocerciasis prevalence estimate for 2017.

METHODS AND ANALYSIS

Study site and study population

The study will be carried out in the Mahenge area of the Ulanga distict, Morogoro region in south-eastern Tanzania, a mountainous area with fast flowing rivers. The population lives on subsistence agriculture, livestock breeding, and also mining is practiced. Those villages that had high epilepsy prevalence during the 1989 study will be selected for this presented study, namely Mdindo, Vigoi and Misegezi [44].

Epilepsy and NS prevalence / incidence study

Study design

This study is designed as cross-sectional, population-based survey. A two-stage approach will be applied for case identification within the villages. The gold-standard in neuro-epidemiological surveys to identify epilepsy cases in LMICs is the door-to-door approach and this will be applied [45]. All inhabitants of the selected villages will be eligible for participation and will be included in the questionnaire screening survey. Due to well described limitations of questionnaire studies on epilepsy (stigma leading to concealment of cases, recall bias, absence of clear terminology for epilepsy and seizures) [3, 45], and to increase sensitivity of case ascertainment, key informants who are likely to be aware of persons with epilepsy in the village will additionally be consulted [45]. These may be health workers, traditional healers, teachers, community leaders or such [46]. In a second stage, suspected cases of all forms of epilepsy identified during the household screening survey will be further invited for clinical examination by a neurologist. The examination will include neurological tests and a detailed interview for case verification. For all suspected epilepsy cases their serological status will be determined using onchocerciasis rapid diagnostic test (Ov16 RDT) (Standard diagnostics, Inc., Gyeonggi-do, Republic of Korea).

Sample size calculation

According to Rwiza et al., 1992, the average prevalence of epilepsy in five villages with high prevalence was 1.313% (Table 1). If we assume a reduction in the prevalence by 33.3% to be able to compare the prevalence at a power of 80% and 95% confidence level, the minimal sample size of 4,746 individuals will be required. Assuming a participation of 80%, a minimum source population of 5,933 is necessary to optain optimal sample size. Since 1989 the population in the villages with high epilepsy prevalence has increased (Table 3). For this survey we will include only the population or part of the population of the villages with the highest epilepsy prevalence in 1989. The total population of the selected villages of Mdindo, Vigoi and Misegezi is about 6'600.

Table 3. List of the study villages in the Mahenge highlands, Tanzania, and their population size in 1989 and in 2016.

Name of Village (1989)	Population size (1989)	Population size (2016)
Mdindo	539	1536
Vigoi**	1822	2572
Misegezi	1667	3658*
Matumbala[#]	-----	1426
Total	**6977**	**9192**

**Population projection based on the growth rate of Vigoi village;* **Vigoi has undergone a village separation and was split in two villages in 2010 (Vigoi and [#]Matumbala)

Data collection at community level

The community survey will commence by a questionnaire interview with the village authorities on demographic topics, and with village health workerw to address general questions on the status of epilepsy and epilepsy treatment in the village. Following, a complete door-to-door active screening for suspected epilepsy cases at village level will be performed. The interviewer team will be trained on how to conduct the active search for epilepsy cases using a pre-tested, validated screening questionnaire targeting epilepsy by 5 specific questions [47, 48]. The questionnaire will be translated in Kiswahili and will include the locally used terms for the two respective conditions (epilepsy: *kifafa,* NS: *kusinzia kichwa*). To ascertain completeness and to ensure the best collaboration with the village population, the interviewer team will be accompagnied to all households by local village health workers in each individual village. Together with the screening data, the geographical coordinates of the participating households will be collected for the mapping and geospatial analysis of cases (proximity to rivers, potential clustering).

Case verification and validation

All suspected epilepsy cases identified during the door-to-door survey will be verified by a medical doctor/neurologist. The neurologist will perform a detailed anamnesis on all suspected

NS and epilepsy cases. In case of confirmation of the epilepsy and / or NS diagnosis, the neurologist will perform a medical examination and administer a detailed questionnaire on the type of epilepsy. Newly diagnosed epilepsy cases will be referred to an epilepsy treatment centre. In case the person is already followed in a treatment centre, permission will be asked to review the medical information available in the treatment centre to record epilepsy diagnosis and epilepsy treatment history.

Definitions:

- A case of epilepsy will be defined according to the International League Against Epilepsy (ILAE) guidelines as a patient who had (1) at least two times, unprovoked and without fever, lost consciousness with convulsions with a minimal time difference of 24h between the two events or (2) one unprovoked seizure and a probability of future seizures similar to the general recurrence risk after 2 unprovoked seizures [49, 50].
- A case will be considered as active epilepsy if the patient is receiving epilepsy treatment or, if without anti-epileptic treatment, the patient presented at least one seizure during the last 5 years.
- A case of suspected NS will be defined as a person who presented with episodes of decreased consciousness during which the head dropped forward repeatedly.
- New cases of epilepsy will be defined as cases that appeared within the last 12 month proceeding the study period.

Onchocerciasis prevalence study

Study design

This study includes two parts. I part one, the aim is to determine the onchocerciasis prevalence in the selected villages after 20 years of CDTi by performing the WHO proposed REMO methodology [51]. In brief, in each study village, 50 adults aged at least 20 years old and resident in the community for at least 10 years, will be invited to participate. They will be examined for the

presence of onchocerciasis nodules (subcutaneous nodules or deep, painless, firm, mobile nodules over bony prominences: pelvic girdle, costal grid, knees, and skull).

The second part aims at determining the level of ongoing transmission of onchocerciasis in the selected villages. Therefore, serological testing for onchocerciasis will be done in all children at ages 7-10 years old, using the onchocerciasis specific Ov16 RDT (Standard diagnostics, Inc., Gyeonggi-do, Republic of Korea). According to the National Census survey in 2012, the population of children aged 7-10 years represented 11.6% of the population in Ulanga district. Estimated population of children aged 7-10 years from the four selected villages is 1065 of which 746 (~70%) are anticipated to participate in the survey giving a power of 85% in detecting the prevalence between 0.8 to 2% at 5% significance level (Table 4).

Table 4. Estimated number of children aged 7 to 10 years old in the study villages in the Ulanga district, Tanzania, in 2016.

Name of Village	Population size (1989)	Population size (2016)	Estimated Population aged 7-10 yrs	Estimated 80% of children (7-10 yrs)
Mdindo	539	1536	178	143
Vigoi**	1822	2572	298	239
Misegezi	1667	3658*	424*	339*
Matumbala[#]	-----	1426	165	132
Total	**7007**	**9192**	**1065**	**745**

**Population projection based on the growth rate of Vigoi village* **Vigoi has undergone a village separation and was split in two villages in 2010 (Vigoi and [#]Matumbala)

Data collection procedures

Data collection will be done using tablet computers. All data collection forms are developed in the open source software 'Open Data Kit' (ODK, https://opendatakit.org/). Interviewers will be trained in how to perform tablet computer based surveys. A technical data coordinator is assigned to

guarantee completeness and quality of data, and to assure daily data transfer from each tablet computer to the central server for data security.

Data management and analysis

The prevalence of epilepsy and NS will be computed as a ratio of the number of epilepsy and NS cases per total number of people registered in the households visited. The epidemiological distribution of families with and without epilepsy will be compared. The ratio of new epilepsy and NS cases per number of people registered in the household will be compared between villages and correlated with onchocerciasis prevalence data. Demographic and clinical characteristics of persons with epilepsy having a positive Ov16 serology will be compared to epilepsy cases with negative Ov16 serology.

Data storage and handling

All data files will be centralised and stored in a secure central server. Name-linked information on participants and ID codes will remain confidential and will be used only to communicate clinical results to participants for their respective treatments.

Ethical considerations

The study will be carried out adhering to the principles of the Declaration of Helsinki; to all other applicable regulations, and according to established international scientific standards.

After having optainded ethics approval from the responsible ethics committees in Belgium and Tanzania, before the activities start, the research team will hold meetings with village and community leaders and health workers of the selected villages. The procedure, purpose and specific aim of the present study will be explained and discussed in regard to the potential risks and benefits for the community. Community leaders, village health workers and researcher will maintain the initially established communication for the entire duration of the study. The dissemination of results will be organized in a similar way as the initial meeting.

As approved by the relevant ethics committees, only participants who provide written informed consent will be enrolled in the study. Participant information sheets and consent forms will be

available in English and Kiswahili. In case of illiteracy, information sheets and consent forms will be read to the participant in the presence of a withness. All participants are permitted to withdraw from the study activities, without reason, at any time. All personal information, samples and test results will be encoded and treated confidentially. Spatial information will be presented in a way that no individual data can be extrapolated. People identified with untreated epilepsy or with interrupted treatment will be referred to the treatment centre and receive advice for care and support.

DISCUSSION

The central research question of this study is to determine whether mass drug administration of ivermectin using the CDTi methodology has the potential to prevent the onset of onchocerciasis associated epilepsy. The expected results will contribute to a better understanding of the linkage between the onset of epilepsy and NS in particular, onchocerciasis and the impact of CDTi. In northern Uganda, an NS epidemic stopped after introducing a programme combining CDTi and larviciding of the main rivers. This study will be the first to investigate systematically whether CDTi alone may reduce epilepsy in an onchocerciasis endemic region.

With the Ov16 serological survey among children between the age 7-10 we will be able to estimate the ongoing transmission of onchocerciasis in the Mahenge area after 20 years of CDTi. So far, the onchocerciasis control program in the study area was monitored based on annual ivermectin coverage data provided by the community directed distributors of ivermectin. With this study we will obtain ivermectin coverage data by interviewing the population. Moreover, by performing an Ov16 seroprevalence study among children under the age of 10 years we will obtain a real-time estimate of the level of ongoing transmission of onchocerciasis. Hopefully, results will show a low Ov16 seroprevalence in children and a decreased prevalence of epilepsy since 1989. In case a high Ov16 seroprevalence is found this will suggest that the CDTi programme was performing suboptimal and/or that ivermectin resisitance may have developed.

In pre- and post-CDTi comparison it is possible that observed differences in epilepsy prevalence and incidence are not related to the intervention (CDTi) but to some of the other factors (e.g. those mentioned in Fig. 1) that might have changed over time. However, a site visit to the Mahenge study site revealed that the village population had increased by a factor of 3, but there was no important in- or out migration or any other major change in lifestyle of the population or another major environmental change. In all the villages included in the study, such potential changes will be carefully assessed to control for potential confounding factors. Families who migrated into the study area after the implementation of CDTi will not be included in the analyses.

Outlook

This study aims to unreveal the potential effect of CDTi on reducing the incidence of epilepsy in onchocerciasis endemic areas. The results will provide an evidence base for to strengthen CDTi programs for the elimination goal for onchocerciasis and that has the power to prevent new cases of epilepsy associated to onchocerciasis. The results and lessons learned from this study will be published in open access journals, as well as presented at conferences and shared with all interested health authorities in Tanzania and beyond.

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Authors' contribution

All listed authors contributed to the development of the study design, essential study documents and study tools. According to their different areas of expertise, the authors critically revised specific parts of this manuscript: HG wrote the manuscript; HG, BM, PS, RC developed the study protocol; WM, MM, WM developed and approved the neurological study protocol and the survey

tools; HG, BM, AK, developed the methodology and tools for the digital data collection; HG, BM, RC visited the study sites.

Competing interests

The authors declare that they have no competing interests.

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Ethics approval

The protocol has received ethical approval from the ethics committee of the University of Antwerp, Antwerp, Belgium (29.08.2016) and the National Institut of Medical Research (NIMR) ethical committee Dar es Salaam, Tanzania (24.08.2016).

Data sharing statement

The data for this study will be collected in 2017 and published in peer-reviewed open access journals. Additionally, data will be extracted from a published article (Rwiza et al., 1992) as described in details the methods section.

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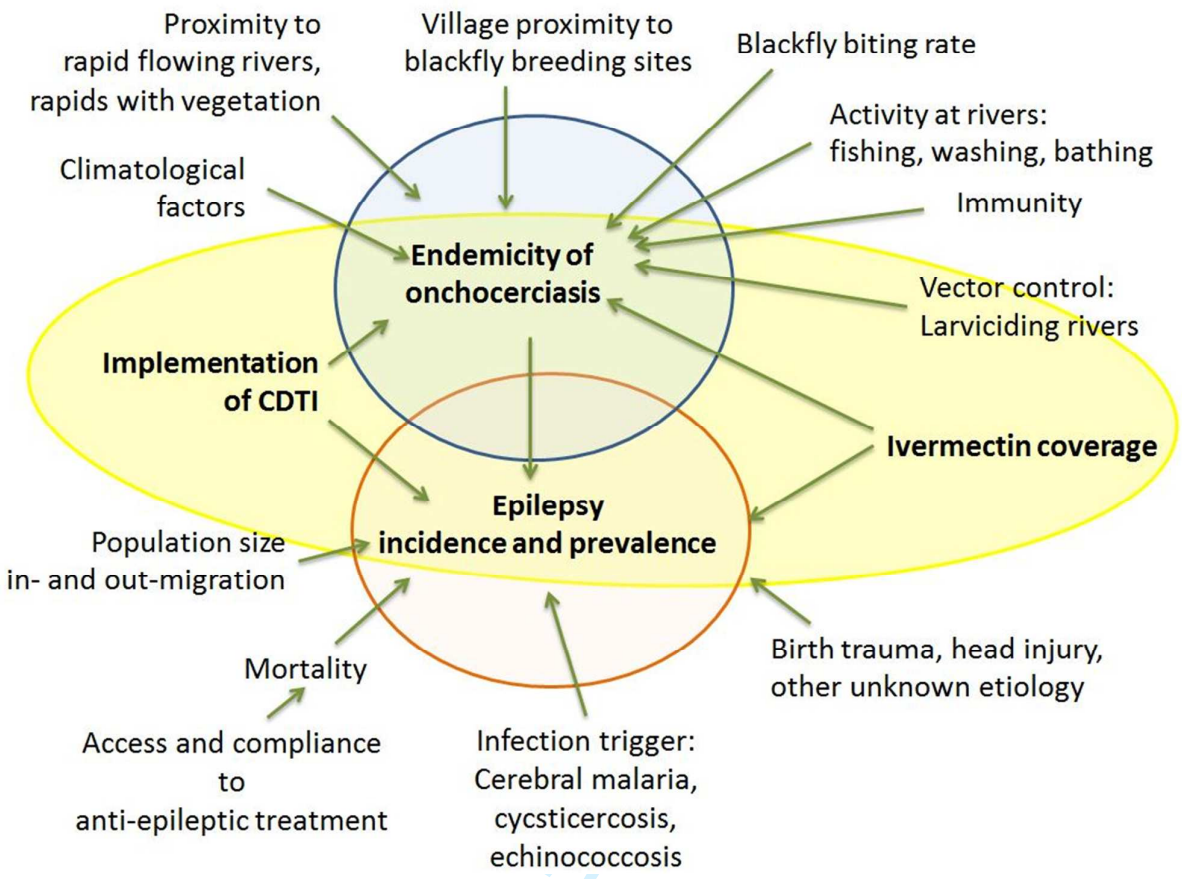

Figure 1. There is an extensive network of controllable and uncontrollable factors influencing the level of onchocerciasis endemicity and prevalence and incidence of associated epilepsy. This project investigates the effect of community directed treatment with ivermectin on the endemicity of onchocerciasis and the incidence and prevalence of epilepsy.

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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

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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

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ABSTRACT

Introduction

Worldwide, there are an estimated 50 million people affected by epilepsy. Its etiology is manifold, and parasitic infections play an important role, specifically onchocerciasis. From onchocerciasis endemic areas a distinctive form of epilepsy has been described as nodding syndrome, affecting children and causing nodding seizures, mental retardation and debilitating physical development. Onchocerciasis is treatable with ivermectin. Control programs using community directed treatment with ivermectin (CDTI) are implemented in endemic countries. This study is designed to contribute to a better understanding of the linkage between the onset of epilepsy, onchocerciasis and CDTI. Comparing the epidemiological data on epilepsy and onchocerciasis from pre-CDTI and 20 years after its introduction will allow identifying a potential impact of ivermectin on the onset of epilepsy.

Methods and analysis

The study will be conducted in the Mahenge highlands in Tanzania. Study site selection is based on an in-depth study on epilepsy in that area dating from 1989. CDTI was introduced in 1997. By a door-to-door approach, the population will be screened for epilepsy using a validated questionnaire. Suspected cases will be invited for a neurological examination for case verification. Onchocerciasis prevalence will be assessed by a rapid epidemiological assessment for prevalence. As an indicator for ongoing transmission, children younger than 10 years of age will be tested for Ov16 antibodies. Ivermectin use will be assessed at household level. Epilepsy data will be analysed in comparison to the 1989 data to reveal pre- and post-CDTI implementation prevalence and incidence.

Ethics and dissemination

The protocol has received ethical approval from the ethics committees of the University of Antwerp, Belgium and of the National Institut of Medical Research (NIMR), Dar es Salaam,

Tanzania. The findings will be published in peer-reviewed journals, and presented to the health authorities in Tanzania, at national, regional and village level.

Strengths and limitations of this study:

- This study will allow comparison of large scale population based data on epilepsy epidemiology in Mahenge over 28 years.
- This comparison will answer key questions on the potential impact of community directed treatment with ivermectin (CDTI) targeting onchocerciasis on epilepsy prevalence and incidence.
- The pre-CDTI epilepsy survey in Tanzania dates from 1989 and adjusting for potential confounding factors other than CDTI that may influence epilepsy incidence and prevalence in the area need to be carefully assessed.
- Minor differences in the study methodology used in 1989 and 2017 will limit the data comparison to prevalence and incidence at village level.
- Focusing on villages with high epilepsy burden in the past may lead to an overestimation of the potential impact of CDTI.

Key words

Epilepsy

Nodding disease

Onchocericasis

Ivermectin

Tanzania

INTRODUCTION

Epilepsy in onchocerciasis endemic regions in Africa

Epilepsy is a chronic disease estimated to affect 50 million people worldwide according to World Health Organisation (WHO).[1] In general, higher prevalence and incidence are reported from those populations living in low and middle income countries (LMICs) when compared to industrialized countries.[2 3] In fact, more than 85% of the global burden of epilepsy occurs in the people living in LMICs.[4 5] The etiology of epilepsy is very diverse and not yet fully understood. Besides birth trauma and head injury involving the brain, infectious diseases can trigger epilepsy as well. Several parasitic infections are associated with epilepsy such as cerebral malaria, neurocysticercosis, echinococcosis, and onchocerciasis.[6-8] Many of these epilepsy cases could be prevented through timely anti-parasitic treatment. Epilepsy manifests in a variety of seizure types and degrees of intensity.[9] In Tanzania, Uganda and South Sudan, a distinct form of epilepsy has been described as nodding syndrome (NS).[10-12] NS is a debilitating epileptic disorder mainly affecting children at the ages of 3 and 18 years.[13] The seizures are characterized by a brief loss of muscle-tone in the neck, leading to repetitive head-nodding.[14] NS is often associated with cognitive decline, and sometimes with stunted growth.[15] So far, NS is solely described in onchocerciasis endemic areas.[10] Since its first description from Tanzania in the 1960s until the until the mid-1990s NS was a rare condition in African countries.[16 17]A NS epidemic has been observed in the past two decades in northern Uganda and in neighboring South Sudan.[18 15] The weight of the public health burden caused by NS 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 affected by epilepsy of the NS type, resulting in one in six children of the village suffering from epilepsy.[19]

Health systems services in remote rural regions in Africa are rarely capable to provide continous anti-epileptic treatment to those patients in need.[5] Further more, most health care workers lack training to diagnose and treat persons with epilepsy adequately. In many societies in Africa, and in addition to the clinical burden, epilepsy is perceived as a possession by evil spirits and hence bears a stigma that puts the diseased individual and his family at risk for social isolation.[20]

Moreover, the family's economy is negatively impacted by the disease, since an epileptic family member needs specific care and supervision, detaining care takers from their subsistence duties.[21]

NS only occurs in onchocerciasis endemic areas. An epidemiological association between epilepsy and onchocerciasis was first reported from western Uganda in the early 1990ties.[22 23] A case-control study performed in 1991-92 in the Mbam valley in Cameroon demonstrated a significantly higher microfilarial load in persons with epilepsy than in controls.[24] Study results from other onchocerciasis-endemic African countries underline this association.[8 25] Case-control studies in northern Uganda and South Sudan focusing on NS patients produced similar results, showing a higher prevalence of onchocerciasis in NS cases compared to non-epileptic controls.[15 18] It is, however, unclear how onchocerciasis might cause NS. Although the eye and the optical nerve are affected when onchocerciasis causes blindness, microfilariae and adult *Onchocerca volvulus* worms are not generally considered to be able to invade the central nervous system. Recent research hypothesises that an immunological cross-reaction of onchocerciasis-specific antibodies may provoke a neurotoxic reaction and trigger NS.[26]

Onchocerciasis, a treatable neglected tropical disease facing obstacles on the road to elimination

Onchocerciasis is a parasitic disease caused by an infection with the the worm *O. volvulus* whose filarial larvae are transmitted by blackflies (*Simulidae* spp.). In the final host, humans, the adult female worms encapsulate in the subcutaneous tissue forming visually detectable nodules. Each female worm releases up to one thousand microfilariae per day. These microfilariae provoke itching, dermatitis and – if left untreated – blindness, which led to the disease also being called river blindness. Blackflies get infected with microfilariae when biting infected humans in proximity to fast flowing rivers, the breeding site of the blackflies. After passing through several larval stages, infected blackflies spread the parasite by biting other people. Onchocerciasis is

treatable with ivermectin.[27] The drug has a twofold mechanism of action: (i) it kills the microfilariae and (ii) it inhibits their release by the adult female worm for up to two years after a single dose treatment.[28] Hence, ivermectin has a strong impact on reducing transmission. However, ivermectin is not lethal to adult worms and infected persons have to take it annually for up to 15 years.[29] Onchocerciasis is a priority disease scheduled for elimination by 2025 by the WHO. Today, 99% of the globally 37 million people infested live in Africa.[1] In 1995, the African Program for Onchocerciasis Control (APOC) was initiated for the implementation of the onchocerciasis control programme based on community directed treatment with ivermectin (CDTI). APOC was coordinating these activities in endemic areas of 22 African countries.[30] Since May 2016, CDTI control programmes are integrated in the WHO Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN).[31] CDTI overcomes the limited performance of weak health systems in rural areas by using an active, strategic involvement of the community.[32] To reach the entire population, ivermectin distribution is organized by trained volunteers in each village, resulting in a large geographical coverage. CDTI, in certain regions combined with the control of the blackfly by larviciding of breeding sites (i.e. fast flowing rivers), resulted in a measurable impact reflected by a remarkable transmission reduction in the past 20 years.[33] But success and effectiveness of these targeted interventions lack comprehensiveness. Certain onchocerciasis endemic regions remain undersupplied or unreachable for CDTI due to political instability, insecurity or armed conflicts.[34] As a consequence in the war-affected regions of South Sudan and northern Uganda, onchocerciasis control measures were stopped or implementation started only recently. Moreover, misconceptions and the fear of adverse effects result in suboptimal therapeutic coverage and reduce the effectiveness of the control program.[35] Adding to the complexity of onchocerciasis control, in regions where *Loa loa* and onchocerciasis are co-endemic, ivermectin may cause severe adverse effects (encephalopathy, coma or death) in *Loa loa* infected individuals with high microfilariae load.[36 37] Thus, CDTI implementation in such regions requires additional precautions.[38] This is the case in certain regions of Cameroon and the

DRC.[39] Compliance to CDTI programs can also decrease over the years since less direct positive effects can be observed when onchocerciasis prevalence drops, and healthy feeling people may not appreciate the importance of continuing repeated treatment.[40]

The potential of CDTI to reduce the incidence of epilepsy in onchocerciasis endemic regions

The endemicity of onchocerciasis and the prevalence and incidence of epilepsy are influenced by a multitude of factors (Figure 1). These factors are of uncontrollable nature (climate, environment, ecology), and of controllable nature (prevention and intervention programs, access to health care and treatment). The controllable factors can be addressed. I.e. onchocerciasis cases can be cured and hence new onchocerciasis associated epilepsy (OAE) cases can be prevented.

<<<<<<Figure 1 near here>>>>>>

The two heavily affected regions of the last decades, Northern Uganda and South Sudan, were – and South Sudan still is - affected by armed conflicts. In 2012, after the conflict ended in northern Uganda, the NS epidemic was investigated. Following on, epilepsy treatment was provided, biannual CDTI was implemented and larviciding of major rivers was carried out.[41] Since 2013 the NS epidemic in northern Uganda has reportedly been halted.[42] In contrast in South Sudan, where CDTI was stopped because of insecurity, new cases of NS continue to appear.[19] Based on the observations from northern Uganda, it has been suggested that ivermectin may reduce the incidence of NS and other forms of epilepsy in onchocerciasis endemic areas.[43] Considering that 30% of the globally 37 million individuals infected with onchocerciasis do not have access to effective treatment, and that 1% of those develop epilepsy (equivalent to the estimated excess

prevalence of epilepsy over non-onchocerciasis areas) the number of excess cases of epilepsy attributed to onchocerciasis could exceed 100'000.[17]

Recent observations in the DRC and northern Uganda suggest that optimal treatment coverage of ivermectin may stop the incidence of NS and other forms of OAE.[43]

Aim

The population based study from 1989 by Rwiza et al. established the baseline prevalence of all forms of epilepsy in the Ulanga district, Tanzania.[44] With this 2017 population based study we aim to further evaluate the prevalence and the incidence rate of new epilepsy cases, including a complete case ascertainment of all forms of epilepsy encountered, and compare the data to the 1989 data (Table 1).

Table 1. The pre- and post-CDTI epilepsy study periods and estimated epilepsy prevalence and incidence in the Mahenge area, Ulanga district in Tanzania.

	Mahenge area, Ulanga district, Tanzania	
Year of epilepsy study data collection	1989[44]	2017
Status of CDTI at study year	Pre-CDTI	Post-CDTI (ongoing since 1997)
Estimated epilepsy prevalence	20.2/1000	14/1000*
Estimated epilepsy incidence	146/100'000	81.7/100'000**

*Median epilepsy prevalence and **incidence in low and middle income countries [43]

Objective

The main objective of this study is to identify the potential impact of long-term onchocerciasis control measures using CDTI on the prevalence and incidence of epilepsy in selected villages in the Mahenge area of the Ulanga district in Tanzania.

Specific objectives

1. To determine the prevalence of all forms of epilepsy in selected villages of the Mahenge area and compare the related data from 2017 to the 1989 data.
2. To determine the incidence rate of annual new onset cases of all forms of epilepsy in the Mahenge area and compare the related data from 2017 to the 1989 data.
3. To assess the ivermectin coverage and the onchocerciasis prevalence with a REMO assessment in selected villages of the Mahenge area.
4. To determine the level of onchocerciasis transmission by a serological survey using a rapid test among children in selected villages of the Mahenge area.
5. To investigate the potential difference in clinical appearance of epilepsy in patients with negative onchocerciasis serology to patients with positive onchocerciasis serology.

METHODS AND ANALYSIS

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. Data collection is carried out between January and September 2017. Analysis is planned to be finalized by end of 2017, to allow publishing of the results in 2018.

Study site

The study will be carried out in the Mahenge area of the Ulanga distict, Morogoro region in south-eastern Tanzania, a mountainous area with fast flowing rivers.

Epilepsy in the Mahenge area

In the 1960s, Aall-Jilnek was the first to report a high burden of epilepsy in the Mahenge area, and also described the first NS cases.[16] In 1989, Rwiza et al. carried out a population based survey to determine prevalence and incidence of epilepsy in the Ulanga district and found an annual incidence of 73.3 new cases in 100 000. The prevalence was 10.2 cases in 1000, and

varied between villages from 5.1 to 37.1 cases in 1000. Those villages with the highest prevalence were located in the Mahenge highlands (Table 2).[44]

Table 2. Population size and prevalence of epilepsy in those villages of the Ulanga district, Tanzania, showing the highest prevalence in 1989, as reported by Rwiza et al. in 1992

Name of Village	Population size	Number of Epilepsy cases	Prevalence per 1000 people
Sali$^{\delta}$	1282	14	10.9
Mdindo$^{\delta}$	539	20	37.1
Vigoi$^{\delta}$	1822	23	12.6
Lupiro	1697	17	10
Misegezi	1667	18	10.8
Total	**7007**	**92**	**13.13**

$^{\delta}$Located in Mahenge highlands

Onchocerciasis and CDTI in the Mahenge area

The Morogoro region is among the five regions where onchocerciais is endemic. The region presents four foci: Uluguru mountain, Ulanga/Mahenge, Kilosa and the Nguru mountain focus.[45] The Ulanga district, specifically the Mahenge area, was known for its high endemicity of onchocerciasis since the early last century.[46] Mass drug administration using annual CDTI was introduced in the Mahenge area in 1997.[47] Before CDTI was implemented, 58.6% of a sample of 482 inhabitants of the Mahenge area were found to have a microfilariae positive skin snip.[48] Twelve years later, in 2009 during the rapid epidemiological mapping of onchocerciasis (REMO) in 10 villages in the Mahenge area, the percentage of persons with a microfilariae positive skin snip has dropped to 21.9%, with a mean village prevalence of 8.3%.[33]

Study population

Those villages that had high epilepsy prevalence during the 1989 study will be selected for this study, namely Mdindo, Vigoi and Misegezi.[44] Of these villages, the entire population will be included in the study.

Epilepsy and NS prevalence / incidence study

Study design

The study is designed as cross-sectional, population-based study, following atwo-stage approach for epilepsy case identification at village level. The gold-standard in neuro-epidemiological surveys to identify epilepsy cases in LMICs is the door-to-door approach and this will be applied.[49] All inhabitants of the selected villages will be eligible for participation and will be included in the questionnaire screening survey. Due to well described limitations of questionnaire studies on epilepsy (stigma leading to concealment of cases, recall bias, absence of clear terminology for epilepsy and seizures) and to increase sensitivity of case ascertainment, key informants who are likely to be aware of persons with epilepsy in the village will additionally be consulted [3] [49] These may be health workers, traditional healers, teachers, or community leaders.[50]

In a second stage, suspected cases of all forms of epilepsy identified during the household screening survey will be further invited for clinical examination by a neurologist. The examination will include neurological tests and a detailed interview for case verification. In verified epilepsy cases the onset of the seizures will be determined and possible etiologic factors will be investigated, such as birth trauma, head injury, meningo-encephalitis, cerebral malaria, neurocystocercosis, and the history of onchocerciasis. For all suspected epilepsy cases their serological status will be determined using onchocerciasis rapid diagnostic test (Ov16 RDT) (Standard diagnostics, Inc., Gyeonggi-do, Republic of Korea).

Sample size calculation

According to Rwiza et al., 1992, the average prevalence of epilepsy in five villages with high prevalence was 1.313% (Table 2). If we assume a reduction in the prevalence by 33.3% to be able to compare the prevalence at a power of 80% and 95% confidence level, the minimal sample size of 4,746 individuals will be required. Assuming a participation of 80%, a minimum source population of 5,933 is necessary to obtain optimal sample size. Since 1989 the population in the villages with high epilepsy prevalence has increased (Table 3). For this survey we will include only the population of the villages with the highest epilepsy prevalence in 1989. The total population of the selected villages of Mdindo, Vigoi and Misegezi in 2016 is about 7,766.

Table 3. List of the study villages in the Mahenge highlands, Tanzania, and their population size in 1989 and in 2016.

Name of Village (1989)	Population size (1989)	Population size (2016)
Mdindo	539	1536
Vigoi**	1822	2572
Misegezi	1667	3658*
Total	**4028**	**7766**

**Population projection based on the growth rate of Vigoi village;*

Data collection at community level

The community survey will commence by a questionnaire interview with the village authorities on demographic topics, and with village health workers to address general questions on the status of epilepsy and epilepsy treatment in the village. Following, a complete door-to-door active screening for suspected epilepsy cases at village level will be performed. The interview team will be trained on how to conduct the active search for epilepsy cases using a pre-tested, validated screening questionnaire targeting epilepsy by 5 specific questions (provided in the supplementary material).[51 52] The questionnaire will be translated in Kiswahili and will include the

locally used terms for the two respective conditions (epilepsy: *kifafa,* NS: *kusinzia kichwa*). To ascertain completeness and to ensure the best collaboration with the village population, the interviewer team will be accompanied to all households by local village health workers in each individual village. Together with the screening data, the geographical coordinates of the participating households will be collected for the mapping and geospatial analysis of cases (proximity to rivers, potential clustering).

Case verification and validation

All suspected epilepsy cases identified during the door-to-door survey will be verified by a neurologist. The neurologist will perform a detailed anamnesis on all suspected NS and epilepsy cases. In case of confirmation of the epilepsy and / or NS diagnosis, the neurologist will perform a medical examination and administer a detailed questionnaire on the type of epilepsy. Newly diagnosed epilepsy cases will be referred to an epilepsy treatment centre. In case the person is already followed in a treatment centre, permission will be asked to review the medical information available in the treatment centre to record epilepsy diagnosis and epilepsy treatment history.

Definitions:

- A case of epilepsy will be defined according to the International League Against Epilepsy (ILAE) guidelines as a patient who had (1) at least two times, unprovoked and without fever, lost consciousness with convulsions with a minimal time difference of 24h between the two events or (2) one unprovoked seizure and a probability of future seizures similar to the general recurrence risk after 2 unprovoked seizures.[53 54]
- A case will be considered as active epilepsy if the patient is receiving epilepsy treatment or, if without anti-epileptic treatment, the patient presented at least one seizure during the last 5 years.
- A case of suspected NS will be defined as a person who presented with episodes of decreased consciousness during which the head dropped forward repeatedly.

- New cases of epilepsy will be defined as cases that appeared within the last 12 month proceeding the study period.

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 presented seperately.

Onchocerciasis prevalence study

Study design

This study includes two approaches. In one approach, the aim is to determine the onchocerciasis prevalence in the selected villages after 20 years of CDTI by performing the WHO proposed REMO methodology.[55] In brief, in each study village, 50 adults aged at least 20 years old and resident in the community for at least 10 years, will be invited to participate. They will be examined for the presence of onchocerciasis nodules (subcutaneous nodules or deep, painless, firm, mobile nodules over bony prominences: pelvic girdle, costal grid, knees, and skull). Although pre-control onchocerciasis prevalence data from Mahenge is not available at village level, onchocerciasis transmission in the study villages is shown by an entomological study carried out in the 1960ties and found a high number of the blackflies contained infective L3 stage parasites in their heads.[56]

The second part aims at determining the level of transmission of onchocerciasis in the selected villages. Therefore, serological testing for onchocerciasis will be done in all children at ages 7-10 years old, using the onchocerciasis specific Ov16 RDT (Standard diagnostics, Inc., Gyeonggi-do, Republic of Korea). According to the National Census survey in 2012, the population of children aged 7-10 years represented 11.6% of the population in Ulanga district. Estimated population of children aged 7-10 years from the four selected villages is 900 of which 722 (~80%) are anticipated to participate in the survey giving a power of 85% in detecting the prevalence between 0.8 to 2% at 5% significance level (Table 4).

Table 4. Estimated number of children aged 7 to 10 years old in the study villages in the Ulanga district, Tanzania, in 2016.

Name of Village	Population size (2016)	Estimated Population aged 7-10 yrs	Estimation of participating children (80%)
Mdindo	1536	178	143
Vigoi**	2572	298	239
Misegezi	3658*	424*	340*
Total	**7766**	**900**	**722**

Data collection procedures

Data collection will be done using tablet computers. All data collection forms will be developed in the open source software 'Open Data Kit' (ODK, https://opendatakit.org/). Interviewers will be trained in how to perform tablet computer based surveys. A technical data coordinator will be assigned to guarantee completeness and quality of data, and to assure daily data transfer from each tablet computer to the central server for data security.

Data management and analysis

The prevalence of epilepsy and NS will be computed as a ratio of the number of epilepsy and NS cases per total number of people registered in the households visited. The incidence of new cases of epilepsy is defined as the number of persons who developed epileptic seizures within two years preceding the study, divided by twice the population size, assuming that the change in population within the two years has a minimal effect on the incidence. Prevalenc and incidence will be compared between villages and to the 1989 data. Ivermectin treatment coverage and onchocerciasis prevalence will be calculated. Demographic and clinical characteristics of persons with epilepsy having a positive Ov16 serology will be compared to epilepsy cases with negative Ov16 serology. Ivermectin treatment coverage, epilepsy prevalence and incidence and OV16 positivity rate among children 7-10 years old will be compared among villages, weighted for the

difference in population size between villages. Odds ratios will be calculated for epilepsy cases with positive Ov16 results, and the association of Ov16 positivity and epilepsy will be analysed by age group.

Ethics

The protocol has received ethical approval from the ethics committee of the University of Antwerp, Antwerp, Belgium (29.08.2016) and the National Institut of Medical Research (NIMR) ethical committee Dar es Salaam, Tanzania (24.08.2016). Before the activities start, the research team will hold meetings with community leaders and health workers of the selected villages. The procedure, purpose and specific aim of the present study will be explained and discussed in regard to the potential risks and benefits for the community. Community leaders, village health workers and researcher will maintain the initially established communication for the entire duration of the study. The dissemination of results will be organized in a similar way as the initial meeting.

As approved by the relevant ethics committees, only participants who provide written informed consent will be enrolled in the study. Participant information sheets and consent forms will be available in English and Kiswahili. In case of illiteracy, information sheets and consent forms will be read to the participant in the presence of a witness. All participants are permitted to withdraw from the study activities, without reason, at any time. All personal information, samples and test results will be encoded and treated confidentially. People identified with untreated epilepsy or with interrupted treatment will be referred to the treatment centre and receive advice for care and support.

Data storage and handling

All data files will be centralised and stored in a secured central server. Name-linked information on participants and ID codes will remain confidential and will be used only to communicate clinical results to participants for their respective treatments.

DISCUSSION

The central research question of this study is to determine whether mass drug administration of ivermectin using the CDTI methodology has the potential to prevent the onset of onchocerciasis associated epilepsy. The expected results will contribute to a better understanding of the linkage between the onset of epilepsy and NS in particular, onchocerciasis and the impact of CDTI. In northern Uganda, an NS epidemic stopped after introducing a programme combining CDTI and larviciding of the main rivers. This study will be the first to investigate systematically whether CDTI alone may reduce epilepsy in an onchocerciasis endemic region.

With the Ov16 serological survey among children between the ages 7-10 we will be able to estimate the ongoing transmission of onchocerciasis in the Mahenge area after 20 years of CDTI. So far, the onchocerciasis control program in the study area was monitored based on annual ivermectin treatment coverage data provided by the community directed distributors of ivermectin. With this study we will obtain ivermectin treatment coverage data by interviewing the population. Moreover, by performing an Ov16 seroprevalence study among children under the age of 10 years we will obtain a real-time estimate of the level of ongoing transmission of onchocerciasis. Hopefully, results will show a low Ov16 seroprevalence in children and a decreased prevalence of epilepsy since 1989. In case a high Ov16 seroprevalence is found this will suggest that the CDTI programme was performing suboptimal and/or that ivermectin resisitance may have developed. It may also indicate that CDTI should be combined with larviciding rivers to reduce blackfly abundance. There is a possibility that we may not be able to show an impact of CDTI because in case of high level exposure to infectious blackflies the administration of ivermectin only once a year may not be sufficient to decrease onchocerciasis transmission considerably. Moreover in pre- and post-CDTI comparison it is possible that observed differences in epilepsy prevalence and incidence are not related to the intervention (CDTI) but to some of the other factors (e.g. those mentioned in Fig. 1) that might have changed

over time. However, a site visit to the Mahenge study site revealed that the village population had increased by a factor of 3, but there was no important in- or out migration or any other major change in lifestyle of the population or another major environmental change. In all the villages included in the study, such potential changes will be carefully assessed to control for potential confounding factors. Families who migrated into the study area after the implementation of CDTI will not be included in the analyses.

Outlook

This study aims to unravel the potential impact of CDTI on reducing the incidence of epilepsy in onchocerciasis endemic areas. The results will provide an evidence base to strengthen CDTI programs for the elimination goal for onchocerciasis and that has the power to prevent new cases of epilepsy associated to onchocerciasis. The results and lessons learned from this study will be disseminated by publications in open access journals, as well as presentations at scientific conferences and shared with all interested health authorities in Tanzania and beyond.

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Authors' contribution

All listed authors contributed to the development of the study design, essential study documents and study tools. According to their different areas of expertise, the authors critically revised specific parts of this manuscript: HG wrote the manuscript; HG, BM, PS, RC developed the study protocol; WM, MM, WM developed and approved the neurological study protocol and the survey tools; HG, BM, AK, developed the methodology and tools for the digital data collection; HG, BM, RC visited the study sites.

Data sharing statement

Data will be available from the Global Health Institute of the University of Antwerp at https://pintra.uantwerpen.be/webapps/cmsmain/webui/_xy-914103_1-t_KW7pxaf1

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Competing interests

The authors declare that they have no competing interests.

Figure caption

Figure 1. There is an extensive network of controllable and uncontrollable factors influencing the level of onchocerciasis endemicity and prevalence and incidence of associated epilepsy.

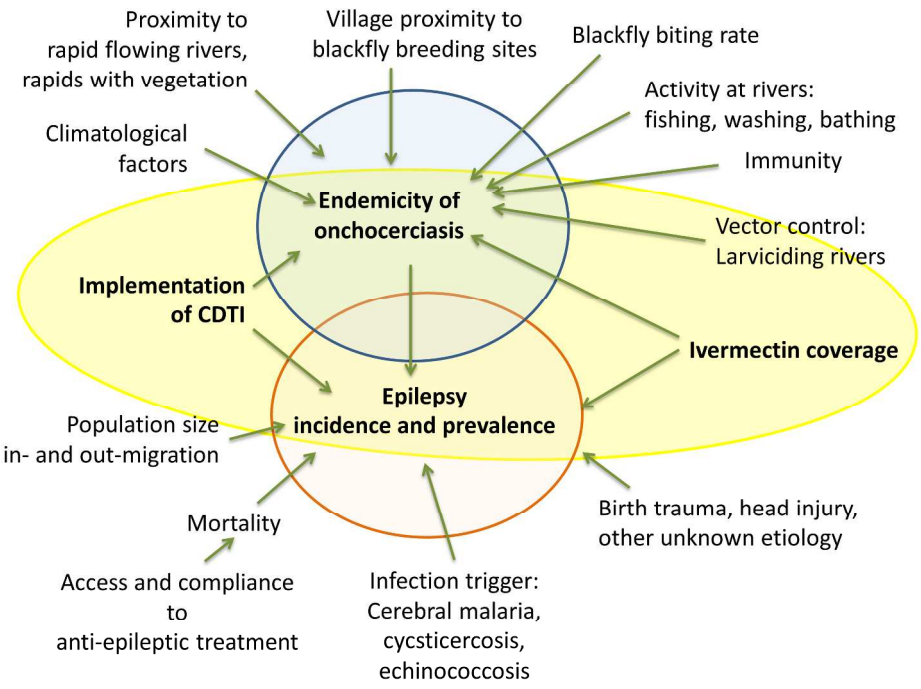

Figure 1. There is an extensive network of controllable and uncontrollable factors influencing the level of onchocerciasis endemicity and prevalence and incidence of associated epilepsy.

254x190mm (300 x 300 DPI)

SUPPLEMENTARY ANNEX

5 QUESTIONS TARGETED FOR THEW SCREENING OF EPILEPSY CASES

If at least one of the 5 questions is answered with YES, the person will be invited to participate in the neurological examination for case verification.

QUESTION 1

Have you ever lost consciousness and experienced:

a) Loss of bladder control? ☐YES ☐NO ☐DON'T KNOW

b) Foam at the mouth? ☐YES ☐NO ☐DON'T KNOW

QUESTION 2

Have you ever experienced absence(s) or sudden loss(es) of contact with the surroundings, for a short duration of time? ☐YES ☐NO ☐DON'T KNOW

QUESTION 3

Have you ever experienced sudden, uncontrollable twitching or shaking of your arms, legs or head, for a period of a few minutes? ☐YES ☐NO ☐DON'T KNOW

QUESTION 4

Do you sometimes experience sudden and brief bodily sensations, see or hear things that are not there, or smell strange odours? ☐YES ☐NO ☐DON'T KNOW

QUESTION 5

Have you ever been told that you are suffering from epilepsy or that you have already had epileptic fits? ☐YES ☐NO ☐DON'T KNOW

BMJ Open

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

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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

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ABSTRACT

Introduction

Worldwide, there are an estimated 50 million people affected by epilepsy. Its etiology is manifold, and parasitic infections play an important role, specifically onchocerciasis. From onchocerciasis endemic areas a distinctive form of epilepsy has been described as nodding syndrome, affecting children and causing nodding seizures, mental retardation and debilitating physical development. Onchocerciasis is treatable with ivermectin. Control programs using community directed treatment with ivermectin (CDTI) are implemented in endemic countries. This study is designed to contribute to a better understanding of the linkage between the onset of epilepsy, onchocerciasis and CDTI. Comparing the epidemiological data on epilepsy and onchocerciasis from pre-CDTI and 20 years after its introduction will allow identifying a potential impact of ivermectin on the onset of epilepsy.

Methods and analysis

The study will be conducted in the Mahenge highlands in Tanzania. Study site selection is based on an in-depth study on epilepsy in that area dating from 1989. CDTI was introduced in 1997. By a door-to-door approach, the population will be screened for epilepsy using a validated questionnaire. Suspected cases will be invited for a neurological examination for case verification. Onchocerciasis prevalence will be assessed by a rapid epidemiological assessment for prevalence. As an indicator for ongoing transmission, children younger than 10 years of age will be tested for Ov16 antibodies. Ivermectin use will be assessed at household level. Epilepsy data will be analysed in comparison to the 1989 data to reveal pre- and post-CDTI implementation prevalence and incidence.

Ethics and dissemination

The protocol has received ethical approval from the ethics committees of the University of Antwerp, Belgium and of the National Institut of Medical Research (NIMR), Dar es Salaam,

Tanzania. The findings will be published in peer-reviewed journals, and presented to the health authorities in Tanzania, at national, regional and village level.

Strengths and limitations of this study:

- This study will allow comparison of large scale population based data on epilepsy epidemiology in Mahenge over a period of 28 years.
- The strength of the study is that it is a first study to follow-up epilepsy epidemiology in the Mahenge area since the introduction of community directed treatment with ivermectin.
- Comparaison of the data obtained in 1989 and 2017 will be challenged by the slightly different study methodologies used.
- The study design is limited in accounting for potential confounding factors other than CDTI that may influence epilepsy incidence and prevalence.
- Focusing on villages with high epilepsy burden in the past may lead to an overestimation of the potential impact of CDTI.

Key words

Epilepsy

Nodding disease

Onchocerciasis

Ivermectin

Tanzania

INTRODUCTION

Epilepsy in onchocerciasis endemic regions in Africa

Epilepsy is a chronic disease estimated to affect 50 million people worldwide according to World Health Organisation (WHO).[1] In general, higher prevalence and incidence are reported from

those populations living in low and middle income countries (LMICs) when compared to industrialized countries.[2 3] In fact, more than 85% of the global burden of epilepsy occurs in the people living in LMICs.[4 5] The etiology of epilepsy is very diverse and not yet fully understood. Besides birth trauma and head injury involving the brain, infectious diseases can trigger epilepsy as well. Several parasitic infections are associated with epilepsy such as cerebral malaria, neurocysticercosis, echinococcosis, and onchocerciasis.[6-8] Many of these epilepsy cases could be prevented through timely anti-parasitic treatment. Epilepsy manifests in a variety of seizure types and degrees of intensity.[9] In the 1960 inTanzania, Aall-Jillek was the first to describe an unusual form of epileptic seizures characterized by nodding movements of the head.[10 11] Later, this distinct form of epilepsy has been described also in Uganda and South Sudan, and has been named as nodding syndrome (NS).[12 13] NS is a debilitating epileptic disorder mainly affecting children at the ages of 3 and 18 years.[14] The seizures are characterized by a brief loss of muscle-tone in the neck, leading to repetitive head-nodding.[15] NS is often associated with cognitive decline, and sometimes with stunted growth.[16] So far, NS is solely described in onchocerciasis endemic areas.[12] A NS epidemic has been observed in the past two decades in northern Uganda and in neighboring South Sudan.[17 16] 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.[18]

Health systems services in remote rural regions in Africa are rarely capable to provide continous anti-epileptic treatment to those patients in need.[5] Further more, most health care workers lack training to diagnose and treat persons with epilepsy adequately. In many societies in Africa, and in addition to the clinical burden, epilepsy is perceived as a possession by evil spirits and hence bears a stigma that puts the diseased individual and his family at risk for social isolation.[19] Moreover, the family's economy is negatively impacted by the disease, since an epileptic family

member needs specific care and supervision, detaining care takers from their subsistence duties.[20]

NS only occurs in onchocerciasis endemic areas. An epidemiological association between epilepsy and onchocerciasis was first reported from western Uganda in the early 1990s.[21 22] A case-control study performed in 1991-92 in the Mbam valley in Cameroon demonstrated a significantly higher microfilarial load in persons with epilepsy than in controls.[23] Study results from other onchocerciasis-endemic African countries underline this association.[8 24] To describe this epidemiological phenomenon the term onchocerciasis associated epilepsy (OAE) was proposed by Kaiser and colleagues.[24] Case-control studies in northern Uganda and South Sudan focusing on NS patients produced similar results, showing a higher prevalence of onchocerciasis in NS cases compared to non-epileptic controls.[16 17] It is, however, unclear how onchocerciasis might cause NS. Although the eye and the optical nerve are affected when onchocerciasis causes blindness, microfilariae and adult *Onchocerca volvulus* worms are not generally considered to be able to invade the central nervous system. Recent research hypothesises that an immunological cross-reaction of onchocerciasis-specific antibodies may provoke a neurotoxic reaction and trigger NS.[25]

Onchocerciasis, a treatable neglected tropical disease facing obstacles on the road to elimination

Onchocerciasis is a parasitic disease caused by an infection with the the worm *O. volvulus* whose filarial larvae are transmitted by blackflies (*Simulidae* spp.). In the final host, humans, the adult female worms encapsulate in the subcutaneous tissue forming visually detectable nodules. Each female worm releases up to one thousand microfilariae per day. These microfilariae provoke itching, dermatitis and – if left untreated – blindness, which led to the disease also being called river blindness. Blackflies get infected with microfilariae when biting infected humans in proximity to fast flowing rivers, the breeding site of the blackflies. After passing through several

larval stages, infected blackflies spread the parasite by biting other people. Onchocerciasis is treatable with ivermectin.[26] The drug has a twofold mechanism of action: (i) it kills the microfilariae and (ii) inhibits their release by the adult female worm for up to two years after a single dose treatment.[27] Hence, ivermectin has a strong impact on reducing transmission. However, ivermectin is not lethal to adult worms and infected persons have to take it annually for up to 15 years.[28] Onchocerciasis is a priority disease scheduled for elimination by 2025 by the WHO. Today, 99% of the globally 37 million people infested live in Africa.[1] In 1995, the African Program for Onchocerciasis Control (APOC) was initiated for the implementation of the onchocerciasis control programme based on community directed treatment with ivermectin (CDTI). APOC was coordinating these activities in endemic areas of 22 African countries.[29] Since May 2016, CDTI control programmes are integrated in the WHO Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN).[30] CDTI overcomes the limited performance of weak health systems in rural areas by using an active, strategic involvement of the community.[31] To reach the entire population, ivermectin distribution is organized by trained volunteers in each village, resulting in a large geographical coverage. CDTI, in certain regions combined with the control of the blackfly by larviciding of breeding sites (i.e. fast flowing rivers), resulted in a measurable impact reflected by a remarkable transmission reduction in the past 20 years.[32] But success and effectiveness of these targeted interventions lack comprehensiveness. Certain onchocerciasis endemic regions remain undersupplied or unreachable for CDTI due to political instability, insecurity or armed conflicts.[33] As a consequence in the war-affected regions of South Sudan and northern Uganda, onchocerciasis control measures were stopped or implementation started only recently. Moreover, misconceptions and the fear of adverse effects result in suboptimal therapeutic coverage and reduce the effectiveness of the control program.[34] Adding to the complexity of onchocerciasis control, in regions where *Loa loa* and onchocerciasis are co-endemic, ivermectin may cause severe adverse effects (encephalopathy, coma or death) in infected individuals harbouring a high *Loa loa* microfilariae load.[35] [36] Thus, CDTI

implementation in such regions requires additional precautions.[37] This is the case in certain regions of Cameroon and the Democratic Republic of Congo (DRC).[38] Compliance to CDTI programs can also decrease over the years since less direct positive effects can be observed when onchocerciasis prevalence drops, and healthy feeling people may not appreciate the importance of continuing repeated treatment.[39]

The potential of CDTI to reduce the incidence of epilepsy in onchocerciasis endemic regions

The endemicity of onchocerciasis and the prevalence and incidence of epilepsy are influenced by a multitude of factors (Figure 1). These factors are of uncontrollable nature (climate, environment, ecology), and of controllable nature (prevention and intervention programs, access to health care and treatment).

<<<<<<Figure 1 near here>>>>>>

The two heavily affected regions of the last decades, Northern Uganda and South Sudan, were – and South Sudan still is - affected by armed conflicts. In 2012, after the conflict ended in northern Uganda, the NS epidemic was investigated. Following on, epilepsy treatment was provided, biannual CDTI was implemented and larviciding of major rivers was carried out.[40] Since 2013 the NS epidemic in northern Uganda has reportedly been halted.[41] In contrast in South Sudan, where CDTI was stopped because of insecurity, new cases of NS continue to appear.[18] Based on the observations from northern Uganda, it has been suggested that ivermectin may reduce the incidence of NS and other forms of epilepsy in onchocerciasis endemic areas.[42] Considering that 30% of the globally 37 million individuals infected with onchocerciasis do not have access to effective treatment, and that 1% of those develop epilepsy (equivalent to the estimated excess

prevalence of epilepsy over non-onchocerciasis areas) the number of excess cases of epilepsy attributed to onchocerciasis most likely exceeds 100'000.[1 7]

Recent observations in the DRC and northern Uganda suggest that optimal treatment coverage of ivermectin may stop the incidence of NS and other forms of OAE.[42]

Aim

The population based study from 1989 by Rwiza et al. established the baseline prevalence of all forms of epilepsy in the Ulanga district, Tanzania.[43] With this 2017 population based study, we aim to further evaluate the prevalence and incidence rates of new epilepsy cases, including a complete case ascertainment of all forms of epilepsy encountered, and compare the data to the 1989 data (Table 1).

Table 1. The pre- and post-CDTI epilepsy study periods and estimated epilepsy prevalence and incidence in the Mahenge area, Ulanga district in Tanzania.

	Mahenge area, Ulanga district, Tanzania	
Year of epilepsy study data collection	1989[43]	2017
Status of CDTI at study year	Pre-CDTI	Post-CDTI (ongoing since 1997)
Estimated epilepsy prevalence	20.2/1'000	14/1'000*
Estimated epilepsy incidence	146/100'000	81.7/100'000**

*Median epilepsy prevalence and **incidence in low and middle income countries [4 3]

Objective

The main objective of this study is to identify the potential impact of long-term onchocerciasis control using CDTI on the prevalence and incidence of epilepsy in selected villages in the Mahenge area of the Ulanga district in Tanzania.

Specific objectives

1. To determine the prevalence of all forms of epilepsy in selected villages of the Mahenge area and compare the related data from 2017 to the 1989 data.
2. To determine the incidence rate of annual new onset cases of all forms of epilepsy in the Mahenge area and compare the related data from 2017 to the 1989 data.
3. To assess the ivermectin coverage and the onchocerciasis prevalence with a REMO assessment in selected villages of the Mahenge area.
4. To determine the level of onchocerciasis transmission by a serological survey using a rapid test among children in selected villages of the Mahenge area.
5. To investigate the potential difference in clinical appearance of epilepsy in patients with negative onchocerciasis serology to patients with positive onchocerciasis serology.

METHODS AND ANALYSIS

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. Data collection was carried out between January and September 2017. Analysis is planned to be finalized by end of 2017, to allow publishing of the results in 2018.

Study site

The study is taking place in the Mahenge area of the Ulanga distict, Morogoro region in south-eastern Tanzania, a mountainous area with fast flowing rivers.

Epilepsy in the Mahenge area

In the 1960s, Aall-Jilnek was the first to report a high burden of epilepsy in the Mahenge area, and also described the first NS cases.[10] In 1989, Rwiza et al. carried out a population based survey to determine prevalence and incidence of epilepsy in the Ulanga district and found an annual incidence of 73.3 new cases in 100'000. The prevalence was 10.2 cases in 1000, and

varied between villages from 5.1 to 37.1 cases in 1'000. Those villages with the highest prevalence were located in the Mahenge highlands (Table 2).[43]

Table 2. Population size and prevalence of epilepsy in some villages of the Ulanga district, Tanzania, showing the highest prevalence in 1989, as reported by Rwiza et al. in 1992

Name of Village	Population size	Number of Epilepsy cases	Prevalence per 1000 people
Sali$^{\delta}$	1'282	14	10.9
Mdindo$^{\delta}$	539	20	37.1
Vigoi$^{\delta}$	1'822	23	12.6
Lupiro	1'697	17	10
Misegezi	1'667	18	10.8
Total	**7'007**	**92**	**13.13**

$^{\delta}$Located in Mahenge highlands

Onchocerciasis and CDTI in the Mahenge area

The Morogoro region is among the five regions where onchocerciais is endemic in Tanzania. The region presents four foci: Uluguru mountain, Ulanga/Mahenge, Kilosa and the Nguru mountain foci.[44] The Ulanga district, specifically the Mahenge area, was known for its high endemicity of onchocerciasis since the early last century.[45] Mass drug administration using annual CDTI was introduced in the Mahenge area in 1997.[46] Before CDTI was implemented, 58.6% of a sample of 482 inhabitants of the Mahenge area were found to have a microfilariae positive skin snip.[47] Twelve years later (in 2009) the rapid epidemiological mapping of onchocerciasis (REMO) in 10 villages in the Mahenge area revealed that the percentage of persons with a microfilariae positive skin snip has dropped to 21.9%, with a mean village prevalence of 8.3%.[32]

Study population

The three villages Mdindo, Vigoi and Misegezi that had high epilepsy prevalence during the 1989 study will be selected for this study.[43] The entire population of each of these villages will be included in the study.

Epilepsy and NS prevalence / incidence study

Study design

The study is designed as a population-based cross-sectional study, following a two-stage approach for epilepsy case identification at village level. The gold-standard in neuro-epidemiological surveys to identify epilepsy cases in LMICs, the door-to-door approach, will be applied.[48] All inhabitants of the selected villages will be eligible for participation and will be included in the questionnaire screening survey. Due to well described limitations of questionnaire studies on epilepsy (stigma leading to concealment of cases, recall bias, absence of clear terminology for epilepsy and seizures) and to increase sensitivity of case ascertainment, key informants who are likely to be aware of persons with epilepsy in the village will additionally be consulted.[3] [48] These may be health workers, traditional healers, teachers, or community leaders.[49]

In a second stage, persons with suspected epilepsy identified during the household screening survey will be further invited for clinical examination by a neurologist. The examination will include neurological tests and a detailed interview for case verification. In verified epilepsy cases the onset of the seizures will be determined and possible etiologic factors will be investigated, such as birth trauma, head injury, meningo-encephalitis, cerebral malaria, neurocystocercosis, and the history of onchocerciasis. For persons with suspected epilepsy their serological status will be determined using onchocerciasis rapid diagnostic test (Ov16 RDT) (Standard diagnostics, Inc., Gyeonggi-do, Republic of Korea).

Sample size calculation

According to Rwiza et al. (1992) the average prevalence of epilepsy in five villages with high prevalence was 1.313% (Table 2). If we assume a reduction in the prevalence by 33.3% to be able to compare the prevalence at a power of 80% and 95% confidence level, the minimal sample size of 4,746 individuals will be required. Assuming a participation of 80%, a minimum source population of 5,933 is necessary to obtain optimal sample size. Since 1989 the population in the villages with high epilepsy prevalence has increased (Table 3). For this survey we will include only the population of the villages with the highest epilepsy prevalence in 1989. The total population of the selected villages of Mdindo, Vigoi and Misegezi in 2016 is about 7,766.

Table 3. List of the study villages in the Mahenge highlands, Tanzania, and their population size in 1989 and in 2016.

Name of Village (1989)	Population size (1989)	Population size (2016)
Mdindo	539	1'536
Vigoi	1'822	2'572
Misegezi	1'667	3'658*
Total	**4'028**	**7'766**

**Population projection based on the growth rate of Vigoi village*

Data collection at community level

The community survey will commence by a questionnaire interview with the village authorities on demographic topics, and with village health workers to address general questions on the status of epilepsy and epilepsy treatment in the village. A complete door-to-door active screening for persons with suspected epilepsy at village level will be performed. The interview team will be trained on how to conduct the active search for epilepsy cases using a pre-tested, validated screening questionnaire targeting epilepsy by 5 specific questions (provided in the supplementary material).[50 51] For validation, the questionnaire will be translated to Kiswahili,

pretested and retranslated to English. The locally used terms for the two respective conditions will be used (epilepsy: *kifafa,* NS: *kusinzia kichwa*). To ascertain completeness and to ensure the best collaboration with the village population, the interviewer team will be accompanied to all households by local village health workers. The geographical coordinates of the participating households will be collected for the mapping and geospatial analysis of cases (proximity to rivers, potential clustering).

Case verification and validation

All persons with suspected epilepsy identified during the door-to-door survey will be verified by a neurologist. The neurologist will perform a detailed anamnesis on all persons with suspected NS and epilepsy. In case of confirmation of the epilepsy, the neurologist will perform a medical examination and administer a detailed questionnaire on the type of epilepsy. Newly diagnosed epilepsy cases will be referred to an epilepsy treatment centre. In case the person is already followed in a treatment centre, permission will be asked to review the medical information available in the treatment centre to record epilepsy diagnosis and epilepsy treatment history.

Definitions:

- A case of epilepsy will be defined according to the International League Against Epilepsy (ILAE) guidelines as a patient who had at least two times nonfebrile seizures unrelated to any acute metabolic disorder or to withdrawal of alcohol or drugs., lost consciousness with convulsions with a minimal time difference of 24h between the two events.[52]
- A case will be considered as active epilepsy if the patient is receiving epilepsy treatment or, if without anti-epileptic treatment, the patient presented at least one seizure during the last 5 years.
- A case of suspected NS will be defined as a person who presented with episodes of decreased consciousness during which the head dropped forward repeatedly.
- New cases of epilepsy will be defined as cases that appeared within the last 12 month proceeding the study period.

To allow for comparison, the epilepsy case definition applied here is identical to the definition applied in 1989 by Rwiza *et al*.[43]

Onchocerciasis prevalence study

Study design

This study includes two approaches. In one approach, the aim is to determine the onchocerciasis prevalence in the selected villages after 20 years of CDTI by performing the WHO proposed REMO methodology.[53] In brief, in each study village, 50 adults aged at least 20 years old and resident in the community for at least 10 years will be invited to participate. They will be examined for the presence of onchocerciasis nodules (subcutaneous nodules or deep, painless, firm, mobile nodules over bony prominences: pelvic girdle, costal grid, knees, and skull). Although pre-control onchocerciasis prevalence data from the study villages is not available, a study from 1966 carried out in three villages in Mahenge found a skin snip positivity rate of 43%, 60% and 65%, respectively.[54]

The second part aims at determining the level of transmission of onchocerciasis in the selected villages. Therefore, serological testing for onchocerciasis will be done in all children at ages 7-10 years old, using the onchocerciasis specific Ov16 RDT (Standard diagnostics, Inc., Gyeonggi-do, Republic of Korea). According to the National Census survey in 2012, the population of children aged 7-10 years represented 11.6% of the population in Ulanga district. Estimated population of children aged 7-10 years from the four selected villages is 900 of which 722 (~80%) are anticipated to participate in the survey giving a power of 85% in detecting the prevalence between 0.8 to 2% at 5% significance level (Table 4).

Table 4. Estimated number of children aged 7 to 10 years old in the study villages in the Ulanga district, Tanzania, in 2016.

Name of Village	Population size (2016)	Estimated Population aged 7-10 yrs	Estimation of participating children (80%)
Mdindo	1'536	178	143
Vigoi	2'572	298	239
Misegezi	3'658*	424*	340*
Total	**7'766**	**900**	**722**

**Population projection based on the growth rate of Vigoi village*

Data collection procedures

Data collection will be done using numeric tablets. All data collection forms will be developed in the open source software 'Open Data Kit' (ODK, https://opendatakit.org/). Interviewers will be trained in how to perform tablet-based surveys. A technical data coordinator will be assigned to guarantee completeness and quality of data, and to assure daily data transfer from each tablet to the central server for data security.

Data management and analysis

The prevalence of epilepsy and NS will be computed as a ratio of the number of epilepsy and NS cases per total number of people registered in the households visited, respectively. The incidence of new cases of epilepsy is defined as the number of persons who developed epileptic seizures within two years preceding the study, divided by twice the population size, assuming that the change in population within the two years has a minimal effect on the incidence. Results will be presented accompanied with 95% confidence interval (95%CI), and P-value<0.05 level of significance. Prevalence and incidence will be compared between villages and to the 1989 data. Ivermectin treatment coverage and onchocerciasis prevalence will be calculated. Proportions will be compared using χ^2-test, while means will be compared using t-tests. Demographic and clinical characteristics of persons with epilepsy having a positive Ov16 serology will be compared to

epilepsy cases with negative Ov16 serology. Ivermectin treatment coverage, epilepsy prevalence and incidence and OV16 positivity rate among children 7-10 years old will be compared among villages, weighted for the difference in population size between villages. Odds ratios will be calculated for epilepsy cases with positive Ov16 results, and the association of Ov16 positivity and epilepsy will be analysed by age group.

Ethics

The protocol has received ethical approval from the ethics committee of the University of Antwerp, Antwerp, Belgium (29.08.2016) and the National Institute of Medical Research (NIMR) ethical committee Dar es Salaam, Tanzania (24.08.2016). Before the activities start, the research team will hold meetings with community leaders and health workers of the selected villages. The procedure, purpose and specific aim of the present study will be explained and discussed with regard to the potential risks and benefits for the community. Community leaders, village health workers and researchers will maintain the initially established communication for the entire duration of the study. The dissemination of results will be organized in a similar way as the initial meeting.

As approved by the relevant ethics committees, only participants who provide written informed consent will be enrolled in the study. Participant information sheets and consent forms will be available in English and Kiswahili. In case of illiteracy, information sheets and consent forms will be read to the participant in the presence of a witness. All participants are permitted to withdraw from the study activities, without reason, at any time. All personal information, samples and test results will be encoded and treated confidentially. People identified with untreated epilepsy or with interrupted treatment will be referred to the treatment centre and receive advice for care and support.

Data storage and handling

All data files will be centralised and stored in a secured central server. Name-linked information on participants and ID codes will remain confidential and will be used only to communicate clinical results to participants for their respective treatments.

DISCUSSION

The central research question of this study is to determine whether mass drug administration of ivermectin using CDTI has the potential to prevent the onset of OAE. The expected results will contribute to a better understanding of the linkage between the onset of epilepsy and NS in particular, onchocerciasis and the impact of CDTI. In northern Uganda, an NS epidemic has been halted after introducing a programme combining CDTI and larviciding of the main rivers. This study will be the first to investigate systematically whether CDTI alone may reduce epilepsy in an onchocerciasis endemic region. To do so pre- and post-CDTI epilepsy prevalence and incidence data will be compared. Concurrently, with the Ov16 serological survey among children between the ages 7-10 years, we will be able to assess wether the transmission of onchocerciasis is ongoing in the Mahenge area after 20 years of CDTI. So far, the onchocerciasis control program in the study area was monitored based on annual ivermectin, treatment coverage data being provided by the community directed distributors of ivermectin. With this study we will obtain ivermectin treatment coverage data by interviewing the population. Moreover, by performing an Ov16 seroprevalence study among children under the age of 10 years we will obtain a real-time estimate of the level of ongoing transmission of onchocerciasis. The hypothesis is to find a low Ov16 seroprevalence in children and a decreased prevalence of epilepsy since 1989. In case a high Ov16 seroprevalence is found this will suggest that the CDTI programme was performing suboptimal and/or that ivermectin resisitance may have developed. It might therefore be useful to combine CDTI with larviciding rivers to reduce blackfly abundance.

This study also has limitations. The methods used will not allow for measuring onchocerciasis infection intensity, one of the main factors influencing the development of OAE. There is a

possibility that it may not be possible to show an impact of CDTI because in case of high level exposure to infectious blackflies the administration of ivermectin only once a year may not be sufficient to decrease onchocerciasis transmission considerably. Moreover, in pre- and post-CDTI comparison it is possible that observed differences in epilepsy prevalence and incidence are not related to the intervention (CDTI) but to some of the other factors (e.g. those mentioned in Fig. 1) that might have changed over time. However, a site visit to the Mahenge study site revealed that the village population had increased by a factor of 3, but there was no important in- or out migration or any other major change in lifestyle of the population or another major environmental change. In all the villages included in the study, such potential changes will be carefully assessed to control for potential confounding factors. Families who migrated into the study area after the implementation of CDTI will not be included in the analyses.

Outlook

This study aims to unravel the potential impact of CDTI on reducing the incidence of epilepsy in onchocerciasis endemic areas. Study results may provide evidence that strengthening CDTI programs could prevent the onset of OAE. The results and lessons learned from this study will be disseminated by publications in open access journals, as well as presentations at scientific conferences and shared with all interested health authorities in Tanzania and beyond.

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Authors' contribution

All listed authors contributed to the development of the study design, essential study documents and study tools. According to their different areas of expertise, the authors critically revised specific parts of this manuscript: HG wrote the manuscript; HG, BM, PS, RC developed the study protocol; WM, MM, WM developed and approved the neurological study protocol and the survey tools; HG, BM, AK, developed the methodology and tools for the digital data collection; HG, BM, RC visited the study sites.

Data sharing statement

Data will be available from the Global Health Institute of the University of Antwerp at https://pintra.uantwerpen.be/webapps/cmsmain/webui/_xy-914103_1-t_KW7pxaf1

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Competing interests

The authors declare that they have no competing interests.

Figure caption

Figure 1. Onchocerciasis endemicity and prevalence and incidence of associated epilepsy are influenced by an extensive network of controllable and uncontrollable factors.

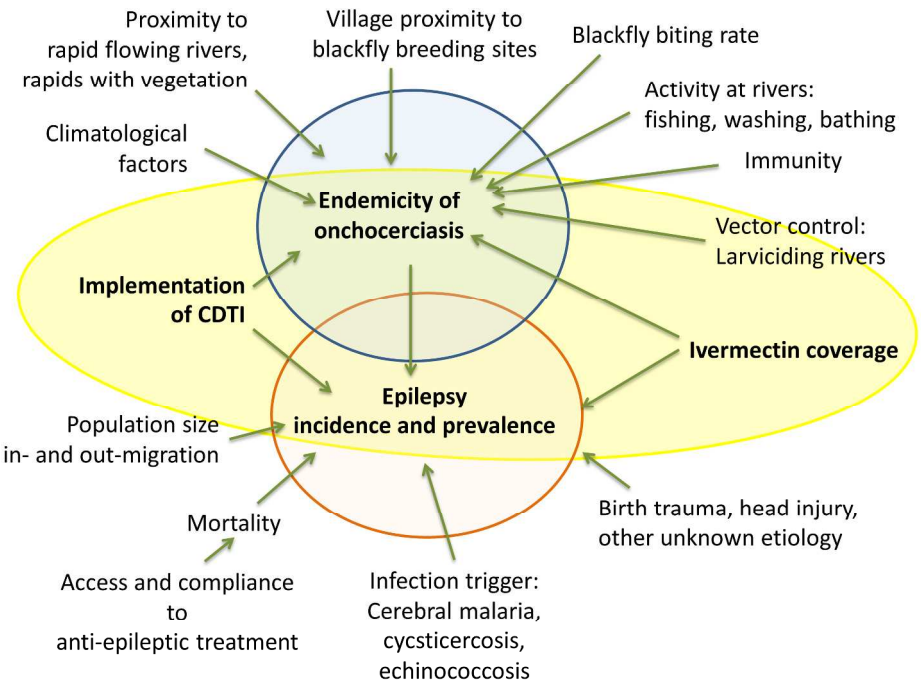

Figure 1. There is an extensive network of controllable and uncontrollable factors influencing the level of onchocerciasis endemicity and prevalence and incidence of associated epilepsy.

254x190mm (300 x 300 DPI)

SUPPLEMENTARY ANNEX

5 QUESTIONS TARGETED FOR THEW SCREENING OF EPILEPSY CASES

If at least one of the 5 questions is answered with YES, the person will be invited to participate in the neurological examination for case verification.

QUESTION 1

Have you ever lost consciousness and experienced:

a) Loss of bladder control? ☐YES ☐NO ☐DON'T KNOW

b) Foam at the mouth? ☐YES ☐NO ☐DON'T KNOW

QUESTION 2

Have you ever experienced absence(s) or sudden loss(es) of contact with the surroundings, for a short duration of time? ☐YES ☐NO ☐DON'T KNOW

QUESTION 3

Have you ever experienced sudden, uncontrollable twitching or shaking of your arms, legs or head, for a period of a few minutes? ☐YES ☐NO ☐DON'T KNOW

QUESTION 4

Do you sometimes experience sudden and brief bodily sensations, see or hear things that are not there, or smell strange odours? ☐YES ☐NO ☐DON'T KNOW

QUESTION 5

Have you ever been told that you are suffering from epilepsy or that you have already had epileptic fits? ☐YES ☐NO ☐DON'T KNOW

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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

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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

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ABSTRACT

Introduction

Worldwide, there are an estimated 50 million people affected by epilepsy. Its etiology is manifold, and parasitic infections play an important role, specifically onchocerciasis. In onchocerciasis endemic areas, a distinctive form of epilepsy has been described as nodding syndrome, affecting children and causing nodding seizures, mental retardation and debilitating physical development. Onchocerciasis control programs using community directed treatment with ivermectin (CDTI) are implemented in endemic countries. This study is designed to contribute to a better understanding of the linkage between the onset of epilepsy, onchocerciasis and CDTI. Comparing the epidemiological data on epilepsy and onchocerciasis from pre-CDTI and 20 years after its introduction will allow identifying a potential impact of ivermectin on the onset of epilepsy.

Methods and analysis

The study will be conducted in the Mahenge highlands in Tanzania. Study site selection is based on an in-depth study on epilepsy in that area dating from 1989. CDTI was introduced in 1997. By a door-to-door approach, the population will be screened for epilepsy using a validated questionnaire. Suspected cases will be invited for a neurological examination for case verification. Onchocerciasis prevalence will be assessed by a rapid epidemiological assessment. As an indicator for ongoing transmission, children younger than 10 years of age will be tested for Ov16 antibodies. Ivermectin use will be assessed at household level. Epilepsy data will be analysed in comparison to the 1989 data to reveal pre- and post-CDTI prevalence and incidence.

Ethics and dissemination

The protocol has received ethical approval from the ethics committees of the University of Antwerp, Belgium and of the National Institut of Medical Research (NIMR), Dar es Salaam, Tanzania. The findings will be published in peer-reviewed journals, and presented to the health authorities in Tanzania, at national, regional and village level.

Strengths and limitations of this study:

- This study will allow comparison of large scale population based data on epilepsy epidemiology in Mahenge over a period of 28 years.
- The strength of the study is that it is a first study to follow-up epilepsy epidemiology in the Mahenge area since the introduction of community directed treatment with ivermectin.
- The comparaison of the data obtained in 1989 and 2017 will be challenged by the slightly different study methodologies used.
- The study design is limited in accounting for potential confounding factors other than CDTI that may influence epilepsy incidence and prevalence.
- Focusing on villages with high epilepsy burden in the past may lead to an overestimation of the potential impact of CDTI.

Key words

Epilepsy

Nodding disease

Onchocerciasis

Ivermectin

Tanzania

INTRODUCTION

Epilepsy in onchocerciasis endemic regions in Africa

Epilepsy is a chronic disease estimated to affect 50 million people worldwide according to World Health Organisation (WHO).[1] In general, higher prevalence and incidence are reported among those populations living in low and middle income countries (LMICs) when compared to industrialized countries.[2 3] In fact, more than 85% of the global burden of epilepsy occurs in people living in LMICs.[4 5] The etiology of epilepsy is very diverse and not yet fully understood.

Besides birth trauma and head injury involving the brain, infectious diseases can trigger epilepsy as well. Several parasitic infections are associated with epilepsy such as cerebral malaria, neurocysticercosis, echinococcosis, and onchocerciasis.[6-8] Many of these epilepsy cases could be prevented through timely anti-parasitic treatment. Epilepsy manifests in a variety of seizure types and intensity.[9] In the 1960 in Tanzania, Aall-Jillek was the first to describe an unusual form of epileptic seizures characterized by nodding movements of the head.[10 11] Later, this distinct form of epilepsy has been described also in Uganda and South Sudan, and has been named as nodding syndrome (NS).[12 13] NS is a debilitating epileptic disorder mainly affecting children at the ages of 3 and 18 years.[14] The seizures are characterized by a brief loss of muscle-tone in the neck, leading to repetitive head-nodding.[15] NS is often associated with cognitive decline, and sometimes with stunted growth.[16] So far, NS is solely described in onchocerciasis endemic areas.[12] A NS epidemic has been observed in the past two decades in northern Uganda and in neighboring South Sudan.[17 16] The weight of the public health burden caused by epilepsy in onchocerciasis endemic regions can be illustrated by the situation in the village Mvolo (West Equatorial State in South Sudan) over 50% of the families had at least one child with epilepsy, resulting in one in six children of the village suffering from epilepsy.[18]

Health systems services in remote rural regions in Africa are rarely capable to provide continous anti-epileptic treatment to those patients in need.[5] Further more, most health care workers lack training to diagnose and treat persons with epilepsy adequately. In addition to its clinical burden, epilepsy is perceived as a possession by evil spirits in many African societies and hence bears a stigma that puts the diseased individual and his family at risk for social isolation.[19] Moreover, the family's economy is negatively impacted by the disease, since an epileptic family member needs specific care and supervision, detaining care takers from their subsistence duties.[20]

NS only occurs in onchocerciasis endemic areas. An epidemiological association between epilepsy and onchocerciasis was first reported from western Uganda in the early 1990s.[21 22] A case-control study performed in 1991-92 in the Mbam valley in Cameroon demonstrated a

significantly higher microfilarial load in persons with epilepsy than in controls.[23] Study results from other onchocerciasis-endemic African countries underline this association.[8 24] To describe this epidemiological phenomenon the term onchocerciasis associated epilepsy (OAE) was proposed by Kaiser and colleagues.[24] Case-control studies in northern Uganda and South Sudan focusing on NS patients produced similar results, showing a higher prevalence of onchocerciasis in NS cases compared to non-epileptic controls.[16 17] It is, however, unclear how onchocerciasis might cause NS. Although the eye and the optical nerve are affected when onchocerciasis causes blindness, microfilariae and adult *Onchocerca volvulus* worms are not generally considered to be able to invade the central nervous system. Recent research hypothesises that an immunological cross-reaction of onchocerciasis-specific antibodies may provoke a neurotoxic reaction and trigger NS.[25]

Onchocerciasis, a treatable neglected tropical disease facing obstacles on the road to elimination

Onchocerciasis is a parasitic disease caused by an infection with the worm *O. volvulus* whose filarial larvae are transmitted by blackflies (*Simulidae* spp.). In the final host (humans) the adult female worms encapsulate in the subcutaneous tissue forming visually detectable nodules. Each female worm releases up to one thousand microfilariae per day. These microfilariae provoke itching, dermatitis and – if left untreated – blindness, leading to the disease name river blindness. Blackflies get infected with microfilariae when biting infected humans in proximity to fast flowing rivers, the breeding site of the blackflies. After passing through several larval stages, infected blackflies disseminate the parasite by biting other people. Onchocerciasis is treatable with ivermectin.[26] The drug has a twofold mechanism of action: (i) it kills the microfilariae and (ii) inhibits their release by the adult female worm for several months up to two years after a single dose treatment.[27] Hence, ivermectin has a strong impact on reducing transmission. However, ivermectin is not lethal to adult worms and infected persons have to be treated annually for up to

15 years.[28] Onchocerciasis is a priority disease scheduled for elimination by 2025 by the WHO. Today, 99% of the globally 37 million people infested live in Africa.[1] In 1995, the African Program for Onchocerciasis Control (APOC) was initiated for the implementation of the onchocerciasis control programme based on community directed treatment with ivermectin (CDTI). APOC was coordinating these activities in endemic areas of 22 African countries.[29] Since May 2016, CDTI control programmes are integrated in the WHO Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN).[30] CDTI overcomes the limited performance of weak health systems in rural areas by using an active strategic involvement of the community.[31] To reach the entire population, ivermectin distribution is organized by trained volunteers in each village, resulting in a large geographical coverage. CDTI, in certain regions combined with the control of the blackfly by larviciding of breeding sites (i.e. fast flowing rivers), resulted in a measurable impact reflected by a remarkable transmission reduction in the past 20 years.[32] But success and effectiveness of these targeted interventions lack comprehensiveness. Certain onchocerciasis endemic regions remain undersupplied or unreachable for CDTI due to political instability, insecurity or armed conflicts.[33] As a consequence in the war-affected regions of South Sudan and northern Uganda, onchocerciasis control measures were stopped or implementation started only recently. Moreover, misconceptions and the fear of adverse effects result in suboptimal therapeutic coverage and reduce the effectiveness of the control program.[34] Adding to the complexity of onchocerciasis control, in regions where *Loa loa* and onchocerciasis are co-endemic, ivermectin may cause severe adverse effects (encephalopathy, coma or death) in infected individuals harbouring a high *Loa loa* microfilariae load.[35 36] Thus, CDTI implementation in such regions requires additional precautions.[37] This is the case in certain regions of Cameroon and the Democratic Republic of Congo (DRC).[38] Compliance to CDTI programs can also decrease over the years since less direct positive effects can be observed when onchocerciasis prevalence drops, and healthy feeling people may not appreciate the importance of continuing repeated treatment.[39]

The potential of CDTI to reduce the incidence of epilepsy in onchocerciasis endemic regions

The endemicity of onchocerciasis and the prevalence and incidence of epilepsy are influenced by a multitude of factors (Figure 1). These factors are of uncontrollable nature (climate, environment, ecology), and of controllable nature (prevention and intervention programs, access to health care and treatment).

<<<<<<Figure 1 near here>>>>>>

The two heavily affected regions of the last decades, Northern Uganda and South Sudan, were – and South Sudan still is - affected by armed conflicts. In 2012, after the conflict ended in northern Uganda, the NS epidemic was investigated. Following on, epilepsy treatment was provided, biannual CDTI was implemented and larviciding of major rivers was carried out.[40] Since 2013 the NS epidemic in northern Uganda has reportedly been halted.[41] In contrast in South Sudan, where CDTI was stopped because of insecurity, new cases of NS continue to appear.[18] Based on the observations from northern Uganda, it has been suggested that ivermectin may reduce the incidence of NS and other forms of epilepsy in onchocerciasis endemic areas.[42] Considering that 30% of the globally 37 million individuals infected with onchocerciasis do not have access to effective treatment, and that 1% of those develop epilepsy (equivalent to the estimated excess prevalence of epilepsy over non-onchocerciasis areas) the number of excess cases of epilepsy attributed to onchocerciasis most likely exceeds 100'000.[1 7]

Recent observations in the DRC and northern Uganda suggest that optimal treatment coverage of ivermectin may stop the incidence of NS and other forms of OAE.[42]

Aim

The population based study from 1989 by Rwiza et al. established the baseline prevalence of all forms of epilepsy in the Ulanga district, Tanzania.[43] This population-based study aims to further evaluate the prevalence and incidence rates of new epilepsy cases, including a complete case ascertainment of all forms of epilepsy encountered, and compare the data to the 1989 data (Table 1).

Table 1. The pre- and post-CDTI epilepsy study periods and estimated epilepsy prevalence and incidence in the Mahenge area, Ulanga district in Tanzania.

	Mahenge area, Ulanga district, Tanzania	
Year of epilepsy study data collection	1989[43]	2017
Status of CDTI at study year	Pre-CDTI	Post-CDTI (ongoing since 1997)
Estimated epilepsy prevalence	20.2/1'000	14/1'000*
Estimated epilepsy incidence	146/100'000	81.7/100'000**

*Median epilepsy prevalence and **incidence in low and middle income countries [4 3]

Objective

The main objective of this study is to identify the potential impact of long-term onchocerciasis control using CDTI on the prevalence and incidence of epilepsy in selected villages in the Mahenge area of the Ulanga district in Tanzania.

Specific objectives

1. To determine the prevalence of all forms of epilepsy in selected villages of the Mahenge area and compare the related data from 2017 to the 1989 data.

2. To determine the incidence rate of annual new onset cases of all forms of epilepsy in the Mahenge area and compare the related data from 2017 to the 1989 data.

3. To assess the ivermectin coverage and the onchocerciasis prevalence with a REMO assessment in selected villages of the Mahenge area.

4. To determine the level of onchocerciasis transmission by a serological survey using a rapid test among children in selected villages of the Mahenge area.

5. To investigate the potential difference in clinical appearance of epilepsy in patients with negative onchocerciasis serology to patients with positive onchocerciasis serology.

METHODS AND ANALYSIS

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. Data collection was carried out between January and September 2017. Analysis is planned to be finalized by end of 2017, to allow publishing of the results in 2018.

Study site

The study is taking place in the Mahenge area of the Ulanga distict, Morogoro region in south-eastern Tanzania, a mountainous area with fast flowing rivers.

Epilepsy in the Mahenge area

In the 1960s, Aall-Jilnek was the first to report a high burden of epilepsy in the Mahenge area, and also described the first NS cases.[10] In 1989, Rwiza et al. carried out a population based survey to determine prevalence and incidence of epilepsy in the Ulanga district and found an annual incidence of 73.3 new cases in 100'000. The prevalence was 10.2 cases in 1000, and varied between villages from 5.1 to 37.1 cases in 1'000. Those villages with the highest prevalence were located in the Mahenge highlands (Table 2).[43]

Table 2. Population size and prevalence of epilepsy in some villages of the Ulanga district, Tanzania, showing the highest prevalence in 1989, as reported by Rwiza et al. in 1992

Name of Village	Population size	Number of Epilepsy cases	Prevalence per 1000 people
Sali$^{\delta}$	1'282	14	10.9
Mdindo$^{\delta}$	539	20	37.1
Vigoi$^{\delta}$	1'822	23	12.6
Lupiro	1'697	17	10
Misegezi	1'667	18	10.8
Total	**7'007**	**92**	**13.13**

$^{\delta}$Located in Mahenge highlands

Onchocerciasis and CDTI in the Mahenge area

The Morogoro region is among the five regions where onchocerciais is endemic in Tanzania. The region presents four foci: Uluguru mountain, Ulanga/Mahenge, Kilosa and the Nguru mountain foci.[44] The Ulanga district, specifically the Mahenge area, was known for its high endemicity of onchocerciasis since the early last century.[45] Mass drug administration using annual CDTI was introduced in the Mahenge area in 1997.[46] Before CDTI was implemented, 58.6% of the 482 inhabitants of the Mahenge area tested were found to have a microfilariae positive skin snip.[47] Twelve years later (in 2009) the rapid epidemiological mapping of onchocerciasis (REMO) in 10 villages in the Mahenge area revealed that the percentage of persons with a microfilariae positive skin snip has dropped to 21.9%, with a mean prevalence of 8.3%.[32]

Study population

The three villages Mdindo, Vigoi and Misegezi that had high epilepsy prevalence during the 1989 study will be selected for this study.[43] The entire population of each of these villages will be included in the study.

Epilepsy and NS prevalence / incidence study

Study design

The study is designed as a population-based cross-sectional study, following a two-stage approach for epilepsy case identification at village level. The gold-standard in neuro-epidemiological surveys to identify epilepsy cases in LMICs, the door-to-door approach, will be applied.[48] All inhabitants of the selected villages will be eligible for participation and will be included in the questionnaire screening survey. Due to well described limitations of questionnaire studies on epilepsy (stigma leading to concealment of cases, recall bias, absence of clear terminology for epilepsy and seizures) and to increase sensitivity of case ascertainment, key informants who are likely to be aware of persons with epilepsy in the village will additionally be consulted.[3] [48] These may be health workers, traditional healers, teachers, or community leaders.[49]

In a second stage, persons with suspected epilepsy identified during the household screening survey will be further invited for clinical examination by a neurologist. The examination will include neurological tests and a detailed interview for case verification. In verified epilepsy cases the onset of the seizures will be determined and possible etiologic factors will be investigated, such as birth trauma, head injury, meningo-encephalitis, cerebral malaria, neurocystocercosis, and the history of onchocerciasis. For persons with suspected epilepsy their serological status will be determined using onchocerciasis rapid diagnostic test (Ov16 RDT) (Standard diagnostics, Inc., Gyeonggi-do, Republic of Korea).

Sample size calculation

According to Rwiza et al. (1992) the average prevalence of epilepsy in five villages with high prevalence was 1.313% (Table 2). If we assume a reduction in the prevalence by 33.3% to be able to compare the prevalence at a power of 80% and 95% confidence level, the minimal sample size of 4,746 individuals will be required. Assuming a participation of 80%, a minimum source population of 5,933 is necessary to obtain optimal sample size. Since 1989 the population in the villages with high epilepsy prevalence has increased (Table 3).

For this survey we will include only the population of the villages with the highest epilepsy prevalence in 1989. The total population of the selected villages of Mdindo, Vigoi and Misegezi in 2016 is about 7,766.

Table 3. List of the study villages in the Mahenge highlands, Tanzania, and their population size in 1989 and in 2016.

Name of Village	Population size (1989)	Population size (2016)
Mdindo	539	1'536
Vigoi	1'822	2'572
Misegezi	1'667	3'658*
Total	**4'028**	**7'766**

**Population projection based on the growth rate of Vigoi village*

Data collection at community level

The community survey will commence by a questionnaire interview with the village authorities on demographic topics, and with village health workers to address general questions on the status of epilepsy and epilepsy treatment in the village. A complete door-to-door active screening for persons with suspected epilepsy at village level will be performed. The interview team will be trained on how to conduct the active search for epilepsy cases using a pre-tested, validated screening questionnaire targeting epilepsy by 5 specific questions (provided in the supplementary material).[50 51] For validation, the questionnaire will be translated to Kiswahili, pretested and retranslated to English. The locally used terms for the two respective conditions will be used (epilepsy: *kifafa,* NS: *kusinzia kichwa*). To ascertain completeness and to ensure the best collaboration with the village population, the interviewer team will be accompanied to all households by local village health workers. The geographical coordinates of the participating households will be collected for the mapping and geospatial analysis of cases (proximity to rivers, potential clustering).

Case verification and validation

All persons with suspected epilepsy identified during the door-to-door survey will be verified by a neurologist. The neurologist will perform a detailed anamnesis on all persons with suspected NS and epilepsy. In case of confirmation of the epilepsy, the neurologist will perform a medical examination and administer a detailed questionnaire on the type of epilepsy. Newly diagnosed epilepsy cases will be referred to an epilepsy treatment centre. In case the person is already followed in a treatment centre, permission will be asked to review the medical information available in the treatment centre to record epilepsy diagnosis and epilepsy treatment history.

Definitions:

- 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 to the current guidelines of the International League Against Epilepsy (ILAE) for an operational definition of epilepsy and to the definition used by Rwiza et al in their baseline study performed in 1989. [43 49 52]
- A case will be considered as active epilepsy if the patient is receiving epilepsy treatment or, if without anti-epileptic treatment, the patient presented at least one seizure during the last 5 years.
- A case of suspected NS will be defined as a person who presented with episodes of decreased consciousness during which the head dropped forward repeatedly.
- New cases of epilepsy will be defined as cases that appeared within the last 12 month preceeding the study period.

Onchocerciasis prevalence study

Study design

This study includes two approaches. The aim of the first approach will be to determine the onchocerciasis prevalence in the selected villages after 20 years of CDTI by performing the WHO proposed REMO methodology.[53] In brief, in each study village, 50 adults aged at least 20 years old and resident in the community for at least 10 years will be invited to participate. They will be examined for the presence of onchocerciasis nodules (subcutaneous nodules or deep, painless, firm, mobile nodules located over bony prominences: pelvic girdle, costal grid, knees, and skull). Although pre-control onchocerciasis prevalence data from the study villages is not available, a study from 1966 carried out in three villages in Mahenge found a skin snip positivity rate ranging from 43to 65%.[54]

The second approach aims at determining the level of transmission of onchocerciasis in the selected villages. Therefore, serological testing for onchocerciasis will be done in all children aged 7-10 years old, using the onchocerciasis specific Ov16 RDT (Standard diagnostics, Inc., Gyeonggi-do, Republic of Korea). According to the National Census survey in 2012, the population of children aged 7-10 years represented 11.6% of the population in Ulanga district. Estimated population of children aged 7-10 years from the four selected villages is 900 of which 722 (~80%) are anticipated to participate in the survey giving a power of 85% in detecting the prevalence between 0.8 to 2% at 5% significance level (Table 4).

Table 4. Estimated number of children aged 7 to 10 years old in the study villages in the Ulanga district, Tanzania, in 2016.

Name of Village	Population size (2016)	Estimated Population aged 7-10 yrs	Estimation of participating children (80%)
Mdindo	1’536	178	143
Vigoi	2’572	298	239
Misegezi	3’658*	424*	340*
Total	**7’766**	**900**	**722**

*Population projection based on the growth rate of Vigoi village

Data collection procedures

Data collection will be done using numeric tablets. All data collection forms will be developed in the open source software 'Open Data Kit' (ODK, https://opendatakit.org/). Interviewers will be trained in how to perform tablet-based surveys. A technical data coordinator will be assigned to guarantee completeness and quality of data, and to assure daily data transfer from each tablet to the central server for data security.

Data management and analysis

The prevalence of epilepsy and NS will be computed as a ratio of the number of epilepsy and NS cases per total number of people registered in the households visited, respectively. The incidence of new cases of epilepsy is defined as the number of persons who developed epileptic seizures within two years preceding the study, divided by twice the population size, assuming that the change in population within the two years has a minimal effect on the incidence. Results will be presented accompanied with 95% confidence interval (95%CI), and P-value<0.05 level of significance. Prevalence and incidence will be compared between villages and to the 1989 data. Ivermectin treatment coverage and onchocerciasis prevalence will be calculated. Proportions will be compared using χ^2-test, while means will be compared using t-tests. Demographic and clinical characteristics of persons with epilepsy having a positive Ov16 serology will be compared to epilepsy cases with negative Ov16 serology. Ivermectin treatment coverage, epilepsy prevalence and incidence and OV16 positivity rate among children aged 7-10 years old will be compared among villages, weighted for the difference in population size between villages. Odds ratios will be calculated for epilepsy cases with positive Ov16 results, and the association of Ov16 positivity and epilepsy will be analysed by age group.

Ethics

The protocol has received ethical approval from the ethics committee of the University of Antwerp, Antwerp, Belgium (29.08.2016) and the National Institute of Medical Research (NIMR) ethical committee Dar es Salaam, Tanzania (24.08.2016). Before the activities start, the research team will hold meetings with community leaders and health workers of the selected villages. The procedure, purpose and specific aim of the study will be explained and discussed with regard to the potential risks and benefits for the community. Community leaders, village health workers and researchers will maintain the initially established communication for the entire duration of the study. The dissemination of results will be organized in a similar way as the initial meeting.

As approved by the relevant ethics committees, only participants who provide written informed consent will be enrolled in the study. Participant information sheets and consent forms will be available in English and Kiswahili. In case of illiteracy, information sheets and consent forms will be read to the participant in the presence of a witness. All participants will be permitted to withdraw from the study activities, without reason, at any time. All personal information, samples and test results will be encoded and treated confidentially. People identified with untreated epilepsy or with interrupted treatment will be referred to the treatment centre and will receive advice for care and support.

Data storage and handling

All data files will be centralised and stored in a secured central server. Name-linked information on participants and ID codes will remain confidential and will be used only to communicate clinical results to participants for their respective treatments.

DISCUSSION

The central research question of this study is to determine whether mass ivermectin administration using CDTI has the potential to prevent the onset of OAE. The expected results will contribute to a better understanding of the linkage between the onset of epilepsy and NS in particular, onchocerciasis and the impact of CDTI. In northern Uganda, an NS epidemic has been

halted after introducing a programme combining CDTI and larviciding of the main rivers. This study will be the first to investigate systematically whether CDTI alone may reduce epilepsy in an onchocerciasis endemic region. To do so pre- and post-CDTI epilepsy prevalence and incidence data will be compared. Concurrently, with the Ov16 serological survey among children aged 7-10 years, it will be possible to assess wether the transmission of onchocerciasis is ongoing in the Mahenge area after 20 years of CDTI. So far, the onchocerciasis control program in the study area was monitored based on annual ivermectin distribution, treatment coverage data being provided by the community distributors. Also, ivermectin treatment coverage data will be assessed in the framework of this study by interviewing the population. Moreover, by performing an Ov16 seroprevalence study among children under 10 years of age, a real-time estimate of the level of ongoing transmission of onchocerciasis will be evaluated. The hypothesis is to find a low Ov16 seroprevalence in children and a decreased prevalence of epilepsy since 1989. In case a high Ov16 seroprevalence is found, this will suggest that the CDTI programme was performing suboptimal and/or that ivermectin resisitance may have developed. It might therefore be useful to combine CDTI with larviciding rivers to reduce blackfly abundance.

This study also has limitations. The methods used will not allow for measuring onchocerciasis infection intensity, one of the main factors influencing the development of OAE. There is a possibility that it may not be possible to show an impact of CDTI because in case of high level exposure to infectious blackflies the administration of ivermectin only once a year may not be sufficient to considerably decrease onchocerciasis transmission. Moreover, in pre- and post-CDTI comparison it is possible that observed differences in epilepsy prevalence and incidence are not related to the intervention (CDTI) but to some of the other factors (e.g. those mentioned in Fig. 1) that might have changed over time. However, a site visit to the Mahenge study site revealed that the village population had increased by a factor of 3, but there was no important in- or out migration or any other major change in lifestyle of the population or another major environmental change. In all the villages included in the study, such potential changes will be

carefully assessed to control for potential confounding factors. Families who migrated into the study area after the implementation of CDTI will not be included in the analyses.

Outlook

This study aims to unravel the potential impact of CDTI on reducing the incidence of epilepsy in onchocerciasis endemic areas. Study results may provide evidence that strengthening CDTI programs could prevent the onset of OAE. The results and lessons learned from this study will be disseminated by publications in open access journals, as well as presentations at scientific conferences and shared with all interested health authorities in Tanzania and beyond.

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Authors' contribution

All listed authors contributed to the development of the study design, essential study documents and study tools. According to their different areas of expertise, the authors critically revised specific parts of this manuscript: HG wrote the manuscript; HG, BM, PS, RC developed the study protocol; WM, MM, WM developed and approved the neurological study protocol and the survey tools; HG, BM, AK, developed the methodology and tools for the digital data collection; HG, BM, RC visited the study sites.

Data sharing statement

Data will be available from the Global Health Institute of the University of Antwerp at https://pintra.uantwerpen.be/webapps/cmsmain/webui/_xy-914103_1-t_KW7pxaf1

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Competing interests

The authors declare that they have no competing interests.

Figure caption

Figure 1. Onchocerciasis endemicity and prevalence and incidence of associated epilepsy are influenced by an extensive network of controllable and uncontrollable factors.

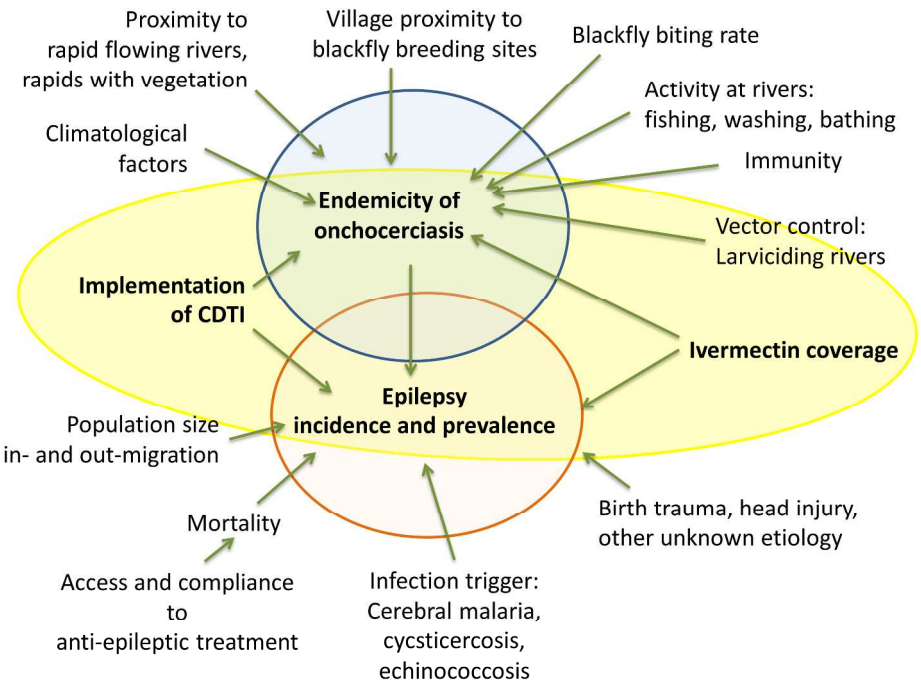

Figure 1. Onchocerciasis endemicity and prevalence and incidence of associated epilepsy are influenced by an extensive network of controllable and uncontrollable factors.

254x190mm (300 x 300 DPI)

SUPPLEMENTARY ANNEX

5 QUESTIONS TARGETED FOR THEW SCREENING OF EPILEPSY CASES

If at least one of the 5 questions is answered with YES, the person will be invited to participate in the neurological examination for case verification.

QUESTION 1

Have you ever lost consciousness and experienced:

a) Loss of bladder control? ☐YES ☐NO ☐DON'T KNOW

b) Foam at the mouth? ☐YES ☐NO ☐DON'T KNOW

QUESTION 2

Have you ever experienced absence(s) or sudden loss(es) of contact with the surroundings, for a short duration of time? ☐YES ☐NO ☐DON'T KNOW

QUESTION 3

Have you ever experienced sudden, uncontrollable twitching or shaking of your arms, legs or head, for a period of a few minutes? ☐YES ☐NO ☐DON'T KNOW

QUESTION 4

Do you sometimes experience sudden and brief bodily sensations, see or hear things that are not there, or smell strange odours? ☐YES ☐NO ☐DON'T KNOW

QUESTION 5

Have you ever been told that you are suffering from epilepsy or that you have already had epileptic fits? ☐YES ☐NO ☐DON'T KNOW