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A Systematic Review of the Geographic Locations and Epidemiologic Features of Nodding Syndrome

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# A Systematic Review of the Geographic Locations and Epidemiologic Features of Nodding Syndrome

By

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2011

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An abstract of
A thesis submitted to the Faculty of the
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## Abstract

A Systematic Review Of The Geographic Locations And
Epidemiologic Features Of Nodding Syndrome
By Anne Abbate

OBJECTIVE: To review and clarify the geographic locations and the epidemiological features of nodding syndrome within and between countries that currently or previously reported cases of nodding syndrome or conditions with clinical presentations similar to nodding syndrome.

METHODS: A retrospective analysis was conducted to evaluate the case status of reported nodding syndrome cases from each country where they have been reported. Published clinical and epidemiological descriptions of these reported cases were reviewed and cross-referenced with the current nodding syndrome case definition to evaluate the true case status. The retrospective analysis was used to support the subsequent review of nodding syndrome risk factors. The strength and consistency of exposure data within and between countries with true nodding syndrome cases was evaluated through intra-country and cross-country analyses.

RESULTS: Cases of nodding syndrome or conditions with clinical manifestations similar to nodding syndrome have been reported from South Sudan, Uganda, Tanzania, Liberia, Cameroon, Taiwan, and Britain. Of these countries with reported cases, only South Sudan and Uganda met the criteria to be classified as confirmed case countries. Tanzania, Liberia, and Cameroon have suspected cases of nodding syndrome. Many risk factors for nodding syndrome were found to be supported by consistent and valid test results in the intra-country analysis. When evaluating common risk factors in the cross-country analysis of confirmed case countries, onchocerciasis was the only supported risk factor; current stunting and current wasting were partially supported. In the cross-country analysis of suspected case countries, onchocerciasis was the only supported risk factor; having a family history of epilepsy was partially supported. Across all case countries, onchocerciasis was the only supported risk factor.

CONCLUSIONS: Suspected nodding syndrome cases in Tanzania, Liberia, and Cameroon warrant further investigation in order to determine the true nature of the suspected illnesses. The intra-country and cross-country analyses indicate that further investigation into the relationship between onchocerciasis and nodding syndrome is justified. Additionally, future investigations should seek to evaluate whether stunting, wasting, and/or a positive family history of epilepsy are risk factors for nodding syndrome as they were determined to be partially supported risk factors in the cross-country analyses.

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# CHAPTER 1: Introduction

## 1.1 Background

Nodding syndrome (NS) cases were first reported as a distinct, unknown entity to international public health authorities in 1997 by a physician working in refugee camps within southern Sudan[1]. In 2002, the World Health Organization (WHO) led a field investigation in southern Sudan and confirmed that communities were experiencing an outbreak of a previously undescribed disease. This illness was first called "nodding disease" because it was characterized by repetitive, involuntary head nodding and impaired consciousness that was often triggered by the presence of food, cold drink, or cold weather. It is now known that this "head nodding" motion is the manifestation of atonic seizure activity [2]. The illness almost exclusively attacked children and adolescents, usually between the ages of 3 to 18. After the disease onset, it was noted that these previously developmentally normal children regressed developmentally and experience dramatic cognitive decline and stunting and wasting. They also experienced psychiatric symptoms such as depression or hallucinations and progressively worsening seizures manifestations [2]. By 2003, nearly 300 cases of nodding syndrome had been reported from isolated areas of South Sudan[3].

Shortly after the outbreak was confirmed in South Sudan, Ministries of Health in northern Uganda reported in 2009 that they too were experiencing an outbreak of "nodding disease" and had identified over 2,000 cases [4]. By February of 2012, that number had risen to 3,097 cases and included 170 deaths[4]. In 2005, 62 cases of nodding syndrome were diagnosed in Tanzania but it is estimated that at least several hundred children are affected within the entire country

[5].Most recently it was estimated that between 3,000-8,000 children in South Sudan, Uganda, and Tanzania are affected by this illness [6].

Many affected families in South Sudan and Uganda have been reported to tie their children with nodding syndrome to the family's hut by the child's ankle when adults are working. This is done out of fear that the child may wander and become lost, fall into fires, or drown [7]. Although no one has been reported to have died from the disease itself, children with nodding syndrome require constant supervision or restraint because many affected children have suffered fatal injuries during seizures.

Despite numerous investigations including case-control studies, case-studies, neurological examinations, and other clinical evaluations, the etiology of nodding syndrome remains unknown. Local healthcare providers have reported that epileptic symptoms can often be well-managed with antiepileptic medications, especially sodium valproate [8]. Unfortunately, most affected areas are also extremely resource poor and most cases remain untreated.

These "nodding disease" outbreaks quickly gained international media attention for being a bizarre, devastating illness of unknown etiology [9-12]. International researchers began noting that nodding syndrome or conditions with clinical presentations similar to nodding syndrome had been documented in Britain as early as 1903[13], in Tanzania in 1962[14], from Liberia in the 1983's[5, 15], from Uganda as early as 1994[16], from Cameroon in 2004 [17], and most recently from Taiwan in 2006[18]. Parallels between nodding syndrome and Nakalanga syndrome, an endemic form of dwarfism with epilepsy manifestations that was documented in Ugandan communities as early as 1903[19], have also been frequently discussed in nodding syndrome literature [14, 20].

## 1.2 Scope of Problem

A major challenge to researchers is that much of the literature published on nodding syndrome contains conflicting results with regard to case cluster locations and history, environmental exposures, the clinical manifestations of the disease, etc. These inconsistencies require review and clarification so that the strength of etiological hypotheses may be objectively evaluated and guide the focus of future research.

It was only very recently, in July of 2012 that an internationally agreed upon term for the illness, nodding syndrome, case definition was decided upon. The formal case definition was agreed upon at the first International Scientific Meeting on Nodding Syndrome in Kampala, Uganda in August 2012 [22]. The meeting report has still not been made publically available and the full, official nodding syndrome case definition was only recently reproduced in a 2014 literature review[6]. As a consequence, to date, no published nodding syndrome investigations have used or tested the full, official case definition in their study. A variety of case definitions have been used, based off of unique clinical descriptions that have been noted as characteristic of the illness but the official case-definition as of 2012 is as follows:

### *The Internationally Agreed Upon Nodding Syndrome Case Definition [6, 21]*

**A Suspected Nodding Syndrome Case:** Reported head nodding in a previously normal person. Head nodding is defined as repetitive, involuntary drops of the head to the chest on two or more occasions.

**A Probable Nodding Syndrome Case:** Suspect case of head nodding with

Both of the following major criteria:

1. Age at onset of nodding between 3 and 18 years old
2. Frequency of nodding 5 to 20 per minute

Plus at least one of the following minor criteria:

1. Other neurological abnormalities (cognitive decline, school dropout due to cognitive/behavioural problems, other seizures or neurological abnormalities)
2. Clustering in space or time with similar cases

3. Triggered by food and/or cold weather
4. Stunting or wasting
5. Delayed sexual or physical development
6. Psychiatric symptoms.

**A Confirmed Nodding Syndrome Case:** Is a Probable case plus a documented nodding episode that is observed by trained healthcare worker, or videotaped, or EEG/EMG

Although nodding syndrome cases have been reported from a number of different countries, there are no publications documenting that these cases were diagnosed using the current case definition. Consequently, it is impossible to know the nature of these reported cases and establish if they are true cases of nodding syndrome, as defined by the current case definition.

Currently, reported cases of nodding syndrome have only been formally investigated in northern Uganda, South Sudan, or Tanzania syndrome cases [6]. If it could be established that cases of nodding syndrome do, in fact, exist outside of the current geographic focuses then such evidence would warrant designating time and resources to formally investigate these lesser known cases. Such cases could provide invaluable insight and a new perspective to researchers working to discover the etiology of nodding syndrome.. An initial means of evaluating the reported case clusters would be to review the clinical symptoms and patient histories that have been published and attempt to retroactively diagnose cases according the internationally agreed upon nodding syndrome case definition.

Determining the true nature of reported nodding syndrome cases is a research priority because much of the etiological research on Nodding syndrome focuses on environmental exposures and infectious etiology. In this context, information relating to the locations of case clusters is considered to be of significance. If nodding syndrome is caused by exposure to one or more environmental factors or infectious agents then it is reasonable to expect that this variable

should be present in all nodding syndrome affected areas. Clarification as to where and when nodding syndrome cases have been documented will enable researchers to evaluate the validity and plausibility of risk factors for true cases of nodding syndrome and proposed etiological hypotheses.

A systematic review of nodding syndrome risk factors is necessary in order to elucidate the validity proposed etiological exposures. A large number of etiological hypotheses have been proposed and investigated in an attempt to understand the etiology this illness. These risk factors include infections (parasitic, viral, bacterial, prions), dietary/nutritional exposures or deficiencies, demographic and genetic risk factors, physical and psychological trauma, and toxic environmental exposures[6]. Most of these investigations have yielded negative or conflicting results. Such inconsistencies hinder research progress because they foster confusion and uncertainty with regard to progress that has been made. Review, evaluation, and clarification of risk factor results from specific locations and their findings may help to provide support for previous results and hypotheses, help to rule out contradicting hypotheses, and generate new etiological ideas and discussion.

For example, nodding syndrome investigations in South Sudan, northern Uganda, and Tanzania have repeatedly documented a significant association between nodding syndrome and onchocerciasis infection [22]. Other parasites endemic to regions of Uganda, South Sudan, and Tanzania have also been proposed and investigated, with conflicting results. If it can be confirmed that case clusters of nodding syndrome are occurring in areas that are not endemic for onchocerciasis, or that are not endemic for other parasites that have been investigated and proposed, then this information would warrant reevaluating any etiological hypotheses reliant on onchocerciasis infection or infection by other specified parasites.

### 1.2.1 Problem Statement

Much of the literature on nodding syndrome contains conflicting or uncertain results with regard to the location of true nodding syndrome cases (as defined by the international nodding syndrome case definition) and the validity of investigated risk factors for the illness. These geographic and epidemiologic inconsistencies require review and clarification so that the strength of proposed etiological hypotheses may be more objectively evaluated and guide the focus of future research.

## 1.3 Purpose of Study

This systematic review will review and evaluate all aspects of nodding syndrome research related to reported case cluster locations, investigated risk factors, clinical manifestations of the illness, and laboratory results. Clinical and epidemiological descriptions in nodding syndrome literature will be cross referenced with the current nodding syndrome case definition to evaluate the status of cases from each country where nodding syndrome has been reported. Additionally, a list of significant risk factors and etiological hypotheses from each case cluster location will be compiled. Information relevant to each risk factor or etiological hypothesis will be cross referenced with information from other case cluster locations to conduct a cross country analysis of risk factors.

## 1.4 Research Questions

This study seeks to answer the following research questions:

1.) Which countries have or have had true cases of nodding syndrome, as defined by the international case definition?

2.) Which risk factors have been investigated within each country that has true cases of nodding syndrome and what is the relevance of these risk factors?

3.) Which risk factors have been investigated across multiple counties that have true cases of nodding syndrome and what is the relevance of these risk factors?

## 1.5 Significance of the Study

Nodding syndrome continues to devastate an increasing number of affected children and families, as researchers indicate that the disease incidence and prevalence is on the rise [23]. Conflicting geographic and epidemiological information published in nodding syndrome literature factors challenge the validity of evidence that has been produced thus far and foster scientific uncertainty. These inconsistencies require review and clarification so that the strength of etiological hypotheses may be objectively evaluated and guide the focus of future research. This systematic review will provide clarification as to where true cases of nodding syndrome have been reported and which risk factors have emerged as significant and consistent within and between geographic areas. This study will also discuss, based on the results, which etiological hypotheses have the most support within and between geographic areas.

# CHAPTER 2: Review of the Literature

## 2.1 Clinical Features of Nodding Syndrome

Nodding syndrome is a degenerative, neurological disease of unknown etiology that has been diagnosed in children and adolescents in South Sudan, Northern Uganda, and Southern Tanzania. Nodding syndrome is characterized by repetitive, involuntary head nodding that is often triggered by the presence of food, cold drink, or cold weather. Clinically, the motion of involuntary head nodding has been observed as the manifestation of a variety of different neurological conditions (ie narcolepsy attacks, tics) and even across species. Involuntary head nodding has even been observed in horses with a condition called Equine Head-Shaking syndrome. It is thought to be a reflex that is triggered by seasonal changes[24]. What distinguishes the "head nodding" motion observed in nodding syndrome patients, however, is that this "head nodding" is the manifestation of atonic seizure activity [1]. Onset of head nodding occurs in previously developmentally normal children and adolescents between the ages of 3 to 18 years old. After the disease onset, patients often experience dramatic cognitive decline, psychiatric symptoms such as depression or hallucinations, stunting and wasting, and progressively worsening seizures manifestations, among other symptoms.

### 2.1.1 Burden of Disease/Prognosis

It estimated that anywhere between 3,000-8,000 children in South Sudan, Uganda, and Tanzania are affected by nodding syndrome [6]. In 2003, nearly 300 cases of nodding syndrome had been reported from South Sudan [3]. In 2009, the Ugandan Ministry of Health released a statement that approximately 2,000 children were affected in Uganda[25]; by February of 2012, that number had risen to 3,097 cases and included 170

deaths[26]. In 2005, 62 cases were documented in Tanzania but it is estimated that at least several hundred children are affected within the entire country [5].

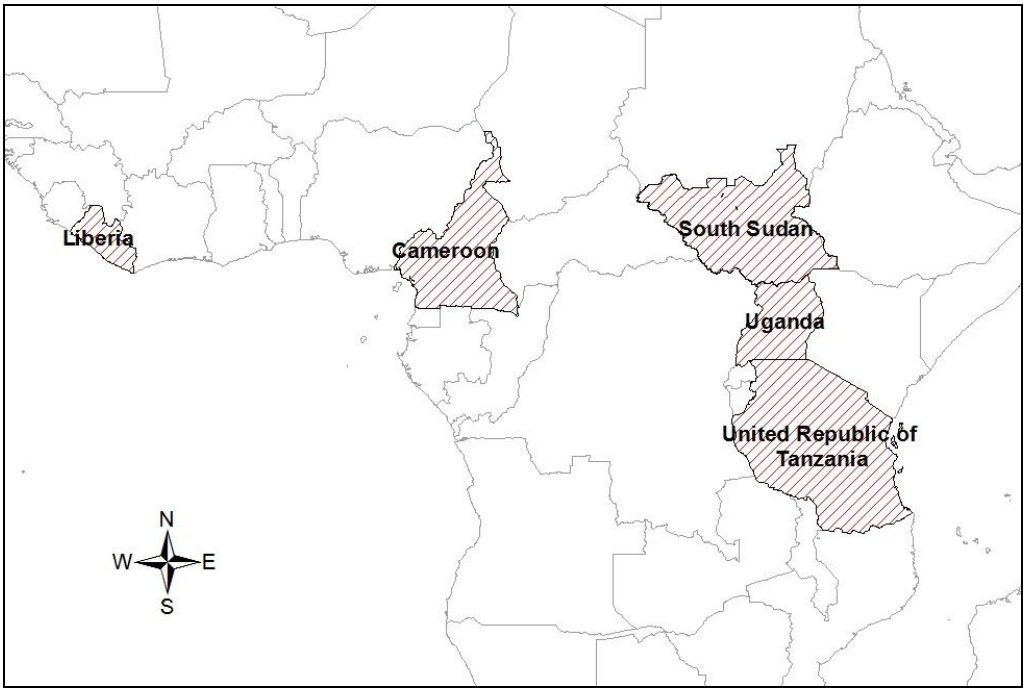
Children with nodding syndrome require constant supervision or restraint because many affected children have suffered fatal injuries during seizures. Many families tie affected children to the family hut by the child's ankle when adults are working. This is done out of fear that the child may wander and become lost, fall into fires, or drown [7]. Currently, there is still no cure and the etiology of the disease remains unknown. Local healthcare providers have reported that epileptic symptoms can often be well-managed with antiepileptic medications, especially sodium valproate[8]. Unfortunately, most affected areas are also extremely resource poor and most cases remain untreated.

## 2.2 Locations of Nodding Syndrome Case clusters

Nodding syndrome or conditions with clinical presentations similar to nodding syndrome have been identified in nodding syndrome literature as being documented inBritain as early as 1903[13], in Tanzania in 1962[14], from Liberia in the 1983's[5, 15], from Uganda as early as 1994[16], Sudan in 1997 [1], from Cameroon in 2004 [17], and most recently in Taiwain[18]. To date, however, outbreaks of nodding syndrome have only been confirmed by international health authorities in South Sudan and Uganda[15, 27, 28] and the full international nodding syndrome case definition has not been applied to diagnose nodding syndrome cases in any investigative studies that have been published thus far. Currently, because no cases have been diagnosed using the standardized case definition, it is difficult to definitively state which locations are experiencing true cases of nodding syndrome, as it is currently being described. Better knowledge of the nature and location of true nodding syndrome cases, as defined by the current

case definition, will help to clarify which countries have or do not have true cases or case clusters of nodding syndrome and in which countries further investigation is required.

**Figure 1. Countries where cases of Nodding syndrome have been reported in Africa from 1964-2014**




Note: Original map, created by author in ArcGIS 10.1 using CDC EpiInfo shapefiles

### 2.2.1 South Sudan

Cases of nodding syndrome in southern Sudan, now the Republic of South Sudan, were first reported in 1997 by a missionary physician and surgeon, Dr. Warren Cooper. Dr. Cooper was working for Samaritan's Purse at Lui Hospital in Lui Village, Western Equatoria State when he was first presented with the bizarre condition [29]. Dr. Cooper stated in a New York Times interview that he had paid little mind to the condition when he was first presented with cases with the explanation that, "It was not something that (he) could surgically treat," [29]. After he began seeing more and more cases, however, he notified international public health authorities of his findings [1].

In September 2001, WHO's Disease Early Warning and Response Network (WHO-EWARN) responded to the disease reports. They performed the first assessments

in Lui and Amadi villages in Mundri County. WHO-EWARN confirmed the presence of a disease [10] but, to Dr. Cooper's surprise, were not able to assign a diagnosis. Dr. Cooper explained to Dr. Marc Lacey in his interview, "I thought these people would come and immediately recognize it and say to me, `You idiot,' …But they said they had never seen anything like it,"[29].

Two months later in November 2001, the Carter Center and HealthNet International conducted a case-control investigation in Lui and Amadi. They sought to evaluate if there could be an association between onchocerciasis and nodding syndrome. Although onchocerciasis has since been established as one of strongest and most consistent associations in nodding syndrome research [22], it is unknown why this particular relationship was initially of interest to investigators at that time. The investigation may have been motivated by previous epidemiology studies that had demonstrated correlations between elevated epilepsy and onchocerciasis prevalence rates [30], or it may have been motivated by convenience.

The skin snips used in the case-control study had been collected through the evaluation component of the Carter Center and HealthNet International's onchocerciasis control program [15]. The onchocerciasis control program in Western Equatoria, southern Sudan was first started in 1995 during a three month conflict cease-fire that had been negotiated by former President Jimmy Carter [31]. It was during the 1995 cease-fire that Ivermectin, the drug of choice for onchocerciasis treatment, was first distributed.

During the course of the investigation, the Carter Center and HealthNet International research team was discovered and made contact with another investigation close by. A WHO led team was also conducting a field investigation in Lui in January

2002 to assess the nodding syndrome outbreak [15]. Although, initially, the WHO team did not have a protocol or intentions to initiate a case-control study, by the end of their first week in Lui a decision was made by the team to undertake a small study. Their rational was that security concerns in southern Sudan, which was currently in the midst of an ongoing civil war and experiencing periodic aerial bombardments in the area, would likely prevent opportunities to conduct an investigation in the near future [15]. The results from Carter Center/HealthNet International studies and from the WHO study were published together in one paper, although, unfortunately, neither study was able to identify the source or cause of the outbreak. According to information provided by patient's caretakers, the first case of nodding syndrome among patients enrolled in the WHO study appeared in 1991. Village elders reported that they had never seen cases prior to the most recent displacement in the early 1990's [15].

In April 2002, WHO recruited a neurologist to conduct EEG examinations on 31 children with nodding syndrome in Mundri County. The neurologist reported that children with nodding syndrome in southern Sudan demonstrated abnormal electrophysiological and showed evidence of progressive epileptic encephalopathy, however, a specific neurological diagnosis was not assigned [10]. Affected communities in the area began reporting concerns to health officials that the disease could be spreading to other new areas and to healthy children. Fortunately, according to a 2006 report from Samaritan's Purse to WHO, the incidence rate appeared to relatively steady. They reported that in Lui and Amadi, where they worked, "new cases of nodding syndrome did continue to appear sporadically but that there had been no marked increase in the number of cases," [10].

In September 2010, the Ministry of Health (MoH) of the newly established Republic of South Sudan conducted a series of focus group interviews with affected communities and families in Witto Payam, Western Equatoria. The goal of these focus groups was to gain a better understanding of when the disease first appeared, a description of conditions and resources available during the civil war, and what the community believes causes nodding syndrome [32].

Most of the patients examined by the MoH research team were between 5-20 years old and the majority of those were between the ages of 10-15.The community described two seizure disorders in the area; generalized epilepsy preceded by an aura and nodding syndrome. They reported that head nodding seizures were usually precipitated by the sight of food and that the disease is degenerative. Over time, children who initially only had head nodding seizures will eventually also experience generalized or tonic-clonic seizures.

Among the explanations and theories proposed by the community for the emergency of nodding syndrome were; contaminated relief food, childhood vaccinations, supernatural forces, intermarriage between IDPs and the local community, and "large or small flies which had invaded the areas of wartime displacement. They had observed that people became blind when bitten by these flies,"[32]. The authors of the publication did not describe which, if any, specific relief food contaminants were identified as possible culprits. The contaminants could include anything from mold, insects, toxins, pesticides, preservatives, unsanitary water, human or animal waste, etc. but without specific leads it is difficult to follow-up on.

The suggestion that "small flies" could be the source of the outbreak may strengthen what is already known about the role of *Simulium* flies and onchocerciasis in relation to nodding syndrome but this description also raises new questions. The statement that "large and small flies *invaded* the areas of wartime displacement," implies that they were not endemic to the area prior to the settlement of displacement camps. If the small, blindness causing flies are indeed the *Simulium* flies which transmit onchocerciasis, these anecdotes contradict epidemiological surveys which report that onchocerciasis had been endemic to Western Equatoria in South Sudan for quite some time [33].

Another interesting point for discussion is the description of "large flies." These flies could easily be from the drosophila genus, species of which are often referred to as the common house fly. Large numbers of drosophila fly species are often observed in settings where hygiene and sanitation have been compromised, such as an IDP camp. It is also possible that the "large flies," especially if they are biting large flies, could be from the genus Chrysops. Chrysops flies transmit the filarial parasite loa loa, commonly referred to as the African eye worm, which is also endemic to Western Equatoria [34]. Common names for Chrysops species include the horsefly, deer fly, or yellow fly.

Unfortunately, as with investigations conducted prior to the MoH investigation, researchers were unable to determine the source of the outbreak. Information gathered from the focus groups was also insufficient to generate credible theories for the disease's emergence. Two months after the MoH investigation, the Government of South Sudan contacted the US Centers for Disease Control and Prevention (CDC) and requested

investigative assistance. Unfortunately, the CDC investigative team was unable to travel to South Sudan until in May 2011 because visa and security concerns.

When the CDC team arrived in South Sudan, they conducted a case-series and a case-control study. They also verified that the outbreak in Western Equatoria region shared similar features with the nodding syndrome outbreak in Uganda that CDC had investigated two years prior, in 2009 [27]. As in previous studies, CDC found that there was an overall association between onchocerciasis and nodding syndrome. In the CDC Morbidity and Mortality Weekly Report (MMWR) it was reported that initial analyses did not find associations with any other risk factors that were investigated. Ongoing conflict and civil unrest in South Sudan has hindered follow-up investigations since 2011 but other researchers have indicated that, as of 2012, new incident cases are still being reported from the area [15].

### 2.2.2 Uganda

Patients with head nodding seizures in Uganda were first examined in 1994 by the German neurologist, Dr. Christoph Kaiser. Between 1994-1996, Dr. Kaiser examined and diagnosed the specific conditions of 91 epilepsy patients from Kabende parish in Karabole district, Western Uganda. Of the 91 patients examined, 19 patients had seizure manifestations that Dr. Kaiser was unable to classify. It had been observed among community members that 15 of those patients with unclassifiable seizures experienced, "seizure(s) characterized by one or several repetitive head movements or "head nodding" which was usually accompanied by impaired responsiveness lasting from a few seconds up to several minutes," [16].

Dr. Kaiser observed seizures in three of the patients with reported head nodding; one patient was sitting up during their epileptic episode and the other two were lying down. The patient who was observed while sitting up was a ten year old boy. He was not connected to an EEG for epileptiform observation at the time but Dr. Kaiser observed that during the epilepsic episode the boy had, "had repeated episodes of uttering a short sound ("like a hickup"), which was accompanied by a short single jerk of the upper body and followed by a dropping head movement and a few seconds of impaired responsiveness." Overall he described this seizure episode as having clonic and atonic features and proposed that it could be classified as myoclonic-astatic. He did not witness head nodding motions in the two patients who were laying down and classified their seizures as complex partial seizure types, [16].

Although nodding syndrome was not officially reported from northern Uganda by the Uganda government until 2009, there are many individuals who contend that the outbreak in northern Uganda began around the same time as the outbreak in South Sudan. Village elders in northern Uganda have reported that the earliest case of nodding syndrome in emerged in 1997 [2, 23, 28]. In a newspaper interview, a Kitgum village health team member, Mr. Joe Otto said that the disease was first noticed by Kitgum health workers in 1998. He insists that cases were reported to the District Health Officer at that time but that their report was not taken seriously. This is why, he claims, that the disease was not recognized by the Ugandan Ministry of Health (MoH) until 2009 [35].

When the Ugandan Ministry of Health requested international investigative assistance for the nodding syndrome outbreak in 2009, it was also reported that there were approximately 2,000 potential cases of Nodding syndrome in Uganda [28]. By

February of 2012, that number had risen to 3,097 cases and included 170 deaths [4]. The presence of the outbreak was confirmed by the Ugandan Ministry of Health, WHO, and by CDC. New cases are still being reported from the northern region of Uganda [2, 22, 36].

### 2.2.3 Tanzania

Epileptic patients with a "nodding head," were first reported from Mahenge region in southern Tanzania by the Norwegian physician, Dr. Louise Jilek-Aall in 1965. Dr. Jilek-Aall specifies that, "to describe this condition the narrator always let his head fall forward on to his chest," [37]. Many researchers assert that Jilek-Aall first documented patients with head nodding seizures in her 1962 article, "Epilepsy in Tanganyika" [14, 20, 38]. That article body itself, however, contains no mention of head nodding or atypical seizure activity in patients [39]. The article mentions an appendix that was originally published along with the article in 1962 but it does not appear to have been published since. Consequently, a copy of the appendix could not be obtained to confirm that nodding syndrome had been documented in patients prior to 1965.

It is also unclear as to when head nodding seizures first affected the Mahenge region or if they had always been a feature of epilepsy that area. In 1968, Dr. Wolfgang Jilek, Dr. Jilek-Aall's husband, reported that, "the Wapogoro are alarmed about a noticeable increase in epileptic conditions among their younger generation," and describes head nodding seizures as a feature of epilepsy among the Wapogoro that only occurs only in children [40]. It is not specified, however, whether cases of head nodding made up the majority of new incident cases or if the incidence of all epileptic conditions rose, and head nodding seizures along with them.

In a 1979 article, Dr. Jilek-Aall specifies that 33 of the epilepsy patients she examined first experienced "nodding of the head" before the onset of puberty

Head nodding seizures in patients from Mahenge region were documented throughout the late 1970's by Dr. Jilek all and then again in 1992 during a genetic and etiological study led by American statistician and geneticist, Dr. Rosalind Neuman. Dr. Neuman and her co-authors, including Dr. Jilek-Aall describe head nodding symptoms as non-convulsive seizures that are observed in patients of the Wapogoro tribe who have "kifafa", or epilepsy. The local name for the head nodding seizures among the Wapogoro is '*amesinzia kichwa'*, which is Swahili for 'nodding of the head' [40]. Neuman states that head nodding symptoms are, "feared and recognized by the Wapogoro as indicating that a child either has or is about to develop kifafa (ie. epilepsy)," [41]. This description is consistent with the onset of nodding syndrome observed in South Sudan and Uganda [2].

After the Nodding syndrome outbreaks in South Sudan and Uganda gained international media attention in the 2000's, researchers once again turned their attention to head nodding seizures in Tanzania. Nodding syndrome or head nodding symptoms were documented again in Tanzania in 2008 by Dr. Andrea Winkler [5] and have continued to be a major focus of investigations in this area ever since [38, 42]. In these more recent investigations,

### 2.2.4 Liberia

In 1983, "head nodding seizures" were reported as a feature of a distinct seizure disorder among children in the Bassa and Kpelle tribes located in Grand Bassa County, Liberia [43, 44]. The head nodding seizures were described by the Dutch physician, Dr. Fransje van der Waals who observed rhythmic "complex partial seizures (that) were

mainly characterized by dorsoventral movements of the head and progressed in 64% of cases to general tonic-clonic convulsion,"

Among affected communities the translated name for the head nodding seizure disorder was, 'to drop the head in the pan,' in reference to the fact that this seizure was usually noticed during social gatherings such as meals [43]. However, the relationship between meals and head nodding seizures were viewed as coincidental by the population. Food was not identified as a trigger for the head nodding seizures, as it has been in South Sudan and Uganda. Photic stimulation was the primary trigger identified for head nodding seizures in Liberia. Although the local description of the seizure disorder would imply that the presentation of food is responsible for triggering attacks, Dr. van Der Waals deduced that head nodding seizures are witnessed at social gatherings and meals because they "reflect the precipitation of seizures by looking into the fire," [44].

According to clan chiefs, town chiefs, and elders, the first known cases of epilepsy in the area, locally known as 'Sii' or 'See-see', developed in the 1950's. Epilepsy then reportedly, "gradually increased in frequency in the 1960's and spread through the whole clan in the 1970's," [45]. This anecdotal description was supported by an epidemiological survey of the area which documented an overall increased incidence rate of epilepsy cases, especially among patients with partial seizures. Epilepsy prevalence in the area was estimated to be 28 per 1,000 people but in select areas could be as high as 190 per 1,000 [45].

### 2.2.5 Cameroon

In Cameroon, epileptics are referred to as, "Ils qui tombent", translated as, "those who fall." In 2008, a research team comprised of four Italian pediatric neurologists and

two trained healthcare workers from a local epilepsy service conducted a six-week study in Littoral Provence of Cameroon to study the prevalence, nature, and etiology of epilepsy in the area [17]. During the case control portion of the study, the team identified four patients with head nodding seizures during the course of their 19-patient. The head nodding seizures were described by caretakers of patients who had histories of partial complex seizures. They reported that, during the head nodding seizures, the patient would have impaired responsiveness for up to several minutes. The EEG records of patients with reported head nodding seizures were, "characterized by focal epileptiform activity and non-specific focal slow wave activity," [17]. Most of the patients with head nodding reported experiencing seizures on a daily basis.

The researchers also assessed the prevalence of epilepsy and onchocerciasis within the community. Epilepsy prevalence was evaluated through door to door household surveys. A rapid epidemiological assessment of onchocerciasis prevalence for the general population was estimated by evaluating the prevalence of nodules in adult men (PNAM). The crude epilepsy prevalence rate in Littoral Provence was calculated to be 105 per 1,000 people. Onchocerciasis was determined to be hyperendemic to the area as indicated by the high PNAM rate of 62.5%.

### 2.2.6 Taiwan

In 2006, a Taiwanese physician published a case report of a 31 year old female patient suffering from an autosomal recessive movement disorder, neurocanthocytosis. This illness is typically caused by a mutation in the CHAH gene on the 9$^{th}$ chromosome but cases can appear sporadically as well. Similar to nodding syndrome, patients with neurocanthocytosis suffer from involuntary movements including seizures and cognitive decline in the form of dementia. Laboratory tests for neurocanthocytosis also give results

with elevated creatine kinase levels and peripheral blood smears which contain a large number of acanthocytes, from which the name neurocanthocytosis is derived[18]. Acanthocytes are abnormal red blood cells that have spiked cell membranes. They are also referred to as spur cells[46].

This particular case was considered unusual because, in addition to the classic symptoms of neurocanthocytosis, this patient also experience prominent truncal tics. These truncal tics were described as "intermittent head nodding jerks." The head nodding tics were not diagnosed as seizures, however, and the patient's symptoms were effectively managed with medications[18]

## 2.3 Prominent Etiological Risk Factors

A large number of etiological hypotheses have been proposed and investigated in an attempt to understand the etiology of nodding syndrome. Proposed exposures include infections (parasitic, viral, bacterial, prions), dietary/nutritional exposures or deficiencies, demographic and genetic risk factors, physical and psychological trauma, and toxic environmental exposures[6]. Most of these investigated risk factors have yielded negative or conflicting results. This hampers research progress because the large number of conflicting results in nodding syndrome literature because foster confusion and uncertainty in regard to the validity of published data. Therefore, a systematic review nodding syndrome risk factors is necessary to provide clarification and elucidate the validity proposed environmental exposures. This will be done by evaluating the strength and consistency of investigated exposures within and between countries with confirmed cases of nodding syndrome. Furthermore, the strength and consistency of investigated exposures will be also evaluated within and between countries where there are probable or suspected cases

of nodding syndrome, as defined and determined in accordance with the current case definition. Finally, the strength and consistency of common investigated exposures will be evaluated between countries with confirmed cases of nodding syndrome and countries with suspected or probable cases of nodding syndrome.

### 2.3.1 Onchocerciasis

The association between nodding syndrome and onchocerciasis infection has emerged as the strongest and most consistent exposure in nodding syndrome investigations [47]. Most children (between 54-93%) with nodding syndrome produce positive skin-snip tests for onchocercaisis infection, the gold standard for onchocerciasis testing [48]. Even within select sample populations within case-control studies where onchocerciasis infection rates were not statistically significant in nodding syndrome cases compared to healthy controls, onchocerciasis was considered hyperendemic to the area and more than half of the cases (and controls) had onchocercaisis infections [17, 49].

The significance of onchocercaisis' connection to nodding syndrome is not yet understood as there is no evidence to support that *Onchocerca vulvus*, the parasitic nematode responsible for Onchocercaisis infection, is capable of causing nodding syndrome symptoms. Onchocerciasis, commonly known as river blindness, is caused by infection with the parasitic, filarial nematode, *onchocerca volvulus*. Infection with the parasite can result in raised, itchy nodules under an infected person's skin and blindness. Onchocerciasis is spread the black *Simulium* fly, which carries larval onchocerca vululus and breeds near fast moving streams and rivers. Transmission occurs when the *Simulium* fly bites an individual to take a blood meal and, in doing so, injects the onchocerca microvilarae into that person's blood. Over time, the microfilarae mature to become large filarial nematodes, which concentrate in the skin of infected individuals and cause itchy

nodules. *Wolbachia*, is an endosymbiotic bacterium in onchocerca vulvus, and is responsible for triggering an inflammation reaction in the cornea of the host which eventually causes blindness [50].

Laboratory testing to search for microfilarae in the cerebrospinal fluid of nodding syndrome patients have all been negative [5]. Additionally, onchocerciasis is endemic to many other countries and regions that have never been affected by nodding syndrome.

#### 2.3.1.a Precedent for Onchocerciasis as a Risk Factor: Epilepsy

Since the 1970's, however, onchocerciasis researchers have observed a positive correlation between general epilepsy rates and onchocerciasis prevalence [30], not just with nodding syndrome. A study published in 1996 examined the association between onchocerciasis and epilepsy in Burundi. The author found a significant association between onchocerciasis and epilepsy (81.8% cases versus 68.3% of controls, p-value <0.05). He also identified nine epilepsy patients with severe growth retardation. He diagnosed them as being Nakalanga dwarves, all of whom tested positive for onchocerciasis [51].

During the 1994 epilepsy study conducted by Dr. Kaiser in Karabole District, Western Uganda, the authors also conducted a 38 patient, matched case-control study to evaluate the association between epilepsy and onchocerciasis. When onchocerciasis was diagnosed using skin snips, the association with epilepsy was not significant (OR=1.68 (CI 0.6-4.57) p-value=0.31). When onchocerciasis was diagnosed by detecting subcutaneous nodules, however, the correlation was more pronounced (OR=2.77 (95%CI 0.92-8.33) p-value=0.065). He proposed that this difference could reflect the role of infection intensity in the development of "onchocerciasis related epilepsy," [52]. T.

solium infection was likely ruled out as cause of the skin nodules because, out of the 61 patients tested in the study, T. solium, test results were only positive for one patient and borderline in three others[16].

When Kaiser evaluated the association between epilepsy and onchocerciasis in an ecological study in the same area he found that the prevalence of epilepsy followed the distribution of onchocerciasis in Kabende parish of Karabole district, Western Uganda. Epilepsy prevalence was significantly higher in the three villages with the highest levels of onchocerciasis prevalence compared to the other nine villages in Kabende parish (p-value < 0.0001) [53]. A more recent meta-analysis has echoed these findings and found that, on average, epilepsy prevalence increases 0.4% for each 10% increase in onchocerciasis prevalence. Based on these results the authors suggest that the burden of onchocerciasis infection may need to be reevaluated to include the burden of epilepsy possibly resulting from infection [54].

#### 2.3.1.a. Precedent for Onchocerciasis as a Risk Factor: Nakalanga Syndrome

Nakalanga syndrome is a form of dwarfism that was once considered to be geographically confined and endemic to parts of eastern Uganda from 1902-1964 [19, 55]. The affected area was also known to be hyperendemic for onchocerciasis and was considered to be a major factor by many in the development of the disease [19]. Since the nodding syndrome outbreak in South Sudan and northern Uganda, many researchers have noted parallels between the two diseases [5, 20] but none have described them in detail.

As with nodding syndrome, Nakalanga syndrome was noted to affect previously normal, healthy children who were living in areas that were considered hyperendemic for onchocerciasis. After the onset of Nakalanga syndrome, children gradually became weak

and malnourished despite the availability of food and the resources to thrive, as demonstrated in their siblings. Developmental milestones such as walking, talking, and self-feeding were delayed in children who had early onset or could regress if a child experienced later disease onset. It was also noted that puberty was delayed in children who experienced disease onset prior to the onset of puberty. The disease was first described in detail in 1950 by the British physician, Dr. Alan B. Raper:

> The child becomes gradually weak and thin; it does not play normally but sits listlessly, preferably in the sun. It frequently cries for food, but on receiving it the child merely toys with it and makes little attempt to eat…He is distinctly undersized, appearing several years younger than his reported age…Inability to hold up the head is taken as a sign of severe affection; one informant described how certain cases would let the head fall forwards while eating, and be quite incapable of raising it again, until a friend raised it manually for the patient…A notable feature is the day-to-day variation in well-being. For a day or two the child will be listless and complain feebly of vague pains, the next day he will have recovered spontaneously, only to relapse some days later (pg 343-347).

Additionally, when Raper and his colleagues admitted seven Nakalanga children to a local hospital for observation, two of those children were observed to suffer from general epilepsy. Laboratory testing and experimental diets ruled out hypoglycemia and renal failure as a cause of Nakalanga. It was, however, determined by Raper that onchocerciasis and anterior pituitary failure were at play in the development of the disease. All seven cases admitted to the hospital had positive diagnoses for onchocerciasis, both by skin snip and nodule examination. Furthermore, community assessments found that Nakalanga patients appeared to be disproportionally affected by onchocerciasis relative to their age-matched, healthy peers. Raper's findings were supported by interviews with community members, all of whom held firm convictions that Nakalanga syndrome was caused by the bite of the *Simulium* fly. A local legend even attributed the disease to a stream god named, "Nakalanga" who would strike healthy

children living near the stream. Parents in the community would often leave gifts to the stream god in the hope that their children would be spared. Children of all social classes and families in the communities were considered to be at risk. Once affected by the disease, the community believed that the only course of action was to remove the child from the affected area [19].

Reports of Nakalanga syndrome from eastern Uganda were published frequently until 1964. Around that time, the affected areas were being treated with the larvicide DDT as part of an onchocerciasis vector control strategy [55]. After the vector was successfully eliminated, Nakalanga syndrome was not reported from eastern Uganda again. It would appear that Nakalanga syndrome had also been eliminated, along with the *Simulium* fly in eastern Uganda, until cases of Nakalanga syndrome were reported from western Uganda in 1992 [56] and again in 1996 [55, 57]. Aside from a personal communication between Raper and a Mr. G.R. Barnley, who reported to Raper that he had encountered stunted, malnourished, children with disproportionally large heads during a *Simulium* survey at Bugoye in western Uganda [19], this is the first time that Nakalanga syndrome or a condition like it has been reported from western Uganda. There were also reports that Nakalanga syndrome was affecting Burundi, the neighboring nation to the west, at this same time [51].

In 1991, a psychiatrist stationed in Kampala, Dr. E. Ovuga, initiated a study to examine patients with epilepsy and retarded growth in Kyarusozi sub-county Karabole district, western Uganda. It had been observed previously by one of Dr. Ovuga's coauthors that, in addition to the area being hyperendemic for onchocerciasis (with

prevalence rates as high as 80-95% [58]), there was an unusually high number of patients with epilepsy in the area.

Through house-hold surveys, Ovuga identified 217 patients from Kyarusozi sub-county who suffered from epilepsy and/or retarded growth. He noted that retarded growth caused by Simulium flies was referred to as Nakalanga syndrome in Luganda, the national language, or as *Ekihuruka* or *Ekinginda* in Rutooro, the local language in Karabole district. The translation of *Ekihuruka* is "failure to grow."

As with nodding syndrome patients, approximately 91% of the patients identified through Ovuga's household survey were younger than 19 years old. Assuming a causal relationship between epilepsy/retarded growth and onchocerciasis, Ovuga postulated that this age distribution could attributed this age distribution to the fact that the population may not have been exposed to onchocerciasis previously; as they were relatively new to the area and had resettled from southern Uganda in the 1960's. This is an interesting point, as many investigations have found that most children affected by nodding syndrome are also internally displaced persons [15, 59].

Ovuga identified 126 patients with a primary diagnosis of epilepsy. Of those, 88% tested positive for onchocerciasis via skin snip. He identified 67 patients whose primary diagnosis was severe growth retardation/growth arrest, 87% of those patients tested positive for onchocerciasis infection. Additionally, there were 24 patients who suffered from both epilepsy and growth arrest, all of whom tested positive for onchocerciasis infection.

Many of the patients with epilepsy or retarded growth were also diagnosed with or reported severe psychological symptoms. These symptoms were observed more often in patients with both retarded growth and epilepsy than in children with retarded growth or epilepsy alone. Parents of children with comorbid conditions reported that their children did not eat well, were physically weak, socially withdrawn, unable to engage in play or other normal activities, slept excessively, often screaming in their sleep, they sometimes had chest pain, and were forgetful. During physical examination of these patients, Ovuga noted that all were both stunted and wasted and that many had pigeon chest, enlarged thyroid glands, and poorly developed secondary sex characteristics [56].

In 1994, however, the German neurologist, Dr. Christoph Kaiser, did explicitly assign a diagnosis of Nakalanga syndrome to patients with growth retardation in the Kabende parish within Karabole district. Between March and June 1994, Dr. Kaiser conducted a population-wide epilepsy prevalence survey in Kabende parish. Out of the 61 epilepsy patients identified in that survey, 12 were also diagnosed as having Nakalanga syndrome. These patients were significantly shorter than patients without Nakalanaga syndrome ($p<0.001$) and had a significantly higher mortality ratio than other epileptic patients in the study (Standard Mortality Ratio=21.4) [60]. For the next years later, at regular 6-month intervals, Kaiser followed up on epilepsy patients in that area. In 1996 he evaluated the clinical and electro-clinical features of the 61 patients identified in 1994 and 30 additional epileptic patients that had been identified during the regular follow up periods. During the evaluation period, 15 patients were reported to have seizures characterized by head nodding. Kaiser observed one of these head nodding seizures in a 10 year old boy and described that the boy had, “repeated episodes of

uttering a short sound ("like a hickup"), which was accompanied by a short single jerk of the upper body and followed by a dropping head movement and a few seconds of impaired responsiveness[16]."

Unfortunately, Kaiser does not provide explicit descriptions of the seizures experienced by the 12 Nakalanga patients evaluated so it is unknown whether any of the Nakalanga patients reported having head nodding seizures. Regardless, his findings are intriguing. Previously it was thought that the connection between Nakalanga syndrome and Nodding syndrome was weak because the two conditions were spatially and temporally distant. Upon evaluation of cases in Karabole district, however, it appears that, in addition to sharing many clinical features (ie epilepsy, stunting/wasting, psychological symptoms, etc.), the two conditions may have both been present in Kabende parish at the same time and even in the same study [16].

Nakalanga patients in Karabole district also share the same significant, risk factor as patients with Nodding syndrome. At the same time that Kaiser was evaluating epileptic patients in Kabarole district, another researcher, Kipp, was conducting a case-control study in Karabole district. In the case-control, the 31 case patients were individuals who had been identified by community members having Nakalanga syndrome. Characteristic of Nakalanga syndrome, cases weighed significantly less and were significantly shorter than their age-matched peers. Additionally, all 31 cases and 22/28 controls tested positive for onchocerciasis infection via skin snip (p-value=0.008) [55].

Mass ivermectin treatment

#### 2.3.1.b. Response to Onchocerciasis as a Risk Factor: Vector Control

The strong association between nodding syndrome and onchocerciasis infection has motivated Ugandan communities and the media to pressure the Ugandan government to conduct widespread aerial larvicide spraying with the goal of killing off the *Simulium* fly that transmits onchocerciasis [61]. In March of 2012, President Museveni pledged that the government would provide helicopters for aerial spraying of the *Simulium* fly over areas affected by nodding syndrome [61]. By June of 2012, the Ugandan government had laid out a plan to begin mapping high risk areas so that they could begin spraying affected areas by August of 2012 [62]. Approximately $100,000 was released to fund *Simulium* vector survey [63]. After a slight delay, the Ugandan government conducted an aerial larvicide treatment over Pader, Uganda in December 2012 [64]. Pader is heavily affected by nodding syndrome and epilepsy. Media sources have reported that between 2009 and July 2012 there have been 4,998 cases of nodding syndrome reported and 351 deaths [65]. When aerial spraying over Pader was conducted in December 22012, media sources announced that the Ministry of Health also intended to spray over Gulu, Kitgum, Lamwo, Oyam, Lira and Amuru districts in the coming month and a half [20]. However, it is unclear as to whether or not these treatments have been conducted yet. According to the Ministry of Health, plans to spray along Pager and Aswa River in Lamwo have been finalized and scheduled to occur in November/December of 2013 [36].

Scheduled spraying in other districts may have been delayed and/or incorporated into the President's five-year master plan to eradicate or control all neglected tropical diseases (NTDs) that are endemic to Uganda. The plan, called ENVISION, was officially launched on October 5, 2013. The master plan is funded by the Ugandan government and

USAID. Programs under the master plan will be carried out by RTI International and the Carter Center [66]

# CHAPTER 3: Methods

This systematic review will conduct three major analyses; a retrospective analysis of the nature of reported nodding syndrome case clusters, intra-country analyses of investigated risk factors, and a cross-country analysis of investigated risk factors. The retrospective analysis will evaluate the nature of reported NS case clusters from each location by comparing the case cluster descriptions to the current nodding syndrome case definition [6, 21]. This retrospective analysis will help to establish if cases in a location can be classified as "Suspect", "Probable", or "Confirmed" Nodding syndrome cases, according to the current case definition.

A systematic review of nodding syndrome risk factors is necessary to provide clarification and elucidate the validity proposed environmental exposures. This will be accomplished by systematically reviewing and evaluating the strength and consistency of investigated exposures within and between countries with true cases of nodding syndrome. The intra-country analysis, will evaluate the strength and consistency of risk factors which have been investigated in connection to nodding syndrome within each country where a risk factor has been evaluated. These risk factors include investigated exposures and laboratory test results. The third analysis, the cross-country analysis, will use the results of the retrospective and intra-country analyses to evaluate the consistency of common risk factors across multiple countries with reported cases of nodding syndrome.

## 3.1 Exclusion and Inclusion Criteria

### 3.1.1 Items to be Included for Review

Reports and descriptions of all clinical features, laboratory test results, and etiological exposures specifically observed or described in NS patients were included for review and analysis. Additionally, if a specific clinical feature (ex: age of disease onset), laboratory test result, or etiological exposure result was not reported for just nodding syndrome patients but was described for an entire study population (ex: the age of onset for all epilepsy patients participating in a study) in which the nodding syndrome patients were included, then the description was still included for review and represented as a reported feature of nodding syndrome. Although descriptions of results/items for entire study populations are not precise representations of nodding syndrome, given the limited availability of NS descriptions reported from certain locations, it was decided that these descriptions still provided the most accurate information that was available. Each description of a study population that was extrapolated to be representative of nodding syndrome cases will be noted within the tables they are published.

### 3.1.2 Reviewed Items to be Excluded from Analysis

Clinical features, laboratory test results, or exposure data which are collected for review will be classified as “Inconclusive” and thereby excluded from analysis if, in the original study, the method used to collect the data was less sensitive and therefore inferior to the currently recognized gold standard for that particular type of testing. For example, the gold standard for onchocerciasis infection testing in individuals is a skin snip [67]. Any tests for onchocerciasis that are known to be less sensitive than skin snips yet were performed in a study to diagnose individuals will not be considered valid tests and in their reported association with nodding syndrome will be marked as “Not Valid”.

They will still be included in results because they will have been evaluated but they not be included in the analysis.

Additionally, any risk factors which were evaluated but not compared to a control group will similarly be considered inconclusive assessments of a risk factor, marked as "Not Valid", and classified as "Inconclusive".

## 3.2 Retrospective Analysis of the Nature of Reported NS Case Clusters

Literature on nodding syndrome, including case-control studies, population surveys, clinical assessment, commentaries, and literatures views were reviewed to compile a list of all potential NS case cluster locations and dates that had been mentioned by authors. A thorough review of the literature was conducted to search for as much information as possible with respect to each potential case cluster location that had been reported in other articles. The potential case clusters being reviewed were considered individual, isolated, clusters of cases if they were separated spatially. Spatial separation was defined as being separated by national border.

The current nodding syndrome case-definition was created by collaborating NS researchers at the First Annual Scientific Meeting on Nodding Syndrome in Kampala, Uganda in July 2012 (unpublished) [21] and republished in a recent NS review[6]. The current case for nodding syndrome is as follows;

**Suspected case:** Reported head nodding in a previously normal person. Head nodding is defined as repetitive, involuntary drops of the head to the chest on two or more occasions.

**Probable case:** Suspect case of head nodding with

Both of the following major criteria:

3. Age at onset of nodding between 3 and 18 years old
4. Frequency of nodding 5 to 20 per minute

Plus at least one of the following minor criteria:

7. Other neurological abnormalities (cognitive decline, school dropout due to cognitive/behavioural problems, other seizures or neurological abnormalities)
8. Clustering in space or time with similar cases
9. Triggered by food and/or cold weather
10. Stunting or wasting
11. Delayed sexual or physical development
12. Psychiatric symptoms.

**Confirmed case:** Is a Probable case plus a documented nodding episode that is observed by trained healthcare worker, or videotaped, or EEG/EMG [6, 21]

The case definition was used to create a diagnostic sheet for this study. On the diagnostic sheet, the case definition criterions were numbered and tabulated. The numbered criterions correspond to a flow chart that was created to facilitate classification of potential cases as "not a case", "suspected case", "probable case", or "confirmed case", in accordance with directions outlined in the international NS case-definition.

These case-definition criterions were cross-referenced and applied to the clinical and epidemiological features of each potential, isolated case cluster that had been described in the collected literature. Criterions for each potential case cluster could be met by one patient being described in an article, by many patients who met several separate criteria within an article, or by patients from many different articles about the same potential cluster.

This method is unique from the typical application of a case-definition, where one patient must meet all of the necessary criteria on his own in order to be considered a case. However, though unorthodox, this retrospective application of the case-definition to multiple patients or sources was considered to be the only viable option to attempt to evaluate the nature of potential case clusters retrospectively. This is because available articles and reports rarely give an

adequate description of any one patient's symptoms for an individual to meet more than one criterion.

Each case definition criterion that was explicitly met by a patient or patients described in the literature, was marked as a "match" on the diagnostic form. If a symptom or description in the literature was not entirely consistent with a case definition criterion and could be subject to interpretation it was marked as a "partial match" on the diagnostic form. Criterion that were not met by a description in the literature were considered to be unmet and were left blank on the diagnostic form

The status of the potential case clusters being reviewed were classified as "not a NS case cluster", a "suspected NS case cluster", a "probable NS case cluster", or a "confirmed NS case cluster" by using the numbered criterion that were matched by descriptions and following the diagnostic form's flow chart. Only criterions that were met completely and were marked as a "match" on the diagnostic form were counted towards case status classification.

The percentage of case-definition criteria that were met for each potential case cluster location was also evaluated. Major and minor criterions were given equal weight when calculating percentage match. Case clusters that were marked as a "match" for every case definition criterion were considered a 100% match; those where only half of the case definition criteria were marked off as being a "match" were considered to have an overall score of 50%. Case cluster locations that received both "match" and "partial match" grades received upper and lower scores. The lower score considers only the "match" marks and the upper score counts both "match" and "partial match" marks. For example, if a potential case cluster location received "match" marks for half of the case definition criterion and "partial match" for the other half of

the criterion then they were consider to have met between 50%-100% of the case definition criterion. The average of the two scores will be presented along with the upper and lower scores but only the average score will be used for discussion purposes.

## 3.3 Intra-Country Analysis of Risk Factors

A list will be compiled for all clinical features, laboratory test results, and etiological exposure data that have been reported as risk factors for nodding syndrome. The goal of intra-country analysis is to evaluate the strength and consistency of risk factors within a country with reported cases of NS. To accomplish this, lists of investigated risk factors will be compiled and reviewed, by country. The strength and consistency of those risk factors in association with NS will be evaluated by classifying the risk factors as either "Supported", "Inconclusive", or "Not Supported" country according to the following classification scheme:

A risk factor will be classified as **"Supported"** if

- The risk factor is statistically significant according to the reported odds ratio and (when applicable) adjusted odd's ratio or has statistically significant p-value for the crude and adjusted model, when applicable.
    - The 95% confidence intervals for the reported odd's ratio and adjusted odd's ratio (when applicable) in the original publication intersected the null
    - Or, if a p-value<0.05 was reported in the original publication
- There are multiple investigations with conflicting association results for a risk factor(s) but overall there is support for the risk factor because at least one

investigation determined that the risk factor was significant and the other(s) found it to have the potential for clinically significance.

- If at least one investigation p-value<0.05 and the conflicting investigation(s) reported 0.05< p-value <0.10.

A risk factor will be classified as **"Inconclusive"** if

- The reported association is not valid. An association will not be considered valid if the diagnostic test used to test for the risk factor is less sensitive than the current gold standard of testing.
- The risk factor has a statistically significant OR but not a statistically significant AOR.
- The risk factor has the potential to have clinical significance despite not being statistically significant
  - 0.10> p-value >0.05.
- There are multiple investigations for a risk factor and results directly contradict each other.
  - If at least one investigation p-value<0.05 and the conflicting investigation(s) reported p-value>0.10.
  - If one investigation published a negative, statistically significant association between NS and a risk factor and another investigation publishes a positive, statistically significant association between NS and that same risk factor.

A risk factor will be classified as **"Not Supported"** if

- The reported risk factor is not statistically significant according to the reported odds ratio or clinically significant according to the reported p-value. That is if
  - The 95% confidence intervals for the reported odd's ratio and adjusted odd's ratio (when applicable) in the original publication intersected the null
  - Or, if a p-value>0.10 was reported in the original publication
- There are multiple investigations with conflicting association results for a risk factor(s) so that overall there is very little support for the risk factor.
  - If at least one investigation p>0.10 and the conflicting investigation(s) reported 0.05< p-value <0.10.

## 3.4 Cross-Country Analysis of Risk Factors

The results from the retrospective analysis and the intra-country analyses will be used to conduct a multi-level cross-country analysis of risk factors. Three levels of cross-country analysis will be conducted:

1. A cross-country comparison of risk factors between countries where cases of NS have been reported and confirmed through the retrospective analysis
2. A cross-country comparison of risk factors between countries where cases of NS have been reported but were not classified as confirmed in the retrospective analysis.
3. A cross-country comparison of risk factors between countries with confirmed NS case clusters and those countries where cases of NS were reported but not confirmed in the retrospective analysis.

During the cross-analyses, Nodding syndrome risk factors which have been investigated in multiple countries will be classified to determine if a risk factor can be considered Supported, Partially Supported, Inconclusive, Partially Ruled-Out, or Ruled Out. The risk factors will be classified according to this classification scheme:

Risk factors will be classified as **"Supported"** in a cross-country analysis if

- all of the countries in comparison had classified that risk factor "Supported" during the intra-country analysis.

Risk factors will be classified as **"Partially Supported"** in a cross-country analysis if

- at least one country classified the risk factor as "Supported" but at least one other classified the risk factor as "Inconclusive"
    - Additionally, in order to be classified as "Partially Supported", the trend of association for the risk factor in question must not be in contradiction. In other words, when evaluating whether a risk factor can be classified as "Partially Supported" overall, the risk factor in question cannot have been found to have a protective effect in one country and but pose a greater risk of disease in another. In such a case the risk factor will be classified overall as "Inconclusive".

Risk factors will also be classified as **"Inconclusive"** in a cross-country analysis if

- they were classified as "Inconclusive" during the intra-country analysis by all countries in the cross-country analysis
- Or, they were classified as "Supported" in at least one country's intra-country analysis and classified as "Not Supported" in another.

Risk factors will be classified as **"Partially Ruled Out"** in a cross-country analysis if

- they have been classified as “Inconclusive” in at least one country’s intra-country analysis and “Not Supported” in at least one other.

Risk factors will be classified as **“Ruled Out”** in a cross-country analysis if

- they were classified as “Not Supported” in the intra-country analysis of all countries being compared in the cross-c Britain as early as 1903[13], in Tanzania in 1962[14], from Liberia in the 1983’s[5, 15], from Uganda as early as 1994[16], Sudan in 1997 [1], from Cameroon in 2004 [17].

# CHAPTER 4: Results

## 4.1 Results of Retrospective Analysis of the Nature of Reported NS Case clusters

Potential nodding syndrome case clusters locations that were identified in NS literature include: Britain [13], Tanzania [14], Liberia [5, 15], Uganda [16], Cameroon [17], and Taiwan [18]. Neither of the cases in Britain nor the case in Taiwan met the first case the first case-definition criterion, "Head nodding reported by caretaker in a previously normal person,"[21] because those reported cases had preexisting health conditions and so were not considered "previously normal". They were therefore not considered to be cases of nodding syndrome. The cases from South Sudan and Uganda were determined to have "Confirmed" case status and those from Tanzania, Liberia, and Cameroon had "Suspected" case status. See Table 4.1 in Appendix for detailed overview.

**Table 4. Summary of Results for Retrospective Analysis**

| **Country** | South Sudan | Uganda | Tanzania | Liberia | Cameroon | Taiwan | Britain |
|---|---|---|---|---|---|---|---|
| **Dates Cases Reported** | 1997-Present | 1994-Present | 1965-Present | 1983 | 2008 | 2006 | 1909 |
| **# Case Definition Criteria Partially Met** | - | - | 3 | 1 | 0 | 0 | 0 |
| **# Case Definition Criteria Completely Met** | 10 | 10 | 5 | 6 | 5 | 0 | 0 |
| **% Case Criteria Met** | 100% | 100% | 65% (50-80%) | 65% (60-70%) | 50% | 0% | 0% |
| **Case Status** | Confirmed | Confirmed | Suspected | Suspected | Suspected | Not a | Not a |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | Case | Case |

### 4.1.1 Retrospective Analysis: South Sudan (See Table 4.2 in Appendix)

Cases of nodding syndrome were first reported from the region that is now considered to be part of the Republic of South Sudan in 1997 by a missionary physician working at a refugee camp. Investigations launched by the Carter Center [15], WHO, and CDC [68] verified the presence of cases in Western Equatoria region within South Sudan. Researchers indicate that, as of 2012, new incident cases are still being reported from the area [15]. Based on the descriptive data provided [15, 68], South Sudan met 100% of all case-definition criteria outlined and the case cluster was classified as a "Confirmed NS Case cluster" by researchers.

### 4.1.2 Retrospective Analysis: Uganda (See Table 4.3 in Appendix)

Approximately 2,000 potential cases of Nodding syndrome were reported from Uganda in 2009 [28]. The presence of the outbreak was confirmed by the Ugandan Ministry of Health, WHO, and by CDC. New cases are still being reported from the northern region of Uganda [2, 22, 36]. As with South Sudan, the characteristics of the cases from NS investigations in Uganda match 100% of the case definition criteria [2, 13, 23]. The 2009-Present NS case cluster in Uganda was classified as a "Confirmed NS Case cluster."

### 4.1.3 Retrospective Analysis: Tanzania (See Table 4.4 in Appendix)

Epileptic patients with a "nodding head" were first reported from Mahenge region in southern Tanzania by Dr. Louise Jilek-Aall in 1965. Dr. Jilek-Aall specifies that, "to describe this condition the narrator always let his head fall forward on to his chest," [37]. This description is consistent with the first case-definition criterion. In a 1979 article, Dr.

Jilek-Aall specifies that 33 of the epilepsy patients she examined first experienced "nodding of the head" before the onset of puberty; meeting the second criteria which states that the onset of head nodding symptoms must occur between the ages of 3-18 years. The third criterion, "During a nodding episode the patient drops their head at a rate of 5-20 times per minute," was not completely satisfied by descriptions in the literature. Dr. Jilek-Aall describes one nodding episode that she observed and stated, "A boy of seven came with his mother to consult me. I observed that his head constantly dropped to his chest so that he almost lost his balance and stumbled."[37] It can be inferred that this patient's head nodding was repetitive in nature which further supports the first criterion. Dr. Wolfgang Jilek, Dr. Louise Jilek-Aall's husband, gave a slightly more detailed description of the head nodding attacks stating,

> "The descriptive term used by the local people is amesinzia kichwa, Swahili for 'nodding of the head.' This type of seizure only occurred in children and was often a precursor of later gran mal, although some children continued nodding with concomitant major convulsions. The child suddenly drops his head to the chest, then raises it up again. Sometimes a series of such nods is observed; the child may drop his head lower and lower until it touches the ground, then lift it up quickly until a new series of nods forces the head down again. In rare cases the head was pulled to either side.

However, because it is impossible to know with certainty that the patient described dropped their head 5-20 times per minute, as mandated in the case-definition, we cannot confidently determine that criterion three was met. No other description was found in the literature that could clarify the exact rate at which patients in this area and in this time period were dropping their head per minute. According to the international case-definition, both the second and third criterion must be met in order for a case, or in this instance a case cluster, to be elevated from "Suspected NS Case cluster" to "Probable NS Case cluster."

Additionally, all three criteria must be met in order for any minor criteria, numbered four to ten, to count towards a case cluster being classified as a "Confirmed NS Case cluster" [21]. The descriptions found in the literature were sufficient to determine that this case cluster meets the first case- definition criterion and can therefore, at the very least, be classified as a "Suspected NS Case cluster" according to the classification criteria. In addition to criterion one and two being met, minor criterions numbered five, six, and ten were also completely met. Criterion number three was a partial match because, although the rate of head nodding was not explicitly stated, it could potentially be inferred by some to be adequate. Criterions four, seven, and nine were considered to be a partial matches because the description given was not specific to nodding syndrome patients but was representative of the study populations, of which the nodding syndrome patients were included. No descriptions could be found in the literature which to support criterion eight. Many of the criterion first filled by descriptions in early literature were echoed in recent publications by Winkler et al [5, 69]. Overall, 70% of the criterion for the "Suspected NS Case cluster" in Tanzania between 1965-2014 met the current NS case-definition.

#### 4.1.4 Retrospective Analysis: Liberia (See Table 4.5 in Appendix)

Between October of 1981 and May of 1982, "head nodding seizures" were encountered and subsequently reported as a feature of a distinct seizure disorder among children in Grand Bassa County, Liberia [43-45]. The head nodding seizures were described by Dr. Van Der Waals who observed rhythmic "complex partial seizures (that) were mainly characterized by dorsoventral movements of the head and progressed in 64% of cases to general tonic-clonic convulsion," [44], a description consistent with case

definition criterions one and four. The mean age of head nodding onset was reported to be 9.18 years, with slight differences between boys and girls, fulfilling case criterion two.

Case criterion number three was not met because a description of the rate at which head nodding occurred was lacking from the literature. Case criterions four, five, nine, and ten were completely met. Overall the head nodding occurrence in Liberia could only be classified as a, "Suspected NS Case cluster," with a 65% match to the overall case definition criteria. Published descriptions of epilepsy in Liberia could not be found after 1983.

#### 4.1.5 Retrospective Analysis: Cameroon (See Table 4.6 in Appendix)

In 2008, a research team, comprised of four Italian pediatric neurologists and two trained healthcare workers from a local epilepsy service, identified four patients who reported experiencing head nodding seizures in Littoral Provence of Cameroon [17]. The mean age of all epileptic patients in the study, including the four patients with head nodding seizures, was 14 years old. All of the study participants had experienced their first seizure after age 8, consistent with case criterion two. Case criterion five was also completely met and criterion nine and ten were partially met. Overall the case cluster was classified as a "Suspected NS Case cluster" with a 40% match rate.

## 4.2. Results of Intra-Country Analysis of Risk Factors

#### 4.2.1. Intra-Country Analysis: South Sudan (See Table 4.7 in Appendix)

Risk factors that were statistically significant exposures for nodding syndrome in South Sudan and, when replicated, had consistent results across separate NS investigations in South Sudan were classified as "Supported". These supported risk factors are

1. Onchocerciasis infection
2. A negative history of measles illness
3. Experiencing a hunger gap during the first two years of life
4. Consuming serena type sorghum

Risk factors that were classified as "Inconclusive" include several parasitic infections (trypanosomiasis, loa loa, M. perstans, lymphatic filariasis), dietary and nutrition exposures (baboon meat, red sorghum, and vitamin B6 deficiency), and clinical characteristics (current stunting and current wasting). All of the parasitic infections that were classified as inconclusive were classified as such, regardless of reported statistical significant, because the testing methods were not valid. The parasitological test results are the product of two case-control studies, one in Lui and the other in Amadi, South Sudan in 2001. According to the original publication, "these studies used skin snips collected by the Carter Center and HealthNet International," [15]. None of the parasitic infections that were classified as inconclusive should have been diagnosed using skin snips.

Although there is no gold standard for trypanosomiasis diagnosis, however, trypanosomiasis can only be detected in lymph tissue, blood, or cerebrospinal fluid [70]. The gold standard for loa loa diagnostic testing is the detection of microfilaria in blood films. Loa loa exhibits diurnal periodicity so blood collection must be done during the daytime, between 10:00am and 2:00pm local time [71, 72]. Mansonella perstans does not exhibit periodicity and so can be diagnosed by detecting microfilaria on blood smears from blood samples collected at any time of day[73]. The gold standard for lymphatic filariasis testing requires nocturnal blood collection [74, 75].

The clinical characteristics that were classified as inconclusive risk factors were "current wasting" and "current stunting." Wasting and stunting have repeatedly been described as symptoms of nodding syndrome and are even included in the NS case definition as diagnostic criterions. This being said, investigations in South Sudan have not established that children with nodding syndrome are consistently and/or significantly more stunted and wasted than their healthy peers. Overall, there is a weakly positive trend between nodding syndrome and current wasting but it is not statistically significant. There are conflicting results regarding the association between nodding syndrome and current stunting. A 2002 investigation in Lui, South Sudan did not find that current stunting was a statistically significant risk factor for nodding syndrome[22] (not published in original article[15]). A 2011 CDC-led case-control study, however, did find stunting to be a statistically significant risk factor for nodding syndrome [22, 27].

Dietary and nutritional factors that were classified as inconclusive risk factors include consumption of baboon meat, consumption of red sorghum, and vitamin B6 deficiency. Both consumption of baboon meat and vitamin B6 deficiency had a weakly positive trends which have the potential for clinical significance despite not being statistically significant. Red sorghum was classified as inconclusive because two separate studies gave conflicting results for questions asked about red sorghum consumption. In the 2002 WHO study, investigators asked specifically about Serena type sorghum. Serena is a type of red sorghum and was found to be a statistically significant risk factor [15]. However, in the 2011 CDC case-control study, investigators did not ask about specific varieties of red sorghum but asked about red sorghum in general. They did not find a difference in red sorghum consumption between cases and controls [22, 27].

### 4.2.2 Intra-Country Analysis: Uganda (See Table 4.8 in Appendix)

Nodding syndrome risk factors investigated in Uganda which were statistically significant and (when applicable) consistent across separate NS investigations in Uganda were classified as "Supported. These supported NS risk factors from Ugandan investigations include:

1. Onchocerciasis infection
2. Use of crushed roots as traditional/herbal medicine
3. Social exposures including exposure to munitions, exposure to gun raids, and poor school attendance
4. Having a sibling with nodding syndrome
5. Clinical characteristics including current stunting, current wasting, low serum sodium levels, low serum chloride levels, and a high serum anion gap
6. Developmental histories including abnormal social, physical, and cognitive childhood development.

Risk factors that were classified as inconclusive include a positive history of measles or malaria, having auditory hallucinations, previously having received treatment for onchocerciasis infection, and being born prematurely. Despite having statistically significant odds ratios, a positive history of a measles infection and clinical symptoms of auditory hallucinations were classified as inconclusive risk factors because they did not retain their statistical significance after being adjusted for age. The risk factors "history of malaria" and "previously received treatment for onchocerciasis" had conflicting results across separate investigations in Uganda. With both exposures, one study found the exposure to be of statistical significance but another did not. Premature birth was

classified as an inconclusive risk factor as well because it considered to be of potential clinical significance ($p<0.10$).

### 4.2.3 Intra-Country Analysis: Tanzania (See Table 4.9 in Appendix)

Nodding syndrome risk factors which were classified as supported among investigations in Tanzania include

1. Onchocerciasis infection
2. Clinical characteristics of intraparenchymal pathologies and gliotic lessons (as seen on MRI scans)

The comorbid condition of having both an onchocerciasis infection and intraparenchymal pathologies was classified as an inconclusive risk factor for NS in Tanzania because $p<0.10$. A family history of epilepsy was also classified as an inconclusive variable because, although two investigations in Tanzania asserted that family history for epilepsy was a significant risk factor, neither of the two studies validated their assertion by comparing case family data to a control group.

### 4.2.4 Intra-Country Analysis: Liberia (See Table 4.10 in Appendix)

None of the reported risk factors for head nodding seizures in Liberia were compared against control groups during the two investigations in 1983. As a result, all tests for reported risk factors for head nodding seizures in Liberia were considered to be not valid and the risk factor results were classified as inconclusive. These risk factors include family history for epilepsy, clinical characteristics of head nodding seizures (photic trigger, intellectual deficits, psychological disturbances, pyramidal signs, extrapyramidal signs, enlarged spleen, enlarged liver, and anemia), and febrile illness.

#### 4.2.5 Intra-Country Analysis: Cameroon (See Table 4.11 in Appendix)

To date, no investigations have investigated risk factors specific for nodding syndrome or head nodding seizures in Cameroon. Investigations for epilepsy risk factors, however, have been conducted in Cameroon. Supported risk factors for epilepsy in Cameroon include

1. Onchocerciasis infection
2. Positive family history for epilepsy
3. Not consuming alcohol
4. Reduced reproductive activity in females with epilepsy
5. And not having completed more than 3 years of school

On an ecological level, a positive correlation was found between onchocerciasis prevalence and epilepsy prevalence in communities. Also, a positive correlation between epilepsy prevalence in community and the distance of that community from Mbam River was found. Risk factors which were not supported in Cameroon include the presence of skin nodules, abnormal sleep patterns, previously receiving treatment with Ivermectin for onchocerciasis, adverse reactions (oedema and/or itching), a history of febrile convulsions, cola consumption, having an unrestricted or pork-free diet, a history of measles, other (unspecified) parasitological diseases and currently being employed or a student.

## 4.3 Cross-Country Analysis of Risk Factors

#### 4.3.1 Cross-Country Analysis: Confirmed Nodding Syndrome Case Locations (See Table 4.12 in Appendix)

A cross-country analysis was conducted which compared data on potential NS risk factors between countries where cases of NS have been reported and subsequently

confirmed through the retrospective analysis. Countries with case clusters that were classified as confirmed in the retrospective analysis are South Sudan and Uganda. The only risk factor that was classified as "Supported" in the cross-analysis between South Sudan and Uganda was the positive association between onchocerciasis and nodding syndrome. Risk factors that which were classified as "Partially Supported" between South Sudan and Uganda were the positive associations between current stunting and NS and that between current wasting and NS.

Risk factors which were classified as "Inconclusive" because they were they were classified as "Supported" in at least one country's intra-country analysis and classified as "Not Supported" in another include crushed root consumption, history of malnutrition, and exposure to munitions. A previous measles infection was classified as "Inconclusive" risk factor as well despite being classified in the intra-country analyses as "Supported" and "Inconclusive" for South Sudan and Uganda, respectively. Measles was classified as "Inconclusive" in the cross-country analysis and not as "Partially Supported" because it had conflicting association trends between South Sudan and Uganda. In South Sudan, measles was identified as a statistically significant, protective exposure for NS and thus classified as a "Supported" risk factor with a positive association. When measles was evaluated for Uganda's intra-country analysis, however, the results were classified as "Inconclusive- Conflicting (Positive/None)". One study found that a previous measles illness had a positive association with NS and was therefore associated with an increase risk of illness but the other study did not find an association with measles and NS at all.

All risk factors which were classified as "Partially Ruled-Out" were classified as such because they were classified as "Not Supported" in one intra-country analysis and

"Inconclusive" in another. "Partially Ruled-Out" risk factors include internal displacement history, vitamin b6 deficiency, consumption of red sorghum, and having previously received Ivermectin for onchocerciasis treatment.

All risk factors which were classified as "Ruled-Out" were classified as such because they were included but classified "Not Supported" both intra-country analyses. "Ruled-Out" risk factors include abnormal urine mercury levels, abnormal urine thiocyanates levels, consumption of river fish, consumption of seeds meant for planting, and vitamin B12 deficiencies.

### 4.3.2 Cross-Country Analysis: Non-confirmed NS Case Cluster Locations (See Table 4.13 in Appendix)

A cross-country analysis was conducted which compared data on potential NS risk factors between countries where cases of NS have been reported but not classified as "Confirmed" through the retrospective analysis. Countries with case clusters that were not classified as confirmed in the retrospective analysis are Tanzania, Liberia, and Cameroon, all of which have suspected cases of nodding syndrome. As with the cross-country analysis of Confirmed locations, the only risk factor that was classified as "Supported" in the cross-analysis of Tanzania, Liberia, and Cameroon was the positive association between onchocerciasis and nodding syndrome. The only risk factor that was classified as "Partially Supported" was the weakly positive association between NS and having a family history for epilepsy. Reports from both Tanzania and Liberia have asserted that there is a positive association between nodding syndrome or head nodding seizures and having a family history of epilepsy. This being said, in the intra-country analyses for both nations, the risk factor was classified as "Inconclusive" because a control group was not used to test the statistical significance of this risk factor. In

Cameroon's intra-country analysis, the reports provided were not specific to nodding syndrome risk factors but represented an entire population of epilepsy patients. Family history was considered to be a "Significant" risk factor in Cameroon's intra-country analysis. Therefore, overall, in the cross-country analysis of family history of epilepsy as a risk factor for NS in Tanzania, Liberia, and Cameroon (all of which were determined to have "Suspected" case clusters of NS), family history was classified as "Partially Supported." The only other common risk factor from the intra-country analyses was having a history of febrile illness with or without convulsions. This risk factor was ruled out.

### 4.3.3 Cross-Country Analysis: Confirmed and Unconfirmed NS Case cluster Locations (See Table 4.14 in Appendix)

A cross-country comparison of risk factors was conducted between countries with confirmed NS case clusters and those countries where cases of NS not classified as confirmed in the retrospective analysis. The only common risk factors between at least three of the five countries were onchocerciasis infection and a previous history of measles. Onchocerciasis infection was classified as "Supported" and a previous history of measles was classified as "Inconclusive."

# CHAPTER 5: Discussion and Recommendations

The objective of this systematic review was to review and clarify the geographic locations and the epidemiological features of nodding syndrome within and between countries that currently or previously have reported cases of nodding syndrome or conditions with clinical presentations similar to nodding syndrome.

A retrospective analysis was conducted to evaluate the status of reported nodding syndrome cases from each country where cases of nodding syndrome or conditions with similar manifestations to nodding syndrome have been reported. Published clinical and epidemiological descriptions of these reported cases were reviewed and cross-referenced with the current nodding syndrome case definition in order to evaluate the true status of reported cases. Clarification as to where and when true nodding syndrome cases have been documented was necessary in order to accurately evaluate the validity of disease risk factors which have been investigated. The retrospective analysis was used to support the subsequent systematic review of nodding syndrome risk factors. The strength and consistency of exposure data within and between countries with true nodding syndrome cases was then evaluated through intra-country and cross-country analyses.

## 5.1 Discussion of the Retrospective Analysis

Case of nodding syndrome or conditions with clinical manifestations similar to nodding syndrome have been reported from South Sudan, Uganda, Tanzania, Liberia, Cameroon, Taiwan, and Britain. Of these countries with reported cases, only South Sudan and Uganda have confirmed cases of nodding syndrome. Tanzania, Liberia, and Cameroon have suspected cases of

nodding syndrome. The reported cases of head nodding in Taiwan and Britain are not consistent with the nodding syndrome case definition.

Cases of nodding syndrome in Tanzania, Liberia, and Cameroon were classified as suspected and not probable or confirmed because explicit descriptions of the rate at which head nods occurred during head nodding seizures in these countries were lacking. If such descriptions had been provided and were consistent with the case definition criteria (head drops at a rate of 5-20 times per minute) all three of these suspected case countries had sufficient additional criteria already met so that they would have been classified as confirmed case countries.

Our classification of Tanzania as a suspected case country and not a probable or confirmed case country contradicts findings by Spencer et al.[76]. Spencer and his contributing authors also conducted a retrospective analysis of head nodding in Tanzania and determined that cases in Tanzania met the criteria to be classified as a probable case country. This indicates that, although not explicitly stated in the publication, Spencer and his colleagues were able to establish the rate of head nods during a head nodding seizure and that this rate was consistent with the case definition criteria of 5-20 nods per minute. This difference in findings may have been due to differences in materials used. While this study was limited to collecting descriptions from publications, Spencer was able to analyze the detailed case records of approximately 180 patients with reported head nodding from Tanzania. Furthermore, one of Spencer's co-authors, Dr. Jilek-Aall was the diagnosing physician for all of the patients whose records were being reviewed and may have been able to confirm the rate of head nods from memory.

This raises questions, however, as to why Spencer and his colleagues did not classify Tanzania as a confirmed case country. If there was sufficient evidence to support a probable case

status, then the only additional criteria that needed to be fulfilled in order to be classified as a confirmed case country was that the head nodding seizures has been witnessed by a health care professional. The very nature of their methodology implies that this criteria was met; Spencer states that the head nodding seizures described in the case-records (the source of their data) were the product of observations by Dr. Jilek-Aall, a physician.

## 5.2 Discussion of Risk Factor Analyses

In the individual intra-country analyses, a large number of risk factors for nodding syndrome were found to be well-supported by valid testing measures and were consistent across multiple investigations within the same country. In the cross-country analysis between countries with confirmed cases of nodding syndrome, however, onchocerciasis infection was determined to be the only supported risk factor; current stunting and current wasting were partially supported risk factors. In the cross-country analysis between countries with suspected cases of nodding syndrome, onchocerciasis infection was determined to be the only supported risk factor; having at least one family member with epilepsy was a partially supported risk factor. In the overall cross-country analysis, between countries with either confirmed or suspected cases of nodding syndrome, onchocerciasis infection was the only supported risk factor.

The results of the risk factor analysis indicate that further, more in-depth research to investigate the relationship between onchocerciasis and nodding syndrome is justified. Additionally, future investigations in Tanzania, Liberia, and Cameroon should evaluate whether stunting and wasting is a feature and/or risk factor for nodding syndrome, as it appears to be in South Sudan and Uganda. Likewise, future investigations in South Sudan and Uganda should evaluate the potential influence of a positive family history of epilepsy as a risk factor for nodding syndrome. Specific epilepsy genes and rare variant genes were tested for in one affected

child from South Sudan and one from Uganda with deep exome sequencing but no genes were identified[6]. It is possible that, if genetics do influence the development of nodding syndrome, that this previously undescribed seizure disorder is caused by one or more previously undescribed epilepsy genes.

## 5.3 Etiological Hypotheses

The proposed and investigated etiological hypotheses appear to follow different lines of reasoning depending on the location of reported cases. When nodding syndrome was first reported from southern Sudan and northern Uganda in the late 1990's and 2000's it appeared to be a distinct, previously undescribed illness. Affected communities, nearly all of which were internally displaced persons[15] [59] as a result of the long standing conflict in southern Sudan and northern Uganda, attested that the illness was a new phenomena and hadn't been seen in previous generations. The concurrent timing of the disease emergence and the ongoing conflict, in addition to the fact that the disease appeared to be localized to conflict affected areas, motivated communities and researchers to reasonably suspect that nodding syndrome was caused by one or more exposures that arose as a consequence of war. This line of reasoning is reflected in questions asked during case-control studies in South Sudan and Uganda.

In Tanzania, Liberia, and Cameroon the etiological hypotheses have centered around the observation that the clusters of cases are geographically contained. As a result, researchers have looked primarily at factors related to the geographic isolation of certain populations (population genetics, health disparities, cultural exposures) and environmental factors such as endemic parasites. Surprisingly, these two different perspectives have yielded different, yet complimentary results.

### 5.3.1 Toxic Encephalopathy

A long list of potential toxic exposures has been explored in connection to nodding syndrome in South Sudan and Uganda. Although accusations were never verified, many affected communities and media sources speculated that the illness could have been caused by chemical or biological weapons used by the Lord's Resistance Army [77]. Case-control studies in South Sudan and Uganda have asked about exposure to munitions but the results have not been consistent between the two countries. Researchers did not find an association between exposure to munitions and nodding syndrome in South Sudan but a statistically significant association was found in a Ugandan case control study (see Table 4.12). When the authors of the Ugandan study discussed the association between nodding syndrome and munitions, however, they expressed doubts that the munitions exposure indicated exposure to toxic chemicals. In addition to the fact that focus groups had never been able to identify specific toxic munitions, most participants in the study had indicated that the munition they had been exposed to were guns (gun raids were also a statistically significant risk factor in this study)[59]. Overall in the cross-country analysis, munitions were determined to be an inconclusive risk factor. Risk factors which are classified as inconclusive typically warrant further investigation, however, given what is known about this exposure, the proposed etiological hypothesis of toxic munitions as a cause of nodding syndrome can most likely be safely ruled out.

Artificial or naturally occurring toxic compounds in foods or water have also been investigated in South Sudan and northern Uganda as potential etiological exposures with little promise. In northern Uganda, questions were asked to determine if exposure to a particular domestic water source or swimming area was associated with an increase risk of nodding syndrome but none emerged as significant (see Table 4.8). There was also speculation that necessity may have driven individuals to eat pesticide-treated seeds that had been distributed by

relief organizations. This was tested in case control studies but no associations were found between eating planting seeds and nodding syndrome, neither in South Sudan nor Uganda (see Table 4.12). Likewise, no associations were found for consumption of supplementary foods nor exposure to spoiled relief goods (see Table 4.8).

The potential for exposure to naturally occurring toxins in foods have also been explored. Case-control studies have asked about consumption of different sorghum types (ripe and unripe), river fish, cawa, insects, cassava, and traditional/herbal medicines such as crushed roots, broths, crushed leaves, crushed flowers. Cassava and sorghum are drought-resistant crops that can contain toxic levels of cyanide and nitrates in extreme drought or when harvested before they are ripe [78]. Consumption of serena sorghum, a type of red sorghum, was identified as a significant exposure among cases in South Sudan. When red sorghum in general was asked about in Uganda, however, no association was found. No association was found for unripe cassava consumption either. This hypothesis was evaluated further with urinalysis of cases and controls in South Sudan and northern Uganda for cyanide poisoning. Case and control groups had equal percentages of abnormal test results for case controls conducted in both countries (see Table 4.12). Therefore, although serena sorghum was identified as a statistically significant risk factor in South Sudan, laboratory tests from both South Sudan and northern Uganda have conclusively ruled out cyanide poisoning as a consequence of sorghum or cassava consumption as a cause of nodding syndrome. Crushed roots are the only other food that has been identified as a statistically significant exposure for nodding syndrome. In the case control study which identified crushed root consumption as a significant risk factor, interviews with participants indicated that crushed roots were not consumed prior to the onset of the illness but were used as a traditional herbal treatment for the disease. Additional urinalysis testing has been conducted to

test for arsenic, mercury, and copper with all negative results (see Tables 4.7 and 4.8). Overall, etiological hypotheses related to toxin exposures that have been proposed thus far have almost conclusively been ruled out.

### 5.3.2 Psychological and Physical Trauma as a Result of Living in Conflict Zones

Etiological hypothesis related to exposures stemming from physical and psychological trauma as a result of living in conflict zones have also been explored. The pathways in which psychological trauma can manifest as physiological illness can be the most difficult to predict or understand but the indicators of extreme psychological trauma are more easily measured. Exposure to gun raids was found to be a statistically significant exposure in Ugandan children with nodding syndrome, as were abnormal social, physical, and cognitive childhood development histories (see Table 4.8). Internal displacement history was not found to be a significant risk factor in South Sudan and was determined to be an inconclusive risk factor in Uganda because it had not been evaluated against a control group (100% of cases and controls in the CDC Ugandan case-control study were internally displaced persons). Although it's staggering that 59% of cases and 49% of controls in Uganda reported that they had experienced the abduction of a family member, this particular psychological trauma was not a statistically significant risk factor for nodding syndrome. Overall, the only traumatic psychological exposure that is of statistical significance is a history of being exposed to gun raids. The precedent for physiological damage to occur as a response to psychological trauma has been well documented in psychology but there are a number of questions which must be answered before psychological trauma can be established as the cause of nodding syndrome. For example, if nodding syndrome is caused by psychological distress, how and why is it that gun raids cause the most significant trauma over other factors such as the abduction of a family member? Furthermore, psychological trauma is suffered by millions of people living in conflict zones around the world, how and why

did it only cause nodding syndrome in the conflict zones of South Sudan and northern Uganda? And finally, if psychological trauma as a consequence of living in conflict zones is the causative factor for the development of nodding syndrome, how can the emergence of nodding syndrome in non-conflict zones such as Tanzania, pre-civil war Liberia, and Cameroon be adequately explained?

Physical trauma during multiple life stages has been investigated as a potential etiological exposure in South Sudan and northern Uganda. Head injury is a known, universal risk factor for seizures but was ruled out as a potential cause of nodding syndrome when it was reported in fewer than 2% of cases in Uganda. Most children with nodding syndrome were conceived, born, and raised in the midst of the 20 year conflict in South Sudan and northern Uganda. This reality spawned the need to rule out the potential for fetal trauma, however, only 2% of case mothers reported experiencing a major illness or adverse medication side effect during pregnancy, 95% of case pregnancies were carried to term, and there were no significant demographic or occupational differences between case and control patients that would indicate that health disparities or occupational hazards existed disproportionately during case pregnancies. Overall, etiological hypotheses related to exposures stemming from psychological or physical trauma, as a result of living in conflict zones, have not been well-supported, if at all.

### 5.3.3 Encephalopathy Caused by Nutritional Deficiencies

Stunted growth and wasting were among the earliest clinical features observed in patients with nodding syndrome [1]. Researchers in South Sudan and northern Uganda have sought to understand the relationship between nutrition and nodding syndrome, which has presented as another enigma in this this mysterious illness. Children with nodding syndrome certainly present with features of chronic malnutrition; what is not yet understood is whether nutritional

deficiencies and malnutrition are involved in the etiology of the disease or if they present as features of the illness after disease onset.

During the 2002 WHO-led case control study in Lui, South Sudan, researchers documented that stunting and wasting were features that were more frequent in children with nodding syndrome than in healthy controls[6, 15]. These differences were not statistically significant although differences in wasting had potential clinical relevance (p=0.09). Nine years later, however, when these features were reevaluated in South Sudan during a CDC-led case-control study in 2011, a shift had occurred; differences in wasting between cases and controls were found to be statistically significant and differences in stunting had potential clinical relevance (p=0.06) (See Table 4.7). Stunting and wasting were several times as risk factors for nodding syndrome in northern Uganda (see Table 4.8). The intra-country analysis of risk factors in Uganda determined that, overall, current stunting and wasting are supported, significant risk factors or features of nodding syndrome in affected Ugandan children. The cross-country analysis of common risk factors between South Sudan and Uganda found that, overall, stunting and wasting were Partially Supported risk factors for nodding syndrome in the two countries.

There exists a paradox in the relationship between the symptoms of chronic malnutrition in children with nodding syndrome (stunting and wasting) and the cause of these symptoms. It would seem obvious that stunting and wasting would be the consequence of a history of malnutrition or a hunger gap but this does not appear to be the case in children with nodding syndrome. Histories of nodding syndrome were not identified as significant risk factors for nodding syndrome in either South Sudan [79] nor Uganda. This would indicate, therefore, that malnutrition is most likely not a risk factor for nodding syndrome but rather a symptom of the illness. This conjecture requires further investigation but is supported by anecdotes that affected

children often lose their appetites after the onset of the disease [1, 20, 80]. If anorexia is a true symptom of nodding syndrome rather than malnutrition being a risk factor for the disease, this could be due in part to the fact that food is a frequent trigger for head nodding seizures and makes it challenging for affected children to feed themselves.

Specific micronutrient deficiencies have also been proposed and investigated as risk factors for the disease. Pyridoxine-dependent epilepsy is a rare pediatric seizure disorder resulting from a severe vitamin B6 deficiency. The disease usually manifests in infancy and is due to genetic mutations although very rare cases of idiopathic pyridoxine-dependent epilepsy with later onset have been documented [81]. Vitamin B6 deficiency was identified as a potentially clinically relevant exposure in among nodding syndrome cases in South Sudan ($p=0.06$) although the deficiency was pervasive among both cases(79%) and controls (59%). When vitamin B6 deficiency was tested for again in Uganda, a widespread deficiency was once again observed in cases (73%) and controls (64%) but differences of neither a statistical nor clinical significance were detected. Micronutrient deficiencies were also tested for vitamins A and B12, zinc, selenium, folate, and phosphate but none were found to be of statistical or clinical significance (see Tables 4.8 and 4.12).

### 5.3.4 Infectious Encephalopathy

#### *5.3.4.b Prion Infections*

The neurological symptoms and degenerative nature of nodding syndrome is strikingly similar to prion diseases, especially those described in Chronic Wasting Disease (CWD), which affects North American cervids, (see Table 5 for details) and those described in variant Creutzfedlt-Jakob Disease (vCJD), a non-hereditary form of classic CJD found in humans. A prion is an infectious, misfolded protein that is capable of inducing other, normal proteins in an organism to become misformed and pathogenic.

Prions are the infectious agents responsible for several degenerative neurological diseases called transmissible spongiform encephalopathy's (TSE's). These include: bovine spongiform encephalopathy (BSE), commonly known as mad cow disease; scrapie in sheep; chronic wasting disease (CWD) in North American deer, elk, and moose; and kuru, fatal familial insomnia, and Creutzfeldt-Jakob Disease in humans.

Until very recently, prion diseases were thought to be only be transmitted through genetic inheritance, ingestion of prion contaminated bodily tissues (ie meat, especially central nervous tissue such as brain), or through injection of prion contaminated materials (ie blood transfusions). Nodding syndrome researchers have investigated exposures in South Sudan and northern Uganda which have historically shown to be high risk for contracting prion diseases. In northern Uganda, investigated high risk exposures included consumption of bush meat, rodent brain, guinea fowl brain, and baboon brain, or exposure to unusual illness or death of animals. None of these exposures were found to be statistically or clinically significant. In South Sudan, questions were asked about consumption of baboon meat and consumption of baboon brain. Of the two, only consumption of baboon brain indicated a weakly positive trend of potential clinical significance ($p=0.07$). Regardless of this potentially identified trend, these results in South Sudan do not support an etiological hypothesis of conventional prion transmission. If a prion infection was being transmitted from baboon meat to humans, then we would expect that consumption of baboon brains (which were widely consumed in the study population; 46% cases and 22% of controls) would have demonstrated a much stronger statistical trend than consumption of baboon meat in general.

Such a phenomena was observed during the outbreak of the prion disease, Kuru, among the Fore tribe in Papau, New Guinea between 1920-1950. Kuru was, as with all prion disease, a degenerative, inevitably fatal illness. Its' symptoms were characterized by an abnormal, broad gait, ataxia of the limbs, and shivering tremors (amplified by cold) [82] in the early stages of disease and in advanced stages characterized by dementia and truncal ataxia[83]. It appeared in the 1920's as an isolated, sporadic mutation in among a member in the Fore tribe but was rapidly transmitted a to a large proportion of the rest of the tribe because the Fore practiced endo-cannibalism as a mourning ritual to honor their dead. Women and young children are the most active participants in the mourning process and women were far more likely to eat the brain and other central nervous tissue of the deceased, which contains the highest concentrations of prion proteins. As a result, women and very young children suffered disproportionately from kuru in the Fore tribe.

Although transmission of prion diseases has only been documented vertically, TSE's such as chronic wasting disease challenge this narrow transmission hypothesis. Horizontal transmission of chronic wasting disease has been observed in cervids (deer, moose, elk, etc.) living in close quarters. Cervids are vegetarians and highly unlikely to have ingested infected meat, as was the case with BSE outbreaks. Experts have theorized that the infectious CWD prion may be transmissible through saliva or feces and subsequently ingested by animals eating contaminated grass, however, this is largely conjecture.

Dr. Frank O. Bastian has challenged the prion transmission theory and presented a large volume of evidence to support that a bacterium may be responsible for inducing prion diseases in mammals. As early as 1979, Dr. Bastin identified spiral shaped

inclusions on brain autopsy samples from a patient that had died of Creutzfedlt-Jakob disease. He determined that these were small, cell wall-less bacterium known as *Spiroplasma* [84]. He continued researching *Spiroplasma* and their relationship to CJD and other encephalopathy's [85-87] but it appears his work was overshadowed and largely ignored after Dr. Stanley Prusiner announced the discovery of prions in the mid-1980s. Regardless, Dr. Bastin has continued his research on *Spiroplasma* and prions at Tulane University. Since his initial finding he has repeatedly demonstrated simultaneous *Spiroplasma* infection in the brains of TSE cases[88, 89] (see Table 5.2), induced TSE pathology in previously disease-free rodents, deer, sheep, and goats [90], and has also identified *Spiroplasma* in the eyes of TSE infected animals, recommending that "the eye should be a research focus for future studies of TSE," [91].

As to what role *Spiroplasma* has in TSE pathology, Dr. Bastian stated in a 1987 research paper that the "data suggest(s) that there are conformational similarities among spiroplasma proteins and infection-specific proteins (prions) of the transmissible spongiform encephalopathies" [92]. It was not until he was able to induce TSE formation in animals during his 2007 study that he directly challenged the prion model as the sole causal agent in TSE diseases[90]. His most recent research project in 2012 revealed that biofilm formation is necessary for *Spiroplasma* to become pathogenic in plant, insect, and animal diseases. These results offer a revision to the earlier CWD transmission theory because "the affinity of *spiroplasma* biofilms for mica and nickel… suggest that soil could be a reservoir for these bacteria [93]."

Aside from Dr. Bastian's work, the study of *Spiroplasmas* has largely been restricted to fields related to entomology and botany/agriculture. In addition to infecting

mammals, *Spiroplasma* has commensal, mutualistic, or pathogenic relationships in a variety of insect, arthopod, and plant hosts [94]. Among the most well-known pathologies is corn stunt disease caused by *Spiroplasma kunkelii* and transmitted by feeding grasshoppers[95]. Some infected insect populations are negatively impacted because Spiroplasma infection kills males in the population, resulting in female biased sex-ratios. However, in *Drosophila* black flies infected with Spiroplasma, researchers have observed that the bacterial infection offers the flies protection from parasitic predators such as wasps [96]. Other Spiroplasma hosts include butterflies (*Danaus chrysippus*)[97], ticks(*Ixodes*)[98], nematodes, blackflies(*Drosophila*), and multiple genera of bloodsucking flies including deerflies(*Chrysops*), horseflies(*Tabanus*), and mosquitoes(*Aedes* and *Culex*)[99].

Simultaneous *Spiroplasma* and *Wolbachia* infections are increasingly common in insect hosts and may actually provide fitness advantages. This co-endosymbiotic relationship has been observed in the black fly, *Drosophila* [96, 100, 101], the monarch butterfly (*Danaus plexippus*)[102], ladybird beetles[103], and spider mites(*Tetranychus urticae*) [104]. The precedence of *Spiroplasma* infection and *Wolbachia-Spiroplasma* co-infection in other Diptera (the black fly Order) indicates that it may be possible for onchocerca volvulus, the larval nematodes, which causes on onchocerciasis or for the *Simulium* flies which transmit onchocerciasis to also become infected. *Spiroplasma* infection of African Monarch Butterflies in Uganda was reported in the year 2000 [105], although female-biased sex ratios (indicating Spiroplasma infection) were observed in Uganda as early as 1968 [106]. Recent entomology research in Uganda indicates that *Spiroplasma* infection is becoming increasingly prevalent among the monarch butterfly

population and is now also observed in local *Drosophila* as well [107]. Insect infection has also been investigated in other East African countries including Sudan [105, 106], Tanzania [97], Ghana [105, 106], Kenya [108], and Madagascar [97, 106] but has only been confirmed in Tanzania (2000), Uganda (2000), and Kenya (2009).

#### *5.3.4.c Parasitic Infections*

There are a number of parasitic infections which have been evaluated in association with nodding syndrome because they have the potential to cause neurological symptoms. These parasites include loa loa, lymphatic filariasis, the tapeworm Taenia soleum, and trypanomiasis. Unfortunately, test results for loa loa, lymphatic filariasis, M. perstans, and trypanomiasis in the 2001 southern Sudan case-study were classified as Inconclusive, regardless of the reported statistical significance of the exposures. This was done because the original publication reported that samples of skin snips were used to test for these parasites which is less sensitive than the gold standard for all of these parasites; each requires blood testing conducted at certain times of day. Trypanosmiasis, specifically, antibodies for trypanosome gambiense were tested for again in Ugandan patients and controls with nodding syndrome but none were found. Taenia soleum antibodies were also tested for in the same case-control study but not found(see Table 4.8).

##### *5.3.4.c.1 Onchocerciasis*

The strongest and most consistent exposure in nodding syndrome investigations is the association between nodding syndrome and onchocerciasis. In every country with true cases of nodding syndrome, regardless of country case status, all but Liberia have investigated and established that onchocerciasis is a statistically significant, positively associated risk factor for nodding syndrome. Even in Liberia, despite never having

explored the direct connection between onchocerciasis and head nodding seizures, Grand Bassa county was determined to have among the highest epilepsy rates in Liberia through epilepsy studies and the highest rates of onchocerciasis in the country through onchocerciasis surveys.

All though the onchocerciasis has been established as prominent risk factor for nodding syndrome and epilepsy in ecological studies, identifying potential mechanisms of pathogenesis has proven to be far more difficult. Test for *onchocerca vulvus* nematodes in the cerebrospinal fluid have been negative [14, 109]. However, researchers in Tanzania, who have focused more on ecological exposure hypotheses instead of exposures related to conflict, have established that intraparenchymal and gliotic pathologies on an MRI in patients with nodding syndrome positively correspond with positive onchocerciasis tests. This data indicates that onchocerciasis is somehow playing a role in the development of intraparenchymal and gliotic pathologies.

## 5.4 Recommendations

The results of the intra-country and cross-country analyses have reinforced that onchocerciasis is the most prominent and consistent risk factor for nodding syndrome, regardless of case location or status. Previous studies investigating the relationship between the two have focused exclusively on identifying a pathogenesis wherein *onchocerca volvulus* is the etiological agent. It is in my opinion that this is approach is misguided.

Onchocerciasis research has demonstrated since the early 2000's that *Wolbachia*, the obligate endosymbiotic bacterium inside the *onchocerca volvulus* nematodes, is responsible for the primary inflammatory response in onchocerciasis infection which eventually leads to blindness. In fact, *Wolbachia* appears to be the only virulent component in an onchocerciasis

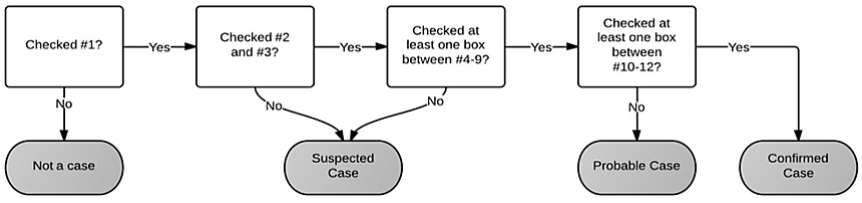
infection[50]. Previously, etiological hypotheses have proposed that maybe onchocerca nematodes are causing epilepsy through inflammation of the central nervous system through the same pathways that it causes blindness through inflammation of the cornea. The flaw of this hypothesis was that *Wolbachia*, and not *onchocerca volvulus*, was responsible for the inflammation response in the human body. Future investigations should explore this hypothesis and others related to endosymbiotic bacterium in *onchocerca volvulus*.

Additionally, future investigations in in all nodding syndrome case countries should seek to evaluate the potential influence of a positive family history of epilepsy as a risk factor for nodding syndrome. A family history of epilepsy was identified as a partially supported common risk factor for all countries with cases of nodding syndrome (see Table 4.14). Even within South Sudan, where a family history of epilepsy has not been investigated as a risk factor, the 2002 WHO-led case control study mentioned that at least 13 families in Lui had more than one child who suffered from the illness.

Specific epilepsy genes and rare variant genes were tested for in one affected child from South Sudan and one from Uganda with deep exome sequencing but no genes were identified[6]. It is possible that, if genetics do influence the development of nodding syndrome, that this previously undescribed seizure disorder is caused by one or more previously undescribed epilepsy genes. Perhaps these "new epilepsy genes" make certain individuals more vulnerable to a dramatic inflammation response during an onchocerciasis infection. Finally, future investigations in Tanzania, Liberia, and Cameroon should evaluate whether stunting and wasting is a feature and/or risk factor for nodding syndrome, as it appears to be a feature more so than a risk factor for nodding syndrome in South Sudan and Uganda.

**Table 4.1 Case Status Analysis: Detailed Summary of Analysis Results for Case Definition Comparison to Reported NS Case cluster Locations (Percent Match and Supported Criteria by Country)**

| | Country | South Sudan (1997-2014) | Uganda (1994-2014) | Tanzania (1965-2014) | Liberia (1983) | Cameroon (2008) | Taiwan (2006) | Britain (1909) |
|---|---|---|---|---|---|---|---|---|
| # | **Case Definition Criteria** | | | | | | | |
| 1 | Head nodding (reported by caretaker) in a previously normal person. Head nodding is defined as repetitive, involuntary drops of the head to the chest on two or more occasions. | √ | √ | √ | √ | √ | | |
| 2 | The patient first experienced head nodding symptoms (above) between the age of 3 and 18 years old | √ | √ | √ | √ | √ | | |
| 3 | During a nodding episode, the patient drops their head at a rate of 5-20 times per minute | √ | √ | | | | | |
| 4 | The patient exhibits other neurological abnormalities (cognitive decline, school dropout due to cognitive/ behavioral problems, other seizures or neurological abnormalities) | √ | √ | √- | √ | | | |
| 5 | This case is one of many also observed in this area or emerging during this time (revised question from criteria "Clustering in space or time with similar cases") | √ | √ | √ | √ | √ | | |
| 6 | Nodding episodes or other seizures are triggered by food and/or cold weather | √ | √ | √ | √- | | | |
| 7 | The patient has stunted growth or appears wasted. | √ | √ | √- | | | | |
| 8 | The patient has delayed sexual or physical development | √ | √ | | | | | |
| 9 | The patient has other psychiatric symptoms | √ | √ | √- | √ | √ | | |
| 10 | A trained healthcare worker has observed a nodding episode by this patient OR a nodding episode has been videotaped OR the patient has had an EEG/EMG during a nodding episode (one or all criteria may apply) | √ | √ | √ | √ | √ | | |
| | **CASE STATUS** | **Confirmed** | **Confirmed** | **Suspected** | **Suspected** | **Suspected** | **Not a case** | **Not a case** |
| | **% Criterion Met** | **100%** | **100%** | **50%-80%** | **60%-70%** | **40-50%** | **0%** | **0%** |




**LEGEND:**

**√ Criterion completely met**
**√- Criterion partially met**

**Table 4.2 Case Status Analysis for South Sudan: Nodding syndrome case definition criteria that were met or partially met by descriptions found in the literature.**

| # | Met | Quote and Source | Case Definition Criteria |
|---|---|---|---|
| 1. | √ | "A case of nodding syndrome was defined as onset of repetitive dropping of the head within the preceding 3 years, as reported by a caregiver," [27] | Head nodding (reported by caretaker) in a previously normal person. Head nodding is defined as repetitive, involuntary drops of the head to the chest on two or more occasions. |
| 2. | √ | "A case of nodding syndrome was defined as onset …. In any previously developmentally normal child aged <18," [27] | The patient first experienced head nodding symptoms (above) between the age of 3 and 18 years old |
| 3. | √ | "Episodes of nodding syndrome consisted of repetitive, involuntary dropping of the head, repeated 10 to 20 times per minute, and continued for 2 to 5 minutes," [15] | During a nodding episode, the patient drops their head at a rate of 5-20 times per minute |
| 4. | √ | "A case of nodding syndrome …. Had at least one other neurologic or cognitive abnormality or seizure type, based upon investigator observation or caregiver history, " [27] | The patient exhibits other neurological abnormalities (cognitive decline, school dropout due to cognitive/ behavioral problems, other seizures or neurological abnormalities) |
| 5. | √ | "The prevalence of Nodding Syndrome in Lui and Amadi was estimated at 2.3% (41/1783) and 6.7% (57/854) respectively," [15] | This case is one of many also observed in this area or emerging during this time (revised question from criteria "Clustering in space or time with similar cases") |
| 6. | √ | "The act of eating local food triggered head-nodding or even a grand mal seizure,"[15] | Nodding episodes or other seizures are triggered by food and/or cold weather |
| 7. | √ | "In some cases physical signs included mental retardation, developmental stunting, dwarfism, and poor development of secondary sexual characteristics," [15] | The patient has stunted growth or appears wasted. |
| 8. | √ | "In some cases physical signs included mental retardation, developmental stunting, dwarfism, and poor development of secondary sexual characteristics," [15] | The patient has delayed sexual or physical development |
| 9. | √ | "Some caretakers reported episodes of sudden shouting or screaming and at times jumping up and running in circles, and several children were reported to have burned themselves during seizures. Agitation, weakness, general body pain, sleepiness during the day, changes in mental ability, itching, and very cold extremities were also reported," [15] | The patient has other psychiatric symptoms |
| 10. | √ | "Nodding episodes were recorded in 3 cases, and nodding activity took the form of an isolated, diffuse delta-theta slow wave increasingly polymorphous as the disease progressed, followed by a brief and small fast discharge," [15] | A trained healthcare worker has observed a nodding episode by this patient OR a nodding episode has been videotaped OR the patient has had an EEG/EMG during a nodding episode (one or all criteria may apply) |

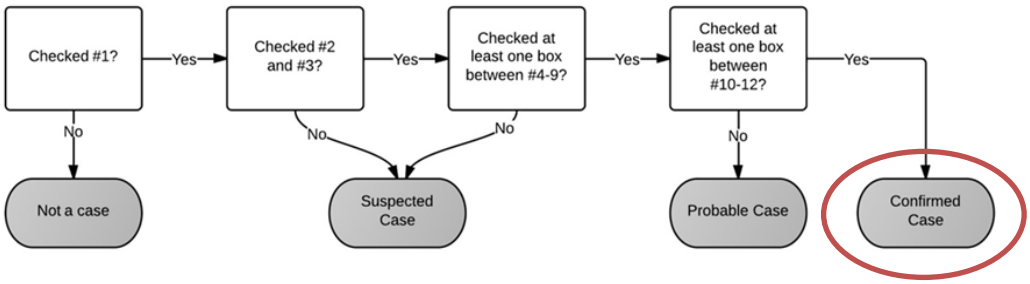


**Table 4.3 Case Status Analysis for Uganda: Nodding syndrome case definition criteria that were met or partially met by descriptions found in the literature.**

| # | Met | Quote and Source | Case Definition Criteria |
|---|---|---|---|
| 1. | √ | "…we defined a case as **a child with nodding seen by investigators or reported by caregivers, and who was previously developmentally and neurologically normal**, with at least one other objective neurological deficit (eg, other fits or seizures, gross cognitive impairment, or focal neurological signs)," [2] | Head nodding (reported by caretaker) in a previously normal person. Head nodding is defined as repetitive, involuntary drops of the head to the chest on two or more occasions. |
| 2. | √ | "The median age at onset of nodding in the 23 children enrolled in the case-series investigation was 8·4 years (range 6–13 years),"[2] | The patient first experienced head nodding symptoms (above) between the age of 3 and 18 years old |
| 3. | √ | "…head nodding occurred 15–20 times per minute," [110] | During a nodding episode, the patient drops their head at a rate of 5-20 times per minute |
| 4. | √ | "…we defined a case as a child with nodding seen by investigators or reported by caregivers, and who was previously developmentally and neurologically normal, **with at least one other objective neurological deficit (eg, other fits or seizures, gross cognitive impairment, or focal neurological signs)**," [2] | The patient exhibits other neurological abnormalities (cognitive decline, school dropout due to cognitive/ behavioral problems, other seizures or neurological abnormalities) |
| 5. | √ | "The overall prevalence in 13 parishes of western Kitgum District was 12 (exact 95% CI = 10·8, 14·2) cases per 1000 five- to fifteen-year-old children, but ranged widely from 0·6 (0, 3·6) to 46 (36·5, 57·7) cases per 1000,"[59] | This case is one of many also observed in this area or emerging during this time (revised question from criteria "Clustering in space or time with similar cases") |
| 6. | √ | "Antecedent stimuli were reported to induce nodding in all but two, most commonly including **meals and exposure to cold weather**," [2] | Nodding episodes or other seizures are triggered by food and/or cold weather |
| 7. | √ | "60% of cases had low height-for-age as compared to 29% of controls (p = 0·003). Cases also had higher frequencies of low BMI-for-age (42%) as compared to controls (13%, p = 0·001),"[59] | The patient has stunted growth or appears wasted. |
| 8. | √ | "60% of cases had low height-for-age as compared to 29% of controls (p = 0·003). Cases also had higher frequencies of low BMI-for-age (42%) as compared to controls (13%, p = 0·001),"[59] | The patient has delayed sexual or physical development |
| 9. | √ | "…we defined a case as **a child with nodding seen by investigators or reported by caregivers, and who was previously developmentally and neurologically normal**, with at least one other objective neurological deficit (eg, other fits or seizures, gross cognitive impairment, or focal neurological signs)," [2] | The patient has other psychiatric symptoms |
| 10. | √ | "Simultaneous video recording allowed for correlation of movements with EEG, electromyography, and ECG,"[2] | A trained healthcare worker has observed a nodding episode by this patient OR a nodding episode has been videotaped OR the patient has had an EEG/EMG during a nodding episode (one or all criteria may apply) |

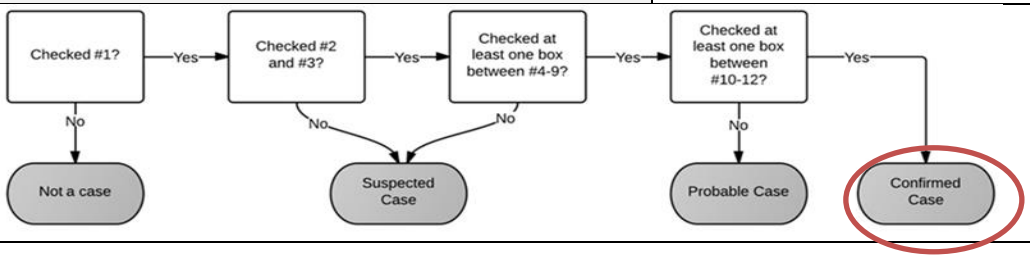


**Table 4.4 Case Status Analysis for Tanzania: Nodding syndrome case definition criteria that were met or partially met by descriptions found in the literature.**

| # | Met | Quote and Source | Case Definition Criteria |
|---|---|---|---|
| 1 | √ | "Eight patients began 2 or 3 months before the major attacks with "nodding head" (to describe this condition the narrator always let his head fall forward on to his chest)," pg.66 [37] | Head nodding (reported by caretaker) in a previously normal person. Head nodding is defined as repetitive, involuntary drops of the head to the chest on two or more occasions. |
| 2 | √ | "However, among the patients we examined were 33 with epilepsia-nutans-type seizures called "nodding of the head" that appeared before puberty,"[111] | The patient first experienced head nodding symptoms (above) between the age of 3 and 18 years old |
| 3 | | "A boy of seven came with his mother to consult me. I observed that his head constantly dropped to his chest, so that he almost lost his balance and stumbled. He was unable to stand quiet for one moment, and his face had a tormented expression," pg 68 [37] | During a nodding episode, the patient drops their head at a rate of 5-20 times per minute |
| 4 | √- | "There are peculiar features that set many kifafa patients apart… (such as) other neurological signs (50 cases) such as weak or asymmetric tendon reflexes or absence of reflexes, markedly asymmetric motor power, occurrence of monopareses, atrophy of certain muscle groups, general psychomotor retardation," [111] | The patient exhibits other neurological abnormalities (cognitive decline, school dropout due to cognitive/ behavioral problems, other seizures or neurological abnormalities) |
| 5 | √ | "In an isolated tribe in the interior of Tanzania the authors found that approximately 200 persons among an intake population of 10,OOO persons presented at the bush clinic with kifafa, a convulsive seizure disorder… A frequent variant of the disease in children involved head nodding, which was often a precursor of later grand mal seizures," [111] | This case is one of many also observed in this area or emerging during this time (revised question from criteria "Clustering in space or time with similar cases") |
| 6 | √ | "Food was the commonest precipitant in all diagnostic groups, quoted by 9 of the 62 patients. In the diagnostic group ''HN plus'' with two other types of seizure, food made up a significant proportion (2/28 ''HN only,'' 4/28 ''HN plus'' with one other type of seizure, 3/6''HN plus'' with two other types of seizure; Fisher's exact test, p=0.035). Two patients with ''HN only'' got their seizures during cold weather or bathing in cold water. No other precipitants were reported." [5] | Nodding episodes or other seizures are triggered by food and/or cold weather |
| 7 | √- | "They suffer even more than their fellows from malnutrition and 4itamin deficiency. Many of them have an unsteady gait and clumsy movements (deficiency of vit. B-complex?)," [37] | The patient has stunted growth or appears wasted. |
| 9 | √- | "Transient psychotic episodes were seen in approximately 20% of all patients,"[111] | The patient has other psychiatric symptoms |
| 10 | √ | "A boy of seven came with his mother to consult me. **I observed that his head constantly dropped to his chest**, so that he almost lost his balance and stumbled. He was unable to stand quiet for one moment, and his face had a tormented expression," pg 68 [37] | A trained healthcare worker has observed a nodding episode by this patient OR a nodding episode has been videotaped OR the patient has had an EEG/EMG during a nodding episode (one or all criteria may apply) |

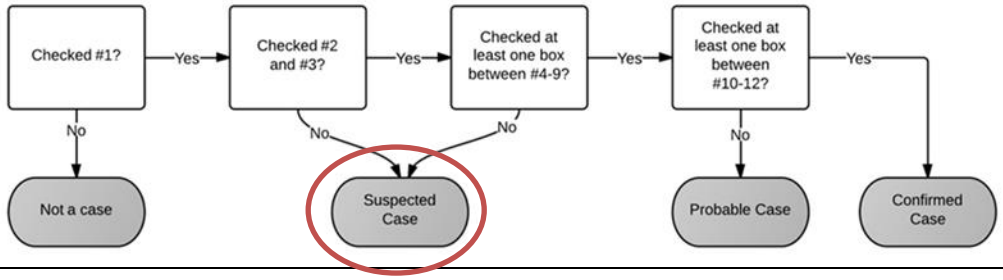


**Table 4.5 Case Status Analysis for Liberia: Nodding syndrome case definition criteria that were met or partially met by descriptions found in the literature.**

| # | Met | Quote and Source | Case Definition Criteria |
|---|---|---|---|
| 1 | √ | "Complex partial seizures were mainly characterized by dorsoventral movements of the head and progressed in 64% of the cases to general tonic-clonic convulsion. The native population recognizes this form of epilepsy as a distinct disorder," [112] | Head nodding (reported by caretaker) in a previously normal person. Head nodding is defined as repetitive, involuntary drops of the head to the chest on two or more occasions. |
| 2 | √ | "…the ages in which the first signs are noticed, differs between the sexes. 36% of the males get epilepsy at prepuberty and during puberty, whereas 42% of the females get the same illness during puberty and late puberty,"[43]<br>"…the mean age of onset in years (± SEM) was 9.18 ± 0.48"[112] | The patient first experienced head nodding symptoms (above) between the age of 3 and 18 years old |
| 4 | √ | "Mental disorders and burns were seen more frequently among patients with generalization and appeared to be linked to attack frequency and duration of the illness,"[112] | The patient exhibits other neurological abnormalities (cognitive decline, school dropout due to cognitive/ behavioral problems, other seizures or neurological abnormalities) |
| 5 | √ | "As computed from the years of onset of the 123 epilepsy cases in our survey, 'See-ee' first occurred in the Wroughbarh Clan in the 1950's, increased gradually in frequency in the 1960's and spread through the whole clan in the 1970's, according to clan chiefs, town chiefs, and elders," [45]<br><br>"In 39 cases (64% (of 77 patients with complex partial seizures)) these seizures evolved to generalized tonic-clonic convulsion. Characteristic for this type of seizure were rhythmic dorsoventral movements of the head," [112] | This case is one of many also observed in this area or emerging during this time (revised question from criteria "Clustering in space or time with similar cases") |
| 6 | √- | "The Bassa and Kpelle distinguish between two types of epilepsy: '*to drop the head in the pan'* and '*the big jerking'*. In Western medical terminology the first type shows similarities with absences or petit mal in children. This type of epilepsy is noticed by the informants mostly during social events of sharing meals together when children are watched more carefully. If a child has absenced during dinner he/she can '*drop the head in the pan*' and often drops the spoon or hands,"[43] | Nodding episodes or other seizures are triggered by food and/or cold weather |
| 9 | √ | "Psychological and intellectual disturbances, and pyramidal and extrapyramidal signs were significantly more frequent in the group of patients with either primary or secondary generalization compared to the series of patients without generalization. In 32% of the patients with complex partial seizures without generalization, extrapyramidal signs appeared early in the course of their illness suggesting that these signs are part of this distinct seizure disorder," [112] | The patient has other psychiatric symptoms |
| 10 | √- | "We witnessed 3 attacks of generalized seizures, 2 of complex partial seizures, and 2 of simple partial seizures in 6 patients," [43] | A trained healthcare worker has observed a nodding episode by this patient OR a nodding episode has been videotaped OR the patient has had an EEG/EMG during a nodding episode (one or all criteria may apply) |

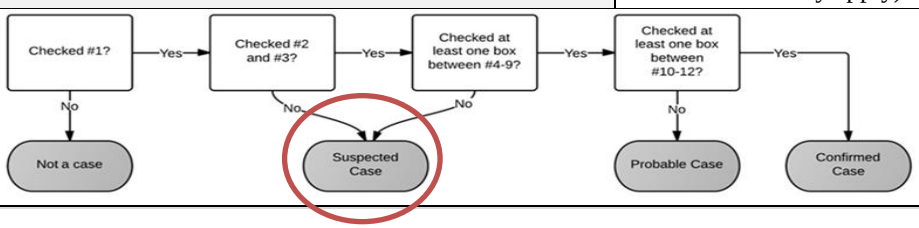
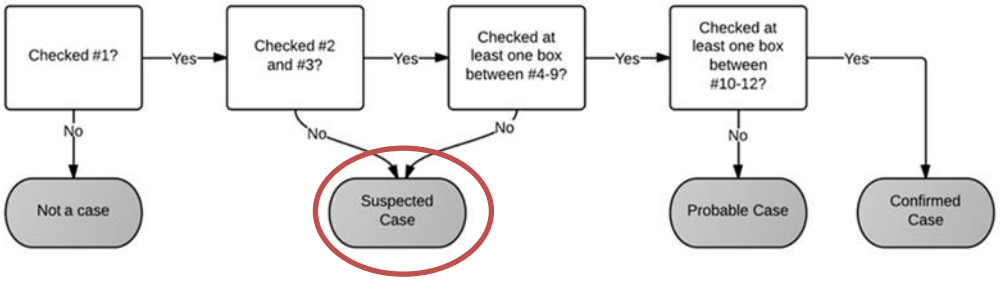


**Table 4.6 Case Status Analysis for Cameroon: Nodding syndrome case definition criteria that were met or partially met by descriptions found in the literature.**

| # | Met | Quote and Source | Case Definition Criteria |
|---|---|---|---|
| 1 | √ | "In 4 patients, with complex partial seizures, the histories gave an account of a seizure characterized by one or several repeated head movements named ''head nodding'' which were usually accompanied by an episode of impaired responsiveness lasting from a few seconds up to several minutes," [17] | Head nodding (reported by caretaker) in a previously normal person. Head nodding is defined as repetitive, involuntary drops of the head to the chest on two or more occasions. |
| 2 | √ | "Mean age of onset of first seizure was 14.7±6.5 years," [17] | The patient first experienced head nodding symptoms (above) between the age of 3 and 18 years old |
| 5 | √ | "The resemblance between the results come out from our study (i.e**., the high prevalence rate in a restricted area**, the clinical characteristics of epileptic seizures, the positive family history for epilepsy and the type of pedigree of a family with epileptic patients) and those come out from the studies done in Tanzania, Liberia, Uganda, and Ethiopia may be accounted for by the presence of an strong interaction between environmental and genetic factors in some areas,"[17] | This case is one of many also observed in this area or emerging during this time (revised question from criteria "Clustering in space or time with similar cases") |
| 9 | √- | "Moreover some inhabitants of the Kéleng village referred transient psychotic episodes among their family members,"[17] | The patient has other psychiatric symptoms |
| 10 | √ | "The 4 patients with ''head nodding'' seizures showed EEG records characterized by focal epileptiform activity and non-specific focal slow wave activity," [17] | A trained healthcare worker has observed a nodding episode by this patient <u>OR</u> a nodding episode has been videotaped <u>OR</u> the patient has had an EEG/EMG during a nodding episode (one or all criteria may apply) |



| Table 4.7 Intra-Country Analysis of Nodding Syndrome Risk Factors Investigated in South Sudan | | | | | | |
|---|---|---|---|---|---|---|
| **Status** | **Risk Factor** | | **Association** | **Cases %** | **Controls %** | **p-value** |
| **Supported** | Parasitic Infections | Onchocerciasis | Positive [15] | 92% | 44% | p=0.01 |
| | | | Positive [27] | 76% | 47% | P=0.02 |
| | Viral/ Bacterial Infections | Measles | Negative [15] | 15% | 58% | p=0.03 |
| | Dietary & Nutrition Factors | Early malnutrition* | Positive †[22, 27] | 24% | 8% | p=0.03 |
| | | Serena type sorghum | Positive [15] | 54% | 16% | p=0.05 |
| **Inconclusive** | Parasitic Infections | Trypanosomiasis | Not valid [15] | - | - | P=0.94 |
| | | Loa loa | Not valid[15] | 0% | 0% | Not defined |
| | | M. perstans | Not valid[15] | - | - | P=0.01 |
| | | Lymphatic filariasis | Not valid [15] | - | - | P=0.47 |
| | Clinical Characteristics | Current wasting | Weakly Positive<br>Weakly Positive†[22, 27]<br>Weakly Positive †[15, 22] | 16% | 3% | p = 0.06 |
| | | | | -1.6‡ | -1.0‡ | p = 0.09 |
| | | Current stunting | Conflicting<br>Positive †[22, 27]<br>None †[15, 22] | 24% | 3% | p=0.03 |
| | | | | -1.5§ | -0.8§ | p = 0.29 |
| | Dietary & Nutrition Factors | Baboon meat | Weakly positive [15] | 69% | 33% | p=0.07 |
| | | Red Sorghum | Conflicting<br>Positive: **[15, 22]<br>None: †[22, 27] | 54% | 16% | p = 0.05 |
| | | | | No difference | | |
| | | Vitamin B6 deficiency | Weakly positive †[22, 27] | 79% | 59% | P=0.06 |
| **Not Supported** | Social Exposures | General demographic characteristics | None[27] | - | - | - |
| | | Parent's occupation | None [27] | - | - | - |
| | | Internal Displacement History | None [15] | 92% | 73% | p=0.36 |
| | | Munitions | None [27] | - | - | - |
| | Family History | Genetic testing for specific epilepsy genes or consistent rare variant genes | None [22] | 0% | - | |
| | Toxic Exposures | Mercury †† | None †[22, 27] | - | - | - |
| | | Thiocyanates †† | None†[22, 27] | 20% | 20% | - |
| | | Arsenic †† | None †[22, 27] | - | - | - |
| | Dietary Exposures | Baboon brain | None [15] | 46% | 22% | p=0.25 |
| | | Pesticide treated seeds | None [15] | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | River fish | None †[22, 27] | - | - | - |
| | | Crushed root | None †[22, 27] | - | - | - |
| | | Unripe sorghum | None [15] | 93% | 83% | p=0.62 |
| | | Cultivation of sorghum crops | None [15] | - | - | - |
| | | Narango type sorghum | None [15] | 84% | 100% | p=0.16 |
| | | Bari type sorghum | None [15] | 69% | 67% | p=0.70 |
| | | Diri type sorghum | None [15] | 84% | 89% | p=1.0 |
| | | Major food is serena type sorghum | None [15] | 23% | 11% | p=0.63 |
| | | Colored seeds | None [15] | 83% | 50% | p=0.11 |
| | | Cawa | None [15] | 85% | 94% | p=0.56 |
| | Viral/ Bacterial Infection | Meningitis | None [15] | 0% | 6% | P=1.00 |
| | Medical History | Prior treatment with ivermectin | None [15] | 62% | 37% | p=0.28‡‡ |
| | Nutritional Factors | Vitamin B12 deficiency | None †[22, 27] | 3% | 0% | - |

* Regarding this risk factor, the question was, "Was there any time from birth to age 2 years old that this child went hungry because he/she did not have food to eat?"
† Risk factor results reported in [3] but not in the original publication, which is cited before [3] for each relevant risk factor
‡ Weight-for-age Z scores
§ Height-for-age Z scores
** The risk factor results "Red Sorghum" for [1, 3] were originally published in [1] as the risk factor results for "Serena type sorghum" on page 245 of that publication. It is apparent from the results published in [2,3] that the authors in [2] may have asked a more broad question about sorghum consumption and that Serena type sorghum is a type of red sorghum.
†† Evaluated with urinary analysis
‡‡ This value is a corrected value which was calculated by using given "n" and percent values from the original publication.

| Table 4.8 Intra-Country Analysis of Nodding Syndrome Risk Factors Investigated in Uganda | | | | | | |
|---|---|---|---|---|---|---|
| **Status** | **Risk Factor** | | **Association** | **Cases %** | **Controls %** | **OR/p-value** |
| **Supported** | Parasitic Infections | Onchocerciasis | Positive* [23] | 95% | 49% | AOR=14 (3, 78) |
| | | | Positive [13] | 77% | 10% | p<0.001 |
| | Traditional/Herbal Medicine Use | Crushed roots | Positive [23] | 39% | 16% | AOR=5 (1, 22) |
| | | | Positive [22] † | 22% | 0% | - |
| | Social Exposures | School Attendance | Negative [23] | 53% | 86% | AOR=0.1 (0, 0.6) |
| | | | Negative [13] | 71% | 0% | p<0.001 |
| | | Munitions | Positive [23] | 71% | 51% | AOR=14 (1, 135) |
| | | Exposure to gun raids | Positive [22] | 54% | 27% | p<0.001 |
| | Family Characteristics | Other children with NS in same family | Positive [13] | 34% | 18% | p=0.001 |
| | Clinical Characteristics | Current Stunting (Low height for age) | Weakly positive [23] | 60% | 29% | AOR=3 ‡(0.8, 8) |
| | | | Positive [22] | 35% | 20% | p=0.05 |
| | | Current Wasting (Low BMI for age) | Weakly positive [23] | 42% | 13% | AOR= 2 (0.5, 7) |
| | | | Positive [22] | 42% | 26% | p=0.03 |
| | | | Weakly positive [13] | 48% | 36% | p=0.087 |
| | | Low serum sodium | Positive[13] | 87% | 25% | p<0.001 |
| | | Low serum chloride | Positive[13] | 17% | 2% | p<0.001 |
| | | High anion gap | Positive [13] | 100% | 0% | p<0.001 |
| | Developmental History | Abnormal social childhood development | Positive [13] | 22% | 0% | p<0.001 |
| | | Abnormal physical childhood development | Positive [13] | 18% | 0% | p<0.001 |
| | | Abnormal cognitive childhood development | Positive[13] | 24% | 0% | p<0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Inconclusive** | Viral/Bacterial Infections | Measles | Weakly positive [23] | 24% | 6% | p=0.02[§] |
| | Social Exposures | Internal Displacement History | Not Valid [13] | 100% | 100% | - |
| | | | Not Valid [23] | 100% | 100% | - |
| | Parasitic Infections | Malaria | None[23] | 43% | 59% | - |
| | | | Positive [13] | 16% | 2% | p=0.002 |
| | Clinical Characteristics | Auditory Hallucinations | Weakly positive [23] | 26% | 0% | - |
| | Medical/Treatment History | Previous treatment for onchocerciasis | None [23] | 33% | 25% | - |
| | | | Negative[**] [13] | 29% | 46% | p=0.01 |
| | | Pregnancy carried to term | Weakly Negative [13] | 95% | 99% | p=0.097 |
| | Social Exposures | Exposure to unusual illness/death of animals | None [23] | 53% | 53% | - |
| | | Family member ever abducted | None [23] | 59% | 49% | - |
| | Viral/Bacterial Infections | Pneumonia | None [23] | 0% | 2% | - |
| | | Multiplex PCR[††] | None [23] | 0% | 0% | - |
| | Parasitic Infections | Tapeworm | None [23] | 0% | 0% | - |
| | | Diarrhea | None [23] | 2% | 4% | - |
| | Dietary Exposures | History of malnutrition | None [23] | 4% | 4% | - |
| | | Seeds meant for planting | None [23] | 61% | 65% | - |
| | | Red sorghum | None [23] | 98% | 100% | - |
| | | Spoiled relief foods | None [23] | 43% | 47% | - |
| | | Supplementary foods | None [23] | 22% | 12% | - |
| | | River fish | None [23] | 96% | 100% | - |
| | | Insects | None [23] | 41% | 33% | - |
| | | Rodent brain | None [23] | 55% | 51% | - |
| | | Guinea fowl brain | None [23] | 8% | 4% | - |
| | | Bush meat | None [23] | 100% | 100% | - |
| | | Cassava | None [23] | 100% | 100% | - |
| | | Domestic water sources [‡‡] | None [23] | - | - | - |
| | Traditional/Herbal Medicine Use | Crushed leaves | None [23] | 8% | 2% | - |
| | | Crushed flowers | None [23] | 0% | 2% | - |
| | | Inhaled medicine | None [23] | 2% | 0% | - |
| | | Lotion | None [23] | 0% | 0% | - |
| | | Broth | None [23] | 0% | 0% | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Medical/ Treatment History | Head injury | None [23] | 2% | 0% | - |
| | | Mother had a major illness during pregnancy | None [13] | 2% | 0% | - |
| | | Mother reported medication with adverse side effects during pregnancy | None [13] | 2% | 0% | - |
| | | Hepatitis E [§§] | None [23] | - | - | - |
| | | *Taenia soleum* antibody | None [23] | 0% | 0% | - |
| | | *Trypanosoma gambiense* antibody | None [23] | 0% | 0% | - |
| | | Vitamin B6 [***] | None [23] | 73% | 64% | - |
| | | Vitamin B12 | None [23] | 8% | 8% | - |
| | | Vitamin A | None [23] | 40% | 33% | - |
| | | Copper | None [23] | 0% | 0% | |
| | | Zinc | None [23] | 47% | 67% | |
| | | Selenium | None [23] | 100% | 100% | |
| | | Creatinine | None [23] | 75% | 71% | |
| | | Urea | None [23] | 16% | 5% | |
| | | Alanine transaminase (ALT) | None [23] | 11% | 7% | |
| | | Aspartate aminotransferases (AST) | None [23] | 59% | 64% | |
| | | Alkaline phosphate | None [23] | 23% | 14% | |
| | | Total Bilirubin | None [23] | 0% | 0% | |
| | | Hemoglobin | None [23] | 49% | 69% | - |
| | | RBC folate | None [23] | 9% | 0% | |
| | | Mercury | None [23] | 0% | 0% | |
| | | Thiocyanate | None [23] | 7% | 7% | |
| | | Homocysteine | None [23] | 0% | 0% | |
| | Family History | Genetic testing for specific epilepsy genes or consistent rare variant genes | None [22] | 0% | - | |
| | Cerebrospinal Fluid Laboratory Tests | Protein | None [23] | 0% | 0% | |
| | | Glucose | None [23] | 7% | 0% | |
| | | Measles PCR | None [23] | 0% | 0% | |
| | | Multiplex PCR | None [23] | 0% | 0% | |

[*] Onchocerciasis testing by skin snip was not significant. Numbers reported are for serum testing of Onchocerca volvulus antibodies OvFAR/MSA by luciferase immunoprecipitation system (LIPS). Serum testing for Onchocerca volvulus antibodies by Ov16 by ELISA were also significant, though less sensitive than LIPS.
[†] No differences in 17 root types before onset of nodding syndrome, root consumption more common after nodding syndrome onset
[‡] Reported OR in Foltz et al., 2013 was significant but the AOR model was not.
[§] Not significant after age adjustment

** Reported in original publication as having a positive association, as indicated by the published OR=1.416. This reported OR is not consistent with percent values given and has been published in this paper to reflect the percent case and control values given.
†† Multiplex PCR tests for 35 groups of family, subfamily or genus nucleic acid targets, covering 19 families of viruses.
‡‡ No association found for questions asked about using water from rivers/streams, boreholes, shallow wells, springs, nor piped water. No associations found for swimming in rivers or ponds either.
§§ Serum analysis
*** Indicates values that where ‘‘low’’ or below normal cut off values and signifies an abnormal test result.

| Table 4.9 Intra-Country Analysis of Nodding Syndrome Risk Factors Investigated in Tanzania | | | | | | |
|---|---|---|---|---|---|---|
| **Status** | **Risk Factor** | | **Association** | **Cases %** | **Controls %** | **p-value** |
| **Supported** | Parasitic Infection | Onchocerciasis | Positive* [109] | 84% | 64% | p=0.005 |
| | Clinical Characteristics | Intraparenchymal pathologies on MRI | Positive [42] | - | - | p=0.01 |
| | | Gliotic lesions on MRI | Positive[42] | - | - | p=0.034 |
| **Inconclusive** | Clinical Characteristics | Intraparenchymal pathologies on MRI and positive onchocerciasis test | Weakly Positive[42] | - | - | p=0.083 |
| | Family History | Patients with at least one relative with epilepsy | Not valid [14] | 90% | - | - |
| | | | Not valid [41] | - | - | - |
| **Not** | Clinical Characteristics | Onchocerca volvulus in CSF | None[109] | 0% | 0% | - |
| | | | None[5] | 0% | 0% | - |

* Percent values and p-value calculated using values reported in table 2 of original publication. P-value given is two sided Mid-P exact. Association evaluated was the association with onchocerciasis among epilepsy patients with head nodding seizures when compared to epilepsy patients without head nodding seizures.

| Table 4.10 Intra-Country Analysis of Head Nodding Seizure* Risk Factors Investigated in Liberia | | | | | | |
|---|---|---|---|---|---|---|
| **Status** | **Risk Factor** | | **Association** | **Cases %** | **Controls %** | **p-value** |
| **Inconclusive** | Family Characteristics | Family history for epilepsy | Unknown[45] | 52%† | - | - |
| | Clinical Characteristics | Photic trigger | Unknown [44] | 26% | - | - |
| | | Intellectual deficits | Unknown [44] | 34% | - | - |
| | | Psychological disturbances | Unknown [44] | 23% | - | - |
| | | Pyramidal signs | Unknown [44] | 10% | - | - |
| | | Extrapyramidal signs | Unknown [44] | 44% | - | - |
| | | Enlarged spleen | Unknown [44] | 62% | - | - |
| | | Enlarged liver | Unknown [44] | 62% | - | - |
| | | Anemia | Unknown [44] | 59% | - | - |
| | Medical/ Treatment History | Antecedent Febrile illness | Unknown [44] | 46% | - | - |

* In the original publication (van der Waals et al., 1983) the authors describe the risk factors investigated for 61 patients with complex partial seizures. In their description they state that, "characteristic for this type of seizure were rhythmic dorsoventral movements of the head," a description that is consistent with head nodding seizures.
† Value given represents all 77 patients in the survey with partial seizures, not just the 61 patients with complex partial seizures.

| Table 4.11 Intra-Country Analysis of Epilepsy Risk Factors Investigated in Cameroon | | | | | | |
|---|---|---|---|---|---|---|
| **Status** | **Risk Factor** | | **Association** | **Cases %** | **Controls %** | **p-value** |
| **Supported** | Parasitic Infection | Onchocerciasis infection | Positive [113] | 288* | 141 | p<0.0001 |
| | | | Not valid† [17] | 61% | 44% | p=0.26‡ |
| | | Correlation between onchocerciasis prevalence and epilepsy prevalence in communities | Positive [113] | $R^2$=0.615 | | p=0.019 |
| | | Correlation between epilepsy prevalence in community and distance of that community from Mbam River | Positive [113] | $R^2$=0.465 | | p=0.026 |
| | Family Characteristics | Family history for epilepsy | Positive [17] | 100% | 69% | p=0.03 |
| | Dietary Factors | Alcohol Consumption | Negative [17] | 11% | 53% | p=0.003 |
| | Milestones | Reproductive activity in females | Negative [17] | 13% | 52% | p=0.03 |
| | | Completed more than 3 years of school | Negative [17] | 72% | 94% | p=0.03 |
| **Not Supported** | Clinical Characteristics | Skin nodules | None [17] | 61% | 44% | p=0.26 |
| | | Normal sleep patterns | None[17] | - | - | - |
| | Medical/ Treatment History | Previous treatment with Ivermectin for onchocerciasis | None [17] | 67% | 72% | - |
| | | Adverse reactions (oedema and/or itching) | None[17] | 33% | 58% | - |
| | | Febrile convulsions | None[17] | 22% | 8% | OR= 3.1 (.07-24) |
| | Dietary Factors | Cola consumption | None[17] | 28% | 53% | p=0.034 |
| | | Unrestricted Diet | None[17] | 100% | 95% | - |
| | | Pork-free | None[17] | 0% | 6% | - |
| | Viral/Bacterial Infection | Measles | None[17] | 28% | 19% | OR=1.6 (.03-7) |
| | Parasitic Infection | Other parasitological diseases | None[17] | 6% | 6% | p=1.0 |
| | Milestones | Currently employed or student | None[17] | 82% | 99% | - |

* Arithmetic mean value

† Onchocerciasis infection in individuals was diagnosed by nodule palpitation which is less sensitive than skin snips, the gold standard diagnostic test for onchocerciasis.

‡ P-value and or specific risk (as worded) not reported in original publication. The p-value given is the 2-tail Mid P Exact value which was calculated using given "n" and "percent values" with OpenEpi software.

| Table 4.12 Cross-Country Analysis of Risk Factors Associated With Nodding Syndrome in Countries With *Confirmed Cases* of Nodding Syndrome | | | |
|---|---|---|---|
| **Overall Status** | **Risk Factor** | **Status and Association of Risk Factor to NS by Country of Investigation (Format: Status-Association)** | |
| | | **South Sudan** | **Uganda** |
| **Supported** | Onchocerciasis Infection | *Supported-Positive* | *Supported-Positive* |
| **Partially Supported** | Current Stunting | *Inconclusive- Weakly Positive* | *Supported- Positive* |
| | Current Wasting | *Inconclusive- Conflicting (Positive/None)* | *Supported- Positive* |
| **Inconclusive** | Previous Measles Infection | *Supported-Negative* | *Inconclusive- Conflicting (None/Positive)* |
| | Crushed Root Consumption | *Not Supported- None* | *Supported- Positive* |
| | History of Malnutrition | *Supported*[1]*- Positive* | *Not Supported- None* |
| | Exposure to Munitions | *Not Supported-None* | *Supported-Positive* |
| **Partially Ruled-Out** | Internal Displacement History | *Not Supported- None* | *Inconclusive- Not Valid* |
| | Vitamin B6 Deficiency | *Inconclusive- Weakly positive* | *Not supported-None* |
| | Red sorghum Consumption | *Inconclusive- Conflicting (Positive/None)* | *Not Supported-None* |
| | Previously received Ivermectin for Onchocerciasis treatment | *Not supported- None* | *Inconclusive- Conflicting (Negative/None)* |
| **Ruled-Out** | Abnormal Urine Mercury Levels | *Not Supported-None* | *Not Supported-None* |
| | Abnormal Urine Thiocyanates Levels | *Not Supported-None* | *Not Supported-None* |
| | Consumption of river fish | *Not Supported-None* | *Not Supported-None* |
| | Consumption of seeds meant for planting | *Not Supported*[2]*-None* | *Not Supported-None* |
| | Vitamin B12 Deficiency | *Not Supported-None* | *Not Supported-None* |

[1] Referred specifically to malnutrition during the first two years of life.
[2] Asked specifically about pesticide treated seeds

| Table 4.13 Cross-Country Analysis of Risk Factors Associated With Nodding Syndrome in Countries Where Cases of Nodding Syndrome Are *Suspected* | | | | |
|---|---|---|---|---|
| **Overall Status** | **Risk Factor** | **Status and Association of Risk Factor to NS by Country of Investigation (Format: Status-Association)** | | |
| | | **Tanzania** | **Liberia** | **Cameroon*** |
| **Supported** | Onchocerciasis Infection | *Supported-Positive* | *N/A* | *Supported-Positive* |
| **Partially Supported** | Family History for Epilepsy | *Inconclusive-Not valid* | *Inconclusive-Not valid* | *Supported- Positive* |
| **Partially Ruled-Out** | Previous Febrile Illness with or without Convulsions | *N/A* | *Inconclusive-Not valid* | *Not Supported-None* |

| Table 4.14 Cross-Country Analysis of Risk Factors Associated With Nodding Syndrome in *All Countries With Cases of Nodding Syndrome* | | | | | | |
|---|---|---|---|---|---|---|
| **Overall Status** | **Risk Factor** | **Status and Association of Risk Factor to NS by Country of Investigation (Format: Status-Association)** | | | | |
| | | **South Sudan** | **Uganda** | **Tanzania** | **Liberia** | **Cameroon*** |
| **Supported** | Onchocerciasis Infection | *Supported-Positive* | *Supported-Positive* | *Supported-Positive* | *N/A* | *Supported-Positive* |
| **Partially Supported** | Family History for Epilepsy | *N/A* | *Supported-Positive* | *Inconclusive-Not valid* | *Inconclusive-Not Valid* | *Supported-Positive* |
| **Inconclusive** | Previous Measles Infection | *Supported-Negative* | *Inconclusive-Conflicting (None/ Positive)* | *N/A* | *N/A* | *Not Supported-None* |

* Results for Cameroon are not specific to nodding syndrome or head nodding seizures.

**Table 5.1** A comparison of symptoms described in Nodding syndrome and those described in the prion disease, Chronic Wasting Disease (CWD).

| | Characteristic | Described in Nodding Syndrome | Described in Chronic Wasting Disease |
|---|---|---|---|
| Behavioral/ Cognitive Symptoms | Head nodding | • "Repetitive quick head drops of irregular frequency" [1] | • "The neurologic signs that accompanied prion disease in sick Tg mice included truncal ataxia and …. head bobbing" [45]<br>• "Behavioral changes also occur in the majority of cases, including... lowering of the head" [46] |
| | Other Seizures | • "Often accompanied by other seizure-like activity, such as convulsions or staring spells." [10]<br>• "Tonic–clonic or psychomotor seizure episodes may develop, often leading to collapse and injuries" [9] | • "Behavioral changes also occur in the majority of cases, including … blank facial expression" [46] |
| | Loss of cognitive abilities | • "Children with nodding did worse on cognitive tasks than did age-matched controls"[1]<br>• "Many decline cognitively, which eventually may lead to mental retardation"[9] | |
| | Selective Anorexia | • "Children with Nodding Syndrome, tend to nod their heads when the patient sees food or when he/she feels cold then will develop a seizure like condition. …this symptom is very unusual as the patients don't appear to suffer from seizures when they are given an unfamiliar food, for example a chocolate or non traditional food." [47] | • "Affected animals continue to eat grain but may show decreased interest in hay."[46] |
| | Excessive salivation | • "Constant flow of saliva is one of the symptoms of nodding disease."[48] | • "Signs observed in deer or elk with CWD include: …increased salivation or drooling"[49] |

| | | | |
|---|---|---|---|
| Physical Symptoms | Ataxia | • "One child with moderate truncal and appendicular ataxia, and one child with hyperreflexia and spastic gait"[1] | • "Signs observed in deer or elk with CWD include:… stumbling, lack of coordination" [49] |
| | Hyper-excitability and walking in circles | • "Some caretakers reported episodes of sudden shouting or screaming and at times jumping up and running in circles… Agitation, weakness, general body pain, sleepiness during the day, changes in mental ability, itching, and very cold extremities were also reported." [50] | • "Behavioral changes also occur in the majority of cases, including … listlessness… and repetitive walking in set patterns within the pen. In elk, behavioral changes may also include hyperexcitability and nervousness." [46] |
| | Malnutrition and Stunting | • "Chronic malnutrition (stunting) is found to be frequent in children affected by nodding syndrome."[51] | • "Signs observed in deer or elk with CWD include: …emaciation (loss of body weight and body condition)" [46] |
| | Brain atrophy | • "MRI in four of five children showed generalized cerebral and cerebellar atrophy." | |

**Table 5.2** A summary of simultaneous *Spiroplasma* infection detected by Dr. Bastian in TSE infected brains.

| **Year** | **Laboratory Test/Method of Observation** | **Disease, Tissue, Animal** | **Number with *Spiroplasma* infection/Number of cases** | **Number with *Spiroplasma* infection/Number of controls** |
|---|---|---|---|---|
| 2004 [21] | PCR amplification of Spiroplasma 16S rDNA | CJD, brain, human | 2/2 | 0/2 (age-matched controls) |
| | | CWD, brain, cervids | 6/7 | 0/7 |
| | | Scrapie, brain, sheep | 8/10 | 0/10 |
| 2001 [20] | PCR amplification of Spiroplasma 16S rDNA | CJD, brain, human | 13/13 | 0/50 |
| | | Scrapie, brain, sheep | 5/9 | 0/50 |

| | | | | |
|---|---|---|---|---|
| 1979 [16] | Electron microscope | CJD, brain, human | 1/1 | 0 |

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