AETIOLOGY OF ENCEPHALITIS AND MENINGITIS IN CHILDREN AGED 1-59 MONTHS ADMITTED TO THE CHILDREN'S HOSPITAL, LUSAKA, ZAMBIA

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A dissertation submitted in partial fulfillment of the requirements for the award of the degree of Master of Medicine in Paediatrics and Child Health

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DECLARATION

This is my original work and has not been presented for an award of a degree in any other University. All the sources used or quoted have been indicated and acknowledged by complete references.

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ABSTRACT

Meningitis and encephalitis are important causes of admissions and mortality in Zambia. There is a worldwide geographical and regional variation in the causative agents. Apart from bacteria, little is known about viral agents that cause the disease in Zambia. To identify the viral and bacterial causative organisms, we conducted a prospective descriptive study at the Children's Hospital, in Lusaka, Zambia.

To determine the causative organisms of viral encephalitis and meningitis, and pyogenic meningitis; determine biochemical and cellular changes in cerebrospinal fluid (CSF) and the associated clinical features in children aged 1-59 months admitted at the Children's Hospital, Lusaka.

Between November 2016 and February 2018, we collected CSF samples and clinical details from children aged 1-59 months with meningitis and encephalitis who met the inclusion criteria. Macroscopic examination, microscopy, bacterial culture, real-time (Multiplex) PCR and biochemistry were performed on the CSF samples.

A total of 106 children were enrolled. The female to male ratio was 1.2:1. The median age of the study patients was 10 months. There were 81 (76.4%) cases with meningitis and 25 (23.6%) with encephalitis. The median duration of symptoms was 3 days. There was only one (0.9%) participant with *Haemophilus influenzae* bacteria detected by both culture and PCR. Two (1.9%) cases had *Neisseria meningitidis* while 5 (4.7%) had *Streptococcus pneumoniae* detected only by PCR. Viruses were only detected in 26.4% (28/106) of the cases. The viral agents detected were *Epstein-Bar virus* (10%) and parvovirus B19, Human herpes virus type 6, Human herpes virus type 7 and CMV at 2.8% each. Viral agents were detected in 64% and 36% of patients with meningitis and encephalitis, respectively. Bacterial agents were detected in 75% and 25% of patients with meningitis and encephalitis respectively. Ninety percent of cases had a history of fever and 50% had a history of a seizure. A raised CSF white blood cell counts (WBC) was significantly associated with case definition of meningitis (P=0.01). Patients that were alive at discharge point had on average 3.6 times increased odds for meningitis case definition (OR = 3.6, CI = 1.96 – 6.68, P-value <0.001).

Viral infections of the central nervous system (CNS) are the commonest causes of both encephalitis and meningitis in children aged 1-59 months admitted at the Children's Hospital in Lusaka, Zambia. The causative agents were not significantly associated with a case definition of encephalitis or meningitis. A raised WBC was significantly associated with meningitis.

Key words: Pyogenic Meningitis, Viral Encephalitis, Lusaka

DEDICATION

I dedicate this work to:

- My participants and their guardians for the opportunity to learn and serve them.
- My lecturers and supervisors for the knowledge I have acquired.
- My family for the support they gave me throughout my career and during the development of this paper.

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LIST OF ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
CDC	Centers for Disease Control and Prevention
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CSO	Central Statistics Office
CMV	Cytomegalovirus
cDNA	Complimentary DNA strand
EBV	Epstein Bar Virus
EPI	Expanded Programme on Immunisations
HHV	Human Herpes Virus
Hib	Haemophilus influenza type b
HIV	Human Immunodeficiency Virus
IMCI	Integrated Management of Childhood Illnesses
IPD	In-Patient Department
LP	Lumbar puncture
MoH	Ministry of Health
PCR	Polymerase Chain Reaction
PCV	Pneumococcal Conjugate Vaccine
OPD	Out-Patient Department
SOPs	Standard Operating Procedures
Spp.	Species
UTH	University Teaching Hospital
VZV	Varicella Zoster Virus
WHO	World Health Organization
ZDHS	Zambia Demographic and Health Survey

CHAPTER ONE: INTRODUCTION

1.1 Background

Despite the discovery of Lumbar Puncture (LP) in the previous millennium, out breaks and sporadic cases of meningitis and encephalitis have continued to cause significant morbidity and mortality.[1,2] Additionally, the incidence and case fatality rates of the affected vary per region, country, pathogen and the affected age group.[3,4,5,6] In the Tropical and Western industrialised countries, the incidence of encephalitis in children has been estimated to be around 10 per 100,000 persons.[5]

Although Africa remains the most affected region in the world[6] and the known variation in the causes, literature is nonexistent on the causes of viral encephalitis and viral meningitis in children admitted at the University Teaching Hospitals' (UTH) Children's Hospital.

Research done in children under the age of five years with bacterial meningitis at the Children's Hospital showed *Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae* as the commonest causes of the disease.[7,8] However, such research did not include a viral component as part of the investigations; viral infections such as enteroviruses and some bacteria such as *Mycoplasma pneumoniae* have been reported to cause meningitis and encephalitis especially in infants and young children.[3,9-13]

The infections of the Central Nervous System (CNS) cause inflammation that may be focal; such as in a cerebral abscess, or diffuse; such as in meningitis (inflammation of the meninges) and encephalitis (inflammation of the brain or spinal cord parenchyma).[9,11,14,15] This diffuse inflammation may also result from noninfectious causes.[11,14,15]

Most of the infectious causes of encephalitis remain unknown; despite extensive investigations, in one study, about 6 in 10 cases of encephalitis had no known cause.[16] Worldwide, the common infectious causes of meningitis include *Streptococcus pneumoniae, Haemophilus influenza, Neisseria meningitidis* and *group B Streptococcus*. However, these causes vary with immunisation trends, geographical location, age of the patient, socioeconomic factors and the climatic

conditions among other factors.[17-26] In this study, both viral and bacterial causes of meningitis and encephalitis were investigated.

From an epidemiological and pathological perspective, meningitis and encephalitis may be described as distinct entities. However, clinically, symptoms overlap, and encephalitis may also present with symptoms of meningeal irritation.[9,11,15,27] The clinical features present in a patient with meningitis and/or encephalitis often depend on the age of the patient, the host response to the ongoing infection, the infecting organism and the duration of the illness.[9,11,28]

The triad or cardinal features of meningitis; meningeal signs, headache, and fever, are a less common finding in younger children than in adults. The symptoms of meningitis and encephalitis may overlap and the younger the child, the less likely that they are to exhibit the classic triad of meningitis.[11,14,29]

Thus, the clinical manifestations in infants and children may include; nuchal rigidity, opisthotonos, bulging fontanelle, convulsions, altered sensorium, coma, fever or hypothermia. On the other hand, infants below three (3) months may present with non-specific symptoms such as; poor feeding, change in sleeping, vomiting, diarrhea, jaundice, hypothermia, fever, lethargy, restlessness, irritability, respiratory distress and/or a high-pitched cry.[9,11,14,21,28,29]

In a study at UTH, Lusaka, in 2002, the commonest presentation in children under the age of five years who presented with meningitis were; fever (96.7%), neck rigidity (69.6%), excessive crying (59.8%), seizures (56.5%) and loss of appetite.[7] However, the study did not try to link the clinical features with the commonest and likely aetiological agent.

The clinical features of viral CNS infections depend on the specific site that has been affected. Clinical features of meningitis, like the ones listed above, may be present if the meninges are affected. Involvement of the brain parenchyma presents with features of encephalitis which include fever, nausea, vomiting, headache, seizures or altered level of consciousness.[9,27,30,31]

The diagnosis of Acute Bacterial Meningitis (ABM) and viral encephalitis is based on the history from the patient and/or guardian, examination of a patient and the

performance of an LP and examination of the cerebrospinal fluid (CSF).[9,11,14,28] The latter is more useful in making a definitive diagnosis. It involves isolation or detection of the pathogens from the CSF and demonstration of meningeal inflammation revealed by CSF cellular pleocytosis, and alterations of the protein levels and glucose levels.[11,15,27,31]

Haematological changes in a full blood count (FBC), and imaging (e.g. computed tomography (CT) and magnetic resonance imaging (MRI)) assist in making a diagnosis and/or identifying the complications.[9,27,28,31]

According to the World Health Organisation (WHO), and as used in a study by Nhantumbo et al. (2015),[32] a suspected case of ABM is defined as a 'child aged < 5 years with sudden onset of fever (>38.5°C rectal or 38.0°C axillary) and one of the following signs: neck stiffness or flaccid neck, bulging fontanelle, convulsion, irritability, or drowsiness'.

Like bacterial meningitis, the diagnosis of encephalitis is made based on the clinical and laboratory findings. Neuroimaging and electrophysiological findings may add another perspective to the diagnosis of encephalitis by supporting a diagnosis of an aetiology or identify alternative conditions that may mimic encephalitis.[27,31] In March 2012, the International Encephalitis Consortium developed consensus guidelines to aid in clinical diagnosis and evaluation of children with suspected encephalitis. Table 1 below summarises the diagnostic criteria for encephalitis.

Table 1. Diagnostic criteria for encephalitis (International Encephalitis Consortium).

Major criterion (required)
Patients presenting to medical attention with altered mental status (defined as decreased or altered level of consciousness, lethargy or personality change) lasting ≥24 hours with no alternative cause identified.
Minor Criteria (2 required for possible encephalitis; ≥3 required for probable or confirmed encephalitis):
Documented fever ≥38°C within the 72 h before or after presentation.
Generalized or partial seizures not fully attributable to a pre-existing seizure disorder.
New onset of focal neurologic findings.
CSF WBC count ≥5/cubic mm.
Abnormality of brain parenchyma on neuroimaging suggestive of encephalitis that is either new from prior studies or appears acute in onset.
Abnormality on electroencephalography that is consistent with encephalitis and not attributable to another cause.

Adapted from reference 31.

The present study sought to identify the causes of encephalitis and meningitis in children aged 1 to 59 months presenting at UTHs' Children's Hospital. A cumulative sample of children presenting with features of encephalitis and/or meningitis who met the inclusion criteria and whose parents agreed to participate in the study and consented for LP in the emergence room from 2016 and 2018, were recruited into the study.

1.2 Statement of the problem

Approximately 2.9 percent of the under-five year children admitted to Children's Hospital, in either 2013 and 2014 (252 and 220, respectively), had encephalitis and/or meningitis.[33] Despite this low prevalence, the mortality and morbidity patterns of these diseases has been shown to be high with case fatality rates of 50-70%.[3,4,6]

Furthermore, although the literature reviewed show a wide geographical and regional variation in the causes of these diseases[3-6,34-37], literature is scarce on the causative organisms of viral encephalitis and viral meningitis in children aged 1-59 months presenting at the Children's Hospital, Lusaka, Zambia (south-central Africa).

In ABM, the timely diagnosis of these infections is a challenge as the microbiological methods used take more than 24 hours to isolate the causative organism. Delayed identification of the likely causative organism in both viral and bacterial CNS infection has been shown to lead to poor patients' outcome or death.[3,4,6,9,11,28,34]

The research data done locally in 1975,1997, 2002 and the current PBM surveillance data focused more on the bacterial causative organisms, common clinical features and/or the antimicrobial sensitivity patterns.[7,8,38,39] Therefore, it was timely that a study that establishes the viral causes and links the clinical features to the likely aetiology was conducted.

1.3 Rationale of the study

Due to the worldwide geographical and regional variation in the causes of encephalitis and meningitis and the limited literature regarding the common microbial causes of meningoencephalitis in Zambia, this study was undertaken to determine viral as well as bacterial causes of encephalitis and meningitis at UTHs' Children's Hospital, Lusaka.

The CSF cellularity vary depending on the pathology and causative organisms of meningitis or encephalitis. In ABM, the CSF is mostly cloudy with an increased WBC count; neutrophils predominate.[4,11,13,28] In acute viral encephalitis and/or meningitis, the increased WBC counts show more of lymphocytic predominance.[9,11,13,27,40] Various changes occur in specific infections such as in mumps encephalitis.[9,41]

Documentation of the CSF changes will have a significant contribution to the knowledge regarding the identification of the specific CSF changes associated with certain bacterial or viral aetiology of meningitis and encephalitis at the Children's Hospital.

In the post *Haemophilus influenzae* type b (Hib) vaccine and Pneumococcal Conjugate Vaccine (PCV10), introduced in 2004 and 2013, respectively, in Zambia[26,42] and the worldwide reported changes in antibiotic susceptibility to antibiotics of the community isolated pathogens[43], documentation of the current, (i) viral and bacterial causes, (ii) antibiotic susceptibility, (iii) link of clinical features to the likely causative organisms, could play a significant role in the clinical management of patients.

When the microbial methods delay establishing the likely causative organism, the correlation between the patients' pattern of clinical features or the CSF changes and the likely aetiology if established could be important in the clinical management of patients and may improve patient outcomes.

Based on the literature reviewed, it is important to know the pathogens of encephalitis and/or meningitis in children aged 1 to 59 months presenting at the Children's Hospital. Additionally, the pathogens' associated CSF biochemical changes and the pattern of clinical features if identified could play a role in the clinical management of patients.

1.4 Research question

What are the causative organisms of viral encephalitis and pyogenic meningitis in children admitted at the Children's Hospital, Lusaka, Zambia?

1.5 Study objectives

1.5.1 Overall objective

To identify the causative organisms of viral encephalitis and pyogenic meningitis in children aged 1-59 months admitted to the Children's Hospital, Lusaka.

1.5.2 Specific objectives

The specific objectives were as follows;

a) Determine the aetiology of viral encephalitis and meningitis in children aged 1-59 months admitted to the Children's Hospital.
b) Determine the aetiology of pyogenic meningitis in children aged 1-59 months admitted to the Children's Hospital in the vaccine era.
c) Determine the biochemical and cellular CSF changes in children presenting with pyogenic meningitis and viral encephalitis.
d) Determine the clinical features associated with the common aetiology of viral encephalitis and pyogenic meningitis.

CHAPTER TWO: LITERATURE REVIEW

2.1 Geographical variation in the aetiology of meningitis and encephalitis

The causes of encephalitis vary worldwide according to the geographical region.[13,34,35,36] In Ireland, between 2005-2008, Herpes simplex virus (HSV) accounted for the highest number (40%) of viral encephalitis infections followed by Varicella zoster virus (VZV) (27%) and mumps virus (12%) in children below 4 years of age while enteroviruses accounted for the highest number of viral meningitis cases (61% which included Coxsackie A and B).[35]

In the United States of America (USA), the causes of encephalitis admissions between 2000-2010 in children aged 1-4 years were enteroviruses and VZV accounting for 0.51 per 100,000 of population, and HSV which accounted for 0.34 per 100,000 populations among this age group.[36]

On the other hand, in a study to establish the viral aetiology of encephalitis in children below the age of 16 years in southern Vietnam, the commonest causes were Japanese encephalitis virus (26%), enteroviruses (9.3%), dengue virus (4.6%) and HSV (0.5%).[34]

In Africa, some studies have documented West Nile virus to be the commonest cause of endemic encephalitis in northern Africa[12] and others have reported rabies in some paediatric populations.[37]

Like encephalitis, bacterial causes of meningitis vary greatly worldwide. In the USA, *Streptococcus pneumoniae* and *Neisseria meningitidis* are the commonest causes of meningitis in children beyond the neonatal period.[28] The children who are under five years and adolescents have the highest incidence rates.[3,4,21] Furthermore, meningococcal meningitis is highest in the meningococcal belt of sub-Saharan Africa (from Ethiopia in the east to Senegal in the west)[4,21,23], though this is likely to change with the introduction of meningococcal conjugate vaccine in the Expanded Programme on Immunisations (EPI) programmes.[22]

In a study in Papua New Guinea, *Streptococcus pneumoniae* and Hib accounted for 88% of the CSF isolates among the paediatric patients admitted with meningitis.[44] Furthermore, a study in Mozambique to establish the causes of meningitis in children

showed that *Neisseria meningitidis*, *Streptococcus pneumoniae* and Hib accounted for 5.4%, 4.8% and 3.6% of the CSF isolates, respectively.[45]

In a study to analyse the indications and results of CSF examinations in East Africa, *Streptococcus pneumoniae* and *Salmonella species* were found to be the commonest causes of pyogenic meningitis among children above two months of age.[46] On the other hand, in Malawi, *Streptococcus pneumoniae, Hib* and *Neisseria meningitidis* were found to be the commonest causes of bacterial meningitis in children aged between 2 months and 13 years; 40%, 28% and 11% respectively[47] prior to the introduction of PCV in 2011.[26]

In 2002, a study in Zambia showed that *Streptococcus pneumoniae* accounted for 61% of the CSF isolates in patients with meningitis. The other causes included *Haemophilus influenzae* (19%), *Neisseria meningitidis* (9.8%) and salmonella spp. (4.9%).[7] Prior to the foregoing, a study done in 1975 had shown *Neisseria meningitidis* and pneumococcus as the commonest causes of meningitis.[39] These studies predominantly looked at the bacterial causes of meningitis.

Documentation of the causes of encephalitis and meningitis in children presenting at the Children's Hospital would contribute to knowledge regarding the commonest pathogens causing these infections in children in the post PCV and Hib vaccine era.

2.2 Cerebrospinal fluid changes in meningitis and encephalitis

A pattern of CSF changes may occur with various groups of organisms infecting the CNS as will be shown below.

ABM usually has cloudy CSF appearances with a high CSF opening pressure, leucocyte count (with neutrophilic predominance) and protein content. In addition, the glucose levels are usually decreased.[4,11,13,25]

In acute viral meningitis and meningoencephalitis, the CSF pressure might be slightly elevated or normal. Furthermore, it is rare for the CSF leucocyte count to be greater than 1000 cells/mm^3 and the protein content to be above 200mg/dl. The glucose levels are usually normal and rarely altered.[9,11,13,27,40] It is important to measure serum glucose levels close to CSF collection as the normal CSF glucose levels of about two thirds of the serum levels may be altered in CNS infections.[11,15,27,31]

The CSF findings which are different from the above description can occur. For example, in eastern equine encephalitis, the leucocyte counts may be over 1000 cells/dl high with polymorphonuclear cell predominance in the early stages of the disease and lymphocyte predominance later.[9,11,48] It has also been found that although the CSF glucose levels are generally within normal range in viral CNS infection, 20-30% of patients infected with mumps virus have a decreased glucose level.[9,41]

The CSF commonly appears turbid in ABM than in viral CNS infections. Turbidity of the CSF is mainly due to raised CSF leucocyte counts exceeding 200-400 cells/mm^3. [11,28] In bacterial CNS infections CSF WBCs is usually raised with a neutrophilic predominance.[28]

In bacterial meningitis where antibiotics have been given prior to CSF examination, bacterial Gram stains or cultures may be negative. In such situations, rapid bacterial antigen testing may be preferred or polymerase chain reaction (PCR).[11,28,32,49] Of note is that even with otherwise normal CSF results, the sample should be sent for cultures as about 10% of children with meningitis may have normal CSF findings.[49]

There are no data from Zambia that describe the CSF changes in viral encephalitis.

2.3 Clinical sequelae of meningitis and encephalitis

The prognosis in viral and bacterial meningitis and/or encephalitis vary depending with the cause, the severity of the clinical illness, and the age of the child.[3,9,11,34]

Severe sequelae are most noted in HSV infection.[3,11] By contrast, the sequelae from enterovirus infection may be subtle with spontaneous recovery in some patients.[11,40] However, HSV infection in encephalitis without treatment is associated with mortality rates which may be as high as 70%.[3,9] It is also estimated that about 10% of children below two years of age with CNS enterovirus infections may have complications such as coma and increased intracranial pressure (ICP).[9,11,30]

Neurological sequelae such as motor incoordination, deafness, visual disturbances, behavioural disturbances and convulsive disturbances occur in viral and bacterial CNS infections. Thus, follow-up in patients recovering from these infections should include neurodevelopmental, auditory and visual assessments to identify these complications.[3,9,11,36]

In marked or severe brain parenchymal involvement, the prognosis has been documented to be very poor.[9,11,27]

In the Zambian context, literature that describe the prognosis or prognostic indicators is almost nonexistent. It is hoped that this study will provide information on the prognosis of viral meningitis and encephalitis.

2.4 Prevention of meningitis and encephalitis

Some of the causes of viral CNS infections such as polio, measles, mumps and rubella have been eliminated, in countries such as the USA and Europe, through successful vaccinations.[20,50]

Arthropod born viruses (arboviruses) may be successfully controlled, and incidences of the diseases reduced, by vector control measures such as spraying and eradication of the insect breeding sites.[11]

Rabies encephalitis has been reported in a study in Malawi.[37] In rabies encephalitis, the vaccination of the domestic animals against rabies have been shown to significantly reduce the incidence of the disease in humans.[51]

The specific control measures above may only be instituted where specific aetiology of meningitis and/or encephalitis are known. Therefore, a study that establishes the common viral and bacterial causes of encephalitis and/or meningitis was important so that the necessary preventive measures may be put in place (where it is possible) such as in Mumps and Rabies.

CHAPTER THREE: METHODOLOGY

3.1 Research design

A prospective descriptive design was used in this study.

3.2 Study location

The study was conducted at the UTHs' Children's Hospital, Lusaka. The UTH is the country's largest tertiary referral hospital located approximately 4 kilometers east of the city centre and receives referrals from all over the country.[52]

The Children's Hospital has several wards managed by eight units, each headed by a consultant. In 2014, the department had a 352 bed spaces and attended to 33,706 patients seeking medical care in the Out-Patient Department (OPD).[33,52]

In 2013 and 2014, 16,191 and 16,440 children, respectively, were admitted in the Children's Hospital. Approximately 2.9 percent of the total admissions during this period, had encephalitis and/or meningitis.[33]

3.3 Target and study population

The target population for the study were children aged 1-59 months admitted at the Children's Hospital between November 2016 and November 2017 with acute encephalitis and meningitis. Children aged 1-59 months were chosen as they are, most times, admitted where the study was conducted. However, the period was extended to February 2018 as only 67% of the expected number of participants were enrolled by November month end. The respondents were the parents and/or guardians to the children.

The study population were those who had consented for LP, a CSF sample successfully drawn, and had a clinical diagnosis of encephalitis and/or meningitis; based on the case definitions of encephalitis and meningitis in children (based on the consensus statement of the International Encephalitis Consortium[31] and a WHO case definition of ABM in under-five children.[32]

3.4 Sampling method

A cumulative sample was used in this study. Children aged 1 to 59 months admitted to the paediatric wards of the Children's Hospital with encephalitis and meningitis, during the study period who meet the inclusion criteria of the study, were enrolled.

3.5 Sample size

The proportion of encephalitis and meningitis in children below the age of 5 years, in 2014 and 2015 was estimated to be 2.9 per cent of the total admissions.

This study was designed to allow a sampling error of 5% and the power of 95%. The sample size was estimated as follows:

$N=[z^2p(1-p)]/e^2$

Where:

N= the sample size, z=1.96 (the p value at 95% confidence limit), p=the proportion of children with encephalitis and/or meningitis (2.92%) and e= 3.5%; the margin of error.

Therefore, $n= [1.96^2x0.029(1-0.0292)]/0.035^2$= 89+40% sampling error= 125 participants.

The 40% sampling error was the estimated proportion of the patients who might refuse to participate or to have an LP done.

The sample size was 125 participants.

3.6 Eligibility criteria

3.6.1 Inclusion criteria

The inclusion criteria for this study was as follows:

(a) All children aged 1 to 59 months admitted between December 2016 and February 2018 with a clinical diagnosis acute encephalitis and/or meningitis, based on the case definitions of encephalitis and meningitis in children; based on the consensus statement of the International Encephalitis Consortium[31] and a WHO case definition of ABM in under-five children.[32]

(b) Participants whose legal guardian or parent consented for LP and had CSF successfully drawn by the attending physician.

(c) Children whose parents or legal guardian made a written informed consent to participate in the study.

3.6.2 Exclusion criteria

The following were excluded from participating in the study:

(a) Children with pre-existing neurological conditions.

(b) Children who are not aged 1 to 59 months at the time of the study.

3.7 Data collection tools

A clinical case record form was used to collect demographic and clinical data, including CSF microscopy, biochemistry, culture and sensitivity, and PCR results.

3.8 Data collection procedure and processing

The study was conducted with the help of all the unit doctors, nurses, ward clerks and potters in the Out-Patient Department (OPD) and In-Patient Departments (IPD), including the laboratory staff.

3.8.1 Recruitment and enrollment of participants

Children who met the inclusion criteria following clinical evaluation and had an LP, according to the hospital protocols, were identified. Once identified and ascertained that they met the inclusion criteria, a participant information sheet (Appendix 1) was given to the parents or guardians to read on their own or was read to them if they are unable to read.

For those who agreed to participate in the study, they were requested to sign (or give a thumb print) a consent form (Appendix 2) and then enrolled in the study. Those who refused to participate were reassured that their child would receive the same standard of medical care available in the hospital.

The CSF specimen collected were labeled and attached to a duly filled-in form, and sent to the laboratory for analysis, while the patient was admitted and started on acyclovir and/or intravenous antibiotics as may be prescribed by the unit doctors.

3.8.2 Collection and transport of specimen to the laboratory

The CSF collected, under aseptic conditions, was put in two plain sterile (red-topped) bottles; the first bottle was for biochemistry and the other for microbiology and PCR analysis. A total of 1.5 milliliters of CSF was collected in each of the two bottles.

The investigator ensured that the CSF specimen reached the laboratory within one hour of collection. This is because these meningitis-causing organisms such as *Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis* are fragile organisms, and any delay to transport to the laboratory would lead to no growth of the bacteria on culture.[4]

3.8.3 Laboratory investigations

The CSF samples were processed for bacteriological examination, standard biochemical analysis and subjected to a Multiplex PCR for the identification of both viral and bacterial infectious causes. In addition, macroscopic and microscopic examination in the laboratory was done on the CSF samples for colour/turbidity or presence of blood.

These are outlined in detail as follows:

3.8.3.1 *Bacterial analysis and Cryptococcal species identification*

Identification of the organisms in the CSF sample was as follows:

a) Gram stain was done on all CSF samples. Culture and isolation of the organisms on Blood Agar, Chocolate Agar and MacConkey Agar was done. The organisms (*Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, and other bacteria*) found in the CSF cultures were then identified using standard bacteriological techniques.
b) Indian ink was used to identify *Cryptococcus* species.
c) Ziehl Nielsen stain was planned to be used to identify Mycobacterium tuberculosis. However, it was not done due to small volumes of CSF collected.

The standard CSF cultures were done on three different media; (i) Blood Agar, (ii) Chocolate Agar and (iii) MacConkey Agar and followed up as stated below. This was done according to the standard operating procedures (SOPs) in the UTH's Bacteriology Laboratory.[53]

On the first day, the CSF was inoculated on the three-different media and incubated between 35°C - 37°C, in 5-10% carbon dioxide for 18-24hours.

On the second day, the plates were then examined for growth of the organisms. If there was growth, we proceeded with identifying and sub-culturing on fresh media for purity of the organisms. If there was no growth, the plates were re-incubated for another 24 hours.

On the third day, antibiotic susceptibility tests were done on purified growth according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. If there was growth on the re-incubated culture plates, we proceeded with identifying the organisms and testing for their antibiotic susceptibility.[4,53]

3.8.3.2 *Real-time multiplex PCR analysis*

The multiplex PCR was used on the CSF samples for the identification of (i) HSV 1/2, (ii) VZV, (iii) Enteroviruses, (iv) Adenovirus (AV), (v) VZV, (vi) Par echovirus, (vii) Epstein Bar virus, (viii) Human herpes virus 6 and 7, (ix) Parvovirus B19, (x) CMV, and the bacterial meningeal pathogens; (i) *Streptococcus pneumoniae, Meningococcal meningitis and Haemophilus influenzae*.

The PCR reagents used were FTD Neuro9 and FTD Bacterial meningitis manufactured by Fast Tract Diagnostics, Luxembourg s.a.r.l.[54,55]

The CSF samples for PCR analysis were processed in batches of 30 or 62 per run using a real-time multiplex PCR (ABI® 7500) machine per the reagents and equipment manufacturer's instructions as follows:

Before the samples can be run, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) was extracted from the CSF samples using NucliSENS® easyMAG® (bioMérieux) machine.[54,55]

To detect the viral pathogens, the viral RNA was transcribed into cDNA using specific primer mediated reverse transcription step which was followed immediately in the same tube by polymerase chain reaction (PCR). This amplified the DNA in the tube. To detect the bacterial and DNA viral meningeal pathogens, the DNA of different pathogens was amplified in the test tubes by the PCR. The presence of specific pathogen sequences in the reaction was detected by an increase in the fluorescence observed and reported as a cycle threshold by the real time thermocycler.[54,55]

The PCR machine takes about 2 hours to run the above processes and detect the pathogens, if any, in the CSF.

3.8.3.3 *CSF cell counts*

The CSF cell counts was done using the Improved Neubauer Counting Chamber. The cell counts were reported per cubic millimeters. The CSF cell differential counts were done using 2% acetic acid tinted with Crystal violet or Gentian violet and reported as percentages[4,53].

3.8.3.4 *CSF biochemical analysis*

The standard biochemical analysis for protein, chloride and glucose were done on the CSF samples using automated equipment.

3.8.3.5 *Quality control*

Quality control measures were performed on all equipment used. These measures included equipment calibrations and analytical control runs before using them according to the UTH's quality control guidelines to ensure that accurate and reliable results are generated.

3.8.4 Clinical data

A detailed demographic and clinical information (the symptoms, signs and the disease outcomes) were collected on clinical case record forms (Appendix 3).

3.9 Ethical considerations

Ethical approval was obtained from the Excellence in Research Ethics Science (ERES) Converge (IRB No. 00005948, FWA No. 00011697), Lusaka, Zambia.

Permission to collect data from the Children's Hospital was sought from the Ministry of Health (MoH).

Before the recruitment and collection of data from the children and their guardians, permission was sought from the National Health Research Authority (NHRA) through the National Health Research Ethics Board (NHREB) as the requirement in Zambia for all research involving human participants.

A participant information sheet (Appendix 1) was given or read out to parents or guardians whose children met the recruitment criteria in a language they understood. The participant information sheet included information on the benefits of participating in the study: the presentation of patients may benefit would be patients in the future; and the risks of participating in the study: minimal risks such as questions which might be personal or intrusive. In addition, the purpose and nature of the study was explained.

No consent was obtained for the collection of the CSF for analysis, as it was assumed that they would have consented for LP as part of the clinical management of their children. Furthermore, the study did not involve any procedures that may alter the disease outcome.

Those who agreed to participate in the study were recruited, then requested to sign a consent form (Appendix 2) or give a thumb print to allow the research team to collect data on sociodemographic and the CSF for analysis. Those who refused to participate were reassured that their child will receive the same standard of medical care available in the hospital for all patients.

Good clinical practice was used by the researchers always. Study participants were assured of anonymity and confidentiality by assigning them with a unique code number.

During the data collection process, the researcher ensured that all research data and tools were kept under lock and key. Access to the data was limited to the researcher and his assistants.

3.10 Statistical analysis of data

The data collected was cleaned, summarised and analysed using Epi info™ version 7 for Windows, IBM SPSS Statistics™ version 21.0 and Microsoft Excel for Office 2016.

IBM SPSS™ version 21.0 was used for statistical analysis and to produce some graphical output. All statistical tests were done at 5% significance level. Independent samples t-test was used to compare mean values between groups.

The Pearson's chi-squared test was used for comparison of proportions between groups and Fisher's exact test was used when one or more of the cells had an expected frequency of five or less. Some variable categories with less frequency were collapsed together accordingly.

Study variables were checked for evidence of co-linearity based on a correlation coefficient of >0.8. The relationship between study variables and case definitions was examined using backward logistic regression. The selections for entry into the logistic regression model was considered at level $p<0.20$ or known clinical significance from literature.

3.11 Limitations of the study

The following were the limitations of this study:

- The study was based at the Children's Hospital in Lusaka. Only the patients who clinicians at the lower level facilities felt needed tertiary services were captured. Therefore, the sample might not have been representative of all the patients with meningitis and/or encephalitis in Lusaka.
- The laboratory results were limited to what was available at the Children's Hospital. Because of this, some of the patients did not have both biochemistry and bacteriology studies done.
- The participants were also followed up over a short duration of time. A surveillance study over a longer period would be necessary to capture a larger sample size and define the prognosis of meningitis and encephalitis.

CHAPTER FOUR: RESULTS

During the study period, November 2016 to February 2018, a total of 15,258 children were admitted. 270 of those admitted had provisional diagnosis of meningitis, meningoencephalitis and/or encephalitis. 106 (106/270, 39.3%) of those admitted with meningitis and/or encephalitis were included into the study. A total of 164 (60.7%) did not meet the inclusion criteria, or their guardian did not consent to participate.

4.1 Participants' characteristics

Data on 106 children was analysed for the study. This comprised 49 (46.2%) males and 57 (53.8%) females. The difference in sex proportional distribution was not statistically significant (P = 0.44). The mean age of the study patients was 15.7 months (SD = 15.43) [Figure 1]. The minimum and maximum ages in months of the study patients were 1 and 58 respectively, while the median age was 10 months [Figure 1].

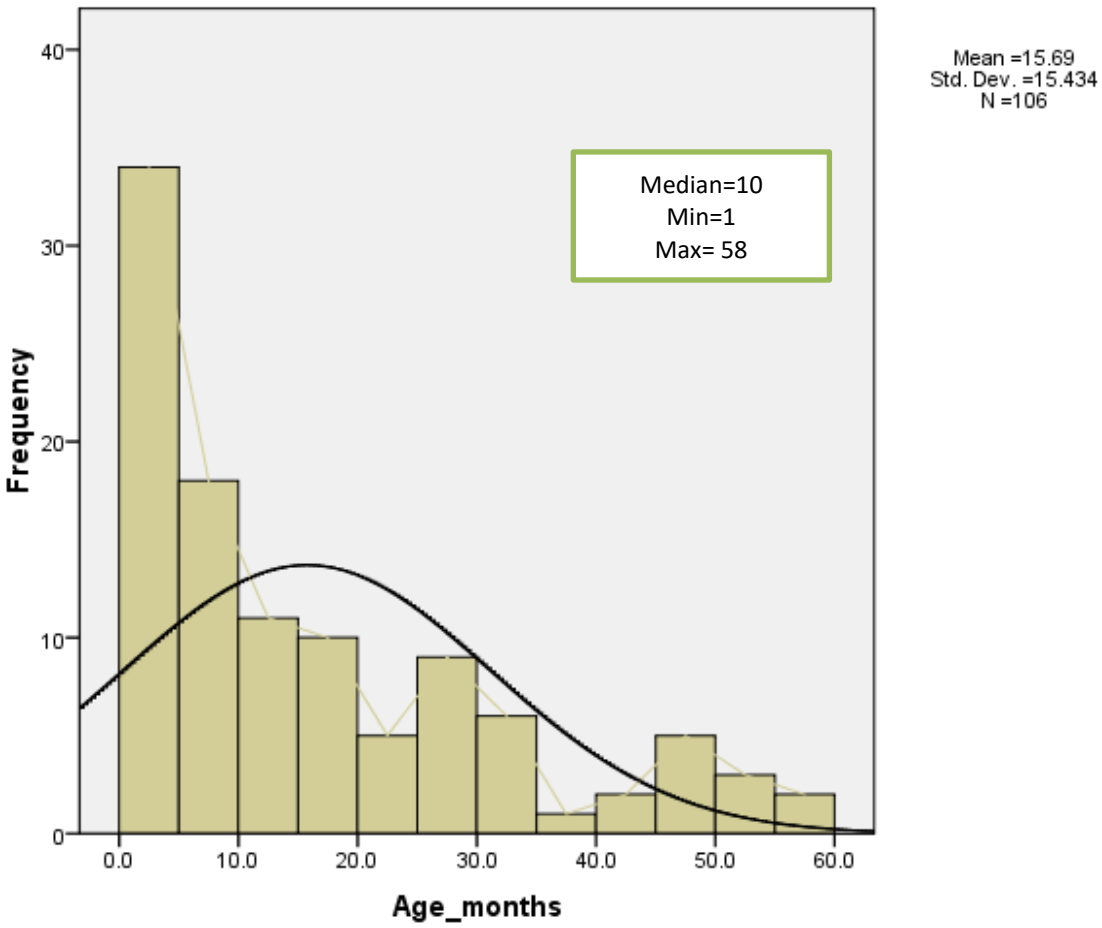

Figure 1. Study patient age distribution [minimum=1, maximum=58, median=10]

There were 81 (76.4%) participants with meningitis and 25 (23.6%) with encephalitis, and this difference in proportional distribution was significant (P<0.001). The median duration of symptoms was 3 days while the mean was 4.8 days (SD = 5.22). About 50% of the participants had symptoms of a seizure, but the greater majority, over 90%, had fever [Table 2].

Table 2. Participants' demographic and clinical characteristics

Variable	Frequency	Percent
Sex		
Male	49	46.2
Female	57	53.8
Case definition		
Encephalitis	25	23.6
Meningitis	81	76.4
Related symptoms		
Seizures	57	53.8
Unable to feed or drink	39	36.8
Fever	99	93.4
Prostration	20	18.9
Petechial	2	1.9
Difficulties breathing	28	26.4
Stridor	4	3.8
Altered consciousness	26	24.5
Bulging fontanelle	10	9.4
Neck stiffness	28	26.4
Dehydration	17	16.0
Chest indrawing	6	5.7
Fast breathing	12	11.3
Antimicrobial drugs given		
Yes	63	59.4
No	21	19.8
Unknown	22	20.8
Patient outcome at discharge		
Alive	93	87.7
Died	13	12.3

There were 63 (59.4%) participants that received antimicrobial drugs while 21 (19.8%) did not receive antimicrobials [Table 2] prior to admission. The proportional difference between participants who received antimicrobial drugs was statistically significant (P<0.001).

Majority of the participants, 89 (84%), had received the DPT-HepB-Hib vaccine, and this was significant (P<0.001). About 12.3% received 1 dose, 22.8% received 2 doses and 64.9% received 3 doses. A significantly greater proportion (P<0.001) of the participants, 84 (79.2%), also received the pneumococcal conjugate vaccine, and about 25.5% had received a single dose, 19.6% received 2 doses, and 54.9% received 3 doses [Table 3].

Table 3 Participants' vaccination characteristics

Variable	Frequency	Percent
Pneumococcal conjugate vaccine (PCV)		
Yes	84	79.2
No	12	11.3
Unknown	10	9.4
DPT-Hep B-Hib vaccine		
Yes	89	84
No	9	8.5
Unknown	8	7.5
Oral Polio Vaccine		
Yes	89	84
No	10	9.4
Unknown	7	6.6

There were similarly 89 (84%) participants that received the Oral Polio vaccine, and about 22.9% received 1 dose, 14.6% received 2 doses, and 62.5% received 3 doses. Similarly, 94 (88.7%) received the Rota virus vaccine and 84.7% received 1 dose and 15.3% received 2 doses [Table 3].

There were 84 (79.2%) participants who received BCG vaccine, and 20.8% had received a single dose, 69.8% received 2 doses, and 9.4% received 3 doses.

Figure 2 shows the participants outcome status at discharge from the study. There were 93 (87.7%) participants alive and 13 (12.3%) died, and this proportion difference was significant (P<0.001).

4.2 Laboratory results and clinical features

Figure 3 shows the CSF appearance, about 52% of the patients had clear CSF and about 9% was blood stained.

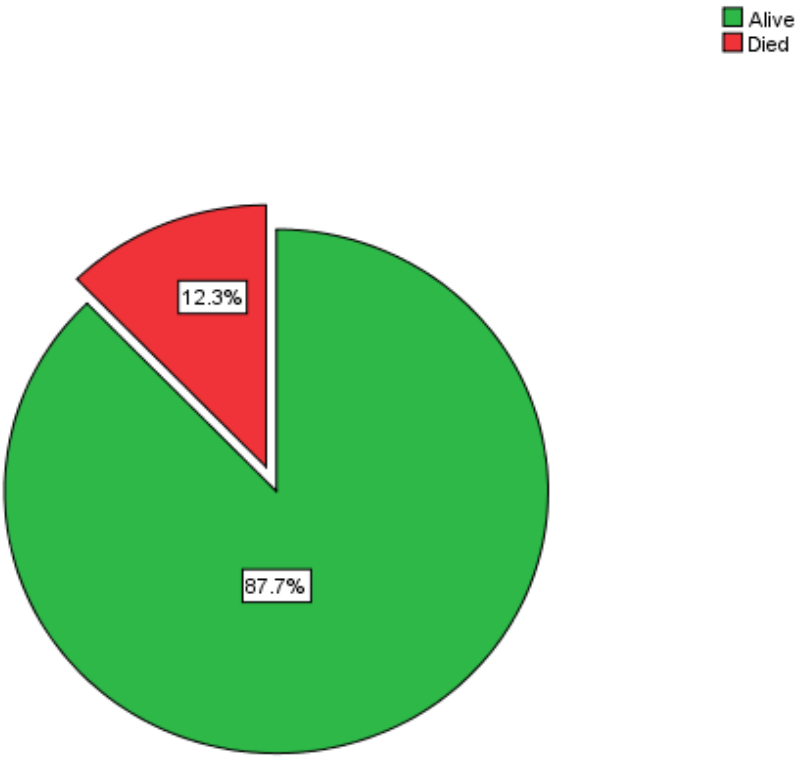

Figure 2. Participants' outcome pie chart

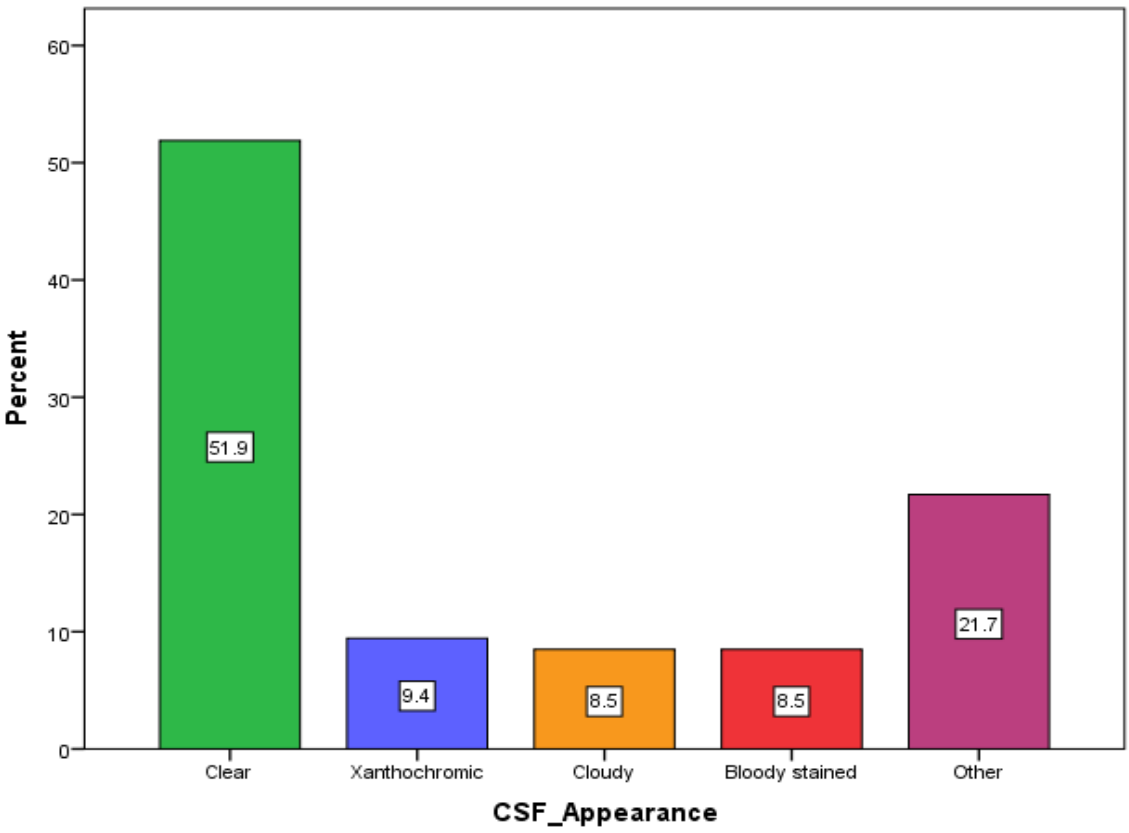

Figure 3. CSF Appearance bar chart

The WBC was skewed to the right of the median 0 cells/mm^3 (IQR = 11). Protein CSF was also skewed to the right with median 0.37 g/l (IQR = 0.55) [Table 4].

Table 4. Participants' laboratory characteristics

Variable	Frequency	Percent
CSF Appearance		
Clear	55	51.9
Xanthochromic	10	9.4
Cloudy	9	8.5
Bloody stained	9	8.5
Other	23	21.7
Viral organisms		
HSV1	1	0.9
EBV	11	10.4
PV B19	3	2.8
HHV6	3	2.8
HHV7	3	2.8
CMV	3	2.8
AV	2	1.9
Age		
(mean, SD)	15.7 (15.43)	
Duration of symptoms		
Median (mean, SD)	3 (4.8, 5.22)	
WBC		
(mean, SD)	0.0 (11)	
Protein CSF		
(mean, SD)	0.37 (0.55)	

There was only one (0.9%) participant with *Haemophilus influenzae* bacteria detected by both microscopy and PCR. Two (1.9%) participants had *Neisseria meningitidis* while 5 (4.7%) had *Streptococcus pneumoniae* detected only by PCR.

The most common viral agent detected by PCR was EBV (10%). Others were Parvovirus, Human herpes virus type 6, Human herpes virus type 7 and CMV at 2.8% each [Figure 4].

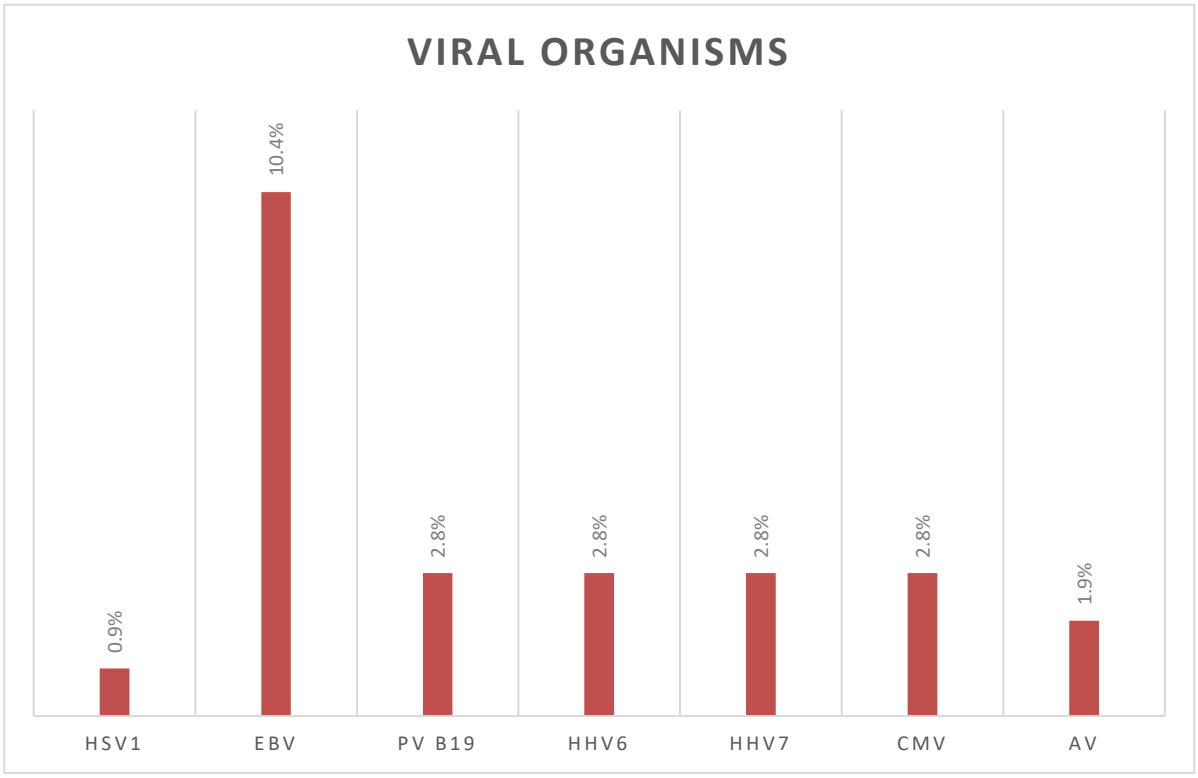

Figure 4. Viral organisms' distribution

Bacterial and viral co-infections were established in 3.8% (4/106) of the participants with meningitis and 0.9% (1/106) with encephalitis. Due to the small number of organisms detected, analysis of the significance of these associations could not be done.

Viral and bacterial agents' detection per case definitions are as shown in figures 5 and 6, respectively. The distribution of organisms between encephalitis and meningitis was similar for both bacteria and viruses.

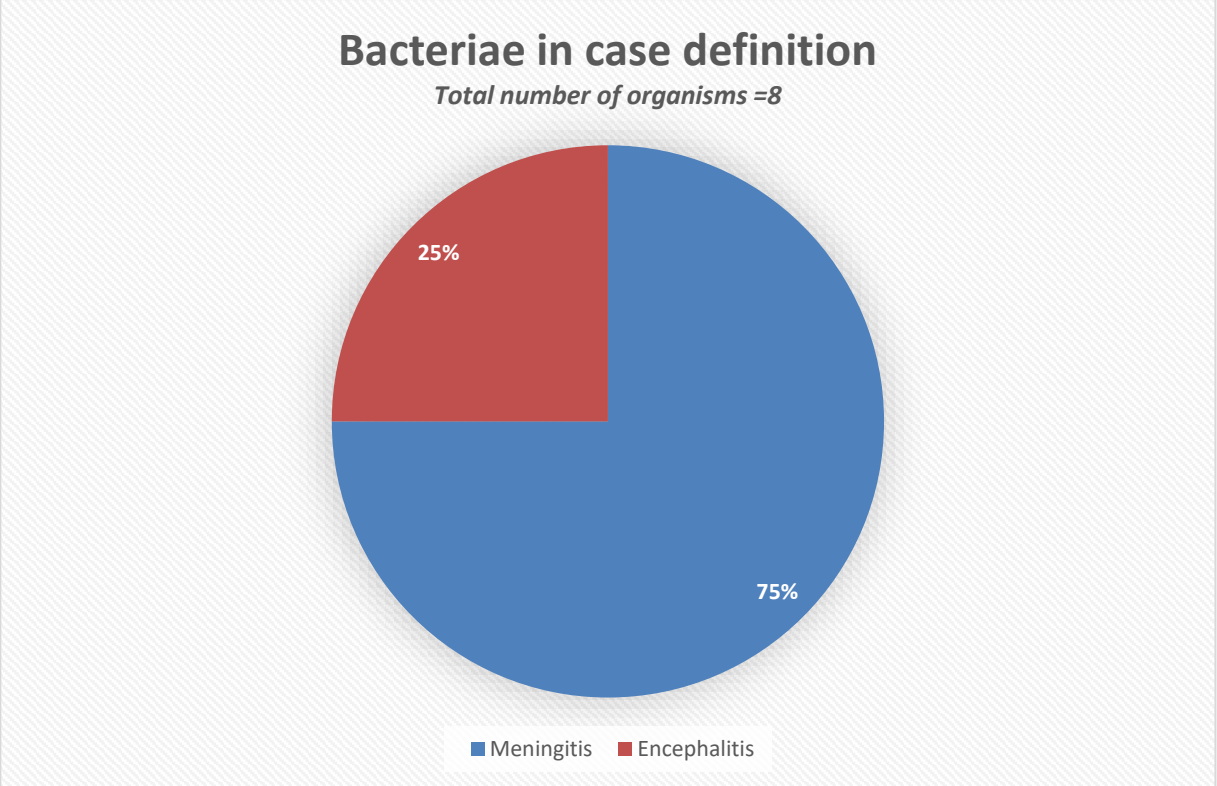

Figure 5. Bacterial organisms' association with case definition

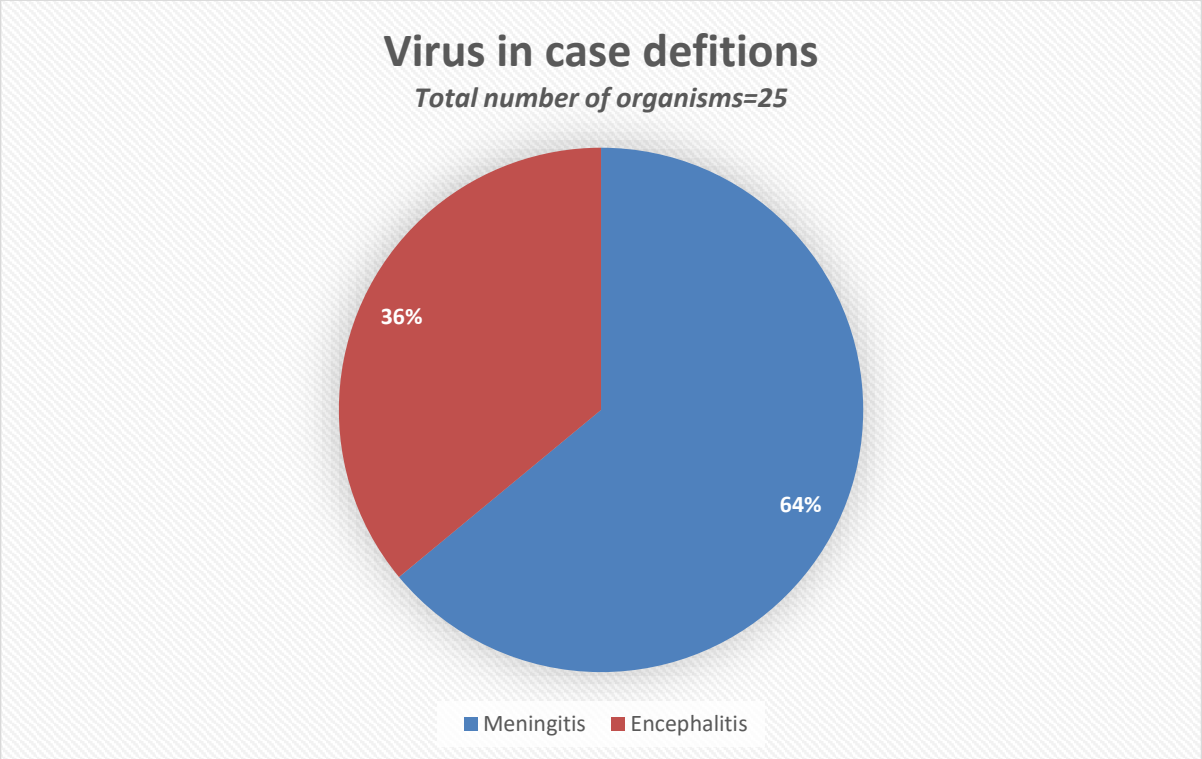

Figure 6. Viral organisms' association with case definition

Almost 80% of the participants had received PCV, DPT-Hep-B-Hib and OPV [Table 3]. There was no association between a case definition and participants' vaccination status [Table 5].

Table 5. Bivariate analysis for case definition association (Clinical features and outcome)

Variable	Encephalitis		Meningitis		P-value
	n	%	n	%	
Sex					
Male	14	56.0%	35	43.2%	0.26
Female	11	44.0%	46	56.8%	
Seizures					
Yes	19	86.4%	38	67.9%	0.1
No	3	13.6%	18	32.1%	
Fever					
Yes	24	96.0%	75	97.4%	0.99
No	1	4.0%	2	2.6%	
Altered consciousness					
Yes	8	40.0%	18	24.7%	0.18
No	12	60.0%	55	75.3%	
Neck Stiffness					
Yes	6	25.0%	22	28.2%	0.76
No	18	75.0%	56	71.8%	
DPT-Hep B-Hib vaccine					
Yes	22	95.7%	67	89.3%	0.68
No	1	4.3%	8	10.7%	
PCV					
Yes	20	83.3%	64	88.9%	0.49
No	4	16.7%	8	11.1%	
Rota vaccine					
Yes	23	92.0%	71	97.3%	0.27
No	2	8.0%	2	2.7%	
Patient outcome at discharge					
Alive	19	76.0%	74	91.4%	0.07
Died	6	24.0%	7	8.6%	

4.3 Bivariate Analysis

Table 6 shows bivariate analysis for case definition association. At 5% significance level, only the WBC was significantly associated with the case definition (P=0.01).

Table 6. Bivariate analysis for case definition association (Laboratory findings)

Variable	Encephalitis		Meningitis		P-value
	n	%	n	%	
CSF Appearance					
Clear	16	64.0%	39	48.1%	0.59
Xanthochromic	1	4.0%	9	11.1%	
Cloudy	1	4.0%	8	9.9%	
Bloody stained	1	4.0%	8	9.9%	
Other	6	24.0%	17	21.0%	
EBV					
No	23	92.0%	72	88.9%	0.99
Yes	2	8.0%	9	11.1%	
HSV1					
No	25	100.0%	80	98.8%	0.99
Yes	0	0.0%	1	1.2%	
Parechovirus					
No	25	100.0%	78	96.3%	0.99
Yes	0	0.0%	3	3.7%	
HHV6					
No	24	96.0%	79	97.5%	0.56
Yes	1	4.0%	2	2.5%	
HHV7					
No	23	92.0%	80	98.8%	0.14
Yes	2	8.0%	1	1.2%	
CMV					
No	24	96.0%	79	97.5%	0.56
Yes	1	4.0%	2	2.5%	
Age					
(mean, SD)	19.8 (20.24)		14.4 (13.52)		0.22
WBC					
(mean, SD)	3.9 (14.88)		91.5 (303.42)		0.01
Protein CSF					
(mean, SD)	0.85 (0.87)		0.70 (1.09)		0.67

4.4 Logistic regression

Logistic regression analysis results predicting meningitis are shown in Table 7 below. Patients with seizures had an average of 86% reduced odds for meningitis case definition (OR = 0.14, CI = 0.02 – 1.13, P-value = 0.065). Removing the constant from the prediction model, the outcome remained the only important variable. Patients that were alive at the point of discharge had on average 3.6 times increased odds for meningitis case definition (OR = 3.6, CI = 1.96 – 6.68, P-value <0.001) [Table 7b].

Table 7a. Logistic regression predicting meningitis case definition

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age_months	-.026	.021	1.472	1	.225	.975	.935	1.016
	Sex(1)	-.467	.670	.486	1	.486	.627	.168	2.332
	Seizures(1)	-1.863	1.163	2.566	1	.109	.155	.016	1.517
	AlteredConsciouness(1)	.012	.677	.000	1	.986	1.012	.268	3.813
	Outcome(1)	.551	1.343	.168	1	.682	1.735	.125	24.146
	WBCcellsmm3	.012	.017	.461	1	.497	1.012	.978	1.046
	Constant	2.647	1.651	2.571	1	.109	14.113		
Step 6[a]	Seizures(1)	-1.990	1.079	3.402	1	.065	.137	.017	1.133
	Constant	2.890	1.027	7.915	1	.005	18.000		

a. Variable(s) entered on step 1: Age_months, Sex, Seizures, AlteredConsciouness, Outcome, WBCcellsmm3.

Table 7b. Logistic regression predicting meningitis case definition

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age_months	-.015	.020	.544	1	.461	.985	.947	1.025
	Sex(1)	-.450	.671	.449	1	.503	.638	.171	2.377
	Seizures(1)	-.991	.865	1.313	1	.252	.371	.068	2.022
	AlteredConsciouness(1)	.232	.653	.127	1	.722	1.262	.351	4.535
	Outcome(1)	2.140	.859	6.212	1	.013	8.501	1.580	45.748
	WBCcellsmm3	.020	.023	.720	1	.396	1.020	.974	1.068
Step 6[a]	Outcome(1)	1.285	.313	16.820	1	.000	3.615	1.956	6.682

a. Variable(s) entered on step 1: Age_months, Sex, Seizures, AlteredConsciouness, Outcome, WBCcellsmm3.

4.5 Antibiotic sensitivity of the bacterial isolates

This could not be done on the bacterial isolates as only one sample of the 106 had an organism (*Haemophilus influenza*) detected by culture. In addition, there was no antibiotic cartridges for sensitivity tests to be done.

CHAPTER FIVE: DISCUSSION

5.1 Aetiology of pyogenic meningitis, and viral encephalitis and/or meningitis in children aged 1-59 months

This study has demonstrated that bacterial pathogens have significantly reduced at the Children's Hospital in Lusaka. The bacterial pathogens were only established in 7.5% of the participants. There was one (0.9%; 1/106 participants) participant with *Haemophilus influenzae* bacteria detected by both microscopy and PCR. Two (1.9%; 2/106 patients) participants had *Neisseria meningitidis* while 5 (4.7%; 5/106 patients) had *Streptococcus pneumoniae* detected only by PCR.

Kankasa (1997)[8], in a study at UTH, found 59% bacterial causes of meningitis in the children enrolled into the study. A study done by Kabamba (2002)[7] showed that *Streptococcus pneumoniae* accounted for 61% of the CSF isolates in patients with meningitis. The other causes included *Haemophilus influenzae* (19%), *Neisseria meningitidis* (9.8%) and salmonella spp. (4.9%).[7] Prior to the foregoing, a study done in 1975 had shown *Neisseria meningitidis* and pneumococcus as the commonest causes of meningitis.[39] These studies show that, though the prevalence of the causative agent has reduced in our study, the causative organisms have remained the same. This is most likely due to the introduction of Hib and PCV in the EPI program.[20,26]

Studies done in Mozambique[45] and Papua New Guinea (East Africa)[44] showed *Streptococcus pneumoniae* as a common cause of meningitis. This is a fact even though, arguably, the incidences have significantly reduced since the introduction of vaccines and modernisation of the health care system over the last decades. Furthermore, current low prevalence of bacterial causes of meningitis and encephalitis (59% in 1997[8] to the current 7.5%) could be attributed to the same.

The ability of vaccines to avert disease can be seen in the WHO publication[20] that showed that despite the low coverage of PCV, by 2014, the vaccine was able to significantly reduce disease and deaths from *Streptococcus pneumoniae*. It is recommended that further studies be done to verify this association in children presenting at the Children's Hospital in Lusaka, Zambia.

With PCR technique, seven (7) more organisms including the one isolated by culture alone were identified. It has been found that where antibiotics had been given prior to CSF examination, Gram stains or cultures may be negative. In such situations, PCR may be preferred to identify the causative organisms.[11,28,32,49] In Zambia, Integrated Management of Childhood Illnesses (IMCI) is widely implemented and any child who presents to a health facility with suspected meningitis receives a parenteral dose of antibiotics before being referred. This could have contributed to the negative CSF culture results.

Viral aetiological agents were established in 26.4% (28/106 patients) of the cases. The most common virus detected in the cases with encephalitis and meningitis, was EBV (10%). This was similar to the study in the USA[36] which showed that about 25% of the admissions were due to viral aetiology. However, viruses accounted for a higher proportion of cases in a study done in Vietnam (41%).[34] The study done in Vietnam[34] had cases aged between 0-16 years. There have been no studies done in sub-Saharan Africa analysing causes of viral encephalitis in CSF.

In the current study, the case in which *Haemophilus influenzae* was detected by both PCR and culture had a clinical diagnosis of encephalitis.

Bivariate analysis to determine whether a viral cause was associated with either a case of encephalitis or meningitis did not show statistical significance for either clinical diagnosis.

The study highlighted the challenge of establishing the aetiology of meningitis and encephalitis. Despite the use of molecular methods in this study, the likely aetiology was not established in the over 70% of the cases. Furthermore, the significant decrease in the bacterial causes of CNS infections such as meningitis and encephalitis were also noted.

5.2 The biochemical and cellular CSF changes in children presenting with pyogenic meningitis and viral encephalitis

There is a documented association of clear CSF with viral meningitis and/or encephalitis. CSF is more commonly turbid in ABM than in viral CNS infections. It is thought that turbidity of the CSF is to a large extent due to CSF leucocyte counts exceeding 200-400 cells/mm^3. [11,28] In bacterial CNS infections, CSF WBCs is usually

raised with a neutrophilic predominance.[28] Bivariate analysis did not suggest a correlation between CSF appearance and case definition of either encephalitis or meningitis [Table 6].

At 5% significance level, only the increase in WBC count was significantly associated with a case definition of meningitis (P=0.01). The opposite was true for encephalitis (mean=3.9 vs 91.5). Further association with lymphocytosis or polymorphs could not be done as most of the CSF studies didn't have differential counts.

A raised CSF protein was not significantly associated with the occurrence of meningitis or encephalitis (mean, SD; 0.85,0.87 vs 0.70,1.09. P=0.67).

In viral meningitis and meningoencephalitis, it is rare for the CSF protein content to be above 200mg/dl.[9,11,13,27] This is not consistent with this study findings that an elevated CSF protein level was not significantly associated with the case definition of viral meningitis or encephalitis [Table 6].

Other biochemical changes such as CSF chloride and glucose levels could not be evaluated as more than 80% of the study patients did not have these tests done on the CSF samples due to unavailability of reagents to do such test.

5.3 The clinical features associated with the common aetiology of viral encephalitis and pyogenic meningitis

Among the 106 patients with meningitis or encephalitis, 93.4% presented with fever, 53.8% had seizures, 36.8% had inability to drink, 26.4% had difficulties in breathing, 26.4% had neck stiffness and 18.9% had prostration as the commonest clinical symptoms at presentation. This is consistent with the finding of Kankasa[8] in 1997 (age range one month to 15 years) at the UTH Department of paediatrics that fever, neck stiffness and anorexia were the commonest findings in patients with meningitis at 99.9%, 96.9% and 88.5% respectively. A subsequent study by Kabamba in 2002 (age range 1-59 months), in the same department, had a similar finding.[7] Therefore, it is important to take note of the findings especially in the management of these disease entities in a clinical setting or in making local treatment guidelines.

Patients with seizures had an average of 86% reduced odds for meningitis case definition [OR = 0.14, CI = 0.02 – 1.13, P-value = 0.065]. When the constant was removed from the prediction model, the outcome remained the only important

variable [Table 5]. Therefore, the presence of seizure may increase the likelihood of a diagnosis of encephalitis than meningitis.

Although the occurrence of seizures remains a common finding in most CNS infections[3,9,11,34,36], this finding could be significant in the distinction of encephalitis and meningitis in the clinical setting.

5.4 Other findings of the study

Patients that were alive at point of discharge from the study had on average 3.6 times increased odds for meningitis case definition (OR = 3.6, CI = 1.96 – 6.68, P-value <0.001) [Table 5]. The prognosis of meningitis and/or encephalitis is known to vary with causative organism, the severity of the clinical illness, and the age of the child.[3,9,11,34]

Severe sequelae were found in HSV infection.[3,11,34] However, CNS infection with enteroviruses may have subtle illness with spontaneous recovery in some patients.[11,40] Notably, in CNS HSV infection without treatment, mortality rates may be as high as 70%.[9,34] However, in this study, there was only one case of HSV infection [Table 6]. In addition, further studies may be required to establish why mortality was higher in the patients with encephalitis than in those with meningitis.

In this study, EBV is the most prevalent viral agent at 10% and there is no specific antiviral agent compared to pyogenic meningitis where there are effective antibiotics. Furthermore, HSV responds to Acyclovir which is readily available. HSV was detected at a low percentage of 0.9% [Table 4].

Bivariate analysis for case definition association occurrence and DPT-HepB-Hib and Pneumococcal vaccination was statistically not significant. Vaccines are documented to significantly reduce disease and deaths in vaccine preventable diseases.[20] The finding in this study could be possible due to the low bacterial organisms compared to the viral agents detected (7.5% vs 26.4%). DPT-HepB-Hib and Pneumococcal vaccines target Hib and *Streptococcal pneumoniae* respectively.

CHAPTER SIX

6.1 Conclusions

Viral infections of the CNS are the commonest causes of both encephalitis and meningitis in children aged 1-59 months at the Children's Hospital in Lusaka, Zambia.

The causative agents identified, and clinical features, were not associated with a diagnosis of encephalitis or meningitis.

A raised WBC was significantly associated with meningitis.

Despite adequate treatment, patients with encephalitis were less likely to be alive at discharge.

The real-time (Multiplex) PCR significantly increased the detection of microbial pathogens than in previous studies where PCR was not used.

6.2 Recommendations

Following this study, the following recommendations are hereby made:

I. Further studies be conducted with broader diagnostic assays to establish the likely aetiology in the over 70% of the patients without a known infectious cause.
II. Molecular detection by PCR should be provided for the diagnosis of meningoencephalitis in children presenting to the hospital.
III. A surveillance study over a longer duration with CSF bacteriological, biochemical and Real-Time PCR studies should be conducted to evaluate the commonest organisms in children with encephalitis and meningitis in Zambia.
IV. Clinicians should collect blood and CSF for culture and PCR in every child presenting with suspected CNS infection who are likely to have had antibiotics prior to LP.

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APPENDICES

Appendix 1: Participant information sheet for parents or guardians

TITLE OF STUDY:
Aetiology of encephalitis and meningitis in children aged 1-59 months at the Children's Hospital, Lusaka, Zambia

Introduction

My name is Dr Akakambama Imamba, a postgraduate student at the University of Zambia, School of Medicine. I thank you for your willingness to know more about this study.

This information sheet explains why i intend to conduct this study, what your participation would involve, what the benefits and risks to your child might be, and what would happen after the study ends. I will give a general explanation and will answer any questions you may have after you have read this information sheet.

Your child is invited to participate in the study because s/he is being treated for an infection of the brain and/or spinal cord which doctors call meningitis, encephalitis or meningoencephalitis. The information you will share with the research team will help the clinicians at the University Teaching Hospital, Department of Paediatric and Child Health improve the care of patients in the future as this study may tell them the commonest organisms which cause the disease.

Should you wish to withdraw from the study at any time during the participation of your child, you are free to do so and your child will continue to receive the same standard medical care available in the hospital.

Purpose of the study

The purpose of the study is to identify the organisms that cause infections of the brain (doctors call this infection encephalitis and/or meningitis) in children admitted at the University Teaching Hospital, Department of Paediatrics and Child Health, in Lusaka.

Risks or discomfort

There are very minimal risks to participating in the study. The questions which you will be asked are not personally intrusive or embarrassing. When you accept to participate, the i will collect the cerebrospinal fluid of your child collected by a clinician, where it was clinically indicated and for which you would have consented to as part of the investigations of your child's illness. In addition, the study will not involve any procedures that may alter the disease outcome, cause physical pain or harm, and all patients will continue to be monitored closely while on treatment in accordance with the hospital protocols applicable to all patients with meningitis and/or encephalitis.

Confidentiality

No personal identifiers will be put on the data collection tools that will be used to collect information from the participants during or after the study. We will also ensure that all research data and tools are kept under lock and key with access only to the researcher and his assistant.

Together we will hold a shared confidentiality regarding the information that will be collected; neither you nor the study team may share what we will discuss with other persons, as doing so will be a breach of confidentiality.

Benefits of participating in Study

The study team will slightly hasten the time required for the results of the cerebrospinal fluid collected to be made available to the clinicians. In addition, there will be some additional test which the research will provide to detect the organisms which cause the disease. Furthermore, the information collected from this study will be used to generate results that will be used to better manage similar disease in patients in the future.

This study is being conducted in partial fulfillment of the award of the degree of Master of Medicine in Paediatrics and Child Health of the University of Zambia, School of Medicine.

Who to contact for clarifications about this research

If you have any questions, concerns or complaints about this study, at any stage, please contact:

1. **The Chairperson**,
   The ERES Converge IRB
   33 Joseph Mwilwa Road
   Rhodes Park, Lusaka.
   Telephone +260-955155633/4
   Email: eresconverge@yahoo.co.uk
   IRB No. 00005948
   FWA No. 00011697

   Or

2. **Researcher**
   Akakambama Imamba
   University of Zambia, School of Medicine
   Ridge Way Campus
   P.O. Box 50110
   Lusaka.
   Email: akaimamba@gmail.com or akaimamba@hotmail.com
   Mobile Phone No. +260 969491828/+260977488710

Appendix 2: Informed consent form

TITLE OF STUDY: **Aetiology of encephalitis and meningitis in children aged 1-59 months at the Children's Hospital, Lusaka, Zambia**

Tick in the space or thumb print (if you are unable to write) on the space provided.

I the undersigned acknowledge that I have read the participant information sheet, or it has been read to me. An opportunity was accorded to me to ask questions regarding this study and they were answered to my satisfaction.	
I also understand that participation is voluntary.	
I have also been informed that my child will continue to receive the standard care available in the hospital should I wish to withdraw my participation at any time in the study.	
I therefore voluntarily consent to allow my child to participate in this study	

Parent or guardian's signature.

Print name	Sign or thumb print	Date (dd/mm/yy)

Witness

Print name	Sign	Date (dd/mm/yy)

Person administering this consent:

I hereby acknowledge that I have given a verbal explanation of the study to the participant and I have answered the participant's questions about the study to the best of my ability and knowledge.

Print name	Sign	Date (dd/mm/yy)

NOTE: *This informed consent form should be signed and dated in duplicate. One copy shall remain with the participants' guardian or parent and the other shall remain with the research team.*

For more information, contact:

1. The ERES Converge IRB
   33 Joseph Mwilwa Road
   Rhodes Park
   Lusaka
   Telephone +260-955155633/4
   Email: eresconverge@yahoo.co.uk
   IRB No. 00005948
   FWA No. 00011697

   Or

2. **Researcher**
   Akakambama Imamba
   University of Zambia, School of Medicine
   Ridge Way Campus
   P.O. Box 50110
   Lusaka.
   Email: akaimamba@gmail.com or akaimamba@hotmail.com
   Mobile Phone No. +260 969491828/+260977488710
   Tel: +260 211 293553/293698

Appendix 3: Clinical case record form

CLINICAL CASE RECORD FORM

1. DEMOGRAPHIC INFORMATION

Patient I.D: _____ Medical Record No: _____

Date of Admission: _____/_____/_____ Sex: ☐ Male ☐ Female

Participant's D.O.B: ____/_____/_____ Age**:** _____ (in Months)

Race of Participant: ☐ Asian ☐ Black ☐ White ☐Unknown ☐ others

If others, specify: _____

Residential Address: _____

2. ADMISSION INFORMATION

Suspected case meets the case definition of encephalitis or meningitis? ☐ Yes ☐ No

If yes, state case definition: ☐ Encephalitis ☐ Meningitis ☐ Other

If other, specify: _____

Admission diagnosis:

a. Suspected pneumonia ☐Yes ☐No
b. Suspected meningitis ☐Yes ☐No
c. Suspected encephalitis/meningoencephalitis ☐Yes ☐No
d. Suspected septicaemia ☐Yes ☐No
e. Other ☐Yes ☐No

Specify: _____

3. CLINICAL INFORMATION

Date of onset of symptoms:

Duration of symptoms: ____ ☐ Years ☐ Months ☐ Weeks ☐ Hours

Symptoms related to current illness (mark x on all applicable):

a.	Seizures /Convulsions	☐ Yes ☐ No ☐ Unknown
b.	Unable to feed or drink:	☐ Yes ☐ No ☐ Unknown
c.	Fever:	☐ Yes ☐ No ☐ Unknown

d.	Prostration/lethargy:	☐ Yes ☐ No ☐ Unknown
e.	Petechial/purpuric rush	☐ Yes ☐ No ☐ Unknown
f.	Difficulties in breathing:	☐ Yes ☐ No ☐ Unknown
g.	Stridor:	☐ Yes ☐ No ☐ Unknown
h.	Altered consciousness:	☐ Yes ☐ No ☐ Unknown
i.	Bulging fontanelle:	☐ Yes ☐ No ☐ Unknown
j.	Neck stiffness:	☐ Yes ☐ No ☐ Unknown
k.	Cough:	☐ Yes ☐ No ☐ Unknown
l.	Dehydration:	☐ Yes ☐ No ☐ Unknown
m.	Chest indrawing:	☐ Yes ☐ No ☐ Unknown
n.	Fast breathing:	☐ Yes ☐ No ☐ Unknown
o.	Others	☐ Yes ☐ No ☐ Unknown

Others specify: _____

Were any antimicrobial drugs given prior to admission? ☐ Yes ☐ No ☐ Unknown

If yes, please specify: _____

4. CSF COLLECTION

CSF collected? ☐ Yes ☐ No

If yes, state date: ____/_____/_____ Time: _____hr/mm

If not collected, state reasons: _____

5. VACCINATION HISTORY

a. DPT-Hep B-Hib vaccine status: ☐ Vaccinated ☐ Not Vaccinated

Has patient received routine vaccinations? ☐ Yes ☐ No ☐ Unknown
(If yes record the # and dates of doses)

Number of doses: ☐ One (1) ☐ Two (2) ☐ Three (3)

Date for Dose #1: ___/___/_____

Date for Dose #2: ___/___/_____

Date for Dose #3:___/___/_____

Source of information:

☐ U-5 Card ☐ Medical Records ☐ Verbal ☐ Unknown

b. Oral Polio Vaccine (or IPV) status: ☐ Vaccinated ☐ Not Vaccinated

Number of doses: ☐ One (1) ☐ Two (2) ☐ Three (3)

Date for Dose #1: ___/___/_____

Date for Dose #2: ___/___/_____

Date for Dose #3: ___/___/_____

Source of information:

☐ U-5 Card ☐ Medical Records ☐ Verbal ☐ Unknown

c. Pneumococcal vaccine status: ☐ Vaccinated ☐ Not Vaccinated

Number of doses: ☐ One (1) ☐ Two (2) ☐ Three (3)

Date for Dose #1: ___/___/_____

Date for Dose #2: ___/___/_____

Date for Dose #3: ___/___/_____

Source of information:

☐ U-5 Card ☐ Medical Records ☐ Verbal ☐ Unknown

d. Rota vaccine status: ☐ Vaccinated ☐ Not Vaccinated

Number of doses: ☐ One (1) ☐ Two (2) ☐ Three (3)

Date for Dose #1: ___/___/_____

Date for Dose #2: ___/___/_____

Date for Dose #3: ___/___/_____

Source of information:

☐ U-5 Card ☐ Medical Records ☐ Verbal ☐ Unknown

e. Bacillus Calmette-Guérin (BCG) vaccine status:
☐ Vaccinated ☐ Not Vaccinated

Number of doses: ☐ One (1) ☐ Two (2) ☐ Three (3)

Date for Dose #1: ___/___/_____

Date for Dose #2: ___/___/_____

Date for Dose #3: ___/___/_____

Source of information:

☐ U-5 Card ☐ Medical Records ☐ Verbal ☐ Unknown

6. PATIENT OUTCOME

Date of discharge (or transfer or death): _____/____/____

Outcome at discharge

Alive: ☐ Yes ☐ No ☐ Unknown

Died: ☐ Yes ☐ No ☐ Unknown

Transferred: ☐ Yes ☐ No ☐ Unknown

LAMA: ☐ Yes ☐ No ☐ Unknown

Pending discharge: ☐ Yes ☐ No ☐ Unknown

7. LABORATORY RESULTS

Specimen ID: _____

Date CSF received in the Lab: _____ Time: _____hr/mm

CSF processed: ☐ Yes ☐ No ☐ Unknown

If No, state reasons: _____

CSF appearance (select one)

Clear: ☐ Yes ☐ No ☐ Unknown
Xanthochromic: ☐ Yes ☐ No ☐ Unknown
Cloudy: ☐ Yes ☐ No ☐ Unknown
Bloody stained: ☐ Yes ☐ No ☐ Unknown
Other, specify: ☐ Yes ☐ No ☐ Unknown

_____

CSF cell count

WBC (cells/mm^3): _____ Differential count: _____

CSF Biochemistry

Protein (g/l): _____Glucose (mmol/l): _____Chloride: _____

Gram stain done? ☐ Yes ☐ No ☐ Unknown

Gram stain organism seen

Gram neg diplococci: ☐ Yes ☐ No ☐ Unknown
Gram neg coccobacilli: ☐ Yes ☐ No ☐ Unknown
Gram neg rods: ☐ Yes ☐ No ☐ Unknown
Gram pos cocci pairs: ☐ Yes ☐ No ☐ Unknown
Gram pos cocci cluster: ☐ Yes ☐ No ☐ Unknown

Others specify: _____

Gram stain result

Gram positive: ☐ Yes ☐ No ☐ Unknown
Gram negative: ☐ Yes ☐ No ☐ Unknown
No organisms seen: ☐ Yes ☐ No ☐ Unknown

Indian ink
Cryptococci organism seen ☐ Yes ☐ No ☐ Unknown

AFB seen? ☐ Yes ☐ No ☐ Unknown

Culture done? ☐ Yes ☐ No ☐ Unknown

CSF culture results *(Tick all applicable)*

H. influenza: ☐ Yes ☐ No ☐ Unknown
S. pneumoniae: ☐ Yes ☐ No ☐ Unknown
N. meningitidis: ☐ Yes ☐ No ☐ Unknown
Other organism: ☐ Yes ☐ No ☐ Unknown

Negative/no growth: ☐ Yes ☐ No ☐ Unknown
Culture not done: ☐ Yes ☐ No ☐ Unknown
Inconclusive: ☐ Yes ☐ No ☐ Unknown
If other, ☐ Yes ☐ No ☐ Unknown
Please specify: _____

Antibiotic susceptibility of the isolates

Organism	AMP	CRO	CHL	PEN	IMP	Other

AMP-Ampicillin, CRO-ceftriaxone, CHL-Chloramphenicol, PEN-Penicillin, IMP-imipenem

Real-time PCR done? ☐ Yes ☐ No ☐ Unknown

Real-time PCR results *(Tick all applicable)*

Bacterial organisms

H. influenzae:	☐ Yes ☐ No ☐ Unknown
S. pneumoniae:	☐ Yes ☐ No ☐ Unknown
N. meningitidis:	☐ Yes ☐ No ☐ Unknown
M. tuberculosis:	☐ Yes ☐ No ☐ Unknown
Salmonella spp.:	☐ Yes ☐ No ☐ Unknown
E. coli:	☐ Yes ☐ No ☐ Unknown
Negative:	☐ Yes ☐ No ☐ Unknown
Other:	☐ Yes ☐ No ☐ Unknown

If other, specify: _____

Viral organisms

HSV1:	☐ Yes ☐ No ☐ Unknown
HSV2:	☐ Yes ☐ No ☐ Unknown
Mumps virus:	☐ Yes ☐ No ☐ Unknown
VZV:	☐ Yes ☐ No ☐ Unknown
EBV:	☐ Yes ☐ No ☐ Unknown
Par echovirus:	☐ Yes ☐ No ☐ Unknown
Enterovirus:	☐ Yes ☐ No ☐ Unknown
Negative:	☐ Yes ☐ No ☐ Unknown
Other:	☐ Yes ☐ No ☐ Unknown

If other, specify: _____

-End of form-