UNIVERSITY OF RWANDA

INVESTIGATION OF EARLY LIFE EXPOSURE TO INFECTIOUS PATHOGENS AND THEIR ASSOCIATION WITH MICROCEPHALY IN RWANDAN CHILDREN

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INVESTIGATION OF EARLY LIFE EXPOSURE TO INFECTIOUS PATHOGENS AND THEIR ASSOCIATION WITH MICROCEPHALY IN RWANDAN CHILDREN

By

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DECLARATION

I, Marie Viviane AKIMANA, hereby declare that this dissertation, entitled **"Investigation of early life exposure to infectious pathogens and their association with microcephaly in Rwandan children"** is the result of my work and has not been submitted for any other degree at the University of Rwanda or any other institution.

Marie Viviane AKIMANA

Signed: 

Date: The 31st July 2025

DEDICATION

I dedicate this work to God Almighty, who has been my source of strength, wisdom, and perseverance. To my husband, Eric IZERIMANA for his love and support through it all. To my children IZERIMANA Ian Ervin and IZERIMANA Mariella Elvie for their love, patience, and motivation. To my mother, whose love, prayers, and sacrifices made this achievement possible, to my siblings for their love and support, and to my late father, whose belief in the education power continues to inspire and motivate me.

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Marie Viviane AKIMANA

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LIST OF ABBREVIATIONS

CDC: Centers for Disease Control and Prevention.

CHEU: Children exposed to HIV and uninfected.

CHUU: Children unexposed to HIV and Uninfected.

CMV: Cytomegalovirus.

CNS: Central Nervous System.

CRS: Congenital Rubella Syndrome.

CZS: Congenital Zika syndrome.

DNA: Deoxyribonucleic acid.

EDTA: Ethylenediamine tetraacetic acid.

FFPE: Formalin Fixed Paraffin-Embedded Tissue

HCs: Hofbauer cells

IgM: Immunoglobulin M

IgG: Immunoglobulin G

PPE: Personal Protective Equipment.

PCR: Polymerase Chain Reaction.

MTCT: Mother-to-child transmission.

Nabs: Neutralizing Antibodies.

NPCs: Neural Progenitor cells.

NDDs: Neurodevelopmental Disorders.

RNA: Ribonucleic acid

RUV: Rubella Virus.

RT-PCR: Reverse transcriptase polymerase chain reaction.

ZIKV : Zika Virus.

HIV: Human Immunodeficiency Virus.

WHO: World Health Organization

ABSTRACT

Background: Microcephaly is a clinical condition characterized by a head circumference more than two standard deviations below the mean for an individual's age and sex. This condition can result from various risk factors, including exposure to infectious pathogens. However, there is a paucity of data describing the association between early-life exposure to infectious pathogens and microcephaly among Rwandan children.

Aim: This study aimed to investigate early-life exposure to infectious pathogens in Rwandan children and evaluate their potential association with microcephaly.

Method: This cross-sectional case-control study included 120 participants (30 children with microcephaly and their mothers as case and 30 age-matched healthy children and their mothers as controls). The study was conducted from November 2023 to July 2025 at the University Teaching Hospital of Kigali. IgM and IgG antibodies against *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV), Herpes simplex virus, Zika virus, and Human Immunodeficiency Virus (HIV) were tested using rapid diagnostic tests. Data were analyzed using SPSS version 28, with statistical significance set at $p < 0.05$.

Results: Children with microcephaly had significantly smaller head circumferences (P = 0.001), lower birth weights (P = 0.026), and shorter heights (P = 0.003) than the controls. Maternal infection during pregnancy was significantly linked to microcephaly (OR=4.33, 95%CI:1.20–15.61; P=0.02). Serological analysis showed a higher positivity rate of IgG for Rubella among children with microcephaly (22.5%) than controls (13.3%) (OR=7.875; 95% CI: 1.958–31.675; P=0.004), and CMV IgG 7.5% in case vs 0% in controls, (OR=27, 95% CI 1.5-488; P=0.02). Similarly, 40% of case mothers tested positive for Rubella IgG versus 8.3% of controls (Odds Ratio=4.8; 95% CI: 2.1–10.9; P<0.0001), and CMV IgG positivity was 23.3% in case mothers compared to 0% in controls (OR=53.6; 95% CI: 3-957; P=0.007).

Conclusion: This study highlights early life exposure to infectious pathogens, specifically Rubella and CMV, in children with microcephaly in Rwanda, suggesting that infectious disease prevention before conception or during pregnancy is very important in preventing congenital defects.

Key words: Microcephaly, early life exposure, infectious pathogens, congenital infections.

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CHAPTER I: INTRODUCTION

1.1. Background of the study

Microcephaly is a clinical finding characterized by a head circumference more than two standard deviations below the mean for age and sex matched populations, resulting from abnormal brain development that may occur during fetal life or shortly after birth, and associated with long-term consequences, including developmental delays, intellectual disability, epilepsy, vision problems, and motor impairment(Hanzlik E, 2017).

The cause of microcephaly is multifactorial, including genetic abnormalities, toxic exposures, prenatal malnutrition, vascular insults, and infectious causes(Racicot K, 2017). Among the most recognized infectious contributors are pathogens grouped under the TORCH acronym -Toxoplasma *gondii*, other (HIV, Zika virus…), Rubella virus, Cytomegalovirus, and Herpes simplex virus(Hunsperger et al., 2024)(Hanzlik E, 2017). These pathogens are capable of passing through the placenta, which can lead to structural brain damage through direct neural damage, immune-mediated inflammation, and placental insufficiency mechanisms (Neu et al., 2015).

Globally, multiple studies have demonstrated a strong association between congenital infections and microcephaly. A multi-center retrospective analysis in Germany by Devakumar et al. showed that 25 of 403(6.2%) cases of microcephaly with an identified cause were due to maternal infections during pregnancy(Devakumar et al., 2018). In 2020, an association between congenital cytomegalovirus and the prevalence at birth of microcephaly in the United States study by Chelsea et al reported that Congenital cytomegalovirus infection increases the prevalence of microcephaly at birth by over sevenfold(Messinger CJ, Lipsitch M, Bateman BT, He M, Huybrechts KF, MacDonald S, Mogun H, Mott K, 2020).

Despite these findings, data from sub-Saharan Africa, and Rwanda in particular, remain limited. So, understanding early exposure to infectious pathogens among Rwandan children is essential to identify the relationship between such exposures and the development of microcephaly. This knowledge is critical for enhancing antenatal care strategies and designing effective preventive interventions, including early diagnostic follow-up.

In this study, we investigated early-life exposure to infectious pathogens by evaluating serological evidence of TORCH infections, Zika virus, and HIV in both children with or without microcephaly and their mothers.

1.2. Problem statement

The research-based evidence suggests that congenital infections during pregnancy play a pivotal role in the development of microcephaly. As shown by Elisabeth et al., 35–87% of microcephaly cases occurring during their investigation in northeast Brazil were attributable to the Zika virus (Krow-Lucal et al., 2018). Chess et al. reported 32% of microcephaly cases in children with congenital rubella syndrome among 383 microcephaly-suspected cases in sentinel sites in five African countries. In 2024, Gundeslioglu et al. reported 11% of microcephaly cases in eighteen children with congenital toxoplasmosis in Turkey (Gundeslioglu et al., 2024).

Despite Rwanda's burden of infectious diseases among pregnant women, highlighted by Esperance et al. in their study on seroprevalence and risk factors of *Toxoplasma gondii* infection in pregnant women attending antenatal care in Kigali, Rwanda, which reported a 12.2% seroprevalence of *Toxoplasma gondii* among 384 pregnant women(Murebwayire et al., 2017), the exposure to infectious agents and their association to microcephaly development in Rwandan children with microcephaly remains poorly explored and underreported, with a lack of systematic investigation. This gap hinders the development of early, effective clinical management protocols and systematic, targeted preventive strategies for microcephaly in Rwanda.

This study aimed to address this gap by investigating early exposure to infectious pathogens in Rwandan children with microcephaly using serologic diagnostic methods, thereby providing valuable data in understanding the status of early exposure to infectious pathogens of Rwandan children with microcephaly and their association with the development of microcephaly.

1.3 Research Questions

- What is the seroprevalence of *Toxoplasma gondii*, Rubella virus, Cytomegalovirus, Herpes simplex virus, Zika virus, and HIV in Rwandan children with or without microcephaly?
- What is the seroprevalence of *Toxoplasma gondii*, Rubella virus, Cytomegalovirus, Herpes simplex virus, Zika virus, and HIV in mothers of children with or without microcephaly in Rwanda?
- Is the early life exposure to *Toxoplasma gondii*, Rubella virus, Cytomegalovirus, Herpes simplex virus, Zika virus, and HIV pathogens associated with microcephaly development in Rwandan children?

1.4. Objectives

1.4.1. Main objective

To investigate the early life exposure to infectious pathogens and their association with microcephaly in Rwandan children.

1.4.2. Specific Objectives

- To determine the seroprevalence of *Toxoplasma gondii*, Rubella virus, Cytomegalovirus, Herpes simplex virus, Zika virus, and HIV in Rwandan children with or without microcephaly.
- To determine the seroprevalence of *Toxoplasma gondii*, Rubella virus, Cytomegalovirus, Herpes simplex virus, Zika virus, and HIV in mothers of children with or without microcephaly in Rwanda.
- To evaluate the association of early life exposure to infectious pathogens and microcephaly in Rwandan children.

CHAPTER 2. LITERATURE REVIEW

2.1. Introduction

Microcephaly is an abnormally small head, characterized by an occipital-frontal head circumference more than two standard deviations below the mean for a child's age and sex, reflecting the underlying brain development and growth problems during pregnancy or in early postnatal stages. It may lead to neurological complications, including intellectual disability, motor skills difficulties, seizures, as well as various other cognitive and physical impairments (Watemberg N, Silver S, Harel S, 2002)(CDC, 2016b).

Microcephaly has diverse etiologies, classified into genetic and non-genetic causes. Among the non-genetic preventable causes of microcephaly, congenital infections, particularly those categorized under the TORCH group of infections —Toxoplasma gondii, Rubella virus, Cytomegalovirus, and Herpes Simplex virus —along with Zika virus and Human Immunodeficiency virus, which target specifically the nervous system and can cause severe fetal brain damage if maternal infection occurs during early pregnancy, especially in the first and early second trimesters and are of significant public health concern(Neu et al., 2015)(Neu et al., 2015). If these pathogens cross the placental barrier, they may result in severe outcomes, including microcephaly, intrauterine growth restriction, or fetal death(Racicot K, 2017).

The prevalence of microcephaly due to infectious causes varies significantly across developing countries, with the highest rates reported in regions affected by the Zika virus(Recaioglu Kolk, 2023). Exposure to ZIKV during pregnancy lead to microcephaly in 4–6% of cases, with the most severe outcome being Congenital Zika syndrome (CZS), which has a 5–14% risk of developing among fetuses (Merfeld et al., 2017). A matched case-control study by Ticiane et al. found that pregnant women who experienced symptoms associated with Zika virus during pregnancy had tenfold higher odds of delivering newborns with microcephaly compared to asymptomatic women(Henriques et al., 2017). A study by de Araújo et al. in Brazil, reported association between microcephaly and congenital Zika virus(de Araújo et al., 2018). Microcephaly occurs also in about 5%-15% symptomatic infants with congenital toxoplasmosis (Salma et al., 2024) .

HIV infection has also been linked to an increased risk of microcephaly. Evans et al. reported that 11.1% of HIV infected children, compared with 5.4% of HIV unexposed infants in the perinatal period, had microcephaly at birth, with an elevated incidence persisting among those infected in utero and intrapartum during follow-up in Zimbabwe(Evans et al., 2016).

Rubella virus has also been associated with the occurrence of microcephaly, as shown in a study by Masresha et al., where 34% of 119 confirmed congenital rubella syndrome (CRS) cases had microcephaly (Masresha et al., 2018a). A strong correlation exists between microcephaly and congenital cytomegalovirus (cCMV) infection. A large population-based cohort study of over 2 million pregnancies by Messinger et al. (2020) found that cCMV increased the risk of microcephaly at birth by at least 7-fold compared to non-infected infants (Messinger et al., 2020). Additionally, approximately 4% of neonatal herpes simplex virus(HSV) infections result in microcephaly at birth (Ethawi et al., 2023).

Studying the association between early life exposure to infectious pathogens and microcephaly in Rwandan children was critically important due to the burden of infectious diseases. While comprehensive data on microcephaly prevalence specifically in Rwanda is underreported, the known impact of congenital infections such as toxoplasmosis, rubella, cytomegalovirus, Zika virus, and HIV on fetal brain development highlights the need for focused investigations within this context.

2.2. Mechanisms of vertical transmission and fetal brain damage

The placenta acts as a crucial immunological and physical barrier to protect the fetus from maternal infections (Messinger et al., 2020). The placental-fetal interface and the fetal blood-brain barrier creates a bilayer defense against microbial invasion in the fetal environment (Ethawi et al., 2023). However, some pathogens have developed mechanisms to cross this barrier through blood circulation spread or ascending infection from the genital tract, leading to fetal infection(Kumar et al., 2022) . Research-based evidences indicate that TORCH pathogens like CMV and Zika virus replicate within placental tissues, forming a reservoir and elevating the risk of fetal exposure. Neuroprogenitor cells are infected, once these pathogens reach the fetal environment, and trigger immune-mediated inflammation, or cause placental insufficiency, blocking brain development(Megli & Coyne, 2022) .

2.3. Microcephaly infectious causes.

2.3.1. Toxoplasma gondii

Toxoplasma gondii is an obligate intracellular protozoan parasite causing toxoplasmosis, transmitted to humans primarily through ingestion of undercooked or raw meat or contaminated water and food with oocysts shed by cats(J G Montoya, 2004). Congenital toxoplasmosis happens when a pregnant woman contracts a primary infection, allowing the parasites to cross the placenta, especially during the first or second trimester. This can lead to severe fetal complications, including microcephaly, chorioretinitis, ventriculomegaly, intracranial calcifications, and, less frequently, hydrocephaly(Berrébi et al., 2010)(Reem N. Said, Mayssa M. Zaki, Manal B. Abdelrazik, 2011).

The prevalence of toxoplasmosis infection among pregnant women in Africa is high, particularly in Central and Eastern Africa(Mulu Gelaw et al., 2024). A study in Northern Tanzania reported a seroprevalence of 44.5%, with 40.2% of the women being IgG positive and 9.1% being IgM positive among pregnant women (Paul et al., 2018).In Rwanda, Murebwayire et al. found a 12.2%. overall seroprevalence in pregnant women, with 9.6% of women showing evidence of past infection (IgG) and 3.9% positive for IgM, indicating recent infection (Murebwayire et al.,2014).

Congenital toxoplasmosis pathophysiology is due to the parasite's ability to pass through the placenta and infect fetal tissue, particularly when maternal immunity is reduced or in the absence of early treatment(Kieffer F, 2013)(Kota AS, 2023)(Bollani L, Auriti C, Achille C, Garofoli F, De Rose DU, Meroni V, Salvatori G, 2022). Gestational age increases the transmission risk approximately 30% in the second trimester and up to 70% in the third trimester, due to changes in placental permeability and maternal parasitemia (Robert-Gangneux F, 2012)(Cruz et al., 2007). Infection leads to severe inflammation, necrosis, and structural brain abnormalities, including microcephaly, hydrocephalus, seizures, and psychomotor delays(Khan K, 2018)(Remington JS, Wilson CB, Nizet V, Klein JO, 2010). Laboratory diagnosis involves serological detection of specific antibodies (IgG, IgM, IgA) and PCR testing of bodily fluids for *Toxoplasma gondii* DNA, and persistent IgG or detection of IgM/IgA antibodies after birth confirms congenital infection (Kota AS, 2023).

2.3.2. Rubella Virus

Rubella virus, a member of the *Togaviridae* family, *Rubivirus* genus, causes Rubella or German measles, which may be acquired congenitally or postnatally (Sahil Batra, 2018). It is transmitted by respiratory droplets from infected individuals, and its infection during early pregnancy is a concern due to its teratogenic effects on the developing fetus, causing congenital rubella syndrome(CRS) (Gubio AB, Mamman AI, Abdul M, 2019). CRS can lead to microcephaly in 10%, hearing loss in 90%, cardiac anomalies in 50%, mental retardation in 40%, ophthalmologic anomalies in 40%, jaundice, and developmental delays in the affected infant (Frenkel et al., 2018).

Rubella pathophysiology involves initial replication in the nasopharyngeal lymphoid tissue, followed by viremia approximately 5-7 days post-exposure. Systemically spread virus can cross the placenta, causing direct cellular damage to fetal tissues by inducing mitotic arrest and cell death, especially when maternal infection occurs early in pregnancy (Banatvala & Brown, 2004)(CDC, 2024c).

Rubella virus laboratory diagnosis includes the detection of rubella-specific IgM in suspected cases and IgG to determine immunity status. Molecular methods such as RT-PCR or virus isolation can be used for detecting rubella RNA in acute cases, with sample collection ideally occurring soon after symptom onset (CDC, 2024b). The presence of rubella-specific IgG indicates past infection or rubella vaccine and serves as a marker of immunity(CDC, 2024d).

Rubella virus remains a public health concern in Sub-Saharan Africa, where seroprevalence studies indicate ongoing exposure among pregnant women. A meta-analysis study by Zemenu et al. found that the pooled seroprevalence of anti-RV IgG among pregnant women in Sub-Saharan Africa was 89.0%, suggesting prior exposure or vaccination, while the anti-RV IgM prevalence stood at 5.1%, indicating recent infections (Kassa ZY, Hussen S, 2020). In the seroprevalence and risk factors for rubella infection in pregnant women attending a tertiary hospital in Kano-Nigeria study, Safiya et al found that the overall rubella seroprevalence was 68.7%, with 58.4% testing positive for IgM and 37.3% for IgG, notably 26.4% had both antibodies, suggesting active or recent infection (Zahradeen SU, Muhammad ID, Adamou N, Rabiu A, Yusuf MA, Shuaibu SAD, 2023).

2.3.3. Cytomegalovirus

Cytomegalovirus(CMV), a double-stranded DNA virus of the herpesvirus family, is among the most prevalent causes of vertical infections worldwide and the most recognized TORCH infection causing microcephaly(Noyola et al., 2001)(Megli & Coyne, 2022). CMV primary maternal infection during the first trimester of pregnancy causes up to 40% risk of vertical transmission (Lenore, 2018), and the greatest risk of fetal damage occurs during the early stages of organ development (Adams Waldorf & McAdams, 2013). In Africa, the prevalence of CMV IgG among pregnant women was reported to be high(87.4%, range of 72%-100%), as well as high congenital cytomegalovirus prevalence in newborns(3.3%, range of 1.3%-6.3%)(Hailemariam et al., 2021).

Serious complications can be caused by congenital CMV, including microcephaly, intrauterine growth restriction, hearing and vision loss, and structural brain abnormalities(Fisher S, Genbacev O, Maidji E, 2000)(Weisblum et al., 2014)(Boppana SB, Fowler KB, Pass RF, Rivera LB, Bradford RD, Lakeman FD, 2005). Around 10% of infants with CMV often present with microcephaly, low birth weight, petechiae, hepatosplenomegaly, and hearing loss(Gordon et al., 2021).

Research indicates that placental pericytes and trophoblasts are targeted by CMV to gain fetal access, leading to CNS damage during early fetal development (David et al.,2017). Primary maternal infection during early pregnancy poses an increased risk of long-term adverse central nervous system (CNS) outcomes, likely due to infection and injury to the developing, immature CNS(Daiminger et al., 2005). A cohort study by Chelsea et al. found CMV to be the strongest risk factor for microcephaly in the U.S, increasing risk at least sevenfold and highlighting the need for more focused public health interventions (Recaioglu et al.,2023).

CMV laboratory diagnosis involves both serological and molecular testing. CMV specific IgM antibodies in the mother may suggest recent infection, a positive test for CMV IgG indicates that a person was infected with CMV at some time during their life while the presence of CMV DNA in amniotic fluid, blood, urine or saliva via polymerase chain reaction confirms active infection in the fetus or newborn(Daiminger et al., 2005)(CDC, 2024a).

2.3.4. Zika Virus

Zika virus(ZIKV) is a mosquito-borne flavivirus transmitted primarily by bites from *Aedes aegypti* and *Aedes albopictus*, but can also spread from mother to fetus , sexual contact, blood transfusion and organ transplantation (CDC, 2016a)(WHO, 2022) . Congenital zika syndrome, a severe condition marked by spontaneous miscarriage or fetal anomalies such as microcephaly, can result from vertical transmission during pregnancy (Scotto et al., 2024).

Infection during the first trimester is especially harmful, causing brain malformations, cortical thinning, eye abnormalities, joint contractures, and hypertonia resulting from impaired neuronal migration and programmed cell death of neural progenitor cells(Moore et al., 2017)(Wheeler et al .,2020). ZIKV exhibits a preference for infecting neural stem cells, astrocytes, oligodendrocyte precursor cells, and microglia, playing a key role in its neuropathogenic effects (Petitt et al., 2017). Children exposed to ZIKV in utero might develop structural anormalies and functional disabilities secondary to central nervous damage, known as congenital Zika syndrome whose most common clinical feature is microcephaly(Marbán-Castro et al., 2021).

Worldwide attention was taken to the viruses' association with microcephaly, after the 2015-2016 outbreak in Brazil, leading the WHO to declare it a public health emergency(Rawal et al ., 2016). Subsequent research, including seroprevalence studies in Guinea-Bissau and an epidemiological study in Brazil, established further the link between maternal ZIKV infection and birth defects such as microcephaly and congenital Zika virus infection (Devakumar et al., 2018) (Frenkel et al.,2018). ZIKV mother to child transmission can occur in 20 to 30% of cases and cause severe fetal and child defects(Marbán-Castro et al., 2021).

ZIKV crosses the placenta via several pathways, including infection of Hofbauer cells, apoptosis of placental trophoblast, vascular injury, direct infection of the amniochorionic membrane(Wen et al., 2017)(Miner et al., 2016) (Delorme-Axford E, Sadovsky Y, 2014).Replication of ZIKV within Hofbauer cells triggers inflammatory responses that damages the structural integrity of the maternal-fetal interface, resulting in indirect harmful effects on neural progenitor cells in the developing fetal brain(Wen et al., 2017)(Petitt et al., 2017).

Figure 1 demonstrating how zika virus passes through the placenta to infect the fetus after mother's infection.

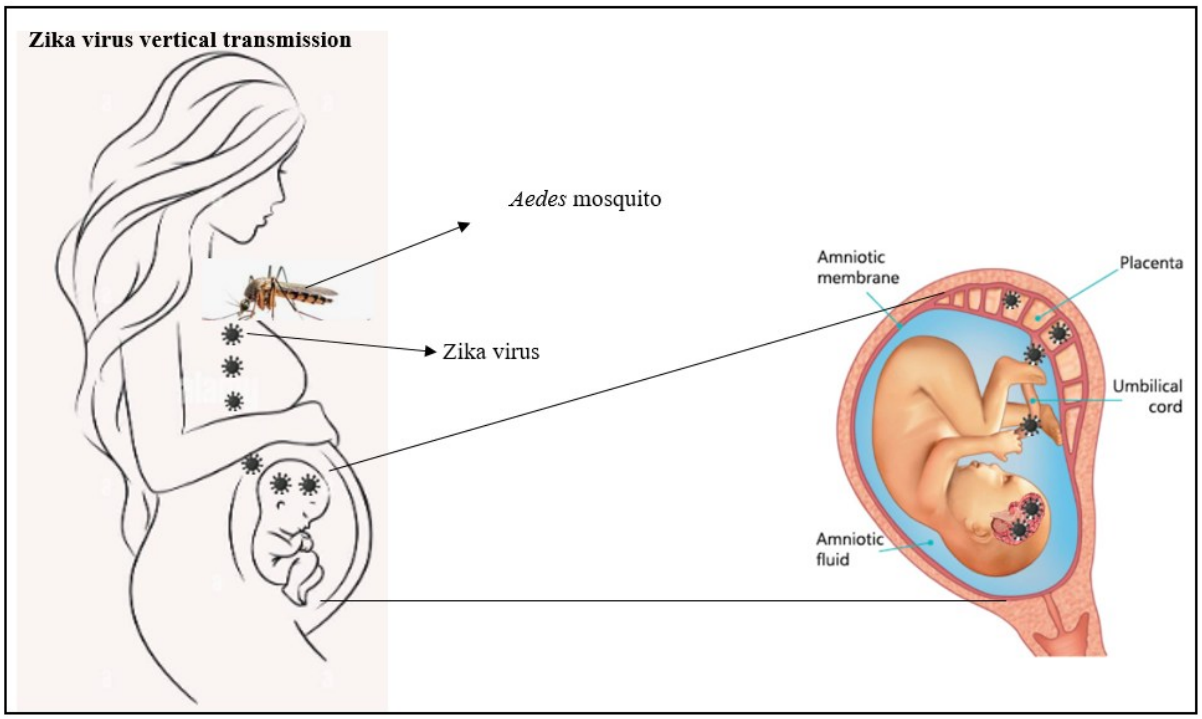

Figure 1. ZIKV vertical transmission.

Experimental research using organoids systems, animal models, and autopsies of stillborn infants has detected ZIKV in both placental and fetal central nervous system tissues, proving strong evidence of the virus's role in the development of microcephaly (Noronha Ld, Zanluca C, Azevedo ML, Luz KG, 2016)(Martines et al., 2016). The virus predominantly infects neural progenitor cells(NPCs), inducing S-phase cell arrest and apoptosis, which disrupts neurogenesis and contributes to microcephaly(Tang et al., 2016). The presence of viral RNA and antigens in amniotic fluid, placenta, and fetal brain classifies ZIKV as a pathogen capable of transplacental neuroinvasion (Bhatnagar et al.,2017). Laboratory diagnosis of ZIKV is done using both molecular and serologic methods(Ecdc, 2016).

2.3.5. Herpes simplex virus

Herpes simplex virus(HSV) is a Deoxyribonucleic acid(DNA) virus in the *Herpesviridae* family, which causes congenital herpes simplex virus infection, resulting in severe neurological complications, including microcephaly in 4% of all neonatal HSV infections associated with brain destruction, calcifications, and encephalitis(Shen et al., 2021).

HSV infection of the newborn can be acquired in utero, intrapartum, or postnatally(Kathleen M. Gutierrez b Richard J. Whitley b Ann M. Arvin, 2018). In pregnant women, infection with HSV is shed at genital sites and can be transmitted to the infant during delivery, or through cervical shedding of virus after a primary episode of genital HSV in the third trimester(Ethawi et al., 2023).

The finding that genital HSV infection and neonatal HSV infections were most often due to HSV-2 suggesting a cause and effect relationship between the two entities, and that causal relationship was strengthened by detection of the virus in the maternal genital tract at the time of delivery, indicating that acquisition of the virus occurs by contact with infected genital secretions during birth(Kathleen et al., 2018).

The definitive laboratory diagnostic method remains virus isolation in appropriate samples. A swab of skin vesicles is done if skin lesions are present, transported in appropriate virus transport media to a diagnostic virology laboratory, and direct immunofluorescence staining test of the skin lesions scrapings is performed to detect the presence of virus in infected cells or enzyme immunoassays is performed for viral proteins detection. Cerebrospinal fluid, stool ,urine ,throat, nasopharynx and conjunctivae are also used for virus detection by detecting HSV DNA by polymerase chain reaction(Kathleen M. Gutierrez b Richard J. Whitley b Ann M. Arvin, 2018).

2.3.6. Human Immunodeficiency Virus

Human immunodeficiency virus(HIV)targets the immune system, and its most advanced stage is acquired immunodeficiency syndrome (AIDS)(WHO, 2024). It is transmitted through body fluids of an infected person, including blood, breast milk, semen, and vaginal fluids, and vertically from mother to child during pregnancy, delivery, or breastfeeding(WHO, 2023)(Johnson et al., 2021). Vertical transmission is facilitated by hofbauer cells in the placenta, which express HIV co-receptors on their cell surface, which allow viral passage into fetal circulation (Johnson et al., 2021)(Marlin R, Nugeyre MT, de Truchis C, Berkane N, Gervaise A, Barré-Sinoussi F, 2009). Immune function, brain development and growth may be impaired by exposure to HIV and antiretroviral during fetal development (Bulterys MA, Njuguna I, Mahy M, Gulaid LA, Powis KM, Wedderburn CJ, 2023). Studies have shown that HIV exposed but uninfected children face increased risks of adverse outcomes, including impaired neurodevelopment(Slogrove et al., 2018).

Research by Evans et al and Macmillan et al. found lower head circumferences and increased microcephaly in HIV infected infants, confirming the virus's impact on brain development (Evans et al., 2016)(Macmillan et al., 2001).

CHAPTER 3. METHODOLOGY

3.1. Study area

This study was conducted at the University Teaching Hospital of Kigali, also known as Centre Hospitalier Universitaire de Kigali (CHUK), in the pediatrics department. CHUK is the largest referral hospital in the country, with a capacity of around 500 beds. It provides quality healthcare to the population, as well as training, clinical research, and technical support to district hospitals. CHUK is located in the Nyarugenge District of Kigali City.

3.2. Study design

This was a cross-sectional, case-control analytical study design that investigated the infectious pathogens exposure among 0-24 months' Rwandan children with (case) or without (control) microcephaly and evaluated its potential association with microcephaly development by assessing both infection status (TORCH, Zika, and HIV) and microcephaly status simultaneously at a single point in time. It was conducted between November 2023 to July 2025.

3.3. Sampling rationale

The study used a case-control design to investigate the association between early-life exposure to infectious pathogens and microcephaly in Rwandan children. Cases were selected to represent infants and young children with clinical microcephaly, confirmed by anthropometric assessment according to WHO child growth standards (head circumference<-2SD).

Controls were age-matched children from the same geographic area who lacked microcephaly or other congenital anomalies to minimize confounding by environmental or socio-economic factors.

3.3.1. Target population

The study population was infants and young children aged 0 to 24 months presenting with a head circumference less than two standard deviations from the mean for age and sex, as defined by the WHO Child Growth Standard ("Head Circumference-for-Age GIRLS," 2007)(Boys, n.d.), and their mothers were identified by geneticists in the study team by clinical and anthropometric assessment.

3.3.2. Selection of study population

3.3.2.1. Inclusion criteria

a. Cases

Children aged 0-24 months diagnosed with microcephaly attending CHUK pediatrics/genetic services, born to mothers residing in Rwanda during pregnancy, and their mothers, and whose parents provided written informed consent, with available of comprehensive clinical and anthropometric data were included.

b. Controls

Age-matched children (0–24 months) without clinical or anthropometric evidence of microcephaly, congenital anomalies, or neurodevelopmental disorders, confirmed by a clinical geneticist or pediatric assessment, and their mothers, residing in the same geographic area as the cases for comparable environmental and exposure risks, with informed consent obtained from a parent or legal guardian, and available clinical records were included.

3.3.2.2 Exclusion criteria

a. Cases

Children with no confirmed microcephaly diagnosis or those with known acquired causes of microcephaly (e.g., birth asphyxia, brain injury, meningitis infections), known genetic syndromes or chromosomal abnormalities strongly associated with microcephaly to avoid confounding by non-infectious etiologies or those with incomplete clinical records or a lack of parental/legal guardian consent.

b. Controls

Children with known genetic syndromes, chromosomal abnormalities or clinical signs suggestive of neurodevelopmental delay, congenital anomalies or associated with microcephaly, known history of exposures strongly linked to microcephaly or brain abnormalities (e.g., maternal alcohol/drug abuse during pregnancy) or those with incomplete or missing clinical records or a lack of parental /legal guardian consent.

3.3.3. Sample size of the study

The sample size for this study was calculated using the formula from Kelsey et al. (Jennifer L.Kelsey, Alice S.Whittemore, Alfred S.Evans, 1996)

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \cdot [p_1(1-p_1) + p_2(1-p_2)]}{(p_1 - p_2)^2}$$

Wherein: n is the required number of subjects per group (cases and controls).

$Z_{\alpha/2}$is the Z score for type I error (1.96 for α=0.05).

Z_{β} is the Z score for power (0.84 for 80% power).

p_1=Proportion of exposure in controls.

p_2=Proportion of exposure in cases.

From the literature exposure in controls(p_1) =15%(0.15)

Exposure in cases(p_2) =45%(0.45)

$$\mathbf{n} = \frac{[(1.96+0.84)^2 [0.15(1-0.15)+0.45(1-0.45)]}{(0.15-0.45)^2}$$

, n=32. 7≈33. The minimum required sample size was 33 Rwandan children with microcephaly and 33 controls.

3.4. Data Collection tools, methods, and procedures.

3.4. 1. Clinical data collection

Clinical assessment of the patient, including medical history, physical examination, neurological evaluations, developmental history and a detailed maternal history, including information about maternal infections during pregnancy, exposure to toxoplasma gondii, zika virus, cytomegalovirus, HIV and Rubella virus, vaccination history, prenatal care received, and any inquiry about any symptoms of infections during pregnancy, was obtained from the geneticist consulting the study participant using study data collection self-designed tool.

3.4.2. Patient peripheral blood sample collection

5 ml of the participant's peripheral blood was collected in red dry blood collection tubes, and serum was generated after centrifugation at 3000 round per minute/3minutes.

3.4.3 Laboratory Procedure

The serological specific rapid tests for *Toxoplasma gondii*, Rubella Virus, CMV, Herpes simplex virus, Zika virus IgG and IgM detection, and HIV rapid test detection were conducted in the CHUK laboratory department.

3.4.3.1. TORCH IgG+IgM Whole Blood Cassette test

TORCH IgG+IgM combo rapid test cassette, a rapid lateral flow chromatographic immunoassay for the qualitative detection and differentiation of IgG and IgM antibodies to *Toxoplasma gondii*(Toxo), rubella virus, cytomegalovirus, and herpes simplex virus 1/2 in human serum generated in response to the infection with each TORCH pathogen, was used. Twenty microliters (20µL) of the serum sample was dispensed into the sample well with a capillary tube, followed by 2 drops (about 80 µL) of sample diluent. The results were read at exactly 10 minutes, interpreted, reported, and all samples and materials used to perform the test were disposed as bio-hazardous.

When three lines appeared, one colored line in the control line region(c) and another colored line(s) appeared in the test line region(s), IgM and IgG antibodies to Toxo, Rubella, CMV, and /or HSV1/2, the test result was reported positive for specific pathogen IgM and IgG.

When one color appeared in the control line region(c), another line appeared in the IgM region or the IgG antibody to Toxo, Rubella, CMV, and /or HSV1/2 region, the test result was reported positive for specific pathogen IgM or IgG. The test was reported negative, when only one colored line appeared in the control line region(c) without any line appearing in the test line regions (IgM and IgG)

3.4.3.2. ZIKA IgG+IgM Whole Blood Cassette

Zika IgG+IgM whole blood cassette, a lateral flow chromatographic immunoassay for the qualitative detection and differentiation of IgG and IgM antibodies to Zika virus in human serum, plasma, or whole blood for Zika infection diagnosis, was used. Twenty microliters (20µL) of the serum sample were dispensed into the sample well with a capillary tube, followed by 2 drops (about 80 µL) of sample diluent. The result was read at exactly 10 minutes, interpreted, reported, and all samples and materials used to perform the test were disposed of as bio-hazardous.

When three lines appeared, one colored line in the control line region(c) and another colored line(s) appeared in the test line region(s), IgM and IgG antibodies to Zika virus, the test result was reported positive for IgM and IgG. When one color appeared in the control line region(c), another line appeared in the IgM region or the IgG antibody to Zika virus region, the test result was reported positive for IgM or IgG. The test was reported negative when only one colored line appeared in the control line region(c) without any line appearing in the test line regions (IgM and IgG).

3.4.3.2. Determine HIV1/2 early detection rapid test.

Determine HIV1/2 early detection rapid test, an immunochromatographic test, was used to detect antibodies to HIV-1 and HIV-2 and HIV-1 p24 antigen in human serum, for the diagnosis of infection with HIV-1 and HIV-2. Fifty microliters (50 μL) of serum sample was applied to the sample pad using a precision pipette, and the results were read after 20 minutes, interpreted, reported, and all samples and materials used to perform the test were disposed of as bio-hazardous. When red bars appear in both the control window (labeled C) and/or in the antibody window (labeled AB) of the strip, the antigen window labeled AG of the strip indicates the test was reported positive.

3.5. Statistical analysis

Data were entered and filtered in an Excel sheet and then imported into IBM SPSS Statistics version 25 for all statistical analyses. Categorical variables, including serological test results IgM/IgG status for Toxoplasma gondii, Rubella Virus, Cytomegalovirus, Herpes simplex virus, Zika virus, and HIV) and the presence of microcephaly, were summarized as frequencies and percentages. Cases were defined as children with microcephaly, while controls were age-matched children without microcephaly.

To determine the seroprevalence of each pathogen among Rwandan children with microcephaly and their respective control groups, descriptive statistics, cross-tabulations were used. Chi-square test and Fisher's exact tests were used to evaluate any statistical significance between variables in cases and controls, and an independent sample t-test was used to compare the means of height and head circumference between cases and controls. For the evaluation of the association between exposure to infectious pathogens and the occurrence of microcephaly, binary logistic regression analysis was conducted.

To evaluate the association between infectious pathogens and microcephaly while controlling for confounding by stratifying the data into homogeneous strata the Mantel-Haenszel test was run.

The dependent variable was the presence of microcephaly, and the independent variables were serological results for the investigated pathogens. The strength of association was expressed as odds ratios (ORs) with 95% confidence intervals (CIs), and the statistical significance was set at $p<0.05$ for all tests.

3.6. Ethical consideration

3.6.1. Ethical approval

Ethical clearance and ethical renewal were obtained from the College of Medicine and Health Sciences Institutional Review Board with reference numbers 298/CMHS IRB/2023 and 500/CMHS IRB/2023, respectively, and from the CHUK ethics committee with reference number EC/CHUK/171/2023.

3.6.2. Informed consent

Before participation in the study, written informed consent was obtained from all study participants' parents. Participants were informed about the study purpose, procedures, potential risks, and benefits in a language they understood (Kinyarwanda). I read aloud in Kinyarwanda the consent form for illiterate participants, before signing it, and a copy of the signed consent was provided to each study participant. Voluntary participation, with assurance that refusal to participate or withdrawal from the study at any stage would not affect the standard of care or any services the participants receive, was emphasized.

3.6.3. Confidentiality

The study participants' national identification numbers and names were hidden in a database only accessible to the researchers and supervisors because in this study, we handled biological information of the participants (blood), which needed special ethical considerations. Each participant was given a study identification number(code), which was used for their blood samples labelling.

Strict confidentiality measures were implemented, including anonymizing participant data and assigning unique study identification codes to maintain privacy during data collection and analysis, study findings, and reporting.

CHAPTER 4. RESULTS

4.1. Demographic characteristics of study participants

This study included 120 participants, with 60 cases and 60 controls. Among the cases, 30 were children with microcephaly, and 30 were their mothers. Similarly, among the controls, 30 were children and 30 were their mothers. The demographic characteristics of the participants are summarized in Table 1. Females were 83.3%, with 17.5% case children,25% case mothers,15.8% controls, and 25% controls 'mothers. Males comprised 16.7% of the cases, with 7.5% of the case children and 9.2% of the control children. The mean age of children was 11.4 months in the cases and 10.6 months in the controls. Mothers in the case group had a mean age of 31, while those in the control group had a mean age of 40 years.

Table 1. Demographic characteristics of study participants.

Characteristics		**Case**		**Control**	
		Children (%)	**Mother (%)**	**Children (%)**	**Mother (%)**
Gender (n=120)	Female (83.3%)	21(17.5%)	30(25%)	19(15.8%)	30(25%)
	Male (16.7%)	9(7.5%%)	0	11(9.2%)	0
Children's age in Months (n=60)	< 6 Months	6 (5%)		8 (6.6%)	
	6-11 months	10(8.3%)		9 (7.5%)	
	12-24 Months	14 (11.6%)		13 (10.8%)	
	Mean/ Median	11.4/11		10.6/11	
Mothers' age in years (n=60)	18-25	8(13.3%)		2(3.3%)	
	26-35	12(20%)		5(8.3%)	
	>35	10(16.6%)		23(38.3%)	
	Mean/Median	31/32		40/43	

Demographic characteristics of children and their mothers stratified by case (microcephaly) and control status. Data are presented as frequencies and percentages (%) for categorical variables and mean/median for continuous age variables. The total number of cases is 60, and the total number of controls is 60.

4.2. Clinical characteristics of study participants

The clinical characteristics of children in the case and control groups are presented in Table 2. The mean head circumference in children with microcephaly was 39.8cm and 44cm in children without microcephaly(P=0.001). The birth weight mean for cases was 2.7kg and 3kg in controls (P=0.026). Cases and controls born by normal delivery were 31.6% and 33.3% respectively, while 18.3% cases and 16.6% controls were born by caesarian section.10% cases and 5% controls were premature at birth,38.3% cases and 45% controls were mature at birth, and 1.6% cases were born post-term. Complications at birth were only observed in cases, with 16.6% neurological complications,10% perinatal complications,18.3%systemic/congenital complications, and 5% combined complications. CT scans in cases showed 5% structural brain malformations and 11.6% acquired brain injury or lesions. The exposure to infection during pregnancy and microcephaly occurrence in children were statistically significant (P=0.02).

Table 2.Clinical characteristics of the study subject

Clinical characteristics		Case	Control	P. value
Head circumference(cm)	Mean/Median/SD	39.8/39.5/3.7	44/44/5.4	**0.001**
Height(cm)	Mean/Median/SD	60.1/60/10.9	68.3/71.5/9.6	**0.003**
Birth weight	Mean/Median/SD	2.7/2.8/0.7	3/3/0.6	**0.026**
Mode of delivery (n=60)	Normal delivery	19(31.6%)	20(33.3 %)	
	Caesarian section	11(18.3%)	10(16.6%)	
Maturity at birth (n=60)	Preterm	6(10%)	3(5%)	
	Term	23(38.3%)	27(45%)	
	Post term	1(1.6%)	0	
Complications at birth (n=60)	Neurological complications	10(16.6%)	0	
	Perinatal complication (%)	6(10%)	0	
	Systemic/congenital complications	11(18.3%)	0	
	Combined complications	3(5%)	0	
Physical examination(n=60)	Isolated microcephaly	3(10%)	0	
	Microcephaly with dysmorphic features	8(13.2%)	0	
	Microcephaly with developmental and neurological delay	19(31.6%)	0	
Radiological investigation (CT scan) (n=60)	Normal findings	2(3.3%)	0	
	Structural brain malformation	3(5%)	0	
	Acquired brain injury or destructive lesions	7(11.6%)	0	
	Unknown	8(13.3%)	0	
History of infection during pregnancy(n=60)	No	18(41.6%)	26(48.3%)	
	Yes	12(8.3%)	4(1.6%)	
	P value			**0.02**

CT=Computed Tomography. Data are presented as frequencies and percentages (%), and a p-value less than 0.05 was considered statistically significant.

4.3. Seroprevalence of infectious pathogens in case and control

The seroprevalence of IgM and IgG antibodies for Toxoplasma gondii (Toxo), Rubella virus, Cytomegalovirus (CMV), Herpes simplex virus (HSV) types 1 and 2, Zika virus (ZIKV), and HIV Ab/Ag in cases, controls, and their mothers is presented in Figure 2. In children with microcephaly and healthy controls, the prevalence of Rubella IgG was 45% and 26.6%, respectively, and Rubella IgM was 1.6% in controls only. In cases only, the prevalence of CMV IgM and IgG was 3.3% and 15%, respectively, and 1.6% for HIV. In case mothers and control mothers, the prevalence of Toxo IgG was 8.3% and 6.6%, respectively, and Rubella IgG was 40% and 8.3%, respectively. CMV IgG (23.3%), HSV1/2 IgM (1.6%), HSV1/2 IgG (6.6%), and HIV (1.6%) were found only in case mothers. Figure 2

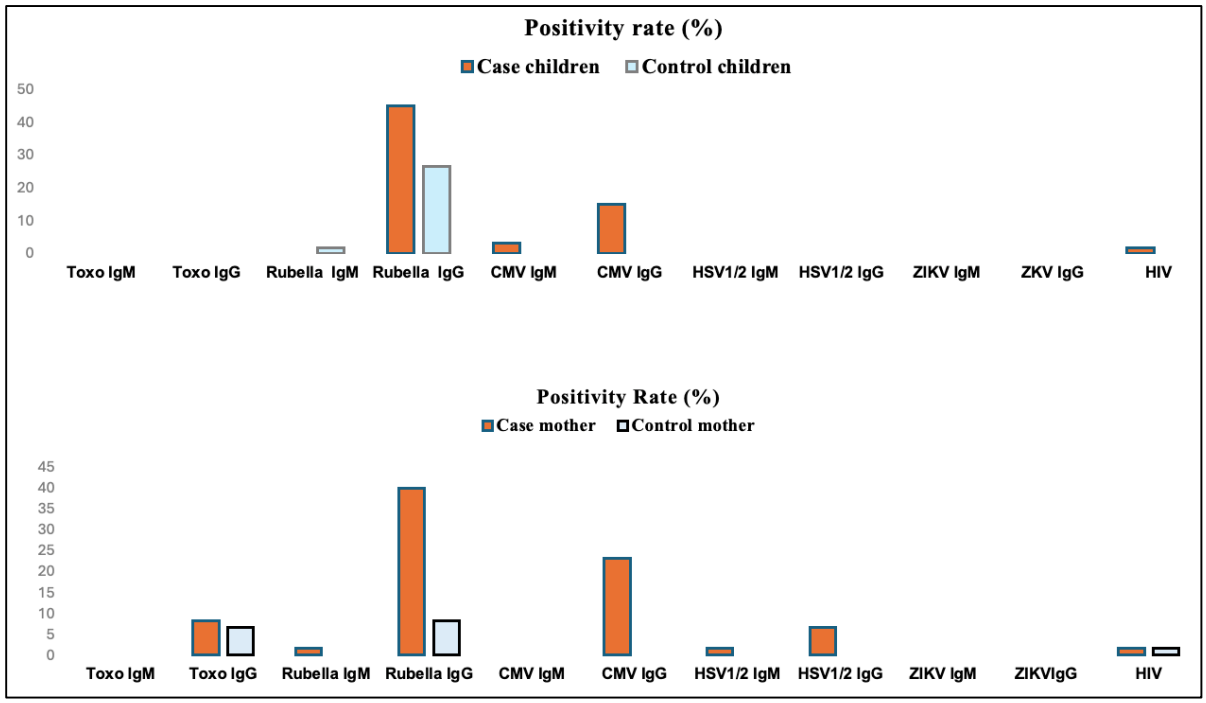

Figure 2.Seroprevalence of infectious pathogens in case and controls(n=120). IgM=Immunoglobulin M, IgG=Immunoglobulin G, CMV=Cytomegalovirus, Rubella=Rubella virus, Toxo=Toxoplasma gondii, HSV 1/2=Herpes Simplex virus, ZIKV=Zika Virus, HIV=human Immunodeficiency virus. The x-axis lists specific IgM and IgG for Toxo, Rubella, CMV, HSV1/2, ZIKV, and HIV. The Y-axis displays the percentage of individuals in each group (cases, mothers of cases, controls, mothers of controls) who tested positive for the indicated infectious disease marker.

4.4. Comparison of seropositivity between cases and controls

The Figure below shows the comparison of seropositivity rates of infectious pathogens in all cases (children and their mothers; n=60) and controls (children and their mothers; n=60). Toxo IgG was 8.3% in the case group and 6.7% in the control group. Rubella IgG was high (85%) in the case group compared to the control group (35%). Rubella IgM was 1.7 % in both the case and control groups. Only in cases CMV IgG was 38.3%, CMV IgM (3.3%), HSV IgG (6.7%), and HSV IgM (1. 7%).HIV seropositivity was 3.3% and 1.7% in the case and control groups, respectively. Figure 3

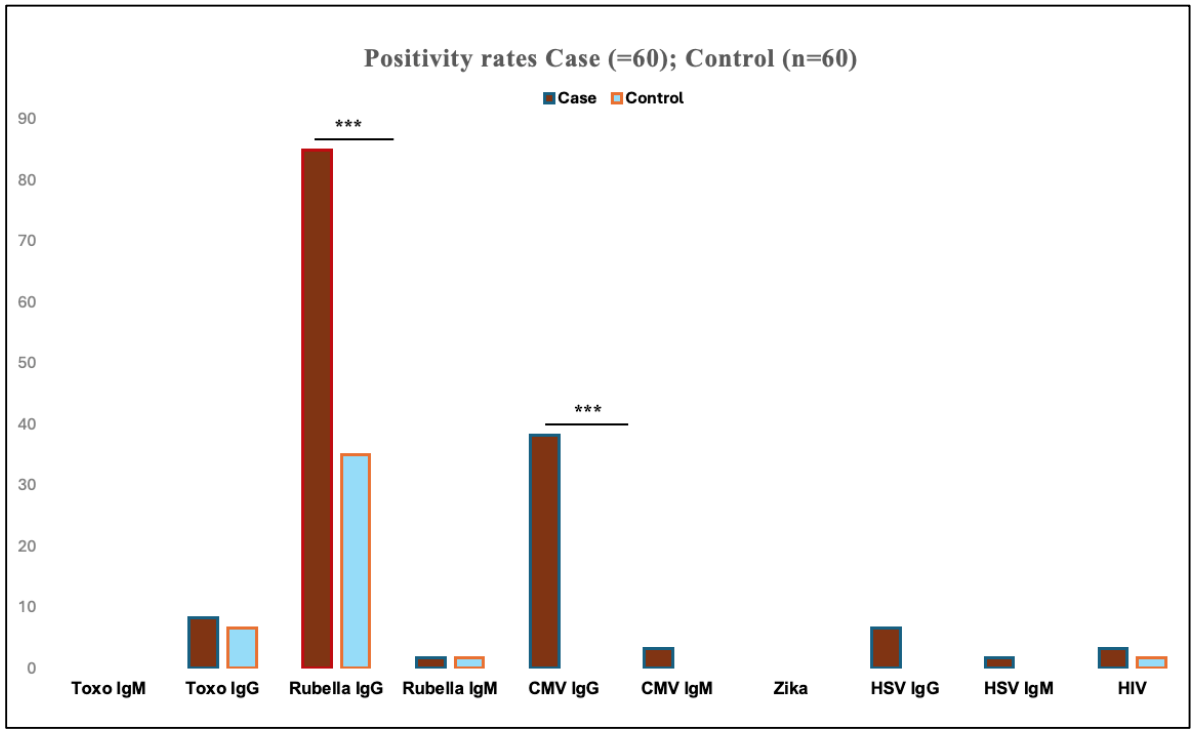

Figure 3.Comparison of seropositivity between cases and controls. *p -value less than 0.05 was considered statistically significant. 0.01=*, 0.001=**, ***=<0.001.* IgM=Immunoglobulin M, IgG=Immunoglobulin G, CMV=Cytomegalovirus, Rubella=Rubella virus, Toxo=Toxoplasma gondii, HSV 1/2=Herpes Simplex virus, ZIKV=Zika Virus, HIV=human Immunodeficiency virus. The x-axis lists specific IgM and IgG for Toxo, Rubella, CMV, HSV1/2, ZIKV, and HIV. The x-axis lists specific IgM and IgG for Toxo, Rubella, CMV, HSV1/2, ZIKV, and HIV. The Y-axis displays the percentage of individuals in each group (all cases and all controls) who tested positive for the indicated infectious disease IgM and IgG antibodies.

4.5. Association between microcephaly and infectious pathogens

Figure 4 illustrates the association between microcephaly and seropositivity for IgM and IgG antibodies against Rubella virus, Cytomegalovirus (CMV), and HIV in children. The positivity rate for Rubella IgG was notably higher among case children (27%) compared to control children (13.3%) (OR = 1.7, 95% CI 1.2–2.4, P = 0.002). After controlling for confounding factors, the Mantel-Haenszel test results were as follows (OR = 7.875, 95% CI 1.958-31.675, $p = 0.004$). CMV IgG was detected in 7.5% of case children but in none of the control children (OR = 27, 95% CI 1.5-488, $p = 0.02$). Additionally, the positivity rates for CMV IgM and HIV among cases were 1.6% and 0.83%, respectively. Figure 4.

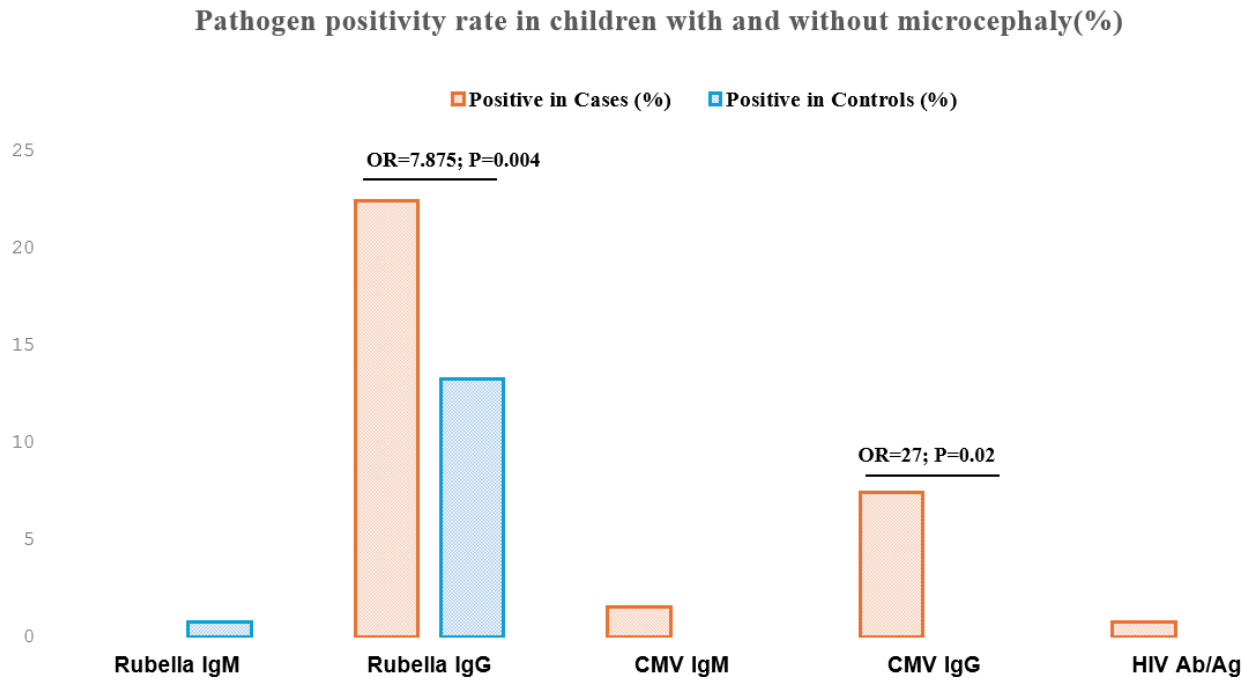

Figure 4. Association between microcephaly and infectious pathogens. $p<0.05$ and Odds Ratio (OR) >1 are considered significant. The x-axis shows specific IgM and IgG for Rubella, CMV, and HIV. The y-axis indicates the percentage of children with or without microcephaly who tested positive for the respective infectious disease IgM and IgG antibodies.

CHAPTER 5. DISCUSSION

This study investigated early exposure to key infectious pathogens, including *Toxoplasma gondii*, Rubella virus, Cytomegalovirus, Herpes simplex virus, Zika virus, HIV, and their association with microcephaly by evaluating IgM and IgG seropositivity in thirty Rwandan children with microcephaly and their mothers compared to thirty Rwandan children without microcephaly and their mothers. Rubella IgG was significantly higher in children with microcephaly (22.5%) compared to control children (13.3%) (OR = 1.7, 95% CI 1.2–2.4, P = 0.002). After controlling for confounding factors, the Mantel-Haenszel test results were as follows (OR = 7.875, 95% CI 1.958-31.675, p = 0.004). Similarly, CMV IgG positivity, absent in controls, was elevated in cases (7.5 %) (OR=27; 95% CI: 1.5-488; P=0.02). The exposure to infections during pregnancy was statistically associated with the occurrence of microcephaly (OR=4.33, 95%CI:1.20–15.61; P=0.02).

In our study, the majority were female (83.3%), with 17.5% cases and 15.8 % controls. Males accounted for 16.7 % overall, with 7.5% cases and 9.2 % controls. The mean age of children was 11.4 months (median 11months) for cases and 10.6 months for controls (median 11 months), indicating that both groups were appropriately age-matched. Mothers of cases and controls have a mean age of 31.5 and 40, respectively. This finding aligns with a study by Liu et al. (2019), which demonstrated that young maternal age, particularly below 25 years, was associated with 30% increased risk of mild microcephaly (AOR 1.3,95% CI 1.1 to 1.4)(Liu et al., 2019). These results suggest that younger maternal age may be a potential risk factor for microcephaly, but further investigation with a larger sample size is needed to clarify the underlying mechanisms.

Cases have smaller head circumferences compared to controls, with a mean of 39.8cm versus 44cm (p=0.001), consistent with the clinical definition of microcephaly characterized by head circumference measurements below two standard deviations of the mean for age and sex(Ashwal S, Michelson D, Plawner L, 2009). These findings are also consistent with previous research showing an association between microcephaly and reduced head circumference and birth weight. Liu et al. (2019) reported a high prevalence of small head circumference in children with microcephaly in southern China(Liu et al., 2019). Similarly, birth weights were significantly lower in cases with a mean of 2.7kg versus the control's mean of 3kg(p=0.026). Rosnah et al. (2018) study demonstrated that low birth weight was the most significant predictor for microcephaly (adjusted OR 12.14,95%CI 10.80-13.65)(Sutan et al., 2018).

Additionally, this study found a significant difference in height between children with(cases) or without(controls) microcephaly, with cases having a mean height of 60.1 cm compared to 68.3 cm in controls (p=0.003). This aligns with research-based evidence indicating that children with microcephaly frequently have concurrent developmental limitations, including decreased stature, which could be a sign of systemic growth disorders(da Costa et al., 2022). Preterm birth occurred in 10% cases compared to 5% of controls. Although the odds of being born preterm were 2.35 times higher among children with microcephaly compared to controls (OR 2.35,95%CI 0.53-10.47), this difference was not statistically significant (Fisher's Exact Test, p=0.291). These findings suggest a possible association, but are not conclusive due to the limited sample size. Notably a study by Songying et al (2021), investigating the prevalence of congenital microcephaly and its risk factors in an area at risk of zika outbreaks, including 46,610 live births in which 154 had microcephaly, reported a significantly higher risk of microcephaly in infants born to women who had preterm labor (OR 16.38,95%CI 11.81-22.71), considering preterm labor as microcephaly risk factor(Shen et al.,2021).

In this study, neurological complications at birth (seizures, poor feeding, abnormal tone, developmental delay) were observed in 16.6% of children under 2 years with microcephaly, with no such findings in controls, suggesting vulnerability of the affected group to neurological insults potentially linked to intrauterine or perinatal infections. A national surveillance study from the UK highlighted that the majority of children with severe microcephaly had neurological abnormalities and developmental delays, aligning with prior evidence that congenital or perinatal brain injuries manifest early with significant neurodisability in microcephaly(Knowles et al., 2023).

Perinatal birth complications including birth asphyxia and respiratory distress, were found in 10% of cases, and systemic/congenital complications including congenital blindness, congenital heart defect, neonatal jaundice, and congenital rubella syndrome, were observed in 18.3% of cases, consistent with known patterns where congenital infections increase risk of perinatal damage and systemic manifestations(Lanzieri TM, Dollard SC, Bialek SR, 2014).

Computed Tomography(CT)scan results showed 5% structural malformations (brain atrophy) and 11.6% acquired brain injury or destructive lesions(lissencephaly, hypoplasia of the corpus callosum, encephalomalacia, periventricular and deep white matter calcification), which often result from disrupted brain development due to intrauterine infections(Devakumar et al., 2018).

This study's results showed a difference in maternal infection during pregnancy between cases and controls, with 12 (20%) cases and 4 (6.7%) controls reporting exposure to infection during pregnancy. A statistically significant association between maternal history of infection exposure during pregnancy and the presence of microcephaly (OR = 4.33, 95% CI: 1.20–15.61; P = 0.02) was found.

IgG positivity, indicative of past exposure or maternal antibody transfer, varied, with Rubella IgG being present in 45% of case children compared to 26.6% of control children. Children who tested positive for Rubella IgG had 1.7 times higher odds of being microcephalic compared to Rubella IgG negative children (OR=1.7,95% CI:1.183 - 2.408, p=0.002). Below 6 months old, 5% cases were all positive for Rubella IgG, suggesting congenital rubella infection. The result aligns with existing evidence indicating that congenital rubella infection is a teratogenic factor capable of causing neurological abnormalities, including microcephaly, especially when maternal infection occurs during the first trimester of pregnancy(De Melo et al., 2025) and also microcephaly has been reported in 32% of congenital rubella cases in African sentinel surveillance sites across Tanzania, Rwanda, Zimbabwe, Zambia and Burkina Faso(Masresha et al., 2018b). Rubella IgG positivity was also higher in case mothers (40%) vs controls (8.3%), and significantly associated with increased odds of being a case mother (OR = 4.8,95%CI:2.11 to 10.9), while Rubella IgG negativity was associated with decreased odds (OR=0.24, 95%CI:0.115-0.50).

CMV IgG was found in 15% of case children but absent in controls, and a statistically significant association was observed between CMV IgG serostatus and microcephaly (OR=27, 95% CI 1.5-488; P=0.02). CMV IgG positivity was high in case mothers (23.3. %) compared to controls (0%) and statistically significant (OR=53.6; 95% CI: 3-957; P=0.007) supporting a potential link between maternal CMV exposure and microcephaly risk consistent with established literature demonstrating an increased risk of microcephaly with congenital CMV infection(Messinger CJ, Lipsitch M, Bateman BT, He M, Huybrechts KF, MacDonald S, Mogun H, Mott K, 2020).

These findings are consistent with the Chelsea et al. (2020) study, which revealed that the prevalence of microcephaly among newborns with and without a congenital cytomegalovirus diagnosis was 655 and 2.8 per 10,000 live births, respectively (OR=232; 95% CI: 154-350) (93) and Moodley et al., study conducted in South Africa which reported 25.2% microcephaly at birth and 46.6% postnatal microcephaly cases in 135 CMV infected infants(Moodley et al., 2024). The absence of IgM and IgG antibodies for ZIKV in both groups suggests no ongoing, recent, or past exposure to Zika virus infection in the studied cohort.

The Mantel-Haenszel test was conducted to assess the potential influence of confounding factors on the association between infectious pathogen exposure and microcephaly development in Rwandan children. The results showed a significant odds ratio of 0.127 (95%CI 0.032-0.511, P=0.004), indicating that confounders were unlikely to have biased the observed relationship, and this strengthened the validity of the association observed in this study between early-life infectious pathogens exposure and microcephaly in Rwandan children.

This study faced several limitations that could introduce biases impacting the validity and generalizability of the findings. First, maternal pregnancy histories regarding infectious exposures were limited and relied on retrospective self-reporting and the lack of laboratory confirmation during pregnancy, leading to potential recall bias, misclassification of exposure status, and further restricting the accuracy of exposure assessment, possibly diluting true associations or causing underestimation of infectious risk.

The study's small sample size, inherent to the rarity of microcephaly, limited statistical power, and the generalizability of results to the broader population, and the cross-sectional design, limited follow-up, and prevented assessment of microcephaly progression or delayed manifestations, introducing potential surveillance bias and underestimating disease burden.

Additionally, measurement bias may have occurred due to inconsistent and non-standardized head circumference and height measurements at birth, potentially causing misclassification of microcephaly cases and controls. Also, the serological analyses could not distinguish rubella maternal IgG from vaccine-induced or infection-acquired IgG in children, complicating the interpretation of antibody positivity in relation to early-life infectious pathogens exposure. Such limitations may have led to exposure misclassification and biased association estimates.

Finally, reliance on Computed Tomography imaging without more sensitive modalities like Magnetic Resonance imaging(MRI) underestimated subtle brain abnormalities, resulting in diagnostic bias and misclassification of neurodevelopmental outcomes.

CHAPTER 6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This cross-sectional case-control study finding provides evidence that exposure to infectious pathogens in pregnancy is statistically significantly associated with the occurrence of microcephaly in Rwandan children under two years of age. Our findings demonstrate that maternal infections, particularly with Rubella virus and Cytomegalovirus, are linked to increased odds of microcephaly, supported by serological and clinical data, which is consistent with broader epidemiological evidence indicating congenital infections account for a significant proportion of microcephaly cases worldwide.

6.2. Recommendations

To enhance the prevention, early diagnosis and management of congenital infections linked to microcephaly, it is recommended that the Rwanda ministry of health establish a nationwide, well-integrated maternal pregnancy history database accessible across all healthcare facilities, which would systematically record and track critical data such as gestational age, maternal infections and other relevant pregnancy information to support comprehensive maternal and child health monitoring.

Routine maternal screening for key congenital infections, including Cytomegalovirus, Rubella, *Toxoplasma gondii,* and Herpes simplex virus, should be integrated during antenatal care to facilitate early identification and intervention. Furthermore, the introduction of amniotic fluid testing is recommended for the early detection of congenital infections, particularly in pregnancies classified as high-risk.

Medical researchers should do large longitudinal cohort studies to identify causal links between specific infections and microcephaly and to assess long-term impacts. Also, long-term monitoring programs should be developed to evaluate neurological, developmental, and growth outcomes in affected children and those born to infected mothers. Finally, community education on infection prevention during pregnancy should be prioritized, emphasizing hygiene practices to reduce risks such as CMV and toxoplasmosis.

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APPENDICES

Appendix I. Ethical Approvals

UNIVERSITY of RWANDA

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DIRECTORATE OF RESEARCH & INNOVATION

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 27th /June/2023

Prof. Annette Uwineza
School of Medicine and Health Sciences, CMHS, UR

Approval Notice: No 298/CMHS IRB/2023

Your Project Title ***"Genomic and Environmental factors of neurodevelopmental disorders in Rwandan Children"*** has been evaluated by CMHS Institutional Review Board.

Name of Members	Institute	Involved in the decision		
		Yes	No (Reason)	
			Absent	Withdrawn from the proceeding
Assoc. Prof. Stefan JANSEN	UR-CMHS	X		
Assoc. Prof. Donatilla MUKAMANA	UR-CMHS	X		
Dr Danilo Melanes ZAMBRANO	UR-CMHS	X		
Prof Peace UWAMBAYE	UR-CMHS	X		
Dr Nuhu ASSUMAN	UR-CMHS	X		
Dr Moussa HAKIZIMANA	UR-CMHS	X		
Dr. Oliva BAZIRETE	UR-CMHS	X		
Dr. Judith MUKAMULIGO	UR-CMHS	X		
Dr. Eugene RUTAYISIRE	UR-CMHS	X		
Dr Innocent HAHIRWA	UR-CMHS	X		
Assoc. Prof. Eugene RUTEMBESA	UR-CMHS	X		
Dr Isiaka ABDULLATEEF	UR-CMHS	X		
Assoc. Prof. Aimable MUSAFIRI	UR-CMHS	X		
Mr Sunday Francois Xavier	UR-CMHS	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 26th June 2023, **Approval has been granted to your study.**
Please note that approval of the protocol and consent form is valid for **12 months**.

Email: researchcenter@ur.ac.rw P.O Box 3286 Kigali, Rwanda www.ur.ac.rw

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 27th /June /2023

Expiration date: The 27th /June /2024

Prof Stefan JANSEN
Ag. Chairperson Institutional Review Board,
College of Medicine and Health Sciences, UR

Cc:

- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

UNIVERSITY of RWANDA

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DIRECTORATE OF RESEARCH & INNOVATION

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, the 24th/06/2024

Ass. Prof. Annette UWINEZA, PhD
School of Medicine and Pharmacy, CMHS, UR

Notice of Renewal of Approval for Research Project: No 500/CMHS IRB/2024

Your Project title ***"Genomic and Environmental factors of neurodevelopmental disorders in Rwandan Children"*** has been evaluated by CMHS Institutional Review Board.

		Involved in the decision		
			No (Reason)	
Name of Members	**Institute**	**Yes**	**Absent**	**Withdrawn from the proceeding**
Assoc. Prof. Stefan JANSEN	UR-CMHS	X		
Prof. Donatilla MUKAMANA	UR-CMHS	X		
Dr Danilo Melanes ZAMBRANO	UR-CMHS	X		
Dr Peace UWAMBAYE	UR-CMHS	X		
Dr Nuhu ASSUMAN	UR-CMHS	X		
Dr Moussa HAKIZIMANA	UR-CMHS	X		
Dr. Oliva BAZIRETE	UR-CMHS	X		
Dr. Judith MUKAMULIGO	UR-CMHS	X		
Dr. Eugene RUTAYISIRE	UR-CMHS	X		
Dr Innocent HAHIRWA	UR-CMHS	X		
Prof. Eugene RUTEMBESA	UR-CMHS	X		
Assoc Dr Isiaka ABDULLATEEF	UR-CMHS	X		
Prof. Aimable MUSAFIRI	UR-CMHS	X		
Mr Sunday Francois Xavier	UR-CMHS	X		

After reviewing your protocol, **Continuation of Approval has been granted to your study.**

Please note that approval of the protocol and consent form is valid for **12 months**.

Email: researchcenter@ur.ac.rw P.O Box 3286 Kigali, Rwanda www.ur.ac.rw

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrollment of participants
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval.
5. Failure to submit a continuing review application will result in termination of the study.
6. Notify the Rwanda National Ethics committee once the study is finished.

Sincerely,

Date of Approval: June24th, 2024
Expiration date: June 24th , 2025

Assoc. Prof Stefan JANSEN
Ag. Chairperson Institutional Review Board,
College of Medicine and Health Sciences, UR

Cc:

- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Innovations, UR

CENTRE HOSPITALIER UNIVERSITAIRE
UNIVERSITY TEACHING HOSPITAL

Quality Health Care
Training & Research

Ethics Committee / Comité d'éthique

17th Nov,2023 *Ref.:EC/CHUK/171/2023*

Review Approval Notice

Dear Annette UWINEZA,

*Your research project: "**Genomic and Environmental factors of neurodevelopmental disorders in Rwandan Children: (GENNEURWA)** "*

During the meeting of the Ethics Committee of University Teaching Hospital of Kigali (CHUK) that was held on 17th Nov,2023 to evaluate your request for ethical approval of the above mentioned research project, we are pleased to inform you that the Ethics Committee/CHUK has approved your research project.

You are required to present the results of your study to CHUK Ethics Committee before publication by using this link:www.chuk.rw/research/fullreport/?appid=938&&chuk.

PS: Please note that the present approval is valid for 12 months.

Yours sincerely,

Dr Emmanuel Rusingiza Kamanzi
The Chairperson, Ethics Committee,
University Teaching Hospital of Kigali

ETHICS COMMITTEE
CHUK

Scan code to verify.

" University teaching hospital of Kigali Ethics committee operates according to standard operating procedures (Sops) which are updated on an annual basis and in compliance with GCP and Ethics guidelines and regulations "

CENTRE HOSPITALIER UNIVERSITAIRE
UNIVERSITY TEACHING HOSPITAL

Quality Health Care
Training & Research

Ethics Committee / Comité d'éthique

13th Feb,2025 *Ref.:EC/CHUK/1/171/2023*

Review Approval Notice

Dear Annette UWINEZA,

Your research project: ***"Genomic and Environmental factors of neurodevelopmental disorders in Rwandan Children: (GENNEURWA) "***

During the meeting of the Ethics Committee of University Teaching Hospital of Kigali (CHUK) that was held on 13th Feb,2025 to evaluate your request for ethical approval of the above mentioned research project, we are pleased to inform you that the Ethics Committee/CHUK has approved your renewal to this research project.

You are required to present the results of your study to CHUK Ethics Committee before publication by using this link:www.chuk.rw/research/fullreport/?appid=1010&&chuk.

PS: Please note that the present approval is valid for 12 months.

Yours sincerely,

Dr Emmanuel Rusingiza Kamanzi
The Chairperson, Ethics Committee,
University Teaching Hospital of Kigali

ETHICS COMMITTEE
CHUK

Scan code to verify.

" University teaching hospital of Kigali Ethics committee operates according to standard operating procedures (Sops) which are updated on an annual basis and in compliance with GCP and Ethics guidelines and regulations "

Appendix 2. Questionnaire

Confidential

Identification

Record ID	_____
Participant Study ID	_____ (Participant Study ID)
Father's Participant ID	_____ (Father Participant Study ID)
Mother's Participant ID	_____ (Mother's Participant ID)
Date of Birth	_____
Gender	○ Female ○ Male ○ Not reported
Where is your primary home location? (Confirm from medical records) Country:	○ Rwanda ○ Burundi ○ DRC ○ Other
Specify if other country of residence	_____
Province	○ Kigali ○ Eastern ○ Western ○ Southern ○ Northern

District	◯ Bugesera ◯ Burera ◯ Gakenke ◯ Gasabo ◯ Gatsibo ◯ Gicumbi ◯ Gisagara ◯ Huye ◯ Kamonyi ◯ Karongi ◯ Kayonza ◯ Kicukiro ◯ Kirehe ◯ Muhanga ◯ Musanze ◯ Ngoma ◯ Ngororero ◯ Nyabihu ◯ Nyagatare ◯ Nyamagabe ◯ Nyamasheke ◯ Nyanza ◯ Nyarugenge ◯ Nyaruguru ◯ Rubavu ◯ Ruhango ◯ Rulindo ◯ Rusizi ◯ Rutsiro ◯ Rwamagana
Sector	_____
Telephone number	_____
Site of consultation	_____
Date of consultation	_____ (date of consultation)

Prenatal and Neonatal Period

What is the chief complaint ?	_____
Did the mother have any problem during the pregnancy?	◯ Yes ◯ No (Probleme during pregnancy)
Which problem did you have during pregnancy?	_____
Birth weight (in gm)	_____ (Birth Weight)
What was the APGAR Score ?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ Unknwon
Mode of delivery	◯ normal delivery ◯ forceps ◯ vacuum ◯ cesarean section (mode of delivery)
Maturity at birth	◯ Premature ◯ Term ◯ Post-term.
Place of Birth	◯ Home ◯ Health center ◯ Hospital
Which health Center/hospital ?	_____
Did he/she cry immediately after birth?	◯ Yes ◯ No ◯ Don't know
if no, after how long he cried?	_____
Did he/she need resuscitation ?	◯ Yes ◯ No ◯ Don't know

Confidential

If yes, why?	_____
Did he/she have hypotonia ?	○ Yes ○ No ○ Don't know
Did he/she have jaundice ?	○ Yes ○ No ○ Don't know
Did he/she have seizure ?	○ Yes ○ No ○ Don't know
Did he/she have fever ?	○ Yes ○ No ○ Don't know
Provide any additional information of the neonatal period	_____

Personal Medical History

Did he/she have a normal psychomotor development?	◯ Yes ◯ No
At which age did he/she sit ?	_____
At which age did he/she crawl ?	_____
At which age did he/she walk ?	_____
Did he/she have any disease during childhood?	◯ Yes ◯ No
If yes, which one?	_____
Did he/she have epilepsy?	◯ Yes ◯ No
If yes, which type of epilepsy ?	_____
Did he/she have history of head trauma ?	_____
Did he/she have history of any surgery ?	◯ yes ◯ No
If yes , what type of surgery ? At which age ?	_____

Family History

Age of the mother at the time of birth	_____
Age of the father at the time birth	_____
Parents have any consanguinity	◯ Yes ◯ No
If yes which degree?	◯ 1st degree ◯ 2nd degree ◯ 3rd degree ◯ Other
What is the other degree of consanguinity ?	_____
Mother's history of miscarriage	◯ Yes ◯ No ◯ Don't know
If yes, how many miscarriages ?	_____
Any history of previous genetic disease in the family	◯ Yes ◯ No ◯ Don't know
Which genetic disease ? who was/were affected in the family?	_____
Any history of congenital malformation in the family?	◯ Yes ◯ No ◯ Don't know
Which congenital malformation ? who was/were affected in the family?	_____

Pedigree (Three generations)

Physical examination

Weight (kg):	_____
Height (cm):	_____
Head Circumference (cm):	_____ (Head Circumference)
Presence of dysmorphic features	○ Yes ○ No
If yes , describe dysmorphic features	_____
Presence of any associated anomaly ?	○ Yes ○ No
If yes, does the patient have following cardiovascular diseases?	☐ Unknown cyanotic congenital heart disease ☐ Unknown acyanotic congenital heart disease ☐ Atrial Septal Defect ☐ Atrioventricular septal defect ☐ Coarctation of aorta ☐ Dextrocadia ☐ Diaphragmatic hernia ☐ Hypoplastic left heart syndrome ☐ Patent Ductus Arteriosus ☐ Pulmonary valve atresia ☐ Pulmonary valve stenosis ☐ Situs invertus ☐ Tetralogy Of Fallot ☐ Total anomalous pulmonary venous connection ☐ Transposition of the Great Arteries ☐ Ventricular Septal Defect ☐ Other cardiovascular anomaly
What type of other cardiovascular anomaly	_____
If yes does he/she have face or head anomaly?	☐ Anencephaly ☐ Anophthalmos ☐ Anotia (absent ear)/Microtia (incompletely formed ear) ☐ Choanal Atresia ☐ Cleft palate and/or lip ☐ Congenital cataract ☐ Craniosynostosis ☐ Encephalocele ☐ Holoprosencephaly ☐ Hydrocephalus ☐ Microcephaly ☐ Microphthalmos ☐ Porencephaly ☐ Other anomaly of face/head

Other anomaly of face/head	_____
if yes Endocrine system abnormality	☐ Hypothyroidism ☐ Other endocrine anomaly
Other endocrine anomalies	_____
Reproductive system	☐ Indeterminate sex (ambiguous genitalia) ☐ Other reproductive anomaly
Other reproductive anomaly	_____
Gastrointestinal system anomaly	☐ Atresia of bile ducts (biliary atresia) ☐ Atresia of oesophagus with tracheo-oesophageal fistula ☐ Atresia of oesophagus without fistula ☐ Congenital absence, atresia or stenosis of anus ☐ Congenital absence, atresia or stenosis of small intestine ☐ Gastroschisis ☐ Omphalocele ☐ Prune belly syndrome ☐ Stenosis of the colon or intesine ☐ Other GI anomaly
Other GI anomaly	_____
Presence of Urinary system anomaly	☐ Exstrophy of urinary bladder ☐ Hydroureter ☐ Hypospadias ☐ Multicystic renal dysplasia ☐ Polycystic kidneys ☐ Posterior urethral valves ☐ Renal agenesis/hypoplasia ☐ Other urinary system anomaly
Other urinary system anomaly	_____
Musculoskeletal system	☐ Achondroplasia ☐ Arthrogryposis multiplex congenita ☐ Club foot (e.g. Talipes equinovarus ☐ Osteogenesis imperfecta ☐ Polydactyly ☐ Syndactyly ☐ Unspecificied congenital malformation of limb(s) ☐ Other MSK anomaly
Other MSK anomaly	_____
Nervous system anomaly	☐ Neural tube defects ☐ Other nervous system anomaly

Other NS anomaly	_____
Other Anomalies	_____
Degree of Intellectual disability/	○ Mild ○ Moderate ○ Severe Proufound ○ No intellectual disability
Degree of Developmental delay	○ Mild ○ Moderate ○ Severe ○ Profound ○ Normal development
Type of Neurodevelopmental disorder:	○ Global developmental delay isolated. ○ Global developmental delay isolated and congenital anomalies ○ intellectual disability isolated ○ intellectual disability isolated and congenital anomalies ○ Microcephaly ○ Autism spectrum disorders ○ Others
Other type of NDD	_____
Genetic investigation	☐ Karyotype ☐ Array_CGH(microarry) ☐ Sanger sequencing ☐ Whole exome sequencing ☐ Whole genome sequencing ☐ X-fragile detection
Karyotype result /or other genetic result	_____
Other investigations	☐ Cardiac ultrasound ☐ Brain CT Scan ☐ MRI ☐ EEG ☐ EMG ☐ Biology (specify if abnormal) ☐ Other
Give the result of the investigation if available	_____

Appendix3.Informed consent form

ARISE-GENNEURWA

Urupapuro rwo kwemera kugira uruhare mu bushakashatsi- Umubyeyi

Izina ry' ubushakashatsi : Gusuzuma Uturemangingo Twose Tw'umubiri N'impamvu Ziterwa N'ibidukikije mu bana bavukanye uburwayi bw'ubwonko mu Rwanda .

Mwaramutse,
Nitwa Profeseri Annette UWINEZA,nkaba ndi muganga ukurikirana indwara z'uruhererekane mu miryango mu bitaro bikuru bya Kigali (CHUK),nkaba n'umwarimu muri Kaminuza Nkuru y'u Rwanda, nkaba ndimo gukora ubushakashatsi mfatanyije n'abandi bashakashatsi b'abanyarwanda ndetse n'abo mu Bubiligi. Ubushakashatsi bwacu bugamije kumenya ubwoko bw'uturemangingo tudakunze kuboneka dutera kudakura cg kudakora neza kw'ubwonko hifashishijwe uburyo bwo gusoma utunyabugingo twose tw'umuntu.
Bityo rero, watoranijwe mu kugira ngo witabire ubu bushakashatsi. Ubu bushakashatsi bwemejwe na komite ishinzwe kurengera abakorwaho ubushakashatsi y'ishuri ry'ubuvuzi muri Kaminuza y'u Rwanda, ibitaro bya kaminuza bya Kigali (CHUK) na Butare (CHUB), ibitaro bya Gisirikare by'u Rwanda n'ibitaro by'indwara zo mu mutwe byigisha bya Ndera .

A. Ubu bushakashatsi bugamije iki ?
Intego y'ubu bushakashatsi n'ugukusanya amakuru yose ku cyaba gitera kuvukana uburwayi bw'ubwonko biturutse ku ndwara z'uruhererekane , kugira ngo ayo makuru abashe gukoreshwa mu kumva neza ibimenyetso byazo kandi no kumenya amavu n'amavuko y'izo ndwara, kugirango abaganga babashe kurushaho kuzisobanukirwa no kubasha kuzivura.
Kubera iyo mpamvu dushobora kubasaba gusuzuma idosiye yanyu yo kwa muganga (kureba imiterere yanyu) no kubafatira amaraso. Ibizava muri ubu bushakashatsi bizabikwa mu gitabo kandi bizakoreshwa gusa mu gusuzuma indwara z'uruhererekane.

B. Kubera iki twaguhisemo kugirango winjire muri ubu bushakashatsi ?
Watoranijwe kugira uruhare muri ubu bushakashatsi kubera ko wowe cyangwa umuntu wo mu muryango wawe afite uburwayi bw'ubwonko yavukanye akaba ashobora kuba yarabutewe n'indwara y'uruhererekane. Namwe mwerekanye ko mwifuza kurushaho kumva icyaba cyarateye iyo ndwara kandi ubumenyi buzavamo buzatuma murushaho kumva amavu n'amavuko y'iyo ndwara.
Ubushakashatsi bugirwamo uruhare gusa n'ubyifuza niyo mpamvu tubasaba gufata umwanya mukabitekerezaho binashobotse mukabiganira n'abo mu muryango wanyu. Kugira uruhare muri ubu bushakashatsi nta gahato karimo kandi uburenganzira bw'ikiremwa muntu buzubahirizwa.

C. Ni iki nasabwa gukora igihe nemeye kugira uruhare muri ubu bushakashatsi ?
Tuzakorana nawe amasaha abiri tugusaba kuduha amakuru akenewe muri ubu bushakashatsi. Uzatwemerera kandi tugufate amaraso. Ubusanzwe uburyo ubu bushakashatsi buzakorwa bimeze nk'ibyo wakorerwa ugiye kwisuzumisha cyangwa gusuzumisha umwana wawe kugirango avurwe

izo ndwara z'uruhererekane. Kandi kutagira uruhare muri ubu bushakashatsi ntibyabuza ko wavurwa cyangwa umwana wawe yavurwa igihe aje kwa muganga. Nugira uruhare muri ubu bushakashatsi tuzagufatira ibipimo nk'ibyo umuntu uje kwivuza bisanzwe. Iyo uje kwivuza cyangwa kuvuzwa tureba imiterere yanyu, tukabafatira amafoto (kugirango tubashe kwerekana neza imiterere yanyu, turabasuzuma, tukababaza indwara mwarwaye , n'ibindi byabaranze kuva mu buto bwanyu, tukababaza ibibazo k' umuryango wanyu (abana mufite, niba hari abafite ibibazo nk'ibyanyu, ...) tukabafatira amaraso, nibiba ngombwa dushobora gufatira n'ababyeyi banyu.

D. Amaraso bazamfatira azamara iki ?

Amaraso muzafatirwa azakoreshwa kugirango dukuremo utunyangingo fatizo (ADN) kugirango tubone uko dusuzuma izo ndwara z'uruhererekane. ADN ni ijambo ry'igifaransa rikoreshwa risobanura utunyabuzima tuba mu mubiri wacu dufite amakuru y'ibyo tuba twarakuye ku babyeyi bacu cyangwa kubandi bantu tugira icyo dupfana kw'isano y'amaraso. Iyo ADN ifite utwo tunyabuzima bita « gènes » dutuma tumera uko turi nko kuba muremure, mugufi, inzobe, igikara , etc, ariko dushobora gutuma tugira indwara zimwe na zimwe nk'aho usanga abantu bo muryango umwe bafite indwara zimwe twatanga urugero aho usanga mu muryango bafite ubumuga bw'uruhu, cyangwa ubugufi bukabije. Iyo ADN yanyu izasuzumwa kugirango tubashe kubona izo ndwara z'uruhererekane.

E. Ni igihe kingana gute nzaguma muri ubwo bushakashatsi ?

Ubushakashatsi bwinshi ku ndwara z'uruhererakane, busuzuma ADN bushobora kumara igihe kirekire. Nibyo kubera hagenda havumburwa ubundi buryo buhanitse bwo kubona izo ndwara dushobora kongera gukoresha ADN yanyu. Muri ubu bushakashatsi tuzabafatira amaraso inshuro imwe gusa , tuzohereza ADN yanyu mu bindi bigo by'ubuvuzi bifite ikoranabuhanga riteye imbere.

F. Ni iyihe nyungu iri mu kwitabira ubu bushakashatsi?

Kugira uruhare muri ubu bushakashatsi bigufitiye inyungu zikurikira: Ubu bushakashatsi buzafasha gusuzuma neza uburwayi hanyuma buguhe amakuru y'ukuri yateye indwara umwana wawe afite. Amakuru azavamo azafasha kandi abaganga kumenya uko bita ku mwana wawe bityo bibafashe kuzamura imibereho ye. Ku babyeyi bizabafasha kumenya ingaruka zishobora kuba ku bandi bana muzabyara. Bizagirira akamaro kandi n'abandi barwayi muhuje indwara batari muri ubu bushakashatsi.

G. Ni izihe ngaruka zishoboka zijyanye n'ubu bushakashatsi ?

Ingaruka kuri wowe nk'uwitabiriye ubu bushakashatsi ni nke. Mu ikusanyamakuru, ushobora gusabwa kudusangiza amakuru wumva yihariye kuri wowe. Ibibazo bimwe na bimwe bishobora kugukomeretsa/kugukoza isoni, ndetse singombwa ko usubiza ibibazo ibyo ari byo byose utifuza gusubiza. Bishobora kubaho ko nanone ibibazo bimwe na bimwe biguhungubanya mu mutima no mu ntekerezo. Ushobora gufata akaruhuhuko tugakomeza nyuma.Na none mu gufatirwa amaraso

ushobora kubabara, kubyimba, ariko hazafatwa ingamba kugira ngo ibyo byavuzwe haruguru bitabaho.

H. Ninde uzagira uburenganzira bwo kubona no gukoresha amakuru yanjye?

Amaraso yawe azakusanyirizwa mu ducupa twabugenewe kandi amazina yawe ntazaba ariho, kuburyo ntamuntu uri gupima amaraso ushobora kukumenya nk'umuntu ku giti cye. Tugusezeranyije mu buryo busesuye kudatangaza ibyawe. Abashakashatsi bonyine barebwa n'ubu bushakashatsi nkuko byavuzwe haruguru nibo bashobora kugera ku mubare w'ibanga uhuye n'amakuru ku buzima bwawe. Tukwijeje ko ibisubizo byawe bizafatwa mu ibanga risesuye no mu buryo butekanye ndetse n'amazina yawe ntazigera agaragara aho ariho hose mu gihe cyo gutangaza ibyavuye mu bushakashatsi kandi nta muntu numwe bizasangizwa keretse abashakashatsi bari muri uyu mushinga w'ubushakashatsi.amaraso yanyu n'ibiyagize ntibizakoreshwa mu nyungu zindi zitari izatangajwe mbere.

I. Ese nshobora kwitabira ku bushake no kwikura mu bushakashatsi

Kwitabira kwawe ni ku bushake, ushobora guhitamo kutitabira ubu bushakashatsi cyangwa kubwikuramo igihe icyo aricyo cyose, ukabimenyesha gusa ikipe y'ubushakashatsi. Turifuza kukumenyesha nanone ko niba uhisemo kutitabira ubu bushakashatsi, nta ngaruka bizakugiraho nko kudahabwa ubuvuzi usanzwe uhabwa kandi wari ubwemerewe. Niba ufashe icyemezo cyo kwikura muri ubu bushakashatsi, abashakashatsi bazakubaza niba amakuru yari yamaze gukusanywa kuri wowe ashobora gukoreshwa.

J. Ni gute nzabona amakuru ku byavuye mu bushakashatsi ?

Muri uyu mushinga w'ubushakashatsi, tugamije gusesengura ibizami by'amaraso n'amakuru, bishobora gufata amezi 6 mbere yuko tumenya ko ibyavuyemo bifite igisobanuro icyo ari cyo cyose. Niba tubonye ibisubizo ku turemangingo bifite igisobanuro cyihariye kandi kijyanye n'ubuzima bwawe, ibi bisubizo uzabihabwa tumaze kugirana ikiganiro gikwiye kirebana n'igisobanuro cyabyo.

K. Nzahemberwa kwitabira ubu bushakashatsi?

Nta gihembo kigenewe abazitabira ubu bushakashatsi. Cyakora itsinda ry'ubushakashatsi rizagenera buri mubyeyi wese waje amafaranga ibihumbi bitanu (5000 Frw) agenewe igihe yamaranye n'umushakashatsi.

L. Ni nde nahamagara igihe bibaye ngombwa?

Niba ufite ikibazo icyo aricyo cyose kijyanye n'ubu bushakashatsi, ntuzagire impungenge zo guhamagara abayobozi b'uyu mushinga w'ubushakashatsi Prof. Annette UWINEZA (+250)788741577),Dr Norbert DUKUZE (+250781268520), or Olivier HAKIZIMANA (+250788622969) cyangwa umuyobozi mukuru wa komite ishinzwe kurengera abakorerwaho ubushakashatsi muri Kaminuza y'u Rwanda ishami ry'ubuvuzi Prof. Stefan Jassen

(+250)784575900 cyangwa ugahamagara umunyamabanga wa komite Mr Sunday Francois Xavier + 250 788563311.

Kwemera kwitabira ubushakashatsi kubushake

Ibibazo byanjye kuri ubu bushakashatsi byasubijwe na----------
Nasomye(cyangwa nasomewe) ndetse numvise uruhare rwanjye muri ubu bushakashatsi, kandi nanumvise ko igihe icyo ari cyo cyose nakenera kwikuramo nabikora nta bisobanuro kandi nta ngaruka byagira mu kwitabwaho mu by'ubuzima. Kandi nemeye ko umwana wanjye yinjira mu bushakashatsi.

Ahagenewe umukono n'amakuru k'uwitabiriye ubushakashatsi

1.....
Amazina y'umwana

2..... .....
Amazina y'umubyeyi cyangwa umurera Umukono, itariki, ahantu

3. ....
Amazina y'Umushakashatsi Umukono, itariki, ahantu