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The University of the Witwatersrand

School of Public Health



PERCENTAGE POSITIVITY AND DETERMINANTS OF CYTOMEGALOVIRUS INFECTION AND IMMUNITY IN THE PUBLIC SECTOR OF SOUTH AFRICA, 2007–2021: A TIME SERIES ANALYSIS

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of MSc Epidemiology of Infectious Diseases

Johannesburg, 2023

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

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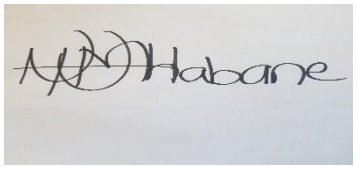

Nelisiwe Lynneth Mhlabane

6 June 2023

Dedication

To Avuyile and Sivuyile, the two beats deep within my heart, this is for you. God has fulfilled his promise.

Abstract

Cytomegalovirus (CMV) infection is common in all age groups but is more prevalent in women of childbearing age and neonates. If left untreated, CMV can cause birth defects, recurrent infections, and death. Understanding the burden of CMV disease and its risk factors is important to instigate preventive measures. Despite global research conducted on CMV, there is still a paucity of studies conducted in South Africa that focus on CMV current infection and its determinants as well as immunity to CMV. The goal of this study was to determine the prevalence of CMV infection and immunity and the factors that influence them in South Africa's public sector between 2007 and 2021.

Methods: An analytical cross-sectional study was conducted using the results of CMV tests carried out on patient samples. The included test results were obtained from the National Health Laboratory Services and included results from all South African provinces from 2007 to 2021. The data were extracted from the National Health Laboratory Services Corporate Data Warehouse. Records of participants of all age groups whose data were available at the National Health Laboratory Services Corporate Data Warehouse were obtained. Patient demographic details and laboratory results were extracted into data collection tables. Data was cleaned and analysed using Stata 17. The CMV results considered in the analysis were serological (immunoglobulin M [IgM] and Immunoglobulin G [IgG] and molecular polymerase chain reaction tests carried out for routine CMV diagnosis. Immunoglobulin M, polymerase chain reaction, and IgG seroconversion are markers of current CMV infection. A positive IgG antibody result is a marker of immunity (previous infection). A current CMV infection was defined as a positive CMV immunoglobulin M result, a CMV IgG seroconversion from negative to positive within three months of testing, or a positive CMV polymerase chain reaction result. Descriptive statistical analysis along with multivariable logistic regression analyses was used using age, sex, province, year and HIV.

Results: A total of 432,170 records were analysed for CMV infection from 2007 to 2021. Among those with available CMV IgG test results, 97.84% (190,933/197,157) tested positive. For those with available CMV immunoglobulin M test results, 5.85% (16,850/288,267) tested positive. Overall, 4.40% (19,006/432,170) of records had

evidence of a current CMV infection. Stratified analysis by sex showed similar proportions of current CMV infection for men (4.48%; 7,534/155,515) and women (4.08%; 10,649/261,310). Individuals aged 0–1 year had the highest proportion of current CMV infection at 7.35% (7,682/104,510), while those aged 2–15 years had the lowest proportion at 2.74% (979/35,686). Limpopo had the highest proportion of current CMV infections with 5.23%, (1,586/30,340), and the Western Cape had the lowest CMV current infection with 2.24% (788/35,114). In adjusted analysis, age and province were significantly associated with current CMV infection. Using individuals 0–1 year as the reference, all other age groups were less likely to test positive for CMV; the age group 2–15 years had the lowest CMV current infection (AOR = 0.34; 95% CI = 0.32–0.36). Limpopo (AOR = 1.91; 95%CI = 1.76–2.0; p = <0.0018) had the highest odds of current CMV infection while Western Cape had the lowest odds (AOR = 0.66; 95%CI = 0.60–0.73; p = <0.001). HIV infection, sex, and season were not associated with current CMV infection in the adjusted analysis.

Regarding CMV immunity, the age group 26–35 years had the highest number of individuals who were IgG positive (99.18%; 45,845/46,223). The Northern Cape province had the highest concentration of IgG-positive individuals (98.81%; 407/4,460), and the year 2012 had the largest percentage of IgG-positive individuals (98.29%; 11,607/11,809).

Conclusion: Age and place of residence (province) were associated with current CMV infection. Cytomegalovirus seroprevalence did not differ by gender. We found that a significant proportion of children are not susceptible to CMV infection. More recent data would be useful to evaluate CMV infection in the South African populace and to give a clearer idea of the epidemiology of CMV infection. Meantime,, without an effective CMV vaccine, the major preventive measure is educating people about CMV risk mitigation measures. Cytomegalovirus infection vaccines are still in the early phases of development, and our study contributes to identifying a potential target age for vaccination.

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List of Abbreviations

AOR	Adjusted Odds Ratio
CDW	Corporate Data Warehouse
CMV	Cytomegalovirus
DNA	Deoxyribonucleic Acid
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NHLS	National Health Laboratory Services
OR	Odds Ratio
PCR	Polymerase Chain Reaction
USA	United States of America
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION

1.1 Introduction

Cytomegalovirus (CMV) infection poses a major diagnostic problem because of a variety of clinical manifestations, resulting in late or incorrect diagnoses and unfavourable health outcomes [1]. It is essential to understand the frequency and risk factors for CMV infections in people of different ages and social and economic orientations because some factors can represent a period of amplified CMV transmission and acquisition. To prevent reinfection and the further spread of CMV, successful public health initiatives and control measures are required.

1.2 Background

The WHO and the Centers for Disease Prevention and Control state that CMV infects all groups of individuals regardless of age, gender, and race, with 33% of children getting infected by their fifth birthday and over 50% of adults getting infected by their fortieth birthday [2]. The prevalence of CMV increases with age as infection is persistence and life-long. Cytomegalovirus is more prevalent in immune compromised individuals and neonates [3]. Cytomegalovirus infections can be asymptomatic or severe, with fatal outcomes in immunocompromised patients, including those with HIV coinfections, pregnant women, neonates, organ transplant recipients, and blood transfusion recipients [2,4]. If untreated, it can be fatal, causing congenital disabilities, recurrent infections, and mortality [2].

Furthermore, CMV infections pose major diagnostic problems because of a variety of disease manifestations, such as hearing loss, intellectual disability, vision problems, seizures, lack of coordination, and weakness of muscles, resulting in late or incorrect diagnosis and unfavourable health effects [4,5]. Although some studies attempted to find the prevalence of CMV in pregnant women and children, not many focused on its prevalence and determinants in the general population [6,7]. Since CMV is among the major causes of ill-health, it is important to study its positivity and determinants to establish focused health promotion and preventive measures in high-risk populations, thus preventing further propagation [4,6]. With the possibility of an upcoming effective vaccine, it is vital to understand the frequency and contributing factors of CMV infections in different age, social, and economic groups because there could be

amplified CMV transmission in certain groups [2,8]. Assessments of the national distribution of CMV could further assist in understanding the burden of disease and inform ongoing vaccine development efforts [9]. Numerous vaccines against CMV are being developed, since they are essential to induce immunity in seronegative people and boost immunity in seropositive individuals [10].

1.3 Literature Review

1.3.1 Virology

Cytomegalovirus is one of the eight human herpesviruses and a member of the Herpesviridae class of viruses [2]. It is an enveloped double-stranded DNA virus that is able to induce chronic or dormant disease in the infected individual. CMV-seropositive patients develop an immune response with neutralizing antibodies targeting the envelope glycoproteins. Recently, recombinant forms of these glycoproteins have emerged as possible aspirants for subunit vaccine development [4,11].

1.3.2 Incidence and prevalence of Cytomegalovirus infection

Cytomegalovirus is a common infection globally, with an estimated incidence of 0.3%–2.3% per 100,000 persons and seroprevalence ranging between 45% and 100% [6]. In Europe, the USA, and Australia, CMV seroprevalence among the adult population varies and is estimated at between 35% and 78% [8]. Contrastingly, CMV is exceedingly prevalent in developing countries, particularly sub-Saharan Africa, with a seropositivity of almost 100% in adults [8]. Zuhair et al. conducted a systematic review and meta-analysis to estimate the global seroprevalence of CMV and estimated it to be 86%, 83%, and 87% in women of childbearing age, the general population, and organ/blood donors, respectively [3]. The highest seroprevalence was in sub-Saharan Africa (90%) and the lowest was in the European region (66%) [3]. The CMV seroprevalence surveys estimate the burden of infection through either CMV immunoglobulin G (IgG) or immunoglobulin M (IgM). IgG tests are used to detect previous CMV infection, and current infection is detected through CMV IgM testing or CMV polymerase chain reaction (PCR) tests [5]. In a study by Adane et al., 83% of samples were positive for IgG CMV antibodies, 14% positive for CMV IgM, and 24% positive for both CMV IgM an IgG antibodies [6]. In Africa, the prevalence of CMV IgG

was estimated to range from 72% to 97.5% and of CMV IgM antibodies from 0% to 15.5% [5]. A study in Western India reported that 83% had CMV IgG antibodies, 9.5% had CMV IgM antibodies, and 16% were positive for both CMV IgG and IgM antibodies [10]. A cross-sectional study in Ethiopia reported a seropositivity for CMV IgG antibodies of 88.6% and 8.2% for CMV IgM [9]. A cross-sectional study to determine the prevalence of congenital CMV infection among 302 babies born at a tertiary hospital in South Africa estimated a prevalence of 6% [8].

1.3.3 Pathophysiology

1.3.3.1 Transmission

Cytomegalovirus infected individuals may spread the virus through body fluids, such as saliva, urine, blood, tears, semen, and breast milk, which has the implications of vertical and horizontal transmission that is exacerbated by sub-clinical infection/mild disease in the majority of immune competent infected individuals [12]. The implications of CMV reinfection in congenital CMV may be more severe [4]. Cytomegalovirus vertical transmission refers to Mother-to-child transmission (MTCT) which occurs transplacental (congenital infection), during birth and through breast milk. Prevention is vital since congenital infection, is an important cause of disability due to hearing loss, impaired vision, cognitive impairment, and neuromotor deficits [2,4,12]

Horizontal transmission of CMV infection is transmitted through sexual activities in adults (semen and cervical secretions) and contact with urine or saliva from young children or saliva from adults. Infection is often transmitted among children and from young children to parents. One of the highest risk populations for acquisition of this virus is therefore an HCMV naïve pregnant woman who is a parent or caregiver of young children. [2,4,12]

Studies showed Factors associated with increased risk of CMV infection includes immune compromise (immune deficiency), low socio-economic status (lower income settings), poor hygiene (2, 4). Congenital CMV is more prevalent in populations that have a high CMV seroprevalence. Cytomegalovirus infection risk increases with individuals who engage in unprotected sexual activity with multiple partners, sharing drinks, organ transplants, and blood transfusions [4,6]. Most individuals develop natural immunity to CMV after exposure because the body produces antibodies to fight

against the virus and prevent reinfection. Primary infection is when CMV infection occurs in a previously seronegative person. Secondary infection occurs when intermittent excretion of the virus in the presence of host immunity and may be due to either reactivation of an endogenous virus or exposure to a new virus strain from an exogenous source. Reactivation during pregnancy is less likely to cause severe congenital infection compared to Primary infection. (4)

1.3.3.2 Symptoms

The incubation period for CMV transmission is between four and eight weeks [8,7]. Cytomegalovirus infections are often asymptomatic [7]; however, some individuals may experience mild symptoms. Babies with congenital CMV and who are immunocompromised at birth may present with the following symptoms: premature birth, low birth weight of less than 3 kg, yellow skin and eyes (jaundice), enlarged and poorly functioning liver, purple skin splotches and/or rash, a peculiarly small head (microcephaly), enlarged spleen, pneumonia, and seizures. It has been noted that most babies who have congenital CMV appear healthy at birth and that a few will develop symptoms over time; some symptoms can even appear years after birth. This indicates that delayed diagnosis is due to the fact that signs like hearing loss or developmental delay cannot be diagnosed early after birth because of the nature of the tests required to ascertain them [7]. People with low immune systems might experience serious problems that affect the eyes, lungs, liver, oesophagus, stomach, intestines, and brain function [7]. Additionally, general symptoms for healthy adults may include, fatigue, fever, a throat infection, and muscle aches [7].

The complications of CMV infection vary from person to person and depend on a person’s immune status. In rare circumstances, CMV causes a healthy adult to develop mononucleosis, and problems with the digestive system, liver, brain, and nervous system [1]. Weakened immune status may lead to vision loss due to inflammation of the light-sensing layer of the eye (retinitis); digestive system problems, including inflammation of the colon (colitis), oesophagus (esophagitis), and liver (hepatitis); nervous system problems, including brain inflammation (encephalitis); and pneumonia [7]. Complications in babies can include hearing loss, intellectual disability, vision problems, seizures, a lack of coordination, and weakness or problems using muscles.

1.3.3.3 Diagnosis of Cytomegalovirus

The standard and routine laboratory test for the diagnosis of CMV infection is PCR, depending on the clinical context, whereby a saliva specimen with urine is collected and tested as a confirmatory test [13]. In addition to this, the recommend tests includes using a PCR assay in blood, virus isolation by culture and CMV DNA pcr using DBS [13]. The urine confirmatory test is important because most CMV-seropositive mothers shed the virus in their breast milk, which may lead to false-positive CMV results on saliva specimens collected soon after the baby has been breastfed [14]. PCR is an extensively used, rapid sensitive method of detecting CMV that is centred on the amplification of viral nucleic acids. The methods typically target the main immediate early and late antigen genes in their well conserved regions [15]. Numerous other genes have been used as targets for the detection of CMV DNA, which can be extracted from whole blood, plasma, leucocytes, tissue biopsies, urine, and cerebrospinal fluid [11].

Serological tests that detect CMV IgM and IgG antibodies are broadly available and valuable for determining whether a patient has or has had a CMV infection [16,17]. The most commonly used serologic test for measuring CMV antibodies is the enzyme-linked immunosorbent assay [20]. A CMV IgM positive test is an indicator of recent or acute infection. A positive CMV IgG test is an indicator of a previous infection; except in children under 12 months who still have maternal antibodies circulating in their system [8]. Measuring CMV IgG antibodies in paired samples taken one to three months apart is useful to diagnose primary infection because seroconversion (first sample IgG negative and second sample IgG positive) is clear proof of current primary infection [18]. The occurrence of CMV IgM antibodies cannot be used in isolation to establish primary CMV infection since IgM can also be detected during a secondary CMV infection [18].

1.3.3.4 Treatment

Cytomegalovirus infection has no cure, but a diversity of antivirals such as valganciclovir, ganciclovir, foscarnet, and cidofovir are used to treat the infection [17]. Access to a suitable diagnosis and antiviral treatment is challenging in resource limited locations. Antiviral treatment is commonly reserved for people with either a weak immune system or a CMV viral load greater than 1,000 copies/ml [19]. Additionally,

CMV antiviral therapy is undoubtedly encouraged for those in whom aggressive end-organ disease is alleged or inveterate [20]. A preventive vaccine is the most robust imminent approach to reduce CMV-associated morbidity and mortality [21]. Two approaches have been used to develop CMV vaccines. The first vaccine is a live attenuated Towne virus, which has displayed weak protection in a human challenge study [22]. In an efficacy study [23], this vaccine failed to provide protection in CMV seronegative women of child bearing age. The second technique is an antigen centred method employing recombinant gB/MF59, which demonstrated more protection with about 50% effectiveness against CMV infections in a Phase II trial in seronegative women [7] and about 50% efficacy in controlling viremia in seronegative recipients of sero-positive organ transplants [24]. Most vaccine trials were piloted in Europe but resulted in limited success [25]. Although an effective CMV vaccine to prevent congenital CMV infections is taking precedence, its effects might be far-reaching in developing countries, especially in communities co-infected with the HIV virus [23].

1.3.3.5 Prevention

Personal hygiene is the best prevention against CMV [7]. Washing hands with soap and running water is especially important when disposing of diapers, tissues, and other items that have been contaminated with bodily fluids and when one is in contact with young children's saliva, tears, urine, or other oral secretions. In addition, CMV spreads through sharing food or drinking out of the same glass as others. Moreover, practicing safe sex by wearing a condom during sexual contact to prevent spreading CMV through semen and vaginal fluids is essential [7]. People with weakened immunity, may benefit from taking antiviral medication to prevent CMV disease. A study reported the availability of experimental vaccines being tested for women of childbearing age [2]. These vaccines may be useful in preventing the transmission of CMV infection in mothers and infants and minimising the risk of babies developing disabilities [7].

1.4 Problem Statement

Cytomegalovirus infection can be recurrent in adults and is a leading cause of ill-health among all age groups worldwide. Cytomegalovirus infections in pregnant women can be fatal and lead to congenital CMV infections in the foetus [26,27]. Several CMV vaccines are currently in clinical development, and there is a need to understand the burden of CMV to plan vaccine introduction following regulatory approval. There is

information on the prevalence of CMV infections and its determinants by continent or region, but no specific data for South Africa [1]. This study therefore sought to determine the percentage of current CMV infection (Igm, PCR and IgG seroconversion, IgG seroprevalence (immunity) and determinants of CMV infection in the public sector of South Africa to enable the implementation of tailor-made interventions to curb its spread.

1.5 Justification

Appreciating the burden of CMV current infection or infection and its determinants is a major step in establishing focused health promotion and preventive measures in high-risk populations [2,4]. With the possibility of a vaccine becoming available, it is important to understand the frequency and risk factors for CMV infections in different ages and social groups because amplified CMV transmission could occur in certain population groups [5]. The high-risk groups could possibly be the target groups for future vaccinations. Knowing the prevalence of CMV will inform policymakers on establishing target populations, especially by age, for CMV interventions, including future vaccines. Enhanced routine and focused screening can be concentrated on high-risk populations in order to control infections.

1.6 Research Question

The following research question guided the study: What is the percentage of CMV current infection, factors associated with CMV current infection and immunity in the public sector of South Africa?

1.7 Study Aim

The study aimed to determine the percentage of CMV current infections and immunity and to examine its determinants in the public sector of South Africa between 2007 and 2021.

1.8 Objectives

1. To determine the percentage of current CMV infections in the public sector of South Africa between 2007 and 2021;
2. To determine the percentage of CMV immunity in the public sector of South Africa between 2007 and 2021; and

3. To determine the factors associated with current CMV infection and immunity in the public sector of South Africa between 2007 and 2021.

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CHAPTER 2: SUBMISSIBLE MANUSCRIPT

Cytomegalovirus Percentage Positivity, Its Determinants and Immunity in the Public Sector of South Africa, 2007‑2021: A Time Series Analysis

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2.1 Abstract

Background: Cytomegalovirus (CMV) infections pose major diagnostic problems due to various disease manifestations that result in late or incorrect diagnoses and unfavourable health effects. This study aimed to determine the percentage of current CMV infections in different age groups of the public sector in South Africa and examine the risk factors for infection.

Methods: This was an analytical cross-sectional study. Current CMV was defined as evidence of CMV immunoglobulin M positive, CMV immunoglobulin G seroconversion from negative to positive, or polymerase chain reaction positive result in infants. The percentage of current CMV infection was calculated using Stata, and a univariate and multivariate logistic regression analysis was performed to determine the factors associated with current CMV infections.

Results: A total of 432,170 records were analysed for CMV for the period 2007–2021. Overall, the proportion with a current CMV infection was 4.40% (19,006/432,170). The age group 0–1 year had the highest proportion of current CMV infection at 7.35% (7,682/104,510), and the age group 2–15 years had the lowest CMV current infection at 2.74% (979/35,686). There was variability by province, and Limpopo had the highest current CMV infection proportions at 5.23%, (1,586/30,340) while the Western Cape had the lowest at 2.24% (788/35,114). In adjusted analysis, age and province were significantly associated with current CMV infection. Using individuals 0–1 year as the reference, all other age groups were less likely to test positive for CMV, and the age group 2–15 years had the lowest CMV current infection (AOR = 0.34; 95% CI = 0.32–

0.36). Limpopo (AOR = 1.91; 95%CI = 1.76–2.0; p = <0.0018) had the highest odds of current CMV infection while Western Cape had the lowest odds (AOR = 0.66; 95%CI = 0.60–0.73; p = <0.001). HIV infection, sex, and season were not associated with current CMV infection in the adjusted analysis.

Conclusion: The findings showed that age and place of residence (province) are significant risk factors for CMV infection in South Africa. This knowledge will help focus preventive measures to these at-risk population groups. The combination of preventive measures and therapeutic techniques such as health education, CMV awareness, early screening and diagnosis, and the introduction of a preventive vaccine will decrease the burden of CMV infection in South Africa.

2.2 Introduction/Background

Cytomegalovirus (CMV) is a significant public health concern worldwide with a prevalence of almost 100% in Asia and Africa and above 75% in other continents [1]. The Centers for Disease Control and Prevention and the WHO stated that CMV infects everyone regardless of age, gender, and race, with 33% of children getting infected by their fifth birthday and over 50% of adults getting infected by their fortieth birthday [2]. Cytomegalovirus infections can be asymptomatic or severe with fatal outcomes in immunocompromised patients, including those with HIV coinfections, pregnant women, neonates, organ transplant recipients, and blood transfusion recipients [1]. Cytomegalovirus infections pose major diagnostic problems due to various disease manifestations that result in late or incorrect diagnoses and unfavourable health effects [3,4].

The transmission of CMV includes contact with infected saliva, urine, and blood. CMV can also be transmitted through contact with cervical secretions and semen during sexual contact [5]. Studies showed that the risk factors for CMV infection range from immune status, living in groups, socio-economic status, age, gender, comorbidity with other sexually transmitted infections, education, sexual activity with multiple partners, sharing drinks, organ transplants, and blood transfusions [6,7]. Although some studies have been done to find the prevalence of CMV in pregnant women and children, few focused on current CMV infection positivity and determinants in the general population [8,9,10].

Since CMV causes ill-health in all age groups worldwide, focusing on CMV infection prevention is a significant step in establishing focused health promotion and preventive measures in high-risk populations, and thus, preventing further propagation [6,7]. With an upcoming effective vaccine, it is essential to understand the frequency and risk factors for CMV infections in people of different ages and social and economic orientations, some of which can represent a period of amplified CMV transmission and acquisition [5]. The high-risk groups may be the target groups for vaccination and catch-up vaccination. Therefore, the current study aimed to determine the percentage of current CMV infections in different age groups of the public sector in South Africa and examine some of the risk factors for infection.

2.3 Methods

2.3.1 Study design

This was an analytical cross-sectional study.

2.3.2 Study setting

Routine data stored at the National Health Laboratory Services (NHLS) Corporate Data Warehouse (CDW) were used. Public hospital laboratories across different provinces in South Africa perform CMV diagnostic tests and send their results to the NHLS-CDW data base. These results from 2007 to 2021 were accessed from the NHLS-CDW database and used in this study. Cytomegalovirus positive results considered in the analysis were those obtained through serological testing (immunoglobulin M [IgM] and immunoglobulin G seroconversion [IgG]) and molecular testing (polymerase chain reaction [PCR]). Records having HIV status were also reported, and viral load results were used to confirm HIV positive status.

2.3.3 Study population

The study included participants of all age groups who tested for CMV and whose laboratory results and data were available and accessible on the NHLS-CDW data base.

2.3.4 Sampling

A convenient sampling approach was used by including all test results during the study period. Cytomegalovirus infection results obtained through serological (IgM and IgG) and molecular (PCR) testing were included.

2.4 Data Collection

Secondary data were collected by reviewing laboratory results on CMV in the NHLS-CDW. Upon accessing the laboratory results, each patient's results were assigned a different study number to maintain patient anonymity. Patient demographic details (age, gender, and province) and CMV laboratory results were retrieved and entered into data collection tables. A current infection was defined as a positive CMV IgM result or a positive CMV PCR result. Participants with only a positive IgG result were classified as having evidence of past infection. Participants were considered to have had a recent infection if their CMV IgG negative result changed to IgM positive within 3 months.

2.5 Data Management

The data were checked for completeness and consistency. Duplicates were dropped. Data were extracted into Microsoft Excel 2020 and cleaned by removing missing variables, dropping variables, and re-creating variables. Data analysis was done using Stata 17 software [11].

2.6 Statistical Analysis

The study outcome variable was CMV infection. Cytomegalovirus current infection was defined as IgM pos /pcr pos and IgG seroconversion and CMV immunity defined as IgG positive test result. The outcome was binary since a result could be either positive or negative. Inconclusive (equivocal) results were included in the descriptive analysis but not in the regression analysis.

2.7 Ethical Considerations

Permission was sought from the gate keepers at the NHLS-CDW to access the data on CMV laboratory results from 2007 to 2021. Ethical approval for this study was

sought from the University of Witwatersrand Human Research Ethics Committee (reference number PR2225906).

2.8 Results

2.8.1 Patient socio-demographic characteristics

Table 1 is a summary of CMV infection by different characteristics for the period 2007–2021. A total of 432,170 records were assessed and analysed for CMV for the period 2007–2021, yielding an overall prevalence of 4.40% (19,006/432,170). About 36% (155,515/432,170) of the total records were from men. Out of the 155,515 male records, 4.84% (7,534/155,515) tested positive for CMV, compared to 4.08% (10,649/261,310) of women. Considering age, the age group of 0–1 year had the most records, accounting for 24.2% (104,510/432,170) of the total. Out of the records from the age group 0–1 year, 7.35% (7,682/104,510) were positive for CMV. Age group 2–15 years (2.74%; 979/35,686) recorded the lowest CMV current infection.

Limpopo province had 7% (30,340/432,170) records and a total CMV current infection of 5.23% (1,586/30,340), while Western Cape had 8% (35,114/432,170) records and recorded the lowest CMV current infection of 2.24% (788/35,114). The year 2021 contributed 9.5% (40,873/432,170) of records with a CMV current infection of 4.10% (1,677/40,873). The year 2007 contributed 2.6% (11,241/432,170).

Lastly, HIV results were available for a total of 395,802 records. Among these, 3.86% (1,390/36,027) tested positive for CMV. We found that HIV status was not associated with CMV infection.

Table 1: Characteristics of patients tested for CMV in South Africa, 2007–2021

		CMV positive n (%)	CMV negative n (%)	Total tested n (%)	P-value
Sex					<0.001
	Male	7,534 (4.84)	147,981 (95.16)	155,515 (36)	
	Female	10,649 (4.08)	250,661 (95.92)	261,310 (60.5)	
	Unknown	823 (5.36)	14,522 (94.64)	15,345 (3.5)	
	Total	19,006 (4.40)	413,164 (95.60)	432,170 (100)	
Age					<0.001
	0–1 yr.	7,682 (7.35)	96,828 (92.65)	104,510	
	2–15 yr.	979 (2.74)	34,707 (97.26)	35,686	
	16–25 yr.	1,719 (2.81)	59,507 (97.19)	61,226	
	26–35 yr.	2,934 (3.16)	89,899 (96.84)	92,833	

		CMV positive n (%)	CMV negative n (%)	Total tested n (%)	P-value
	36–45 yr.	2,025 (3.67)	53,090 (96.33)	55,115	
	>45 yr.	2,113 (4.19)	48,368 (95.81)	50,481	
	Unknown	1,554 (4.81)	30,765 (95.19)	32,319	
Province					<0.001
	Eastern Cape	994 (3.27)	29,438 (96.73)	30,432	
	Free State	554 (2.88)	18,672 (97.12)	19,226	
	Gauteng	3,988 (4.67)	81,485 (95.33)	85,473	
	KwaZulu-Natal	9,142 (5.06)	171,393 (94.94)	180,535	
	Limpopo	1,586 (5.23)	28,754 (94.77)	30,340	
	Mpumalanga	907 (4.04)	21,526 (95.96)	22,433	
	North West	618 (4.03)	14,699 (95.97)	15,317	
	Northern Cape	232 (3.04)	7,394 (96.96)	7,626	
	Western Cape	788 (2.24)	34,326 (97.76)	35,114	
	Unknown	197 (3.47)	5,477 (96.53)	5,674	
Year					<0.001
	2007	135 (1.20)	11,106 (98.80)	11,241	
	2008	171 (1.44)	11,672 (98.56)	11,843	
	2009	179 (1.49)	11,811 (98.51)	11,990	
	2010	384 (2.06)	18,276 (97.94)	18,660	
	2011	648 (2.39)	26,482 (97.61)	27,130	
	2012	1,064 (3.52)	29,129 (96.48)	30,193	
	2013	1,821 (5.54)	31,039 (94.46)	32,860	
	2014	2,107 (5.84)	33,979 (94.16)	36,086	
	2015	1,862 (5.50)	31,974 (94.50)	33,836	
	2016	1,608 (4.94)	30,919 (95.06)	32,527	
	2017	1,796 (5.42)	31,322 (94.58)	33,118	
	2018	1,901 (5.09)	35,422 (94.91)	37,323	
	2019	1,979 (5.09)	36,882 (94.91)	38,861	
	2020	1,67 (4.70)	33,955 (95.30)	35,629	
	2021	1,677 (4.10)	39,196 (95.60)	40,873	
HIV status					<0.001
	Positive	1,390 (3.86)	34,637 (96.14)	36,027	
	Negative	17 (4.99)	324 (95.01)	341	
	Unknown	17,599 (4.45)	378,203 (96.60)	395,802	

2.8.2 Current CMV infection

There were 432,170 patients with at least one result of CMV IgG, CMV IgM, or CMV PCR result between 2007 and 2021. A total of 4.40% (19,006/432,170) had evidence of current CMV infections, and 5.84% (16,850/288,263) of these tested positive for IgM while 6.36% (2,090/32,837) were confirmed by a positive PCR test and 0.34% (66/111,070) had seroconverted IgG results. These results are shown in Table 2.

Table 2: CMV current infection results in South Africa, 2007–2021

	IgM positive	PCR positive in infants	Seroconverted IgG result	Overall current CMV infection
Positive tests	16,850	2,090	66	19,006
Total tested	288,263	32,837	111,070	432,170
Percentage of current CMV infection	5.84%	6.36%	0.1%	4.40%

2.8.3 Cytomegalovirus Immunity status

Cytomegalovirus immunity (CMV IgG Positive test) status was assessed by measuring CMV IgG antibodies. There were 195,157 records of individual patients included in the analysis with at least one result of IgG CMV testing. Among those with available CMV IgG results, 97.84% (190,933/195,157) tested positive. The age group 26–35 years had the highest IgG seroprevalence of 99.18% (45,845/46,223), and the age group 0–1 year had the lowest IgG seroprevalence of 96.6% (33,26/34,597). The Northern Cape province had the highest IgG seroprevalence of 98.81% (4,407/4,460), and the Western Cape province had the lowest IgG seroprevalence of 96.55% (8,837/9,153). Lastly, the year 2012 had the highest proportion of IgG positive (IgG seroprevalence) 98.29% (11,607/11,809). Table 3 show the results for IgG seroprevalence.

Table 3: CMV immunity (IgG) by age, sex, province, and year for South Africa, 2007–2021

		IgG positive n (%)	IgG negative n (%)	Total	P-value
Age	0–1yr	33,26 (96.16)	1,329 (3.84)	34,597 (100)	<0.001
	2–15yrs	931 (6.02)	14,529 (93.98)	15,460 (100)	
	16–25yrs	28,189 (98.83)	334 (1.17)	28,52 (100)	
	26–35yrs	45,845 (99.18)	378 (0.82)	46,223 (100)	
	36–45yrs	26,854 (99.11)	240 (0.89)	27,094 (100)	
	>45yrs	24,260 (98.66)	330 (1.34)	24,590 (100)	
Sex	Male	65,395 (97.00)	2,025 (3.00)	67,420 (100)	<0.001
	Female	120,201	1,705	121,906	

		IgG positive n (%)	IgG negative n (%)	Total	P-value
		(98,60)	(1,40)	(100)	
Province	Eastern Cape	13,180 (97.11)	392 (2.89)	13,572 (100)	<0.001
	Free State	10,959 (97.93)	232 (2.07)	11,191 (100)	
	Gauteng	53,780 (97.68)	1,277 (2.32)	55,057 (100)	
	KwaZulu-Natal	54,187 (98.32)	925 (1.68)	55,112 (100)	
	Limpopo	21,741 (98.63)	302 (1.37)	22,043 (100)	
	Mpumalanga	13,129 (97,95)	275 (2.05)	13,404 (100)	
	North West	9,735 (98,49)	149 (1.51)	9,884 (100)	
	Northern Cape	4,407 (98,81)	53 (1.19)	4,460 (100)	
	Western Cape	8,837 (96,55)	316 (3.45)	9,153 (100)	
Year	2007	2,632 (97.63)	64 (2.37)	2,696 (100)	<0.001
	2008	1,931 (96.55)	69 (3.45)	2,000 (100)	
	2009	2,352 (96.28)	91 (3.72)	2,443 (100)	
	2010	5,806 (97.69)	137 (2.31)	5,943 (100)	
	2011	8,472 (98.02)	171 (3.45)	2,000 (100)	
	2012	11,607 (98.29)	202 (1.71)	11,809 (100)	
	2013	19,037 (98.01)	386 (1.99)	19,423 (100)	
	2014	20,674 (97.98)	427 (2.02)	21,101 (100)	
	2015	13,751 (97.66)	329 (2.34)	14,080 (100)	
	2016	14,929 (97.49)	384 (2.51)	15,313 (100)	
	2017	16,479 (97.60)	406 (2.40)	16,885 (100)	
	2018	18,399 (97.91)	392 (2.09)	18,791 (100)	
	2019	18,143 (97.68)	430 (2.32)	18,573 (100)	
	2020	17,085 (98.16)	320 (1.84)	17,405 (100)	
	2021	19,636 (97.93)	416 (2.07)	20,052 (100)	

2.8.4 Time series analysis for CMV infections by age, sex, and province

A Stata analysis was done for CMV current infections by age, sex, and province, and the results showed a higher proportion of CMV current infection in the 0–1 year age group 24.18% (104,510/432,170). Women had a higher positivity overtime compared

to men 35.98% (155,515/432,170), as shown in Table 4. Limpopo province had the highest positivity of 5.23% (1,586/30,340). Overall trends from the years 2007–2021 showed no variation in CMV current infection between age, sex, and province.

Table 4: Time series analysis table for CMV infection by age, sex, and province of origin of the CMV records for South Africa, 2007–2021

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total
Age	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
0–1 yr.	2,379 (2.28)	2,650 (22.38)	2,709 (22.59)	3,724 (19.96)	5,531 (20.39)	6,245 (20.68)	6,976 (21.23)	8,040 (22.28)	8,207 (24.26)	7,592 (23.34)	8,062 (24.34)	9,556 (25.60)	10,394 (26.75)	10,240 (28.74)	12,205 (29.86)	104,510 (24.18)
2–15 yrs.	940 (8.36)	1,034 (8.73)	1,134 (9.46)	1,585 (8.49)	2,268 (8.36)	2,644 (8.76)	2,702 (8.22)	3,226 (8.94)	2,905 (8.59)	2,900 (8.92)	2,961 (8.94)	3,164 (8.48)	3,099 (7.97)	2,419 (6.79)	2,705 (6.62)	35,686 (8.26)
16–25 yrs.	1,942 (17.28)	2,057 (17.37)	1,990 (16.60)	2,817 (15.10)	4,045 (14.91)	4,663 (15.44)	4,745 (14.44)	5,011 (13.89)	4,552 (13.45)	4,644 (14.28)	4,850 (14.64)	5,022 (13.46)	5,042 (12.97)	4,582 (12.86)	5,264 (12.88)	61,226 (14.17)
26–35 yrs.	2,591 (23.05)	2,776 (23.44)	2,878 (24.00)	4,002 (21.45)	5,540 (20.42)	6,069 (20.10)	6,728 (20.47)	7,469 (20.70)	7,224 (21.35)	7,144 (21.96)	7,456 (22.51)	8,377 (22.44)	8,275 (21.29)	7,771 (21.81)	8,533 (20.88)	92,833 (21.48)
36–45 yrs.	1,541 (13.71)	1,545 (13.05)	1,610 (13.43)	2,191 (11.74)	3,024 (11.15)	3,531 (11.69)	3,992 (12.15)	4,436 (12.29)	4,489 (13.27)	4,375 (13.45)	4,153 (12.54)	4,949 (13.26)	5,117 (13.17)	4,744 (13.31)	5,418 (13.26)	55,115 (12.75)
>45 yrs.	1,182 (10.52)	1,146 (9.68)	1,182 (9.86)	1,798 (9.64)	2,676 (9.86)	3,378 (11.19)	3,728 (11.35)	4,254 (11.79)	4,230 (12.50)	4,139 (12.72)	4,117 (12.43)	4,612 (12.36)	5,038 (12.96)	4,317 (12.12)	4,684 (11.46)	50,481 (11.68)
Unknown	666 (5.92)	635 (5.36)	487 (4.06)	2,543 (13.63)	4,046 (14.91)	3,663 (12.13)	3,989 (12.14)	3,650 (10.11)	2,229 (6.59)	1,733 (5.33)	1,519 (4.59)	1,643 (4.40)	1,896 (4.88)	1,556 (4.37)	2,064 (5.05)	32,319 (7.48)
Sex								Grand total					432,170 (100)			
Female	7,162 (63.71)	7,498 (63.31)	7,314 (61.00)	11,424 (61.22)	16,285 (60.03)	18,048 (59.78)	19,453 (59.20)	21,402 (59.31)	20,186 (59.66)	19,344 (59.47)	20,177 (60.92)	22,923 (61.42)	23,545 (60.59)	21,470 (60.26)	25,079 (61.36)	261,310 (60.46)
Male	3,697 (32.89)	3,976 (33.57)	4,329 (36.11)	6,684 (35.82)	9,961 (36.72)	10,992 (36.41)	11,922 (36.28)	13,311 (36.89)	12,412 (36.68)	12,020 (36.95)	11,814 (35.67)	13,251 (35.50)	13,933 (35.85)	12,880 (36.15)	14,333 (35.07)	155,515 (35.98)
Unknown	382 (3.40)	369 (3.12)	347 (2.89)	552 (2.96)	884 (3.26)	1,153 (3.82)	1,485 (4.52)	1,373 (3.80)	1,238 (3.66)	1,163 (3.58)	1,127 (3.40)	1,149 (3.08)	1,383 (3.56)	1,279 (3.59)	1,461 (3.57)	15,345 (3.55)
Province								Grand total					432,170 (100)			

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total
Eastern cape	986 (8.77)	1,089 (9.20)	1,187 (9.90)	1,378 (7.38)	1,757 (6.48)	2,299 (7.61)	2,586 (7.87)	2,489 (6.90)	1,934 (5.72)	1,818 (5.59)	1,768 (5.34)	2,776 (7.44)	2,757 (7.09)	2,399 (6.73)	3,209 (7.85)	30,432 (7.04)
Free State	956 (8.50)	892 (7.53)	710 (5.92)	1,011 (5.42)	1,122 (4.14)	1,375 (4.55)	1,357 (4.13)	1,356 (3.76)	1,276 (3.77)	1,099 (3.38)	1,167 (3.52)	1,477 (3.96)	1,546 (3.98)	1,680 (4.72)	2,202 (5.39)	19,226 (4.45)
Gauteng	3,377 (30.04)	3,675 (31.03)	4,153 (34.64)	4,660 (24.97)	4,978 (18.35)	4,871 (16.13)	4,645 (14.14)	5,314 (14.73)	6,192 (18.30)	6,385 (19.63)	7,037 (21.25)	8,071 (21.62)	7,624 (19.62)	6,992 (19.62)	7,499 (18.35)	85,473 (19.78)
KwaZulu-Natal	57 (0.51)	112 (0.95)	99 (0.83)	5,438 (29.14)	12,212 (45.01)	13,793 (45.68)	17,323 (52.72)	19,195 (53.19)	16,235 (47.98)	15,271 (46.95)	14,489 (43.75)	15,657 (41.95)	17,207 (44.28)	15,567 (43.69)	17,880 (43.75)	180,535 (41.77)
Limpopo	1,890 (16.81)	1,992 (16.82)	1,696 (14.15)	1,384 (7.42)	1,576 (5.81)	1,306 (4.33)	1,505 (4.58)	2,079 (5.76)	2,081 (6.15)	2,238 (6.88)	2,690 (8.12)	2,744 (7.35)	2,572 (6.62)	2,388 (6.70)	2,199 (5.38)	30,340 (7.02)
Mpumalanga	693 (6.16)	715 (6.04)	685 (5.71)	1,324 (7.10)	1,881 (6.93)	2,160 (7.15)	1,394 (4.24)	1,470 (4.07)	1,636 (4.84)	1,610 (4.95)	1,510 (4.56)	1,596 (4.28)	1,769 (4.55	1,772 (4.97)	2,218 (5.43)	22,433 (5.19)
North West	557 (4.96)	446 (3.77)	442 (3.69)	578 (3.10)	538 (1.98)	618 (2.05)	612 (1.86)	811 (2.25)	1,018 (3.01)	1,003 (3.08)	1,326 (4.00)	1,781 (4.77)	1,845 (4.75)	1,688 (4.74)	2,054 (5.03)	15,317 (3.54)
Northern Cape	310 (2.76)	267 (2.25)	315 (2.63)	292 (1.56)	503 (1.85)	688 (2.28)	524 (1.59)	569 (1.58)	657 (1.94)	701 (2.16)	621 (1.88)	588 (1.58)	504 (1.30)	506 (1.42)	581 (1.42)	7,626 (1.76)
Western Cape	1,819 (16.18)	2,024 (17.09)	2,002 (16.70)	1,766 (9.46)	1,716 (6.33)	2,212 (7.33)	2,700 (8.22)	2,689 (7.45)	2,678 (7.91)	2,286 (7.03)	2,415 (7.29)	2,514 (6.74)	2,864 (7.37)	2,493 (7.00)	2,936 (7.18)	35,114 (8.13)
Unknown	596 (5.30)	631 (5.33)	701 (5.85)	829 (4.44)	847 (3.12)	871 (2.88)	214 (0.65)	114 (0.32)	129 (0.38)	116 (0.36)	95 (0.29)	119 (0.32)	173 (0.45)	144 (0.40)	95 (0.23)	5,674 (1.31)
							Grand total						432,170 (100)			

2.8.5 Cytomegalovirus current infection by provinces

Figure 1, shows the distribution of positive CMV tests in all nine provinces of South Africa. Limpopo (5.23%) had the highest CMV current infection and the Western Cape (2.24%) recorded the lowest current CMV infection.

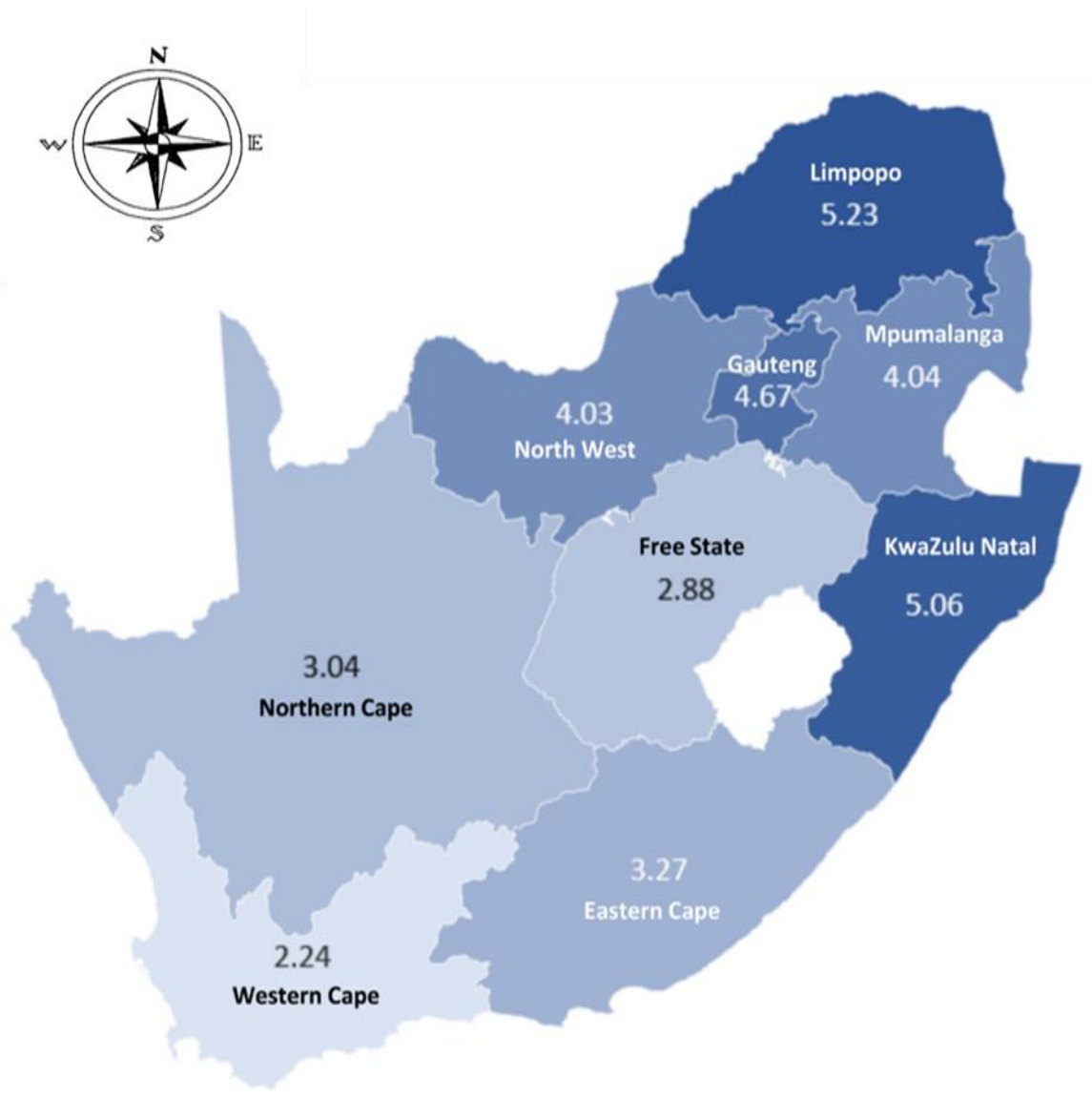

Figure 1: Map of CMV current infection by province for South Africa

2.8.6 Cytomegalovirus infection over time

Figure 2 shows a steady increase in CMV cases from 2007 to 2009 followed by a sharp decrease in 2010. In 2011, there was a sharp increase, which continued through to 2014, after which it remained almost constant until 2018. There was a sharp

decrease of cases in 2019 followed by an increase in 2020 and a further decrease in 2021.

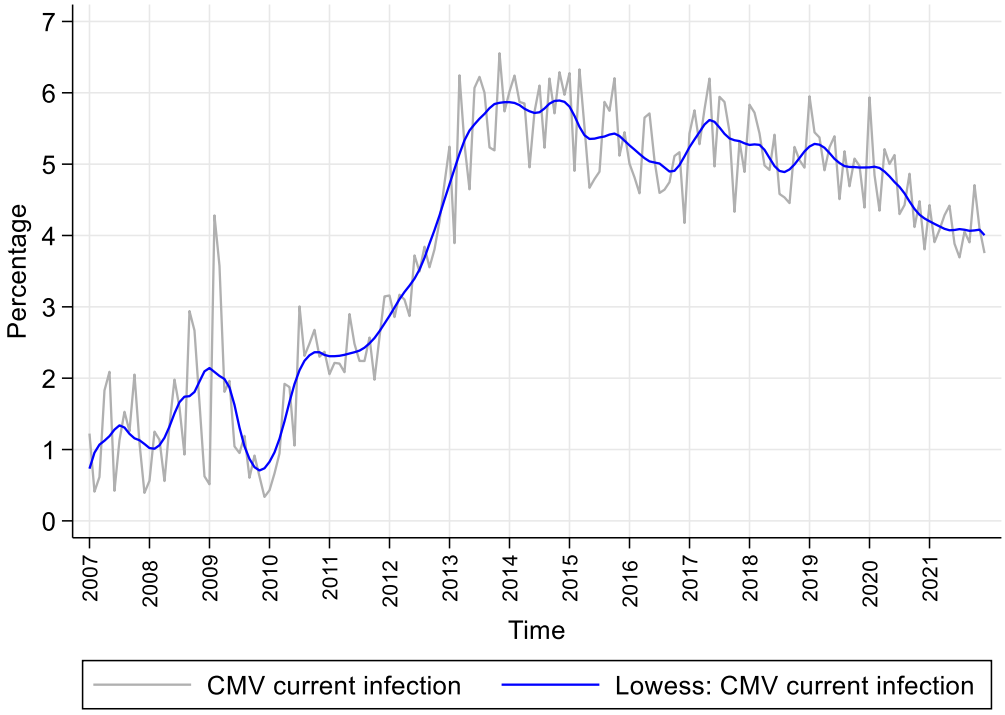

Figure 2: Description of trends in CMV current infection in South Africa from 2007 to 2021

2.8.7 Univariate analysis of association between variables and CMV (IgM and PCR) positivity

A univariate logistic regression analysis was conducted to identify individual factors associated with CMV current infection, and it showed that compared to men, women were less likely to test positive for CMV. This difference was statistically significant (OR = 0.83; 95% CI = 0.81–0.86; p = 0.001). The age group 0–1 year had the highest odds of testing positive for CMV. CMV current infection differed between provinces, and there was no clear trend in CMV current infection by provinces. Limpopo had the highest CMV current infection (OR = 1.63; 95% CI = 1.51–1.78; p = <0.001). CMV current infection was not associated with HIV status.

2.8.8 Multivariate analysis of association between variables and CMV

Multivariate logistic regression was conducted to identify factors associated with CMV current infection after adjusting for other variables. In the adjusted analysis, gender (p = 0.088) was not associated with CMV infection. Age (p = <0.001) and province (p = <0.001) were associated with CMV current infection. All age groups had a higher odd of CMV current infection compared to the age group 0–1 year, and the values of the odds ratio increased with age. Province (p = <0.001) was associated with CMV current infection.

Table 5: CMV current infection from 2007 to 2021

	Crude OR (95% CI)	P-value	Adjusted OR (95% CI) *	P-value
Sex				
Male	1	<0.001	1	0.088
Female	0.83 (0.81–0.86)		1.02 (0.99–1.06)	
Age				
0–1 yr.	1	<0.001	1	<0.001
2–15 yrs.	0.36 (0.33–0.38)		0.34 (0.32–0.36)	
16–25 yrs.	0.36 (0.34–0.38)		0.35 (0.33–0.37)	
26–35 yrs.	0.41 (0.39–0.43)		0.39 (0.37–0.41)	
36–45 yrs.	0.48 (0.46–0.51)		0.45 (0.43–0.47)	
>45 yrs.	0.55 (0.52–0.58)		0.51 (0.49–0.54)	
Province				
Eastern Cape	1	<0.001	1	<0.001
Free State	0.88 (0.80–0.98)		0.99 (0.89–1.11)	
Gauteng	1.45 (1.35–1.56)		1.54 (1.43–1.66)	
KwaZulu-Natal	1.58 (1.48–1.69)		1.35 (1.26–1.45)	
Limpopo	1.63 (1.51–1.78)		1.91 (1.76–2.08)	
Mpumalanga	1.25 (1.14–1.40)		1.37 (1.25–1.51)	
North West	1.25 (1.12–1.40)		1.40 (1.26–1.56)	
Northern Cape	0.93 (0.80–1.10)		1.13 (0.97–1.31)	
Western Cape	0.68 (0.62–0.75)		0.66 (0.60–0.73)	
HIV				
Negative	1	0.303		
Positive	0.76 (0.47–1.23)			
Year				
2007	1	<0.001	1	<0.001
2008	1.21 (0.96–1.51)		1.20 (0.95–1.51)	
2009	1.25 (1.00–1.56)		1.23 (0.98–1.54)	
2010	1.73 (1.42–2.11)		1.69 (1.38–2.05)	
2011	2.01 (1.67–2.43)		1.94 (1.61–2.35)	
2012	3.00 (2.51–3.60)		2.98 (2.49–3.58)	
2013	4.83 (4.05–5.76)		4.85 (4.06–5.79)	

	Crude OR (95% CI)	P-value	Adjusted OR (95% CI) *	P-value
2014	5.10 (4.28–6.08)		5.02 (4.21–6.00)	
2015	4.79 (4.02–5.71)		4.64 (3.88–5.54)	
2016	4.28 (3.58–5.11)		4.16 (3.48–4.97)	
2017	4.72 (3.95–5.62)		4.53 (3.80–5.41)	
2018	4.41 (3.70–5.26)		4.21 (3.53–5.03)	
2019	4.41 (3.70–5.26)		4.17 (3.50–4.98)	
2020	4.06 (3.40–4.84)		3.76 (3.15–4.49)	
2021	3.52 (2.95–4.20)		3.26 (2.73–3.90)	

2.9 Discussion

Cytomegalovirus infections are common globally and associated with fatal outcomes, especially in infants and immunocompromised patients. According to the Centers for Disease Prevention and Control and the WHO, CMV infects different age groups, and approximately 33% of children have been infected by their fifth birthday [12]. The current study was an all-inclusive national, illustrative CMV sero-survey of the population of South Africa.

Immune responses to CMV infection vary between individuals [13]. A healthy person infected with CMV is more likely to be asymptomatic [14,15]. We found that the age group 26–35 years old had the highest proportion of individuals who were IgG positive to CMV. IgG is usually considered the marker for immunity however, having a positive IgG does not mean one is immune from reinfection. A similar study conducted in Nigeria also found that the age group 26–35 years had the highest CMV IgG immunity [13]. The seropositivity for CMV IgG antibodies was found to be high in women. Contrary to our study, a study done in Nigeria reported that the seropositivity of IgG antibodies was the same in both men and women [13]. In the current study, the year 2012 had the highest percentage of people who tested positive for CMV IgG antibodies.

In the current study, multiple factors (age, sex, province, year and HIV) were investigated for their association with CMV infection. Age was a prominent independent factor connected with CMV current infection. However, other studies used a different categorisation of age groups [10,13] and reported higher seroprevalence in older ages, but the current study had higher seroprevalence in the 0–1 year age group. The increase of CMV current infection with age has been documented and is an outcome of cumulative exposure to CMV through life [14].

The findings of the current study showed that men had a higher CMV current infection than women. The proportion of positives in males was higher than females. This is contrary to most published work where CMV seroprevalence was reported to be higher in women [14–16]. Nevertheless, this shows that exposure to CMV could be moderately different among sexes. In some research to assess factors connected with CMV current infection, sex was adjusted for but not separately analysed. According to these studies, the variation in CMV current infection for gender could be due to differences in role patterns and behaviours, such as women being more involved in caring for children and physically incapacitated extended family. The variation found by our study could have been due to inconsistencies in results reporting, demographic data capturing, and differences in data quality management among provinces.

This study showed significant regional differences in the CMV current infection in South Africa, congruent with what has been reported in other countries [16]. The reason for this is unclear, and additional behavioural and lifestyle data are crucial to investigate the cause of these provincial differences [18]. The CMV current infection was high in provinces that are predominantly urban, and this could be due to the centralisation of testing platforms and resources in urban areas [19]. There are also more health facilities in urban areas than rural areas, which adds to the volume of tests conducted [20]. The trends over the years show great variations in CMV current infection. The low current cmv positivity recorded in 2007 to 2009 could be due to lack of testing platforms and poor technology compared to recent years [21]. Additionally, data recording and storage were dominantly paper based in the early years, which is prone to incomplete records and losing records [22]. The almost constant current CMV infection between 2014 and 2018 could be attributed to the decentralisation of laboratory tests in South Africa with the provision of testing platforms and reagents [23]. The sharp decrease noted from 2019 to 2020 may be due to the restrictions, lockdowns, and curfews implemented because of the COVID-19 pandemic [24,25].

Conclusion

In conclusion, this study included population-based CMV current infection data conducted on a large sample that is representative of the population of South Africa. This population-based CMV current infection time analysis is an essential source for

epidemiological modelling and a source of baseline data for longitudinal surveys in the future.

The data show that a significant proportion of children and urban populations are susceptible to CMV infection. Additional seroprevalence research with more recent and thorough data is necessary to evaluate CMV current infection in the South African population and to better understand the epidemiology of CMV infection. In the meantime, without an effective CMV vaccine, the major preventive measure is educating people about CMV risk reduction measures.

2.10 References

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CHAPTER 3: EXTENDED METHODS AND RESULTS

3.1 Introduction

This chapter explains study methods and results that were not covered in Chapter 2. This chapter firstly explores cytomegalovirus (CMV) infection seasonal variation, and secondly, CMV infection is stratified by comparing the distribution of CMV using year, age, and sex over the whole study period.

3.2 Cytomegalovirus infection by Season

We explored CMV current infection by seasons. South Africa has four seasons, namely spring in September, October, and November; summer in December, January, and February; autumn in March, April, and May; and winter in June, July, and August. Figure 3 shows that most records were from patients tested in spring (26.6%; 114,907/432,170) with a CMV current infection of 4.42% (5,080/19,006). Patients tested in winter (25.2%; 108,962/432,170) had the lowest CMV current infection at 4.33% (4,714/19,006).

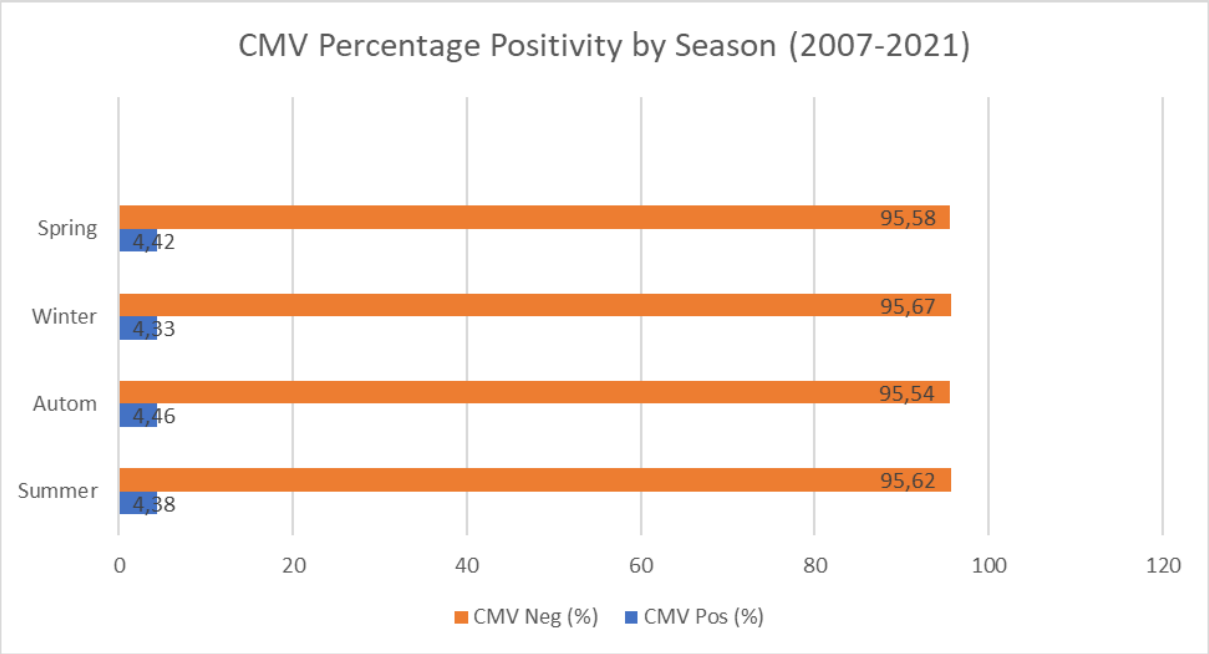

Figure 3: Graph showing CMV current infection (immunoglobulin M and PCR) for the period 2007–2021

3.3 Distribution of CMV Infection Using Sex and Age from 2007–2021

Our study also explored CMV current infection percentage difference between men and women over the years. The distribution of CMV infections by time shows that there was a sharp increase in cases in both men and women from 2007 to 2013. For both genders the cases then decreased remarkably between 2014 and 2021. Men had more current CMV infection cases compared to women for the majority of the study period (Figure 4).

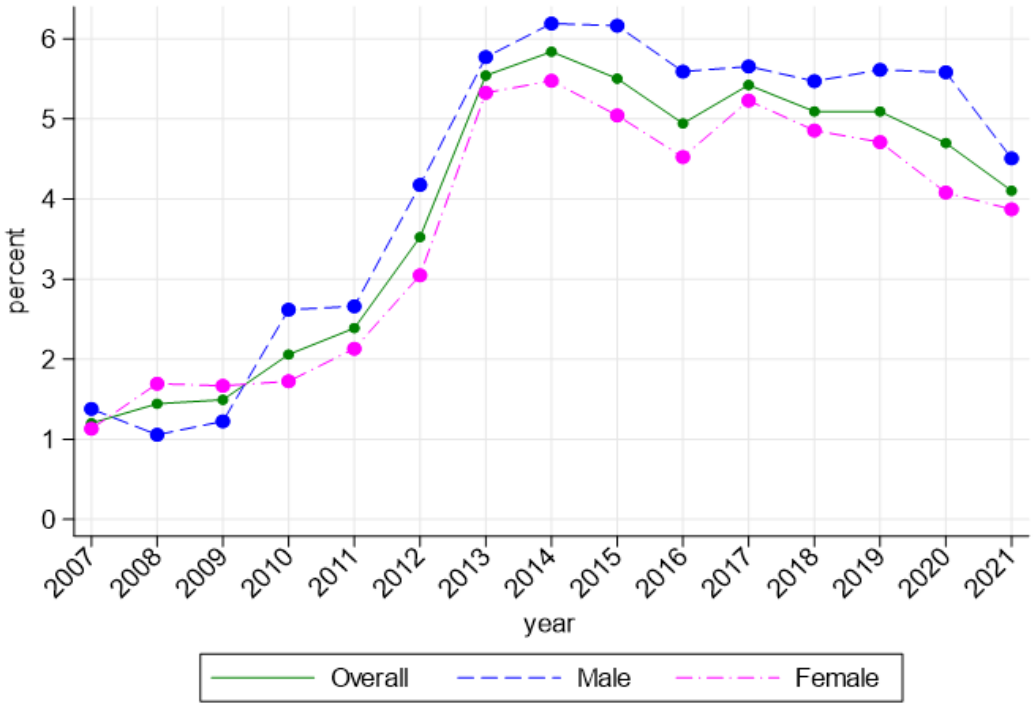

Figure 4: Trends of CMV current infection between men and women in South Africa. 2007–2021

3.4 Distribution of CMV Infection Using Sex and Age from 2007 to 2021

The current CMV infection percentage between different age groups was similar between 2007 and 2009 and ranged between 0% and 2%. Almost all age groups saw an increase in CMV current infection between 2009 and 2014. However, the age group 0–1 year old had the highest spike in CMV current infection, peaking around 11%. Similarly, almost all age groups saw a constant decline in CMV current infection from 2014 to 2021 (Figure 5).

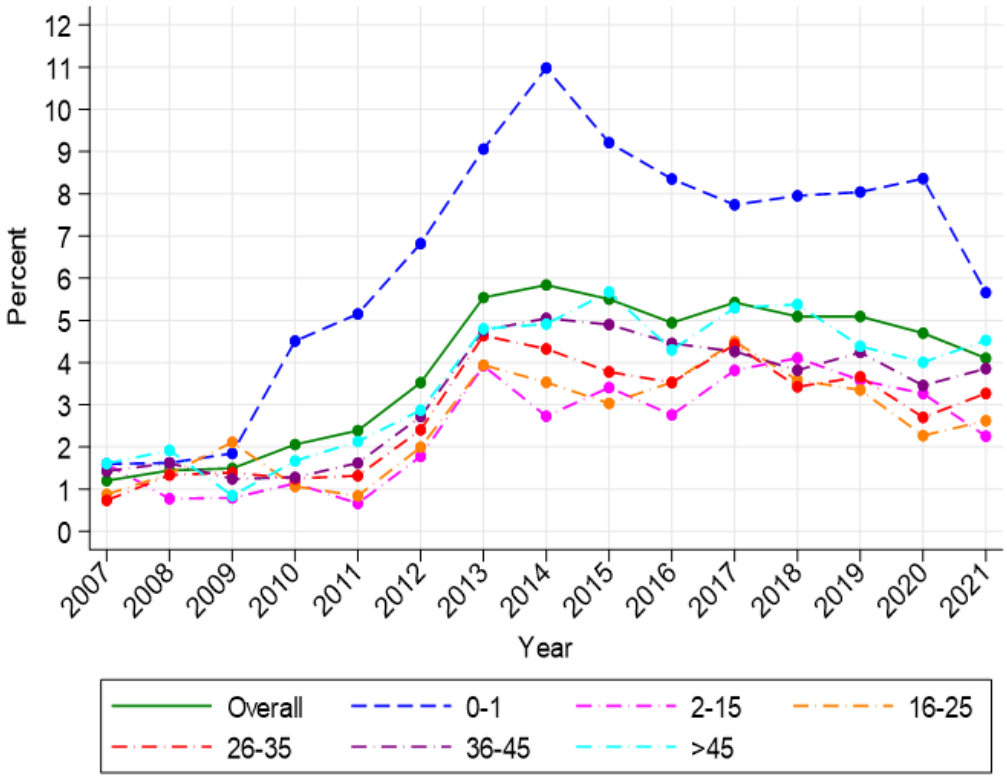

Figure 5: Cytomegalovirus current infection by year and age group in South Africa, 2007–2021

CHAPTER 4: OVERALL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

4.1 Overall Discussion

This study aimed to determine CMV (IgG seroprevalence) and current CMV infection (IgM, PCR, IgG seroconversion) in South Africa as well as its associated risk factors. Age and geographical location were associated with current CMV infection.

An interesting finding of this study was that season was not associated with CMV current infection. There was no seasonal trend on CMV current infection, but autumn and spring had the highest current CMV infection while winter had the least current CMV infection. Three studies done in Malaysia, Brazil, and South Africa had contrasting findings and reported that seasonality is a common feature of viral respiratory infections [2,3]. A study conducted in KwaZulu-Natal among children 0–5 years old reported that there was no association between CMV and season [4].

An important finding that aligns with the primary objective of the study was that current infection in our study was very low compared to what is reported in other studies which could be justified by the fact that we did not have clinical information to confirm if the data contained CMV cases which were symptomatic. It is possible that many of these tests were done for screening purposes, hence the low current CMV infection.

The findings show risk factors for CMV specific to South Africa, which will help develop preventive therapeutic techniques to ease the burden of CMV. It will also contribute noble knowledge to the field. CMV vaccines are still in the early phases of development, and our study contributes to identifying a potential target age for vaccination. The implications for clinicians and policy makers are to consider the cost-effectiveness of vaccines, since they are known to reduce CMV infection [5,6]

4.2 Recommendations

Existing CMV infection prevention measures should be enforced targeted towards prevention of CMV Congenital Infection in children. Meanwhile, it is very important for the high authorities and the department of Health to target CMV preventive measures in provinces with the high current CMV infection. CMV screening across all age groups

and health awareness about CMV infection risk reduction measures should be put into place, to reduce the burden while considering availability of resources.

4.3 Strengths of the Study

The main strength of this study is that to determine CMV current infection it used a representative population making use of South African Public Health facilities. Different demographic data enabled us to compare CMV infection trends and CMV current infection across all provinces. The Corporate Data Warehouse data covered all public health laboratory data in all provinces across South Africa. Although this was a huge data set, it was easy to clean, merge, and link the CMV data with HIV data.

4.4 Limitations

This study was not without limitations, especially because it was a retrospective study. Patient demographic details (race) and socio-economic status was missing on patient results, and this affected the analysis of the association between these variables and CMV infection and immunity. To reduce observation and selection bias, data needed to have similar laboratory conditions, methods, materials, sample type, and equipment. Moreover, having homogenous and uniform data is essential for a true reflection of the results of the analysis. It was also difficult to ascertain how primary data were collected, why data were collected, and which variables were used. Furthermore, some provinces did not record all data that were required for the analysis, such as patient sex and age, type of test done, HIV status, and results. The data set showed marked evidence that testing and reporting standards are not the same across the different provinces. Inconsistency or variable testing patterns of immunoglobulin G, immunoglobulin M, and polymerase chain reaction showed that the ability to generalise the results may not be truly representative of the whole South African population because the data only entail results of only the public sector of South Africa. Lastly, data that are analysed over a long period of time may prime bias because of change in data storage systems or data mismanagement. The study was subject to mistakes where other risk factors could have been present that were not measured, such as distinguishing whether the effect of provinces being urban or rural made it impossible to determine causation and temporal relationships. However, a weighting factor was not implemented in the analyses, leading to some bias, especially for the provincial current CMV infection in CMV current infection. Lastly, our study was

not able to ascertain why the CMV testing was done (diagnosis purposes, random testing, sub population including healthy people (Asymptomatic) or people with symptoms) which affects the external validity or the applicability of our study.

4.5 Conclusion

This study determined the CMV (IgG seroprevalence) and current CMV infection (IgM, PCR, IgG seroconversion) in South Africa and some risk factors for CMV. Age and geographical location were found to impact the prevalence of CMV. The findings showed risk factors for current CMV infection specific to South Africa, which will help develop preventive therapeutic techniques to ease the burden of CMV infection. It will also contribute noble knowledge to the field. It is essential to study current CMV infection and risks of CMV disease-specific to our population so that we may develop techniques to diagnose, manage, treat, prevent, and eradicate the disease.

4.6 References

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Appendix A: University of Witwatersrand Ethics Approval

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Ms N Mhlabane-Fakudze

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220205

NAME: (Principal Investigator)	Ms N Mhlabane-Fakudze
DEPARTMENT:	School of Public Health Division of Epidemiology and Biostatistics Medical School University
PROJECT TITLE:	*Prevalence and determinants of Cytomegalovirus infection and immunity in the public sector of South Africa, 2007-2021: a time series analysis*
DATE CONSIDERED:	2022/02/25
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr NV Motaze and Ms R Mapuroma
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/05/03

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **February** and therefore reports and re-certification will be due in the month of **February** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

2022/05/03
Date

Appendix B: Change of Title of Research Letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

10 November 2022
Person No: 2108595
TAA

Ms NL Mhlabane
Plot 130 Camel-foot Lane
P.O.BOX 2151
Matsapha
M202
Swaziland

Dear Ms Nelisiwe Mhlabane

Master of Science in Epidemiology: Change of title of research

I am pleased to inform you that the following change in the title of your Research Report for the degree of **Master of Science in Epidemiology** has been approved:

From:	**Prevalence and determinants of Cytomegalovirus Infection and Immunity in the Public Sector of South Africa, 2007-2021: A Time Series Analysis**
To:	**Percentage positivity and determinants of cytomegalovirus infection and immunity in the public sector of South Africa, 2007 - 2021: A time series analysis**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix C: Guidelines for the Pan African Medical Journal



As of November 19, 2009

Instructions for authors

This guide aims to providing support for an effective online submission process of your manuscript to the Pan African Medical Journal (PAMJ).

Outlines

Submission of a manuscript

Organisation of full-length papers

Title page

Abstract

Keywords

Abbreviations

Background

Methods

Results

Discussion

Conclusions

Acknowledgements

Competing interests

Authors contributions

Tables and figures

Additional material(if any)

References

Appendix D: National Health Laboratory Services Approval Letter

Academic Affairs and Research
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Email: babatyi.kgokong@nhls.ac.za
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05 July 2022

Applicant: Nelisiwe Fakudze
Institution: University of the Witwatersrand
E-mail Address: 2108595@students.wits.ac.za
Cell: 078 154 4668

CC: Nkengafac Motaze, Relebogile Mapuroma

Project Title: Project Title Prevalence and determinants of Cytomegalovirus Infection and Immunity in the Public Sector of South Africa, 2007-2021: A Time Series Analysis
Reference Number: PR2225906

Research Application Type(s):
1. Request for Data

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentionedresearch using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available ***without patient names***.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
5. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
6. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – https://aarms.nhls.ac.za.
7. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
8. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

pp. Dr. Babatyi Malope-Kgokong
05/07/2022

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix E: Turnitin Report

Supervisor's Signature: 

Supervisor's Signature: 

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