SEROPREVALENCE AND INCIDENCE OF TOXOPLASMA GONDII, RUBELLA AND CYTOMEGALOVIRUS AMONG NAMIBIAN WOMEN OF CHILDBEARING POTENTIAL

by

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes 3 original papers published in peer-reviewed journals and 1 unpublished paper. The development and writing of the papers (published and unpublished) were the principle responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

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ABSTRACT

Introduction:

Data on the prevalence or incidence of congenital infections in Namibia are limited. Therefore, this study aimed to determine the prevalence and model the incidence of three important vertically transmitted infectious diseases: *Toxoplasma gondii* (*T. gondii*), rubella and cytomegalovirus (CMV) infections in women of childbearing potential in Namibia.

Methodology:

Three hundred and forty-four consenting women attending public antenatal care in Windhoek were included in the study. Clotted blood was collected, and a questionnaire included demographic data, immunization and obstetric history data as well as information on the exposure to risk factors. Seroprevalences of IgG against *T. gondii*, rubella and CMV and specific IgM antibodies against CMV were determined. *T. gondii* IgM and *T. gondii* and CMV IgG avidity were determined with ELISA. Statistics: Fisher's exact test was used for categorical associations and Kruskal Wallis test for continuous variables.

Results:

Anti-*T. gondii* IgG was found in 9 (2.61%) pregnant women. There was no association of anti-*T. gondii* IgG with demographic characteristics or exposure to risk factors. Anti-*T. gondii* IgM was positive in 1 (0.3 %) woman while 3 (0.9 %) women had borderline positive results. Specific IgG avidity was equivocal and high in 33% and 67% of seropositive women.

Seroprevalence of rubella did not increase with age and the overall seroprevalence of specific IgG was 95.9%. The majority of the participants had never been vaccinated against rubella infection. The percentage of women with IgG levels of <10 IU/ml, 10-14.9 IU/ml and > 15 IU/ml were 2.0%, 2.0% and 95.9% respectively. An overall anti-rubella IgG mean level of 164.5 IU/ml (95% CI 150.4-178.7) was found in five age groups, namely 15-20 (195.8; 159-232); 21-25 (167.8; 143-193); 26-30 (165.2; 136-195); 31-35 (147.6; 116-179) and 36-47 (150.4; 107-194). Demographic factors like maternal

age, gestational age and immunization history did not show significant associations with anti-rubella IgG levels.

Seroprevalence of anti-CMV IgG among pregnant women was 100%. Eleven participants (3.2%) had a positive or equivocal anti-CMV IgM result. Specific IgG avidity was high in all of these cases. Neither maternal age nor gestational age was associated with a positive or equivocal zone IgM result.

Incidences of infections could not be modelled due to either a very high or very low prevalence across age ranges.

Conclusion:

Seroprevalence of anti-*T. gondii* IgG is much lower in central urban Namibia than in other developing countries. Investigation into specific IgM seropositivity and IgG avidity showed that pregnant women in the central region of Namibia are at low risk of vertical transmission and development of congenital toxoplasmosis.

A high percentage of pregnant women in the study were immune to rubella virus despite no history of vaccination. This is likely due to a high rate of natural infection with rubella of children before reaching child-bearing potential.

This was the first study to investigate seroprevalence of CMV in Namibia. The high seroprevalence of CMV suggests a risk of reinfection or reactivation rather than primary CMV infection in pregnancy. Further studies are needed to determine the prevalence of congenital CMV in Namibia.

ABSTRAK

Inleiding:

Data oor die voorkoms of insidensie van aangebore infeksies in Namibië is beperk. Hierdie studie het ten doel gehad om die voorkoms te bepaal en die insidensie te modelleer van drie belangrike vertikaal oordraagbare aansteeklike siektes: *Toksoplasma gondii* (*T. gondii*), rubella en sitomegalovirus (CMV) infeksies in vroue van vrugbare potensiaal in Namibië.

Metodiek:

Driehonderd vier-en-veertig instemmende vroue wat die openbare voorgeboortekliniek in Windhoek besoek het, is in die studie ingesluit. Stolboed is versamel en 'n vraelys het demografiese data, inentingsgeskiedenis en verloskundige geskiedenis sowel as inligting oor blootstelling aan risikofaktore ingesluit. Seroprevalensie van IgG teen *T. gondii*, rubella en CMV en spesifieke IgM teenliggampies teen CMV is bepaal. T. gondii IgM en *T. gondii* en CMV IgG aviditeit is bepaal met 'n ELISA. Statistiek: Fisher se eksakte toets is gebruik vir kategoriese data en die Kruskal Wallis-toets vir aaneenlopende waardes.

Resultate:

Anti-*T. gondii* IgG is in 9 (2.61%) swanger vroue gevind. Daar was geen assosiasie van anti-*T. gondii* IgG met demografiese eienskappe of blootstelling aan risikofaktore nie. Anti-*T. gondii* IgM was positief in 1 (0.3 %) vrou terwyl 3 (0.9 %) vroue grenslyn anti-*T. gondii* IgM resultate getoon het. Spesifieke IgG aviditeit was dubbelsinnig en hoog in 33% en 67% van seropositiewe vroue.

Seroprevalensie van rubella het nie met ouderdom toegeneem nie. Die meerderheid van die deelnemers het geen rubella-inentingsgeskiedenis gehad nie. Die persentasie vroue met IgG vlakke van <10 IU/ml, 10-14.9 IU/ml en > 15 IU/ml was 2.0%, 2.0% en 95.9% respektiewelik. 'n Algehele anti-rubella IgG gemiddelde vlak van 164.5 IU/ml (95% CI 150.4-178.7) is gevind in vyf ouderdomsgroepe, naamlik 15-20 (195.8; 159-232); 21-25 (167.8; 143-193); 26-30 (165.2; 136-195); 31-35 (147.6; 116-179) en 36-47 (150.4; 107-194). Demografiese faktore soos moederlike

ouderdom, swangerskapouderdom en inentingsgeskiedenis het nie beduidende assosiasies met hoë vlakke van anti-rubella IgG getoon nie.

Seroprevalensie van anti-CMV IgG in swanger vroue was 100%. Elf deelnemers (3.2%) het 'n positiewe of dubbelsinnige anti-CMV IgM resultaat getoon. Spesifieke IgG aviditeit was hoog in al hierdie gevalle. Moederlike ouderdom of swangerskapouderdom was nie positief geassosieer met 'n positiewe of dubbelsinnige IgM resultaat nie.

Insidensie van infeksies kon nie bepaal word nie weens of baie hoë of baie lae voorkoms oor ouderdomskategorieë.

Gevolgtrekking:

Seroprevalensie van anti-*T. gondii* IgG is baie laer in sentraal stedelike Namibië as in ander ontwikkelende lande. Ondersoek na spesifieke IgM seropositiwiteit en IgG aviditeit het getoon dat swanger vroue in die sentrale streek van Namibië 'n lae risiko vir vertikale oordrag en gevolglike aangebore toksoplasmose het.

'n Hoë persentasie van swanger vroue in die studie was immuun teen rubella virus ten spyte van geen rubella inentingeskiedenis. Dit is waarskynlik as gevolg van 'n hoë koers van natuurlike infeksies met rubella onder kinders voordat vrugbare ouderdom bereik word.

Hierdie was die eerste studie wat die seroprevalensie van CMV in Namibië ondersoek het. Die hoë seroprevalense dui op 'n groter kans vir herinfeksie of heraktivering as vir primêre CMV infeksie. Verdere studies is nodig om die voorkoms van aangebore CMV infeksies in Namibië te ondersoek.

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Dedication

I dedicate this study to all children born in Namibia with congenital infections which might have detrimental effects on their future development, quality of life, economic contribution and social welfare.

Silence

The gentle silence of the dandelion seed
floating on
a wisp of warm air

of the violet
opening
to the morning sun

of the motion of
the butterfly's wings
as it sucks the nectar
of a cherry blossom

The silence is broken
by the cries
of all the infants destroyed
and the anguish of their mothers
whose tears cannot reverse
what the organism has wrought

The quiet silence of the womb
harbouring an unborn child
awaiting its first breath
of deserved normality

Not the sad silence
of deafness
or
of retardation
or
of blindness
Nor of the sad silence
of the grave

How can we silence the sad silence?

Jack (2005)

This poem was published in Infectious diseases of the fetus and newborn infant, Remington JS, Klein JO, Wilson CB, Nizet V, Maldonado YA, editors, 7th ed. p vi; Philadelphia: Saunders. Copyright Elsevier (2011).

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Abbreviations

CDC:	Centers for Disease Control and Prevention
CID:	Cytomegalic inclusion disease
CMV:	Cytomegalovirus
cCMV:	Congenital cytomegalovirus infection
CNS:	Central Nervous System
CRS:	Congenital rubella syndrome
CSF:	Cerebrospinal fluid
CT:	Congenital toxoplasmosis
DBS:	Dried blood spot
DNA:	Deoxyribonucleic acid
dsDNA:	Double stranded DNA
DRC:	Democratic Republic of Congo
EIA:	Enzyme immuno assay
ELISA:	Enzyme-linked immunosorbent assay
EPI:	Expanded programme for immunization
FDA:	Food and Drug Administration
HSV:	Herpes simplex virus
EBV:	Epstein-Barr virus
HIV:	Human immunodeficiency virus
IgA:	Immunoglobulin A
IgE:	Immunoglobulin E
IgG:	Immunoglobulin G
IgM:	Immunoglobulin M
MMR:	Measles-mumps-rubella vaccination

MR:	Measles-rubella vaccination
MoHSS:	Ministry of Health and Social Services in Namibia
NCRST:	National Commission on Research, Science and Technology in Namibia
NDP 3:	National Development Plan 3
NHLS:	National Health Laboratory Systems in South Africa
NUST:	Namibia University of Science and Technology (Previously Polytechnic of Namibia
PCR:	Polymerase chain reaction
RNA:	Ribonucleic acid
RPR:	Rapid plasma reagin test for syphilis
SANAS:	South African National Accreditation Systems
SIA:	Supplementary immunization activity
SPSS:	Statistical Package for Social Sciences
T cell:	T lymphocyte
T. gondii:	*Toxoplasma gondii.* Alternatively, *T. gondii* infection is indicated as toxoplasmosis
TOP:	Termination of pregnancy
UK:	United Kingdom
USA:	United States of America
WHO:	World Health Organization

Chapter 1: Introduction

1.1 Overview of the study

1.1.1 Background

This study assessed the prevalence and incidence of three infectious agents, which are associated with congenital infection, among pregnant women attending the antenatal clinic at Windhoek Central Hospital in 2016. We were initially interested in directly assessing congenital infections among neonates born in Central Namibia in 2016. However, this was not regarded as feasible due to logistical difficulties and a limited budget not allowing for the testing of neonates shortly after birth.

The term "congenital infections" refers to those infections that are transmitted vertically with most severe complications when occurring during the period of organogenesis – resulting in congenital infections associated with abnormal intrauterine development.

Perinatal infections are infections that are transmitted just before, during or just after birth, resulting in neonatal infection that could be acute: e.g. group B *Streptococcus; Listeria; E. coli;* varicella or herpes simplex or result in chronic infection e.g. hepatitis B and C viral infections.

Congenital infection is a well-described cause of stillbirths, as well as perinatal morbidity. The epidemiology varies in different parts of the world while the burden is greatest in developing countries. Common congenital infections, associated with intrautine tranmission include *Treponema pallidum,* resulting in congenital syphilis, human cytomegalovirus, *Toxoplasma gondii,* rubella, HIV, other herpes viruses such as varicella or herpes simplex viruses and enteroviruses. Recently a newly emerging infection in the New World, Zika virus, has been found to cause congenital infections. One of the most common congenital infections in sub-Saharan Africa has been congenital syphilis. Congenital syphilis has been described as one of the most important congenital infections in Southern Africa and is being addressed by maternal screening and treatment. It was rare in most developed countries with a slight increase recently in several European countries. Despite screening programs which reduced the prevalence, in large parts of the world and specifically Sub-Saharan

Africa congenital syphilis remains a public health problem although antenatal screening programmes for pregnant women are combined with cost-effective treatment with penicillin. In developing countries, antenatal care screening needs to be strengthened by implementation of point-of-care decentralized screening and treatment and alternative innovative applications to control congenital syphilis [155,156].

The point of departure for this study was the diagnostic testing for maternal and congenital infections which were known in previous years as the TORCH screen. The TORCH screen was well-known in clinical laboratories in Namibia and appeared on the repertoire of tests offered to clinicians. The acronym "TORCH" was first used in 1971 to indicate *Toxoplasma gondii (T. gondii)*, rubella, cytomegalovirus (CMV) and herpes simplex virus. Later the ''O'' indicated other infections like hepatitis viruses, human immunodeficiency virus (HIV), varicella and parvovirus B19. In 1993, it was suggested in the United Kingdom (UK) that the term ''TORCH'' be abolished due to the unreliable nature of Immunoglobulin M (IgM) results in congenital infections. Over the last decade there has been significant improvement in diagnostic and imaging techniques [1,2].

This study aimed to investigate the prevalence and incidence of agents listed in the TORCH screen and included three infectious agents originating from the initial TORCH screen, namely *T. gondii*, rubella and CMV. Herpes simplex virus was not included due to the similarity in latency and reactivation to CMV which is also in the family of herpes viruses. *T. gondii* was of interest because the Faculty of Health and Applied Sciences at Namibia University of Science and Technology (NUST) hosts a niche research area for zoonotic diseases. Rubella is the main vaccine-preventable cause of birth defects. Cytomegalovirus is the leading cause of congenital infection and of sensorineural hearing loss in neonates.

Zika virus is the newest maternal infection associated with a high risk of congenital infection and complications. The high occurrence of babies born with microcephaly coincided with an outbreak of Zika virus in South America and the Caribbean in 2015. Subsequently systematic epidemiological studies and experimental studies in primates have substantiated Zika virus as the probable cause of microcephaly [3,4,5].

Reduction of the under-five mortality rate by two thirds, between 1990 and 2015, was one of the United Nations Millennium Development Goals (Target 4.A). This meant that health systems internationally and especially in sub-Saharan Africa had to address factors contributing to the high under-five mortality and morbidity. In Namibia, the under-five mortality rate is 49.8 per 1000 live births and the neonatal mortality rate is 21.8 per 1000 live births [6]. Namibia has identified the need to enhance the health of pregnant women and children, and to lower rates of perinatal deaths as stipulated in the National Development Plan 3 (NDP 3). So far, the Ministry of Health and Social Services (MoHSS) has managed to improve mortality rates of pregnant women and children, by mainly focusing on services rendered at primary health care centres. Much more work in this field needs to be done to enable achievement of the goals set by NDP 3. The Faculty of Health and Applied Sciences at the Namibia University of Science and Technology (NUST) responded to this need by establishing a Centre for Maternal and Child Health. The aim of this centre is mainly to investigate the burden of Group B *Streptococcus* infections in pregnant women, which is associated with high neonatal mortality due to pneumonia and meningitis and high morbidity due to neurodevelopmental sequelae in those who survive. However, infections that can be vertically transmitted through early intrauterine infection may also contribute to the burden of infectious diseases in pregnant women, the foetus and child after birth when suffering from the complications of a congenital infection.

Transmission of pathogens may occur through the placenta before birth (intrauterine), during the birth process through contact with blood and vaginal secretions (perinatally) or postnatally through breast milk.

Early diagnosis of congenital infections may allow for interventions that may modify the outcome or prevent complications [1]. Preferably, pre-conception screening programmes should be in place to identify pregnancies at risk of transmitting congenital infections, but these are found in developed countries only. For example, as part of family planning or before considering infertility treatment, it is essential that vaccination of women not immune to rubella or varicella is offered. Aggressive screening programmes in developed countries have led to a decrease in both maternal infections

and congenital infections [1] but this may be more difficult to achieve in resource-constrained settings. Well-managed screening programmes may, however, prove cost-effective in some settings.

In this project, a pilot study was conducted to determine the prevalence of toxoplasmosis among blood donors in Namibia. Results from this study were used to determine sample size for the study in the pregnant population. This study focused on pregnant women attending the antenatal clinic at Windhoek Central Hospital. The aim of the study was to establish the exposure to toxoplasmosis and CMV infection, as well as anti-rubella immunity in this study group. Blood samples were taken from voluntary, consenting participants in each of the three trimesters of pregnancy. Participants were requested to fill a questionnaire with demographic information, history of previous pregnancies and information about exposure to risk factors.

In addition to testing of serum samples for immunoglobulin G (IgG) and IgM antibodies, IgG avidity was tested to determine whether IgM positive individuals had recent infections. The avidity test assesses the combined affinity of binding between antibodies and antigens in the assay, and a low avidity indicates a recent primary infection, whereas a high avidity indicates a longstanding or secondary infection. In pregnant women, it is important to distinguish between older infections and new infections since transmission to the foetus is more likely during an early primary infection before neutralizing antibodies are present. In the case of toxoplasmosis, IgM antibodies persist for twelve to eighteen months which means that IgM antibodies alone do not indicate a current infection. This challenge can be addressed by implementing the IgG avidity test, which would distinguish a recent (low avidity) from a longstanding (high avidity) infection.

In case of a positive rubella IgM test, rubella IgG avidity is valuable in discriminating recent acute infections (low avidity) from re-infections (high avidity), as recent infections hold a much higher risk of transmission and may require termination of pregnancy.

CMV IgM assays are prone to false positivity and unless there is a high pre-test probability have low positive predictive values. When used in conjunction with avidity testing it is more informative: a positive IgM with a low IgG avidity would support recent primary infection whereas a positive IgM

and an intermediate IgG avidity may suggest the possibility of re-infection, and a high avidity may suggest possible virus reactivation.

The prevalence of toxoplasmosis in human study groups in Namibia has been documented in one previous article [7], with two more articles on toxoplasmosis in groups investigating its zoonotic potential [8,9]. Toxoplasmosis in pregnant women has not been reported, neither the prevalence of CMV infection in any study group in Namibia. The only information available on rubella immunity in Namibia is from a study done on HIV sentinel survey samples collected from pregnant women [10]. There is no national surveillance system for rubella in Namibia, but measles negative cases are referred for rubella testing as part of the routine screening for measles in the national measles surveillance programme of the MoHSS in collaboration with the World Health Organisation (WHO).

1.1.2 Benefits for Namibia

This study aimed to shed light on the extent of maternal infectious diseases in central Namibia (Khomas region). This information might guide further interventions in the health care system to improve diagnosis and treatment of infectious diseases which affect pregnant women and their foetuses. The outcome of the investigation into rubella immunity among pregnant women could evaluate vaccine coverage and further guide the immunisation programme of the Ministry of Health and Social Services. The ultimate goal would ideally be to enhance screening for infectious diseases among pregnant women, should this study identify a need for that. Effective prevention, diagnosis and treatment of infections in pregnant women would impact on the prevalence and severity of congenital infections. This may save a lot of money for the health care and social systems in Namibia in terms of management of children with disabilities.

1.1.3 Place of Research

The study was performed in Windhoek, Namibia. Samples were collected at the antenatal clinic of the Windhoek Central Hospital. This facility serves as a primary health care centre for the surrounding Khomas region.

1.1.4 Research problem / Central research theme

The screening panel for congenital infections used by local laboratories is only requested in cases of symptomatic maternal or congenital infection. Many infections in pregnant women in central Namibia may never be diagnosed, resulting in detrimental or fatal effects on the foetus. Except for Jacobs & Mason (1978) [7], no recent studies have determined the burden of *T. gondii* infection in Namibia. No information is available on the prevalence of CMV infection among pregnant women or any other population in Namibia. Only one previous study reported on rubella immunity among pregnant women in Namibia.

1.2 Overview of infections in pregnant women and congenital infections

1.2.1 Concepts in congenital infections

Pregnant women are constantly exposed to infectious agents circulating in their community. An additional risk factor is their association with young children which increases their exposure to infectious agents. Maternal infections are mainly through the oral or respiratory route and resolve with or without symptoms in immunocompetent women. If the infection enters the maternal bloodstream it can be transmitted to the foetus [11].

When microorganisms invade the maternal blood stream, four consequences are possible: (1) placental infection without infection of the foetus; (2) foetal infection without infection of the placenta; (3) neither foetal nor placental infection; (4) infection of both placenta and the foetus. Invasion of the maternal bloodstream by microorganisms is common, yet in most cases neither foetal nor placental infections result. In cases where both placenta and foetus are infected, the infection might spread from the placenta by emboli from necrotized placental tissue or through contaminated amniotic fluid ingested by the foetus [11].

1.2.2 Developmental immunology

The human foetus and neonate are susceptible to infectious agents which may not cause significant disease in immunocompetent more mature individuals, due to underdevelopment of the foetal immune system.

Host defence against specific classes of neonatal pathogens include the interaction between the foetal immune system and *T. gondii*. Information regarding the innate immune response in the foetus and neonate against the parasite is limited. Neonatal monocytes can ingest and kill *T. gondii*, and macrophages from neonates can kill or restrict the growth of *T. gondii* as effectively as adult cells. However, it is indicated that other deficiencies in the neonatal innate immune system contribute to increased susceptibility of the human foetus to *T. gondii*. Another contributing factor could be

differences in T lymphocyte (T cell) responses between the foetus or neonate and adults. Foetal and neonatal antigen presenting cells produce less cytokines to activate helper T cells [12].

The underdeveloped immune system of the foetus renders it more susceptible to infections incurred intrauterine when transmitted from the mother. The foetus and young child have high susceptibility to intracellular pathogens indicating different T cell-mediated immune responses from adults [152]. A number of viruses, including CMV, HIV and herpes simplex type 2 cause more severe or rapidly progressing disease in early life compared to later life. The immaturity of the neonatal immune system could be ascribed to defects in conventional T cells, especially CD4 T cells, and impaired dendritic cell responses. Probably in all species, immature T cells develop in the foetus with T cell receptors which are different from those found in adults [152]. Vermijlen et. al. (2010) reported that CMV infection in utero changes the T cell compartments. Foetal T cells generated in utero during CMV infection are equipped with a range of antiviral effector mechanisms, including interferon production and granule-mediated cytotoxicity. T cell clones generated from CMV-infected newborns killed CMV-infected cells and limited CMV replication in vitro [152]. Interferon and cytokines appear to be upregulated in rubella-infected human foetal cells, which could disrupt developing and differentiating cells and thus contribute to congenital defects [153].

1.2.3 Contributing factors to congenital infections and neonatal deaths

In addition to direct infectious causes of neonatal deaths, a multitude of indirect causes contribute to the high neonatal mortality rate in developing countries. These contributing factors may be socioeconomic or medical by nature and include lack of clean water and sanitation and inadequate access to high-quality medical care. If untreated, infections in newborns can fast become life-threatening or lead to adverse effects lasting for life [13]. Lack of well-managed vaccination programmes with high coverage, also play a role in child mortality and morbidity in developing countries, e.g. in the case of the preventable infection with rubella virus.

1.2.4. Prevention of congenital complications by maternal and neonatal infections

Prevention of infections in newborns can in some instances be achieved by better maternal care – preventive and curative. Furthermore, capacity to diagnose and treat neonatal infections should be built. Interventions for prevention of infectious congenital diseases can be classified as follows:

(1) primary prevention of maternal infection during pregnancy;

(2) secondary prevention, or reduction of risk of foetal infection and disease once maternal infection has been acquired in pregnancy; and

(3) tertiary prevention, defined as reduction in the risk of neonates being affected by the agent once infected [14].

Approved treatment regimens for pregnant women are not always available, once maternal CMV or rubella is diagnosed no treatment has been shown to change the disease course. No therapies have been approved to treat intra-uterine CMV infection. In some cases, commonly used medication is toxic to the foetus when administered to pregnant women. In other cases, like rubella, congenital infections can best be prevented by well-managed vaccination programmes where vaccines are available. Education of pregnant women on lifestyle interventions like good hygiene is sometimes the best practice to prevent congenital infections. In France, reduction of maternal toxoplasmosis has greatly been attributed to better education regarding hygiene practices to prevent infection [15]. Behavioural strategies are also recommended by Centers for Disease Control and Prevention (CDC) to prevent CMV infection [16]. Promotion of hygiene is a cost-effective way of preventing infectious diseases [17].

1.3   Toxoplasma gondii

1.3.1 Parasite description

Toxoplasma gondii (*T. gondii*) is an apicomplexan protozoan parasite with the only definitive hosts being cat species. Genetic diversity among *T. gondii* includes three lineages, namely type I, II and III which predominates in different combinations in geographical areas. *T. gondii* exists in three forms outside the cat intestine, namely the oocysts, the bradyzoites and the tachyzoites [18].

1.3.2 Morphology and biology

Tachyzoites are oval with one end pointed and one end rounded. They are 4-8 µm long. Tachyzoites require an intracellular habitat to survive and multiply, and very few survive the gastric environment of the host. Once in a vacuole in the host cell the parasites multiply by nuclear divisions and form a large collection within the host cell. During the acute stage of infection tachyzoites invade every kind of mammalian cell [18].

During the first initial acute phase after ingestion of the parasites, tachyzoites prevail which are responsible for parasitaemia and systemic infection. The second phase is characterized by the transformation of rapidly dividing tachyzoites in slowly dividing bradyzoites. In the third stage cysts in tissues represent the latent phase of infection. The tissue cyst is formed within the host cell and may be up to 200 µm in size. Cysts occur in any type of tissue, but are found mainly in brain, heart and skeletal muscle tissue where they persist for life to form a latent infection. Cysts in the brain are found within neurons [18].

Oocysts are shed by cats and excretion may last up to two weeks. In 1-5 days, the oocysts sporulate and become infectious. In a favourable environment like warm, moist soil they may remain infectious for up to a year [18].

1.3.3 Epidemiology in Africa

Prevalence of toxoplasmosis varies widely in different populations, and this may be due to environmental factors or differences in culture and eating habits [19]. While the prevalence of toxoplasmosis among pregnant women in Austria has declined from 50% at the end of the 1970s to

35% in recent years and declined in humans and pigs in the United States of America (USA), recent reports on seroprevalence in Africa remain scarce [20,21]. Since the first report on the prevalence of *T. gondii* in Africa between 1971 and 2012, there has been a decline in seroprevalence from the northern to the southern, and from the western to the eastern regions of the continent. Toxoplasmosis rates are highest in the northern part of Africa while there has been a decreasing trend over time in northern, southern and eastern Africa [22]. While a considerable reservoir of *T. gondii* prevails among wild animals, toxoplasmosis remains a threat to the health of humans, especially immunocompromised individuals like HIV positive patients [23,24].

Table 1 summarizes the seroprevalence of *T. gondii* in developing countries.

Table 1. Prevalence of Toxoplasmosis in developing countries

Country	Year Published	Seroprevalence %	Population	Method	Reference
Sudan	1991	41.7	General population	LAT	Abdel-Hameed [37]
Tanzania	1995	35	Pregnant women	Sabin-Feldman dye test	Doehring [38]
Benin	1995	53.6	Pregnant women	ELISA	Rodier [28]
India	2004	IgG 45 IgM 3.3 Low avidity in 1.1	Pregnant women	ELISA	Singh [27]
Ethiopia	2007	60	Urban	MDAT	Negash [32]
Nigeria	2007	20.8	Healthy individuals		Uneke [36]
Tanzania	2009	46	Occupationally exposed	LAT	Swai [39]
Brazil	2010	0.06	Neonates	DBS, ELISA	Neto [25]
Brazil	2010	IgG 62 IgM 3.4	Pregnant women	Patient records	Dos Santos Goncalves [26]
Gabon	2010	56	Pregnant women		Mickoto [33]
Mozambique	2010	18.7	Pregnant women	ELISA	Sitoe [34]
Nigeria	2011	IgG 32.6 IgM 7.6	Pregnant women	ELISA	Deji-Agboola [35]
Egypt	2012	IgG 67.5 IgM 2.8 0.3 low avidity	Pregnant women	ELFA	El Deeb [31]
Benin	2014	IgG 30 IgM 0.4	Pregnant women	ELISA	De Paschale [29]
Congo	2014	IgG 80.3 IgM 4.4 11.8 low avidity	Pregnant women	ELFA	Doudou [30]
Ethiopia	2015	18.5	Pregnant women	LAT	Awoke [40]

In 1978 Jacobs and Mason reported seroprevalence of antibodies against *T. gondii* in 12% of blood donors in Windhoek area, while a study in South Africa reported toxoplasmosis in 9.8% and 6.4% of HIV-positive and HIV-negative subjects respectively [7,41].

Information on the prevalence of congenital toxoplasmosis remains scarce and it is not well-documented, especially in developing countries. In 2001 data from the New England Regional Newborn Screening Program suggested congenital *T. gondii* infection in 1 in 10 000 live births. Given about 4 million births in the USA each year, an estimated 400-4000 infants are born each year with congenital toxoplasmosis [42]. Torgerson & Mastroiacovo (2013) used mathematical modelling and information from a literature review to estimate the global burden of congenital toxoplasmosis (CT). The global estimated incidence of congenital toxoplasmosis (CT) is 1.5 cases per 1000 live births or 190 100 annual cases. The European CDC reported a rate of 288 cases of CT in Europe in 2015 [151]. The incidence of CT in the WHO Afro E region is 2.4 per 1000 live births or 37 000 cases annually [43].

1.3.4 Lifecycle and transmission

T. gondii is an obligate intracellular parasite with the only definitive hosts being cat species [18]. Sexual reproduction only takes place in the intestine of the cat family, where oocysts are produced after ingestion of any of the three forms mentioned. Within 3-15 days from infection gametocytes appear in the small intestine. After zygote and oocyst formation no further development takes place in the intestine of the cat. Oocysts are passed out of the gut with the faeces with peak oocyst production between days 5 and 8. Oocysts are shed in the faeces for a period of 7-20 days. Up to 10 million oocysts might be shed in one day. When sporulated oocysts are ingested they are infective to the intermediate hosts and give rise to the extra-intestinal forms. Tissue cysts form in muscle and brain tissue of the host, which in the case of rodents might serve as food source for the definitive host. Humans can be regarded as accidental dead-end intermediate hosts and could be infected by accidently ingesting cat feaces, or through contaminated food. Proper storage and cooking of meat should be effective to prevent infection in humans. Oocysts do not sporulate below 4° C or above 37° C. Freezing and thawing, heating above 66° C, and dessication destroy tissue cysts in meat products [18].

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Infection occurs via contamination of soil and water and the parasite can be found in all mammals, some reptiles and even sea otters. Humans are intermediate hosts. Infections in humans occur through ingestion of raw or undercooked meat, unpasteurised milk or contaminated water or living with cats and inadvertently ingesting eggs from cat faeces. It can also be acquired through organ transplant or blood transfusion. Toxoplasmosis can be transmitted from a mother to a foetus and cause deafness, blindness, mental retardation, physical impairment or even stillbirth. If a pregnant woman is diagnosed with *T. gondii* infection, treatment is available although the success of the treatment will depend on a number of variables [18].

Figure 1 depicts the life cycle of *T. gondii* with the different forms of the parasite and how it can be transmitted to a foetus [150].
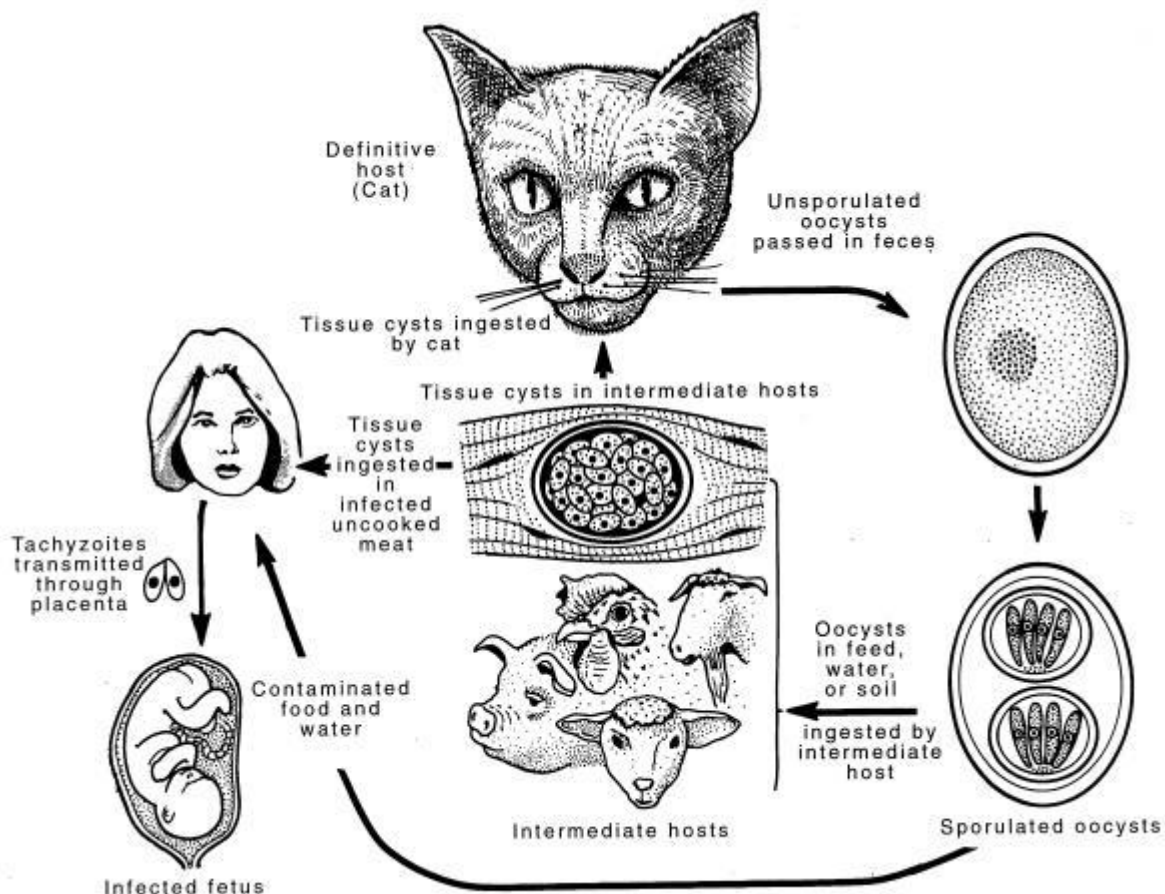
Figure 1. Life cycle of *Toxoplasma gondii* [150]

1.3.5 Immune response to *T. gondii* infection

After local invasion through the intestine, the organisms are either phagocytosed or they invade cells directly where they form intracellular cysts. Human monocytes and neutrophils kill the majority of parasites, but some may persist within macrophages which move into the tissues. *T. gondii* invades every organ and tissue of the human host. Once there are too many parasites in an intracellular vacuole, the cell disrupts. Tissue destruction is limited by cell-mediated, as well as humoral immune responses. The intracellular location of parasites mostly protects them against antibodies and cysts persist for life [18].

In immunocompetent patients *T. gondii* induces a strong immune response. During infection, *T. gondii* spreads through the blood circulation and establishes in cysts in organs like the brain and heart. The first response to acute infection is by the innate immune system and is mediated by cells like neutrophils, monocytes, macrophages and dendritic cells which release interleukins (IL-12). This is followed by an adaptive immune response where CD4 T cells facilitate a strong response by CD8 T cells releasing interferons which suppress intracellular parasite replication. The parasites are able to evade the host immune response by: (i) modulating host signalling pathways by down-regulating interleukin (IL-12) and by blocking up-regulation of genes responsible for production of interferon; (ii) inhibition of apoptosis in host cells infected with *T. gondii*; and (iii) evading intracellular death in neutrophils and macrophages [145]. Following acute infection is the latent infection characterized by exhaustion of the CD4 T cell and CD8 T cell responses towards the parasite [142], resulting in clinical recovery in most individuals. However, the infection is not erradicated and cysts persist as it enters a latent stage, which in immunocompetent women would not result in vertical transmission.

1.3.6 Pathology

Cysts formed in the brain tissue may rupture and form new cysts in the nearby tissue through the "leakage" of bradyzoites. The necrosis of brain tissue seen in newborns with CT is characterised by lesions in the cortex and basal ganglia. Zones of necrosis may undergo calcification. In the eye, the parasites first lodges in the retina and causes inflammation followed by destruction of the tissues in the retina and optical nerve [18].

1.3.7 Congenital toxoplasmosis

The second or subacute stage is considered of importance in the development of congenital toxoplasmosis [18]. Congenital transmission of *T. gondii* from an infected mother to her foetus was the first form of vertical transmission of any infection to be described in the literature. Immunocompetent and immunocompromised women contracting *T. gondii* infection for the first time, during pregnancy run a risk of transmitting the infection to the foetus due to the parasite crossing the placenta from the maternal blood. A host that is immunocompetent acquires life-long immunity against toxoplasmosis when infected with *T. gondii*. A new *T. gondii* infection contracted 4 – 6 months before conception will usually not result in vertical transmission of toxoplasmosis on subsequent exposures. In immunocompromised women who are HIV positive, *T. gondii* infection may reactivate with resulting vertical transmission [19,44].

Only about 30% of infants born to mothers who had seroconversion in pregnancy acquire prenatal infection. If the infection in a pregnant woman is not treated, the risk of intrauterine infection increases with gestational age, i.e. from 14% for primary maternal infection in the first trimester, to 59% for primary maternal infection in the last trimester. [19]. The later in gestation of primary infection, the higher the risk of transmission to the foetus. However, when transmission occurs early in gestation the risk of symptomatic infection and severe disease is high [45].

The highest risk for a mother of giving birth to a child with severe congenital infection is when maternal infection occurs at 10-20 weeks of gestation. When maternal infection is acquired after the 24th week of gestation congenital infection is frequent but mainly mild [18].

Reactivation of latent infection causes symptoms in immune compromised patients [19]. Given the high prevalence of HIV infection in pregnant women in Africa, it is advisable to follow pregnancies and CT infections in women who were seropositive at conception as well. A study in Brazil found that among 49 foetuses born to HIV-1 infected mothers with chronic *T. gondii* infection, 3 children were diagnosed with CT. It was suggested that the CT could be attributed to reactivation of maternal *T. gondii* infection during pregnancy. However, there was no difference in the rate of vertical transmission between HIV-1 infected and uninfected pregnant women in Brazil [44].

1.3.8 Symptoms and signs

Most healthy people, including pregnant women, are asymptomatic. Mild flu-like symptoms may occur and may be accompanied by symptoms of infectious mononucleosis-like lymphadenopathy [2]. Effects on the foetus vary with different stages of primary maternal infection with *T. gondii* and are more severe when the pregnant woman is infected early in pregnancy. Clinical signs of toxoplasmosis in neonates include the classical triad namely intracranial calcification, hydrocephalus and chorioretinitis. About 10% of prenatal infections result in abortion or neonatal death. The surviving infants suffer from CT presenting with progressive neurologic complications like mental retardation, learning problems, hearing and visual impairment or seizures and require special management with regards to residency and education. Most children (70-90%) who have been infected before birth might be asymptomatic at birth, but will develop complications associated with toxoplasmosis (chorioretinitis, motor and cerebellar dysfunction, microcephaly, seizures, mental retardation or sensorineural hearing loss) later in life [1,19,46,47]. More severe signs and symptoms usually indicate *T. gondii* infection early in pregnancy, while infections contracted in the third trimester of pregnancy show no symptoms at birth [1]. Clinical trials indicate that early treatment of these children can decrease manifestations of disease and improve the quality of life [19].

1.3.9 Diagnosis of toxoplasmosis

Serological screening of pregnant women is an effective way to prevent transmission of toxoplasmosis to the foetus and practical approaches based on serologic data have been proposed. Villard et. al. (2016) proposed two approaches to serologic testing, with the first including fast or automated screening while the second is used for confirmatory testing [48]. In the USA IgG and IgM antibodies against *T. gondii* are determined with enzyme-linked immunosorbent assays (ELISA) and in cases of seroconversion followed up with amniocentesis and polymerase chain reaction (PCR) to determine whether the foetus had been infected. Laboratory testing for toxoplasmosis in pregnant women poses a specific challenge since it is difficult to distinguish a past infection from a new infection due to sustained persistence of IgM (up to two years after acute infection) antibodies in some persons [1,47]. Additional IgM may be produced when dormant *T. gondii* is reactivated or when a person has a chronic infection [49]. Use of immunoglobulin A (IgA) and immunoglobulin E (IgE) *T.*

gondii antibodies have been evaluated but the recommendation is that determination of *T. gondii* specific IgG avidity in patients with detectable IgM antibodies is the most accurate way to determine the stage of infection [50].

Antibody avidity is the compound value for binding strength among a pool of specific antibodies and antigens. It normally increases as an immune response proceeds and matures. Avidity testing has diagnostic value, especially when a decision needs to be taken about the risk of neonatal infection during pregnancy. For determination of specific IgG avidity commercially available enzyme-linked immunosorbert assays (ELISA) are used. Chaotropic agents such as urea reduce effective binding between specific antibodies in patient's serum and antigens coated in the wells of the ELISA plate and are employed to break the antigen-antibody bonds. Addition of urea would therefore indicate the strength of the antigen-antibody binding when compared to the addition of a buffer. Diluted patients' serum is run in duplicate following the protocol from the manufacturer. An additional incubation step is inserted with either urea or phosphate buffered saline (PBS) added to the antigen-bound antibodies from the patient's serum in the respective wells. The avidity result is obtained by dividing the values for Absorbances (Urea/PBS) for duplicate tests run with and without urea incubation. Ratios of 0% to 100% could be obtained and clinical interpretation (low, equivocal and high) are made according to manufacturer's recommendations, preferably based on validation against a reference panel [146]. Low avidity would indicate a recent infection with low binding strength between antibodies and antigens while a past infection would be characterised by high avidity. Liesenfeld et. al. reported that the IgG avidity test on a single sample taken in the first trimester of pregnancy has great value, as being indicative of recent infection, in the diagnosis of *Toxoplasma gondii* infection in pregnant women [51].

A challenge with the IgG antibody test in pregnant women is that during reactivation of *T. gondii* in HIV-infected women, IgG does not show a rise in titre even when the infants are infected [44]. An alternative strategy is to screen neonates for CT and in the USA, this is mostly done on dried blood spots which are processed for use in ELISA or PCR [19].

Recently, Interferon-gamma release assay (IGRA), a T-cell-based test, was introduced as an in vitro test for detection of *T. gondii* infection. Few studies have investigated the potential role of cellular

immunity in diagnosis of toxoplasmosis. IGRA accurately distinguished infected from uninfected individuals, showing strong lymphocyte activation after in vitro stimulation with *T. gondii* antigens, even during the first days of life. IGRA is an easy-operation and low-cost method to measure cell mediated immunity against *T. gondii*. The results of a review underline the importance of evaluating cellular immunity to establish an early diagnosis particularly for congenital toxoplasmosis. Therefore, ELISA-based IGRA holds the potential to become a useful diagnostic tool for early detection of *T. gondii* infection [154].

1.3.10 Treatment

Most healthy individuals with toxoplasmosis do not require treatment. Treatment may however be required for people with ocular disease, people with compromised immune systems and in pregnant women and neonates. The folate pathway, involved with DNA synthesis, is the main target for anti-*T. gondii* drugs. Drugs often used are pyrimethamine and sulfadiazine, plus folinic acid to limit toxic effects. Pyrimethamine and trimethoprime inhibits an enzyme which is necessary for synthesis of thymidine. It has been used since the 1950's as antimalarial drug but was discontinued due to resistance which developed in malaria parasites. Anti-malarial drugs have been used for treatment of *T. gondii* infection since both organisms are protozoan parasites. Unfortunately, the drug has limited seletctivity for the folate metabolic pathway of the parasite and also affects the human host. Taken alone, pyrimethamine has limited potency, thus it is administered in combination with sulfonamides which block another enzyme in the folate pathway [157].

Current treatment regimes have side effects due to myelotoxicity. This is even worse for patients who have been congenitally infected who usually need prolonged treatment. Most of all, no current drug is able to eliminate *T. gondii* cysts from the infected host, which remain quiescent, provided that the immune system is strong enough to hamper their reactivation into tachyzoites [157].

Current treatment options available for *T. gondii* infection are limited and many have drawbacks of high toxicity and low tolerability. No Food and Drug Administration (FDA)-approved treatment is available for pregnant women, who are a high-risk population due to transplacental transmission.

There is therefore a need for clinical studies of investigational compounds, which demonstrate *in vitro* growth inhibition of of the parasite [52].

Administration of antimalarial drugs to pregnant women to treat *T. gondii* infection during gestation is a challenge. Pyrimethamine inhibits synthesis of folic acid, thus producing reversible depression of the bone marrow. Reversible neutropenia is the most frequent toxic effect, although platelet suppression and anaemia may occur as well. Folinic acid has been used to protect the bone marrow from toxic effects of pyrimethamine [18]. Pregnant women are routinely given folic acid supplementation to prevent teratogenic risks like neural tube defects in their infants. However, high doses of folic acid counteract the effect of sulfadoxine-pyrimethamine. Therefore, it is recommended by CDC that women take only the recommended 0.4 mg daily dose of folic acid.

Sulfonamide administration to pregnant women is controversial since it is believed to be teratogenic. Sulfonamides readily cross the placenta and thus could act as folate antagonists in humans. Folate is critical for the development of organs, especially neural tube development. Therefore, there is concern about use of sulfonamides early in pregnancy. However, it remains unclear from previous studies whether exposure to sulfonamides during pregnancy increases the risk of congenital anomalies. A comprehensive study from 2001 to 2008 among 500 000 pregnancies in the USA found no greater risk of congenital anomalies among infants exposed to pyrimethamine-sulfonamide during the firs trimester than among those exposed to non-teratogenic anti-bacterials (penicillin or cyclosporin) or in absence of any drug exposure during the same time period [158].

Prenatal treatment is aimed at prevention of materno-foetal transmission of parasites and/or reduction of foetal damage [157]. The benefit of prenatal treatment is debated in the literature. A prerequisite is to know the accurate time of maternal infection, which is achievable only in countries with serological screening programmes in pregnant women, i.e. a limited number of European countries [157]. Women in these countries infected during pregnancy (or around conception) are generally offered spiramycin, a potent macrolide antibiotic that concentrates in the placenta but does not cross it. It has a low rate of adverse effects but is not effective for treatment of established foetal infection. Treatment with pyrimethamine-sulfonamide then follows after 16 weeks of gestation onwards, when foetal infection has been established with amnioscentesis and PCR [157].

Spiramycin has been used in foeto-maternal toxoplasmosis prevention and treatment in Canada, Latin America and Europe for decades but is classified as "experimental therapy" in the U S. Spiramycin monotherapy is effective if administered early in pregnancy as a preventive measure but not after foetal exposure to the infection. The efficacy of other foetal-maternal treatments like azithromycin, clarithromycin, dapsone and cotrimoxazole is still being debated. In France 24% of children born to infected women who were treated with pyrimethamine and sulfadoxine were diagnosed with congenital infection [19].

New therapies for toxoplasmosis are being investigated and include quinone derivatives, which are fairly safe and effective treatment against protozoans like malaria and *T. gondii*. It targets mitochondrial respiration in the parasites. When treated with quinoline, 40-45% of parasites lost their apicoplasts [53]. Atovaquone has however, not been approved for use in pregnant women. Another compound for possible use in pregnant women is diclazuril (a triazinone antiprotozoal) which targets the apicoplast in *T. gondii*. An apicoplast is an extra-nuclear deoxyribonucleic acid (DNA) organelle, which is essential for survival of the parasite [19]. Without the apicoplast the parasites cannot invade the host cells.

For congenitally infected infants identified before birth, either symptomatic or asymptomatic at birth, postnatal treatment for 12 months with pyrimethamine and sulfadiazine is indicated. Folinic acid is also administered to counteract toxic effects of the medication on red blood cell production. Children should be followed up with serial ophthalmologic, auditory and neurologic examinations after treatment is discontinued [1].

1.3.11 Prevention

Congenital toxoplasmosis can best be prevented by ensuring that pregnant women who are seronegative do not get infected, or by treatment of pregnant women who are diagnosed with a primary infection as reflected in seroconversion. Regulated routine screening programmes for toxoplasmosis have been introduced in many European countries (Austria, France, Italy and Portugal) and the USA (only recommended, not routine). These include either diagnosis of primary

maternal infection with *T. gondii* during pregnancy or detection of congenital infections in neonates at birth [19].

There is no vaccine available for *T. gondii*. Especially pregnant women and immunocompromised individuals should be educated on life-style habits, which can prevent infection with the parasite. These include proper cooking of meat, washing of hands after handling meat, washing of fruit and vegetables, and special precaution when handling cat litter. Despite the challenges mentioned in Section 1.3.10, some European countries do administer treatment to infected pregnant women to prevent congenital infections and complications. Infection in foetuses can be prevented by serologic testing to identify women at risk, and spiramycin treatment during pregnancy, which lead to an approximate 60% reduction in contracting the infection by the foetus, and also to a marked reduction in illness in the foetus and infant [18,42]. In European countries where prenatal screening for *T. gondii* is mandatory by law (France, Austria, Belgium), rates and severity of congenital infection have been reduced. For populations with greater than 1 case of congenital toxoplasmosis per 10 000 live births, (or 2 infected mothers per 10 000) maternal serologic screening is a cost saving strategy. It is also argued that prevention options like preconception education reduce the prevalence of *T. gondii* in these countries [42,144].

1.3.12 Risk factors

Risk factors for acquiring *T. gondii* infection include the following: working with or ingesting raw or undercooked meat, not washing raw fruit and vegetables, and handling cat litter. Keeping cats and dogs are risk factors according to Cong et. al. (2015). *T. gondii* can also be acquired through blood transfusion or organ transplantion [54]. Poor obstetric history has also been associated with an increased risk of *T. gondii* in pregnant women: toxoplasmosis was found to be more common in women who had two or more abortions than in women with no history of poor obstetric outcomes [55].

1.4   Rubella virus

1.4.1 Virus description

Rubella is a single stranded ribonucleic acid (RNA) virus [1]. It is a member of the *Togaviridae* family [56]. The term "toga" refers to the structure of the virus which looks like it carries a cloak in the form of an envelope around the core containing RNA.

1.4.2 Morphology and biology

The rubella virus particle is generally a spherical structure, 50-70 nm in diameter. The central nucleoid is surrounded by a single layer envelope. The free virus contains positive strand RNA within its core, from where structural proteins are translated. Three major structural proteins are E1, E2 and C. E1 and E2 are envelope proteins and are responsible for the spikes protruding from the spherical structure. They are involved in cell entry. The E1 envelope glycoprotein shows remarkable antigenic stability [56].

Humans are the only known host for rubella virus. Infections typically peaks in spring but could circulate any time of the year. As congenitally infected infants excrete rubella virus for prolonged periods, it is possible that congenitally infected infants may provide a reservoir for the virus [56].

1.4.3 Epidemiology in developing countries

Single cases of rubella can occur at any time, but there can be outbreaks involving schools or other large groups of children. Adults (teachers and parents) may also be infected in these outbreaks. There is a seasonal pattern, with rubella activity peaking in late spring / summer in neighbouring South Africa [57]. A study in Zimbabwe using measles case definition-based surveillance revealed that of the 2302 measles IgM-negative samples received over a five-year period, 865 (37.6%) were rubella IgM-positive. Ninety-eight percent of confirmed rubella cases were children younger than 15 years of age [58].

Following rubella epidemics, many infants with congenital rubella syndrome (CRS) have been identified [57,59]. Global incidence of CRS is about 100 000 cases annually, mostly in developing countries [60,61].

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In many developing countries, CRS is an underrecognized public health problem [62]. In South Africa it is suspected that most cases of CRS go undetected. The exact incidence of congenital rubella syndrome in South Africa is not known, but it is estimated at about 660 cases per annum. There is evidence that CRS may be largely undetected, especially in the upper socio-economic group [63]. While neighbouring Zimbabwe is rated by the United Nations as one of the countries highest affected by rubella infection, no data were available for Namibia in 2015 when this study was designed.

Immunity to rubella virus might be due to natural infection or vaccination. While many developing countries are still struggling to introduce routine anti-rubella vaccination programmes, immunity to rubella virus is mostly attributed to natural infection, mostly during early childhood. The naturally acquired immunity to rubella in women of childbearing age in South Africa has been estimated at over 90%.[64].

Table 2 summarizes the seroprevalene of rubella virus in developing countries.

Table 2. Immunity to rubella virus in developing countries

Country	Year	Immune %	Population	Method	Reference
Benin	1995	85.8	Pregnant women	ELISA	Rodier [28]
Ghana	1997	92.6	Pregnant women	ELISA	Lawn [70]
Mozambique	2002	95.3	Pregnant women	ELISA	Barreto [74]
South Africa	2005	97.5	Pregnant women	ELISA	Corcoran [57]
DRC	2009	84	Pregnant women	ELISA	Alleman [73]
Nigeria	2010	97.9	Pregnant women	ELISA	Amina [69]
Namibia	2010	85	Pregnant women	ELISA	Jonas [10]
Brazil	2010	IgG 93.1	Pregnant women	Patient records	Dos Santos Goncalves [26]
Sudan	2011	72	Pregnant women	ELISA	Hamdan [67]
Nigeria	2011	53	Pregnant women	ELISA	Onakewhor [68]
Morocco	2012	90.2	Pregnant women	ELISA	Belefquih [65]
Burkino Faso	2012	77.0	Pregnant women	ELISA	Linguissi [71]
Iran	2013	96	Pregnant women	ELISA	Honarvar [66]
Nigeria	2013	91.54	Pegnant women	ELISA	Adewumi [75]
Benin	2014	94	Pregnant women	ELISA	De Paschale [29]
Democratic Republic of Congo	2014	58.97	Pregnant women	ELISA	Zanga [72]

1.4.4 Transmission

Transmission of rubella infection occurs via droplet transmission while the virus is detectable in the nasopharynx of the host for one week before and one week after onset of the rash [1]. In pregnant women, primary infection with rubella causes viraemia. The virus then spreads from the blood to the placenta and may infect the foetus. Reinfection with rubella virus in a pregnant woman rarely causes intrauterine transmission [56]. CRS develops in 90% of infants exposed to the rubella virus during the first trimester of pregnancy, and in 20-40% of infants exposed during early second trimester, the latter usually resulting in deafness only [56,60]. There is a greater risk (80-90%) of transmission from a mother with primary rubella infection in the first trimester of pregnancy and vertical transmission during this period is associated with the most severe sequelae at birth [1].

1.4.5 Pathology

Little information is available on pathology of rubella infection acquired after birth, since patients seldom die of the mild disease. In adults, there is a cellular immune response involving lymphocytes, as well as a local immune response in the throat producing IgA. It is possible that lack of the local IgA response in cases of rubella vaccination, plays a role in increasing incidence of reinfection in vaccinated individuals [56].

Congenital infection with the rubella virus causes damage to cells, and it also has an effect on cell division. Therefore, it is of clinical importance in the developing embryo / foetus. The pathological effect of the virus causes progressive necrotizing vasculitis and inflammation in focal areas. It is postulated that emboli from the small blood vessels in the placenta break loose and lodge in organs of the foetus. These emboli might also cause damage to the foetus by obstructing small blood vessels. Furthermore, cytolysis with necrosis has been seen in many organs in the foetus including the inner ear (organ of Corti) [1,56].

1.4.6 Symptoms and signs

Maternal symptoms include a mild rash, viral pneumonia and infection of the meninges and brain [2]. During an outbreak in Australia in 1940, Sir Norman Gregg was the first to document the

devastating effects of rubella infection on infants exposed in the first trimester of pregnancy. He noted that affected infants presented with ocular defects and cardiac lesions [56].

Today it is known CRS is associated with cataracts and other visual impairments, cardiac defects, deafness, microcephaly, and mental retardation. Classically CRS leads to the triad including deafness (80%), congenital cardiac disease (50-70%) and cataracts (30%) [1,56]. Delayed manifestation of symptoms includes the development of insulin-dependent diabetes mellitus, thyroid disorders, hypertension and behavioural disorders [1,2]. In one study 68% of infected newborns had no symptoms at birth. They were followed for the first five years of life and 71% developed manifestations of infection. It is said that CR infection is a silent, progressive disease [56].

1.4.7 Diagnosis of rubella infection

Enzyme immunoassays (EIA) are the most commonly used and widely available diagnostic test for rubella IgG and IgM antibodies. EIAs are sensitive and relatively easy to perform. The IgG level is important for indicating susceptibility to re-infection which might occur in women with IgG levels below 10 IU/ml. EIA is the preferred testing method for IgM, using the capture technique [76]. In contrast, indirect IgM assays on blood of affected infants may give false negative results due to competition with high concentrations of maternal IgG.

It is important to differentiate between an asymptomatic primary rubella infection and re-infection, because IgM together with IgG may be present in both cases [77]. Re-infections are mostly asymptomatic and pose a low risk to the foetus. Re-infection of vaccinated women is usually accompanied by the detection of IgM and IgG. Additional tests used in the clinical diagnosis of rubella, are IgG avidity testing and reverse transcriptase PCR in nasopharyngeal swabs, urine, cerebrospinal fluid (CSF) and blood at birth. Most pathology laboratories have developed a rubella testing algorithm including quantitative cut-off's for IgG antibodies used for reporting and clinical decision making [78]. Rubella testing poses a challenge in low resource settings due to the following problems which might arise: false positive rubella IgM results; no access to other assays, such as rubella IgG avidity; limited experience of diagnosis and its pitfalls (e.g. persistent specific IgM) and misinterpretation of laboratory results [79].

1.4.8 Treatment

There is no specific treatment for infected children available. All infants with CRS are considered contagious till the age of one year by which time viral shedding in urine and other body fluids has usually ceased [1], although rubella RNA has been demonstrated in ocular fluid from a 28-year old woman who was born with CRS.

1.4.9 Prevention

Prevention of rubella infection is by vaccination of all adults and children in a population. It is important that women of childbearing potential show evidence of rubella immunity, to control the incidence of CRS. It is recommended that pregnant women who are not immune or have immunity, which is not protective against rubella infection, take post-partum rubella vaccination. However, even in immune subjects, due to previous infection or vaccination there is an increase in susceptibility to rubella infection over time. The screening cut-off level (IgG<10 IU/ml) used to identify women at risk of transmitting rubella infection to a foetus was determined in 1995, based on early epidemiological studies. The Rubella Sub-Committee of the National Committee for Clinical Laboratory Standards have proposed that the level to define rubella immunity be lowered from 15 to 10 IU/ml. Proven rubella infections have, however, occurred in persons with titres above 15 IU/ml [85].

The WHO's Measles and Rubella Initiative aims to eliminate both diseases from at least five of its six regions by 2020 [80]. The availability of a vaccine against rubella virus has decreased infections in pregnant women significantly [81]. One of the two approaches in rubella vaccination programmes is to immunize adolescent girls or women of childbearing age, or both groups [66]. Congenital rubella syndrome is completely preventable if an effective vaccination programme in both private and public sectors is in place [60]. However, given the increase in susceptibility to rubella infection among women of childbearing age in countries with rubella vaccination programmes, this might need further intervention by health authorities in the form of revised vaccination efforts. Nearly a decade after Iran's nationwide measles-rubella vaccination campaign for the population aged 5–25 years, most pregnant women up to 34 years of age had humoral immunity against rubella. The authors recommend rubella immunity screening or catch-up immunization for women older than 35 years who wish to become pregnant [66]. A study in England evaluated the current rubella control

programme to inform future planning and found that over a six-year period (2004-2009) the frequency of women with anti-rubella IgG <10 IU/ml increased by 60%. Susceptibility to rubella infection should be monitored, in order to identify high-risk groups, evaluate control measures and to inform policy and service planning [82].

In South Africa, Schoub et. al. (2009) postulated that limited administration of measles-mumps-rubella (MMR) vaccine (not routinely administered in the public sector) in infancy may reduce childhood rubella outbreaks, which would have otherwise resulted in high levels of natural immunity and thereby gives rise to the development of a group of susceptible females reaching childbearing age. These females might be exposed to rubella circulating amongst individuals in the public sector who have not been vaccinated. Therefore, incomplete rubella vaccination could paradoxically result in an increased occurance of CRS. Currently routine rubella vaccination does not form part of the Expanded Programme for Immunization (EPI) in South Africa. It is suggested that selective immunisation of girls before puberty should be instituted together with a routine rubella immunisation programme of infants to forestall a possible future outbreak of CRS, as occurred in Greece in 1993 [63].

In Namibia, since 2005, the measles surveillance programme of the MoHSS in association with the WHO includes rubella testing for measles negative cases. Few positive rubella cases were recorded in the years preceding this study with a spike in cases in 2015.

Since 2016 routine rubella vaccination forms part of the vaccination schedule of children in the public sector (measles-rubella [MR] at 15 months for male and female children). In 2016 the MoHSS conducted a national Special Immunization Activity (SIA) whereby male and female individuals aged 9 months to 39 years received MR vaccination. The SIA excluded pregnant women.

Insufficient rubella vaccination coverage results in continued disease transmission [83]. There is an urgent need to collect appropriate data to estimate the cost-effectiveness of a potential global rubella control programme [62,84].

1.4.10 Risk factors

Since the incidence of natural rubella infection peaks at the age of 5-7 years in Africa, a risk factor for acquiring rubella by unvaccinated individuals is close association with children.

1.5   Cytomegalovirus

1.5.1 Virus description

Human cytomegalovirus (CMV) belongs to the *Herpesviridae* family, *Betaherpesvirinae* subfamily and is also referred to as Human betaherpesvirus 5 [86,143]. It has a large dsDNA genome (about 235 000 bp). Due to numerous mechanisms to modulate the host immune response, infection persist lifelong in humans. While the evolution of CMV has been well described, research in the last decade outlined the molecular diversity of CMV strains among hosts (interhost variability) as well as within the same host (intra-host variability) [87]. The virus also evolves on much shorter time scales, in the order of days or months [87]. Compartmentalization of CMV persisting in different tissues (peripheral blood and distal compartments) and undergoing mutations while replicating is offered as an explanation for intra-host molecular diversity. A case has been described where drug-resistance to ganciclovir has been found in the blood of a patient while sensitivity prevailed in the CSF [87].

1.5.2 Virus properties, replication and immune response

Infection with CMV results in the formation of greatly enlarged (cytomegalic) cells with nuclear and cytoplasmic inclusions. The large cells with owl's eye appearance was first described in 1881 in the kidney of a stillborn infant with congenital syphilis. The virus was first called the cytomegalic inclusion disease (CID) and later salivary gland virus. CMV is the largest and structurally most complex virus in the family of *Herpesviridae*. It has three identifiable regions: the capsid containing the double stranded DNA (dsDNA) viral genome, the tegument and the envelope. The structure of the capsid is very much similar to that of Herpes simplex virus (HSV), although it houses 60% more material than HSV. Numerous CMV proteins include epitopes targeted by CD8 and CD4 T cells. It was reported that in an infected host up to 15% of total T cell reactivity is directed at CMV antigens and that CMV-

specific T cell responses in seropositive subjects were enormous, comprising on average ~10% of both the CD4⁺ and CD8⁺ memory compartments in blood [147]. Cytomegalovirus infections of immunocompetent hosts are characterized by a dynamic, life-long interaction in which host immune responses, particularly of T cells, restrain viral replication and prevent disease but do not eliminate the virus or preclude transmission. The biology of CMV infection in immunocompetent populations can be explained as an evolutionarily "negotiated" balance between viral mechanisms of pathogenesis, persistence, and immune evasion and the host cellular immune response [147]. Once infected, the host retains the infection as a persistent infection, due to immune evasion by the virus. Limited information is available on the numerous proteins in the envelope and integument. Envelope glycoproteins play an important role in early virus infection and are a potential target for vaccine development [86].

Replication of CMV starts when the virus attaches to the host cell membrane. Specific receptors have not been identified beyond doubt. The capsid containing the DNA is transported to the host cell nucleus. Viral replication takes place in the nucleus of the host cell and then the new viruses are assembled in the cytoplasm. Studies on tissues from autopsies have demonstrated CMV virus in almost every cell type of the host [86].

1.5.3 Epidemiology

 Cytomegaloviruses are highly species specific, and humans are the only reservoir of human cytomegalovirus. Infection with CMV is endemic and does not show a seasonal pattern. Seroprevalence differs greatly according to socioeconomic status, ethnic background and geographic location. Of clinical importance is the seroprevalence of CMV among women of childbearing age [86].

Prevalence of CMV infection is about 50% in Europe and North America, while it is close to 100% in developing countries in South America and Africa [88,89]. In sub-Saharan Africa, South America and Asia, 95-100% of pre-school children were seropositive for CMV, while surveys in Great Britain and the US found less than 20% of children of similar age to be seropositive [86].

Table 3 summarizes the prevalence of CMV in developing countries.

Table 3. Prevalence of CMV in developing countries

Country	Year	Seroprevalence	Population	Method	Reference
Benin	1995	IgG 97.2 IgM 2.9	Pregnant women	ELISA	Rodier [28]
Sudan		IgG 77 IgG 95	Blood donors Pregnant women	ELISA	Kafi [89]
Ghana	2008	77.6 59.2	Blood donors HIV patients	ELISA	Adjei [90]
Nigeria	2009	IgG 96 IgM 19.5	Blood donors	ELISA	Akinbami [91]
Iran	2012	IgG 69.6 IgM 2.5	Pregnant women	ELISA	Bagheri [96]
Saudi Arabia	2013	60 82.9	Pregnant women Blood donors	ELISA	Al-Jiffri [92]
South Africa	2014	2.9	Newborns from HIV-positive mothers	Saliva PCR	Manicklal [93]
Mexico	2014	IgG 65.5 IgM 0.0	Pregnant women	ELISA	Alvarado-Esquivel [95]
Iraq	2014	IgG 93.5 IgM 9.6	Pregnant women	ELISA	Aljumaili [97]
Kenya	2014	IgG 77.3 IgM 8.1	Pregnant women	ELISA	Maingi [98]
Sudan	2016	IgG 98.9 IgM 1.1	Pregnant women	Automated immunoanalyser	Altayeb [94]
Ethiopia	2016	IgG 88.5 IgM 15.5	Pregnant women	ELISA	Mamuye [99]

Prevalence of congenital cytomegalovirus (cCMV) infection was found to be 4.6 /1000 births in Sweden and 3.2/1000 births in London [100]. Bonalumi et. al. (2011) reported an incidence of 0.3 – 2.4 percent in developed countries [101]. The incidence of cCMV infection is highest in developing countries (1 – 5 percent of births) and can most likely be attributed to non-primary maternal infections [93,102]. A study in South Africa showed that 2.9% of babies born to HIV-positive mothers had cCMV [93].

1.5.4 Vertical transmission and congenital infection

CMV can be found in body fluids, genital tract secretions and breast milk. It is generally believed that CMV is acquired at mucosal sites. Infections can also be blood-borne in transfusion products or organ transplants [86]. In children and adults, transmission of CMV infection is through breastfeeding, sexual contact and spread from children through contact with body fluids [92]. Vertical transmission of CMV virus may occur during pregnancy via the placenta; during birth via contact with the birth canal or after birth through breast milk [88,103].

CMV infection in pregnant women can either be primary or recurrent. Primary infection is when a person gets infected with the CMV virus for the first time and can be demonstrated by seroconversion (appearance of antibodies which were not previously present). Recurrent infection is described as a past infection and includes, reactivation of latent infections and reinfection with a different CMV strain. During latency there is no viral replication, and the virus remains dormant in mononuclear leukocytes and cells of organs like the kidneys and heart. The virus may start replicating at any point in time, causing a reactivation of the infection [88].

In pregnancy, it is useful to distinguish between the types of infections. Primary maternal infection leads to 20-75 per cent of exposed foetuses having cCMV infection while only 1 – 2.2 per cent is infected in reactivated CMV [101,104]. In primary maternal infection 32.3 percent of congenitally infected neonates are symptomatic at birth while it is 1.4 per cent in case of recurrent infection [105]. Primary maternal infection is also associated with increased risk of sequelae associated with CMV infection compared to recurrent infection [105,106]. However, many congenitally infected babies do not develop abnormalities. Kenneson & Canon (2007) reviewed CMV screening on foetuses and/or

infants and found that 11% of those born with cCMV were symptomatic [105]. Congenitally infected newborns are at risk for serious long-term sequelae regardless of the presence or absence of symptoms at birth [88].

Pregnant women who become infected during the first 16 weeks of pregnancy are more likely to transmit the virus to the foetus than women who are infected later in pregnancy. Early infections are associated with a higher occurrence of central nervous system (CNS) sequelae, essentially sensorineural hearing loss and long-term disability [88,103,106].

Recent data demonstrate similar risk of developing sequelae, especially hearing loss, in infants born to mothers with CMV primary and re-infection. There is increasing evidence that non-primary maternal infection could lead to symptomatic and severe outcomes. Recent studies in developed- as well as developing countries have shown that symptomatic infection occurs with similar frequency in children born to women with primary CMV infection and those born to women who were CMV seroimmune before pregnancy (nonprimary infection). In addition, the severity of newborn disease and the rates of CMV-associated sensori-neural hearing loss also do not differ between primary and nonprimary infection groups [86]. This underscores the importance of screening for congenital cCMV infection in resource-poor settings. In well-resourced settings, it is believed that primary maternal infection drives cCMV infection – however in resource limited settings with a high prevalence and incidence re-infections may contribute to cCMV [101,107]. More recent studies show that women who are seropositive can become re-infected with a different strain of CMV and give birth to infected infants [108].

Congenital infection, as a result of recurrent infection in a seropositive mother, is common in settings with high seroprevalence of CMV. A study in Ivory Coast where seroprevalence of CMV is 100%, found a prevalence of cCMV infection in 1.4% of live births [108].

Intrauterine transmission of CMV after reinfection or reactivation in immune women can possibly explain the direct relationship between seroprevalence of CMV and incidence of cCMV infection in different countries [86,105]. One study outlined the paradox of maternal CMV infection as a risk factor for cCMV and used a prediction model to show that for a range of population prevalences of

CMV, nonprimary maternal infections are responsible for the majority of cCMV infections and related hearing loss [109]. This finding is supported by Yamamoto et. al. (2011) who found that among 10 children with sensorineural hearing loss, 6 were born to mothers with non-primary CMV infection [108]. Although the individual risk of transmission and cCMV during primary infection is much higher than with recurrent infections, recurrent infections are much more common, especially in high prevalence settings [105].

1.5.5 Pathology - Congenital cytomegalovirus infection

Congenital CMV infection is assumed to take place through the placenta [86]. Once transmitted to the foetus, the virus initially infects endothelial cells, and then other target tissues. Consequences of placental infection range from no foetal infection to foetal death [88,103].

Neutrophils do not support viral replication in the host but may carry CMV to different sites within the human body. Macrophages derived from blood monocytes have been shown to harbour CMV. Endothelial cells in vascular beds support replication of the virus. Cytoplasmic changes are characterized by very large cells, 20-35 µm in diameter, and with inclusions in the cytoplasm and nucleus. Large inclusions are separated from the nuclear envelope by a clear zone giving the cells an owl's eye appearance. CMV may spread to all organs of the body (disseminated disease), or can more commonly be found in the CNS, haematopoietic system, kidneys, gastrointestinal tract and lungs. CMV can be found in the placentas of most congenitally infected infants. The most significant feature is the presence of inclusion-bearing cells in endothelial cells or in cells attached to the capillary walls [86].

1.5.6 Symptoms and signs

Ninety percent of adult primary CMV infections are asymptomatic, but may be associated with fatigue, myalgia and fever. cCMV is the most common of all intrauterine infections and should be considered in all neonates with symptoms of congenital infection [2]. CMV-infected infants showing no symptoms at birth are also at risk of developing complications later [105]. CMV infection in the first trimester of pregnancy is known to cause congenital malformation, especially of the CNS.

Cytomegalovirus has been identified as the main cause of non-inherited hearing loss while long-term CNS sequelae include seizures and motor and visual defects [88,101,110].

Symptomatic as well as asymptomatic cCMV cause hearing loss in children. A study in Flaanders established cCMV at birth with PCR or viral culture on urine. In the group with symptomatic cCMV 63% had hearing loss while in the group with asymptomatic cCMV 8% had hearing loss. Delayed onset hearing loss occurred in 10.6% of symptomatic and 7.8% of asymptomatic cCMV cases [111].

In 1990 CDC established a national surveillance system to monitor cCMV infections. In the first four years 285 cases were reported. It was reported that petechiae was the most common clinical symptom, presenting in 50% of cases. It was often seen together with hepatosplenomegaly, intracranial calcification, microcephaly and thrombocytopaenia [86,112,113,114].

Due to variations in epidemiology and seropositivity in women of childbearing age, prevalence of cCMV infection vary between 0.15 – 2.0 percent in different countries. Ten to twenty percent of all children with cCMV infections, show signs of neurological damage when followed up [101,102], while Enders et. al. (2001) reported that 57.6 percent of congenitally infected live-born infants had symptoms of varying degree [104].

Differential diagnosis is sometimes necessary since clinical signs like cataracts, congenital heart defects, certain types of rash, certain lesions to the retina and absence of cerebral calcifications are more likely to occur with CRS than with cCMV. Almost all the manifestations described for symptomatic cCMV infection are also described in CT. The calcifications in the CNS are usually scattered through the brain in toxoplasmosis, while they surround the ventricles in CMV infection. Lesions to the retina cannot be distinguished but they are usually accompanied by other manifestations in the case of cCMV infection [86].

1.5.7 Diagnosis of cytomegalovirus infection

Laboratory methods used can detect the virus itself (CMV DNA assays, electron microscopy and viral culture) or detect antibodies produced in response to CMV virus (serological assays). Methodology can also be divided into screening and confirmatory tests. For cCMV infection,

serological testing is used for screening while the PCR of CMV DNA is used for confirmation and for risk stratification [115].

IgG and IgM serology of a pregnant woman can identify pregnancies at risk for transmission of CMV to the foetus. IgG avidity testing adds additional diagnostic value and is complemented by ultrasound in the clinical setting [94,116]. IgM positivity and low IgG avidity at ≤ 14 weeks of gestation is a good indicator of congenital infection [117]. Antibody kinetics as measured by IgG avidity can be useful to predict vertical transmission of CMV. Increase in rate of IgG avidity in consecutive samples is higher in pregnant women with congenitally infected foetuses than in mothers with no infection [118].

Congenital CMV infection is detected by analysis of amniotic fluid, foetal tissue, foetal blood or neonatal samples collected within the first two weeks of life [88]. It is possible to predict the risk of a foetus with cCMV to be born symptomatic. It can be established with a combination of ultrasound, amniocentesis or cordocentesis and laboratory testing of amniotic fluid and foetal blood [119]. Invasive antental sampling is however not readily available in developing countries, and it furthermore poses a risk of foetal infection or abortion. After birth confirmation of cCMV infection requires detection of virus in neonatal blood, urine or saliva by PCR or virus isolation within the first two weeks of life [86]. Saliva or urine are the sample types of choice. Although dried blood spots (DBS) have the benefit of sometimes being available for retrospective diagnosis this sample type has a sensitivity of only about 30% [120].

The best diagnosis of maternal primary infection is by demonstrating seroconversion. CMV IgM is a good indicator of recent CMV infection, but CMV IgM can also be detected during recurrent infection or primary Epstein-Barr virus (EBV) infection due to cross-reactive antigens or polyclonal activation. Detecting maternal CMV IgM does not mean that the foetus will be infected, and further testing is needed to determine whether it is a primary or recurrent infection, or an EBV infection [88]. In pregnant women, CMV DNAemia is detected during primary infection and it might be useful to confirm infection [121]. Cytomegalovirus DNA testing of urine or blood of pregnant women may help to identify pregnant women at risk of transmitting the virus to the foetus [122].

Interferon-gamma release assays for tuberculosis were the first T cell based immunodiagnostic tests in clinical practice. It has rapidly expanded to the diagnosis or risk stratification of other infectious diseases including CMV. It is now used for risk stratification of posttransplant CMV infection [159].

In newborn infants, computed tomographic (CT) scans showed abnormal findings in 70% of children with symptomatic cCMV with intracerebral calcification the most frequent finding [123].

1.5.8 Treatment

There is a lack of studies described in the literature which examined either prophylaxis or treatment interventions for cCMV. There is a need for high-quality clinical trials to demonstrate the benefit of available treatment interventions before they can be recommended for use. This is probably a prerequisite for health systems to cover the cost of such interventions [14]. A few antiviral agents which are systemically administered have been used in clinical trials of life-threatening or sight-threatening cases of CMV infection. Agents licenced for use in immunocompromised patients include ganciclovir, valganciclovir, cidofovir and foscarnet. Foscarnet decreases viral replication by binding at the pyrophosphate binding site and by inhibiting cleavage of the pyrophosphate moiety from deoxynucleotide triphosphates, thereby inhibiting viral DNA polymerase, while ganciclovir acts as a chain terminator during elongation of the newly formed viral DNA. A study on the prevention of hearing loss in congenitally infected infants showed beneficial effects of ganciclovir treatment of infants born with neurological symptoms of CMV infection [14,86]. In an open-label study Leruez-Ville et. al. (2016) provided valacyclovir to pregnant women with moderately infected foetuses and showed that the majority gave birth to asymptomatic neonates. This study showed favourable outcomes in neonates followed for 12 months but need confirmation by randomized trials [124]. Cidofovir is used to treat certain ganciclovir resistant CMV strains, as it does not require phosphorylation by the viral UL97 enzyme. However, these drugs are too toxic to administer during pregnancy.

Diagnosis and treatment of CMV infection is very costly in sub-Saharan Africa and calls are going out for more affordable health solutions. Moreover, there is limited evidence of benefit of antenatal antiviral therapy and due to their toxicity anti-CMV treatments are rarely administered to pregnant

women [14]. Work on new drug development is ongoing and one study showed promising results with a combination of drugs in an *ex vivo* model of floating placental villi in tissue culture [125].

Cytomegalovirus infections reactivate frequently in the immunocompromised host, which makes it a reality in regions hit by high prevalence of HIV infections [126].

1.5.9 Prevention

After 30 years of research, a vaccine to prevent cCMV infection is not available. Despite these challenges, clinical trials with vaccines, prenatal interventions and prolonged postnatal antiviral therapy are underway. Current efforts for elimination of CMV infections are focused on vaccine development, which is regarded as a Level 1 priority by the National Academy of Medicine. This emphasizes the need for more information on the epidemiology and diagnosis of CMV infections in pregnant women and neonates [93,107,148]. Improved understanding of cCMV transmission rates resulting from non-primary maternal infections will provide further evidence to study maternal immunity as a potential target for vaccine development [148].

A protocol has been proposed to identify pregnancies at risk for primary CMV infection [127]. The screening may facilitate prevention of congenital infections by preventing infection of seronegative pregnant women. Pregnant women could be educated about hygiene and behavioural interventions like frequent handwashing, especially if they have regular contact with saliva and urine of small children [128].

1.5.10 Risk factors

A well-documented risk factor for acquisition of CMV is close contact with young children in crèches. This poses a risk for other young children as well as for adults who have contact with these children [129]. A strong background of sexually transmitted diseases as well as greater numbers of lifetime sexual partners show significant correlation with seropositivity. Low socioeconomic status and lower levels of education also appear to be risk factors. Organ transplant and blood transfusion from infected donors are sources of CMV infection [86]. CMV exposure is associated with increasing age [95,130].

A risk factor for vertical transmission of CMV is CMV immune status [131].

Neonates with cCMV are more likely to be co-infected with HIV. The co-infection of HIV and CMV is associated with a high mortality rate, especially in male neonates [114]. Another study in Zimbabwe did not find an association of HIV co-infection in cCMV infection and increased mortality [132].

In conclusion, this study will focus on seroprevalence and incidence of the three infectious agents described above among pregnant women in Namibia. While the description above focused on the seroprevalence of infectious agents, the next section describes the application of mathematical modelling in order to estimate incidence of infection with the three infectious agents among pregnant women.

1.6   Incidence of infections

In order to estimate the risk of pregnant women contracting an infection during pregnancy which could possibly result in neonates born with congenital infections, it is important to obtain information on the number of new infections in a population. Studies on the prevalence of congenital infections are invasive and expensive and an alternative method has been offered using mathematical modelling to estimate incidence of infection [62, 133, 134, 141].

1.6.1 Concept and role of mathematical modelling in public health

Mathematical modelling is a description of a system and the inter relationships of its components using mathematical methods. It makes use of analysis of trends in available data and uses mathematical equations in order to make predictions and forecasts regarding future outcomes or bigger populations. A mathematical model is an explicit mathematical description of the simplified dynamics of a system and allows for conceptual experiments which would be otherwise difficult or impossible. It could also be used to predict the impact of changes on the dynamics of a system [149].

Public health officials can gain a better understanding of infectious disease epidemiology by using mathematical modelling. Mathematical modelling is used in epidemiology and public health to understand the dynamics of infections in a population. It is important for health managers to understand the transmission rates of infectious diseases and the potential impact of infectious disease control programmes. For example, if a decision has to be taken to introduce a national immunization programme in a population of 50 million people, an enormous extrapolation is required to consider the effect of the immunization programme in such a big population [149].

Mathematical modelling using smaller datasets and allowing predictions on the outcomes in the bigger population could be helpful for policy makers in the health sector. For example, in order for

public health managers to evaluate the need for and effectiveness of a national rubella immunization campaign and to plan future interventions like introduction of a routine immunization programme, epidemiological data could be used to apply a mathematical model in providing estimates of prevalence and incidence of rubella in different years.

Another application of mathematical modelling in public health is the evaluation of intervention programmes to reduce infection rates in populations, taking into consideration available data from surveillance systems. In France, incidence and prevalence of *T. gondii* have decreased markedly during the last 30 years [144]. Since 1978, public health authorities in France have implemented a CT prevention programme, including monthly serological screening of all pregnant women, and treatment in case of seroconversion. However, this programme does not include systematic surveillance data on incidence and prevalence. In 2007, a surveillance programme was set up to collect information on cases of CT diagnosed by means of amniocentesis during pregnancy or diagnosis of newborns and infants less than one year old [144].

Nogareda et. al. (2014) used a mathematical model to estimate incidence and prevalence of *T. gondii* infection between 1980 and 2020 in women of childbearing age. They used age- and time-specific seroprevalence data obtained from the National Perinatal Surveys. They also estimated incidence of seroconversion during pregnancy in 2010 using the National Surveillance of Congenital Toxoplasmosis data. Information on 42 208 women aged 15-42 was collected. For women aged 30 years the modelled incidence decreased from 7.5/1000 susceptible women in 1980 to 3.5/1000 in 2000. In 2010 the incidence was 2.4/1000. The predicted incidence and prevalence for 2020 was 1.6/1000 and 27% respectively [144].

Nogareda et. al. (2014) estimated seroconversion in pregnant women and incidence rate per 1000 pregnant women per year. Based on this model and considering the same trend and conditions they estimated that incidence may continue to decline over the following years. This information is essential for epidemiologists and health economists who estimate the impact and the cost-effectiveness of preventive programmes for *T. gondii* [144].

The application of mathematical modelling in this study could thereforeprovide valuable information to policy makers in the health sector about the effectiveness of the *T. gondii* preventive programme among pregnant women in France.

1.6.2. Estimation of incidence of infection in this study

A description of the objectives, methodology used, and suitability of results obtained in the estimation of incidence of *T. gondii*, rubella and CMV among pregnant women attending the antenatal clinic at Windhoek Central Hospital follows in Chapter 3.

The next section describes the study design and methodology for the investigation of seroprevalence and incidence of the three infectious agents.

1.7 Study design and methodology

The overall goal of the study was to determine seroprevalence of three infectious agents among pregnant women in Namibia. The study was a descriptive and quantitative study. It only included pregnant women aged 15-47 years attending the antenatal clinic at Windhoek Central Hospital during September and October 2016.

1.7.1 Rationale and expected results of the study

There is no information available on the prevalence of congenital infections in Namibia. In view of this gap in knowledge in the health sector, the current study aimed at the description of maternal seroprevalence of three major infectious agents responsible for congenital infections in neonates. At the time of study design, only one study on seroprevalence of *T. gondii* had been done in Namibia. No information was available for the other two infectious agents. Expected results of this study were based on literature published in other countries.

Expected result 1

T. gondii infections occur among blood donors in Namibia as well as among pregnant women attending the antenatal clinic of Windhoek Central Hospital and pose a risk of transfusion transmitted infection as well as vertical transmission. Certain risk factors are associated with toxoplasmosis in these populations based on reports from other countries.

Expected result 2

Rubella immune status is high among pregnant women attending the antenatal clinic of Windhoek Central Hospital. This reduces the pregnancies at risk for vertical transmission of rubella although there are pregnant women who are not immune / have low immunity against rubella.

Expected result 3

Previous exposure to CMV infection is very high among pregnant women attending the antenatal clinic of Windhoek Central Hospital. Mainly recurrent maternal infections pose a risk for the development of cCMV infection.

1.7.2 Proposed solution / specific aims / objectives / outcomes

The focus of the research was to begin to unravel the extent to which infectious diseases may be impacting on women of childbearing potential in Namibia.

1.7.2.1 Aims

The aim of this study was to conduct a study on a group of pregnant women to evaluate exposure to infectious agents namely *T. gondii*, rubella and CMV, as well as the level of anti-rubella immunity. The risk of vertical transmission of *T. gondii* and CMV infections was evaluated. Risk factors associated with *T. gondii* and CMV infections were determined. A pilot study had been conducted to determine toxoplasmosis among blood donors in Namibia, in order to get an indication of toxoplasmosis among the broader Namibian population. Results from the pilot study were used to determine sample size for the study among pregnant women. Mathematical modelling was planned to determine the incidence of rubella, *T. gondii* and CMV infection, in order to determine the risk of vertical transmission of these infections.

1.7.2.2 Objectives

The objectives of this study were to test blood samples from study groups in Khomas region to determine the following:

Objective 1:

Pilot study among blood donors in central Namibia (First publication already published and included):

1.   Seroprevalence of IgG antibodies against *T. gondii* to determine previous exposure in the general population of Central Namibia

2.   Seroprevalence of IgM antibodies against *T. gondii* to determine risk of transfusion associated transmission

3.   Risk factors associated with toxoplasmosis in blood donors

The rest of the study was performed among pregnant women attending the antenatal clinic at Windhoek Central Hospital to determine the following (Second publication):

4. Seroprevalence of IgG antibodies against *T. gondii* to determine previous exposure of pregnant women to toxoplasmosis

5. Seroprevalence of IgM antibodies against *T. gondii* among exposed individuals to evaluate risk of vertical transmission

6. Assessment of anti-*T. gondii* IgG avidity among exposed individuals to evaluate risk of vertical transmission.

7. Calculation of any incident cases of *T. gondii* infection by using age stratified IgG seroprevalence data.

8. Analysis of association between seropositivity and exposure to risk factors for *T. gondii* infection to guide future education of pregnant women on prevention strategies

Objective 2 (Third publication)

1. Seroprevalence of IgG antibodies to rubella virus mainly to determine anti-rubella immune status

2. Calculation of any incident cases of rubella infection by using age grouped IgG seroprevalence data.

3. Level of immunity (IgG measured in international units/ml) among patients testing IgG positive to determine whether immune status is actually protective against congenital infection

Objective 3 (Fourth publication)

1. Seroprevalence of IgG antibodies to CMV to determine previous exposure of pregnant women to CMV

2. Seroprevalence of IgM antibodies against CMV to evaluate risk of vertical transmission.

3. Assessment of risk of vertical transmission among CMV IgM positive individuals with the specific IgG avidity test.

4.   Calculation of any incident cases of CMV infection by using age stratified IgG seroprevalence data.

1.7.3 Methodology

1.7.3.1.   Collection of samples

Inclusion criteria: All pregnant women aged 13 years and above attending the antenatal clinic at Windhoek Central Hospital between September and October 2016 were included in the study. Exclusion criteria: Sampling was planned to be age stratified so once the required number of participants in a certain age range had been reached, no further participants would be recruited in that age range.

Sample size was determined according to cost efficiency ensuring optimal benefit in terms of the study objectives from available funding. For sample size calculation, the primary objective used was *T. gondii* prevalence: For toxoplasmosis the expected prevalence was 6% based upon previous publications in Namibia, and in South Africa (4%-8%). At 95% confidence level, it needs a sample size of 542 to achieve a 2% margin of error for this estimate.

Calculation of sample size using Cochrane's formula:

$N = Z^2 P (1 - P) / d^2$

Z = Z value at 5% level = 1.96

P = Prevalence

d = margin of error

$n = 1.96^2 \times 0.06 (1 - 0.06) / 0.02^2$

$= 3.8416 \times 0.06 (0.94) / 0.0004$

$= 542$ samples

This sample size would also allow accurate estimates of prevalence for the other agents.

In order to achieve optimal efficiency to estimate incidence using mathematical modelling a stratified sample across the age range of women of child bearing potential was taken: participants were stratified according to five age groups (13-20, 21-25, 26-30, 31-35, 35-48) and 108 pregnant women were included in each age group. 5 ml of blood was collected from each participant and transported immediately to the laboratory where serum was stored at -80 ⁰C until testing.

1.7.3.2 Analysis of samples

Automated chemiluminescence immunoassays were performed to determine seroprevalence of IgG antibodies to *T. gondii*, Rubella and CMV and IgM antibodies to CMV. Patients testing positive for anti-CMV IgM were tested for CMV IgG avidity. Patients testing positive for anti-*T. gondii* IgG were tested for anti-*T. gondii* IgM followed by IgG avidity testing in patients who tested positive for anti-*T. gondii* IgM.

Anti-*T. gondii* IgG, anti-rubella IgG and anti-CMV IgG and IgM testing was performed in a South African National Accreditaion Systems (SANAS) accredited laboratory participating in an external quality assurance programme, with validation of test methods in place.

1.7.3.3 Questionnaire

A questionnaire assessed demographic information, obstetric history, exposure to risk factors and vaccination history, and was filled by consenting participants.

1.7.3.4 Data analysis

All data were entered into an Excel spread sheet and analysed using Statistical Program for Social Sciences (SPSS) statistical software. Assistance from a statistician was obtained to determine association between seropositivity and exposure to risk factors. Pearson's χ^2 tests or Fisher's exact tests (when values were lower than 5) was used for categorical data. Bivariate analyses and multivariate analysis assessed associations between population characteristics provided in the surveys and seropositivity results. P-values less than 0.05 were considered statistically significant.

1.7.3.5 Involvement of a statistician

Namibia University of Science and Technology provided a mathematician as co-supervisor while assistance from two biostatisticians was also available.

1.7.4 Ethical approval and considerations

All pregnant women attending the antenatal clinic at Windhoek Central Hospital during the period September to October 2016 were approached to participate in the study. The background and objectives of the study were explained to each participant and a recruitment information pamphlet was handed to each patient. Participation was voluntary and informed consent was obtained from each participant and parent/guardian if applicable. Participants were allowed to opt out of the study at any stage. Blood samples were collected, and questionnaires were administered by a nurse registered with the Health Professions Councils of Namibia. No incentive was involved in the donation of a blood sample as the patients were not required to have an additional visit apart from their normal clinic visit. Demographic data and information concerning previous pregnancies, exposure to risk factors and vaccination history were collected with the aid of a questionnaire. Information was kept safe in a building with access control, and confidentiality was maintained throughout the study. Students doing data entry signed a confidentiality statement and were registered with the Allied Health Professions Council of Namibia. Questionnaires carried the patient codes as well as allocated laboratory numbers. Apart from IgG avidity and anti-*T. gondii* IgM analysis, testing was conducted according to standard laboratory procedures in an established diagnostic laboratory. Blood collected was used to test for the infectious diseases as agreed by the participant with no extra testing. Samples were transported between the hospital and the laboratory by Student Medical Laboratory Scientists in a hamper with a biohazard sign. Samples were stored in a building with access control for a period of five years after which they were sent to the incinerator at Katutura Hospital.

Ethical approval for this study was obtained from the Faculty of Health and Applied Sciences of Namibia University of Science and Technology; the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Stellenbosch University as well as the Research Ethics Committee of the MoHSS. Approval letters may be found in Addendums A, B and C.

Chapter 2: Manuscripts prepared for publication

Four manuscripts were prepared during this study. Three have been published in subsidy generating peer reviewed journals.

2.1   First article

Van der Colf BE, Noden BH, Wilkinson R, Chipare I. Low seroprevalence of antibodies to *Toxoplasma gondii* in blood donors in central Namibia. South Afr J Infect Dis 2014;29(3):101-104.

2.1.1   Reflection on first article

2.2   Second article

Van der Colf BE, Van Zyl GU, Noden BH, Ntirampeba D. Seroprevalence of *Toxoplasma gondii* infection among pregnant women in Windhoek, Namibia, 2016. S Afr J Infect Dis. 2020;35(1),a25. https://doi.org/10.4102/sajid.v35i1.25.

2.2.1   Reflection on second article

2.3   Third article

Van der Colf BE, Van Zyl GU, Noden BH, Maposa I. Seroprevalence of rubella among pregnant women after an epidemic in Windhoek, Namibia, 2016. Unpublished.

2.3.1   Reflection on third article

2.4   Fourth article

Van der Colf BE, Van Zyl GU, Mackenzie SBP. Seroprevalence of cytomegalovirus among pregnant women in Windhoek, Namibia, 2016. S Afr J Obstet Gynaecol 2019;25(2):52-55. https://doi.org/10.7196/SAJOG.2019.v25i2.1441

2.4.1   Reflection on fourth article

A description of author contributions for the following articles may be found in Addendum I: Author contributions.

2.1 First Article

Van der Colf BE, Noden BH, Wilkinson R, Chipare I. Low seroprevalence of antibodies to *Toxoplasma gondii* in blood donors in central Namibia. South Afr J Infect Dis 2014;29(3):101-104.

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Low seroprevalence of antibodies to Toxoplasma gondii in blood donors in central Namibia

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Toxoplasma gondii, blood donors, Namibia

Although emphasis has been placed on research relating to human immunodeficiency virus (HIV), tuberculosis and malaria, several researchers in Africa are focusing on other threats to human health, such as neglected tropical diseases. *Toxoplasma gondii* is a possible neglected tropical disease in Namibia, although the country has a diversity of climate, ranging from tropical in the north to semi-desert in the south. Except for one study in 1978, no recent studies have determined the burden of *T. gondii* infection in Namibia. Three hundred and twelve convenience samples were collected from volunteer blood donors in central Namibia. Donors provided informed consent to participate in the study, and 5 ml blood was collected. Demographic information was collected by means of a questionnaire. Serum was analysed using Captia™ *T. gondii* immunoglobulin G (Ig) G enzyme-linked immunosorbent assay (ELISA) kit. Only samples that tested positive or equivocal for IgG antibodies were then tested for IgM antibodies using Captia™ T. gondii IgM ELISA kit. Of the 312 samples, 3 (0.961%) tested positive for IgG antibodies to *T. gondii.* One sample (0.3%) tested positive for IgM antibodies to *T. gondii.* These donors lived in urban areas in central Namibia and interacted regularly with animals, such as cats and dogs. The prevalence of antibodies to *T. gondii* in Namibian blood donors was found to be considerably lower than that reported in other African countries, but comparable to that in a recent report from South Africa. It is notable that most of the donors lived in the arid central regions of Namibia, where the high altitude could also affect parasite survival.

Introduction

It is estimated that a third of the world's population is seropositive for *Toxoplasma gondii*, indicating that people have been infected with the parasite at some stage of their lives.[1-3] Studies to determine the seroprevalence of exposure to *T. gondii* have been conducted in human and animal populations in many countries,[4] including blood donors.[2, 5-13]

The prevalence of toxoplasmosis has been studied in several African countries, and ranges from 7% in Zambia to 80% in Ethiopia.[14] In Tanzania, occupationally exposed individuals were identified with a seroprevalence of 46%, while 35% and 30.9% of pregnant women were seropositive for *T. gondii* antibodies.[15-17] In Nigeria, a seroprevalence of 20.8% was reported in apparently healthy individuals, and that of 60% in urban and peri-urban Ethiopian residents.[18,19]

Recent studies in southern Africa have reported lower rates of seroprevalence of toxoplasmosis than previous studies in the rest of the world. While Kistiah et al noted an unexpectedly low prevalence (6.4% in the general population) in neighbouring South Africa, Sitoe et. al. reported 18.7% in Mozambique.[14,20,21] *T. gondii* is postulated to be one of the neglected tropical diseases in Namibia.[22] Except for Jacobs and Mason, no recent studies have determined the burden of *T. gondii* infection in humans in Namibia.[8]

The objective of this study was to evaluate the exposure by blood donors in Namibia to *T. gondii*. Determining the seroprevalence of antibodies to *T. gondii* in blood donors might provide an indication of the seroprevalence in the broader Namibian population.[2,12] Furthermore, it could give an indication of the risk of transfusion-transmitted toxoplasmosis in Namibian blood donations.

Method

Study design and study population

A cross-sectional, prospective study was performed in voluntary blood donors in central Namibia. Three hundred and twenty-nine blood donors volunteered to participate in the study when they donated blood to the Blood Transfusion Service of Namibia between October 2011 to January 2012. An additional 5 ml of blood was drawn in a plain tube for each donor. The serum was aliquot and stored at −20°C before testing. Each participant received an information sheet describing the

purpose of the research, as well as a questionnaire in English or Afrikaans, from which demographic data and information on their contact with animals were collected. Only samples with an accompanying survey were used for testing. Informed consent was obtained from the donors, participation was anonymous and voluntary, and donors could opt out of the study at any stage. Permission for the study was granted by the Institutional Research and Publication Committee, the Research Committee of the Ministry of Health and Social Services, and by the management of the Blood Transfusion Service of Namibia.

Data collection

The prevalence of IgG antibodies to *T. gondii* was determined in 312 samples using the Captia™ *T. gondii* immunoglobulin (Ig) G enzymelinked immunosorbent assay (ELISA) kit (Trinity Biotech, Bray, Ireland). The manufacturer's protocol was followed to determine the outcome of the test results. Samples with an equivocal IgG result were retested with the same kit and included in the negative results if still equivocal. Serum samples that tested positive or equivocal for IgG antibodies were further analysed using the Captia™ *T. gondii* IgM ELISA kit. Results from the ELISA tests, as well as responses from the questionnaires, were entered into a Microsoft® Excel® spreadsheet. Statistical analysis was performed using SPSS©version 21. An association between the population characteristics in the surveys and the seropositivity results was not calculated owing to a low prevalence of positive results.

Results

Study population

Of a total of 329 samples, only 312 samples were included in the study as accompanying demographic information was lacking for the rest. The majority (61.9%) of donors were male. Their ages ranged from 16-64, with a mean age of 32.2 ± 11.3 years. Most of the donors (75.7%) were aged 40 years and younger. Donors from different regions in Namibia were included in the study, although the majority (86.9%) were from the central region. Most participants (81.1%) indicated that they lived in urban areas, 95.8% with animals and 32% with animals as part of their occupation. 22.1% of the respondents indicated that they owned cats, while 78.2% of respondents were dog

owners. Many respondents were farmers who also had contact with other animals, such as cattle, goats and horses. The demographic distribution of the donors and their exposure to animals is summarised in Table I.

Seroprevalence

Of the 312 samples tested, 3 (1%) were positive and three tested equivocal for IgG antibodies to *T. gondii.* All three IgG-positive donors were urban dwellers in central Namibia and had indicated that they lived with animals, such as cats and dogs. Their ages ranged from 1861. Two of the three IgG-positive donors were women (Table II). Of the six samples tested for IgM antibodies, 1 (0.3% of the total donors) was positive. This donor was a 29-year-old man who owned cats and dogs.

Table I: Characteristics of the study population (n = 312)

Characteristics	n (%)
Gender	
Females	119 (38.1)
Males	193 (61.9)
Age (years)	
20 and younger	38 (12.2)
20-29	105 (33.7)
30-39	93 (29.8)
40-49	54 (17.3)
50 and older	22 (7.1)
Areas*	
North	24 (7.7)
Central	271 (86.9)
South	17 (5.8)
Residence	
Rural	53 (17)
Urban	253 (81.1)
Unknown	6 (1.9)
Interact with animals on a regular basis?	
No	13 (4.2)
Yes	299 (95.8)
Work with animals on a farm?	
No	212 (68)
Yes	100 (32)

*

Regions: North (Caprivi, Ohangwena, Oshikoto, Oshana, Omusati and Kunene);
Central (Otjozondjupa, Erongo, Khomas and Omaheke) and south (Hardap)

Table II: The seroprevalence of immunoglobulin G and immunoglobulin M antibodies to *Toxoplasma gondii* in Namibian blood donors and the corresponding demographic information

IgG	IgM	Age	Gender	Region	Residence	Live with animals	Kinds of animals	Work with animals
Equivocal	Negative	32	Male	Oshikoto	Urban	Yes	Cattle, goats and dogs	No
Positive	Negative	61	Female	Khomas	Unknown	Unknown	Unknown	Unknown
Equivocal	Negative	31	Male	Khomas	Urban	Yes	Dogs	No
Equivocal	Negative	35	Male	Khomas	Urban	Yes	Cattle, goats and dogs	No
Positive	Negative	18	Female	Khomas	Urban	Yes	Cats	No
Positive	Positive	29	Male	Khomas	Urban	Yes	Dogs and cats	No

IgG: immunoglobulin G, IgM: immunoglobulin M

Discussion

The low prevalence of *T. gondii* antibodies in Namibian blood donors was much lower than 7-80% reported in other African countries.[14] However, the prevalence is comparable to that of toxoplasmosis in blood donors in China, which varied from 0.4-20.2%.[23] Alvarado-Esquivel et. al. reported a 7.4% IgG prevalence of *T. gondii* in healthy blood donors in Mexico, with an IgM prevalence of 1.9%.[2] A low prevalence of toxoplasmosis (3% in healthy individuals) was also reported in Thailand.[13]

While no specific studies have been published on toxoplasmosis in humans in Namibia, one study reported its prevalence using Namibian serum samples. Jacobs and Mason carried out an indirect fluorescent antibody survey of the the prevalence of *Toxoplasma* antibodies using sera from southern Africa (Natal, Eastern Cape and Western Cape, South Africa) and south-west Africa (Namibia) and Botswana.[8] The overall prevalence in that study was 20% of 3 379 sera tested. Serum samples were obtained from the San (n = 725) in eastern Namibia, Damara speakers (n = 77) in the Erongo region and urban white residents (n = 261) in the Windhoek area, for the Namibian component of the Jacobs and Mason study. Prevalence rates were 12% in the white residents of Windhoek, 27% in the Damara speakers in Damaraland and 9% in the San. The differences in culture were thought to play a role in the variations in the prevalence of toxoplasmosis in the Namibian groups.[8]

In the current study, the low prevalence in central Namibia indicates a substantial decline from that documented by Jacobs and Mason.[8] This is in line with the trend in Europe and New Zealand, where a decrease in the prevalence of toxoplasmosis has been seen over the past few decades.[12] One of the reasons for this decline could involve the more common use of frozen meat since the consumption of previously frozen meat is associated with a lower risk.[24,25] In Europe and America, the consumption of raw or undercooked meat, as part of cultural practice, is the main risk factor for toxoplasmosis.[25,26] This is not the case in certain populations of southern Africa, although some restaurants serve rare or underdone beef and game steak, particularly popular with tourists. Additionally, the results are comparable to those recently published for South Africa, which also describe a lower seroprevalence of *T. gondii* than that previously reported.[14,21] This could be attributed to common socio-cultural practices and environmental aspects in the region.[5]

Age and the gender distribution of the blood donors compare well to those in other studies. Most of the blood donors were recruited in the Khomas region, where the capital city is 1 650 m above sea level. High altitude is usually associated with a lower prevalence of toxoplasmosis.[2] Furthermore, the exceptionally dry conditions encountered in the central regions of Namibia could have also contributed to low exposure to *Toxoplasma* spp. as oocysts are sensitive to desiccation.[3] However, the situation may differ in other regions, like the Kavango and Caprivi, where warm, humid conditions prevail and communities share water resources with livestock and wild animals.

Five of the possible six *T. gondii*-positive donors engaged in regular interaction with cats and/or dogs, which have been investigated as risk factors for toxoplasmosis, e.g. contact with dogs and farming activities, as well as living with cats at home.[2,27,25] As toxoplasmosis has been reported in domestic and wild animals in Namibia, there is the potential risk of transmission to humans since the reservoir for the parasite exists in the country.[28,29]

However, it is notable that the majority of respondents in this study were dog and/or cat owners, but their exposure to toxoplasmosis was very low. This result is supported by that in another study in which an association between the seroprevalence of *Toxoplasma* spp. and cats in the household or contact with dogs was not found.[27] One limited but important aspect of this study involves the possibility of the transmission of *T. gondii* via the national blood transfusion service. The transmission of *T. gondii* through blood transfusion has been well documented.[30] *T. gondii* may be transmitted through the transfusion of infected blood since trophozoites can survive for several days in blood and blood products, especially leukocytes.[31,32] The parasite can survive in citrated blood stored at 4°C for 50 days. In India, after malaria, toxoplasmosis is the most important protozoan disease that is transmitted by blood transfusion.[5] While the overall risk is probably low for a donor transmission event, the donor who tested positive for IgM antibodies could have potentially been at risk of blood safety being compromised through transfusion, especially if the recipient was a pregnant woman or an HIV-positive patient.[7] *T. gondii* is currently not part of donor blood screening in Namibia, although it has been proposed in other countries, like India, where high rates of toxoplasmosis exist in the population.[5]

The small, non-random sample used for the study made it difficult to identify any regional trends and establish risk factors based on the questionnaire responses. As most of the study population came from the central arid regions, it would be important for future studies to include the northern tropical regions, in order to obtain a holistic epidemiological picture of *T. gondii* in Namibia. However, evaluating the exposure of volunteer blood donors is a valuable indicator of the epidemiology of an infectious disease in the broader population in the same community.[2] Therefore, the low seropositivity in central Namibia indicates that the broader population in this area probably has limited exposure to *T. gondii.*

Conclusion

This study, while regional in nature, demonstrated that *Toxoplasma* spp. occurs in Namibia. Further studies are needed to evaluate the current status of the parasite in human and animal populations. Further point prevalence studies should be conducted in the country, particularly in the areas of high prevalence reported by Jacobs and Mason. Occupational risks for abattoir workers, especially those handling goats and sheep in the slaughtering process, should also be established. It is anticipated that an increase could be indicative of a warming trend accompanied by higher humidity to keep the oocysts from desiccating. Ideally, the prevalence of toxoplasmosis in at-risk groups, such as pregnant women, their neonates and persons living with HIV, should also be evaluated.

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2.1.1 Reflection on first article

This publication is the first on the prevalence of *T. gondii* in Namibia since 1978. Worldwide, prevalence of *T. gondii* has declined over the past decades. Possible reasons could be better controlled facilities to process meat, and practices to freeze meat for storage. Climate change could also play a role with severe droughts on the increase in certain parts of the world like Africa and South America. Oocytes of *T. gondii* cannot survive in dry conditions since they are sensitive to dessication. Namibia has also suffered severe droughts in the past decade. Therefore, there was a need to update information on the prevalence of *T. gondii* in Namibia. Prevalence was expected to be lower than the 12% recorded among blood donors in 1978.

Blood donors were selected as the study group mainly for the convenience of sampling. Another project in the department investigated the prevalence of zoonotic and tick-borne diseases among blood donors in Namibia. The same blood samples could thus be used to study the prevalence of *T. gondii* among blood donors. Therefore, a sample size was not calculated for this study.

This study was a good starting point to pave the way for the actual goal, viz. to study the prevalence of maternal infections including *T. gondii*. Blood donors could give a good indication of the prevalence of an infection in the general population. Data from this study was used to determine the sample size for the bigger study.

Although the results showed a decline in the prevalence of *T. gondii* among blood donors since 1978, the extremely low prevalence (the lowest found in literature) was possibly not representative of the general population. The sampling was biased since blood donors generally have more affluent lifestyles with better hygiene than people in villages in rural areas. The expectation was that the prevalence of *T. gondii* could be higher in rural areas, as well as in parts of Namibia which have a tropical climate.

The next step was to study the prevalence of *T. gondii* among pregnant women attending antenatal care in a public clinic in Windhoek.

2.2   Second article

Van der Colf BE, Van Zyl GU, Noden BH, Ntirampeba D. Seroprevalence of *Toxoplasma gondii* infection among pregnant women in Windhoek, Namibia, 2016. S Afr J Infect Dis. 2020;35(1):a25. https://doi.org/10.4102/sajid.v35i1.25.

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AOSIS

Seroprevalence of *Toxoplasma gondii* infection among pregnant women in Windhoek, Namibia, in 2016

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Background: When a pregnant woman contracts *Toxoplasma gondii* (*T. gondii*) infection during pregnancy, it may be vertically transmitted to the foetus. Information on the incidence of congenital toxoplasmosis (CT) in developing countries is scarce. Most studies focus on the seroprevalence of *T. gondii* infection among pregnant women. This study aimed to determine the seroprevalence of *T. gondii* infection among pregnant women attending public antenatal care in Windhoek, Namibia, in 2016.

Methods: In this descriptive study, 344 urban pregnant women attending public antenatal care were voluntarily enrolled in the study. Seroprevalence of anti-*T. gondii* Immunoglobulin G (IgG) was determined by automated immunoassay. Samples with a positive *T. gondii* IgG result were tested for *T. gondii* Immunoglobulin M (IgM) and specific IgG avidity by using an enzyme-linked immunosorbent assay (ELISA) test. A questionnaire captured demographic data and exposure to risk factors. Data were analysed using Statistical Package for the Social Sciences (SPSS) and R.

Results: Anti-*T. gondii* IgG was found in nine (2.61%) pregnant women. There was no association of anti-*T. gondii* IgG with demographic characteristics or exposure to risk factors. Anti-*T. gondii* IgM was positive in one (0.3%) woman, while three (0.9%) women had borderline anti-*T. gondii* IgM results. Specific IgG avidity was low, equivocal and high in 0%, 33% and 67% of seropositive pregnant women, respectively.

Conclusion: Seroprevalence of anti-*T. gondii* IgG is much lower in Namibia than is reported in other developing countries. Investigation into specific IgM seropositivity and IgG avidity showed that pregnant women in the central region of Namibia are at low risk of vertical transmission and development of CT.

Keywords: *Toxoplasma gondii*; toxoplasmosis; seroprevalence; IgG avidity; pregnant women; Namibia.

Introduction

Toxoplasma gondii (*T. gondii*) is a protozoan parasite with the only definitive hosts being the cat species. Infection occurs via contamination of soil and water, and the parasite can be found in all warm-blooded vertebrates and some reptiles. Humans are intermediate hosts. Infections in humans occur through the ingestion of raw or undercooked meat, unpasteurised milk or contaminated water or by sharing the environment with cats and inadvertently ingesting oocytes from cat faeces. Toxoplasmosis can be transmitted from a mother to a foetus and can cause deafness, blindness, mental retardation, physical impairment or even stillbirth. If a pregnant woman is diagnosed with *T. gondii* infection, antimicrobial treatment is possible, although the success of the treatment will depend on a number of variables like the dose and the route of administration.[1] Congenital toxoplasmosis (CT) may occur in spite of treatment with spiramycin of pregnant women with primary *T. gondii* infection.[2] Furthermore, spiramycin is not available in the public or the private sector in Namibia.

Congenital toxoplasmosis

Immunocompetent and immunocompromised women contracting *T. gondii* infection for the first time during pregnancy run a risk of transmitting the infection to the foetus because of the parasite crossing the placenta from the maternal blood. A host that is immunocompetent acquires life-long immunity against toxoplasmosis when infected with *T. gondii*. A new *T. gondii* infection contracted 4–6 months before conception will usually not result in mother-to-child transmission of toxoplasmosis

Seroprevalence of *Toxoplasma gondii* infection among pregnant women in Windhoek, Namibia, 2016

Abstract

Background*:* When a pregnant woman contracts *Toxoplasma gondii (T. gondii)* infection during pregnancy, it may be vertically transmitted to the foetus. Information on the incidence of congenital toxoplasmosis (CT) in developing countries is scarce. Most studies focus on the seroprevalence of *T. gondii* infection among pregnant women. This study aimed to determine the seroprevalence of *T. gondii* infection among pregnant women attending public antenatal care in Windhoek, Namibia, 2016.

Methods*:* In this descriptive study 344 urban pregnant women attending public antenatal care were voluntarily enrolled in the study. Seroprevalence of anti-*T. gondii* IgG was determined by automated immunoassay. Samples with a positive *T. gondii* IgG result were tested for *T. gondii* IgM and specific IgG avidity by ELISA. A questionnaire captured demographic data and exposure to risk factors. Data were analysed using SPSS and R.

Results*:* Anti-*T. gondii* IgG was found in 9 (2.61%) pregnant women. There was no association of anti-*T. gondii* IgG with demographic characteristics or exposure to risk factors, although a bigger sample size is needed to find possible associations. Anti-*T. gondii* IgM was positive in 1 (0.3 %) woman while 3 (0.9 %) women had borderline anti-*T. gondii* IgM results. Specific IgG avidity was low, equivocal and high in 0%, 33% and 67% of seropositive pregnant women.

Conclusion*:* Seroprevalence of anti-*T. gondii* IgG is much lower in Namibia than is reported in other developing countries. Possible explanations are the arid climate and high altitude of the capital, Windhoek. Investigation into specific IgM seropositivity and IgG avidity showed that pregnant women in the central region of Namibia are at low risk of vertical transmission and development of CT. Further studies are needed in rural areas where seroprevalence of *T. gondii* may be higher.

1. Introduction

Toxoplasma gondii (T. gondii) is a protozoan parasite with the only definitive hosts being cat species. Infection occurs via contamination of soil and water and the parasite can be found in all warm-blooded vertebrates and some reptiles. Humans are intermediate hosts. Infections in humans occur through ingestion of raw or undercooked meat, unpasteurised milk or contaminated water or sharing the environment with cats and inadvertently ingesting oocytes from cat faeces. Toxoplasmosis can be transmitted from a mother to a foetus and cause deafness, blindness, mental retardation, physical impairment or even stillbirth. If a pregnant woman is diagnosed with *T. gondii* infection, antimicrobial treatment is possible, although the success of the treatment will depend on a number of variables like the dose and the route of administration [1]. Congenital toxoplasmosis may occur despite treatment with spiramycin of pregnant women with primary *T. gondii* infection [2]. Furthermore, spiramycin is available in neither the public nor private sector in Namibia.

Congenital toxoplasmosis

Immunocompetent and immunocompromised women contracting *T. gondii* infection for the first time, during pregnancy run a risk of transmitting the infection to the foetus due to the parasite crossing the placenta from the maternal blood. A host that is immunocompetent acquires life-long immunity against toxoplasmosis when infected with *T. gondii*. A new *T. gondii* infection contracted 4 – 6 months before conception will usually not result in mother-to-child transmission of toxoplasmosis on subsequent exposures. In immunocompromised women who are HIV positive, *T. gondii* infection may reactivate with resulting vertical transmission [3,4]. In a study in Brazil, 28% of *T. gondii* IgM-positive pregnant women with unknown HIV status transmitted the infection vertically [2].

Only about 30% of infants born to mothers who had seroconversion in pregnancy acquire prenatal infection. If the infection in a pregnant woman is not treated, the risk of intrauterine infection increases with gestational age, i.e. from 14% for primary maternal infection in the first trimester, to 59% for primary maternal infection in the last trimester. [3]. The later in gestation of primary infection, the higher the risk of transmission to the foetus. However, when transmission occurs early in gestation

the risk of symptomatic infection and severe disease is high [5]. Clinical signs of toxoplasmosis in neonates include the classical triad, namely intracranial calcification, hydrocephalus and retinochoroiditis. About 10% of prenatal infections result in abortion or neonatal death. The surviving infants suffer from progressive neurologic complications like mental retardation, learning problems, hearing and visual impairment or seizures and have special care and education needs. Children who have been infected before birth might be asymptomatic at birth but may develop complications associated with toxoplasmosis later in life [3,6,7]. Clinical trials indicate that early treatment of these children can decrease manifestations of disease and improve the quality of life [3].

Congenital toxoplasmosis (CT) can best be prevented by ensuring that pregnant women who are seronegative do not get infected, or by treatment of pregnant women who are diagnosed with a primary infection, as evident from seroconversion, T. *gondii* IgM positivity and low IgG avidity. Screening programmes for toxoplasmosis have been introduced in many European countries and the USA. These include either diagnosis of primary maternal infection with *T. gondii* during pregnancy or detection of congenital infections in neonates at birth [3]. No routine screening for *T. gondii* is done in Namibia and only two previous studies were done to determine the prevalence of toxoplasmosis among blood donors [8,9]. The aim of this study was to determine the prevalence of antibodies to *T. gondii* among urban pregnant women attending public antenatal care in Windhoek, Namibia, from September to October 2016.

2. Materials and Methods

2.1 Study design

This was a descriptive study aiming to describe the seroprevalence of *T. gondii* among pregnant women in Windhoek, Namibia.

2.2 Sample size

At an expected prevalence of 6% of *T. gondii* infection, using Cochran's formula, a sample size of 542 would provide a precision (error margin, above and below the prevalence estimate) of 2%.

Estimated prevalence was based on two previous publications in Namibia and the seroprevalence in neighbouring South Africa. Funding was available to enrol 344 participants in the study.

2.3 Exclusion and inclusion criteria

All pregnant women attending public antenatal care in Windhoek, Namibia, from 12 September to 11 October 2016 were informed about the study and all women who were identified by the clinic to have their blood drawn for routine antenatal testing, were eligible to participate. Five participants were excluded due to not signing the consent form by omission and one Ovahimba woman was excluded due to not understanding English or Afrikaans.

2.4 Sample collection

Participation in the study was voluntary and written consent was obtained from each participant and guardian where applicable. A venous blood sample was collected from each consenting participant and transported immediately to the testing laboratory. A questionnaire was administered to collect information on demographic characteristics and exposure to risk factors and was filled by consenting participants. Questions were derived from the literature.

2.5 Sample storage

Blood samples were transported to the laboratory within an hour. Serum was stored for not more than a week at 2-8 ⁰C and then at -80 ⁰C until testing.

2.6 Laboratory methods

An automated chemiluminescent microparticle immunoassay was performed in an ISO 15189 accredited laboratory to determine seroprevalence of IgG antibodies to *T. gondii* (Access immunoassay systems, Beckman Coulter Eurocenter SA, 22 rue Juste-Olivier, Case Postale 1044, CH - 1260 Nyon 1, Switzerland). Presence of anti-*T. gondii* IgM antibodies was determined in samples testing positive for specific IgG using an ELISA method (Euroimmun Medizinische Labordiagnostika, D-23560 Lubeck, Seekamp 31, Germany). Cut-off points were calculated by using the ratio of the extinction of the control or patient sample to the extinction of the calibrator. The

extinction value of the calibrator defined the cut-off value as recommended by the manufacturer. IgG avidity was determined in samples testing positive for specific IgG by performing a manual ELISA test with an urea wash step to dissociate antibodies (Euroimmun).

For interpretation of IgG results, recommendations from the manufacturer were followed. Specimens with specific IgG concentration of >10.5 IU/ml were considered positive for *T. gondii* past or current infection. Acute infection with *T. gondii* was determined by the presence of anti-*T. gondii* IgM antibodies and specific IgG antibodies with low avidity. Recommendations from the manufacturer were followed for interpretation of results. For interpretation of IgM results, an extinction ratio (sample relative to calibrator) of <0.8 was considered negative, $\geq$0.8 to <1.1 was borderline and $\geq$1.1 was positive. For interpretation of avidity results, the ratio of the enzyme immuno-assay optical density when urea-treated relative to untreated was used. Low avidity was defined as a ratio of <40% which was indicative of acute *T. gondii* infection. A ratio of 40-60% was considered equivocal while a ratio above 60% was an indication of high avidity antibodies.

2.7 Data analysis

Association of demographic characteristics (maternal age, gestational age and parity) with seroprevalence of *T. gondii* infection among pregnant women was investigated. Association of risk factors (water source, living with cats or dogs, blood transfusion, as well as consumption of untreated water, unpasteurized cow's milk, goat's milk, raw/undercooked meat or unwashed fruit/vegetables) with seroprevalence of *T. gondii* infection was investigated. A case-based description of anti-*T. gondii* IgG and IgM seropositivity and IgG avidity was provided for nine participants who tested IgG positive. Seropositivity among five age groups was evaluated with the assumption that all age groups had had similar exposure to *T. gondii*. All data were entered into an Excel spreadsheet (Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-7329) and analysed using SPSS statistical software (version 24, IBM North America, 590 Madison Avenue, New York, NY 10022) and R version 3.2.2 [10]. Chi-square and Fisher's exact test (when the expected frequency in any cell of the two-by-two table was less than 5) were used to assess associations between population characteristics provided in the surveys and seropositivity results. Multivariate analysis was done with generalized

linear models using binary logistic regression. Beta-values were used to calculate odds ratios of variables while the Wald 95% confidence intervals gave an indication of the width of the odds ratios; p-values less than 0.05 were considered statistically significant.

2.8 Ethical approval

Ethical approval of the study was obtained from the School of Health and Applied Sciences, Polytechnic of Namibia, the Ministry of Health and Social Services in Namibia, and from the Health Research Ethics Committee at Stellenbosch University, Cape Town, South Africa.

3. Results

Three hundred and forty-four pregnant women each donated a blood sample and filled the questionnaire. Maternal age at enrolment ranged between 17 and 47 years with the median age of 27 years. All but two women were residing in the urban area. Forty-one (11.9%) women were in the first trimester, 172 (50%) in the second trimester and 130 (37.8%) in the third trimester of pregnancy upon enrolment in the study. A hundred and twenty-nine (37.5%) of women were primigravidae, 100 (29.1%) had one previous pregnancy while 115 (33.4%) had two or more previous pregnancies.

Overall seroprevalence of anti-*T. gondii* IgG among pregnant women attending public antenatal care in Windhoek was 2.61% (Table 1). Overall susceptibility to *T. gondii* infection was high (n=335; 97.4 %). There was no statistically significant difference between seroprevalence of anti-*T. gondii* IgG in different age groups. There was no statistically significant difference between the occurrences of anti-*T. gondii* IgG in different trimesters of pregnancy. There was no statistically significant difference between the occurrences of anti-*T. gondii* IgG in different parities although the highest observed seroprevalence was in primigravida cases.

Table 1. Association of demographic characteristics with seroprevalence of *Toxoplasma gondii* among urban pregnant women (n=344) attending public antenatal care in Windhoek, Namibia, 2016.

Demographic characteristic	N	IgG +[a] n (%)	Bivariate p-value	Multivariate p-value	Odds Ratio[b]	95% CI[c]
Overall	344	9 (2.61)				
Maternal age						
15-20	38	0 (0)	0.383	0.211	1.08	-0.046-0.209
21-25	108	3 (2.78)				
26-30	96	5 (5.21)				
31-35	59	0 (0)				
36-47	43	1 (2.33)				
Gestational age						
1st trimester	41	0 (0)	0.793	0.999	NA[d]	
2nd trimester	172	5 (2.91)		0.796	0.84	-1.533-1.176
3rd trimester	130	4 (3.08)				
Parity						
Primigravida	129	6 (4.65)	0.220	0.048	12.16	0.022-4.973
1 previous pregnancy	100	2 (2.0)		0.334	3.41	-1.262-3.713
≥2 previous pregnancies	115	1 (0.87)				

[a]Including borderline positive results
[b]Adjusted odds ratios were not calculated as none of the predictors were statistically significant
[c]Ninety-five percent Wald confidence interval
[d]Not available since one predictor equals zero

This study found no association between risk factors and seropositivity of anti-*T. gondii* IgG in pregnant women (Table 2). Available data showed that none of own tap versus communal tap as water source; living with cats or dogs; or blood transfusion was associated with seroprevalence of *T. gondii* infection. Consumption of water from a dam or river; unpasteurized cow's milk, goat's milk; raw/undercooked meat or unwashed fruit/vegetables were not identified as risk factors for *T. gondii* exposure.

Table 2. Association of risk factors with seroprevalence of *Toxoplasma gondii* among urban pregnant women (N=344) attending public antenatal care in Windhoek, Namibia, 2016.

Risk Factor	N	IgG +[a] n (%)	Bivariate p-value	Multivariate p-value	Odds Ratio[b]	95% CI[c]
Water source						
Own tap	226	9 (3.98)	0.185	0.998	NA[d]	
Communal tap	114	0 (0)				
Live with cats						
Yes	45	0 (0)	0.612	0.999	NA	
No	299	9 (3.01)				
Live with dogs						
Yes	122	4 (3.28)	0.726	0.787	1.24	-1.349-1.779
No	222	5 (2.25)				
Received a blood transfusion						
Yes	6	1 (16.67)	0.172	0.417	3.55	-1.794-4.331
No	327	8 (2.45)				
Consumption						
Water from dam or river						
Yes	55	3 (5.45)	0.160	0.088	4.75	-0.235-3.352
No	289	6 (2.08)				
Unpasteurized cow's milk						
Yes	39	3 (7.69)	0.070	0.052	7.30	-0.017-3.992
No	305	6 (1.97)				
Goat's milk						
Yes	7	1 (14.29)	0.171	0.703	1.87	-2.590-3.838
No	337	8 (2.37)				
Raw / undercooked meat						
Yes	97	2 (2.06)	1.000	0.323	2.71	-0.981-2.972
No	247	7 (2.83)				
Unwashed fruit / vegetables						
Yes	83	0 (0)	0.121	0.998	NA	
No	261	9 (3.45)				

[a]Including borderline positive results
[b]Adjusted odds ratio was not calculated since no predictor was statistically significant
[c]Ninety-five percent Wald confidence interval of the odds ratio
[d]Not available since one predictor equals zero

Of the 344 participants, 9 (2.61%) had a positive anti-*T. gondii* IgG result, while 1 (0.3 %) had a positive IgM result and 3 (0.9%) had a borderline-positive IgM result (Table 3). The mean anti-*T. gondii* IgG titre among *T. gondii*-positive pregnant women was 132.1 IU/ml, ranging between 25.9 and 298.2 IU/ml. Specific IgG avidity among infected women was low, equivocal and high in 0 %; 33 % and 67 %, respectively, of positive IgG cases.

Table 3. Anti-*T. gondii* IgG and IgM seropositivity, and IgG avidity among pregnant women.

Case	IgG IU/ml	IgM	IgG avidity
5015	38.8	Neg	High
5022	298.2	Neg	High
5040	168.2	Pos	Equivocal
5050	144.2	Borderline	Equivocal
5116	25.9	Borderline	Equivocal
5145	210.5	Borderline	High
5164	83.3	Neg	High
5320	113.6	Neg	High
5328	106.4	Neg	High

4. Discussion

4.1 Seroprevalence of anti-T. gondii IgG

Very low seroprevalence of anti-*T. gondii* IgG (2.61%) was found among urban pregnant women attending public antenatal care in Windhoek, Namibia, 2016. This is comparable to a previous study in Namibia which found 1% seroprevalence of anti-*T. gondii* among blood donors [9]. Possible reasons for this low seroprevalence are the arid climate and high altitude of the capital city, Windhoek [11]. Oocysts cannot withstand dry conditions which prevail during extended periods of drought in central Namibia. The worst drought since recording of rainfall had started, occurred in Namibia in 2016. The seroprevalence of anti-*T. gondii* found in 2016 was lower than the 12.1% recorded among blood donors in Windhoek in 1978 [8]. This finding is in line with the worldwide trend of decreasing seroprevalence of anti-*T. gondii* over the previous decades [12]. Factors that could contribute to the decreasing trend are urbanization, which is accompanied by better access to safe drinking water and abattoirs, as well as increased use of pasteurized cow's milk.

Being the driest African country south of the Sahara, Namibia shows much lower seroprevalence of

T. gondii infection than in other developing countries (Table 4) [8,13-29].

Table 4. Seroprevalence of *Toxoplasma gondii* in developing countries.

Country	Year published	IgG Seroprevalence %	Population	N	Method	Reference
Namibia	1978	12	Blood donors	261	IFA	Jacobs [8]
Sudan	1991	41.7	General population	386	LAT	Abdel-Hameed [13]
Benin	1995	53.6	Pregnant women	211	ELISA	Rodier [14]
Tanzania	1995	35	Pregnant women	849	Sabin-Feldman dye test	Doehring [15]
India	2004	IgG 45 IgM 3.3 1.1 low avidity	Pregnant women	180	ELISA	Singh [16]
Ethiopia	2007	60	Urban population	65	MDAT	Negash [17]
Nigeria	2007	20.8	Healthy individuals	144		Uneke [18]
Tanzania	2009	46	Occupationally exposed	199	LAT	Swai [19]
Brazil	2010	IgG 62 IgM 3.4	Pregnant women	574	Patient records	Dos Santos Goncalves [20]
Gabon	2010	56	Pregnant women	839	ELFA	Mickoto [21]

Mozambique	2010	18.7	Pregnant women	150	ELISA	Sitoe [22]
South Africa	2011	6.4	General population	376	LAT	Kistiah [23]
Nigeria	2011	IgG 32.6 IgM 7.6	Pregnant women	276	ELISA	Deji-Agboola [24]
Egypt	2012	IgG 67.5 IgM 2.8 0.3 low avidity	Pregnant women	323	ELFA	El Deeb [25]
Benin	2014	IgG 30 IgM 0.4	Pregnant women	283	ELISA	De Paschale [26]
Congo	2014	IgG 80.3 IgM 4.4 11.8 low avidity	Pregnant women	781	ELFA	Doudou [27]
Namibia	2014	1.0	Blood donors	320	ELISA	Van der Colf [9]
Ethiopia	2015	18.5	Pregnant women	384	LAT	Awoke [28]
Mexico	2018	3.6	Women of reproductive age	445	ELISA	Alvarado-Esquivel [29]

It is however comparable to seroprevalence in neighbouring South Africa, with which Namibia shares weather patterns [23]. The seroprevalence is the same as in Mexico with similar arid climate [29]. A review of studies done in Africa found a median seroprevalence of *T. gondii* of 37 %, with rates ranging from 6.4 – 74.5 % [12]. Very little is known about the burden of CT in developing countries.

In Brazil, Neto et al. (2010) found 0.06% of neonates anti-*T. gondii* IgG seropositive [30]. It is postulated that the high burden of CT in South America can be attributed to more pathogenic genotypes that circulate in that part of the world [31].

In Windhoek, 97.4 % of pregnant women attending public antenatal care remain susceptible to *T. gondii*, posing a risk of vertical transmission and CT if they get infected while pregnant. Infection pressure is however low: a large population is at risk, but the probability of exposure is low. In hyperendemic settings, acquisition and immunity is likely before women fall pregnant, whereas models suggest that when the incidence of *T. gondii* infection among pregnant women is 4% per annum the risk of seroconversion during pregnancy and occurrence of CT would be the highest. This correlates with a susceptibility of 40% among pregnant women, which is much lower than the susceptibility found in this study [31].

4.2 Factors associated with seropositivity

There was no significant difference in prevalence of anti-*T. gondii* IgG among five different age groups of pregnant women. Seroprevalence linearly increased in the lowest three age groups (15-20 years, 21-25 years and 26-30 years), only to decrease in older women (31-47 years) (Table 1). Seroprevalence of anti-*T. gondii* IgG increased with gestational age although the difference was not statistically significant. Primigravidae showed the highest seroprevalence of anti-*T. gondii* IgG, which is in line with the finding of higher seroprevalence among younger women.

There was no significant association between risk factors and seroprevalence of *T. gondii* (Table 2). Surprisingly, participants with access to own tap showed the highest seroprevalence of *T. gondii*. However, this association was not significant. Another interesting finding was the lack of association between consumption of unwashed fruit or vegetables and seropositivity while 3.45% of participants denying this consumption showed seropositivity of *T. gondii* infection. Alvarado-Esquivel et. al. (2018) reported a similar finding [29]. These exposures to *T. gondii* could possibly be due to other risk factors like working with raw meat, which were not assessed in our study.

Magwedere et. al. (2012) included a discussion on *T. gondii* in their report on zoonoses posing a possible threat to the wildlife industry in Namibia [32]. Working with raw game meat or consumption

of undercooked game meat could possibly pose a risk of exposure to *T. gondii.* A previous study found presence of *T. gondii* in lions in Namibia. Among a pride of five lions in a private reserve, four showed evidence of *T. gondii* infection [33]. A high prevalence of *T. gondii* was also found in feral cats in the Western Cape Province in South Africa, providing a reservoir for the parasite and potential source of contamination of the environment [34]. In the 1990s a municipality in the Province of British Columbia in western Canada experienced an outbreak of toxoplasmosis, with the municipal water supply implicated as the source of infection [35,36].

4.3 Specific IgM and IgG avidity

Very few pregnant women had possibly been infected during pregnancy so the risk of giving birth to an infant with CT was minimal. Anti-*T. gondii* IgM antibodies and specific IgG avidity were only tested in samples positive for anti-*T. gondii* IgG. Other studies found very low prevalence of samples positive for anti-*T. gondii* IgM only (0.3% [37], 0.9% [38]).

It can thus be concluded that the pregnant women who presented with anti-*T. gondii* IgG antibodies were infected before they became pregnant with low risk of vertical transmission. Avidity testing is widely used in Europe and increasingly in southern Africa. The French National Reference Center for Toxoplasmosis has proposed algorithms for interpretation of serologic testing (IgG, IgM and IgG avidity) for *T. gondii* [39].

The *T. gondii* IgG avidity test is useful to distinguish past from present infection in specific IgM-positive pregnant women. In a study in Brazil presence of anti-*T. gondii* IgM in an immune pregnant woman was often not associated with congenital infection. 20% of infants born to immune mothers with specific IgM antibodies and low avidity IgG antibodies were vertically infected while 8% of infants born to mothers with specific IgM antibodies and intermediate or high IgG avidity were vertically infected [2,40]. Liesenfeld et al. (2001) evaluated the usefulness of testing for IgG avidity in association with *T. gondii* and found that among pregnant women with either positive or equivocal specific IgM, 55.9% had high avidity specific IgG.

4.4 Prevention strategy

The high rate of *T. gondii* susceptibility among pregnant women could justify health education interventions. Women of childbearing potential found to be susceptible to *T. gondii* should be educated on behaviour to prevent infection [41]. A health promotion strategy should be aimed at women of reproductive age, making them aware of preventive behaviour [38].

5. Conclusion

The study group of urban pregnant women attending public antenatal care in Windhoek, Namibia from September to October 2016 had a low prevalence of antibodies to *T. gondii*. This could possibly be attributed to geographical and meteorological aspects. The youngest and oldest age groups were least affected by toxoplasmosis. Health education of pregnant women on preventive measures could minimize the risk of CT. Further studies could investigate the seroprevalence of *T. gondii* in rural areas of Namibia, where higher rates of infection might be found. Future investigations could also be directed towards a one health approach to focus on *T. gondii* infection among wildlife like game and carnivores, and among abattoir workers. Of interest is the lack of information on genotypes of *T. gondii* found in southern Africa.

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2.2.1 Reflection on second article

Funding became available to perform the bigger study, namely the prevalence of infectious diseases among pregnant women attending public antenatal care. Information from the first study, together with seroprevalence data from neighbouring South Africa, was used to determine the sample size. Due to financial constraints, the calculated sample size could however, not be reached.

Initially, the ultimate goal was to assess the prevalence of congenital infections in Windhoek, Namibia. There are, however, no surveillance systems for congenital infections available. It would not be feasible to investigate newborns for CT, hence the shift to the investigation of infectious diseases among pregnant women. Pregnant women can transmit infections vertically although this does not happen in all maternal infections.

The prevalence among pregnant women attending antenatal care was a bit higher than the prevalence found among blood donors. Demographic data suggests that this clinic sees the more affluent population of clients and that another clinic in Windhoek would have yielded a higher seroprevalence since they serve mainly the informal settlements.

Another goal of the study was to determine associations between seropositivity and risk factors which were recorded in a questionnaire. All 9 seropositive individuals had indicated that they do not have cats at home. Owning cats are generally seen as a risk factor for *T. gondii* infection, especially when the owner is handling cat litter. Interestingly, other authors in African countries reported a similar lack of association between owning a cat and *T. gondii* seropositivity [24,27 of the article]. Alvarado-Esquivel et. al. (2018) conducted a study in Mexico in a similar setting to this study (similar sample size, similar seroprevalence of *T. gondii* of 3.7%) and found no association between owning a cat or handling cat litter and seroprevalence [29 of the article]. Cat litter should, however, be handled with caution since presence of *T. gondii* in 20% of privately owned cats' litter was shown with molecular techniques at a teaching hospital in Italy [160 of thesis].

Given the low seroprevalence, however, a very large sample size would have been needed to show meaningful associations. It was concluded that even the calculated sample size would not have had

the desired outcome, which was to assess possible associations with meaningful statistical power. In retrospect, a sample size of 244 was calculated using Cochran's formula and the 2.61% seroprevalence found among pregnant women.

Testing for IgG avidity was a strength of this study since it was done for the first time in Namibia. Avidity testing is widely used in Europe and is gaining support in South Africa. As specific IgM tests are prone to false positivity a low avidity can confirm a recent acute infection in a pregnant woman with a positive *T. gondii* IgM test result.   This would inform whether there is an increased risk of congenital infection. However, termination of pregnancy (TOP) in Namibia, in case of intrauterine infections, is only allowable by law when it is adjudged to cause a serious risk of mental or physical defect to the unborn child and may therefore require additional investigations to determine intrauterine infection and the severity of congenital disease. Furthermore, avidity assays are relatively unknown in the local industry and may not be justified in local diagnostic laboratories due to the small volume of requests expected. It would, however, be good to create awareness among clinicians about the availability of the test in South Africa for possible referral.

It was reasoned that mathematical modelling could be useful to determine the risk of vertical transmission. Unfortunately, the data obtained was not suitable for mathematical modelling since susceptibility did not decrease linearly with age. After initial possible linear increase in prevalence, amongst the younger age groups, the age group of 31 – 35 had no cases with positive *T. gondii* IgG.

The study was meaningful to provide updated data on the seroprevalence of *T. gondii* in Namibia. It will hopefully create awareness of avidity testing among clinicians in order to corroborate a recent infection in *T. gondii* IgM positive cases and confirm this as the likely aetiology in cases with abnormal foetal growth or suggestive abnormalities on sonar.

2.3   Third Article

Van der Colf BE, Van Zyl GU, Noden BH, Maposa I. Seroprevalence of rubella among pregnant women after an epidemic in Windhoek, Namibia, 2016.

It has not yet been accepted for publication or published.

Seroprevalence of rubella among pregnant women after an epidemic, Namibia, 2016

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Abstract

Background: While rubella and congenital rubella syndrome have been eliminated in the Americas through effective vaccination programmes, some countries in Africa are still in the pre-vaccine era. Up until 2016, Namibia did not have an immunization programme for rubella. In 2015, a rubella outbreak was reported in the Khomas region of Namibia.

Objectives: The aim of this study was to determine rubella immunity among urban pregnant women in Windhoek, Namibia, in 2016.

Methods: Three hundred and forty-four pregnant women attending public antenatal care during September and October 2016 were recruited for the study. Seroprevalence was determined with the Access automated rubella IgG assay. A questionnaire was used to capture demographic data, obstetric history and exposure to risk factors.

Results: The percentage of women with IgG levels of <10 IU/ml, 10-14.9 IU/ml and > 15 IU/ml were 2.0%, 2.0% and 95.9% respectively. An overall anti-rubella IgG mean level of 164.5 IU/ml (95% CI 150.4-178.7) was found in five age groups, namely 15-20 (195.8; 159-232); 21-25 (167.8; 143-193); 26-30 (165.2; 136-195); 31-35 (147.6; 116-179) and 36-47 (150.4; 107-194). Parity was significantly associated with high anti-rubella IgG levels (IU/ml) with primigravida showing the highest levels (p = 0.004) while maternal age, gestational age, immunization history and living with children under 5 years were not significant.

Conclusions: A very high percentage of pregnant women in the study were immune to rubella virus despite never receiving rubella vaccination. This is likely due to a high rate of natural infection with rubella of children before reaching child-bearing potential. The overall anti-rubella IgG titre was much higher than the 26.5 IU/ml found in a Canadian cohort of pregnant women where rubella vaccination has been in place since the 1970's. Natural rubella immunity is associated with higher titres that persist longer. The small percentage of pregnant women who remained susceptible to rubella virus infection is within targets set by WHO.

Keywords: Rubella, seroprevalence, pregnant women, vaccination, Namibia

1. Introduction

Since live attenuated rubella vaccines were licenced in the USA in 1969, the incidence of congenital rubella syndrome (CRS) was decreased by well-organized immunisation campaigns [1]. Between 2000 and 2014, there was a worldwide decline of 95% in reported rubella cases even though the number of WHO member states reporting rubella cases increased from 102 to 162. Rubella and CRS interruption was reached in the Americas in 2009 with countries in four WHO regions meeting rubella control and elimination goals by December 2014 [2]. In 2010, the second highest estimated incidence of CRS was in Africa (116 per 100,000 live births). The estimate for Namibia was less than 150 CRS cases per 100,000 births [3]. The future goal is to eliminate measles and rubella in five WHO regions including Africa by 2020 [4-6].

Before 2016, Namibia did not have a rubella immunization programme in the public sector with combined measles, mumps and rubella immunizations (MMR) only given on request in the private sector. The first Namibian measles and rubella special immunization activity (SIA) was conducted in July 2016 by the Ministry of Health and Social Services, in collaboration with WHO, targeting all persons, both male and female, from 9 months to 39 years of age, excluding pregnant women. Routine measles and rubella (MR) vaccination of male and female children at 15 months was also introduced by the Ministry of Health and Social Services. Good coverage by the SIA was expected based on the wide inclusion of gender and age. The WHO is making available data on vaccine coverage by country, but measles and rubella vaccination coverage in Namibia is only included from 2017 onwards [161]. Endorsed by the World Health Assembly in 2012, the Global Vaccine Action Plan calls on all countries to reach ≥90% national coverage with all vaccines in the country's national immunization schedule by 2020. Among countries that offer a second measles vaccine dose during the second year of life, coverage increased from 19% in 2007 to 54% in 2018 [162]. Coverage rate of a previous measles vaccination campaign in Namibia was 76% in 2009 [7].

Currently, the only published record on the seroprevalence of rubella among pregnant women attending public antenatal care in Namibia is from a 2010-report with an overall seroprevalence of 85%. Higher seroprevalence was found in urban than rural populations [8]. At the time of our study,

no other information about the seroprevalence of rubella or the incidence of CRS in the Namibian population was available. This study, therefore, aimed to determine the seroprevalence of rubella among mainly unvaccinated pregnant women who attended a public antenatal clinic in 2016, Windhoek, Namibia.

2. Materials and Methods

This was a descriptive study aiming to describe the rubella immunity status of pregnant women.

2.1 Study population

Women aged 15 years and above were eligible to participate in the study. Three hundred and forty-four pregnant women attending the public antenatal clinic at Windhoek Central Hospital during September and October 2016 were included in the study. A minimum sample size of 234 was calculated using Cochrane's formula and a prevalence rate of 97.5% found in neighbouring South Africa [9]. A 95% confidence level was used, and provision was made for a 2% error. 5 ml of blood was collected from each consenting participant and transported immediately to the laboratory where serum was stored for one week or less at 2-8 ⁰C until testing. A questionnaire was completed by consenting participants which assessed demographic information, obstetric history, exposure to risk factors and vaccination history.

2.2 Laboratory methods

An automated chemiluminescent microparticle immunoassay (Access immunoassay systems, Beckman Coulter Eurocenter SA, 22 rue Juste-Olivier, Case Postale 1044, CH - 1260 Nyon 1, Switzerland) was performed to measure individual rubella IgG concentrations and determine rubella seroprevalence. All testing was performed in an accredited laboratory which operates according to the ISO 15189 standard.

2.3 Interpretation of results

Following the recommendation from the testing laboratory concerning the cut-off level for rubella protection, a rubella IgG value > 15 IU/ml was considered protective. For the purpose of this study, the following IgG cut-off levels were accepted: 0.0-9.9 IU/ml: rubella susceptible; 10-14.9 IU/ml: possibly rubella susceptible; > 15 IU/ml: protected/immune. For results interpretation, the first two groups were taken as susceptible.

Participants were asked whether they had been immunized against measles and/or rubella ("Yes", "No" and "Don't know"). Those who answered "Yes" were further asked when they had been immunized. "In July 2016" was taken as having received the rubella vaccination. Upon recommendation from the Ministry of Health and Social services, "As a child" meant that participants received the measles vaccination and had never been immunized for rubella. "Don't know": It was assumed that pregnant women who did not recall being immunized (unknown immunization history) were not included in the July 2016 national immunization campaign and had never been immunized for rubella. It was further assumed that a person who presented with immunity during the study, without having participated in the SIA, had previous natural infection with rubella virus.

2.4 Data analysis

All data were analysed using SPSS statistical software (IBM version 24, IBM North America, 590 Madison Avenue, New York, NY 10022) and R version 3.2.2 [10]. Fisher's exact test was used to determine associations between socio-demographic characteristics and rubella immune status. The Kruskal Wallis test was used to assess equality of means for IgG antibody titres in IU/ml between different population characteristics provided in the surveys. 95% confidence intervals (CI) were calculated for IgG antibody levels. P values less than 0.05 were considered statistically significant. Correlations were assessed with Spearman's rank correlation. Difference between continuous variables in different categories was assessed with one-way analysis of variance (ANOVA).

2.5 Ethical approval

Ethical approval of the study was obtained from the School of Health and Applied Sciences, Polytechnic of Namibia, the Ministry of Health and Social Services (Reference 17/3/3) and from the Health Research Ethical Committee at Stellenbosch University (Ethics Reference S16/05/092, Institutional Review Board Number IRB0005239).

3. Results

Maternal age at enrolment ranged between 17 and 47 years with the median age 27 years (Table 1). The age group with the highest number of participants (108; 31.4%) was 21-25 years. All but two participants were residing in the urban area. Most participants (172; 50.0%) were in the second trimester of pregnancy. Twenty-four (7.0%) participants indicated that they had been vaccinated in the 2016 SIA. One hundred and sixty-nine (49.1%) participants said they had not been vaccinated for rubella and 151 (43.9%) did not know whether they had been vaccinated for rubella. These two groups were considered not to have received rubella immunizations. Most participants indicated that they did not live with small children in the house. For parity, primigravida was the category with the highest percentage of participants (129; 37.5%), (Table 1).

Table 1

Socio-demographic characteristics of pregnant women (n=344) attending public antenatal care in Windhoek, Namibia, 2016.

Characteristic	N	(%)
Age		
15-20	38	11.0
21-25	108	31.4
26-30	96	27.9
31-35	59	17.2
36-48	43	12.5
Residence		
Urban with own tap	226	65.7
Urban with communal tap	114	33.1
Rural	2	0.6
Gestational age		
First trimester	41	11.9
Second trimester	172	50.0
Third trimester	130	37.8
Unknown	1	0.3
Rubella immunization		
Yes[a]	24	7.0
No	169	49.1
Unknown	151	43.9
Live with children <5 years		
Yes	126	36.6
No	217	63.1
Parity		
Primigravida	129	37.5
One previous pregnancy	100	29.1
Two or more previous pregnancies	115	33.4

[a] July 2016 supplementary immunization activity (SIA)

Overall, seroprevalence of anti-rubella IgG among pregnant women attending public antenatal care in Windhoek was 95.9% while susceptibility to rubella virus infection was low (n=14; 4.1%) (Table 2). The age group with the highest level of rubella immunity (97.9%) was 26-30 years while the age group with the highest susceptibility (8.5%) was 31-35 years. There was no significance in the association of percentage immunity and age groups.

The overall level of anti-rubella IgG titre was very high. Anti-rubella IgG mean concentration (95% CI for mean) was 164.5 (150.41 – 178.66) IU/ml. Participants of 15-20 years had the highest (195.8 IU/ml) mean IgG levels, while participants of 31-35 years had the lowest (147.6 IU/ml). Participants in the first trimester of pregnancy had the highest immunity (presence of IgG 100%).

All participants immunized in July 2016 (7.0%) were immune to rubella virus after the SIA (mean anti-rubella IgG 187.1 IU/ml). Of 169 participants (49.1%) who indicated that they had not been immunized in July 2016, 160 (94.7 %) were immune to rubella virus, while 5.4% remained susceptible. Of 151 participants with unknown immunization history, 146 (96.7%) were immune to rubella virus while 3.3% were susceptible (Table 2). Of these participants, 14 (9.2%) indicated that they were in the first trimester of pregnancy, meaning that they could possibly have been included in the SIA. The majority of participants were already pregnant two months earlier and had not been included in the SIA.

There was a statistically significant difference between the levels of anti-rubella IgG in different parities (p = 0.004) with the highest level of rubella IgG in primigravida cases. As expected, higher parity was significantly associated with a higher age (p<0.001). There was an overall significant negative correlation between rubella IgG concentration and age (p<0.01), although there were no significant differences between the levels of anti-rubella IgG in different age categories (p = 0.085), gestational age (p = 0.215), immunization history (p = 0.439) or living with children (p = 0.319) (Table 2).

Table 2

Association of socio-demographic characteristics with rubella immunity among pregnant women (n=344) attending public antenatal care in Windhoek, Namibia, 2016.

	N	Immune[a]	Susceptible[b]	Probably susceptible[c]	P value	IgG level		P value
		n (%)	n (%)	n (%)		IU/ml	95 %CI	
Overall	344	330 (95.9)	7 (2.0)	7 (2.0)		164.5	150-179	
Maternal age								
15-20	38	37 (97.4)	1 (2.6)	0	0.563	195.8	159-232	0.085
21-25	108	104 (96.3)	1 (0.9)	3 (2.8)		167.8	143-193	
26-30	96	94 (97.9)	1 (1.0)	1 (1.0)		165.2	136-195	
31-35	59	54 (91.5)	3 (5.1)	2 (3.4)		147.6	116-179	
36-48	43	41 (95.3)	1 (2.3)	1 (2.3)		150.4	107-194	
Gestational age								
1st trimester	41	41 (100.0)	0 (0)	0 (0)	0.492	174.6	130-220	0.215
2nd trimester	172	162 (94.2)	6 (3.5)	4 (2.3)		146.4	129-164	
3rd trimester	130	126 (96.9)	1 (0.8)	3 (2.3)		185.3	160-211	

Immunization history

Immunized[d]	24	24 (100)	0 (0)	0 (0)	0.417	187.1	129-246	0.439
Not immunized[d]	169	160 (94.7)	6 (3.6)	3 (1.8)		151.9	134-170	
Unknown	151	146 (96.7)	1 (0.7)	4 (2.6)		175.1	152-199	

Live with children <5 years

Yes	126	120 (95.2)	3 (2.4)	3 (2.4)	0.754	175.6	151-200	0.319
No	217	209 (96.3)	4 (1.8)	4 (1.8)		158.6	141-176	

Parity

Primigravida	129	124 (96.1)	3 (2.3)	2 (1.6)	0.879	190.4	167-214	0.004
1 previous pregnancy	100	96 (96.0)	1 (1.0)	3 (3.0)		146.4	121-172	
≥2 previous pregnancies	115	110 (95.7)	3 (2.6)	2 (1.7)		151.3	127-176	

[a] Rubella IgG ≥15 IU/ml
[b] Rubella IgG <10 IU/ml
[c] Rubella IgG ≥10 < 15 IU/ml

4. Discussion

The results from this study revealed that a vast majority of those tested (89.0% of the study group) were pregnant during the July 2016 national vaccination campaign directed by the Namibian Ministry of Health and had thus been excluded. Results also indicated that most of these participants were immune to rubella virus (n=306; 95.6%). Only 41 (11.9%) of all participants were in the first trimester of pregnancy when they participated in the study so were probably not pregnant at the time of the SIA, and a few of those with unknown vaccination history could have received the rubella vaccination.

High seroprevalence of anti-rubella IgG indicates that most pregnant women were immune with low risk of vertical transmission and development of CRS. It also showed that the seroprevalence of anti-rubella IgG in this region increased from 84% in 2010 [8] to 95.9% among urban pregnant women attending the antenatal clinic at Windhoek Central Hospital. This increase in rubella immunity, however, could not be attributed to the vaccination campaign alone as rubella outbreaks resulting in naturally acquired rubella immunity could have contributed. In 2015, there had been an increase in rubella cases identified in the Khomas region.

Results are in agreement with studies in other parts of the world. Natural infection could result in high seroprevalence in the absence of vaccination. An unvaccinated Turkish pregnant population had a rubella IgG positive rate of 93.8% [11]. A worldwide 16-year review of rubella seroprevalence studies reported seroprevalence rates of 53.0% to 99.3% [12] while a review of African studies in the pre-vaccination era found rubella seroprevalence rates of 52.9% to 97.9% [13]. In this study, seroprevalence of anti-rubella IgG among pregnant women was comparable to other developing countries although lower levels were reported in Sudan, Nigeria and Democratic Republic of Congo [8;9;14-27].

The decline in rubella susceptibility among pregnant women in Windhoek, Namibia from 15.0% in 2010 [8] to 4.1% in 2016 was similar to findings in Brazil where pre-immunization rubella susceptibility among pregnant women was 9.4% versus post-immunization susceptibility of 2.8% [28]. In our study the decrease in susceptibility in a mainly unvaccinated study population was thus comparable to a

vaccinated population. Furthermore, our participants were within the WHO target of <5% rubella susceptibility [28] ,29].

In our setting only 2.0% of participants had IgG < 10 IU/mL whereas in Canada where routine vaccination has been in place since the 1970's 12.4% had IgG < 10 IU/ml [30]. The results in Namibia could possibly be explained by recent rubella outbreaks prior to the SIA, which probably affected the younger age groups. Recent outbreaks could further explain the very high overall level of anti-rubella IgG (164.5 IU/ml) among pregnant women in 2016. In this largely unvaccinated population, the rubella IgG concentration was much higher than the overall mean concentration of 26.5 IU/ml found in the Canadian cohort of pregnant women [30]. This could be since natural infection provide a higher and more long-lasting immunity than vaccination [31,32].

Primigravida had higher anti-rubella IgG levels than multigravida in the study, which can be explained by the fact that younger age was associated with higher levels of anti-rubella IgG. This could possibly be attributed to younger age groups having had more recent rubella infections. This is in contrast with previous studies in Namibia and Spain where primigravidae showed higher susceptibility to rubella [8,33]. IgG levels were not significantly different in pre-defined age categories, gestational ages, immunization history or co-habitation with children which could result in higher exposure to rubella virus.

A limitation of the study might be the lack of evidence-based information on rubella immunization history among participants. It can be concluded that the SIA did not have a meaningful impact on the study. It should also be noted that seroprevalence may be different in other geographical areas of Namibia. Seroprevalence would be artificially high after a recent outbreak in the geographical area studied. Although the study used 10 IU/ml as a cut off and 10-15 IU/ml as doubtful immunity, there is a body of evidence that indicates rubella IgG results less than 10 IU/ml are protective [34,35].

In this study, women of childbearing age who were susceptible to rubella virus infection formed a group of susceptible individuals not reached by the SIA. Even though susceptibility to rubella was < 5%, continued efforts should be made to limit occurrence of CRS in Namibia. Once a rubella immunization initiative is started, it must be maintained to ensure long term maintenance of

vaccination coverage and prevent increase in CRS cases. Incomplete vaccination coverage leads to the paradoxical increase in the proportion of girls reaching puberty still susceptible to rubella [36,37]. Namibia addressed this challenge by introducing routine Measles and rubella (MR) vaccination in the public sector in July 2016 for male and female children.

5. Conclusion

The study group of urban pregnant women attending antenatal care in Windhoek, Namibia from September to October 2016 demonstrated a high prevalence of rubella immunity (IgG > 15 IU/ml), despite only 7% of these women being known to be included in the nationwide SIA of July 2016, with very high proportion of immunity in unvaccinated individuals. This indicated that the majority of pregnant women in this group became immune to rubella due to natural infection. Of interest, the seroprevalence of rubella virus among pregnant women attending the antenatal clinic at Windhoek Central Hospital increased from 84% in 2010 to 95.9% in 2016, which can largely be attributed to natural immunity after recent rubella outbreaks in Namibia. Few individuals in our study had been vaccinated, with the maximum possible proportion that could have been vaccinated being 11.0% (24 plus 14 which is the number in the first trimester with unknown immunization history). Further epidemiological studies could inform policy makers on the need to address persons missed by the SIA, although results from this study do not indicate the need for follow-up national rubella vaccination campaigns. Further studies could be conducted to assess the occurrence of CRS after introduction of rubella containing vaccine into the routine immunization programme in the public sector.

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Conflict of interest

The authors declare no conflict of interest.

Contributions

EVDC was responsible for the conception and design of the study, acquisition of data, analysis and interpretation of data and drafting the article. GUVZ was supervising the project and revised the article critically. BN revised the article critically and advised on data analysis. IM assisted with data analysis and revised the article.

Disclaimer

The views expressed in the submitted article are the authors' own and not an official position of the institution or funder.

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2.3.1 Reflection on third article

The initial goal with this study was to determine the seroprevalence and incidence of rubella among pregnant women in Windhoek, Namibia. At the time when the proposal was developed, routine rubella immunization had not been introduced in the public sector in Namibia. When sampling was due to start, however, the MoHSS announced a SIA for measles and rubella to take place in July 2016. Expanded programmes of immunization (EPI's) and SIAs are both used to increase coverage and the combination should positively impact on herd immunity. More individuals are reached by mass immunization which is a programme to control disease by vaccinating large segments of the population [149]. It may even be possible to create herd immunity so that transmission is completely interrupted because of the high percentage of immune individuals. The natural reproductive number (R0) differs between infectious agents therefore requiring different vaccine coverage rates in order to achieve herd immunity [149]. Compared to rubella, measles has a higher reproductive ratio (R0) which is the number of secondary cases generated from a single infective case introduced in a susceptible population [149]. Therefore, measles vaccination programmes would require higher coverage (more immune individuals) than rubella vaccination programmes in order to achieve herd immunity.

Namibia was one of the first African countries to include a wide age range of 9 months to 39 years male and female in the SIA, supported by WHO. Pregnant women were not immunized during the SIA and thus the majority of participants in this study had missed the immunization campaign. This provided an opportunity to show that there might be a need for follow-up immunization to include these participants who have not been immunized during the SIA.

A major challenge to the study is the fact that many participants could not provide clear information about their immunization history. Questions in the questionnaire included the following: "Were you ever immunised against measles and / or rubella? - Yes, No, Don't know. If yes, when were you immunised? – July 2016, As a child, Don't know". Almost half of participants reported unknown immunization history. Only 24 participants had been immunized in the SIA of July 2016. As childhood vaccination for rubella had not been available in the Namibian public sector prior to July

2016, for the purpose of this study, it was assumed that all the other participants (As a child, Don't know) never received rubella immunization.

High seroprevalence of rubella is thus ascribed to natural infection during previous rubella outbreaks. The 2010 study was performed in antenatal clinics all over the country. This comparison shows the 2010 results in this specific facility, so the comparison to this study is valid.

Socio-demographic characteristics did not show meaningful associations with IgG positivity. Primigravidae had significantly higher specific IgG concentrations in IU/ml than those participants with one or more previous pregnancies. Primigravidae were also younger than multiparous women. Although the decrease in prevalence with increased age category was not statistically significant there was an overall significant negative correlation between rubella IgG concentration and age as continuous variable ($p<0.01$). This can be explained by information loss when binning variables in cateogries. Hence, it is not surprising that the the model including age as a continuous variable was more sensitive to detect a significant drop in seroprevalence with increase age, whereas the difference was not significant when binning age into categories.

Due to the high rate of immunity in all of five age groups, it would not have been meaningful to do mathematical modelling to determine incidence of rubella infection.

The study was meaningful to point out that there is probably no need for follow-up immunization among women who were pregnant during the SIA and therefore did not receive the rubella immunization. It also showed that most participants were immune to rubella in any case, so the risk of vertical transmission to the foetus in case of maternal infection is very low. Moreover, it suggests that vaccination coverage should be maintained at a high level in order not to paradoxically increase the number of susceptible pregnant women.

2.4   Fourth Aricle

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Seroprevalence of cytomegalovirus among pregnant women in Windhoek, Namibia, 2016

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Abstract

Background: Seroprevalence of cytomegalovirus (CMV) is high in developing countries. However, this does not exclude the risk for congenital CMV infection in pregnancies where the mothers have pre-existing immunity.

Objectives: This study aimed to determine the seroprevalence of CMV among pregnant women attending the public antenatal clinic at Windhoek Central Hospital, Namibia, in 2016. Participants were evaluated for recent CMV infections to determine the risk for vertical transmission and congenital CMV infection.

Study design: Three hundred and forty-four pregnant women consented to participate in the study. A blood sample was collected, and demographic information was captured using a questionnaire. Serum was tested for anti-CMV IgG and IgM using an automated chemiluminescence assay. Specific IgG avidity was assessed using an enzyme-linked immunosorbent assay. Data were analysed with SPSS and R. Fisher's exact test was used to determine associations among variables.

Results: Seroprevalence of anti-CMV IgG among pregnant women aged 15-48 years was 100%. Eleven participants (3.2%) had a positive or grey zone anti-CMV IgM result. Specific IgG avidity was high in all of these cases. Neither maternal age nor gestational age was positively associated with a positive or grey zone IgM result. Parity showed an association with seroprevalence of CMV IgM, with the highest seroprevalence observed in women who had one previous pregnancy.

Conclusion: This was the first study to investigate seroprevalence of CMV in Namibia. Despite the high seroprevalence of CMV among pregnant women, the Namibian population might carry the burden of congenital CMV infection among infants. This may contribute to long term disability, especially sensorineural hearing loss. Further studies are needed to determine the prevalence of congenital CMV in Namibia and neonatal surveillance studies may be important to establish the prevalence of congenital CMV disease. Aspects like timing of viral infection should be considered in the design of future studies.

Introduction

Cytomegalovirus (CMV) infection in the first trimester of pregnancy is known to cause congenital malformation, especially of the central nervous system. CMV has been identified as the main cause of non-inherited hearing loss while long-term CNS sequelae include seizures and motor and visual defects.[1-3] Between 10% and 20% of all children with congenital CMV (cCMV) infections may show signs of neurological damage during follow up,[3,4] and Enders *et al.*[5] reported that 57.6% of congenitally infected live-born infants had symptoms of varying degree. Congenitally infected newborns are at risk for serious long-term sequelae regardless of the presence or absence of symptoms at birth.[2,6] Transmission of CMV infection occurs through breastfeeding, sexual contact and contact with body fluids.[7] cCMV infection may develop when a pregnant woman is infected with CMV. Primary or non-primary maternal infection (reactivation or reinfection) at any time during pregnancy can lead to CMV crossing the placenta and infecting the fetus.[8]

Owing to variations in epidemiology and seropositivity in women of childbearing age, prevalence of cCMV infection varies across countries. In the USA, epidemiologic studies suggest that CMV infection occurs in approximately 1% of all births or affects about 40 000 infants annually. These CMV infections are asymptomatic in most cases.[9] Prevalence of cCMV infection was found to be 4.6/1 000 births in Sweden and 3.2/1 000 births in London.[10] Bonalumi *et al.*[3] reported an incidence of 0.3 - 2.4% in developed countries. The incidence of cCMV infection is highest in developing countries (1 - 5% of births) and can most likely be attributed to non-primary maternal infections.[4,11,12]

CMV infection in pregnant women can be either primary or recurrent. Primary infection occurs when a person is infected with the virus for the first time and can be demonstrated by seroconversion (appearance of antibodies that were not previously present). Recurrent infection is described as a past infection and includes reactivation of latent infections and reinfection with a different CMV strain. During latency there is no viral replication, although viral products may be transcribed during this time. The virus remains dormant in mononuclear leukocytes and cells of organs such as the kidneys and heart. The virus may start replicating at any time, causing a reactivation of the infection.[2]

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Recent data demonstrate a similar risk of developing sequelae, especially hearing loss, in infants born to mothers with primary CMV infection or CMV reinfection. There is increasing evidence that non-primary maternal infection could lead to symptomatic and severe outcomes. This underscores the importance of screening for cCMV infection in resource-poor settings. In well-resourced settings, it is believed that primary maternal infection drives cCMV infection; however in resource-limited settings with a high prevalence and incidence, reinfections may contribute to congenital infections.[3,13]

Methods

A total of 344 pregnant women attending public antenatal care in Windhoek, Namibia during 2016 were enrolled in the study. Serum was tested for CMV IgG, CMV IgM (Access immunoassay systems, Beckman Coulter Eurocenter SA, 22 rue Juste-Olivier, Case Postale 1044, CH - 1260 Nyon 1, Switzerland) and IgG avidity (ELISA, Euroimmun Medizinische Labordiagnostika, Germany). The reference range for negative CMV IgG was <6.0 AU/mL. For specific IgM, a grey zone value meant that the value was not negative but also not high enough to confidently call it positive. Software for statistical analysis included SPSS (version 24) and R (version 3.2.2).[14] Fisher's exact test was used to determine p-values while a p-value of <0.05 was considered significant.

Ethical approval

Ethical approval was granted by the School of Health and Applied Sciences, Polytechnic of Namibia; the Namibian Ministry of Health and Social Services (Reference 17/3/3) and by the Health Research Ethics Committee of Stellenbosch University (Ethics Reference S16/05/092, Institutional Review Board Number IRB0005239).

Results

The seroprevalence of anti-CMV IgG was 100% among all participants (Table 1). Results from 11 women (3.2%) were positive (or in the grey zone) for IgM. Neither age group nor gestational age was associated with IgM activation. A significant association was found between parity and CVM IgM seroprevalence, with the highest seroprevalence in women who had one previous pregnancy. This

could possibly be attributed to a higher probability of risky behaviour and higher exposure to CMV in

younger age groups.

Table 1.

Anti-cytomegalovirus IgM in relation to sociodemographic and antenatal characteristics among pregnant women attending public antenatal care in Windhoek, Namibia during 2016 (*N*=344)

Characteristics	IgG-positive samples, *n/N* (%)	IgM-positive samples, *n/N* (%)*	*p*-value
All participants	344/344 (100)	11/344 (3.2)	
Maternal age (years)			
15 – 20	38/38 (100)	1/38 (2.6)	0.613
21 - 25	108/108 (100)	5/108 (4.6)	
26 - 30	96/96 (100)	2/96 (2.1)	
31 - 35	59/59 (100)	3/59 (5.1)	
36 - 48	43/43 (100)	0/43 (0.0)	
Gestational age			
1st trimester	41/41 (100)	1/41 (2.4)	1.000
2nd trimester	172/172 (100)	6/172 (3.5)	
3rd trimester	130/130 (100)	4/130 (3.1)	
Parity			
Primigravida	129/129 (100)	5/129 (3.9)	0.027
1 previous pregnancy	100/100 (100)	6/100 (6.0)	
>2 previous pregnancies	115/115 (100)	0/115 (0.0)	

*Including grey zone results

Evidence of longstanding CMV infection was found in all samples. All IgM-positive samples (*n*=11) showed high-avidity IgG antibodies against CMV, indicating previous infections (Table 2).

Table 2.

Anti-cytomegalovirus IgG and IgM seropositivity, and IgG avidity among pregnant women

Case	IgG concentration (AU/mL)	IgM status	IgG avidity
5011	241.2	Grey zone	High
5014	140.0	Grey zone	High
5020	46.0	Positive	High
5049	>250.0	Grey zone	High
5127	181.7	Positive	High
5133	205.3	Grey zone	High
5165	>250.0	Positive	High
5188	248.3	Grey zone	High
5287	>250.0	Positive	High
5295	213.5	Positive	High
5348	114.0	Grey zone	High

Discussion

Seroprevalence of anti-CMV IgG was higher than the prevalence of CMV infection in Europe and North America (approximately 50%) but similar to the prevalence seen in South America and elsewhere in Africa.[2,7,15-18] In high-prevalence settings, most primary CMV infections occur in infancy. In a recent study in South Africa, polymerase chain reaction (PCR) analysis of saliva samples showed 2.9% of newborns from HIV-positive mothers to be CMV infected.[11] Another recent study in South Africa revealed that cCMV was prevalent in 5.96% of births with no significant association with HIV-status of the mother.[29] A population-based study of 460 healthy infants in Zambia showed 83% CMV seroprevalence by 18 months of age.[19,20] Early infection (i.e. before reaching childbearing potential) could therefore explain the 100% prevalence in our study across age strata.

Despite all pregnant women shown to have pre-existing anti-CMV antibodies, they remain at risk of CMV reactivation or reinfection during pregnancy and subsequent vertical transmission to the fetus. High maternal seroprevalence of CMV therefore does not exclude the threat of cCMV infection. Although individual risk is highest with primary infections, Omoy and Diav-Citrin[21] concluded that cCMV infection occurs in infants born to previously infected mothers. More than 60% of infants infected with CMV *in utero* appear to be born to mothers who were seropositive before conception

and who experienced reactivation of latent virus or reinfection with a new strain during pregnancy. An increasing number of studies show severe sequelae in these infants.[8] Maternal IgG positivity alone does not eliminate the risk of cCMV infection. It is thought that the cytotoxic T-lymphocyte response –another arm of the adaptive immune response – is more important than antibodies in preventing CMV reactivation, and therefore treatment or conditions that result in a decreased cellular immunity is associated with CMV reactivation even in the presence of high titres of IgG.

Leruez-Ville and Ville[22] reported that the burden of cCMV infection can be attributed to secondary maternal infection to a larger extent than previously thought. Unfortunately, there are still no validated tools to accurately diagnose and differentiate maternal reinfection and reactivation.[22]

CMV IgM is a sensitive marker of primary CMV infection but can also be detected during recurrent infection. False positives may develop owing to polyclonal activation or the presence of cross-reactive antigens in the case of a primary Epstein–Barr infection.[23] In the case of a primary CMV infection, the individual risk of transmission is highest, but in high-prevalence settings most cases of true CMV-IgM positivity would be due to secondary infections when the individual transmission risk is lower. Taken together, detecting maternal CMV IgM does not mean that the foetus will be infected, and further testing is warranted in an attempt to differentiate a primary or recurrent infection, or to identify another cause of CMV-IgM reactivity (e.g. primary Epstein–Barr infection).[2,24]

IgG and IgM serology of a pregnant woman can identify pregnancies at risk for transmission of CMV to the foetus. Finding CMV IgM antibodies with low IgG avidity supports a primary infection and a high risk of transmission, whereas in cases with high avidity, the individual risk of transmission is lower.[25] When maternal primary infection has been diagnosed or when high-definition ultrasound and magnetic resonance imaging suggest congenital infection, CMV DNA can be detected by means of PCR using amniotic fluid taken at 21 weeks of gestation.[3] Neonatal diagnosis of cCMV infection is by viral culture or PCR on blood or urine within 2 weeks of birth. Cases with asymptomatic cCMV infection may require more frequent follow-up to diagnose sensorineural hearing loss early and to prevent further deterioration.[12]

However, the costs of comprehensive diagnosis and treatment of CMV infection is prohibitive in sub-Saharan Africa and there is a need for more affordable solutions. Moreover, there is limited evidence of the benefit of antenatal antiviral therapy, which, owing to its toxicity, is seldom considered during pregnancy. In neonates, there is uncertainty about the indications for and duration of therapy.[26] Studies on newer, less toxic drugs are ongoing.[27] CMV reactivation is common in immunocompromised hosts. Therefore, in settings with a high prevalence of HIV infections, the risk of reactivation is increased.[28] Despite these challenges, clinical trials with vaccines, prenatal interventions and prolonged postnatal antiviral therapy are underway. This emphasises the need for more information on the epidemiology and diagnosis of CMV infections in pregnant women and neonates.[13]

Conclusion

All pregnant women in our study presented with IgG antibodies for CMV. Of the women, 11 showed IgM antibodies for CMV, which could have been a result of reactivation of the infection or reinfection with a new strain of CMV. IgG avidity was high in all cases, indicating absence of primary infections. However, pre-existing immunity does not exclude vertical transmission of the virus during pregnancy. Despite the high seroprevalence of CMV among pregnant women, the Namibian population might carry the burden of cCMV infection among infants. This may contribute to long-term disability, especially sensorineural hearing loss. Further studies are needed to determine the prevalence of cCMV in Namibia and neonatal surveillance studies may be important to establish the prevalence of cCMV disease. Availability of this information could lead to health interventions aimed at the reduction of disabilities such as hearing loss. Furthermore, emphasis should be on factors to be considered in designing further studies, for example the timing of viral infection.

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Author contributions. EvdC was responsible for study conceptualisation and design, data collection, analysis and interpretation, and drafting the manuscript. GvZ supervised the project and was responsible for critical revision of the manuscript. SM assisted with sample collection and reviewed the manuscript.

Conflicts of interest. None.

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2.4.1 Reflection on fourth article

This was the first study in Namibia to assess seroprevalence of CMV in any population. Given information in the literature on seroprevalence in other African countries, it was expected to be high in Namibia as well, but the 100% seroprevalence still came as a surprise. The first impression would be that there is no risk of vertical transmission in this population, because of pre-existing seropositivity to CMV. Further investigation of the literature revealed that secondary infections are now getting more attention regarding vertical transmission to the foetus. There is increasing evidence that secondary infections are the most important contributor to cCMV infection, either due to reactivation of maternal infection, or maternal infection with a new strain of CMV.

Most CMV transmission from mothers, either post-natally through breast feeding, which is by far the most common route of mother to child transmission, or intrauterine infection occurs in mothers with reinfection or reactivation of CMV infection.

Avidity testing was performed on all IgM positive and equivocal cases. No case of low avidity was found. It is therefore unlikely that these IgM positive or equivocal cases represented true recent or reactivated infections and were therefore unlikely to transmit CMV. In addition, the IgM assay may have missed some cases of reactivation. CMV IgM assays may be false positive due to a number of factors including polyclonal activation, rheumatoid factor (depending on the assay design), molecular mimicry with cross-reactive antigens such as in the case of a primary Epstein–Barr infection. The latter is, however, unlikely since seropositivity for Epstein-Barr virus is probably also close to 100% in Africa. An IgM capture enzyme immunoassay is preferred as this would reduce the impact of rheumatoid factor and improve the sensitivity of IgM assays in the presence of high concentrations of IgG

Although younger age groups might have a higher risk of sexual CMV reinfection, as the incidence of other sexually transmitted infections, such as HIV is highest in this group, high IgG avidity in all IgM seropositive individuals rather suggests that infections were acquired long ago, possibly in childhood. In conclusion, I could not offer a plausible explanation for the higher IgM prevalence in those participants with one previous pregnancy.

Due to the nature of the data, it would not be possible to do mathematical modelling to determine incidence of CMV infection. None of the participants was susceptible to primary CMV infection, so there is no linear decrease of CMV susceptibility over the five age groups.

More than 60% (and probably close to a 100% in Africa) of infants infected with CMV in utero appear to be born to mothers who were seropositive before conception and who experienced reactivation of latent virus or reinfection with a new strain during pregnancy. In fact, reactivation of CMV in pregnant and breastfeeding mothers is very common.

It can be concluded that despite high seroprevalence of 100%, pregnant women might still run the risk of transmitting the virus to the foetus, and the possibility of cCMV exists. The literature suggests that in high prevalence developing countries about 1-5% of children are born with cCMV, most likely as a result of maternal non-primary infections. Nevertheless, screening programs for cCMV are not in place in the region. Clinicians should be made aware of this, and neonatal assessments should be followed up, especially to detect sensorineural deafness as early as possible to facilitate treatment.

Future perspectives should focus on assessment of cCMV burden in Namibia, especially with technology which uses saliva or urine (rather than blood) for CMV PCR to determine the prevalence of neonatal infection.

2.5   Conclusion

Results presented in this chapter focused on seroprevalence and risk factors associated with *T. gondii*, rubella and CMV infections among two study populations, namely blood donors, and pregnant women in Namibia.

In conclusion of this chapter it can be said that a strength of this study is the three articles which have been published in peer-reviewed journals which are indexed in widely accessed databases such as PubMed (in progress) and African Journals Online.

The next chapter describes the progression and outcome of mathematical modelling applied to the seroprevalence data obtained in this study in order to estimate the rate of new infections in the study population of pregnant women.

Chapter 3: Incidence of Infection

3.1 Definitions

Prevalence of an infectious agent: The percentage of the study group who previously had or currently has an infection with the infectious agent. This can be determined by the presence of specific IgG antibodies.

Incidence of an infectious agent: The rate of new infections among the susceptible population with the infectious agent over a time period. This can be determined prospectively by identifying new infections in previously uninfected individuals or by cross sectional markers of recent infection. However, specific IgM has relatively low accuracy and the period of low IgG avidity varies resulting in inaccurate estimations. Prospective studies are costly and markers of recency, even when accurate may require very large sample sizes to accurately estimate incidence.

Force of infection: Force of infection is a per capita rate of acquisition of infection that gives an indication of the incidence of infection in the seronegative population. Force of infection is estimated as the slope of the log-linear regression line when we have the seronegative prevalence (percentage susceptible) as the dependant variable (Y-axis) and the age as the independent variable (X-axis).

Herd immunity: Herd immunity refers to the percentage of a community that needs to be immune to an infection in order to contain spread of the infection. This concept is used to determine the need to vaccinate a community for an infectious agent like rubella. Rubella is less infectious than measles, so a lower level of herd immunity would be required to interrupt rubella transmission than for measles.

Infectiousness: An infectious agent is highly infectious if the infection spreads fast in a community. The natural reproductive number R_0 is used to measure this, which is the average number of cases infected, in a susceptible population, by a single infected case.

Virulence: Virulence refers to the likelihood that an infectious agent would cause serious disease. Virulence is not related to incidence (rate of new infections) unless only cases with severe disease

qualify for inclusion; infectiousness and virulence are totally different concepts – some viruses are highly virulent but have low infectivity.

Prior infection: Pregnant women may present with specific IgG antibodies in the absence of IgM antibodies, indicating that they had been infected with the infectious agent before becoming pregnant.

Affinity: The binding force between an individual specific antibody and its antigen.

Avidity: Avidity is the net effect of all the different antibodies binding to their specific antigens with a certain affinity.

Vertical transmission: transmission from an infected mother to her child, either in-utero, perinatally of post-partum.

Congenital infection: An infection acquired before birth through vertical transmission from the mother.

Neonate: A newborn baby during the first 28 days of life.

An infection can either be endemic or epidemic. An endemic infection is found consistently over time in a specific population or in a certain geographical area. CMV infections occur worldwide in an endemic pattern and in countries with a high prevalence of *T. gondii* endemic transmission is also observed. Infections with an epidemic pattern of occurrence, however, do not occur evenly spread over time in a population. Rubella is an example of such an infection with a seasonal peak occurrence, with some years having much larger outbreaks than other years.

In case of infections that are associated with protective immunity, in any population there would be three different groups of individuals regarding their immune status to an infectious agent.

1. The first group would be recovered or immune since they may have been naturally infected or if a vaccine is available could have been vaccinated.
2. The second group is currently infected with the agent.
3. The third group are susceptible either because they have never been exposed to the agent or because their immunity has waned (occurs after some cases of vaccination) or in the case

of some chronic or latent infections, the immune response is not fully protective against reinfection or reactivation [81].

Seroprevalence of an agent refers to the first group and may be measured by the presence of IgG antibodies to the agent. The presence of IgG antibodies to an agent indicates past infection and lasts for life. In some cases, like CMV infection, reactivation or reinfection may occur.

Incidence refers to the rate at which new infections occurred in the susceptible population over the study period. Incident cases could be detected by the presence of IgM antibodies against an agent, indicating current or recent infection. Immunoglobulin M antibodies are the first to appear upon exposure to an infectious agent but diminish within a couple of months. However, IgM is not an ideal marker to estimate incidence for the following reasons:

1. it be could also be positive in secondary infections;
2. the duration of IgM positivity is short and therefore estimates of recent infections would require large sample sizes if the incidence is low;
3. IgM testing may be prone to false positivity and the false positive rate could be high relative to the true positive rate when the prevalence of acute infections (i.e. incident cases) is low.

Recent infections could further be corroborated by the presence of specific IgG antibodies with low avidity. Upon exposure to an infectious agent, specific IgG antibodies are formed, which are however not yet exactly fitted for the epitope on the antigen. This would be reflected in antibodies with low affinity – which would result in a low avidity when assayed (avidity is the net effect of individual antibody affinities and is measured by the proportion of antibodies that are disrupted by a chaotropic agent such as urea). With time antibodies develop that have a higher binding affinity. This would be reflected in a high avidity, and thus points at non-recent infection. The mechanism of avidity testing is described in Chapter one, section 1.3.9 Diagnosis of *T. gondii* infection.

Age-prevalence data refer to designation of age groups within the study group. It would require stratification of sample collection to ideally include the same number of participants in each of the selected age groups. After testing of the samples, data analysis could reveal the prevalence or susceptibility to an agent in each age group.

3.2 Objective

Incidence of an infection could give an indication of the force of infection, and how easily it spreads in a community. Measuring the incidence of an infection among pregnant women could be useful to give an indication of the risk of congenital infection occurring. If an agent that is associated with intra-uterine infections shows high incidence in a pregnant population, there would be an increased risk of vertical transmission. It would then follow that the prevalence of congenital infections with adverse sequelae would be high. Knowledge of the incidence of infections would be useful in Namibia since there is no information available on the prevalence on congenital infections.

3.3 Mathematical modelling

The direct measurement of incidence is neither feasible nor practicable as very large sample sizes and repetitive testing would be required to find new cases of infection. Moreover, in the context of a low prevalence of recent infections (incident cases) any assay aimed at detecting these incident cases with specificity that is not very close to 100% would result in the greater proportion of positive tests being false positive. For example, if at any time the expected prevalence of recent infections is 1% (this would translate to an incidence of > 3 % as the expected window for IgM positivity is < 4 months for most agents) and the specificity of a test is 99%, ~50% of positives will be false positive (positive predictive value of ~50%).

Therefore, rather than attempting to directly measure incidence, mathematical models may be helpful in estimating incidence from cross-sectional age-prevalence data. Such a model would hold true if the following conditions are met:

1) The incidence is stable across the age range studied; and

2) the infection does not affect the probability of dying during this observed period.

These assumptions are likely to hold true for CMV and *T. gondii* – as infections are mostly asymptomatic and the incidence is likely not very variable across child-bearing age. However, in the case of rubella an epidemic pattern of transmission may require a large sample size due to the expected variance in incidence. Nevertheless, if the proportion of susceptible individuals and age

shows a significant negative log-linear relationship with high correlation coefficient it is likely that the age-prevalence models would be valid.

It was expected that the prevalence of exposure (presence of specific IgG antibodies) to these agents would increase with age, and it followed that the number of susceptible pregnant women would decrease with age. Studying the relationship between these two variables would enable the mathematician to make predictions on what the incidences of these infections would have been during the study period.

The following calculations can be done to estimate incidence mathematically:

S_j: Number susceptible at time j

j: number of years

i: incidence – number of new cases in the susceptible population

S_0: number of susceptible individuals at start

Susceptible individuals decrease at a constant proportion:

$$S_j = S_0 (1 - i)^j$$

For each interval (year) the ratio of S_j / S_{j-1} = 1-i

As ratio of S_j to S_{j-1} is constant; one has to log transform S_j: $(ln(S_j))$ for linear regression with j:

It follows that the gradient:

$$ln(S_j) - ln(S_{j-1}) = ln(1 - i) = m$$

where m = gradient of regression line for S_j = mj+c

e^{-m} = 1-i

Solve i:

$$i = 1 - e^m$$

Note: important to have enough candidates in each age stratum

3.4 Examples from literature

In a previous study, Cutts and Vynnycky modelled the incidence of congenital rubella syndrome in developing countries. They developed a formula to determine estimates of the incidence rate of infection in pregnant women, as well as estimates of the incidence of congenital rubella syndrome. They tested the formula using data from two big reviews and papers from a Medline search. Included in the research were studies of women attending antenatal clinics. Data were required on the number studied and the numbers seropositive for rubella in at least two age groups between the ages of 15-49 years. There was an excellent agreement between model predictions and the prevalence of seronegativity to rubella virus in different WHO regions. The estimated incidence rate of congenital rubella syndrome in the WHO Africa region was found to be 104 per 100 000 live births. The number of CRS cases in the same region was estimated at 22 471 in 1996 [62,133].

In another study, Colugnati et. al. used CMV seroprevalence data from a national survey to estimate CMV incidence among the general United States population and among pregnant women. They employed models that used age specific CMV seroprevalence data as cumulative markers of previous infections in order to derive mainly the force of infection. Force of infection is a per capita rate of acquisition of infection that gives an indication of the incidence of infection in the seronegative population. Force of infection is estimated as the slope of the log-linear regression line when we have the seronegative prevalence (percentage susceptible) as the dependant variable (Y-axis) and the age as the independent variable (X-axis). Age groups selected for the study were 12-19, 20-29, 30-39 and 40-49. The percentages of seronegative women in these age groups were 42.6, 17.8, 13.4 and 5.3. The corresponding numbers of women with primary infection during live-birth pregnancies were calculated at 4150, 1867, 621 and 14. The force of infection among blacks in the USA was found to be 5.7 – this is the number of primary CMV infections per 100 seronegative persons per year. It was estimated that 27 000 new CMV infections occur among seronegative pregnant women in USA each year [134].

In preparation of this study, age-stratified seroprevalence data in several published studies were investigated, and incidences of infections could be calculated using the mathematical formula

described above. Using an Excel spreadsheet, prevalence % in each age group was tabulated and susceptibility % in each age group was calculated. Susceptibility % was converted to decimals which were used to calculate logarithms of susceptibility. These were used to create a graph with mean age of each age group on the X-axis and logarithm of susceptibility on the Y-axis. The trendline was drawn and the formula y=mx+c was applied in order to find m which was used in the mathematical model to calculate incidence. Line graphs are shown in Figures 2,3 and 4 while Table 4 tabulates data including age intervals used in the calculations.
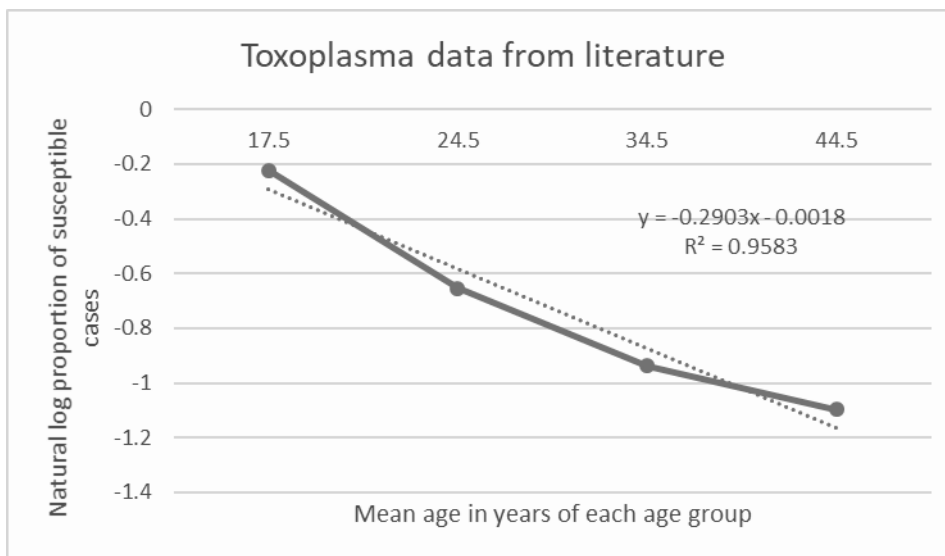
Figure 2. Mathematical modelling of age prevalent *T. gondii* susceptibility from the literature
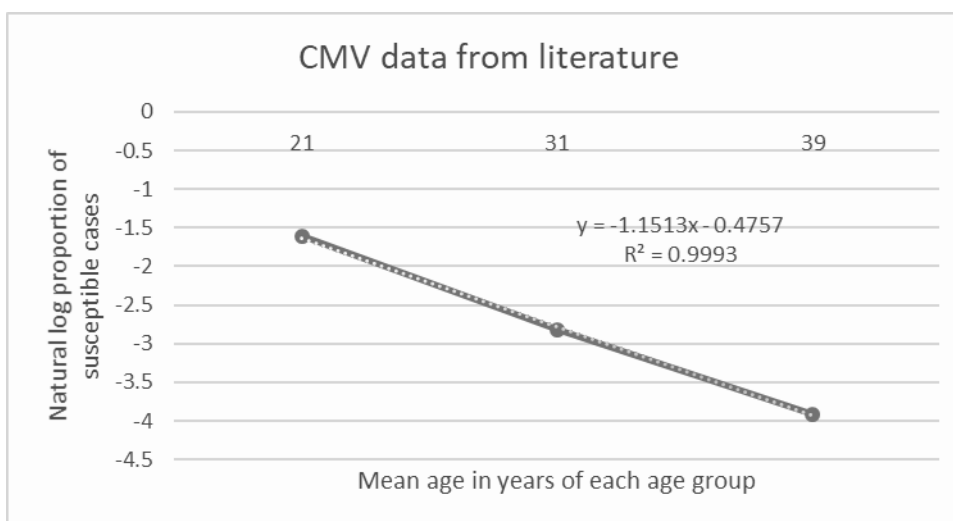
Figure 3. Mathematical modelling of age prevalent CMV susceptibility from the literature
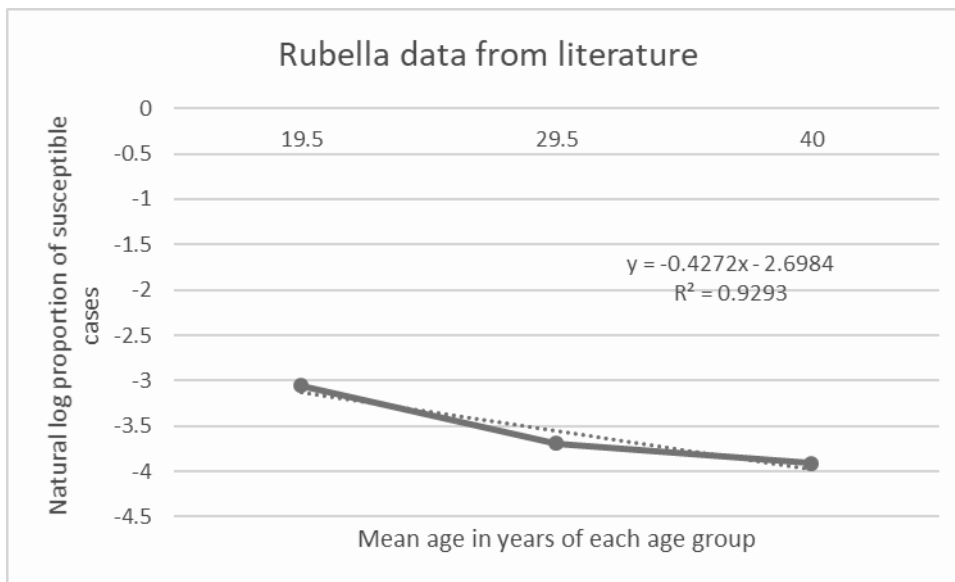
Figure 4. Mathematical modelling of age prevalent rubella susceptibility from the literature

In all these cases the data followed a specific trend with a negative trendline. This means that prevalence increased with age while it follows that susceptibility decreased with age. This is shown by negative slopes (m-values) for all three graphs. When these negative m-values are used in the model, it yields positive incidence values meaning that the probability or risk of new infections occurring in a future time period increases by the calculated incidence rate.

Table 4 depicts data from the literature and mathematical modelling applied to it in order to estimate incidence of infections.

Table 4. Mathematical modelling to calculate incidence from published data

Rodier et. al. [28]: *Toxoplasma gondii*

Age	Expected age	Prevalence %	Susc %	Suscept	LN(susc)
15-19	17.5	20	80	0.8	-0.22314
20-29	24.5	48	52	0.52	-0.65393
30-39	34.5	60.8	39.2	0.392	-0.93649
39+	44.5	66.6	33.4	0.334	-1.09661

	m=slope of graph			y=mx+c	
	-0.2903				

Incidence	0.251960878	25.19%		R^2	0.9583

A study done in India reported by Cannon et. al. [130]: CMV

Age	Expected age	Prevalence	Susc	LN(Susc)
16-25	21	80	0.2	-1.60944
26-35	31	94	0.06	-2.81341
36-42	39	98	0.02	-3.91202

m=slope of graph			-1.1513			
Incidence = 1 - e^m			0.683775	68%	R^2	0.9993

Corcoran [57]: Rubella

Age	Expected age	Prevalence	Susc	LN(Susc)
15-24	19.5	95.3	0.047	-3.05761
25-34	29.5	97.5	0.025	-3.68888
35-45	40	98	0.02	-3.91202

m=slope of graph   -0.4272
Slope: y=mx+c
c= intercept with y axis

Incidence = 1 - e^m			0.347667	34.80%	R^2	0.9293

These calculations using data available from previous studies, showed that the mathematical model could be used to predict incidence of the respective infectious agents. In some studies, however, the age-prevalence data did not show a linear decline in susceptibility. While the data could be used for linear regression analysis in most cases, the assumptions discussed below in 1.6.5 were not always met.

For *T. gondii*, Sitoe et. al. conducted a study among 150 pregnant women in Mozambique. Among HIV-negative women they presented the following IgG prevalences: <17 (1%); 17-22 (9%); 23-27 (20%); 28-35 (22%). Among HIV-positive women: <17 (16%); 17-22 (25%); 23-27 (39%); 28-35 (38%). These data met the requirements of modelling and would give meaningful results since susceptibility declined in a linear fashion over increasing age ranges.

For rubella, Belefquih et. al. [65] presented the following susceptibility data among 2284 pregnant women: 14-24 years (9.1%); 25-34 years (11.0%); 35-44 years (7.9%); >45 years (8.1%). These data would probably result in a low R^2 when modelled. In another study, Onakewhor et. al. [68] found IgG negativity among 270 women: <35 years (47%); >35 years (47%). In this case mathematical modelling was not possible since there was no trend in the susceptibility among age groups. Rodier et. al. [28] presented the following IgG prevalence data: 15-19 (80%); 20-29 (87%); 30-39 (84.7%); >39 (83.3%). Although there was no linear increase in seroprevalence with increasing age, the data met the requirement of decreased susceptibility with age.

For CMV, Rodier et. al. [65] presented the following IgG prevalence data among pregnant women: 15-19 (80%); 20-29 (99%); 30-39 (95.6%); >39 (100%). These data also met the requirement of decreased susceptibility with age.

Mathematical modelling nevertheless poses a solution to the challenging task of determining the incidence of infectious diseases among pregnant women and provides an alternative to costly and invasive methods.

3.5   Assumptions

In order to use the mathematical model to predict incidence of infection, certain assumptions regarding the epidemiology of the three infections studied in this research will be discussed.

3.5.1 Uniform risk

One of the assumptions for mathematical modelling is that the risk of infection is spread uniformly over the test period, or that exposure to the infection is the same over the five age groups [62]. This would probably be true for *T. gondii* and CMV. However, rubella infections are seasonal with most outbreaks in southern Africa occurring during late spring which is during October to November. Chimhuya et. al. [58] reported on rubella incidence rates found in the WHO measles/rubella surveillance programme in Zimbabwe. They reported peaks in late spring with a sharp decline in December through April associated with the rainy season. Laboratory confirmed rubella cases among the suspected cases were 37.5% during the rainy season and 62.5% during the dry season. They further reported that 95% of rubella cases were among children up to 14 years of age, with a peak from 4-14 years. Once the first individuals get infected, the infection spreads rapidly especially among school children and in institutions like hostels. The relative proportions of Rubella infections in various age strata cwould thus be influenced by an epidemic occurring in a specific year, which depending on the transmission setting might have affected younger age groups (attending school) more than older ones The assumption of uniform risk may therefore not hold true.

In the case of CMV, the epidemiology of the infection in sub-Sharan Africa is such that almost all infections had likely beenacquired long before females reached childbearing potential, Therefore, it was not feasible to assess an age seroprevalence relationship. In other geopgraphical settings, where CMV is acquired later in life, through sexual tranmssion, an age seropevalence model may be useful to estimate incidence, although the exposuse risk may not be uniform, as in additional to sexual exposure, exposure to the urine or respiratory secretions of infants or young children, such as in daycare settings may be another source of infection for mothers having young children or pregnant women who are exposed to young children through occupational or social interactions. [128]

3.5.2 Mortality rate

Another assumption is that the infectious agent is not causing a high mortality rate among patients. This needs to be taken into consideration in cases where the infection itself is likely to cause the death of the patient during the test period. In this study, however, the mortality rate due to the infection itself could be negated since the infections are not life-threatening under normal circumstances in healthy individuals.

3.5.3 False-recent results

It is further assumed that the three infectious agents do not show a high rate of false positive results. Some diagnostic tests may show false positive or persistent positive results, especially in the case of specific IgM antibodies. In this study, seroprevalence data were based on IgG antibody testing, which is less prone to false positive results.

3.5.4 Decrease in susceptibility over age

To make mathematical modelling meaningful for the purpose of this study, it was expected that susceptibility to the three agents would decrease linearly over the five age groups selected. Most of the published studies described in 3.4 showed decreasing susceptibility to the three infections among increasing age groups. The expectation for this study was thus that the susceptibility to an agent would decrease with increasing age.

In conclusion, the age-prevalence data found in the literature for the three infectious agents provided meaningful results when incidence was estimated using the mathematical model presented above. However, no age-prevalence data obtained in Namibia of the three infectious agents investigated in this study could be found in the literature. Therefore, this study aimed to determine seroprevalence of *T. gondii*, rubella and CMV in five age groups among pregnant women in Namibia.

3.6   Mathematical modelling using data from this study

For all three infectious agents, one of the objectives was to estimate the incidence of these infections as this would provide an indication of the potential or risk for congenital infections to occur.

During the planning stage a modelling study of published age-prevalence data was done and incidences of the three infections could be calculated using age-prevalence data from published articles. The mathematical model used as well as the suitability for the model of age-prevalence data presented by different authors were described in 3.4. Chapter Three, 3.5, also described certain assumptions or requirements for the data obtained in this study to deliver epidemiologically meaningful results when the model was applied to it. In 3.5.1. the assumption of uniformity of risk was described. In 3.5.4 the expectation that age-prevalence in this study would also follow a specific pattern of prevalence increasing with age was described.

The seroprevalence data obtained in this study were presented in Chapter Two. The data did not show an age-prevalence trend and the incidences obtained when the model was applied, were not epidemiologically meaningful. It was concluded that the model was not applicable to our data since some of the assumptions were not met. The main and most important reason was that the sample size (cases different from 100% or 0 in each age stratum) was too small to calculate this with any precision; secondly no age-prevalence trend could be demonstrated as discussed below.

Unlike the expectation that prevalence of infection would increase with age and susceptibility would decrease with age, both *T. gondii* and rubella showed a decline in seroprevalence with increasing age. This means that the susceptibility of participants increased with age which was contradictory to what was expected. The trendlines on the graphs in Excel showed positive slopes. Results obtained with the model thus yielded a negative incidence rate which indicated decreased risk with increased exposure or increased age.

In the case of *T. gondii* prevalence increased linearly over the first three age groups only to decrease as age increased further. However, considering the small number of cases (9/344) that were *T. gondii* IgG positive random error could play a role and differences in prevalence between the different age strata cannot be ascertained with any precision. Different from published data we did not observe a

decrease in susceptibility with increased age. There could thus have been a cohort effect which indicated that risk of infection was not uniform over the five age groups as it appeared that the risk of infection decreased in older individuals. Urbanization could have played a role with younger individuals growing up in the urban areas and older individuals moving from rural areas. This is contradictory to a previous study in Namibia [7] which reported higher prevalence of *T. gondii* in rural areas. The expectation would be that rural people would be more exposed to *T. gondii* due to their culinary habits. They are more likely to consume untreated water and milk and they frequently handle raw meat during slaughtering of their animals. As there were too few cases with *T. gondii* IgG, our study did not have the statistical power to investigate associations with being seropositive and the lack of age-prevalence trend remains unexplained.

In the case of rubella, seroprevalence among five age groups ranged from 91% to 97% with small differences between age groups. With such a high frequency of immunity and as there were only 7 cases out of 344 who were susceptible to rubella, we could not reliably assess differences in seroprevalence between various age categories. This was unexpectedly high and higher than the expected prevalence and effectively decreased our statistical power to assess differences between groups, and in this instance different age strata. It is therefore not surprising that we failed to observe a consistent increase in seroprevalence of rubella IgG with increased age. A much larger sample size would have been needed to accurately assess differences between age categories, with such a high overall immunity rate.

Mathematically deriving incidence from age- seroprevalence data also assumes that different age cohorts would have had relatively uniform exposure risks with the cumulative risk of exposure and immunity increasing in older individuals. Interestingly, younger individuals had statistically significant higher antibody titres supporting recent infection; a proportion of these infections could have occurred during a recent rubella epidemic.

The Windhoek region has seen rapid recent urbanization and older individuals who moved from less dense rural areas may have had lower exposure risks. However, Alleman et. al. [73] reported 84% rubella seroprevalence in urban settings in Democratic Republic of the Congo (DRC) versus 83% in

rural settings. Chimhuya et. al. ascribed a measles and rubella outbreak in 1977 in Zimbabwe to the movement of people during the war [58]. It seems like the model would be more applicable in stable populations where more uniform risk over age groups would be expected. Chimhuya et. al. further reported a peak of rubella infection in children from 4-14 years. In their report on measles and rubella surveillance data 95% of rubella cases were found in children up to the age of 14 years. This finding is supported by Alleman et. al. [73]. It supports the statement above that younger participants were probably exposed while in school, while older participants probably had less exposure. Children only became liable to attend school after Namibian independence in 1990. Furthermore, the cohort effect found in the rubella data could possibly have been influenced by the recently introduced rubella vaccination, although only 27 of the 344 participants had indicated that they had been vaccinated. Cutts and Vynnycky [62] only included data from studies prior to wide-scale vaccinations in the country when they developed their model.

The mathematical modelling expectation was not realised for CMV since all age groups had seroprevalence of 100%, even though we used an age stratified sample collection to attempt to ensure that we may have some young individuals who were not yet infected. Vynnycky et. al. [141] excluded data sets where the best-fitting force of infection was zero and where the upper limit on the 95% CI was 100%. In this study it was expected that seroprevalence would be high, but the 100% seroprevalence in all age groups was unexpected. This finding is however, in line with a previous finding in Zambia that most children are seropositive for CMV at the age of 18 months [137]. This previous study indicated that individuals in Africa get infected at an early stage of life and would exhibit very high seroprevalence by the time they reach reproductive potential. Another previous study in DRC concurred that most CMV exposure and seroconversion occur before the age of 15 years [73].

It can be concluded that although using data from the literature we were able to model incidence of the three infections, the data obtained in this study were however not sufficient and suitable for age-prevalence models. The first challenge faced was the very low seroprevalence of *T. gondii* IgG and very high prevalence of rubella IgG, with CMV IgG being 100% across all age-strata. Moreover, there

was no increased seroprevalence with age, but in case of both *T. gondii* IgG and rubella, a trend, suggesting that the seroprevalence might decrease with age, which was unexpected.

Alleman et. al. used data from 1605 pregnant women aged 15-46 years to estimate incidence of CRS per 100 000 live births and the number of CRS cases born in 2013 in DRC [73]. In Chapter One of this study data from a study in India [reported in 130] were used to apply the model to CMV infection. Although only a small sample size was used in the study (16-25: N= 172; 26-35: N=119; 36-41 N=49) a perfect fit of $R^2 = 0.9993$ was obtained when we applied the model to the data.

3.7 Conclusion

In summary, datasets obtained in our study proved unsuitable to do mathematical modelling as described in the study design. Although the model could be applied to data from the literature and produced meaningful estimates of incidence rates, the results from this study did not provide answers which could be applied epidemiologically.

Results from the study do, however, contribute to knowledge about seroprevalence of infectious diseases among pregnant women and possible risk of vertical transmission of infections.

The next chapter discusses seroprevalence results obtained in this study and concludes on the meaningfulness of seroprevalence and incidence data presented.

Chapter 4: Overall Discussion and Conclusion

In the absence of a surveillance system for congenital infections in Namibia a study on vertically transmissible infections among pregnant women was conducted. The study focussed on assessment of seroprevalence (IgG) of infections and further aimed to identify pregnancies at risk of vertical transmission of infections by determining IgM and IgG avidity. It also investigated the association of risk factors with infections. The study design included the calculation of incident cases by using age-prevalence data in mathematical modelling in order to assess the probability of congenital infections to occur.

This chapter presents several aspects, including a narrative of the approach followed showing how the methodology relates to the objectives. It is followed by a summary of the main findings relating them to the objectives and an evaluation of the contribution of the study to existing knowledge and contextualization. Limitations of the study are discussed. A description of how the study impacts on health with a focus on Namibia is followed by a reflection on the future perspectives. A conclusion is giving a short summary of main findings and the way forward, highlighting the remaining gaps in research.

4.1   Approach followed

The ultimate goal of the study was to assess the prevalence of congenital infections in Windhoek, Namibia. In the absence of surveillance systems for congenital infections and given the high cost of PCR testing among newborn babies for research purposes, the study focused on seroprevalence of infectious diseases among pregnant women attending public antenatal care. An effort was made to assess risk of vertical transmission through IgM and IgG avidity testing and initially mathematical modelling to calculate incidences of the infections had been planned.

4.1.1 Methods

This was a cross-sectional descriptive study. A descriptive study was selected as the method of choice since the aim of the study was to investigate the immune status of pregnant women and assess their risk of vertical transmission of infection. Participants were recruited prospectively but

samples were collected once only and there were not sufficient funds for follow up testing of mothers and babies. We chose this approach due to the challenge to access patient records and scarcity of information on laboratory tests in a retrospective study. In addition, a retrospective study would have ruled out laboratory testing to determine seroprevalence and risk of vertical transmission and administration of the questionnaire to determine risk factors associated with infection.

A pilot study was performed on a group of blood donors in central Namibia (Van der Colf et al., 2014, attached). Seroprevalence of antibodies to *T. gondii* was 1% in that group, the lowest that could be found in the literature. It was estimated that a higher seroprevalence would be found in the bigger study among pregnant women attending antenatal care, since blood donors originate from a more affluent population while patients attending a public health facility might have different living conditions and culinary habits.

4.1.2 Sample size

The results from the pilot study of toxoplasmosis among blood donors informed the calculation of sample size for the study among pregnant women and gave an indication of toxoplasmosis among the broader Namibian population. This was necessary because the only previous study on *T. gondii* in Namibia (South West Africa) was reported in 1978 and the information was outdated [7]. For calculation of sample size, the prevalence of toxoplasmosis in Namibia (1978 and 2014) was combined with the prevalence found recently in neighbouring South Africa [41] and a prevalence of 6% was used in Cochran's formula. The ideal sample size was calculated to be 542 participants based on seroprevalence of *T. gondii*. Smaller sample sizes were calculated for the other two infectious agents based on the high seroprevalence found in neighbouring South Africa. No information on seroprevalence of rubella and CMVs in Namibia was available at the time of study design. The aim of the study was to include results from 542 participants for all three infectious agents respectively. Eventually a sample size of only 344 could be reached due to lack of funding.

4.1.3 Sample collection

The pilot study was published in 2014 and paved the way for the bigger study involving the seroprevalence and incidence of three infectious agents among pregnant women. In 2015 funding

was obtained for the study among pregnant women. In January 2016 the study was registered for a PhD with Stellenbosch University. Ethics approval was obtained in July 2016 from the MoHSS as well as from the Human Research Ethics Committee of Stellenbosch University. Sample collection took place from September to October 2016 but was terminated due to lack of funding. The funding agency was not in a position to imburse the whole amount which had been allocated to the study.

To facilitate calculation of incidence according to age-prevalence, a stratified sample collection method was used, with the target 108 participants in each of five age groups (13 - 20; 21 - 25; 26 - 30; 31 - 35; 36 - 48). At the time when sample collection was interrupted the first age group of 21 – 25 years had been filled with 107 participants. It means that up till that time, the stratified sampling approach had not been implemented and for the purpose of this study it can be said that sampling was sequencial. The youngest participant was 15 years old and the oldest participant was 47 years old. The mean age of participants was 27 years. All participants gave consent and minors (persons under 18 years) gave assent while consent was obtained from the parent / guardian. In five cases participants did not sign the consent form and these participants were excluded from the study. One Ovahimba woman was excluded due to not being able to understand English or Afrikaans. The remaining 344 participants were included in the study.

4.1.4 Sample analysis

A SANAS accredited pathology practice analysed the samples and all results were received in one week. Testing of *T. gondii* IgM (in IgG positive cases) and IgG avidity (*T. gondii* and CMV) were performed by the researcher in the NUST laboratory which is a teaching laboratory. For the pilot study among blood donors, all sample analysis was performed in the NUST laboratory by the researcher.

4.1.5 Questionnaire

A questionnaire was designed using information from previous publications in the same field (Addendum G). The questionnaire addressed demographic information like maternal age, gestational age, parity and obstetric and perinatal history. It also addressed risk factors like living with animals, consumption of various foods, blood transfusion, living with children under the age of

five and handwashing. The questionnaire was successful to collect demographic data and data on risk factors. It was, however, not able to collect unambiguous data on rubella vaccination history. The reason being the additional goal to do further studies on seroprevalence of measles among pregnant women after completion of the PhD study. The question on rubella vaccination history tried to capture data about measles vaccination as well, rendering it ineffective for the purpose of this study. The essential information from this question is that only 7% of participants had been vaccinated in the SIA due to the rest being pregnant at the time. For the purpose of this study, the SIA was deemed as not having had a significant influence on the results.

Information on risk factors associated with toxoplasmosis were useful for this study. However, information on obstetric history as well as exposure to risk factors could not be fully utilized due to low seropositivity of *T. gondii* IgG. A much larger sample size would be needed in order to have good statistical power to determine meaningful associations. The association of risk factors with CMV infection could not be analysed due to 100% seropositivity.

Overall, as the effective sample size was small (few individuals diferent from the majority: low seroprevalence in the case of *T. gondii* IgG and high or very high in the case of rubella and CMV infection), there was insufficient power to assess the impact of multiple possible risk factors collected through a very comprehensive questionnaire.

4.1.6 Data analysis

Data collected with the questionnaire as well as test results were entered in an Excel spreadsheet and analysed with SPSS version 24 and R. Participants were divided into five age groups. Mean seroprevalence, mean rubella IgG concentration and 95% confidence intervals were calculated. P-values were calculated using the Fisher's exact test with p-value <0.05 deemed significant. The Kruskall-Wallis test was used to determine associations between demographic data and rubella IgG concentrations. This is a non-parametric test and an extension of the Mann Whitney test of ranks. The R statistical package was used in cases where there were too many variables in a model for a Fisher's exact test (typically 2 by 2) to be performed. Involvement of a biostatistician is acknowledged in Addendum I, Author contributions.

It is concluded that the methodology followed was appropriate to address the objectives set during study design. The following section gives a summary of the main findings of the study.

4.2   Summary of Main Findings

This study focused on seroprevalence of three infectious agents namely *T. gondii*, rubella and CMV among pregnant women attending antenatal care at Windhoek Central Hospital, Khomas Region, Namibia. A pilot study was conducted to determine seroprevalence of *T. gondii* among blood donors.

In the first publication, 1% seroprevalence of *T. gondii* was found among blood donors from mainly urban settings in Central Namibia. Of 312 samples tested, 6 had positive or equivocal IgG results. Blood donors had limited exposure to *T. gondii*, and this result could possibly be extrapolated to the broader Namibian population. One blood donor exhibited both IgG and IgM antibodies against *T. gondii* and could possibly pose a risk of transfusion transmitted infection. This donor had indicated that he lived in an urban area and kept cats and dogs. Association of risk factors with *T. gondii* infection could not be determined due to the low rate of positive cases among blood donors.

In the second publication, 2.61% seroprevalence of *T. gondii* was found among pregnant women attending the antenatal clinic at Windhoek Central Hospital. Of 344 participants, 9 had IgG antibodies against *T. gondii* (including borderline positive results). Of these 9 participants, 1 had IgM antibodies against *T. gondii* with IgG avidity giving an equivocal result. The other 8 IgG positive cases also had high or equivocal IgG avidity. This means that there had been limited previous exposure of pregnant women to toxoplasmosis while the risk of vertical transmission of toxoplasmosis was also low. Among 9 risk factors for *T. gondii* infection evaluated, none showed a significant association (p-value <0.05). Surprisingly, all 9 IgG positive participants indicated that they use water from their own tap as opposed to water from a communal tap indicating that risk factors other than those investigated could have contributed to the infection. Calculation of incident cases of *T. gondii* infection by using age stratified IgG seroprevalence data was not possible due to the lack of a linear increase in seroprevalence among pregnant women in five age groups. Seroprevalence increased linearly in the first three age groups, only to decrease after the age of 30. Although the number of infections were too few to make strong conclusions, the lack of increasing seroprevalence with higher age may

suggest that different age cohorts had different exposure risks. Without a relative uniform exposure risk in different age cohorts a mathematical model of age-prevalence ratio to calculate incidence would be invalid.

The third article described the very high overall rubella seroprevalence of 95.9% among pregnant women. There was no statistically significant (p-value <0.05) difference between seroprevalence in five age groups. High seroprevalence and the small number of individuals remaining susceptible might have caused a lack of statistical power to calculate any differences. Seroprevalence in five age groups were 97.4; 96.3; 97.9; 91.5 and 95.3 percent suggesting that younger age groups may have been more affected by rubella outbreaks.   The mean anti-rubella IgG level was highest in the age group of 15 – 20 years (195.8 IU/ml) only to decline to 150.4 IU/ml in the age group of 36 – 48 years. This finding was supported by the finding that primigravidae had significantly (p-value <0.05) higher anti-rubella IgG levels than multiparous women indicating that participants probably acquired naturally infected at a young age. Shortly before the study a rubella outbreak occurred in the Khomas region, and in addition, a national SIA took place including male and female persons aged 9 months to 39 years but excluding pregnant women. Only 7% of participants had been included in the rubella and measles SIA of July 2016 while the rest of the participants were pregnant at the time. Therefore, immunity was ascribed to natural immunity acquired in previous rubella outbreaks. The recent outbreak prior to the study could also explain the very high mean anti-rubella IgG concentration of 164 IU/ml found among participants. IgM and IgG avidity testing were not done for rubella since very few if any incident cases were expected. Rubella is a seasonal infection which occurs mainly in spring and in retrospect, it was found that there had been no outbreak in the spring of 2016. Once again, incidence of rubella infection could not be calculated due to the small proportion of non-immune individuals in each age category. Differences in seroprevalence among the five age strata, although not statistically significant, suggest they did not have similar exposure to rubella infection. Younger age groups are probably more susceptible to rubella infections during outbreaks due to crowded conditions in schools increasing the risk of being infected.

The fourth article focussed on seroprevalence of CMV among pregnant women attending the antenatal clinic. CMV IgG seroprevalence of 100% was found among all five age groups, indicating

comprehensive previous CMV exposure, probably at a young age. All participants were tested for anti-CMV IgM with 11 positive or grey zone results. Of these 11, all had high IgG avidity showing that it had been past infections and not secondary infections (possibly with a different strain) during pregnancy. These pregnant women, therefore, were not at risk for primary infection during pregnancy and consequent cCMV. It was however, noted from the literature that secondary infections during pregnancy result in 1-5% cCMV prevalence in neonates in developing countries where high seroprevalence of CMV prevails. The majority of these cases are however asymptomatic of which a small proportion may have sequelae [100]. Calculation of incident cases of CMV infection by using age stratified IgG seroprevalence data was not possible due to 100% seropositivity in all five age groups. For the same reason association of risk factors like blood transfusion, living with children under the age of five or handwashing practices with CMV infection could not be established.

4.3   Contribution of the study to existing knowledge

Results from this study contribute to knowledge about the prevalence of vertically transmissible infections among pregnant women in Southern Africa. This information is scarce in the region and Africa as a whole, while considerable work in this regard has already been done and reported in Europe, China, northern and southern America as well as India.

The originality of the study revolves around the scarcity or absence of information about the seroprevalence of infectious diseases among pregnant women in Central Namibia or the country as a whole. The two studies on *T. gondii* were the first to investigate seroprevalence of *T. gondii* in Namibia since 1978. It was the first study to determine risk factors associated with toxoplasmosis among pregnant women in Namibia. This was only the second study to determine seroprevalence of rubella among pregnant women in Namibia. This study was the first done in Namibia on seroprevalence of cytomegalovirus in any population.

There had been a lack of local access to IgG avidity testing in Namibia and this had only recently become available. There remains limited availability and it is not clear whether awareness of its usefulness exists among clinicians and pathologists in Namibia.

The study furthermore resulted in four manuscripts which have either been published or have been submitted to peer-reviewed journals. Findings described in these four publications are evaluated and contextualized in this section.

4.3.1 *Toxoplasma gondii*

The two study groups, namely of blood donors in Central Namibia and of urban pregnant women attending public antenatal care in Windhoek, Namibia from September to October 2016 had low prevalence of antibodies to *T. gondii*. This could possibly be attributed to geographical and meteorological aspects.

The first publication on the study among blood donors showed that they had not been exposed to *T. gondii* to a greater extent. One blood donor who had IgM antibodies to *T. gondii* could possibly pose a risk of transfusion transmitted infection. Since he lived in the urban area it is postulated that he got infected by eating undercooked meat. Many restaurants in Windhoek serve venison and it is popular to order it rare. The very low overall seroprevalence of *T. gondii* among blood donors, however, does not justify routine screening of blood donors for *T. gondii* infection. Therefore, a minimal risk of transfusion transmitted infection remains.

Seroprevalence of *T. gondii* among blood donors could further give an indication of the prevalence of *T. gondii* among the general population of Namibia. The rate of 1% was much lower than the 12% found in the same region in 1978. This finding is in line with the decline in toxoplasmosis found world-wide over the past centuries. This could possibly be due to climate changes like droughts and flash-floods which do not favour the survival of oocytes. Also, freezing facilities for raw meat have become more widely available, eliminating live oocytes from meat products. In addition, access to clean drinking water is hopefully improving world-wide.

In the subsequent study on seroprevalence of *T. gondii* among pregnant women the result was also very low, indicating that residents in the central part of Namibia are not greatly exposed to *T. gondii* while pregnant women carry little risk of transmitting the infection to the foetus. Therefore, low prevalence of CT could be expected in the Khomas region. The youngest and oldest age groups among pregnant women were least affected by toxoplasmosis while the age group 26-30 years had

the highest exposure with seroprevalence of 5.21%. It once again shows that different age groups did not have similar exposure to infection.

Seroprevalence of *T. gondii* in different parts of the world vary greatly. Seroprevalence in Namibia was comparable to that in neighbouring South Africa [41], and the prevalence in Mexico [135], with similar weather patterns. The study in Mexico similarly did not find any association between toxoplasmosis among women and raw meat consumption, cow raw milk consumption, goat raw milk consumption, unwashed raw vegetables consumption or untreated water consumption.

4.3.2 Rubella

In the third manuscript the study group of urban pregnant women attending antenatal care in Windhoek, Namibia from September to October 2016 had high prevalence of rubella immunity (IgG $\geq$ 15 IU/ml), despite only 7% of these women being known to be included in the nationwide SIA of July 2016. This indicates that the majority of pregnant women in this group had rubella immunity probably due to natural infection. A previous study found that seroprevalence of rubella virus among pregnant women attending the same antenatal clinic was 84% in 2010 [10]. In our study seroprevalence had increased to 95.9% in 2016 after an outbreak in 2015 and after the national rubella vaccination campaign in July 2016. As few individuals in our study had been vaccinated, this increase was probably due to naturally acquired immunity obtained during previous rubella outbreaks in Namibia and could not be attributed to the SIA vaccination campaign alone.

In our study, the average level of rubella IgG (IU/ml) was determined in five different age groups with no significant difference of infection among the age groups. The overall level of anti-rubella IgG was much higher than the level found among pregnant women in Canada where rubella vaccination had been implemented in the 1970s [30]. A recent study among pregnant women in Iran found a mean concentration of anti-rubella IgG of 14.9 IU/mL after a nation-wide mass vaccination campaign [66]. It is possible that the anti-rubella IgG concentration in our study was artificially elevated after a recent rubella outbreak. This could explain the extremely high overall anti-rubella IgG concentration in this mainly unvaccinated population. It underscores previous findings that natural immunity elicits a much higher antibody titre than vaccination [136].

Our finding that primigravidae had higher rubella antibody concentrations could indicate that younger age groups had been more affected by recent rubella outbreaks.

Overall, high seroprevalence found in all age groups could indicate low risk of vertical transmission of rubella since very few cases of re-infection in previously immune individuals have been reported. The high seroprevalence found in our study was similar to the seroprevalence reported in South Africa [57] while it is comparable to seroprevalence found in other developing countries.

4.3.3 Cytomegalovirus

The seroprevalence of IgG to CMV was also comparable to the seroprevalence found in other developing countries with one other African country also reporting seroprevalence of 100% [108]. All pregnant women in our study had IgG antibodies for CMV (100% seroprevalence). Five participants had IgM antibodies for CMV with 6 participants having grey zone results. This seropositivity could have been associated with reactivation of the infection, or re-infection with a new strain of CMV or false positive IgM results. IgG avidity was high in all cases indicating absence of primary infections. Pre-existing immunity does, however, not exclude vertical transmission of the virus during pregnancy. Despite the high seroprevalence of CMV among pregnant women, the Namibian population might carry the burden of congenital CMV infection among infants [108,140]. This may contribute to long term disability, especially sensorineural hearing loss [111]. In Windhoek deaf adults are seen begging in parking lots and the question arises as to the cause of the hearing disability. To what extent is deafness in the Namibian population attributable to congenital infection? Further studies are needed to determine the prevalence of cCMV in Namibia and neonatal surveillance studies may be important to establish the prevalence of cCMV disease. Since surveillance studies involving PCR testing would be costly, mathematical modelling could still be considered in future as more epidemiological data become available.

4.3.4 Mathematical modelling

For all three infectious agents, one of the objectives was to calculate incidence of infections in order to get information on the potential for congenital infections to occur. That was the reason for including a stratified sampling protocol, in order to obtain age stratified prevalences which would facilitate

mathematical modelling as described in the literature [144]. Main findings when the model was applied to data obtained in this study, were presented in Chapter three. This objective could not be met due to the nature of the data obtained.

The following section focuses on the strengths and limitations of the study.

4.4 Strengths and Limitations of the Study

The study was successful in describing seroprevalence of three infectious agents among pregnant women in Namibia. This information could be useful to policy makers in the health sector to determine future diagnosis and treatment policies for pregnant women in order to address the possible burden of congenital infections.

4.4.1 Challenges faced

The study did face challenges in terms of financial constraints which made it impossible to reach the carefully calculated sample size. Furthermore, the results were not exactly as expected when the research questions were phrased. In both study populations the seroprevalence of *T. gondii* was very low, making it difficult to reach enough statistical power to establish meaningful associations between risk factors and infection. It is however, postulated that a much larger sample would have been needed in order to be able to establish associations and that the outcome would not have been much different. For the rubella study, the major challenge was ambiguity regarding vaccination history. It is however, concluded that the SIA did not have a meaningful effect on the study. Participants were able to indicate clearly whether they had been included in the SIA since it only took place two months prior to the study. Since no rubella vaccination had been done in the public sector prior to the SIA, the rest of the participants were deemed not to have received any vaccination. The CMV study posed a challenge due to 100% seropositivity among participants. Similar studies in developing countries created the expectation of a high seropositivity in this population, but such comprehensive seropositivity was not expected. Unfortunately, this rendered the study unsuitable to consider risk factors for CMV infection. Finally, the objective of calculating incidence of the three infectious agents among pregnant women could not be attained due to unsuitable data obtained in the study.

4.4.2 Strengths and limitations

A strength of the study is the inclusion of a pilot study of *T. gondii* seroprevalence among blood donors. Results from this study could inform the sample size which would be needed for the consecutive studies in the absence of relevant data from Namibia.

A further strength of this study was the submission of a PhD by publication and due to its nature, it implied the publication of 4 – 5 manuscripts in peer-reviewed journals. The first achievement was the publication of the study among blood donors in a peer-reviewed journal. Three more articles had been prepared for publication of which two have been published by peer-reviewed journals which are indexed in widely accessible databases. Within three months the CMV manuscript had attracted more than 300 views. To further enhance international visibility of study findings, an abstract on the seroprevalence of *T.gondii* among pregnant women had been accepted by the International Conference on the HIV Treatment Pathogenesis and Prevention Research in Resource-Limited Settings (INTEREST) 2020 which is the largest international platform for HIV-related studies from Africa.

It is thus envisaged that results from this study will be useful to clinicians and health administrators within Namibia but also beyond its borders, especially in other resource-limited settings. Furthermore, results from this study contribute to the growing body of evidence-based information becoming available in Namibia. Before Independence in 1990, isolated studies performed by the South African Defence Force based in then South West Africa were published in local journals. It was only after the establishment of the Medical School at the University of Namibia (2009) and the Faculty of Health and Applied Sciences at NUST (2010) that medical research was conducted on limited scale in Namibia. Although a lot of data are available, publication in reputable journals outside the African continent still poses a challenge to many African researchers. Furthermore, securing funding for medical research remains a challenge in economies which are hardly hit by other disasters like the HIV, malaria and tuberculosis burdens and severe droughts.

Several efforts were made to ensure validity and reliability of the study which are described in the next section.

4.4.2.1 Validity and reliability of the study

In the absence of any recent information on the seroprevalence of the three agents in Namibia, it was important to get an indication of the current seroprevalence of at least one of them. Therefore, seroprevalence of *T. gondii* was assessed among blood donors in preparation of the study on seroprevalence of three infectious agents among pregnant women. This information was used in the calculation of the sample size for the bigger study among pregnant women. It also gave a preliminary impression of results which could be expected for toxoplasmosis in the study among pregnant women.

Pregnant women are often used in nation-wide studies to determine seroprevalence of infections. An example is the statistics on HIV infection rates in different regions, which are derived from the testing of blood collected from pregnant women (HIV sentinel surveys). Data obtained in this population are generally accepted as representative of the general population.

Validity and reliability of data were also ensured. As far as validity of the questionnaire is concerned, a pilot assessment was performed with a woman of childbearing potential to ensure that the questions are clear and unambiguous. Data obtained in the questionnaire were entered in a spreadsheet by senior Medical Laboratory Sciences students. Data were encoded and cleaned by the researcher. Spot checks were done to ensure that data were transferred correctly from the questionnaires to the spreadsheet. All positive laboratory results were double checked for correct transcript.

Laboratory testing was done in a SANAS accredited laboratory which operates according to ISO 15189 standards. The test method used for IgG and IgM testing utilizes the latest technology and is seen to be adequately sensitive and specific. For interpretation of results, reference ranges either from the manufacturer of the kits or the pathology laboratory were used.

To ensure validity of statistical analysis, a biostatistician was involved from an early stage in the study design and data analysis of the study. He also critically reviewed the manuscript on rubella before submission to the journal. A second biostatistician assisted with multivariate analysis for *T. gondii* among pregnant women which had been requested by a reviewer.

For all manuscripts, co-authors from other Universities and from industry were involved in the critical review process. Submissions were made to peer-reviewed journals which are listed in widely accessible databases.

4.4.2.2 Limitations of the study

Similar to most studies in health research, there were limitations to the study. Firstly, this study was only done at one public antenatal clinic in Central Namibia, so the data were not representative of the whole country. Especially in the case of *T. gondii* which is dependent on social and environmental factors, other regions of Namibia could yield different results. Namibia has diverse ecosystems ranging from the oldest desert in the world to a semi-tropical climate in the north. Furthermore, rural communities have different lifestyles from urban communities.

The study among blood donors was possibly biased due to blood donors probably living a healthier and more affluent life than citizens in remote villages of the country. An attempt was made to improve on this by conducting the bigger study in a population of state patients attending public health care.

A major setback for the study was the premature termination of funding. This led to a situation where the calculated sample size could not be reached. It is, however, believed that a larger sample size would not have changed the outcome of the study. Due to the very low seroprevalence of *T. gondii* a huge sample size would be needed in order to identify significant associations with risk factors. In Namibia funding and resources are not available to facilitate a study with such a large sample size. For rubella and CMV, seroprevalence was very high or 100% and a larger sample size would not have changed the results.

The validity of the IgG avidity testing could not be established. This was the first time that the methodology was used in Namibia and there are no reference laboratories available in country. Contact was established with a private laboratory in Pretoria in South Africa, who use the same manufacturer's reagents for CMV IgG avidity testing. Furthermore, NHLS laboratories in Cape Town in South Africa were willing to test serum for *T. gondii* IgG avidity. However, regulations regarding export of human samples posed a challenge. Furthermore, integrity of stored serum samples was

compromised due to a power failure in the NUST laboratory during the summer recess. Therefore, efforts to validate the test method for IgG avidity testing were not successful.

For rubella the questions in the questionnaire regarding immunization history were not well designed. The reason for this was the recommendation that the protocol be written in such a way as to facilitate further testing for other infectious agents when this study is completed. The questions on immunizations were thus phrased to include measles immunization history in addition to rubella immunization history. However, for the purpose of this study the researcher focused on the answers to the question whether participants were immunized in July 2016. This was the first rubella immunization campaign in Namibia. Therefore, if they were not immunized in 2016 due to being pregnant at the time, it means that they had never received rubella immunization.

Another limitation for the rubella study was the publication a few weeks before sample collection started, of a comprehensive study on rubella seroprevalence covering the whole country using HIV sentinel survey samples as discussed in Section 4.3.2 of this chapter. Therefore, the current study was not the first to be published on rubella in Namibia as planned in the protocol.

Our findings also highlight how difficult it is to determine incidence of maternal infections that could pose intrauterine infection risks. We attempted to use an age-prevalence model, as this would have been most cost-efficient and affordable, but in the case of CMV, women of child bearing potential had already been infected and we had no reliable way to determine the rate of reinfections (assays to detect these are not freely available and have not been validated).

Another limitation of the age-prevalence ratio approach to determine incidence is that it may only work in populations with relatively high stability and very similar exposure risks amongst age cohorts. The *T. gondii* and rubella age-prevalence trends suggest that the different age cohorts in our study may have had different exposure risks. As developing countries often experience large population movements including immigration or emigration or urbanisation, with accompanied different living conditions, population density and exposure risks, this approach may not be feasible in many developing countries. An alternative approach is to screen large numbers of blood samples that are collected as part of programs such as HIV antenatal seroprevalence studies and attempt to identify

acute or recent acute infections. If one assumes a 1% annual incidence, and IgM remaining detectable for 6 weeks, it would require a cross sectional sample size of > 140 000 for a precision (95% confidence interval) of 30% or smaller around the estimate (binomial probability test). Similarly, prospective cohorts of pregnant women are very costly. Moreover, IgM assays in cases with a low pre-test probability have very low positive predictive values, which would result in many more false positives than true positives. Therefore, additional confirmatory testing of IgM positive samples to determine IgG avidity would be required to partially overcome this. Lastly relying on reporting of congenital infections are also inadequate as this would rely on diagnostic screening of infants to detect congenital infections, which is not feasible in most resource limited settings.

Despite these limitations the study was able to identify areas for further investigation.

4.5 Impact of the study: Health with a focus on Namibia

Results from this study indicates that congenital infections with *T. gondii*, rubella are probably not a huge public health concern in Central Namibia. While there may be an underdiagnosis of mild or asymptomatic congenital CMV in children from mothers with reinfection or reactivation, it is not clear to what extent asymptomatic cCMV impacts on long term child development. We found a low seroprevalence of *T. gondii* and high immunity to rubella and high proportion of CMV seropositivity among pregnant women. It is significant to note that *T. gondii* is not highly prevalent in Central Namibia. The results from this study do not justify routine screening of blood donors or pregnant women for *T. gondii*, although a previous study indicated that maternal screening could save cost in terms of support of disabled persons [42].

The results from the rubella study were useful to inform policy makers that a group of women were missed during the first rubella SIA due to being were pregnant at the time of the SIA. In Central Namibia, however, unvaccinated women exhibited high immunity to rubella, with the percentage of susceptible women within the acceptable range determined by WHO. Women missed during the SIA probably pose minimal risk for CRS in future outbreaks and do not justify follow-up nation-wide rubella vaccination campaigns.

A 100% seroprevalence of CMV among pregnant women probably indicates no further public health action is required. It is, however, evident from previous studies that non-primary CMV infection also poses a risk for cCMV to develop. Policy makers and clinicians should be made aware of this.

There is limited access to IgG avidity testing in many resource-limited settings. Availability of avidity testing may assist clinicians in differentiating risk of congenital infections as primary rubella infection or CMV infection are both associated with an increased risk of transmission to the foetus. Moreover, as IgM assays performed in patients with a low pre-test probability of infection have low positive predictive values a concurrent low avidity would increase support for a recent acute primary infection.

The question could be asked about the application of the CMV IgM and IgG avidity tests in a setting with very high seroprevalence of CMV. According to Prince and Lape-Nixon (2014) the IgG avidity test is useful to determine the time of infection in case of a primary CMV infection during pregnancy. This information is useful to indicate further testing and treatment if it is available. IgM detection is a sensitive marker of primary CMV infection, but its specificity is poor because CMV IgM is also produced during viral reactivation and persists following primary infection in some individuals [163]. CMV IgG avidity is both a sensitive and specific method for identifying a pregnant woman with recent primary infection and thus at increased risk for vertical CMV transmission [163]. Results from our study showed that primary CMV infection is not likely in this study population. Although all CMV IgG avidity tests showed a high avidity, it is possible that reactivation or reinfection with CMV could have occurred. A review of studies in 5 medical centres in Europe summarized the following IgG avidity results among pregnant women: (i) primary infection (seroconversion/virus isolation) >85% low avidity; (ii) past infection (CMV IgG positive IgM negative) >90% high avidity; (iii) CMV reactivation (four-fold increase of CMV IgG levels with or without detectable IgM); or IgG positive IgM positive for more than a year >90% high avidity; (iv) at risk group of pregnant women (first prenatal serum IgG positive IgM positive precluding determination when primary infection occurred) 50% high avidity. All women who had CMV-infected offspring exhibited low CMV IgG avidity [163]. However, two other studies showed that mothers with intermediate or high avidity could also transmit CMV infection to

offspring [163,164]. This finding is supported by the demonstration of cCMV in settings with very high CMV seroprevalence where reactivation of latent infection probably predominates.

In conclusion, there is no routine screening programme for maternal CMV in any country and the reasons are postulated: (i) unjustified pregnancy termination due to anxiety of the mother; (ii) lack of interventions proven to reduce or prevent transmission even in primary infection; (iii) inability of screening programmes to identify the small percentage of women who exhibit CMV antibodies before conception but transmit the infection to their foetuses [163].

4.6   Future perspective

4.6.1 *T. gondii*

Further studies could be conducted on the seroprevalence of *T. gondii* in rural areas in Namibia. *T. gondii* might show higher seroprevalence in rural areas as reported by a previous study in Namibia [7]. Animals which are raised in a free-range setting are more likely to get infected [138] and rural populations live in closer contact with animals than the population studied in this research.

It could be useful to study the prevalence of *T. gondii* in the sub-tropical savannah areas of Namibia. This study was conducted in the dry central area of Namibia and it is expected that seroprevalence of *T. gondii* will be higher in the northern regions where rainfall is higher and more favourable conditions for the survival of oocysts prevail.

Information on genotypes of *T. gondii* circulating in Africa is scarce. It seems like there are two *T. gondii* population structures in Africa: one in desert and semi-arid North and East Africa and one in the tropical zone of Central and West Africa. No genotyping studies of *T. gondii* have been done in Southern Africa [139]. Genotyping of *T. gondii* in Southern Africa is recommended in view of the possible development of a vaccine.

In France, where high seroprevalence of *T. gondii* prevailed in the previous century, the reduction of *T. gondii* prevalence is partly attributed to lower exposure to the parasite due to changes in food

habits (the Ministry of Agriculture and Food reported a 30% decrease in beef consumption between 2000 and 2010) and preventive education [15,144]. Even in a country with low seroprevalence like Namibia, preventive strategies like the education of pregnant women to create awareness of hygienic practices like hand washing and safe water could be promoted. This would prevent primary infection of susceptible pregnant women and reduce the risk of CT with the social and economic burden which it carries. CDC recommendations for prevention of toxoplasmosis in pregnant women can be found at: http://www.cdc.gov/parasites/toxoplasmosis/prevent.html.

Parasites-toxoplasmosis (*Toxoplasma* infection). Available at: http://www.cdc.gov/parasites/toxoplasmosis/gen_info/pregnant.html. Accessed November 2018.

Routine maternal testing for toxoplasmosis could save cost. Despite the low prevalence of toxoplasmosis among pregnant women in the central region of Namibia, routine maternal testing could be indicated should data about the prevalence of CT in Namibia become available in future and should safe and effective treatment for pregnant women be developed and become available in Namibia. Despite the cost of early diagnosis and treatment of toxoplasmosis, it could be a cost-saving strategy in the long run. In European countries like France, where routine maternal screening for *T. gondii* is mandatory by law, a reduction in rates and severity of CT has occurred. In France where relatively high seroprevalence of *T. gondii* prevails, screening for CT is mandatory since 1992. Seroprevalence of *T. gondii* decreased from 84% in the 1960's to 54% in 1995 and further to 44% in 2004 [42,144]. Stillwagon et al. (2011) compared the cost of pre- and postnatal diagnosis and treatment, versus estimates of lifetime societal costs of developmental disabilities. They postulated that for populations with rates of CT greater than 1 infected child per 10 000 live births or 2 infected mothers in 10 000, maternal serologic screening is a cost-effective strategy. This is much lower than the 2.61 infected pregnant women in 100 found in Central Namibia. Although the cost of diagnosis and treatment in Namibia is not known, maternal screening for toxoplasmosis could possibly also save lifelong costs, especially in rural areas where seroprevalence may be higher [7]. Early identification of pregnancies at risk could lead to treatment and possible prevention of CT.

No information on the prevalence of CT in Namibia is available. An important intervention to control the occurrence of CT is the implementation of a CT surveillance system. This is however very rare in developing countries and researchers use data from literature reviews to model incidences of CT. Global estimated incidence of CT is 1.5 cases per 1000 live births or 190 100 annual cases. Incidence of CT in the WHO Afro region is 2.4 per 1000 live births or 37 000 cases annually [43].

Due to the vibrant wild-life industry in Namibia, a One Health-approach should be followed to curb toxoplasmosis in human populations. Closer collaboration between health care providers and veterinarians would be beneficial to address zoonotic diseases like toxoplasmosis.

The findings of this study in the setting of a developing African country are greatly underscored by the European CDC Report on Congenital Toxoplasmosis from 2016 [151]. They raised a concern about the cost benefits for prenatal screening programmes mainly because of the low prevalence of CT in European countries and lack of effective treatment. However, considering recent epidemiological studies in non-reporting countries, changes in eating habits (e.g. the consumption of undercooked meat), increased travel and therapeutic developments, the significance of this infection in European countries warrants a better estimation. Congenital disease should be better investigated to assess severity, urban vs. rural setting and prevalent risk factors in a specific region. Prevention options for CT should be reinforced. Information for pregnant women should include information about *T. gondii* risk factors [151]. Diagnostic capabilities in the clinical as well as in the food sector should be regularly assessed against the changing epidemiology of the disease and circulating pathogen genotypes. They raised a concern about the introduction of exotic genotypes through imported food, causing severe disease in immunocompetent adults as already reported in France. Therefore, the One-Health approach is mentioned [151]. In Namibia, collaboration with CDC could be expanded beyond the classical agenda of HIV, Tuberculosis and malaria, to include zoonotic diseases which are becoming relevant in this time of emerging infections.

4.6.2 Rubella

It is important for public health care providers to ensure that the newly implemented MR vaccination of all children at the age of 15 months be maintained comprehensively to prevent further outbreaks of rubella in future, and to mitigate the risk of CRS.

All available information on the seroprevalence of rubella among pregnant women in Namibia originates from the public sector. Co-ordinated involvement of the private sector is needed to ensure sufficient vaccination coverage to protect the population against CRS.

4.6.3 CMV

Further studies are needed to determine the prevalence of cCMV among newborns in Namibia. Only then can it be established whether public health care interventions are needed to counteract the effect of secondary CMV infection among pregnant women. A recent study in the Eastern Cape in South Africa where high seroprevalence of CMV also prevails, established that 6% of babies born in an academic hospital, were PCR positive for CMV. This finding was similar to results in other resource-limited settings [140].

Further studies in Namibia could also focus on determination of the age of infection with CMV in the population.

4.6.4 Mathematical modelling

Mathematical modelling could be explored in future to determine incidence of CRS, should more seroprevalence data and immunisation coverage data of rubella become available [141]. In the report by Cutts and Vynnycky, estimates of the incidence of infection among pregnant women were calculated using the average susceptibility to infection and the incidence of infection during gestation [62]. Since there is no data available on the occurrence of CRS in Namibia, mathematical modelling could provide valuable information without costly and invasive research methodology.

Given the very low rate of *T. gondii* in Namibia, very large sample sizes would be needed to estimate likely transmission rates to the foetus. In a study in France, where relatively high seroprevalence of *T. gondii* prevails, data from two national surveillance programmes namely the National Perinatal

Surveys and the National Surveillance of Congenital Toxoplasmosis were used. Data were collected from 42 208 pregnant women. In addition, procedures like amnioscentesis are done to establish foetal infection [144]. This is not achievable in low income countries.

4.7   Conclusion

This study investigated the extent of vertically transmissible infections among pregnant women in Central Namibia. Seroprevalence results (IgG) indicated that previous exposure to *T. gondii* was very low among participants. Previous exposure to rubella and CMV had been very high with 95.9% of participants being immune to rubella and 100% of participants found to be CMV IgG seropositive. It is concluded that very low risk of vertical transmission of the infections existed in the study population.

This was further supported by results from IgM and IgG avidity testing which indicated that no current infection with either *T. gondii* or CMV occurred among participants during the study period. Further investigation into the incidence of infections by employing mathematical models did not prove successful due to data being unsuitable for age-prevalence modelling. Participants in five age groups did not exhibit equal exposure to *T. gondii* and rubella and furthermore, the seroprevalence did not follow a linear increase over age as expected and as had been found in previous studies. In the case of CMV 100% seroprevalence in all age groups rendered the data unsuitable for modelling. The ideal method to investigate incidence would have been to use a very large sample size like HIV sentinel survey samples and determine IgG avidity in IgM positive cases. This is, however, not plausible in resource-limited settings.

Another objective which could not be explored to its full potential was the association of risk factors with maternal infection due to either very low or very high seroprevalence found among participants. In the case of rubella, primigravidae showed a positive association with anti-rubella IgG levels indicating that younger age groups had higher antibody titres to rubella. This was attributed to younger persons being more exposed to rubella outbreaks in crowded situations like schools and hostels. Although risk factors for CMV had been included in the questionnaire they could not be explored due to 100% seroprevalence in all age groups.

The very high overall mean rubella IgG concentration found among participants was attributed to natural immunity acquired during previous rubella outbreaks. The mean anti-rubella IgG level among participants was much higher than those reported by two post-vaccination studies in other countries. It underscores the belief that antibody production due to vaccination may wane and it showed that the study population in Namibia was highly protected against future rubella outbreaks.

In conclusion routine testing of pregnant women for *T. gondii*, rubella and CMV IgG may not be justified based on the results of this study. This concurs with recommendations from a previous study done in South Africa [142]. Furthermore, results from this study do not indicate the necessity of follow-up rubella vaccination campaigns after pregnant women missed the SIA in July 2016. This is due to high naturally acquired rubella immunity found in this study. It should, however, be emphasized that the newly introduced rubella vaccination of all children at 15 months be maintained in order to avoid development of the paradoxical effect of insufficient rubella vaccination coverage. Health care providers should also be made aware of the occurrence of cCMV in developing countries despite very high seroprevalence of CMV among pregnant women as described in the literature.

Although a surveillance programme may not be justified, future investigations into congenital infections with *T. gondii*, rubella and CMV might prove useful to establish the extent of congenital infections impacting on the Namibian population. Although this study was able to report on the prevalence of maternal infections with these three agents in Central Namibia, it was for various reasons not completely able to evaluate the risk of vertical transmission and congenital infection. Immunoglobulin M positive cases were few and IgG avidity was high or equivocal in all IgM positive cases indicating low risk of transmission to the foetus. The low positive predictable value of IgM tests for these infections poses a further challenge and much larger samples sizes would be needed to effectively evaluate transmissible intra-uterine infections.

If a better understanding of the extent of infectious diseases amongst pregnant women is achieved, it could lead to improved management of this group of patients and assist to identify possible interventions needed in the Namibian healthcare system. Further investigations are needed to describe occurrence of congenital infections in order to guide future health policies.

Although seroprevalence of *T. gondii* among pregnant women was very low, the preventive effect of hygiene education given by health authorities to susceptible women during pregnancy could be considered.

Better understanding of the immune status of pregnant women might guide expanded immunisation programmes including rubella vaccination, possibly linking it to the national measles campaigns by the MoHSS.

Improved understanding of cCMV transmission rates resulting from non-primary maternal infections will provide further evidence to study maternal immunity as a potential target for vaccine development.

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Addendums

Addendum A: Ethics Approval Polytechnic of Namibia

Addendum B: Ethics Approval MoHSS

Addendum C: Ethics Approval Stellenbosch University

Addendum D: Recruitment Advertising Leaflet

Addendum E: Participant Information Leaflet and Consent Form

Addendum F: Participant Information Leaflet and Assent Form

Addendum G: Questionnaire

Addendum H: Confidentiality Statement for Data Capturing

Addendum I: Author contributions

Addendum A: Ethics Approval Polytechnic of Namibia

POLYTECHNIC OF NAMIBIA
SCHOOL OF HEALTH AND APPLIED SCIENCES

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transforming into

Namibia University
of Science and
Technology

MEMO

Thursday, 15 October 2015

ETHICAL CLEARANCE OF MRS E VAN DER COLF RESEARCH PROJECT

The ad hoc Ethics committee of the School of Health and Applied Sciences have gone through the National Council of Research, Science and Technology, Namibia (NCRST) funded research proposal of Mrs E Van Der Colf of the Department of Health Sciences entitled "Prevalence of *Toxoplasma gondii*, rubella and cytomegalovirus among pregnant women attending the antenatal clinic at Windhoek Central Hospital, Namibia. The following issues and clarifications were made.

Issues and clarifications on:

The questionnaire

1. There was no indication in the questionnaire of the choice of participants to disengage from the research at any stage without consequences.
2. Additional consent from the subject/participant is required to collect samples from the baby even though the participant or subject is positive for a disease.
3. A statement indicating the utilisation of collected blood samples solely for the agreed purpose only.
4. Need to translate the questionnaire into Afrikaans and Oshivambo languages in addition to English.

Additional recommended process/document

1. A participant information sheet (PIS) need to be made available to participants before completion of the questionnaire.
2. A consent form should be prepared.
3. A clarification of transportation mode of samples from the Windhoek Central Hospital to the Polytechnic of Namibia in view of the Human Tissue Act as well as access control measures of stored samples.

Comments/Recommendation

The researcher has complied with all the recommended corrections and ethical approval from the School for the project is hereby granted.

Sincerely,

Professor Sylvester R Moyo
Dean: School of Health and Applied Sciences

POLYTECHNIC OF NAMIBIA
P.O. Box 13388
WINDHOEK

2015 -10- 1 6

SCHOOL: HEALTH & APPLIED SCIENCES
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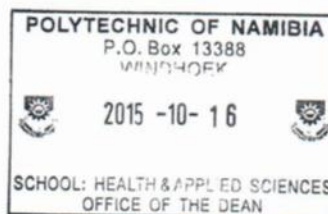
Addendum B: Ethics Approval MoHSS

REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198	Ministerial Building	Tel: 061 – 203 2510
Windhoek	Harvey Street	Fax: 061 – 222558
Namibia	Windhoek	E-mail: Ester.Shaama@mhss.gov.na

OFFICE OF THE PERMANENT SECRETARY

Ref: 17/3/3
Enquiries: Ms. E.N. Nepolo

Date: 13th July 2016

Ms. Elzabe van der Colf
Private Bag 13388
Windhoek
Namibia

Dear Ms. van der Colf

Re: Seroprevalence and incidence of Toxoplasma gondii, rubella and cytomegalovirus in Namibian women of childbearing potential.

1. Reference is made to your application to conduct the above-mentioned study.

2. The proposal has been evaluated and found to have merit.

3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**

3.1 The data to be collected must only be used for completion of your PhD in Medical Virology;

3.2 No other data should be collected other than the data stated in the proposal;

3.3 Stipulated ethical considerations in the protocol related to the protection of Human Subjects' information should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;

3.4 A quarterly report to be submitted to the Ministry's Research Unit;

3.5 Preliminary findings to be submitted upon completion of the study;

3.6 Final report to be submitted upon completion of the study;

3.7 Separate permission should be sought from the Ministry of Health and Social Services for the publication of the findings.

Yours sincerely,

Andreas Mwoombola (Dr)
Permanent Secretary

19 JUL 2016
REPUBLIC OF NAMIBIA

"Health for All"

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Addendum C: Ethics Approval Stellenbosch University

Approval Notice
Response to Modifications- (New Application)

19-Jul-2016
Van Der Colf, Berta BE

Ethics Reference #: S16/05/092

Title: SEROPREVALENCE AND INCIDENCE OF TOXOPLASMA GONDII, RUBELLA AND CYTOMEGALOVIRUS IN

NAMIBIAN WOMEN OF CHILDBEARING POTENTIAL

Dear Mrs Berta Van Der Colf,

The **Response to Modifications - *(New Application)*** received on **15-Jun-2016**, was reviewed by members of **Health Research Ethics Committee 1** via Expedited review procedures on **19-Jul-2016** and was approved.
Please note the following information about your approved research

protocol: Protocol Approval Period: **19-Jul-2016** -**18-Jul-2017**

Please remember to use your **protocol number** (**S16/05/092**) on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review:
Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.
Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States
Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.
For standard HREC forms and documents please visit: www.sun.ac.za/rds

If you have any questions or need further assistance, please contact the HREC office at 219389819.

 Included Documents:

Protocol.pdf

Application   form
signature   page.pdf

Grant contract.pdf CV

S Shivute.pdf

Ethical clearance SHAS.pdf

Recruitment advert.docx

CV U Mungunda.pdf

20160706 MOD Child Assent Form(Eng)

CV A Strauss.pdf

CV G van Zyl.pdf

Protocol Synopsis.pdf

CV S Reju.pdf

Informed Consent General.pdf

20160706 HREC- Modifications Required

Investigator declarations.pdf

Review Report_BvdColf_April2016.docx

CV P Hanghome.pdf

CV E van der Colf.pdf

Checklist.doc

Questionnaire English

1.docx Application

form.docx

20160706 MOD Recruitment advert

Sincerely,

Ashleen Fortuin
HREC Coordinator
Health Research Ethics Committee 1

Addendum D: Recruitment Advertising Leaflet

ΠAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY
Faculty of Health and Applied Sciences
Department of Health Sciences

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Windhoek
NAMIBIA

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F: +264 61 207 9899
E: dhs@nust.na
W: **www.nust.na**

Dear Patient

Did you know that infections other than HIV (for example German measles) can affect you and your unborn child?

Infectious diseases contribute to the high death rate and illness of pregnant women and their children in Africa. Several infectious diseases may be transmitted from a pregnant woman to her unborn child having bad effects on the newborn baby which include deafness, blindness, mental retardation, physical impairment or even spontaneous abortion or stillbirth. Information about these infections is essential for health care workers to either treat or prevent these diseases. Some of these infections can be prevented by vaccination before pregnancy (German measles and chicken pox) or through reduction of exposure risk before or during pregnancy (toxoplasma and cytomegalovirus).

Information on the prevalence of infections in newborn babies in Namibia is limited. Therefore, this study aims to determine the prevalence of some important mother-to-child transmitted infectious diseases in women of childbearing age in Namibia. You are kindly invited to participate in a research project on infectious diseases other than HIV which can be transmitted from a mother to an unborn child.

Please ask the sister for more information and she will hand you an information pamphlet explaining the study to you. She will also explain what you need to do should you choose to participate in the study.

Addendum E: Participant Information Leaflet and Consent Form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT: Seroprevalence and incidence of *Toxoplasma gondii*, rubella and cytomegalovirus in Namibian women of childbearing potential

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR: Ms E van der Colf

ADDRESS: Department of Health Sciences, Faculty of Health and Applied Sciences, Namibia University of Science and Technology

CONTACT NUMBER: 00264 61 207 2872

You are being invited to take part in a research project. It will take about twenty minutes. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research. This study is funded by the National Commission on Research, Science and Technology.

What is this research study all about?

➢ *540 participants will be recruited at the antenatal clinic of Windhoek Central Hospital*

➢ *This is a study on the prevalence of infectious diseases in pregnant women. The infections I am investigating are Toxoplasma gondii (a parasite), rubella (German measles), measles, cytomegalovirus (causes flu-like symptoms), varicella (chicken pox) and hepatitis B virus (jaundice). If a woman contracts one of these infections during pregnancy, the infection can be passed on and have serious effects on the unborn child. It can cause abnormalities like deafness or other disabilities or even stillbirth*

➢ *If you agree to participate, the sister will ask you a few questions and take a blood sample. The blood taken will be used to test for the infectious diseases mentioned*

above with no extra testing. Your blood sample will be stored in a building with access control for a period of five years, after which it will be incinerated.

➢ *108 patients in each age group will be selected, so once that number has been attained, no more patients in that age group will be recruited*

Why have you been invited to participate?

➢ *This study focuses on pregnant women attending the antenatal clinic at Windhoek Central Hospital*

What will your responsibilities be?

➢ *Kindly answer questions in the questionnaire and allow the sister to draw a blood sample.*

Will you benefit from taking part in this research?

➢ *This study will give information to the Ministry of Health and Social Services, on the prevalence of these infections in pregnant women, and hopefully lead to better diagnosis and treatment of these patients, as well as improved immunisation programmes for state patients.*

Are there in risks involved in your taking part in this research?

➢ *There is only minimal risk associated with the collection of a blood sample. This will be done by a qualified phlebotomist/nurse.*

If you do not agree to take part, what alternatives do you have?

➢ *You may discuss it with the doctor at the clinic if you feel that you are at risk for any of these infections.*

Who will have access to your medical records?

The information collected will be treated as confidential and protected. If it is used in a publication or thesis, the identity of the participant will remain anonymous. Student Medical Laboratory Scientists who do data entry will sign a Confidentiality Statement.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

➢ *No medications are involved*

Will you be paid to take part in this study and are there any costs involved?

➢ *No, you will not be paid to take part in the study. There will be no costs involved for you, if you do take part.*

Is there anything else that you should know or do?

➢ You can contact Ms E van der Colf at tel 061-207 2872 if you have any further queries or encounter any problems.

➢ You can contact the Health Research Ethics Committee at 002721-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

➢ You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I …………………………………..…………. agree to take part in a research study entitled "Seroprevalence and incidence of *Toxoplasma gondii*, rubella and cytomegalovirus in Namibian women of childbearing potential".

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.

- I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.

- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.

- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) ....…......……………. on (*date*) …………...……….. 20 .

....
Signature of participant   **Signature of witness**
……………………………………………………………………..
Signature of Parent/Guardian if Participant is under the age of 18 years

Declaration by investigator

I *(name)* …………………………………….……… declare that:

- I explained the information in this document to …………………………………..

- I encouraged him/her to ask questions and took adequate time to answer them.

- I am satisfied that he/she adequately understands all aspects of the research, as discussed above

- I did/did not use a interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) ....……………. on (*date*) ……………..……….. 20 .

....

Signature of investigator   **Signature of witness**

Declaration by interpreter

I (*name*) ………………………………………..……… declare that:

- I assisted the investigator (*name*) ……………………………………. to explain

  the information in this document to (*name of participant*)

  ……………..……………………………. using the language medium of

  Afrikaans/Oshiwambo.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) ....……………. on (*date*) ……………..………………..

....

Signature of interpreter   **Signature of witness**

Addendum F: Participant Information Leaflet and Assent Form

	STELLENBOSCH UNIVERSITY	
	FACULTY OF HEALTH SCIENCES	
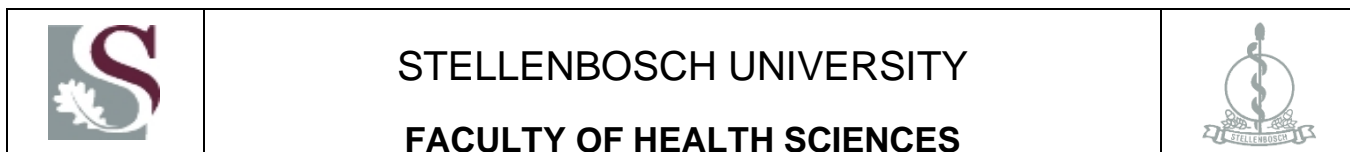
PARTICIPANT INFORMATION LEAFLET AND ASSENT FORM

TITLE OF THE RESEARCH PROJECT: Seroprevalence and incidence of *Toxoplasma gondii*, rubella and cytomegalovirus in Namibian women of childbearing potential.

RESEARCHERS NAME(S): Ms E van der Colf

ADDRESS: Department of Health Sciences, Faculty of Health and Applied Sciences, Namibia University of Science and Technology (NUST)

CONTACT NUMBER: 61 207 2872

What is RESEARCH?

Research is something we do to find new knowledge about the way things (and people) work. We use research projects or studies to help us find out more about disease or illness. Research also helps us to find better ways of helping, or treating children who are sick.

What is this research project all about?
This is a study on the prevalence of infectious diseases in pregnant women. The infections we are investigating are Toxoplasma gondii (a parasite), German measles, measles, cytomegalovirus (causes flu-like symptoms), chicken pox and hepatitis B virus (jaundice). If a woman contracts one of these infections during pregnancy, the infection can be passed on and have serious effects on the unborn child. It can cause abnormalities like deafness or other disabilities or even stillbirth.

Why have I been invited to take part in this research project?
The researchers want to test the blood of pregnant women attending the antenatal clinic at Windhoek Central Hospital, while including people of the age group 13 to 45.

Who is doing the research?
Ms Van der Colf and Professor Reju are lecturers at NUST and we are working with Professor Van Zyl at the University of Stellenbosch and Professor Noden at Oklahoma State University. Once we have the information on the prevalence of infections in pregnant women, we shall share the information in the form of publications and also advise the Ministry of Health and Social Services.

What will happen to me in this study?
If you agree to participate, the sister will ask you a few questions and take a blood sample. The blood taken will be used to test for the infectious diseases mentioned above with no extra testing.

Can anything bad happen to me?

The sister will just give you a small prick to withdraw one blood sample. Please inform your parents should you experience any discomfort after the blood sample has been withdrawn.

Can anything good happen to me?
This study will give information to the Ministry of Health and Social Services, on the prevalence of these infections in pregnant women, and hopefully lead to better diagnosis and treatment of these patients, as well as improved immunisation programmes for state patients.

Will anyone know I am in the study?
The information collected will be treated as confidential and protected. If it is used in a publication or thesis, your identity will not be made known. Information might be given to the NCRST who is funding this research.

Who can I talk to about the study?
You can contact Ms E van der Colf at tel 061-207 2872 if you have any further queries or encounter any problems.

What if I do not want to do this?
Participation is entirely voluntary, and you can refuse to take part even if your parents have agreed to your participation. You can stop being in the study at any time without getting in trouble.

Do you understand this research study and are you willing to take part in it?

| YES | NO |

Has the researcher answered all your questions?

| YES | NO |

Do you understand that you can pull out of the study at any time?

| YES | NO |

_____   _____
Signature of Child

Date

Addendum G: Questionnaire

QUESTIONNAIRE

Lab number

Age ☐

Where do you live?

☐ Windhoek house with own tap

☐ Windhoek house with communal tap

☐ Farm / village

Month of pregnancy

☐ 1 to 3

☐ 4 to 6

☐ 7 to 9

Number of previous pregnancies?

☐ None

☐ One

☐ Two or more

Did you have problems with previous births if you had any?

☐ No problems with previous pregnancies

☐ Premature baby

☐ Baby with jaundice

☐ Emergency caesarean section

☐ Stillbirth / miscarriage

☐ Baby died within four weeks

Do you live with animals around the house? ☐ None

☐ Cat

☐ Dog

☐ Goat

☐ Other

Do you consume any of the following:

☐ Water other than running tap water

☐ Unpasteurized cow's milk or products of it

☐ Goat's milk or products of it

☐ Raw or undercooked meat

☐ Fruit and vegetables which have not been washed

Were you ever immunised against measles and /or rubella?

☐ Yes ☐ No ☐ Don't know

If yes, when were you immunised?

☐ July 2016

☐ As a child

☐ Don't know

Did you ever receive a blood transfusion? ☐ Yes ☐ No

Do you have children less than five years of age in the house? ☐ Yes ☐ No

If yes, kindly record the number and ages:

Number of children less than five years ☐

Age of first child ☐

Age of second child ☐

Age of third child ☐

Age of fourth child ☐

How many times per day do you wash your hands?

Three times or less ☐

More than three times ☐

THANK YOU FOR YOUR TIME!

Addendum H: Confidentiality Statement for Data Capturing

CONFIDENTIALITY STATEMENT

I _____ (full names and surname)

_____ (Identity number)

hereby declare that I am aware of the obligation to keep patient records containing any demographic or other personal and clinical information confidential.

I shall at all times maintain confidentiality when handling patient data for the research project titled:

"Seroprevalence of *Toxoplasma gondii*, rubella and cytomegalovirus among pregnant women attending the antenatal clinic at Windhoek Central Hospital."

I shall not share any information collected during the course of this research project with any other medical staff, fellow students or members of the public.

I shall ensure safekeeping of records at all times, and only use the information as instructed by the researcher, for the purpose of the research project.

Signature   Date_____

Addendum I: Author contributions

Declaration by the candidate:

With regard to Chapter 2, page numbers 50-123 in the dissertation, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Conceptualization and study design; sourcing and management of funding; obtaining ethical clearance; literature review; sample collection (performed by a nurse); sample analysis (partly by Pathcare Laboratories); statistical analysis; preparation of manuscripts; corresponding author for submission to peer-reviewed journals	65

The following co-authors have contributed to Chapter 2, page numbers 50-123 in the dissertation:

Name	e-mail address	Nature of contribution	Extent of contribution (%)
Prof GU van Zyl	guvz@sun.ac.za	Study design; statistical analysis; critical review; preparation of manuscripts	20
Prof BH Noden	Bruce.noden@okstate.edu	Critical review	5
Dr I Maposa	Innocent.maposa@wits.ac.za	Statistical analysis; critical review	3
Dr SBP Mackenzie	mackenziesbp@gmail.com	Sample collection; critical review	3
Mr I Chipare	Israel@bts.com.na	Sample collection; critical review	2
Mr R Wilkinson		Sample collection; critical review	2

Signature of candidate _____

Date: 15 May 2020

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 2, pages 50-123
2. no other authors contributed to Chapter 2, pages 50-123 besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 2, pages 50-123 of this dissertation.

Signature	Institutional affiliation	Date
	Stellenbosch University	
	Oklahoma State University	
	University of Witwatersrand	
	University of Namibia	
	The Blood Transfusion Services of Namibia	
	Retired	

Authors' signatures are in the possession of the candidate and the supervisor.