UNIVERSIDADE D
COIMBRA

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Prevalence and Serological Characterization of Toxoplasmosis, Rubella and Cytomegalovirus Infection in Pregnant Women of Luanda, Angola: Geospatial Analysis of the Infections, its Association with Socio-demographic and clinical determinants

Tese de doutoramento em Ciências Farmacêuticas, na área de especialização em Microbiologia e Parasitologia, orientada pela Professora Doutora Maria do Céu Rodrigues Sousa e apresentada à Faculdade de Farmácia da Universidade de Coimbra

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"*Ninguém escapa ao sonho de voar, de ultrapassar os limites do espaço onde nasceu, de ver novos lugares e novas gentes. Mais saber ver em cada coisa, em cada pessoa, aquele algo que a define como especial, um objecto singular, um amigo é fundamental. Navegar é preciso, reconhecer o valor das coisas e das pessoas, é mais preciso ainda!*"

Antoine de Saint-Exupéry

Agradecimentos

A realização desta tese de doutoramento contou com importantes apoios sem os quais não se teria tornado uma realidade e aos quais estarei eternamente grata:

À Professora Doutora Maria do Céu Rodrigues Sousa, Professora Auxiliar da Faculdade de Farmácia da Universidade de Coimbra, minha eterna orientadora que soube estimular em mim o gosto pela reflexão do tema bem como proporcionou as condições necessárias para a realização deste trabalho.

À Doutora Clarissa Faria pela preciosa ajuda e apoio, foi bom ter-te por perto: contigo partilhei dúvidas, teorias e conhecimentos, que sempre estiveste disponível para ouvir. Mais que tudo, tornaste-te amiga. A ti, muito obrigada.

Ao Doutor Ricardo Almendra do Departamento de Geografia e Turismo da Faculdade de Letras da Universidade de Coimbra, agradeço o seu apoio e disponibilidade.

À Professora Doutora Leonor Martins de Almeida, Professora Catedrática da Faculdade de Farmácia da Universidade de Coimbra, distinta académica, que pela superior qualidade humana e percurso profissional constitui para mim uma referência, agradeço o seu apoio.

Ao Dr. Nuno Cunha, Dr. Frederico Valido e Dra. Sofia Carreiro do serviço de patologia Clínica do Instituto Português de Oncologia-Francisco Gentil de Coimbra, pelo auxílio, disponibilidade e simpatia com que sempre me receberam.

Ao Dr. Duarte do Gabinete de Estatistica e Planeamento do Ministerio da Saúde de Angola, agradeço o seu apoio e disponibilidade.

À Direcção da Clínica Sagrada Esperança, agradeço pela cedência do seu laboratório na execução da parte experimental. Igualmente, agradeço à Dra. Marta Epalanga, Directora Técnica do Laboratório da Clínica Sagrada Esperança, Dra. Verónica Franco Comba e as técnicas Sandra Marisa Garcia, Eva Mendes, entre outros que não menciono os nomes, mas que sabem quem são amigos, que estiveram comigo durante esta fase.

À Dra. Adelaide de Carvalho que enquanto Directora da Maternidade Lucrécia Paim sempre foi sensível a questões ligadas ao ensino e investigação, o meu muito obrigada pelo apoio e disponibilidade institucional e pessoal.

Agradeço à Dra. Joaquina Ferreira responsável do laboratório geral da Maternidade Lucrécia Paim de Luanda pelo apoio e disponibilidade na aquisição de reagentes.

Às Dra (s) Idalma Ramos Junco, Lucia Fernandes Duarte e as técnicas Isabel Costa, Isabel Bravo, Isabel Lima, Rosa das Graças, Anabela, Nzila, Gina, Elisa, Helena Salvador, Ana Lucala entre outros que não menciono os nomes, mas que sabem que são amigos que estiveram comigo durante esta etapa, pela preciosa ajuda e disponibilidade que sempre me dispensaram. A todos os elementos do laboratório central da Maternidade Lucrécia Paim pela disponibilidade e simpatia com que sempre me receberam.

À Dra. Euridece Chongolola chefe do serviço de consultas externa pré-natal da Maternidade Lucrécia Paim pela simpatia e disponibilidade em colaborar.

As enfermeiras Acielinda Feijó, Conceição Quental, Maria da Natividade e Teresa Alfredo do Serviço de Consultas Externas da Maternidade Lucrécia Paim pela Simpatia e apoio na organização das mulheres para o inquérito e recolha da amostra de sangue.

Ao Instituto Nacional de Estatística de Angola, pela cedência de dados relativos ao mapa da província de Luanda e alguns indicadores múltiplos e de saúde que permitiram a distribuição geoespacial da amostra estudada.

Agradeço profundamente à Fundação Calouste Gulbenkian pela concessão da bolsa de Doutoramento no âmbito do Programa de Bolsas de Estudo de Pós-graduação para Estudantes dos PALOP e Timor Leste (66/SBG/18).

Às Dras Maria dos Anjos Valente e Domingas Mariana Tchiweyengue Zamboli, que ao longo destes anos revelaram ser umas grandes amigas, agradeço de todo o coração o apoio e as palavras de carinho, simpatia e disponibilidade.

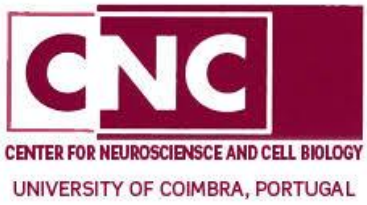
Tendo consciência que sozinha nada disto teria sido possível, dirijo uma palavra especial de apreço ao meu esposo Manuel Vueba, que está sempre presente incondicionamente. Nos momentos mais difíceis, que não foram raros durante este percurso, sempre fez-me acreditar que chegaria ao fim desta difícil, porém gratificante etapa da minha vida; obrigada por acreditares em mim, mesmo nos momentos de maior desalento, sem o seu apoio incondicional e companheirismo não teria provavelmente conseguido percorrer este caminho.

Aos meus filhos Kiesse e Victória pequenos de mais para perceberem os meus momentos de ausência e cansaço, imensamente grandes no amor que me dedicam, fica este trabalho.

A toda a minha família especialmente aos meus pais Amélia Alice Kalassi e João Gaila (in memoriam), aos meus irmãos o meu imenso obrigada pelo apoio incondicional.

This work was performed at the Faculty of Pharmacy, within the activities of the Centre for Neuroscience and Cell Biology of Coimbra, University of Coimbra, Coimbra, Portugal, under the supervision of Professor Doctor Maria do Céu Rodrigues de Sousa.

It was financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme under project CENTRO-01-0145-FEDER-000008:BrainHealth 2020, and through the COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia, I.P., under strategic project POCI-01-0145-FEDER-007440 (UID/NEU/04539/2013); Calouste Gulbenkian Foundation, Lisbon, Portugal for the PhD scholarship grant (ref. 66/SBG /18); Lucrecia Paim Maternity, Clínica Sagrada Esperança; and National Research Institute for Health of Angola and National Institute of Statistics of Angola.



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Abbreviations

ADD	Acute Diarrheal Diseases
ADs	Antigenic Domains
AFP	Alpha Fetoprotein
AIDS	Acquired Immunodeficiency Syndrome
AF	Amniotic Fluid
ART	Antiretrovirals
BCG	Bacillus Calmette-Guérin
CCE	Characteristic Cytopathic Effect
CEDAW	Convention on the Elimination of All Forms of Discrimination Against Women
CMV	Cytomegalovirus
COI	Cut-off index
CNS	Central Nervous System
CRS	Congenital Rubella Syndrome
CT	Congenital Toxoplasmosis
DESC	Economic, Social and Cultural Rights
DNA	DeoxyriboNucleic Acid
DRC	Democratic Republic of Congo
DT	Dye Test
ECL	ElectroChemiLuminescence
EIU	Economist Intelligence Unitt
ELFA	Enzyme-Linked Flourescence Assay
ELISA	Enzyme-Linked Immunosorbent Assay
EV	Epidemiological Surveillence
FDA	Food and Drug Administration
gB	glycoprotein B
GDP	Gross Domestic Product
GEMS	Global Enteric Multicenter Study
GRA	Dense Granule Protein
GVAP	Global Vaccine Action Plan
HAART	Highly Active Antiretroviral Therapy
hCG	Human chorionic gonadotropin
HCMV	Human Cytomegalovirus
HDI	Human Development Index
HDR	Human Development Report
HIG	Human immunoglobulin

HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HBsAg	Hepatitis B virus surface antigen
HCV	Hepatitis C Virus
HDV	Hepatitis D Virus
HEV	Hepatitis E Virus
HIV	Human Immunodeficiency Virus
HLA-DR	Human Leukocyte Antigen – DR isotype
HSV	Herpes Simplex Virus
IFAT	Indirect Fluorescent Antibody Test
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
HIg	Hyperimmune immunoglobulins
IHA	Indirect Haemagglutination
IIBEP	Integrated Survey on the Welfare of the Population
SMHI	Survey of Multiple Health Indicators
INE	Instituto National de Estatistica
NIFAA	National Institute for the Fight Against AIDS
ISAGA	Immunosorbent Agglutination Assay
JRF	Joint Report Form
LAT	Latex Agglutination Test
LPMH	Lucrécia Paim Maternity Hospital
LTBI	Latent tuberculosis infection
MAG	Matrix antigen
MAT	Modified Agglutination Test
MDI	Material Deprivation Index
MIC	Micronemal protein
MH	Ministry of Health
MTCT	Mother-to-Child-Transmission
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
NB	Newborn
NBs	Newborns
NCD_S	Non-communicable chronic Diseases

NCCLS	National Committee for Clinical Laboratory Standards
Nested PCR	Nested Polymerase Chain Reaction
NHS	National Health Service (
NMCP	National Malaria Control Programme
PALOP	Portuguese Speaking African Countries
PC	Pentameric Complex
PCR	Polymerase Chain Reaction
PCR-DNA	Polymerase Chain Reaction - Deoxyribonucleic Acid
PCR-RFLP	Polymerase Chain Reaction - Restriction Fragment Length Polymorphism
PHC	Primary Health Care
PMTCT	Prevention of Mother-to-Child Transmission
NHDP	National of Health Development Plan
UNDP	United Nations Development Program
Post-HCT	Post- Hematopoietic Stem Cell Transplantation
qPCR	Quantitative Real-Time Polymerase Chain Reaction
RDTs	Rapid Diagnostic Tests
RNA	Ribonucleic acid
RT-PCR	Real-time polymerase chain reaction
ROP	Rhoptry Protein
SAG	Surface Antigen Gene
SDZ	Sulfadiazine
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
STIs	Sexually Transmitted Infections
TB	Turberculosis
TORCH	Toxoplasmosis, Other agents (including HIV, syphilis, varicella, and fifth disease), Rubella, Cytomegalovirus, Herpes simplex
UNDP	United Nations Development
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNICEF	United Nations International Children's Emergency Fund
USA	United States of America
VCR	Vaccine Containing Rubella
VZV	Varicella Zoster Virus
WB	Western Blot
WHO	World Health Organization

Abstract

Infections acquired in the uterus or in the postnatal period play a prominent role in perinatal and infant morbidity. Although toxoplasmosis, rubella and cytomegalovirus (CMV) infection are diseases that have been widely studied worldwide, there are few studies carried out in Luanda, Angola. There is only one published work on toxoplasmosis and there are no studies of rubella and CMV infections in the Angola´s population. Therefore, the work aimed to: (i) study the seroprevalence of antibodies IgM and IgG anti-*T. gondii*, anti-Rubella and anti-CMV among pregnant women attended at the Lucrécia Paím Maternity Hospital (LPMH) in Luanda, Angola; (ii) identify maternal primary infection through the profiles of anti-*T. gondii*, Anti-Rubella and anti-CMV antibodies; (iii) dating the primary maternal infection through the IgG avidity test; (iv) perform the diagnosis of congenital infection using molecular techniques (qPCR); (v) studying the geospatial distribution of infections; (vi) assess the socio-demographic risk factors associated with infections by *T. gondii*, and Rubella and CMV virus. A longitudinal study for toxoplasmosis and a prospective transversal study were carried out in the case of Rubella and CMV. Blood samples were collected from August 2016 to May 2017 and specific antibodies anti-*T. gondii*, anti-CMV and anti-Rubella (IgG and IgM) were quantified. The sociodemographic, clinical and behavioral characteristics of the pregnant women were collected using a face-to-face questionnaire after written consent. The spatial distribution was assessed using the Kernel Density Function and the potential risk factors associated with infections were evaluated using bivariate and multivariate binomial logistic regression analysis. Anti-*T. gondii* antibodies were quantified in 878 pregnant women, and 346 (39.4%) samples were IgG positive, 2 (0.2%) positive for IgM and IgG, and 530 (60.4%) negative for both immunoglobulins. In the longitudinal study, a total of 653 pregnant women were followed during pregnancy: 408 (62.5%) were seronegative and, of these, 178 (33.6%) were in the first trimester of pregnancy and 230 (43.4%) in the second quarter. The cross-sectional study included 225 pregnant women in the third trimester, 103 of whom were positive for anti-*T. gondii* antibodies and 122 seronegative. The longitudinal study showed that none of the seronegative women seroconverted during the survey. In the two positive samples for IgM and IgG antibodies, the results of the IgG avidity test (high avidity) showed that it was a chronic infection by *T. gondii*. These results were corroborated by the negative results obtained in the qPCR. Regarding other infections, 226 of pregnant women (25.7%) were positive for hepatitis B, while 118 (13.4%) were HIV-positive The multivariate analysis has shown a significant increased risk for toxoplasmosis in women in the last trimester of

pregnancy (OR 1.457, CI: 1.011–2.102), suffering spontaneous abortion (OR 1.863, CI: 1.014–3.465) and having pets at home (OR 1.658, CI: 1.212–2.269). Also, women who tested positive for hepatitis B (OR 1.375, CI: 1.008–1.874) and HIV (OR 1.833, CI: 1.233–2.730) had a significant increased risk for *T. gondii* infection. There is a significant number of pregnant women in Luanda who are not immunized against toxoplasmosis and, therefore, are at risk of acquiring the primary infection during pregnancy and, consequently, infect the fetus (congenital toxoplasmosis). In the case of the Rubella and CMV study, 396 pregnant women participated in the study. Of the participants, 382 (96.5%) had anti-CMV IgG antibodies, 8 (2.0%) had anti-CMV IgG and IgM antibodies and 6 (1.5%) were seronegative. For Rubella virus, 347 (87.6%) were positive for anti-IgG, 4 (1.0%) positive for anti-IgG and IgM, and 45 (11.4%) were seronegative. The multivariate logistic regression analysis has shown a significant association between Rubella virus infection and pregnant women without child (OR 2.673; CI: 1.026 - 7.007) and suffering spontaneous abortion (OR 3.232; CI: 1.192 - 7.952). In contrast, the level of schooling, residence, occupation, marital status, number of children in the household, basic sanitation, gestational age, history of miscarriages and hepatitis B were not significantly associated with the Rubella virus infection. The seroprevalence of toxoplasmosis, Rubella e CMV infection was similar in most municipalities, however the seroprevalence of toxoplasmosis in municipality of Belas was lower (25.8%; 31 of 120) and multivariate analysis has shown a lower risk for toxoplasmosis in this area (OR 0.471, CI: 0.299–0.728). In conclusion, our study showed that a large number of pregnant women are not immunized for toxoplasmosis and identified the risk factors for this infection in Luanda. We also showed that there is a high seroprevalence of anti-CMV and anti-Rubella antibodies in pregnant women in Luanda. Therefore, it is important a rapid and accurate diagnosis of toxoplasmosis, CMV and Rubella infection in pregnant women to prevent congenital infections. Rubella vaccination should be offered to women non-immune to Rubella. Overall, it would be important to implement national screening for toxoplasmosis, CMV, Rubella and other diseases linked to maternal and child health, as highlighting the need for diagnostic and clinical follow-up of other infectious diseases, such as HIV/SIDA and hepatitis B during pregnancy.

Resumo

As infeções adquiridas no útero ou no período pós-natal desempenham um papel proeminente na morbilidade perinatal e infantil. Apesar da elevada prevalência global da toxoplasmose, rubéola e infeção por citomegalovírus (CMV), a epidemiologia destas infeções tem sido pouco documentada em Luanda, Angola. De facto, existe somente um trabalho publicado sobre a toxoplasmose e não existem estudos de infeções pelos vírus da Rubéola e CMV na população. Portanto, os objetivos do trabalho foram: (i) estudar a seroprevalência de anticorpos IgM e IgG anti-*T. gondii*, anti-vírus da Rubéola e anti-CMV em mulheres grávidas atendidas na Maternidade Lucrécia Paím (MLP) de Luanda; (ii) identificar a primoinfeção materna através dos perfis de anticorpos anti- *T. gondii*, anti-vírus da Rubéola e anti-CMV; (iii) datar a primoinfeção materna através do teste de avidez da IgG; (iv) realizar o diagnóstico da infeção congénita através de técnicas moleculares (qPCR) em fragmentos de placenta; (v) estudar a distribuição geoespacial das infeções; (vi) avaliar os fatores de risco sociodemográficos associados às infeções por *T. gondii*, vírus da Rubéola e CMV. Assim, foi realizado um estudo longitudinal para toxoplasmose e transversal prospectivo no caso da Rubéola e CMV. As amostras de sangue foram recolhidas no período de Agosto de 2016 a Maio de 2017 e os anticorpos específicos anti-*T. gondii*, anti-CMV e anti-virus da Rubéola (IgG e IgM) foram quantificados. As características sociodemográficas, clínicas e comportamentais das gestantes foram recolhidas mediante um questionário *face-to-face* após o consentimento escrito. A distribuição espacial foi avaliada através da função de densidade de Kernel e os fatores de risco potencialmente associados às infeções foram determinados por análise de regressão logística binomial, bivariada e multivariada. Relativamente à Toxoplasmose, os anticorpos anti-*T. gondii* foram quantificados em 878 amostras das quais 346 (39,4%) foram positivas para IgG anti-*T. gondii*, 2 (0,2%) foram positivas para IgM e IgG anti-*T. gondii* e 528 (60,4%) foram negativas para ambas as imunoglobulinas. No estudo longitudinal, um total de 653 gestantes foram acompanhadas durante a gestação: 408 (62,5%) foram seronegativas e, destas, 178 (33,6%) encontravam-se no primeiro trimestre de gestação e 230 (43,4%) no segundo trimestre. No estudo transversal foram incluídas 225 gestantes no terceiro trimestre, sendo 103 seropositivas para anticorpos anti-*T. gondii* e 122 seronegativas. Nas duas amostras positivas para anticorpos IgM e IgG, os resultados do teste de avidez da IgG (alta avidez) mostraram que se tratava de uma infeção latente/crónica por *T. gondii*. Esta conclusão foi corroborada pelos resultados negativos obtidos na qPCR. Assim, existe um número significativo de mulheres grávidas em Luanda que não estão imunizadas contra a

toxoplasmose e, portanto, correm o risco de adquirir a infeção primária durante a gravidez e, consequentemente, infetar o feto (toxoplasmose congénita). Em relação a outras infeções, 226 grávidas (25,7%) foram positivas para o vírus da Hepatite B, enquanto 118 (13,4%) foram positivas para HIV. A análise de regressão logística multivariada mostrou que a idade gestacional (OR 1,457; IC:1,01-2,10), presença de animais domésticos em casa (OR 1,658; IC:1,21-2,26), seropositividade para HIV (OR 1,83; IC: 1,23-2,73) e seropositividade para o vírus d Hepatite B (OR 1,375; IC: 1,00-1,874) são fatores de risco para infeção por *T. gondii*. No caso do estudo do vírus da Rubéola e CMV foram testadas 396 mulheres grávidas. Em relação ao CMV, 382 (96,5%) possuíam anticorpos IgG anti-CMV, 8 (2,0%) anticorpos IgG e IgM e 6 (1,5%) eram seronegativas. Para o vírus da Rubéola, 347 (87,6%) foram positivas para anticorpos IgG, 4 (1,0%) positivas para IgG e IgM e 45 (11,4%) foram seronegativas para os dois anticorpos. A análise de regressão logística multivariada mostrou associação significativa entre infeção pelo vírus em grávidas sem filhos (OR 2,673; IC: 1,026 - 7,007) e que sofreram aborto espontâneo (OR 3,232; IC: 1,192 -7,952). Em contrapartida, escolaridade, residência, ocupação, estado civil, número de filhos em casa, saneamento básico, idade gestacional, história de aborto e vírus da Hepatite B não apresentaram associação significativa com a infeção pelo vírus da Rubéola. A seroprevalência de toxoplasmose, rubéola e infeção por CMV foi semelhante na maioria dos municípios de Luanda, porém a seroprevalência da toxoplasmose no município de Belas foi mais baixa e a análise multivariada mostrou risco menor para a toxoplasmose nessa área (OR 0,471, CI: 0,299–0,728).

Em conclusão, o nosso estudo mostrou que um grande número de mulheres grávidas não está imune para toxoplasmose e identificou os fatores de risco para esta infeção em Luanda. Mostrámos também que, embora exista uma elevada seroprevalência de anticorpos anti-CMV e anti-vírus da Rubéola nas mulheres grávidas em Luanda, um número ainda considerável não está imune ao vírus da Rubéola e, por isso, devem ser vacinadas. Assim, é muito importante o diagnóstico rápido e preciso da Toxoplasmose, Rubéola e infeção por CMV em mulheres grávidas para prevenir as infeções congénitas e preservar a saúde materno-infantil. Globalmente os resultados sustentam a importância da implementação de rastreios nacionais para Toxoplasmose, Rubéola, infeção por CMV e de outras doenças relacionadas com a saúde materno-infantil bem como evidenciam a necessidade de diagnóstico e acompanhamento clínico de outras doenças infeciosas, como HIV/SIDA e Hepatite B, durante a gravidez.

CHAPTER 1

General Introdution

In this chapter are included the review articles:

1. Amélia Nkutxi Vueba and Maria do Céu Sousa (2020). "Diagnosis of toxoplasmosis in humans: advantages and limitations of conventional and innovative methods". (*in preparation*)

2. Amélia Nkutxi Vueba and Maria do Céu Sousa (2020). "Rubella infection: Advances and challenges in the diagnosis and prevention of Congenital Rubella Syndrome". Published in: HSPC, *International Journal of Clinical Virology*. 4: 006-013.

3. Amélia Nkutxi Vueba and Maria do Céu Sousa (2020). "Cytomegalovirus infection: advances and challenges in the diagnosis and prevention of cytomegalovirus infections in various clinical settings". Submitted in: *Pathogens and Global Health (in revision).*

1.1. Pregancy and Infectious Diseases

Infectious diseases change a woman's health and can negatively influence her reproductive function. When associated with pregnancy, they take special importance challenging on three principal questions: the treatment of the mother's disease; the effect of infection during pregnancy; and the influence on the fetus, not only of maternal disease but also of the therapy used. In the case of pregnant women, prenatal examinations are relevant for the prevention of vertical transmission of various infectious agents (Numan et al., 2015). *Toxoplasma gondii* (*T. gondii*), Rubella virus and Cytomegalovirus (CMV) are infectious agents that are part of the TORCH complex, an acronym used to group five infectious diseases that can affect the fetus and the newborn (NB), and which have a similar clinical picture (Numan et al., 2015). Sometimes, these diseases are not associated with symptoms, however can cause spontaneous abortions, sterility, congenital malformations and intrauterine fetal loss (Rasti et al., 2016). The transmission of an infection from the mother to the child can occur congenitally during delivery, before or after birth and through breast milk (Neu et al., 2015). Although these diseases have been widely studied and tests are used frequently in medical practice in many countries, the discussions generated around the feasibility of screening in women of reproductive age and pregnant women is mainly associated with the variability in the prevalence of these diseases in populations (Leeper & Lutzkanin, 2018; Neu et al., 2015; Rasti et al., 2016). However, the evidence for and against screening for these pathologies varies widely when considering public health conditions and population epidemiological data in each country (Nayeri et al., 2020).

1.2. *Toxoplasma gondii* and toxoplasmosis

Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii* (*T. gondii)*, a protozoan of the Phylum Apicomplexa, order Eucoccidia, capable of infecting humans, birds, rodents and other animals, these being its intermediate hosts. Felids, including the domestic cat, are considered the definitive hosts (Montoya & Liesenfeld, 2004).

T. gondii was discovered in Tunisia, North Africa, more precisely in the Pasteur Institute of Tunisby Nicolle and Manceaux, and by Splendore, in Brazil, in 1908 (Nicolle & Manceaux, 1908; Splendore, 1908). During studies on leishmaniosis, Nicolle and Manceaux observed an arc-shaped protozoan in tissues of a rodent, the gundis (*Ctenodactylus gundi*) (Dubey, 1994; Dubey, 2009). It was named *T. gondii* based on its morphology (Toxon: arc, plasma: form) and its host (Rouatbi et al., 2019). *T. gondii* is an obligate intracellular parasite that invades and multiplies in any nucleated cell, parasiting the most diverse tissues of various

mammals and birds. Its life cycle has been known since the late 1960s and features three infecting stages: tachyzoites, bradyzoites and sporozoites (Figure 1).

Although toxoplasmosis is generally asymptomatic for most adults, severe complications may occur in some individuals, including immunocompromised patients such as patients with AIDS, and transplant recipients (Liu et al., 2015). In addition, primary infections of pregnant women are associated with potential congenital infections and abortions, as *T. gondii* may cross the placenta barrier (Montoya & Liesenfeld, 2004).

T. gondii was considered a unique species in the genus *Toxoplasma*. Studies in North America and Europe have identified limited genetic diversity on parasite strains, which have been classified into genetic types I, II and III (Ajzenberg et al., 2010; Howe & Sibley, 1995; Li et al., 2014). In other parts of the globe, such as in South America or Africa, recombinant or atypical strains are associated with ocular toxoplasmosis and severe clinical manifestations in immunocompromised individuals (Porter & Sande, 1992; Previato et al., 2015; Sumeeta & Batra, 2016).

1.2.1. Epidemiology

Toxoplasmosis is one of the most common infections in humans and warm-blooded animals, with a worldwide distribution and great importance in public health (Hill et al. 2005). The disease has been proven in all zoo-geographic areas in about 200 mammalian species (Hill et al., 2005). In animals, toxoplasmosis acquires importance, mainly because when infected they serve as a direct or indirect source of infection to humans, besides causing direct damages to animals of economic interest and pets. The parasite encysts in the musculature and can infect the man by ingestion of raw or undercooked meat (Dubey et al., 2012).

It is estimated that approximately 30% of the human population in the world is chronically infected by *T. gondii* (Moncada & Montoya, 2012; Sun et al., 2013). However, the prevalence of the disease varies widely among countries, with incidence reaching over 95% in some regions (Robert-Gangneux & Dardé, 2012). The prevalence in man depends on climate, socioeconomic level, eating habits, cultural and ethnic practices and presence of cats in home area (Halonen & Weiss, 2013). Unlike many parasites limited to the tropics or subtropics, *T. gondii* can inhabit the most diverse regions of the globe, from the frozen ground of Alaska to the Amazon forest, although climatic factors have an influence on the prevalence of infection, which is lower in very cold climates with ice cycles, in arid areas and at high altitudes as oocysts lose their virulence when dried or frozen (Halonen & Weiss, 2013; Hill et al., 2005; Robert-Gangneux & Dardé, 2012).

1.2.2. Transmission

In humans, *T. gondii* infection usually occurs through the ingestion of mature oocysts (containing sporozoites) or through ingestion of raw or undercooked meat from animals containing tissue cysts with bradyzoites (Figure 1) (Dubey, 2009; Halonen & Weiss, 2013). Another important route of transmission to humans is the transplacental passage of tachyzoites during acute infection in pregnant women (Jones et al., 2014; Jones & Dubey, 2012). This transmission occurs when the pregnant woman acquires toxoplasmosis during pregnancy and presents the acute phase of the disease, being able to transmit toxoplasmosis to the fetus, probably having tachyzoites as forms responsible for the infection (Robert-Gangneux & Dardé, 2012). Also the transmission can occur through organ transplantation and blood transfusion (Alvarado-Esquivel et al., 2018; Dodd, 1998; Liu et al., 2017).

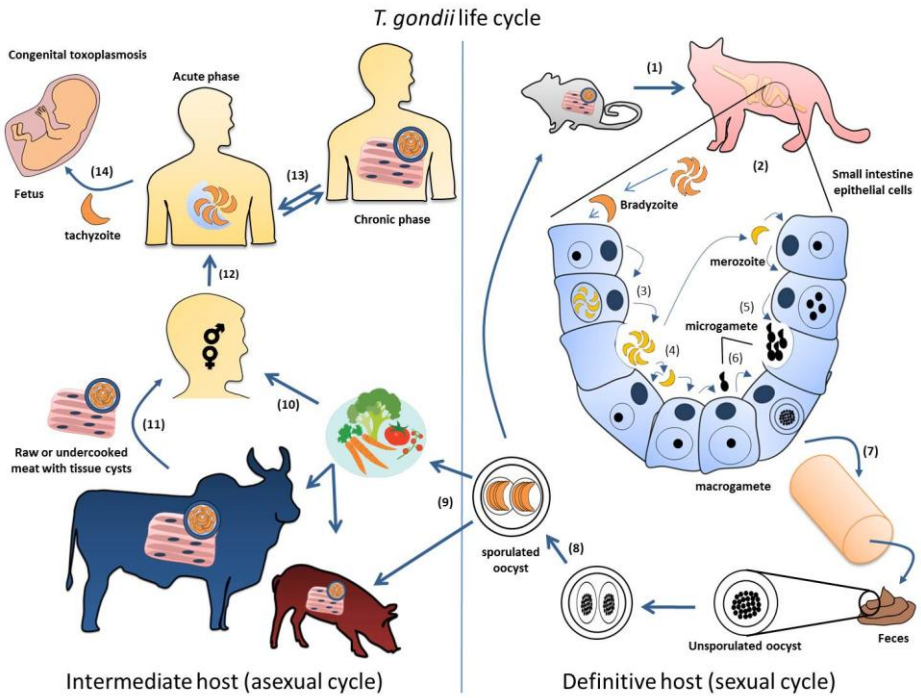

Figure 1: Sexual and asexual phase of *Toxoplasma gondii*. (1) Infection of the definitive host; (2) cyst rupture and infection of intestinal epithelial cells; (3) formation of merozoites; (4,5) the beginning of the sexual phase with the formation of macrogamete and flagellated microgamete; (6) fusion of microgamete and macrogamete; (7) release of the oocyst in the faeces into the environment; (8) oocysts will become infectious, sporulated, and contaminate the environment; (9) sporulated oocysts can cause infection in animals through the consumption of contaminated food and water; (10,11) human infection occurs by eating raw or undercooked meat from infected animals containing cysts;

(12) multiplication of tachyzoites in humans; (13) tachyzoite-bradyzoite differentiation and formation of tissue in the tissue; (14) transplacental transmission of tachyzoites (Source: Duque et al., 2013).

1.2.3. Clinical manifestations

The congenital disease can cause important neonatal changes like ocular lesions, microcephaly, hydrocephalus, cerebral calcifications, psychomotor alterations and mental retardation, making the primary infection on pregnant woman and consequently fetal infection the most serious aspect of human toxoplasmosis (Maldonado et al., 2017; Neu et al., 2015; Wallon & Peyron, 2018). Toxoplasmosis in the immunocompromised can become severe or even deadly. The deficit in the patient's immune system leads to reactivation of the chronic infection, particularly affecting the CSN (Liu et al., 2017; Xavier et al., 2013). In patients with acquired immunodeficiency syndrome (AIDS), toxoplasmosis results, in most cases, from reactivation of a latent toxoplasmosis (bradyzoites can become tachyzoites again), acquired previously (Xavier et al., 2013). The most frequent clinical manifestations are headache, fever and neurological signs. Toxoplasmosis in HIV-positive people can manifest itself in different places such as the CSN, lung, heart and eye. The most frequent is pulmonary toxoplasmosis characterized by pneumonia and respiratory failure.

In cases of immunodepression associated with transplant surgery it is very important to know, in advance, the immunity of the donor and recipient in relation to *T. gondii*. When the non-immune recipient receives a transplant from an immune donor, it is exposed to a risk of severe toxoplasmosis, due to the absence of specific immunity and the therapeutic-induced immunodepression, leading to reactivation of cysts present in the transplanted organ (Derouin & Pelloux, 2008). Another very serious situation occurs when the recipient has a chronic toxoplasmosis, because it is exposed to the reactivation of their own cysts due to therapeutic suppression that weakens their immune system (Derouin & Pelloux, 2008).

1.2.4. Maternal infection versus risk of fetal infection

The diagnosis of toxoplasmosis is usually only performed in people considered at risk (Liu et al., 2015). There are four groups that are considered at risk: pregnant women who acquired the infection during pregnancy; fetuses and newborns who were infected during pregnancy; immunocompromised individuals and people with chorioretinitis. However, the most important thing in medicine is whether the pregnant woman acquires the infection during pregnancy (Leeper & Lutzkanin, 2018).

The risk of fetal infection increases with the time of gestation: 15% if maternal infection occurs at 13 weeks of gestation, to over 70% if infected at 36 weeks (Pappas et al., 2009). Women with chronic *T. gondii* infection do not contaminate their children during the intrauterine period, and there is also no evidence that toxoplasmosis in chronic stage is a cause of abortions. However, the reactivation cases of *T. gondii* infection, caused by maternal immunosuppression, could lead to infection of the fetus during pregnancy (Halonen & Weiss, 2013; Mozzatto & Procianoy, 2003).

In the fetus, the parasite lodges in cells of the phagocytic-mononuclear system, giving rise to intermediate structures called pseudocysts; it then spreads through blood or lymph to any organ or tissue, including the central nervous system and eyes, for which it has a greater tropism (Halonen & Weiss, 2013; Helieh, 2017).

The success of fetal infection depends on factors such as virulence of *T. gondii*, placental development, age of pregnancy and parasitic burden, among others (Neu et al., 2015; Kieffer & Wallon, 2013). During the first trimester of pregnancy, infection by *T. gondii* can lead to fetal death. In the second quarter, it can cause the so-called Sabin tetrad, in which the fetus has hydrocephalus, with macro or microcephaly (in 50% of cases), chorioretinitis (in 90% of infections), brain calcifications (69%) and delayed mental or neurological disorders (in 60% of cases). The newborn may also have initial lesions as miliary nodules spread throughout the brain or around necrotic foci, the cerebral ventricles may be dilated and brain lesions may be calcified (Leeper & Lutzkanin, 2018; Saadatnia & Golkar, 2012). Likewise, other ocular changes still can happen in varying degrees of degeneration as retinal edema, choroidal vascular lesions, neuritis optics, microphthalmos, nystagmus, and strabismus. Among the neurological manifestations, it can be mentioned the psychomotor disorders, seizures and opisthotonus. Children who survive congenital toxoplasmosis are most of the time children mentally retarded (Kieffer & Wallon, 2013; Saadatnia & Golkar, 2012; Tenter et al., 2000).

1.2.5. Laboratory Diagnosis of Toxoplasmosis

Although toxoplasmosis develops immunity in the individual, there is still no specific vaccine available. Therefore, the diagnosis is highly relevant for treatment and prophylaxis purposes. The validation of a diagnosis of *T. gondii* should not be made only on the basis of clinical data; these should be confirmed with laboratory data, since toxoplasmosis is a benign and asymptomatic infection in most cases (Montoya, 2002).

The laboratory diagnosis of *T. gondii* infection can be performed by serological tests (indirect diagnosis) or by parasite isolation, amplification of specific sequences of deoxyribonucleic acid (DNA) and histological studies (direct diagnosis) (Maldonado, 2017; Liu et al., 2015).

The evidence of the parasite by the demonstration of its components as antigens or segments of DNA is of high diagnostic value and has been assuming a prominent position especially in immunocompromised individuals (Bastien, 2002; Robert-Gangneux & Belaz, 2016). Recent advances in the knowledge of the genome of *T. gondii* have made it possible to use qPCR to detect this protozoan (Liu et al., 2015). Real-time PCR (qPCR) has been used in prenatal diagnosis for confirmation of congenital toxoplasmosis.

The methodology used for the laboratory diagnosis of toxoplasmosis diversifies according to some parameters, such as the biological material available, specificity and sensitivity of each technique.

1.2.5.1. Humoral response to *T. gondii* infection and serological diagnostic

In the case of oral transmission mediated by the ingestion of mature oocysts, the immune system develops humoral immunity, firstly with IgM and IgA antibodies production and later with IgG and IgE (Decoster et al., 1995; Murat et al., 2013; Zhang et al., 2016). IgM antibodies appears approximately in the first or second week after infection, peaking in six to eight weeks, when it then declines. However, it may remain in the patient for up to four to six months after the onset of infection by *T. gondii* (stage considered acute or recent) or persist in low titers for more than 12 months, being considered residual IgM (Gras et al., 2004; Liu et al., 2015; Villard et al., 2016). Specific IgG appears after 2 weeks of infection, peaks at 2-3 months, remaing at a plateau level for 6 months and after 1 year starts to slowly decrease to lower levels until the end of infected subject's life (Robert-Gangneux & Dardé, 2012). This stage is equivalent to the chronic or latent stage of the disease due to the persistence of latent cysts in organs. So, a positive IgG result indicates a previous infection but cannot accurately determine the time of infection (Liu et al., 2015; Saadatnia & Golkar, 2012). To determine the moment of toxoplasmic infection, it is necessary to perform serological tests that detect anti-*T. gondii* antibodies of type IgM and IgG, mainly. However, the presence of high levels of IgG, as well as the persistence of IgM for long periods of time, complicates the interpretation of serological tests and the discrimination between acute and chronic cases (Liu et al., 2015; Zhang et al., 2016).

In clinical practice the serological detection of IgM and IgG antibody levels are the basis for identifying the infection and is one of the most commonly used method. Diagnostic strategies for toxoplasmosis are according to the patients' immunological history and clinical settings, including immunocompromised individuals, pregnancy and newborns (Liu et al., 2015; Robert-Gangneux & Dardé, 2012; Villard et al., 2016; Zhang et al., 2016). Primary infection during pregnancy and prenatal diagnosis of congenital toxoplasmosis are the most challenging situations and the interpretation of results is most problematic in these cases. The serological patterns in pregnancy and the newborn include: IgG- and IgM-negative; IgG-negative and IgM-positive; IgG-positive and IgM-negative; and IgG- and IgM-positive (Sensini, 2006). Serological monitoring is necessary to obtain reliable conclusions about the patient's serological status. The challenge lies in interpreting the results for specific IgM in pregnancy (Zhang et al., 2016; Sensini, 2006).

In immunocompromised individuals, the serological diagnosis is difficult to interpret due to immunological imbalance, since the correlation between specific IgG antibody titers and clinical manifestations is not verified. Regarding IgM antibodies, normally they are not detected in these patients (Liu et al., 2017; Xavier et al., 2013).

1.2.5.2. Serological tests

Serology remains the primary approach to establishing a diagnosis of toxoplasmosis. It is based on the research of antibodies of different classes of anti-*T. gondii* (IgG, IgM, IgA and IgE) (Liu et al., 2015; Da Costa et al., 2007; Zhang et al., 2016). The presence of anti-*T. gondii* antibodies in the course of infection allows the analysis of different serological profiles: recent infection; acute phase; old infection; and latency phase or chronic. The first test available to detect specific anti-*T gondii* antibodies was the Sabin-Feldman reaction (dye test; DT). Sixty years after its description, it is still considered a reference test with high rates of sensitivity and specificity (Zhang et al., 2016). However, its use has been restricted by the mandatory use of live toxoplasma, which causes serious biosafety problems (Remington et al., 2004).

Serodiagnosis is widely used in routine examinations of pregnant women to prevent congenital toxoplasmosis. Different techniques have the property of detecting these antibodies in patients' blood. Tests such as enzyme-linked immunosorbent (ELISA), electrochemiluminescence assays (ECLIA), chemiluminescence assays (CIA), indirect fluorescent antibody test (IFAT), immunosorbent agglutination assays (ISAGA), latex agglutination test (LAT), serum IgG avidity test and Western blotting (WB) have been

developed to improve the ability to diagnose *T. gondii* infections (Dard et al., 2016; Liu et al., 2015; Massa et al., 2017; Peretti et al., 2017; Teimouri et al., 2018; Remington et al., 2004). Some of these methods, including DT, IFAT, LAT and ISAGA, are generally reserved for reference laboratories. ELISA and CIA are routine screening methods for specific IgG and IgM in serum and are used primarily in clinical laboratories. There are many commercial kits and automated platforms for using ELISA and CIA methods (Zhang et al., 2016).

Certain limitations of these methods have been evident, since failures to detect IgG and IgM have been described during the active phase of the infection, and it has been shown that these antibodies may not be produced during the first weeks of the parasitemia (Liu et al., 2015). Therefore, the risk of congenital toxoplasmosis may not be detected in these cases. More recently, it has been shown that the avidity of IgG for the *T. gondii* antigen (which measures the binding strength between the antibody and antigen) increases over time (Pomares & Montoya, 2016). This is an informative marker as the low avidity of IgG could be interpreted as an indicator of recent toxoplasmic infection (Montoya & Liesenfeld, 2004; Rossi, 1998). Likewise, the serological diagnosis of toxoplasmosis is complicated in immunocompromised patients, fetuses, newborns, recipients of transplanted organs and especially, in AIDS patients. In these cases, and in pregnant women with a presumptive serological diagnosis of acute phase, direct detection of the parasite can be proposed (Cassaing et al., 2006; Robert-Gangneux & Dardé, 2012). The emergence of the human immunodeficiency virus (HIV) has increased the need for more sensitive and efficient diagnoses for opportunistic infections. Latent toxoplasmosis is at risk of developing into toxoplasmic encephalitis in these patients (Cassaing et al., 2006; Joseph et al., 2002). Equally, the need for early diagnoses to detect the reactivation of latent toxoplasmosis in patients with other types of immunosuppression has increased, such as cancer, organ transplantation and others (Kupferschmidt, 2001; Robert-Gangneux et al., 2017).

Some studies have reported that qualitative measurements of specific IgG antibodies are less problematic than quantitative measurements, and a variety of commercial immunoassay methods and systems can achieve good sensitivity and specificity (Franck et al., 2008; Maudry et al., 2009). According to these studies, IgG results were confirmed by DT or WB, and the number of discrepant samples was low (Zhang et al., 2016). The commercial immunoassay systems analyzed included enzyme immunoassays and fully automated assays, however, it should be noted that the quantitative results were not as good as expected when evaluating the performance of six automated anti-toxoplasma IgG immunoassays (Liu et al., 2015; Maudry et al., 2009). Samples with low levels of specific IgG are often observed in the

clinic, resulting in borderline results, that is defined as a "gray area" by the manufacturers (Robert-Gangneux & Dardé, 2012). These findings suggest that confirmatory tests, such as WB, are needed to identify IgG in the "gray zone" (Zhang et al., 2016).

There are some problems with the specific IgM test for *T. gondii*. Studies have reported a high number of false positive results in pregnant women (Garry et al., 2005; Liesenfeld et al., 1997). This occurrence may be due to performance deficiencies of specific IgM assays or residual IgM in individuals with past infection (Hofgärtner et al., 1997; Liesenfeld et al., 1997). In populations with low prevalence of seroconversion to acute toxoplasmosis, false-positive results are more frequent (Zhang et al., 2016). This shows that commercial trials must consider the balance between sensitivity and specificity when establishing cutoff points within a given population (Hofgärtner et al., 1997; Liesenfeld et al., 1997). Another problem is the low level of detection of specific IgM (Petersen et al., 2005; Roberts et al., 2001). False-negative results from samples with a low level of specific IgM due to a recent infection can result in the misdiagnosis of a newly acquired infection in patients, especially pregnant women (Zhang et al., 2016). False-positive or false-negative results for *Toxoplasma*-specific IgM antibodies highlight the importance of confirmatory tests (Liu et al., 2015).

Since the serological interpretation of IgM results is very complex, the use of these tests in combination with other means of diagnosis, such as IgG avidity detection, is a better tool to confirm a recent or past infection (Sensini, 2006). IgG avidity detection was initially developed as an “internal” method (Hedman et al., 1989) and commercial kits are now available in many countries (Zhang et al., 2016). The high avidity indices exclude a recent infection, while the low avidity indices indicate weak interactions between the antigen and the antibody and could indicate a newly acquired infection (Zhang et al., 2016). Another method depends on analyzing the IgG titer of two samples from the same patient collected at 3-week intervals without treatment. The increase in IgG titers over time suggests a recently acquired infection (Robert-Gangneux & Dardé, 2012). Intertechnical and interlaboratory variability in specific antibody assays for *T. gondii* is frequently observed in the evaluation of automated immunoassays (Maudry et al., 2009). These differences can arise for several reasons: variations in the antigen used by different tests, differences in cut-off values proposed by different manufacturers due to the lack of cooperation and measurement errors made by clinical laboratories (Zhang et al., 2016).

To improve the harmonization and standardization of the detection of antibodies specific to *T. gondii*, it is important to develop reference materials and standards. Although there is the International Standard (IS) released by WHO since 1994, carried out through an

international collaborative study, the results indicate that there is still significant progress to be made towards the harmonization and standardization of immunoassays for *Toxoplasma*-specific IgG detection (Maudry et al., 2009; Rigsby et al., 2004; Zhang et al., 2015). Currently, when two successive titrations from one patient are analyzed, the same commercial assay should be used for both (Zhang et al., 2016).

1.2.5.2.1. Lateral flow immunochromatographic tests: Point-of-Care test

The costs of conventional serological tests for toxoplasmosis often discourage universal adoption of monthly prenatal use in screening programs to prevent congenital toxoplasmosis.

The lateral-flow immunochromatographic test, also called the rapid test, has been an increasingly used tool for the diagnosis of a wide range of biological targets (Katarzyna et al., 2016; O'Farrell, 2009; Pierilli, 2004). Due to its characteristics, this platform is easily adapted for use at home or in the field, not requiring large technological resources or prior technical knowledge, allowing the rapid diagnosis of several pathologies (Gomez et al., 2018; Koczula & Gallotta, 2016). Most developed methods are capable of detecting antibodies or antigens. The advantages of using rapid tests as a diagnostic tool have already been widely disclosed: fast results, relatively low manufacturing cost, high scale production facility, long shelf life, independent use of reading equipment and possibility of using small sample volumes. However, they have limitations related to the production technology, that typically requires long development cycles, and to the performance characteristics as sensitivity and reproducibility (Begeman et al., 2017; Piergili, 2004). Different formats of rapid tests are available in the market and academic research. A good example was the Point-of-Care (POC) test for *T. gondii* infection diagnosis (ICT IgG-IgM POC test) (Begeman et al., 2017). The studies have demonstrated an optimal diagnostic performance for Toxoplasma ICT IgG-IgM POC test (LDBIO Diagnostics, Lyon, France) compared to serological reference tests realized in France and the United States (Begeman et al., 2017; Chapey et al., 2017). In the United States the Toxoplasma ICT IgG-IgM POC test was highly sensitive (100%) and specific (100%) in distinguishing positive IgG / IgM sera from negative sera. In France, performance was also excellent, with 97% sensitivity and 96% specificity and the test was already approved for clinical use (Begeman et al., 2017).

1.2.5.3. Isolation of the parasite

The direct diagnosis is based on the identification of evolutionary forms of the parasite (tachyzoites, bradyzoites and oocysts). Although in recent years the techniques of indirect

diagnosis (serological tests) have already evolved, these should still be complemented by direct diagnostic techniques whenever possible (Bonyadi & Bastani, 2013; Montoya, 2002).

Isolation of *T. gondii* in blood or other body fluids demonstrates that the infection is acute. Isolation of the parasite can be performed by inoculating the biological sample, almost all human tissues or body fluids, into mice or into cell cultures (Bonyadi & Bastani, 2013; Montoya, 2002; Montoya & Liesenfeld, 2004). However, the direct detection of the parasite as an unequivocal diagnosis are difficult to perform, due to the wide dissemination of the parasite and its tropism for several cells of the human organism, generating repeated negativity in the direct search (Pittman & Knoll, 2015; Wong & Remington, 1993). Likewise, it is known that parasitemia is detectable intermittently in some patients (Filice et al., 1993; Hofflin & Remington, 1985; Kompalic-Cristo et al., 2004). Because it is a obligate intracellular parasite, *in vitro* culture is complicated to maintain, has a high cost and requires a long time to provide results, often only being effective in less than 50% of cases. The isolation of the parasite can be done with inoculation in mice, which is more sensitive, but requires three to six weeks and maintenance of animals in vivariums (Grover et al., 1990; Hitt & Filice, 1992; James et al., 1996; Kupferschmidt et al., 2001; Lin et al., 2000).

1.2.5.4. Histopathological examination

In histopathological examinations are used staining techniques, such as hematoxylin-eosin, to observe the presence of *T. gondii* evolutionary forms. The presence of tachyzoites in tissue cuts or body fluids establishes a good diagnosis for acute infection (Eapen et al., 2005; Montoya, 2002).

1.2.5.5. Molecular methods based on the detection of nucleic acids

In 1985, the strategy to amplify a genomic target through DNA replication *in vitro* was described and revolutionized the use of technology that involves nucleic acid (Saiki et al., 1985). Molecular methods are also widely used in addition to conventional serological methods for the diagnosis of toxoplasmosis (Liu et al., 2015).

Conventional methods are limited in prenatal cases or in immunocompromised patients. For example, a primary infection can be accurately diagnosed by serology in a woman during pregnancy, but serology results cannot confirm whether the mother's parasite was transferred for the fetus. However, molecular diagnostic techniques can do so (Liu et al., 2015).

1.2.5.5.1. Polymerase Chain Reaction (PCR)

The laboratory diagnosis of toxoplasmosis can be made using the Polymerase Chain Reaction (PCR) to detect the DNA of the parasite. Due to inherent limitations of traditional diagnostic methods, PCR can be used in addition to serology to diagnose *T. gondii* infection. PCR is an efficient method of *in vitro* enzymatic amplification that allows the specific amplification of DNA from small amounts of starting material in a short period of time (Saiki et al., 1988). Advances in the knowledge of the *T. gondii* genome have made it possible to use PCR to detect the parasite (Ajzenberg, 2010; Bastien, 2002; Wong & Remington, 1993). To achieve high sensitivity, several multiple target genes are generally used for the detection of *T. gondii* in biological samples (Ajzenberg et al., 2016; Liu et al., 2015).

The *T. gondii* parasite was detected by PCR in several living tissues and nucleated cells and in organic liquids such as blood, lymph, saliva, milk, exudates, sperm, amniotic fluid, bronchoalveolar lavage fluid, aqueous humor, and peritoneal fluid (Bastien, 2002; Bou et al., 1999; Burg et al., 1989; Guy et al., 1996; Robert-Gangneux & Belaz, 2016; Roth et al., 1992;) and even in urine (Fuentes et al., 1996). PCR is very sensitive because it can detect the DNA of only one tachyzoite, providing a rapid diagnosis (Robert-Gangneux & Belaz, 2016). Blood samples tested to investigate parasitemia by PCR assays, amplifying segments of *T. gondii* B1 and P30 genes, showed the potential of the technique for the non-invasive diagnosis of disseminated toxoplasmosis (Dupoy-Camet et al., 1993; Filice et al., 1993; Ho-Yen et al., 1992; Weiss, 1995). Different pairs of primers for *in vitro* replication targeting different targets have been used. The first PCR method for the detection of *T. gondii*, targeting the B1 gene, was established in 1989 (Burg et al., 1989). The locus gene B1 (2.2 Kb) is one of the most widely used markers for the detection of *T. gondii* because it is repeated in 35 copies in the genome (Burg et al., 1989; Romand et al., 2004). It has a high sensitivity, being able to detect the DNA of a single organism directly from a crude cell lysate or 10 parasites in the presence of 100,000 human leukocytes (Burg et al., 1989; Grover et al., 1990). This marker has been widely used in the prenatal diagnosis of congenital toxoplasmosis and *T. gondii* infection in immunocompromised patients (Kompalic-Cristo et al., 2007; Lamoril et al., 1996; Matin & Shahbazi, 2017; Reischl et al., 2003). The presence of parasites is rarely detected in blood, therefore blood PCR has a low negative predictive value (Liu et al., 2015; Mousavi et al., 2016). Several other single-copy genes, such as SAG1, SAG2 and GRA1, were also used as PCR targets (Liu et al., 2015). The nested-PCR assay has been reported as the most significant and sensitive assay for diagnosing toxoplasmosis (Homan et al., 2000; Mousavi et al., 2016). To further improve the sensitivity and specificity a nested PCR based on the

repeating element of 529 bp and the ITS-1 sequences were developed (Fallahi et al., 2014). The genetic marker of 529 bp is repeated 300 times in the genome of *T. gondii* presenting high sensitivity for the detection of the parasite (Su et al., 2010). ITS-1 is a target sequence widely used as molecular marker in phylogenetic studies because it is a less conserved region and has significant differences between species (Teixeira et al., 2013).

For a given targeting gene, nested-PCR is more sensitive than conventional PCR (Liu et al., 2015). In a study conducted on children with cancer to identify *T. gondii* infections, the nested-PCR method showed the proper sensitivity and specificity for detecting toxoplasmosis (Fallahi et al., 2014). Finaly, the sequence of the PCR product should be checked to provide adequate diagnostic specificity.

1.2.5.5.2. Real-time PCR

The quantitative real-time PCR (qPCR) technique is highly sensitive, specific and reproducible for the quantitative detection of DNA and RNA (Contini et al., 2005; Romand et al., 2004). The method is more sensitive because it is optimized for maximum efficiency and is more specific because the most formats include specific annealing of at least three oligonucleotides (two primers and one probe). In addition, its reproducibility is related to the fact that the quantification data correspond to the initial exponential phase of the PCR, in which the variability is lower than in the readings of the final cycles. Another advantage is that real-time PCR detection and / or quantification eliminates post-amplification processing, such as electrophoresis, reducing manipulation with unnecessary steps (Contini et al., 2005; Romand et al., 2004). The reaction tubes are not opened from start to finish for further processing, reducing the risk of aerosol contamination of amplified products (Romand et al., 2004; Contini et al., 2005). The qPCR combines conventional PCR methodology with fluorescence DNA detection and quantification. The methodology allows the processes of amplification, detection and quantification of DNA to be performed in a single step, speeding up the results and giving greater precision.

The qPCR has been successfully used to detect *T. gondii* DNA in human blood, cerebrospinal fluid, aqueous humor, amniotic fluid and other samples (Gomez et al., 2019; Liu et al., 2015; Kasper et al., 2009; Kompalic-Cristo et al., 2007). qPCR is also used to evaluate toxoplasmosis progression and treatment efficacy, since it can estimate the intensity of *T. gondii* infection (Menotti et al., 2003).

The qPCR assay using the B1 gene is considered the best-performing technique for the diagnosis of congenital toxoplasmosis, compared to conventional PCR and nested-PCR

(Bastien et al., 2008; Teixeira et al., 2013). As a fast closed tube system, qPCR produces reproducible quantitative results and therefore is suitable for standardization (Bretagne & Costa, 2006; Liu et al., 2015).

Molecular diagnosis of toxoplasmosis is very useful in cases of low parasitemia, where it may be the method of choice, allowing the detection of the parasite and pointing to a more accurate interpretation of the stage of infection by *T. gondii*, indispensable for preventing the birth of a child with congenital toxoplasmosis. On the other hand, in countries where therapeutic abortion is allowed, unnecessary abortions could be avoided (James et al., 1996).

Many authors have evaluated PCR as a diagnostic technique for toxoplasmic encephalitis, pulmonary toxoplasmosis and even congenital toxoplasmosis, using different protocols (Cardona et al., 2011; Robert-Gangneux and Belaz, 2016). These exciting results suggest that fetal infections can be diagnosed early in pregnancy, through the analysis of amniotic fluid, without the need to obtain fetal blood samples or more invasive procedures. The PCR is particularly useful in AIDS patients, since their ability to generate IgM is limited, in addition to the difficulty encountered in interpreting serological studies. Quantitative PCR assays are essential for the diagnosis of toxoplasmosis and therapeutic monitoring (Pomares et al., 2020; Robert-Gangneux and Belaz; 2016).

1.2.6. Imaging techniques

Imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US), are not specific but may facilitate the diagnosis of toxoplasmosis and monitoring the therapeutic effect (Blaakaer, 1986; Liu et al., 2015, Virkola et al., 1997). Because immunocompromised patients often develop brain encephalitis and abscesses when infected with *T. gondii*, CT and MRI can be used to locate the lesions. CT is often used as the initial screening test, and MRI is best suited for the determination of extent of damage (Porter & Sande, 1992). For congenital toxoplasmosis, US are recommended for prenatal diagnosis (Abboud et al., 1995) and CT can detect diffuse hydrocephalus and cerebral calcifications of toxoplasmosis in infants (Collins, & Cromwell, 1980; Liu et al., 2015).

1.2.7. Treatment and follow-up

Before 18 weeks, pregnant women are treated with spiramycin until the diagnosis can be confirmed with ultrasound and PCR laboratory testing. Once the diagnosis is confirmed, patients are treated with a scheme of spiramycin, pyrimethamine, sulfadiazine and folinic acid. Infected children are treated for a total of 12 months with a regime of pyrimethamine,

sulfadiazine and folinic acid (Leeper & Lutzkanin, 2018). One month after treatment, the infant must be reexamined, including serology, head CT and detailed eye / ear / neurological exams (SYROCOT, 2007).

1.2.8. Prevention and control measures

The congenital toxoplasmosis (CT) prevention can be divided into three categories: primary, secondary and tertiary. Primary prevention is basically characterized by education and public health programs, in which pregnant women are advised to avoid contact with potentially contaminated materials (Aguirre et al., 2019). These guidelines when applied in prenatal care contribute to the 63% reduction of the infection in pregnancy (Jones & Dubey, 2010; Mandelbrot, 2012). Secondary prevention consists of trying to avoid transplacental transmission of the parasite by making an early diagnosis of acute infection in the pregnant woman and instituting antiparasitic treatment in order to prevent congenital infection and / or reduce the incidence of severe forms in the child. Tertiary prevention consists of making an early postnatal diagnosis by measuring specific IgA and IgM antibodies in the blood of newborns (NBs), allowing the introduction of a therapeutic regimen to prevent or minimize sequelae (Hill & Dubey, 2002).

Prevention programs are effective in reducing the incidence of toxoplasmosis. However, patients are unaware of the disease, sources of infection and preventive measures. Thus, health education actions are essential and should be directed to the population trying to reduce the prevalence of toxoplasmosis. Prevention and control measures must be directed to transmission routes (Aguirre et al., 2019; Hill & Dubey, 2002). One of the recommended measures is to avoid the consumption of raw or undercooked animal products, such as goats and cattle. However, it is noteworthy that cattle may not be potential sources of infection, as demonstrated by Dubey (Dubey, 2010). The identification of risk factors is crucial for the effective implementation of toxoplasmosis control measures, allowing the general population and people in special situations (susceptible pregnant women, immunocompromised patients) to be guided about primary prevention care (Pleyer et al., 2020; Tenter et al., 2000; Villard et al., 2016). In the prophylaxis related to the ingestion of cysts and tachyzoites, the recommendations are to eat meat well cooked (67º C for 10 min), consume well-cooked or salted sausages (2.5% salt for 48 hours), freeze the meat (- 18ºC for seven days); wash hands and preparation surfaces (boards and knives) after handling raw meat; do not try raw meat; boiling or pasteurizing goat's milk before consumption; perform serological monitoring and treatment of the pregnant woman to avoid transmission to the fetus (Aguirre et al., 2019).

For prevention the infection by ingestion of oocysts, one must: feed cats only with well-cooked food or meat; wash fruits and vegetables well under running water; cleaning cats' litter boxes daily; control flies and cockroaches or other species that can serve as mechanical vectors for *T. gondii*; ingest only treated or boiled water.

Given that in the world there is a wide variation in the types and quantities of meat consumed and the way in which they are prepared, each country must make its own risk assessment and, based on the conclusions, plan management actions for the efficient prevention of infection by *T. gondii* via meat (Aguirre et al., 2019). Another important point is the guidance for owners to verify the origin of cats before adoption or acquisition, thus seeking to prevent the infection of other animals, including man (Pleyer et al., 2020). Moreover, among the forms of parasite control are sanitary handling practices for farm animals, such as the control of rodents and felines on the premises and mainly health education (Hartmann et al., 2013; Perdoncini et al., 2010).

1.3. Rubella

Rubella is caused by a virus of the Togaviridae family, genus *Rubivirus*. It is a benign disease characterized by a macular rash accompanied by low fever, joint pain, pharyngitis and cervical adenopathies. The infection can in some cases be asymptomatic, although it can become severe when it occurs during pregnancy (Lambert et al., 2015). The teratogenic properties of the rubella virus were first discovered in Australia in 1941 by Gregg who associated the occurrence of rubella during pregnancy with the presence of congenital cataracts (Bukasa et al., 2018). The most common form of transmission is through direct contact with droplets with the respiratory secretions of infected people. In addition, transmission can also be congenital (Karacan et al., 2014; Yeshwondm et al., 2016).

The severity of fetal infection is related to the time of pregnancy in which maternal infection occurs, being more severe in the period of organogenesis (1st trimester of pregnancy), due to the high tropism of the virus to fetal tissues (Yeshwondm et al., 2016). Rubella infection in pregnant women can cause devastating results, such as spontaneous abortion, fetal death and birth defects (Winter et al., 2018). If the primary infection is contracted in the first three months, the likelihood of the appearance of “Congenital Rubella Syndrome (CRS)” is high (Karacan et al., 2014; Rasti et al., 2016). A NB with CRS may present major malformations (cataracts, congenital glaucoma, heart disease, deafness, microcephaly, retinopathy), minor type (purple, thrombocytopenia, jaundice splenomegaly in

the first 24 hours of life) or present with jaundice asymptomatic condition at birth, in which later clinical manifestations such as partial deafness or psychomotor delay may appear (Karacan et al., 2014).

CRS remains a public health problem in a significant number of countries. Therefore, global health experts encourage the use of rubella vaccination, with the main objective of preventing CRS (Martínez-Quintana et al., 2015; Winter et al., 2018). Rubella is a vaccine-preventable infection and is considered potentially eradicable. As a result of the vaccination program in many high-income countries and in some low- and middle-income countries, the estimated number of CRS cases has declined globally from around 119000 cases in 1996 to around 105000 cases in 2010 (Tamirat et al., 2017; Vynnycky et al., 2016). The scale vaccination program in the Americas and Europe has managed to dramatically reduce or eliminate both the virus and CRS. In contrast, the highest risk of CRS is found in countries where the rubella-containing vaccine (VCR) has not been introduced in the national immunization program or vaccination coverage is low (Tamirat et al., 2017). There is a growing recognition of the importance of going beyond national analyzes and considering heterogeneity within countries to assess public health interventions with VCR (Winter et al., 2018; Plotkin, 2014). High acceptance can disrupt endemic rubella transmission and prevent CRS cases, as demonstrated by the WHO (Su et al., 2018; Winter et al., 2018). In contrast, countries in the WHO regions of Southeast Asia and Africa, which have been the slowest to add VCR to their national vaccination programs, have the highest incidence of CRS, suffering 84% of the estimated 105.000 global incidence cases of CRS in 2010 (Vynnycky et al., 2016; Winter et al., 2018).

1.3.1. Epidemiology

Rubella occurs worldwide, with a seasonal distribution. The incidence of infection tends to peak in winter in cold or early spring countries and during spring in countries with temperate climates (Zanga et al., 2017). The prevalence estimated through the seropositivity of the population varies between countries according to their geographic characteristics and the outbreaks still occur among unvaccinated individuals (Zanga et al., 2017). In 1969, prior to the introduction of the rubella vaccine, widespread outbreaks generally occurred every 6 - 9 years in the United States of America (USA) and 3-5 years in Europe, mainly affecting children aged 5-9 years. Since the introduction of the vaccine, occurrences have become rare in countries with high rates of infection (Plotkin, 2014; Winter et al., 2018). A rubella pandemic started in Europe between 1962 and 1963 and in the USA between 1964 and 1965.

Among the Americans, there were about 12.5 million cases with 11.000 fetal deaths; about 20.000 newborns were born with malformations compatible with CRS; 11.000 with deafness; 3.500 with blindness; 1.800 with mental retardation and 2.100 died in the neonatal period (Winter et al., 2018). The costs of controlling this epidemic were very high (estimated at approximately two billion dollars), making it one of the biggest epidemics of the disease on record. As consequence of the epidemic, in 1966, rubella and CRS became notification diseases in the USA. In this way, it was possible to establish the registration of cases and the dynamic monitoring of rubella and CRS due to the strategies adopted. These records point to 1969 as the year with the highest number of cases: 57.686. The incidence of rubella until 1968 was 24.4 cases / 100.000 inhabitants, with children aged from 5 to 9 years being the most affected, with 101.3 cases / 100.000 inhabitants (Orenstein et al., 2018). Between 1969 and 1988 there was a 99% reduction in the incidence of rubella. This great decrease was a direct result of the strategies adopted for rubella control, which was accompanied by a reduction in the incidence of CRS. During the 1964 epidemic, this incidence was 16 cases / 100.000 live births, falling to 2.7 cases / 100.000 live births in 1969 (Grant et al., 2019; Wu et al., 2016). In the 1980s and early 1990s, rubella vaccination strategies in the Americas evolved rapidly. The leadership was taken by the English-speaking Caribbean countries, which included rubella vaccine in their efforts to eliminate measles. The Caribbean strategy was effective and included two main components:

a) mass vaccination in both sexes, aged up to 40 years, with the double viral or triple viral vaccine, to provide protection and decrease the risk of pregnant women becoming infected with the rubella virus;

b) the introduction of the rubella vaccine in the regular immunization schedule of children and young people (Su et al., 2018).

The successful implementation of the highly effective VCR (chickenpox, mumps and rubella) can best be exemplified by the experience in Finland, where the 2-dose scheme introduced since 1982 reduced all CRS diseases to 0.1 per 100.000 inhabitants in 1995 (Hui et al., 2017). A more recent example is Spain, where a 54% increase in VCR vaccine coverage from 2003 (one dose) to 2013 (two doses) resulted in 95.5% of mothers and 96.4% of their newborns having protective levels of anti-rubella IgG (Plans et al., 2015). In contrast, ironically, there are emerging reports of decreased rubella immunity and decreased incidence of protective antibody levels (≥ 10 IU / ml) among women born after the introduction of routine universal rubella immunization in European and Asian countries (Hui et al., 2017; O'Connor et al., 2018; Skidmore et al., 2014). In fact, national investigators in Ireland showed

that rubella seronegativity was 14.7% among pregnant women aged <25 years compared to 5.0% of older pregnant women (Hui et al., 2017; O'Connor et al., 2018). The above observations have led to the concern that the CRS vaccination program can only be effective in preventing childhood rubella (Hui et al., 2017; Skidmore et al., 2014). In the USA, after the vaccine was licensed in 1969, the incidence decreased by 99% until reaching 20 years of age and in Chile approximately by 100% (Andrus et al., 2016; Bouthry et al., 2014; Seppälä et al., 2019). In some African countries, rubella seropositivity in women of reproductive age is between 71-99%. Countries with higher incidence included Mozambique (95%) and South Africa (97-98%) (Zanga et al., 2017). In 2011, a measles surveillance campaign implemented in the Kinshasa, capital of Democratic Republic of Congo (DRC), showed that 24% of blood samples screened were positive for rubella IgM antibodies (Pukuta et al., 2016; Zanga et al., 2017). Although knowledge of the geographic distribution of rubella virus genotypes has grown substantially since 2003, the genotypes present in many countries and regions remain unknown (Cheng et al., 2013; Zanga et al., 2017). In 2011, WHO updated the guidelines on the preferred strategy for the introduction of VCR in national vaccination programs and recommended an initial vaccination campaign, aimed at children aged 9 months to 14 years. The Global Vaccine Action Plan 2011-2020 (GVAP), approved by the World Health Assembly in 2012, includes goals to eliminate rubella in at least five of the six WHO regions by 2020 (WHO, 2020; Zanga et al., 2017).

Global coverage with VCR increased from 21% in 2000 to 40% in 2012 and to 47% in 2016. In 2000, just over half (99. 51%) of countries had introduced VCR into their vaccination schedule; by the end of 2012, more than two thirds (132. 68%) of countries were using VCR. By 2014, at the time of the last world update, eight of the other countries introduced VCR, bringing the total number of countries using VCR to 140 (72%) (WHO, 2020; Zanga et al., 2017). VCR was introduced into the routine vaccination schedule in 152 (78%) countries, including 13 (28%) in the African Region, 16 (76%) in the Eastern Mediterranean Region, 8 (73%) in Southeast Asia and all 115 countries in the Region of the Americas, Europe Region and Western Pacific Region. In December 2016, 152 (78%) of the 194 countries introduced VCR into the national immunization calendar, representing an increase from 53 countries since 2000, including 20 countries that introduced VCR after 2012 (WHO, 2020). The rubella and CRS elimination targets were set by the European Region (target date: 2015) and Western Pacific Region (target date to be determined), while the Southeast Asia Region has a rubella and CRS control target (WHO, 2020; Zanga et al., 2017). Neither the African Region nor the Eastern Mediterranean Region has set regional goals or

targets for rubella. Since rubella cases are detected through measles surveillance and because the rubella vaccine is generally administered as a combined measles and rubella vaccine, the elimination activities for both diseases are programmatically linked, and the elimination activities for measles can be used to support rubella elimination (WHO, 2020). Rubella and CRS surveillance is necessary to assess disease burden prior to the introduction of VCR, and to monitor disease burden and epidemiology after their introduction. Also, the surveillance important to identify pregnant women infected with rubella virus that need follow-up to assess pregnancy outcomes and identifying, diagnosing and managing CRS in affected babies. Countries report information on immunization schedules, vaccination campaigns, and number of vaccine doses administered through routine immunization services and other WHO monitoring data in conjunction with the United Nations International Children's Emergency Fund (UNICEF) each year using the Joint Report Form (JRF) 〝 Joint Report Form 〞 (Zanga et al., 2017). Surveillance data, including the number of rubella and CRS cases, are also reported to WHO and UNICEF through the JRF using standard case definitions (WHO, 2020; Zanga et al., 2017).

Routine administration of VCR is recommended as a combined vaccine or simultaneously, at the same visit; this recommendation was implemented in 144 (95%) of the 152 countries that introduced the vaccine. Based on the vaccination programs of each country, the first dose of measles, mumps and rubella vaccine is scheduled for 8-11 months in 27 (18%) countries and at 12-18 months in 125 (83%) countries. VCR is supplied as a vaccine combined with a measles vaccine in 30 countries (20%) and combined with a measles and mumps vaccine (with or without chickenpox vaccine) in 122 countries (80%) (WHO, 2020; Zanga et al., 2017). The WHO recommends that all countries that have not yet introduced rubella vaccine should consider doing so using existing and well-established measles immunization programs. To date, three WHO regions have set goals to eliminate this preventable cause of birth defects. In 2015, the WHO Region of the Americas became the first in the world to be declared free of endemic rubella transmission (WHO, 2020; Zanga et al., 2017). The number of countries that use rubella vaccines in their national program continues to increase steadily. As of December 2016, 152 of the 194 countries had introduced rubella vaccines; however, national coverage ranges from 13% to 99%. Reported rubella cases decreased by 97%, from 670.894 cases in 102 countries in 2000 to 22.361 cases in 165 countries in 2016. CRS rates are highest in WHO regions of Africa and Southeast Asia where vaccination coverage is lowest (WHO, 2020; Zanga et al., 2017). In Angola, there are no known rubella seroprevalence studies in the population. In fact, in April 2012, the Measles

Initiative - now known as the Measles and Rubella Initiative - launched a Global Strategic Plan for Measles and Rubella, covering the period 2012-2020. The Plan includes global targets for 2015 and 2020 (WHO, 2020; Zanga et al., 2017).

1.3.1.1. Transmission

Rubella is transmitted mainly by direct contact with individuals infected by droplets of nasopharyngeal secretions. The upper respiratory tract and nasopharyngeal lymphoid tissue appear to be the first sites of virus replication, and the virus then spreads to regional lymph nodes. Indirect transmission, through contact with objects contaminated with nasopharyngeal secretions, blood and urine is uncommon (Lambert et al., 2015). Rubella is also transmitted via the transplacental route from the mother to the fetus. The child with congenital rubella can eliminate the virus through urine and nasopharyngeal secretions (Yamamoto et al., 2013; Yeshwondm et al., 2016). Rubella's incubation period varies from 12 to 23 days, lasting an average of 17 days. After exposure to the virus, usually occurs a maculopapular rash, first on the face and spreading to the rest of the body (Lambert et al., 2015; Thompson et al., 2017;). The greatest transmissibility is observed in the period between seven days before the appearance of the characteristic rash of the disease until the seventh day after its disappearance. Children with congenital rubella can eliminate the virus for more than 1 year, and transmission is greatest in the first months of life. Passive immunity is acquired by maternal antibodies and active immunity by natural infection or vaccination. Children of immune mothers generally remain protected by maternal antibodies for the first 6 to 9 months of life. Active immunity is long-lasting and is believed to remain lifelong (Thompson et al., 2017).

Transmission of the virus to the fetus occurs after transplacental passage of the virus during maternal viremia. This transmission is directly influenced by gestational age at the time of primary maternal infection. The rate of maternal-fetal transmission is 90% in the first 12 weeks of gestation, with a decline between 12 to 28 weeks of gestational age and increasing again at the end of the 3rd trimester of pregnancy, when it can reach up to 100% of foetuses. In cases of maternal infection (1 to 3% of individuals), replication of the virus in the pharynx persists for a short period and without demonstrable viremia, thus being the insignificant risk to the fetus (Thompson et al., 2017). The infected NB will become a reservoir of the virus, spreading the disease later: viral elimination can occur up to 18 or 24 months of age (80% transmissibility in the first month of life; 62% from the first to the fourth

month; 33% between the fifth and eighth month; 11% between nine and twelve months and 3% in the second year of life) (Thompson et al., 2017).

1.3.2. Clinical manifestations

Rubella is characterized by a diffuse maculopapular and punctiform rash, which begins on the face, scalp and neck, and subsequently spreads to the entire body. Low fever and the presence of retroauricular, cervical and occipital lymphadenopathy, which usually precede the rash (5 to 10 days) are signs that contribute to the differential diagnosis in relation to other rash diseases (Getahun et al., 2016; Lambert et al., 2015). In general, a prodromal period is not observed in children with rubella. Adolescents and adults may present prodromes with low fever, headache, generalized pain (arthralgia and myalgia), conjunctivitis, runny nose and cough. About 25% to 50% of rubella virus infections are subclinical. Viremia occurs for about 7 days before the rash appears which disappears when the humoral immune response begins to develop (Getahun et al., 2016; Grangeot-Keros et al., 2014). Rubella can cause complications, with transient joint involvement, such as arthritis and arthralgia, being the most frequent. Interestingly, these symptoms are more prevalent and severe in women infected with rubella virus than in men (Grangeot-Keros et al., 2014). More serious complications, including thrombocytopenic purpura and post-infectious encephalopathy or encephalomyelitis, are occasionally associated with rubella acquired in the postnatal period. A rare and generally fatal neurodegenerative disease, called progressive rubella panencephalitis, has also been reported as a late complication of rubella in childhood (George et al., 2019).

The main concern represented by rubella is its teratogenicity, with maternal infection in early pregnancy leading to CRS in children (George et al., 2019). There is more than an 80% risk of birth defects when viral infection is acquired in the first 12 weeks of pregnancy (1st trimester) (Boucoiran & Castillo, 2018). The time when the infection occurs during pregnancy can influence the outcome. The earlier in pregnancy the maternal infection occurs, the more severe the damage to the fetus. The risk of fetal infection and the severity of congenital abnormalities decreases after the first trimester; after 17 weeks of gestation, the risk of developing any defects is low (Boucoiran & Castillo, 2018). The effects of rubella infection in pregnancy are unpredictable, ranging from normal birth, spontaneous abortion, death shortly after birth or even birth with simple or combined abnormalities, such as damage to the central nervous system, leading to delayed physical growth and mental, microcephaly, encephalitis, hepatomegaly, cardiac malformations, pneumonia, eye and hearing defects (Yamamoto et al., 2013). Insulin-dependent diabetes mellitus commonly occurs as a late

sequel to CRS, and defects such as deafness may not be detected initially. CRS cases have been reported after maternal reinfection, although this appears to be a rare phenomenon (Grant et al., 2015). As with primary rubella infection, gestational age at the time of reinfection influences the likelihood of fetal abnormalities. No case of rubella reinfection that causes CRS has been reported after 12 weeks (Kaushik et al., 2018).

1.3.3. Diagnosis

Many diseases have a clinical presentation similar to rubella and up to 50% of rubella infections can be sub-clinical (Epidemiology & prevention of Vaccine, 2019). The diagnosis of rubella in general is usually done through the clinical picture. That is later confirmed by laboratory tests, such as the search for anti-rubella antibodies. Laboratory tests, serology and / or viral isolation and PCR, are essential for establishing the diagnosis (Bouthry et al., 2014; Kaushik et al., 2018). Rubella virus can be isolated from nasal fluid, blood, throat, urine and cerebrospinal fluid samples from patients with rubella and CRS. The virus can be isolated from the pharynx 1 week before and up to 2 weeks after the rash starts. Although virus isolation is a diagnosis of rubella infection, viral cultures are laborious and therefore are not done in many laboratories; they are generally not used for the routine diagnosis of rubella. However, viral isolation is an extremely valuable epidemiological tool and should be used in all suspected cases of rubella or CRS (Bouthry et al., 2014). Serology is the most common method of diagnosing rubella. Acute rubella infection can be confirmed by a significant increase in the antibody titre against rubella in serum samples or by the presence of serum anti-rubella IgM. The serum should be collected as soon as possible (within 7 to 10 days) after the onset of the disease and again 14 to 21 days (minimum of 7) days' later (Hübschen et al., 2017).

However, false positives can occur in IgM detection, depending mainly on the methodology used for the detection of immunoglobulins in serum (Hübschen et al., 2017). False positive IgM tests for rubella occurred in people with parvovirus infections, with a positive heterophile test for infectious mononucleosis or with positive rheumatoid factor. The serological tests available for laboratory confirmation of rubella infections vary between laboratories (Epidemiology & prevention of Vaccine, 2019). The ELISA is sensitive, widely available and relatively easy to perform, and can also be modified to measure IgM antibodies. (Epidemiology & prevention of Vaccine, 2019). During prenatal care, it is recommended to request maternal serology for rubella in the first consultation, with the aim of knowing the immune status of the pregnant woman (Hübschen et al., 2017).

1.3.3.1. Differential diagnosis

The differential diagnosis must be made for other febrile exanthematic diseases such as measles, scarlet fever, dengue, sudden rash (children up to 2 years old), infectious erythema, enteroviruses (coxsackie and echo) and also for other diseases that can cause congenital syndromes, such as infectious mononucleosis, toxoplasmosis and cytomegalovirus infection (Lambert et al., 2015).

1.3.3.2. Diagnosis of maternal and fetal infection

In pregnant women, laboratory diagnosis is made by isolating the virus or by serological methods for detecting specific antibodies, and it is necessary to ensure the collection of the blood sample at the first visit. The most used test is the ELISA for the detection of specific IgM and IgG antibodies and / or for the identification of the virus from the secretion of the nasopharynx and urine, until the fifth day, preferably, on the third day of the onset of the rash (Epidemiology & prevention of Vaccine, 2019; Lambert et al., 2015).

In case of contact of the pregnant woman with a suspected rubella patient, blood collection should be done within a short period of time after contact. Samples collected after 28 days, although considered late, should also be sent to the laboratory for IgM research (Bukasa et al., 2018). The rubella virus-specific IgM antibodies appear soon after the onset of the disease. The presence of positive IgM means that there has been a recent infection, but they are generally not detected after 4 to 6 weeks of the rash onset. IgG antibodies usually persist for life. It is important to remember that non-reactive results for IgM do not rule out the possible recent rubella virus infection (Bukasa et al., 2018; Yeshwondm et al., 2016). The prenatal diagnosis aims to clarify with greater accuracy whether or not there was fetal involvement. Thus, the decision for an abortion can be guided more appropriately, based on scientific data and not just on probability. According to the Rubella Control Plan Standard, the diagnosis of pregnant women in prenatal care is indicated when (Bukasa et al., 2018; Yeshwondm et al., 2016):

- Seroconversion is confirmed during the prenatal period, up to the 16th week;
- There is confirmation of the late maternal diagnosis of rubella or contact with the infected patient;
- There is doubt in the maternal diagnosis, but in a period of significant risk for the fetus;
- Reinfection is suspected or confirmed.

The diagnosis of fetal infection can be made through cordocentesis performed after the 22nd week of pregnancy. Before that the immaturity of the fetal immune system combined with the immunological depression caused by the virus can generate false-negative results. The intrauterine diagnosis is based on the finding of changes in fetal blood: positive IgM for rubella; isolation of the virus; or identification of viral Ribonucleic acid (RNA). Some nonspecific signs of the infection may also be present in fetal blood: anemia, thrombocytopenia, elevated Gamma Glutamyl Transferase, Lactic Dehydrogenase and Interferon.

Due to the fact that the vaccine consists of live viruses, there is concern about the theoretical possibility of CRS after inadvertent administration during pregnancy (Bukasa et al., 2018; Yeshwondm et al., 2016). Thus, it is recommended that women who received the vaccine avoid conception for a period of up to one month after the vaccine dose. Although several studies have demonstrated the safety of the vaccine, this procedure would avoid doubts in the diagnosis of any problem that could occur with the NB (Yeshwondm et al., 2016). Women who are pregnant or become pregnant soon (up to 30 days) inadvertently get the vaccine must be carefully monitored by a doctor (Bukasa et al., 2018). As for postnatal diagnosis, the fetus is capable of producing specific antibodies, IgM and IgG for rubella, even before birth. The presence of specific IgM in the NBs blood shows congenital infection, since this immunoglobulin does not cross the placental barrier. In 100% of children with CRS, IgM antibodies can be detected up to the 5th month and in about 60%, between 6 and 12 months, and are rarely detected after the 18th month (Bukasa et al., 2018). Laboratory diagnosis of suspected CRS cases is made by taking a blood sample for investigation shortly after birth, in cases where maternal infection is suspected or confirmed during pregnancy, or at the time of suspected diagnosis in children less than one year of age (Yeshwondm et al., 2016). Serology detects IgM levels in the NB or by monitoring IgG levels for a prolonged period of up to 2 years. Stable or elevated levels of IgG confirm the diagnosis while their drop suggests the presence of maternal IgG (Bukasa et al., 2018).

1.3.3.3. Real-Time PCR

Real-time PCR (RT-PCR) technology is an evolution of the PCR method. Its principle is based on the duplication of DNA strands *in vitro* that can be repeated several times, generating enough DNA to perform several analyzes. With just a single fragment of DNA it is possible to reproduce millions of copies (Namuwulya et al., 2014). RT-PCR tests are much

more sensitive, specific and quick, especially when compared to conventional tests, taking 2 to 3 hours to output the result. They are widely used in the diagnosis of infectious diseases, in which the culture of the causative agents can be very difficult or even impossible (Zheng et al., 2013). The progress in the diagnostic techniques of rubella, has allowed, in addition to serology, the detection of viral RNA in clinical samples by the PCR method, enabling rapid and highly sensitive detection. RT-PCR is currently the most widely used laboratory technique to confirm acute rubella infection (Rota et al., 2011). Suitable samples include throat / nose secretions, nasopharyngeal aspirates, urine or peripheral blood mononuclear cells (Hubschen et al., 2008; Takao et al., 2012).

The diagnosis of congenital rubella infection is mainly based on the detection of rubella virus in Amniotic Fluid (AF) by RT-PCR (Bosma et al., 1995; Macé et al., 2004) (or detection of rubella virus-specific IgM antibody in fetal blood (Daffos et al., 1984; Morgan-Capner et al., 1985). The fast and accurate identification of the rubella virus is very important in pregnant women as it helps to guide prenatal treatment and identify the need for long-term follow-up. In addition, in many countries, maternally rubella clinically confirmed in the first 8 weeks of pregnancy is considered an indication for therapeutic abortion due to the high incidence of birth defects (Curti et al., 2014; Hübschen et al., 2017). The RT-PCR require little manipulation, thus reducing the risk of contamination, and generally reach small fragments, thus allowing detection even in case of partial RNA degradation (Hubschen et al., 2008; Mosquera et al., 2002). The high sensitivity of this technique allows excellent results, but it is also subject to the presence of inhibitors and contamination that can affect its efficiency (Hübschen et al., 2017).

1.3.4. Treatment

Currently, there is no specific treatment for rubella. The signs and symptoms presented must be treated according to the symptomatology and appropriate therapy (Hui et al., 2017). The most important thing, however, will be to promote control and minimize possible sequelae in case of teratogenic effects, with interdisciplinary monitoring therapy (Hui et al., 2017).

1.3.5. Prevention and control

Rubella is a vaccine-preventable infection and considered potentially eradicable (Winter et al., 2018). CRS remains a public health problem in a significant number of countries. Therefore, global health experts encourage the use of rubella vaccination, with the

main objective of preventing CRS (Martínez-Quintana et al., 2015; Winter et al., 2018). Promoting health education in the population, clarifying the population about the importance of vaccinating children and women, is the main control measure to decrease the circulation of the rubella virus, in order to prevent CRS (Hui et al., 2017). Immunity is acquired by natural infection or by vaccination, being durable after natural infection and remaining for almost a lifetime after vaccination. The vaccine is composed of attenuated viruses, grown in rabbit kidney cells or in human diploid cells. It can be produced in monovalent form, associated with measles (viral double) or with measles and mumps (triple viral). The vaccine is presented in lyophilized form, and must be reconstituted for use. After its reconstitution, it must be kept at a positive temperature of 2 ° to 8 ° C, at the local and regional levels. At the central level, the recommended temperature is minus 20 ° C. It must be kept protected from light, so as not to lose activity (Hui et al., 2017). The vaccine is used in a single dose of 0.5 mL subcutaneously Children of immune mothers generally remain protected by maternal antibodies around six to nine months after birth (Hui et al., 2017; Tamirat et al., 2017).

The triple viral vaccine is recommended for all children (two doses), adolescents and adults (men and women), especially women who have not had contact with the disease. Pregnant women should not be vaccinated and vaccinated women should avoid pregnancy until the month following vaccination due to the risk of contamination of the fetus (even if the virus is weakened it can cross the placenta (Epidemiology & prevention of Vaccine, 2019). All infected people should avoid public places (such as schools, work and busy streets) during the disease period. The vaccine is highly effective and is unlikely to generate side effects. Non-immunized adults and adolescents can also get the vaccine (Epidemiology & prevention of Vaccine, 2019). The scale vaccination program in the Americas and Europe has managed to dramatically reduce or eliminate both the virus and CRS. In contrast, the greatest risk of CRS is found in countries where VCR has not been introduced in the national immunization program or the vaccination coverage is low (Tamirat et al., 2017). Despite the high prevalence of rubella in Africa, the infection had never been studied in Angola. Thus, a study was carried out between May 2016 and August 2017, regarding the assessment of rubella seroprevalence in 396 pregnant women in the province of Luanda.

The results showed that there are a high number of women (87.6%) with previous exposure to rubella (Vueba & Sousa, 2018a). In Angola, the rubella vaccine was included, as of the year 2017, in the National Vaccination Calendar, a decision by the Ministry of Health (MINSA) that aims to prevent the increasing birth of children with malformation or with congenital rubella. This decision by MINSA is based on the strategy for the combined

immunization of the vaccine against rubella and measles, because the symptoms of rubella are confused with those of measles and with the naked eye it is not possible to make the distinction correctly, being only possible with laboratory tests, to be distinguished accurately. However, despite the lack of official data on cases of congenital rubella, it is estimated that the situation is not yet "as alarming" according to MINSA of Angola (UNICEF, 2018). It is important to emphasize that the introduction of vaccines in any country depends a lot on the epidemiological situation, on the existence of qualified human resources, on sustainable financing and on cold chain conditions to ensure the conservation and quality of the vaccine to be introduced (MINSA, 2017; UNICEF, 2018).

Despite the progress made on rubella elimination and the significant gains made, rubella elimination is not close to achieving the ambitious targets set in the WHO GVAP 2011-2020. The VCR vaccine is highly effective and safe. Significant health policies and public health campaigns in the Americas have shown that rubella virus transmission can be stopped and the disease eliminated. Additional efforts should be made to ensure that people have the opportunity to be fully immunized according to their countries' immunization schedule. In addition, it would be beneficial to have a non-living viral vaccine that could be used in all patients and populations. On the other hand, further advances are needed in diagnostic tests of high sensitivity and low cost.

It is essential to encourage adherence by countries that do not yet comply with the WHO GVAP, demonstrating the need for global cooperation in creating a rubella-free world. The strategies included in the GVAP are sound, although their full implementation is often limited by the lack of global and national political will reflected in the lack of resources. Reorienting the rubella elimination program in these countries is essential to increase the emphasis on surveillance, so that programmatic and strategic decisions can be guided by the data.

1.4. Human Cytomegalovirus (HCMV)

Human Cytomegalovirus (HCMV), a virus of the Herpesviridae family, has a worldwide distribution and infects humans of all ages and all socioeconomic groups without seasonal or epidemic transmission patterns. HCMV is considered an opportunistic pathogen and can cause serious problems in immunocompromised patients. In addition, it is also seen as an important pathogen, particularly when manifested during pregnancy (Marsico &

Kimberlin, 2017). Mother-to-child transmission of HCMV may occur during pregnancy by via transplacental or during passage through the birth canal by contact with cervical secretions (Yamamoto et al., 1999). Congenital HCMV transmission is usually the result of a primary infection of a seronegative pregnant woman, but a reactivation of a latent infection of a seropositive pregnant woman or reinfection with a new viral strain can also occur, although these cases tend to be rarer (Codaccioni et al., 2019; Hamilton et al., 2014).

Consequences or sequel of congenital HCMV infection include congenital malformations, psychomotor retardation, deafness, foetal death, infant death, and neurological and sensory impairment (Marsico & Kimberlin, 2017). Frequency of vertical transmission and severity of infection are more common in primary maternal infection (Fowler et al., 2017). However, non-primary infection is more common than primary infections and therefore probably contributes to more total cases of congenital HCMV infection and related disability (De Vries et al., 2013; Lanzieri et al., 2014).

Through the expansion of transplantation techniques, HCMV has become an important agent of severe disease associated with significant morbidity and mortality in immunosuppressed patients (Hughes et al., 2016; Selvey et al., 2017). HCMV is also one of the most common opportunistic agents in patients with advanced Acquired HIV infection. The development of HCMV disease in this population occurs concomitantly with an increase in the immunosuppression stage, shown by a decrease in CD4+ T cell count and the presence of HCMV viremia (Grønborg et al., 2017).

The worldwide prevalence of HCMV infection is estimated to range from 40% to 100% in the general population, being directly proportional to age and inversely proportional to the socioeconomic conditions of the population (Griffiths et al., 2015; Gentile et al., 2014). The seropositivity rate in women of reproductive age is estimated to be 40-83% (Johnson & Anderson, 2014; Lanzieri et al., 2014) and the prevalence of congenital HCMV infection has ranged from approximately 0.2% to 2%, with higher overall rates in countries with higher maternal seroprevalence (Lanzieri et al., 2014; Manicklal et al., 2013).

HCMV seroprevalence in developing countries is generally over 90% in adolescence and above 95% in early old age. Consequently, most cases of congenital HCMV infection seems to result from non-primary maternal infection. Moreover, in developing countries the reported prevalence of congenital HCMV infection varies substantially within and between countries, with some reported prevalence's higher as 6 - 14% (Lanzieri et al., 2014; Sharghi et al., 2019;).

Despite the high prevalence of CMV in Africa, the infection has been poorly studied in Angola. We conducted the first CMV seroprevalence study, between May 2016 and August 2017 in 396 pregnant women in Luanda province and the results showed a high number of women (96.5%) seropositive to CMV (Vueba & Sousa, 2018).

Laboratory diagnosis of HCMV infection in immunocompetent is not relevant, whereas in immunocompromised and pregnant women it is of the utmost importance. Laboratory diagnosis during pregnancy has been the subject of several studies aimed at reducing the impact of congenital HCMV infection on public health (Lanzieri et al., 2014; Naing et al., 2016). Laboratory confirmation of maternal HCMV infection during pregnancy entails verifying the possibility of foetal impairment through serology-based techniques (IgM and IgG), the antigenemia (pp65), viral particle detection (PCR-DNA) and histological examination (Naing et al., 2016).

In the absence of an approved HCMV vaccine and effective treatment in the prenatal and postnatal period, the main way to prevent congenital HCMV infection is to prevent transmission of the virus from mother to foetus (Thackeray et al., 2014). In the case of immunocompromised patients, the recognition of the clinical importance of the virus together with the advancement of diagnostic modalities and appropriate therapy can drastically reduce the frequency of complications and the risk of life of these patients, improving their quality of life.

This review focus on HCMV infection highlights the clinical importance of congenital infection and the infection in immunocompromised patients, as well as the progress made in laboratory diagnosis and the overall efforts still needed to achieve an effective vaccine against HCMV infection.

1.4.1. Pathophysiology and epidemiology in HCMV infection

1.4.1.1. Maternal infection

Maternal HCMV infection manifests itself in the same way as in immunocompetent individuals; it is usually asymptomatic. Some studies show that <5% of pregnant women with primary HCMV infection present a mononucleosis-like syndrome with fever, pharyngitis, cervical adenopathy, fatigue, myalgia, headache, hepatosplenomegaly and rash (Johnson & Anderson, 2014; Naing et al., 2016).

The incubation time for HCMV infection is poorly known, but it is estimated to be within 3 to 12 weeks from the moment of virus contamination to the onset of the first signs and symptoms. It should be noted that these are not specific for an HCMV infection (Torii et

al., 2019). The most frequent are fever (present in 42.1% of primary infections and 17.1% of recurrent), asthenia (between 31.4 and 11.4%), myalgias (21.5 and 6.7%), rhino pharyngo-tracheo-bronchitis (42.1 and 29.5%) and pseudogroup syndrome, defined by the coexistence of fever and at least one of the previously mentioned signs (24.5 and 9.5%), lymphocytosis higher or equal to 40% (39.2 and 5.7%) and increased transaminases (35.3 and 3.9%) (Marsico & Kimberlin, 2017; Vide Tavares et al., 2011).

1.4.1.2. Congenital infection

Congenital HCMV infection can be classified according to the presence or absence of symptoms at birth and whether or not these symptoms involve the CNS or sensory organs (Harrison, 2015; Khalil et al., 2017).

Infants with congenital HCMV infection and symptomatic at birth account for 10-25% of all infants infected with this virus and approximately 5-15% die within the first 6 weeks of life, and 40-60% develops long-term sequel (Harrison, 2015; Jückstock et al., 2015; Khalil et al., 2017).

Classic symptoms and complications that can be found in up to 75% of symptomatic new-borns include jaundice, petechial, neurological changes such as microcephaly and intracranial calcifications, ocular complications (blindness), hepatosplenomegaly with varying degrees of severity and deafness (Khalil et al., 2017). In the severe forms, there may occur foetus death, premature birth, presenting signs of fulminant disease like lethargy, convulsions, massive hepatosplenomegaly, thrombocytopenic puerperal and hyperbilirubinemia (Jückstock et al., 2015; Khalil et al., 2017).

Congenital HCMV infection represents a serious public health problem as it causes more problems in foetal development and long-term sequel than Down syndrome, neural tube defects or foetal alcohol syndrome (Khalil et al., 2017; Mestas, 2016; Muldoon et al., 2017). Screening programs for both pregnant women and newborns have not yet been developed or implemented in most countries. One of the biggest complications for its implementation of a neonatal screening program has been the lack of a suitable screening test for high throughput analyses (Marsico & Kimberlin, 2017).

1.4.1.3. Infection in Immunocompromised individuals

With the development of transplantation and chemotherapy techniques, as well as the introduction of new immunosuppressive drugs for cancer treatment, HCMV has become an important causal agent of severe disease in immunodepressed patients (Grønborg et al., 2017).

HCMV has the ability to remain latent in the host cell after an acute infection (Santos et al., 2017). An imbalance between the immune system and the latent virus, caused for example by immunosuppressive therapy, may result in viral reactivation and clinically manifest disease (Santos et al., 2017).

HCMV infection leads to immune dysfunction and is associated with risk of organ rejection in transplantation. Moreover, the therapy used in organ rejection cases increases the risk of HCMV disease exponentially, which explains why post-transplant prophylaxis schemes or preventive therapy are used (Requião-Moura et al., 2015; Santos et al., 2017).

HCMV disease is a highly prevalent complication among kidney transplant patients, although there is wide variation between studies, ranging from 5.8% to 100% (Requião-Moura et al., 2015). There are several reasons for this variation, but the characteristics of the population studied, types of immunosuppression and diagnostic test used are among the main ones. Reports show that in patients admitted to hospitals, the prevalence ranged from 13.3% to 39.2%, while a study conducted in outpatients showed a prevalence of 5.8% (Santos et al., 2017). Risk factors related to HCMV disease after kidney transplantation are mainly the immunosuppression type and the serological status of the donor and recipient against HCMV; the highest risk is characterized with the combination of positive donor with negative recipient (Davi-Neto et al., 2014; Díaz et al., 2014).

HCMV is also one of the most common opportunistic agents in patients with advanced HIV infection. The development of HCMV disease in this population occurs concomitantly with an increase in the immunosuppression stage, manifested by a decrease in CD4+ T cell count and the presence of HCMV viremia (Manicklal et al., 2013). HIV-infected women are usually positive for HCMV and have more frequent HCMV relapses with progressive immune impairment (Bialas et al., 2016; Manicklal et al., 2013). Studies in Europe and America related an increased risk of congenital CMV infection in neonates born to mothers co-infected with HIV-CMV (Manicklal et al., 2013). In addition, HCMV can act as a cofactor for HIV disease progression. The risk of infant mortality increases in infants co-infected with HIV-CMV, and there is accelerated progression of CNS disease in survivors, especially delayed development and worsening motor deficits (Ellington et al., 2016; Manicklal et al., 2013).

The high prevalence of HCMV worldwide makes it difficult to interpret tests that detect HCMV in clinical materials. This is also due to the fact that the virus remains latent, and it is not always possible to implicate the virus detected as the etiological agent of the present disease. Thus, it is important to develop diagnostic techniques to detected

cytomegalovirus disease or the presence of active HCMV infection with viral replication, which is a risk factor for the development of clinical manifestation (Fowler et al., 2017; Manicklal et al., 2013).

1.4.2. Diagnosis of HCMV Infection

Laboratory diagnosis of HCMV infection can be done by several methods, which stand out in clinical practice: serology (IgM and IgG), pp65 antigenemia, PCR-DNA, histological examination, and viral isolation in cell cultures. These tests are very important when it comes to pregnant women or immunocompromised patients, including transplanted ones (Kuessel et al., 2015; Lino et al., 2018).

1.4.2.1. Maternal infection

The diagnosis of maternal HCMV infection is based on serology to detect immunoglobulin IgM and IgG anti-HCMV. The ELISA method is the most widely used, with sensitivity and specificity very close to 100% (Davis et al., 2017; Rajasekariah et al., 2013). Identification of IgG seroconversion confirms primary HCMV infection. However, in the absence of previous serology, the detection of seroconversion is not possible. Therefore, the IgM is used whose presence indicates a potential recent infection (Hughes et al., 2016; Ozgu-erdinc & Sen, 2014). The IgM antibody response begins in the initial days after maternal infection and peaks during the first month before gradually disappearing. IgM can be detected in the first three months after the onset of infection although it may also persist longer (Kuessel et al., 2015; Neu et al., 2015).

To define the occurrence of a primary infection during pregnancy or the risk of intrauterine transmission, other support techniques are used, namely the IgG avidity test (Leruez-Ville et al, 2013). This test classifies recent infections as infections that happened more or less than 3 months prior. The avidity serves to measure of the antigen-antibody binding strength. The weak avidity reveals the few functional affinity of recently produced IgG (Lazzarotto et al., 2014). Thus, at the onset of a primary infection, antibodies show poor avidity for the antigen, but over time this avidity increases. So, infections less than three months old can be distinguished from older infections (Kuessel et al., 2015; Lazzarotto et al., 2014).

The study of these parameters, their characteristics and dynamics, are useful for distinguishing between primary and secondary infection, as well as, for determining the approximate date of maternal infection (Neu et al., 2015).

1.4.2.2. Congenital infection

For all the intrinsic characteristics to foetal life, it is impossible to diagnose an infection only by the symptoms. However, there may be some albeit unspecific signs that may lead one to suspect that an infection exists (Leruez-Ville et al., 2016; Vide Tavares et al., 2011). The best available method for assessing the foetus and its well-being is obstetric ultrasound, being a screening tool for foetal CMV infection (Mestas, 2016). The pathophysiology of this infection allows for predicting the ultrasound images that are classically associated with the lesions (Kuessel et al., 2015; Lazzarotto et al., 2014; Mestas, 2016). However, even in a high-risk population, the predictive value of ultrasound is far from ideal, as a significant number of infected foetuses have no echographic particularities and certain ultrasound anomalies may be transient and have already disappeared at the time that are performed (Muldoon et al., 2017).

The first infected organ is the placenta, where the virus replicates. The placenta acts both as a barrier against HCMV, and as a reservoir that can later release the virus into the foetal circulation (Muldoon et al., 2017). In addition to the placenta, HCMV presents kidney-specific tropism and provokes dispersed calcifications on the liver and lungs (Kuessel et al., 2015; Lazzarotto et al., 2014; Mestas, 2016). Another important fact is the increase in alpha fetoprotein (AFP) and chorionic gonadotropic hormone in the maternal blood.

It is reported that the type of brain injury differs according to the gestational age at which maternal HCMV infection occurred. Magnetic Resonance Imaging (MRI) of the foetal brain is today widely used in multiple indications in prenatal diagnosis (Kuessel et al., 2015; Mestas, 2016). MRI confirms the absence or presence of foetal brain anomaly with excellent predictive value. Today the systematic use of ultrasound (abdominal and endovaginal) and MRI to confirm the presence or absence of such lesions as microcephaly and intracranial calcifications is legitimate (Hughes et al, 2016; Kuessel et al., 2015; Leeper & Lutzkanin, 2018). However, the definitive diagnosis of foetal infection is obtained by culturing the virus or, more often, by amplifying its genome in AF obtained by amniocentesis (Hughes et al., 2016; Kuessel et al., 2015). HCMV colonizes AF through foetal kidney infection following virus replication in the tubular epithelium, followed by excretion in the foetal urine. Following the mother's seroconversion or reactivation, the process leading to urinary excretion of the virus lasts an average of six to eight weeks. For amniocentesis, this break must be respected in order to limit the rate of false negatives. It should be done after 22 weeks of pregnancy so that foetal diuresis is healthy established (Enders et al., 2017; Kuessel et al., 2015). The sensitivity of this technique varies between 50 and 100% and the specificity

between 67 and 100%. On the other hand, viral culture has sensitivity between 50 and 80% for a specificity of 98 to 100%, but the long time required to obtain the result (± 14 days) is discouraging (Enders et al., 2017; Kuessel et al., 2015).

PCR sensitivity varies according to the studies between 40 and 90% and is explained by inappropriate AF harvesting conditions (less than six weeks after seroconversion or before 22 weeks' gestation), but also by the different PCR techniques used (Enders et al., 2017; Kuessel et al., 2015). Detection of the virus or its genome in newborn urine is the reference technique (Cardoso et al., 2015; Emery & Lazzarotto, 2017; Kuessel et al., 2015). When HCMV is positive in AF but negative at birth, we are facing a false positive prenatal diagnosis or one by contamination of AF by maternal blood during amniocentesis if the mother has a positive viraemia at that time (Enders et al., 2017). Another explanation may be the contamination of the AF collected during the laboratory procedure (Khalil et al., 2017). Currently, the automation of DNA extraction before RT-PCR decreases this risk of contamination to a minimum because many hands-on steps are suppressed in DNA automatic extraction (Emery & Lazzarotto, 2017; Kuessel et al., 2015).

Scarce data are available in the literature regarding prenatal diagnosis of HCMV infection from foetal blood. Foetal blood collection by cordocentesis is not recommended for diagnostic purposes, because it is technically more difficult to perform as compared to amniocentesis, it requires an experienced operator and carries a higher risk of foetal loss (between 1 and 3%) (Coll et al, 2009; Enders et al., 2017; Kuessel et al., 2015; Vide Tavares et al., 2011). Therefore, its use should be justified by an additional interest in relation to amniocentesis (Emery & Lazzarotto, 2017). At the diagnostic level, the sensitivity of IgM detection ranges from 50-80% and for the rapid culture viral detection is 16% (Vide Tavares et al., 2011).

1.4.2.3. Infection in Immunocompromised individuals

Because of the importance of differentiating a primary infection from a HCMV reinfection or reactivation in transplant patients, serologic IgG or IgM measurement are not useful in practice because they hardly differentiate primary or late infection (Dioverti and Razonable, 2016). Direct detection of the virus by classical techniques in urine or saliva is a procedure with little clinical value. Moreover, it is technically difficult, expensive and provides results only after 3 to 5 weeks (Paya et al, 1987; Ross et al., 2011). Therefore, the use of rapid, sensitive and specific diagnostic techniques is recommended. Among the techniques, the direct search for HCMV antigens in circulating neutrophils (antigenemia) and

PCR are indicated (Lino et al., 2018; Santos et al., 2017). PCR is an excellent method that can be used to include or exclude diagnosis in immunosuppressed patients with suspected HCMV infection.

High-risk patients, such as bone marrow transplant recipients, HCMV seronegative patients who have received organs from HCMV-positive donors, and patients who have received immunosuppressive therapy, among others, will have greater benefits if they can be monitored by molecular techniques or if this is not possible by antigenemia, taking care to be marked by low cut off levels (Ellington et al., 2016).

1.4.3. Diagnostic tests

The tests currently available in clinical practice to monitor active CMV infection are the detection of antibodies anti-CMV, antigenemia, which detects the presence of phosphoprotein pp65 in peripheral blood leukocytes, and the detection of viral DNA by molecular methods, such as PCR (de Keyzer et al., 2011; Emery et al., 2000).

1.4.3.1. Serology

Detection of anti-CMV antibodies is usually performed by immunoenzymatic assays such as ELISA, ELFA or by chemiluminescence (Lazzarotto et al., 2008).

1.4.3.2. Viral isolation in cell cultures

The virus can be isolated from saliva, urine, liver, adenoids, kidneys and peripheral blood leukocytes. In the case of a NB, cord blood is used (Zeytinoğlu et al., 2019). The standard method for HCMV detection is the inoculation of clinical material into human lung fibroblast cultures followed by observation of the Characteristic Cytopathic Effect (CCE). This technique is quite slow and may take up to two weeks for the onset of CCE (Ng et al., 2014). Therefore, rapid isolation in cell culture, based on stimulation of monolayer infection by low speed centrifugation, and detection of antigens by monoclonal antibodies (Shell vial) was developed (Fowler et al., 2017). The method allows virus detection within 24 to 48 hours but loses sensitivity when compared to traditional isolation. However, viral isolation is less sensitive than modern methods.

1.4.3.3. Antigenemia

Detection of HCMV matrix antigen (pp65) phagocytized by peripheral blood neutrophils (antigenemia) was the first laboratory method routinely used to provide a

quantitative parameter because determine the proportion of pp65 antigen in the nucleus of infected neutrophils. This technique requires the processing of fresh blood and when performed by immunofluorescence produces results dependent on subjective interpretation (Lino et al., 2018; Hughes et al., 2016).

1.4.3.4. Histology

The histological feature of HCMV infection is the cytomegalic cell, which is an enlarged cell containing a dense corpus of basophilic and central intranuclear inclusion in the "owl's eye". These cells can be found in any body tissue and also in the urinary sediment (Levin et al., 2017; Zhou et al., 2016).

1.4.3.5. Molecular methods

In recent years, a variety of molecular methods for HCMV detection have been developed for qualitative and quantitative analysis, such as PCR, a specific nucleic acid-based amplification reaction, having greater sensitivity than viral isolation in cell culture and antigenemia (Griffiths, 2017).

The PCR is a rapid, sensitive and specific method, which allows early diagnosis, avoiding lengthy and invasive investigations, which cause great anxiety to both the patient and physician. The PCR could give qualitative or quantitative results, providing greater flexibility with the materials under test, allowing for storage at -20°C until processing, and the possibility to repeat the tests when doubts arise as to the results (Griffiths, 2017; Morozov et al., 2016). However, PCR is an expensive method and not available in routine laboratories, especially in developing countries.

Solano et al. (2001) and Storch et al. (1994) compared PCR and antigenemia techniques, and observed that the diagnosis by PCR technique is positive 1 week in advance regarding antigenemia. This great PCR sensitivity levels in relation to antigenemia has also been observed in other works (Reina et al., 2014; Storch et al., 1994; Vries et al., 2012). These data reinforce the possibility that, depending on the period in which these tests are performed, a discrepancy of results between the techniques can be found. Furthermore, other studies state that viremia occurs before the onset of symptoms and correlates with the later development of the disease and its severity (Eguchi et al., 2017). In conclusion, antigenemia is less sensitive than molecular methods, but the results offer a good correlation with the clinical manifestations; molecular techniques are more sensitive, but the results can

sometimes be dissociated from the clinic. So, PCR can be used for HCMV diagnosis and the antigenemia in monitoring therapeutic efficacy (Eguchi et al., 2017).

1.4.4. Prevention of HCMV infection

In the absence of an approved HCMV vaccine and effective treatment both in the prenatal and postnatal period, the main way to prevent congenital HCMV infection is to prevent transmission of the virus from mother to foetus (Thackeray et al, 2014). Prevention is essential not only in seronegative but also in HCMV positive pregnant women as reinfection may occur.

Pregnant women should be alert to measures that reduce the risk of acquiring an HCMV infection during pregnancy (CDC, 2019; Thackeray et al., 2017), such as strengthening personal hygiene habits and not sharing food, drink or utensils with children. Several studies give relevance to the education and training of women of childbearing age, pregnant and future pregnant women regarding HCMV infection and its implications. For example, recent studies have shown that training for pregnant women about personal hygiene measures during pregnancy has resulted in decreased HCMV seroconversion rate (Naing et al., 2016).

1.4.4.1. Passive immunization

Passive immunization is the administration of antibodies to a nonimmune individual for the purpose of conferring immediate immunity against an infectious agent. It is usually indicated in situations where active immunization is unavailable, contraindicated, or not administered prior to agent exposure (Jückstock et al., 2015).

In passive immunization the antibodies can be naturally acquired by passing maternal IgG to the foetus via placental and breast milk, or by administering hyperimmune immunoglobulins (IgH) (Jückstock et al., 2015). Immunity acquired following natural HCMV infection prior to pregnancy does not confer complete protection to the foetus against congenital infection. Thus, the administration of IgH in women with primary infection during pregnancy for the prevention of congenital HCMV infection has been the subject of several studies (Jückstock et al., 2015; Minsart et al., 2018; Nigro, 2017). The hyperimmune globulin therapy significantly increases anti-CMV IgG concentration and decreases the number of natural killer cells and Human Leukocyte Antigen – DR isotype (HLA-DR+) cells. Moreover, the passive immunization with IgH was associated with a significantly lower risk of congenital CMV infection and had no adverse effects. Knowledge about the potential efficacy

of preventive or therapeutic administration of IgH should be broadened by multicenter randomized trials, which may be favoured by the implementation of HCMV screening (Nigro, 2017; Revello et al., 2014). However, if ultrasound examinations show signs of foetal injury, or if HCMV is detected in AF, patients should be advised of the possible option of IgH therapy (Nigro, 2017).

Clinical trials have been conducted by administering a HCMV-specific hyperimmunoglobulin (HCMV-HIG) such as HCMV-HIG-CytoGam® and HCMV-Cytotect® aiming to prevent congenital HCMV infection after primary infection during pregnancy (Adler et al., 2013; Nigro, 2017; Revello et al., 2014; Van Zuylen et al., 2014). The results of the two clinical trials, developed in the USA and Europe, may shed light on the efficacy and safety of HCMV-HIG as a means of preventing HCMV transplacental transmission. Although there are no conclusive results from controlled and randomized clinical trials in humans, it is consensual on the part of obstetricians the use HCMV-HIG in cases of ultrasound evidence of foetal infection as an alternative to abortion (Nigro, 2017). The administration of HCMV-HIG for the prevention of intrauterine transmission of HCMV presents some problems, including batch variability, the volumes administered and the difficulty in maintaining an adequate supply of HCMV-HIG.

The glycoprotein B (gB) has been a major target for development of HCMV vaccines and antiviral drugs. Two gB-based vaccines, gB/MF59 (Sanofi) and ASP0113 (Vical, Astellas), and two gB antibodies, LJP538 (Novartis) and TCN202 (Theraclone) have been tested in clinical trials (McVoy et al., 2018; Patel et al., 2016; Ye et al., 2020). The gB-specific antibodies in CMV-infected individuals target five major antigenic domains (ADs) (Potzsch et al., 2011; Ye et al., 2020). The antigenic domain 2 (AD-2), located at the N-terminus of gB, is one of the major ADs targeted by gB-specific antibodies isolated from CMV-infected individuals. AD-2 contains a highly conserved site I epitope (amino acids 68–77) that is targeted by neutralizing antibodies and a strain-specific site II epitope (amino acids 50–54) that is targeted by non-neutralizing antibodies (Meyer et al., 1992; Ye et al., 2020). Several studies implicate gB AD-2 specific antibodies as a correlate of protective immunity against HCMV infection or disease. The magnitude of maternal AD-2 specific antibodies was borderline associated with low risk of congenital CMV infection among HIV-1 exposed infants (Bialas et al., 2016). Despite the importance of gB AD-2, little is known about the neutralization mechanism of gB AD-2 specific antibodies (Ye et al., 2020). The use of a HCMV monoclonal antibody anti-gB (AD-2) with therapeutic efficacy against intrauterine

transmission of HCMV seems a more advantageous alternative to HCMV- HIG (Auerbach et al., 2014).

1.4.4.2. Vaccines

The study of vaccines as a preventive measure against HCMV infections includes congenital infection, begun in the 1970s with the Towne attenuated vaccine (Dasari et al., 2013).

Human CMV contains multiple surface-expressed glycoproteins that play pivotal roles in viral entry, including gB, the complex of glycoprotein M (gM) with glycoprotein N (gN) (gM/gN), the complex of glycoprotein H (gH) with glycoprotein L (gL) (gH/gL), and a pentameric complex (PC) of gH/gL/unique long (UL)128/UL130/UL131A (Tanimura & Yamada, 2018).

Currently, there are three CMV antigens that appear to be of greatest interest to a vaccine development: gB, PC and the pp65 protein. Antibodies to gB are believed to primarily prevent entry into fibroblasts, but also block entry into epithelial and fibroblast cells (Kirchmeier et al., 2014). A gB vaccine has been shown to be highly effective in preventing HMCV disease in organ transplant patients (Bernstein et al., 2016). Moreover, the role of pp65 as an important T cell response inducer is recognized for protecting transplant patients (Smith et al., 2013). As for the demonstration of efficacy in clinical trials, it worth noting that an attenuated virus prevented HCMV disease in renal transplant patients and that the gB subunit moderately decreased HCMV acquisition by seronegative women and reduced disease in the recipient's solid organs (Permar et al., 2018).

Vaccine-driven responses may be challenging to elicit early post- hematopoietic stem cell transplantation (Post-HCT), since the recipient's immune system remains impaired for the initial months post-HCT (Chalandon et al., 2006). Thus, vaccination for preventing infectious diseases in hematopoietic stem cell transplantation recipients are generally recommended to begin no earlier than 6 months' post-procedure, the period of highest risk for CMV infection (Tomblyn et al., 2009). Nonetheless, recent studies have indicated that recovery of pp65 CD8 T cells during the first 65 days post-HCT is associated with protection from CMV related complications (Gratama et al., 2010; Nakamura et al., 2016). The continuing need for a CMV vaccine in the hematopoietic stem cell transplantation setting prompted the development of CMVpp65-A*0201 or CMVPepVax, an investigational CMV peptide vaccine composed of HLA A*0201 pp65495–503 fused with tetanus toxin (Tet) sequence, both of which are known to be universal T helper epitopes (La Rosa et al., 2012; Nakamura et al., 2016). An acceptable

safety profile and vaccine-driven expansion of pp65 T cells in healthy adults supported its further evaluation in hematopoietic stem cell transplantation recipients (Einsele et al., 2002; Gratama et al., 2010; Nakamura et al., 2016).

Prevention of congenital HCMV infection was considered the most relevant and practical final outcome for a phase III putative efficacy study (Permar et al., 2018). Vaccination strategies for HIV-positive women remain unknown and should be informed by studies of immune deficiencies that allow reinfection or transmission of HCMV in pre-existing immunity situations (Kawada et al., 2015).

The results of the efficacy trials should continue to be re-evaluated with a view to defining immunological correlates of vaccine protection; similar to what was sought with partially effective HIV vaccine trials (Nakamura et al., 2016).

1.4.4.3 GB / MF59 subunit vaccine

The gB/MF59 subunit vaccine has in its composition a HCMV gB and adjuvant MF59. The choice of this glycoprotein is related to the fact that it is encoded by a conserved virus gene and induces the production of neutralizing antibodies (Nelson et al., 2018). In contrast with the Towne vaccine, the gB/MF59 vaccine has the ability to induce anti-gB CMV antibody production and cellular immunity (Nelson et al., 2018; Sabbaj et al., 2011; Wang & Fu, 2014). In phase I clinical trials in children and adults, the vaccine was considered safe and immunogenic. The vaccine induced the production of neutralizing antibodies to gB at concentrations similar to those induced by natural infection. The antibody titer decreased rapidly upon completion of immunization; however, it increased rapidly upon administration of an additional dose of vaccine (Schleiss, 2008).

There continues to be a need to define which of the various diagnostic resources for CMV is the most appropriate for a given clinical situation. The determination of anti-HCMV antibodies in pregnant women does not provide enough information for the prognosis of a congenital infection. Ultrasound and MRI are excellent screening methods in the presence of a suspected foetal infection. However, to achieve the definitive diagnosis culturing the virus or amplifying its genome from amniotic fluid is necessary. The confirmation of congenital HCMV infection in newborns is performed by direct methods such as the Shell-Vial technique or the Polymerase Chain Reaction (PCR) in urine or saliva samples. In immunocompromised patients the qualitative PCR is a reliable diagnostic technique and the antigenemia is adequate for monitoring the therapeutic.

1.5. Aim of the thesis

Worldwide there is great interest in perinatal infections, as they can lead to fetal death or congenital defects. Although Toxoplasmosis, Rubella and Cytomegalovirus (CMV) infection are diseases that have been widely studied, there are few studies carried out in Angola. In addition, there are several studies that correlate these infections with it´s clinical manifestations in patients with other diseases that cause depression of the immune system such as Human Immunodeficiency Virus (HIV) and hepatitis. Therefore, this work aimed to: (i) study the seroprevalence of IgM and IgG anti-*T. gondii*, anti-Rubella and anti-CMV antibodies in pregnant women attended at the Lucrécia Paím Maternity Hospital (LPMH) in Luanda; (ii) identify maternal primary infection through the profiles of anti-*T. gondii*, anti-Rubella and anti-CMV antibodies; (iii) dating maternal first infection through the IgG avidity test; (iv) perform the diagnosis of congenital infection using molecular techniques (qPCR); (v) studying the geospatial distribution of infections; (vi) assess the socio-demographic and clinical risk factors associated with infections by *T. gondii*, Rubella and CMV.

Certainly, the knowledge of the the prevalence and geographical distribution of these diseases, as well as the recognition of associated risk factors, are the first steps and a prerequisite for the development of control strategies by the Angolan government. This study will help us to understand the epidemiology of Toxoplasmosis, Rubella and Cytomegalovirus infection in Angola and will elucidate the dynamics of these infections in Luanda population´s

CHAPTER 2

Social Determinants and Indicators of Health in Angola

This chapter correspond to the article:

Amélia N Vueba, Marcelo B Freitas, Clarissa Faria, Maria C Sousa (2020), "Social Determinants and Indicators of Health in Angola", submitted to: *Journal of Public Health Research*

2.1. Abstract

According to the definition of the WHO, the social determinants of health are related to the conditions in which a person lives and works.

Angola has been facing an economic crisis since 2014, development challenges are enormous, including the reducing oil dependence, diversification of the economy, rebuilding infrastructures, increasing institutional capacity, improvement governance system, public finance management and improvement human development indicators. With stagnant of economic growth, the population lives with serious problems such as health, education, water supply and sanitation. In this context, socioeconomic and health indicators are bad and represent a major matter. According to the Human Development Report 2019, in which they were analysed the progress of 189 countries in the area of human development, Angola dropped in the assessment from 147 to 149, with most of the population living in precarious conditions and with a multiplicity of preventable diseases, also demonstrating that the policies adopted by the country do not yet favour a joint in between health and development. This situation requires the country to apply policies for the redistribution of income and health care to the poorest populations.

2.2. Introdution

The global reality of today shows that health and development are inextricably linked. It is enough to recall the WHO Declaration of 1946, which defines health as a complete state of physical, mental and social well-being and not just the absence of illness or infirmity. This definition assumes that an individual's health condition is a complex, multidimensional and dynamic concept. To be characterized, it is necessary to collect information on different aspects that, although they can be considered individually, only when they are the target of a joint analysis provide information to describe the health status of an individual (Working Group for Monitoring Action on the Social Determinants of Health, 2018; WHO, 2011).

The main challenge of studies on the relationships between social determinants and health is to establish a hierarchy of determinations between the most general factors of a social, economic, political nature and the mediations through which these factors affect the health situation of groups and people, since the determination relationship is not a simple direct relationship of cause-effect (Braveman et al., 2011; Thornton et al., 2016). It is through the knowledge of this complex of mediations that one can understand, for example, why there is no constant correlation between the macro indicators of wealth in a society, such as Gross Domestic Product (GDP), with health indicators (WHO, 2011). The study of this chain of mediations also makes possible to identify where and how interventions should be carried out, with the aim of reducing health inequities (Thornton et al., 2016).

In the model proposed by Dahlgren & Whitehead (1991) to understand the social determinants of health (Figure 2), they are represented by five interdependent levels that act directly and indirectly on the health-disease process of groups and individuals. At the most external level are the macro determinants, or socioeconomic, cultural and environmental conditions; at the subsequent level, the intermediate determinants, which are living and working conditions, represented by access to public services, education, housing, sanitation, health, food production, employment and income. From the intermediate level, are the determinants associated with individual lifestyles, located on the border of social microdeterminants, comprised of hereditary factors, age and gender. Still, according to Dahlgren & Whitehead (1991), the understanding of the health-disease process results from the analysis of micro and macro determinants and their forms of correlation and mediation, attributing to the social context the so-called non-communicable diseases.

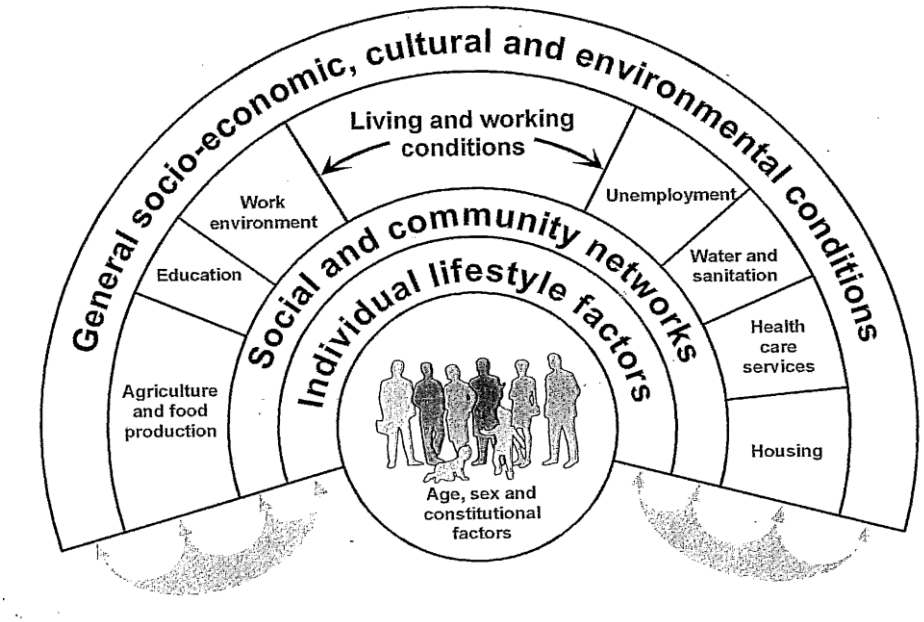

Figure 2: The main determinants of health (Source: Dahlgren & Whitehead, 1991).

The complexity of health is undeniable, regardless of the perspective from which it is approached. International agendas have, over the past few decades, been positioned between a perspective based mostly on medical technology and a position that tries to understand health as a social phenomenon, which implies more complex forms of action (Taylor et al., 2016).

At the international level, it is possible to observe the milestones considered important for health equality, in which events related to health determinants are perceived. Currently, it is unquestionable that social, environmental and economic conditions greatly influence the health conditions of populations (Braveman & Gottlieb, 2014; WHO, 2011). More than half of the influence on individuals' health is based on the conditions in which populations are born, grow, live, work and age (WHO, 2011). Understanding these factors makes it possible to look for appropriate intervention strategies at various levels in order to minimize their harmful effect on health (Braveman & Gottlieb, 2014). The various determinants influence health in different ways, so not all are equally important. Mackenbach's suggest that social factors are important (Mackenbach, 1996; Mackenbach et al., 1989). Environmental factors, those most valued by WHO, determine 25% of the population's health (WHO, 2011). On the other hand, within the social determinants, the healthy lifestyle must have a relevant place because it apparently presents greater health gains and the determinants that should be highlighted are those that cause more social stratification (Gold et al., 2019). According to the health perspective, environmental determinants may include the impact that certain chemical, physical and biological agents have on health (WHO, 2011). There is a concern about air,

water, land, food pollution and, more recently, some global risks, of which the destruction of the ozone layer and climate changes are an example. The relationship between the ability of environmental determinants to influence populations and their socioeconomic development is well known (Gold et al., 2019).

Research has shown that water contaminated by chemicals and heavy metals (both on the surface and in the subsoil) is a factor of great impact on the health of populations. This issue has become a global concern, due to the considerable impact on the environment and consequently on human health (Khan et al., 2013; WHO, 2017). If it is indisputable that the effects of global warming have an impact on the health of populations in the long term, the impact of extreme weather changes appears in the short term. Very intense heat and cold waves can be a lead to the appearance of history of disease cardiovascular, cerebrovascular, respiratory and skin carcinomas, with consequent influence on mortality rates (AghaKouchak et al., 2014; Sun et al., 2019).

Research that seeks to understand the relationship between socioeconomic status and health status is not a recent phenomenon. There are countless factors that contribute to socioeconomic inequalities in the health sector. As an example, material deprivation, health-related behaviours that directly depend on income and unemployment (Ahnquist et al., 2012; Karmakar & Breslin, 2008). The phenomenon of globalization, with the consequent elimination of borders and ease of movement of people and goods, has been criticized for bringing unequal economies and cultures together. This approach tends to produce uneven results not only between different countries, but also within them (Ahnquist et al., 2012; Karmakar & Breslin, 2008).

Countries that show good economic results may have been harmed by the globalization process (Ahnquist et al., 2012). Concern about inequality has been global. Despite the accelerated economic growth of the last decades, the good performance of certain countries, although necessary, has proved to be insufficient in reducing inequalities (Nichols & Taylor, 2018). Health-related behaviours are among the factors often associated with economic determinants. Income may (or may not) allow access to certain behaviours with an impact on the health of individuals (participating in physical activity, food choices). Health indicators may reflect differences in material wealth. To reduce health inequalities requires action to reduce socioeconomic and other inequalities (WHO, 2011). There are other factors that influence health, but these are outweighed by the overwhelming impact of social and economic factors the material, social, political, and cultural conditions that shape our lives and our behaviours. In fact, so close is the link between social conditions and health that the

magnitude of health inequalities is an indicator of the impact of social and economic inequalities on people's lives (WHO, 2011; Commission on Social Determinants of Health, 2008).

A study carried out by the WHO (2012) on health systems in Africa, perceptions and perspectives of communities reveals that they are fragile and have a deficit in the workforce, providing precarious services, information and medical products. Another factor that the study records are the differences between countries and within them, highlighting the inequalities in access and care between the rich, who are generally in power, and those considered poor (Maxwell et al., 2015; WHO, 2012). In this context, the Commission for Social Determinants of Health of WHO states that socio-political and economic forces determine the conditions in which people live and die and how they can access health services.

Sub-Saharan Africa has been striving to develop, representing a region of the world with serious public health problems: the vast majority of people living with HIV are located in low and middle income countries, with about 68% living in Africa. Among this group, 20.6 million live in East and Southern Africa, with about 800,000 new HIV infections per year. Moreover, three countries, South Africa, Zimbabwe and Mozambique, occupy the first three positions in the classification of the 22 countries that most contribute to tuberculosis in the globe (UNAIDS, 2019; WHO, 2019).

The region continues to have high rates of infant and maternal mortality and infectious diseases, which demonstrate and contribute to the low rate of human development. More than 50% of its inhabitants suffer from preventable diseases resulting from poor water quality, such as cholera and childhood diarrhoea. As a consequence, every 30 seconds, an infant death is registered, mainly due to malaria (WHO- Malaria, 2017; Marsh, 1998; Nankabirwa et al., 2014). Although poverty was reduced from 1990 to 2015 in the sub-Saharan region, approximately 41% of its population still lives in extreme poverty, with an income below US $1.25 per day, revealing a serious problem of social inequality (UN, 2015).

Despite the effort of the Angolan government to improve sanitary conditions, the annual report of the United Nations Program for Development (UNPD) shows that problems related to poverty and social inequalities persist (UNDP, 2019). The government of Angola, through the MINSA and its national and international partners as WHO, UNAIDS, National Institute of Statistics, elaborated several documents with strategies to guarantee the sustainability of the health system (PNDS, 2014; PNDS, 2012; WHO, 2016). However, very few concrete measures have been implemented to improve effective access to health care and guarantee the economic and financial sustainability of the health system (WHO, 2016).

The Republic of Angola (Figure 3) is located on the western coast of sub-Saharan Africa, whose main territory is limited to the north and northeast by the Democratic Republic of Congo (DRC), to the east by Zambia, to the south by Namibia and to the west by Atlantic Ocean. It also includes the Cabinda exclave, which borders the Republic of Congo to the north. Apart from the neighbours already mentioned, Angola is the closest country to the British colony of Saint Helena (Vueba, 2018). It is one of the largest countries on the continent, with a surface area of 1.246.700 km^2 with a population density of 22.06 inhabitants / km^2. Angola, like most developing countries, has a very young population (INE, 2018). Currently, about 36% of the population lives below the poverty line and has difficulty accessing to basic public services as potable water, sanitation, energy, health care, education and housing (WHO, 2016).

Figure 3: Map of Angola (Source: https://www.un.org/Depts/Cartographic/map/profile/angola.pdf)

The Angolan state provides health services to the population through the National Health Service (NHS) (PNDS, 2012; WHO, 2016). The provision of services is offered by institutions of state and institutions private for-profit entities. The NHS health care network is made up of 2.356 health units of which 20 central hospitals, 25 provincial hospitals, 165 municipal hospitals, 43 maternal and child centers and 331 health centers and 1650 health posts (PNDS, 2014). It is estimated that 50-60% of the population has access to basic health services, being this is percentage much lower in rural areas, by only 24%, compared with 63%

in urban areas. Profitable private health networks are generally concentrated in major cities (MPA, 2010; PNDS, 2014).

According to the MINSA, the major problems facing the NHS are related to: insufficient health coverage and lack of maintenance of health facilities; reduced health human and technical resources; poor distribution of staff in rural and periurban areas; weaknesses in the Health Management System, including the information system, logistics, communication, access to clean water and energy and insufficient financial resources (MINSA, 2014; PNDS, 2014).

This finding corroborates with WHO which drafted a document based on consultations with key health stakeholders in the country (WHO, 2016). As described in this document, some of the key health sector challenges in Angola have been identified and summarized as follows:

(i) The high index of communicable disease mortality and morbidity, the frequent occurrence of epidemic outbreaks and the accelerated increase in the prevalence of no communicable diseases;

(ii) The increased vulnerability of the country to the occurrence of various adverse health situations due to the strong movement of people, goods and commercial activity in and out of the country, as well as the existence of extensive open borders with various countries;

(iii) Insufficient basic capacities and legislative instruments in the National Health System to implement national and international public health emergency prevention and control measures;

(iv) Reduction in maternal and child mortality rates;

(v) Ensure continuing education and sustainability of human resources and quality health services;

(vi) The coordination and congregation of intra and intersectional efforts that favourably influence the social and economic determinants of health.

There are sociodemographic, political, cultural and economic determinants that relate to health and are closely linked to poverty and can only be overcome by reducing inequalities, gender equity, improving socio-economic development and especially poverty reduction, increased quality and equitable access basic services health.

Underdevelopment plays a nefarious role in the production of diseases for the population, and, consequently, a serious health problem for the population. On the contrary, when development is equitable among populations, it is also expected to improve people's

lives through access to drinking water, decent housing and the suppression of other basic needs. It is also expected that there will be more active participation by the population in the development process and in the better distribution of the goods that result from it. In this context, Gadelha (2009), states that health is a condition of citizenship, an inherent part of development itself, with no country in the world that can be considered developed with poor health.

The objective of this work was to identify which social determinants of health could have the greatest impact on the health of the population.

2.3. Socioeconomic Indicators

2.3.1. Area of Residence

Politically and administratively, Angola is divided into 18 provinces 162 municipalities and 559 communes (Figure 3). It is a multicultural country and has more than 18 national languages, but Portuguese is spoken by the majority (83%) of the population (PNDS, 2012). Demographic indicators are challenges for the sustainable development of the country. According to the last census conducted in 2014 and the Health Indicators Survey (2015-2016), Angola has a population of 25 789 024 inhabitants. The majority (63%) reside in the urban area and 37% live in the rural area (Table 1) (INE, 2016).

Table 1: Population of Angola by area of residence and gender in 2014 (Adapted from INE, 2016).

Area of residence	**Men**		**Woman**		**Total**	
	Number	%	Number	**%**	Number	%
Urban	7 860 614	62.9	8 293 373	62.4	16 153 987	62.6
Rural	4 638 427	37.1	4 996 610	37.6	9 635 037	37.4
Total	12 499 041	100	13 289 983	100	25 789 024	100

About 18 513 994 people, corresponding about three quarters of the population (72%), are concentrated in only 7 provinces of the country. Among these, five are located in the central south region of the country, with 10 059 909 inhabitants, corresponding to two fifths of the country's population (39%). Luanda province (the capital of the country), is the most populous with 6 945 386 people, representing just over a quarter (27%) of the country's population. This is followed by the provinces of Huila, Benguela and Huambo with 2 497 422

(10%), 2 231 385 (9%) and 2 019 555 (8%), respectively (INE, 2016). In Angola, in each square kilometer (Km^2) live 20.7 people. Statistically, Luanda province has the highest population density in the country with 368 inhabitants per Km^2, about 18 times the country average. Benguela and Huambo provinces follow with an average of 70 and 59 inhabitants, respectively, about 3 times higher than the country average (IIMS, 2017; INE, 2016).

2.3.2. Age struture

The age structure of the population in 2014 has marked differences between age groups, showing that the majority of population are quite young (Table 2) (INE, 2016). The age groups of 0-14 years old and 15-24 years old correspond to 65% of the resident population. The gap between young and old is enormous, where only 2% of the population is 65 or older (INE, 2016). The average age of the population is about 20.6 years, with a median of 16 years. The average age of women (21 years) is higher than men (20 years). In fact, Angola is a developing country as a result of high birth and death rates, the average life expectancy is low (52 years), with 51 years for men and 53 years for women and the mortality rate of children under 5 is one of the highest in Africa (194 deaths per 1000 live births) (WHO, 2016).

Table 2: Age structure of Angola population by gender in 2014 (Adapted from INE, 2016).

Age	**Men**		**Woman**		**Total**	
	Number	%	Number	%	Number	%
0-14 years	6 051 650	48.4	6 144 846	46.2	12 196 496	47.3
15-24 years	2 243 399	17.9	2 441 539	18.4	4 684 938	18.2
25-64 years	3 938 886	31.5	4 356 274	32.8	8 295 160	32.2
≥65 years	265 106	2.1	347 325	2.6	612 430	2.4
Total	12 499 041	100	13 289 983	100	25 789 024	100

In general, children who live in the rural areas have four to ten times less possibilities of access to basic services, information and goods than children who live in urban areas. Around 10% of children between 0-17 years are orphans as a consequence of the war, and to some extent due to HIV/AIDS (UNPAF 2015).

2.3.3. Schooling/Education level

In the education sector, Angola in 2012 was considered by United Nations Educational, Scientific and Cultural Organization (UNESCO) to be a low educational development country, ranking 111th out of 120 countries. Actually, UNESCO Education for all attributed a value of 0.685 and a gender parity index of 0.734. Between 2008 and 2013 various international bodies such as the Committee on Economic, Social and Cultural Rights (DESC), the Committee on the Rights of the Child and the Convention on the Elimination of All Forms of Discrimination against Women (CEDAW), expressed their views about this concerns related to the state of the right to education for all Angolans (WHO, 2016). In 2014, the population aged 18 and over who completed the 2nd cycle of secondary education was 13%. On the other hand, the population aged 18 or over who never attended school or did not complete the 1st cycle is 48%. This value increases in age groups 25-64 years and 65 or older compared to the current system (INE, 2016).

2.3.4. Economic growth

The economically active population is less than 50%. Women of reproductive age (15-49 years) constitute about 44% (WHO, 2016; PNDS, 2012). The mean of household aggregates is constituted of 5 persons. Most households are headed by men, with only one in five of them headed by a woman. This demographic pattern has implications for the demand for social services, particularly health and education, as well as for creating job opportunities, considering that less than 50% of this population is economically active INE, 2016; WHO, 2016; PNDS, 2012). In 2014, the number of unemployed covered 1 739 946 individuals, which corresponds to a national unemployment rate of 24.9%. The unemployment rate mainly affects the young population aged 15-24 (INE, 2016)

Angola faces one of the worst economic and social moments in its history. The Economist Intelligence Unit (EIU) predicts Angola's economy will remain in recession, prolonging the negative growth of recent years due to the fall in oil price since summer 2014 (EIU, 2019a). According to the document on quarterly national accounts, Angolan economy had negative growth of 2.5% from January to March 2018, 3.8% in the second quarter, and 1.3% in the third quarter. In the last quarter entered positive territory in the last three months of the year, when it registered an economic expansion of 2.6% (EIU, 2019b). In the first three months of 2019, the Angolan economy returned to negative growth, registering a contraction of economic activity that International Monetary Fund estimates to have been 0.4%, which led

the executive to review, in April, the prospect of growth from 3.2% to 0.4% in 2019 as a whole (EIU, 2019b).

Lack of financial resources has been severely hampering the improvement of economic indicators and has aggravated health indicators.

2.3.5. Basic Sanitation

2.3.5.1. Water

Having adequate drinking water and sanitation services, with broad levels of coverage, contributes to improving the health and well-being of the population. In 2015 about 663 million people worldwide still used an inadequate water source (unprotected wells and springs and surface water), and nearly half lived in sub-Saharan Africa (UNICEF & WHO, 2015). According to the census realized in Angola, in 2014, results indicated that the number households aggregate with access to a water source suitable for consumption is 43.6% (2 417 726 of 5 544 834 households). However, there is a contrast in water supply levels between urban and rural areas of the country; the urban population with access to safe water is around 34.86%, while the rural population is only 8.74% in accordance with census data (INE, 2016).

Unfortunately, a drinking water supply in Angola does not guarantee adequate drinking water consumption, so a safe water treatment system needs to be implemented. At national level, 67% of households do not treat water, 52% in urban areas and 91% in rural areas. In addition, water availability is higher in rural areas (IIMS, 2017).

2.3.5.2. Waste and Sanitary facilities

Despite significant progress in recent years, complex challenges remain in different intervention areas in which are observed still asymmetries in the development of communities between regions and great demographic pressure in urban centers, due to the inequality of opportunities throughout the territory, which causes internal migratory movements to urban centres (UNPAF, 2015; WHO, 2016).

In Angola, 26.3% of households aggregate (1 460 237 of 5 544 834 households) have properly dispose of solid waste / garbage, 57.6% (1 460 237 of 5 544 834) live in urban areas and 42.4% (1 460 237 of 4 084 597) in rural areas. Waste is disposed of outdoors by 13% of households living in urban areas and 87% of residents in rural areas (INE, 2016). Moreover, 60% of households (3 324 389 of 5 544 834 households) have appropriate place for defecation. However, this figure is only 10.1% in the rural area compared to 49.9% in the urban area. Contributing to this gap is the fact that members of 69% of rural-dwelling households

defecate in the grass, undergrowth, or outdoors (IIMS, 2017). Sanitary facility conditions can contribute to disease transmission, so it is important to use appropriate and non-shared sanitation facilities.

2.4. Health Indicators

In public policies, health is considered a human right, in accordance with Article 77 of the Constitution of the Republic of Angola. In that way, the Angolan state ensures that all citizens have the right to medical and health care (MINSA, 2014).

2.4.1. Country Health Profile

Despite a slight improvement in the country's main global health indicators, the health *status* of the Angolan population is characterized by low life expectancy at birth, high maternal and child mortality rates, a high burden of communicable diseases and growing of chronic and degenerative diseases, high incidence of infectious and parasitic diseases, especially large endemics, respiratory and diarrheal diseases, a still high malnutrition level in children under 5 years old, persistent outbreaks of Cholera, Rabies and Measles and an exponential increase in Non-Communicable Chronic Diseases (NCDs) (MINSA, 2014). Communicable diseases are still responsible for more than 50% of deaths in the population as well as premature mortality (MINSA, 2014; PNDS, 2014).

Malaria, diarrheal diseases, acute respiratory infections, measles, tetanus, and perinatal infections constitute more than two thirds of child deaths in Angola (PNDS, 2014). Institutional coverage of childbirth is limited for reasons of geographical, economic and cultural accessibility. In 2009, the Survey of Population Welfare Indicators data reported 45% of deliveries to health facilities. The main causes of direct obstetric mortality are due to bleeding (33%), unsafe abortions (24%), septicaemia (17%), toxaemia (14%) and uterine ruptures (9%). In 2012, malaria accounted for 46% of all deaths and for 56% of reported morbidity cases in the country, according to the Angola Epidemiological Data Processing Center (PNDS, 2014). Malaria represents about 35% of the demand for curative care, 20% of hospitalizations, 40% of perinatal deaths and 25% of maternal mortality (DNSP, Annual Report 2013 and PNCM- DNSP, IIMA Report 2011). In 2013, there were 2 487 306 cases with 6518 deaths (IIMS, 2017; Ljolje et al., 2018).

The ADD ranking second after Malaria. In 2011, the prevalence was 537.575 cases, with 769 deaths and the annual incidence rate was 8.589/100,000 inhabitants. Of the registered cases, the highest incidence was found in the province of Luanda: 135,560 clinical

cases, corresponding to 25.2% of the total (Pelkonen et al., 2018). The highest number of deaths occurred in Huambo Province, with a specific fatality rate of 26% (PNDS, 2012). Angola it has one of the mortality rate the high reported in children under-5 in the world, with 156.9 deaths per 1000 live births (Das et al., 2014; You et al., 2015). The prevalence of diarrhoea is higher in children living in households that have inappropriate and shared toilets than in children in households with appropriate and unshared toilets (IIMS, 2017; Pelkonen et al., 2018).

The epidemiological situation of Tuberculosis (TB) is worrying in Angola, with variations in different regions of the country due to the multiple factors as limited adherence to treatment, inequalities, poverty and discrimination (PNCT, 2016). Tuberculosis is a difficult disease to control. Individual and environmental factors affect adherence to TB treatment that are aggravated by (i) high dropout rates, (ii) difficult access to vulnerable groups, (iii) increased TB/HIV manufacturing, (iv) existence of strains resistant to anti-tuberculosis drugs among others (PNCT, 2016). Tuberculosis has a negative impact on the health and development of communities, mainly affecting the working-age population (15 to 39 years old). The annual incidence of pulmonary tuberculosis has increased since 2009 and in 2013 it reached a rate of 277 cases / 100,000 inhabitants. This situation places Angola among the high-risk countries, being considered an important public health problem with negative consequences for the country's economy (Rando-Segura et al., 2018; WHO, 2016). Tuberculosis is also considered one of the main opportunistic infections of AIDS in Angola (MINSA, 2014; PNDS, 2014).

The HIV epidemic in Angola is considered widespread, with an estimated prevalence of 2.0% in the 15-49-year-old population, lower than in other countries of sub-Saharan region. Currently, national HIV data are derived from the 2016 Multiple Health Indicator Survey. HIV prevalence in adult men (15-49 years old) is estimated at 1.2% and in women 2.6% (Augusto, 2016; INE, 2016). Approximately 223,350 adults and 29,103 children live with HIV/AIDS and 53% of people require antiretroviral therapy (ART) (MINSA, 2014).

The infection for HIV or Tuberculosis has many indirect effects on families and society. For example, families where parents are sick with AIDS and/or tuberculosis are often impoverished families due to unemployment and high medical bills pay (Population Reference Bureau, 2018). When an HIV-positive mother advances towards the final stages of AIDS, her children are 3.5 times more likely to die, whatever their infectious state, and are four times more likely to die when the mother dies (Population Reference Bureau, 2018). A parental loss is an additional evil for families and an extra effort for society. The epidemic has

already produced about 12 million orphans on the African continent; nine percent of all children will have lost at least their father or mother to AIDS, and one in six households with children is treating orphans (WHO, 2001).

2.4.1.1. Fertility

Fertility is an important indicator in the assessment and plays a relevant role in a country's population growth and demographic transition. Despite the state's efforts to ensure gender equality, the current context and state of the national health system remains disadvantageous to women's health, especially of reproductive age, due to the high maternal mortality rate (IIMS, 2017).

Gender inequalities show harmful consequences on women's health and consequently on children, and the male / female ratio of HIV infection is unbalancing to the detriment of the latter (IIBEP, 2010; MPA, 2010). According to definitive 2014 census data, the annual growth rate in Angola is 3.1% and the overall fertility rate is 6.2 being higher in rural areas (8.2) than in urban areas (5.3) that is, on average, women in rural areas have three more children than women in urban areas. Contributing to this situation is the high illiteracy rate (66%) coupled with poor dissemination of contraceptive methods, problems with sex education and low levels of education. From this set of circumstances result pregnancies and unwanted children (INE, 2016).

Child and maternal mortality rate are among the highest in the world. The maternal mortality rate is estimated at 450 deaths per 100.000 live births. Between 2001-2005 and 2011-2015, child mortality decreased from 81 to 44 deaths per 1.000 live births (IIMS, 2017; WHO, 2016). The causes of stillbirth and early neonatal deaths are associated and it can be difficult to distinguish from each other. Since the perinatal mortality rate covers stillbirths and early neonatal deaths, the perinatal mortality rate provides a better measure of the mortality level around childbirth. In the period 2011-2015, the perinatal mortality rate in Angola was 30 deaths in 1.000 pregnancies and the child and juvenile mortality was 68 deaths per 1.000 live births. The main direct causes of under-5 mortalities are the diseases associated with malnutrition (IIMS, 2017; MINSA, 2014; PNUD, 2018; WHO, 2016).

2.4.1.2. Health care services

The Angolan National Health System consists of three levels. The primary level corresponds to the level of Primary Health Care (PHC) - represented by Health Posts/Centres, Municipal Hospitals, nursing posts and medical offices, constitute the population's first point

of contact with the Health System. The secondary level or intermediate level, represented by general hospitals, is the reference level for the first level units. The tertiary level is represented by the differentiated and specialized mono or multipurpose reference hospitals and is the reference level for health units at the secondary level (MINSA, 2014; PNDS, 2012). Despite the established hierarchy, the referral and counter-referral system has not been operational due to several factors, mainly due to the lack of structure of the health system and the consequent reduction of health coverage (Kendall et al., 2014).

2.4.1.3. Maternal and Child Health Care

The health services that a mother receives during pregnancy, childbirth and the period immediately after childbirth are important for the survival and well-being of the mother and child. The Ministry of Health, at its National Health Development Plan (PNDS, 2012-2025), recommends that a pregnant woman should be observed at least in four prenatal consultations in health units by a qualified health professional, with the first consultation taking place during the first three months of pregnancy (UNAIDS, 2019). Several interventions are part of the package of health care and services during pregnancy: assessment of the mother's health status; verification of weight; checking blood pressure; conducting laboratory tests, including testing for Syphilis and HIV; vaccination against tetanus; administration of micronutrients (iron, folic acid and multivitamins); intermittent and preventive malaria therapy; and distribution of mosquito nets treated with long-lasting insecticide. The level of coverage is analysed according to the type of health service to which the woman has access, the number of prenatal consultations during pregnancy, as well as the services and information provided during prenatal care (IIMS, 2017; UNAIDS, 2019).

2.5. Problems in access to Health Care

It is considered that there are three obstacles to seeking health services: (i) the decision to leave home, usually determined by the level of education of women, information on essential family practices and cultural aspects related to “obtaining authorization”; (ii) geographic, transport and financial barriers; (iii) assistance at the health unit. Seven out of ten women reported at least one problem of access to health services. Obtaining money for counselling or treatment (63%) and distance to the health unit (52%) are the most cited problems in accessing health care (IIMS, 2017; UNAIDS, 2019).

The majority of women aged 15-49 (82%) who have had a live child in the past five years have had at least one prenatal consultation with a qualified health professional, with

54% being attended by a registered nurse, 16% by a doctor and 12% by a midwife.[37] Prenatal care assistance, by a qualified health professional, decrease as the number of live births increase; fewer women with six or more children had antenatal consultations in contrast with than women with only one live-born child (IIMS, 2017; PNDS, 2014).

Newborn care is essential to reduce neonatal mortality. In Angola, to prevent, diagnose and treat complications after childbirth, it is recommended that the mother and the newborn have at least one consultation six days after delivery, as this is a critical period for both. About a fifth of newborns received a consultation two days after birth (IIMS, 2017; UNAIDS, 2019).

2.6. Prevention of Mother-to-Child Transmission (PMTCT) Services in Angola.

HIV-1 mother-to-child-transmission (MTCT) is the main mode of infection among the paediatric population and is disproportionately affecting children in impoverished countries. Despite the decline in MTCT rate in recent years in most of the sub-Saharan Africa, it is estimated that 150,000 children became newly infected with HIV in 2015 (Martin et al., 2017; Mustapha et al., 2018). Children infected perinatally are at high risk of rapid disease progression and death during the first year of life without antiretroviral therapy (ART) (Marston et al., 2011).

Given the reported benefits of early ART initiation in reducing HIV-1-related mortality and long-term morbidity and reducing the size of the HIV-1 reservoirs, early HIV-1 diagnoses in newborns represents the critical gateway to timely initiation of life-saving ART (Bitnun et al., 2014; Goga et al., 2016; Martin et al., 2017). Serological assays do not permit the early diagnosis of HIV-1 infection because of the persistence of maternal HIV-1 antibodies in infants during the first 12-18 months of life. The WHO recommends the use of molecular-based virological testing to determine the infection status for HIV-1-exposed infants during the first 4-6 weeks of life or at the earliest opportunity thereafter (Martin et al., 2017).

In Angola between 2005 and 2012, the number of PMTCT sites increased from 9 to 347, and the number of HIV tests performed to pregnant women increased from 12 061 to 314 805. Despite expansion of PMTCT services, urgent action is needed to rapidly scale-up HIV prevention and treatment services for HIV-positive pregnant women and for their children (Augusto, 2016; Lussiana et al., 2012).

Currently in Angola, 34% of pregnant women living with the AIDS receive ART to avoid contaminating the fetus (Augusto, 2016; UNAIDS, 2019). Data from the Joint United Nations Programme on HIV/AIDS (UNAIDS) Program, project the difficulties experienced

by many women in accessing health care. To reverse the effects of the disease, prevention and treatment project was created (UNAIDS, 2019). An African Union initiative "Born Free to Shine" was born out to reduce the rate of HIV contamination from mother to child and is related around the dissemination and mobilization actions (UNAIDS, 2019).

Addressing stigma and discrimination related to the disease, increasing the condom by use by young people (aged 15 to 24) and improving the quality of paediatric care are some of the measures to be implemented. It is estimated that in the world there are 2 million and 100 thousand children with HIV and most of them (65%) live in Sub-Saharan Africa (Augusto, 2016). In Angola, 86% of children infected under the age of 14, are not receiving any antiretroviral treatment (UNAIDS, 2019).

In most African countries there are PMTCT services, but the coverage is limited and its use varies between countries and within each country. In 1998, the first pilot projects started to demonstrate the viability of PMTCT programs in countries with high HIV prevalence. In 2004, only 10% of women were tested for HIV through PMTCT services, and only 8.7% of HIV-positive pregnant women received prophylactic antiretroviral (ARV) treatments globally. In East and Southern Africa, where these services are most needed, 17% of women have been identified as HIV positive through HIV screening in PMTCT services and only 11% of the projected total of pregnant women HIV-positive women received ART prophylaxis (WHO, 2020). In West and Central Africa, coverage is even lower, only 3% of women infected were identified, and only one percent received ART prophylaxis (WHO, 2020). This value does not include women that become infected during pregnancy, when they appear to be especially vulnerable to infections. Some reports suggest that in places where HIV prevalence is high, about five percent of pregnant women can become infected (Elwell, 2016; WHO, 2020). Also the value does not include women who have been tested for HIV in the very early stages of infection, once HIV-specific antibodies are not yet detectable; only detectable through a repeat HIV test at 36 weeks of pregancy or even later. Thirty-nine sub-Saharan countries have implemented PMTCT programs, but only two of them, Botswana and Mauritius have achieved universal coverage (Goga et al., 2016; WHO, 2020).

The provision or acceptance of postnatal service is still a weak point of continued health services for women and their babies. Most women and their newborns are lost to the health system after delivery, but paradoxically, vaccination rates for Bacillus Calmette-Guérin (BCG) and the three doses of diphtheria vaccine, whooping cough and tetanus remain high (76 and 65 percent respectively), suggestting that families are still within reach of formal health services (Goga et al., 2016; WHO, 2020).

A review of the last six years of implementation of the PMTCT shows that progress has been made in some areas and that the most significant progress has been the widespread increase consciousness of the problem of vertical transmission of the HIV, and in the response of government. Despite these encouraging signs, resources and local commitment to improve the quality of services provided to the entire population has been little animators (Elwell, 2016).

2.7. Conclusion

The health status of a population depends not only on the health system but on the combination of various factors such as education and the environment, among others. A country's socioeconomic and health indicators are an important tool for assessing the well-being of a population. Therefore, understanding the health status of a population implies in determining the expression of the social context in which this population lives, works and circulates.

Based on the knowledge about distal determinants (education, work environment, source of income, basic sanitation, housing, social services of health and culture), intermediaries' determinants (lifestyle of individuals, social networks, community) and proximal determinants (age group, sex and hereditary factors) it is possible to create indicators that will allow the establishment of more effective public health policies in the management of health promotion and prevention.

In many countries, public policies are aimed at improving socioeconomic and health indicators. In the case of health, primary prevention programs are implemented for systematic diseases control. However, Angola is still in a "precarious" phase of socio-economic and health development and there is no effective implementation of policies and strategic orientations related for example to primary disease prevention. Several documents have already been prepared by MINSA and its partners such as the Multiple Health Indicators inquiry, health development plan among others.

Developed countries invest a lot of resources in programs and research on prevention, early diagnosis and treatment of diseases. Given the importance of disease control and prevention, it is imperative to find an approach that reaches the entire population of public health systems. However, before implementing a disease control program it is essential to know the epidemiology of these diseases to better define more cost and human resource strategies. Taking in account that Angola is a country with approximately 24 million

inhabitants and it is known that the epidemiology of the infection diseases is very variable in the world, it is essential to obtain information on the epidemiological structure of infections.

The lack of health development in Angola is characterized by the poor quality of life and the lack of basic education capable of leading the population to recognize measures to prevent certain diseases and how to treat them, appealing for deep reflection and an attitude of rapid changes in the area of education and health. Therefore, it is expected that this work can contribute to planning and improvement of health actions in the Angolan population.

CHAPTER 3

Serological prevalence of toxoplasmosis in pregnant women in Luanda (Angola): Geospatial Distribution and Its Association with Socio-demographic and Clinical-obstetric Determinants

This chapter correspond to the article:

Amélia N Vueba, Clarissa P Faria, Ricardo Almendra, Maria C Sousa (2020), "Serological prevalence of toxoplasmosis in pregnant women in Luanda (Angola): Geospatial Distribution and Its Association with Socio-demographic and Clinical-obstetric Determinants", Published in: *PLoS ONE* 15(11): e0241908.

3.1. Abstract

We report a study on toxoplasmosis in pregnant women in Luanda, Angola, determining the seroprevalence, geospatial distribution and its association with socio-economic features, dietary habits and hygiene and health conditions. Anti-*Toxoplasma gondii* (Anti-*T. gondii*) IgG and IgM were quantified in serum samples of women attended at the the Lucrecia Paim Maternity Hospital between May 2016 and August 2017. The IgG avidity test and qPCR assay were used for dating the primary infection. Data were collected by questionnaire after written consent, and spatial distribution was assessed through a Kernel Density Function. The potential risk factors associated with Toxoplasma infection were evaluated using bivariate and multivariate binomial logistic regression analysis. Anti-*T. gondii* antibodies were quantified in 878 pregnant women, and 346 (39.4%) samples were IgG positive, 2 (0.2%) positive for IgM and IgG, and 530 (60.4%) negative for both immunoglobulins. The longitudinal study showed that none of the seronegative women seroconverted during the survey. Regarding other infections, 226 (25.7%) were positive for hepatitis B, while 118 (13.4%) were HIV-positive. The seroprevalence of toxoplasmosis was similar in most municipalities: 43.8% in Cazenga (28 of 64); 42.5% in Viana (88 of 207); 42.3% in Cacuaco (22 of 52); and 41.1% in Luanda (179 of 435). In contrast, the seroprevalence in municipality of Belas was lower (25.8%; 31 of 120) and bivariate and multivariate analysis has shown a lower risk for toxoplasmosis in this area (OR 0.479, CI: 0.305–0.737; OR 0.471, CI: 0.299–0.728). The multivariate analysis has shown a significant increased risk for toxoplasmosis in women in the last trimester of pregnancy (OR 1.457, CI: 1.011–2.102), suffering spontaneous abortion (OR 1.863, CI: 1.014–3.465) and having pets at home (OR 1.658, CI: 1.212–2.269). Also, women who tested positive for hepatitis B (OR 1.375, CI: 1.008–1.874) and HIV (OR 1.833, CI: 1.233– 2.730) had a significant increased risk for *T. gondii* infection. In conclusion, our study showed that a large number of pregnant women are not immunized for toxoplasmosis and identified the risk factors for this infection in Luanda. It is crucial to establish the diagnosis of primary maternal infection as well as the diagnosis of congenital toxoplasmosis. Our results underlined the need for diagnostic and clinical follow-up of toxoplasmosis, HIV and hepatitis B during pregnancy.

3.2. Introduction

Toxoplasmosis is a cosmopolitan zoonosis that can affect humans as well as all warm-blooded animals, including mammals and birds. Toxoplasmosis is an infectious disease caused by *T. gondii*, an obligate intracellular protozoan that has the ability to invade and multiply in any nucleated cell. During its life cycle, it presents three evolutionary forms: (i) the tachyzoite, (rapidly growing life stage) present during acute infection, (ii) the bradyzoite, (slow growing life stage) present during chronic infection in tissue cysts, and (iii) the sporozoite (spore-like form), protected inside an oocyst, shed by feline hosts in feces (Dubey, 2009; Dubey et al., 1998; Montoya & Liesenfeld, 2004).

The most common transmission routes of toxoplasmosis are by oral means, either by eating undercooked contaminated meat that contains cysts or by ingesting water and uncooked foods contaminated with sporulated oocysts, and the congenital pathway, mother-to-child transmission during pregnancy. Less frequent transmission occurs through blood transfusion and organ transplant (Galván-Ramírez et al., 2018; Halonen & Weiss, 2013; Hill & Dubey, 2002; Montoya & Liesenfeld, 2004). Congenital infection is one the most serious forms of toxoplasmosis, occurring during acute toxoplasmosis in a seronegative mother when tachyzoites present in the blood cross the placenta and infect the fetus (Galvan-Ramirez et al., 2012; Hernández-Cortazar et al., 2015; Hill & Dubey, 2002).

Maternal infections are predominantly asymptomatic or cause only mild symptoms, including malaise, night sweats, myalgia, hepatosplenomegaly, swelling of the lymph nodes and fever (Leeper & Lutzkanin, 2018). Frequency of congenital transmission and severity of the infection varies considerably according to the gestation time at which the woman became infected. During the first trimester, transplacental transmission is relatively low (<20%), increasing up to 90% by the end of pregnancy (Ortiz-Alegría et al., 2010; Jones et al., 2003). Nevertheless, frequency of transmission and severity of the disease are inversely related. Congenital infection acquired in the first and second trimesters may result in severe congenital toxoplasmosis with spontaneous abortion, hydrocephaly, cerebral calcifications and mental retardation (Hernández-Cortazar et al., 2015; Montoya & Liesenfeld, 2004). Whilst in late maternal infection (third trimester) the damage tends to be lower. Clinical manifestations range from chorioretinitis (occurring in 90% of cases), learning disability, sensorineural hearing loss, and cerebellar or motor dysfunction (Leeper & Lutzkanin, 2018).

Toxoplasmosis has a cosmopolitan distribution with seroprevalence rates range from 10% to 90% (Pappas et al., 2009). The prevalence of toxoplasmosis varies dramatically between countries and often within different regions of the same country or between different

communities in the same region. Generally, developing countries have a higher incidence than industrialized countries. Areas of high prevalence have been documented in Latin America, Eastern/Central Europe, the Middle East, Southeast Asia and in tropical countries in Africa. Low prevalence has been observed in North America, in South East Asia, in Northern Europe, and in Sahelian countries of Africa (Pappas et al., 2009; Robert-Gangneux & Dardé, 2012).

In Angola, a country in South West Africa with a population of over 25 million, few studies on toxoplasmosis have been conducted in recent years (Lobo et al., 2017; López et al., 1992; Martins & Abranches, 1976). To date, only one study on the prevalence of antibodies to *T. gondii* in pregnant women has been conducted in Luanda (Lobo et al., 2017) and the prevalence of congenital toxoplasmosis is not known. Also, no research to date has been explored the overall seroprevalence of *T. gondii* infection among women in Angola, nor have the risk factors associated with the infection been examined in a regional context.

The objective of this study was to determine the seroprevalence of toxoplasmosis in pregnant women who attended a referral maternity facility located in Luanda (Angola), and to provide a detailed analysis of the geographical distribution. The study also evaluated the influence of demographic variables, socio-economic features, dietary habits, and hygiene and health conditions on the *T. gondii* infection. This knowledge is essential for the development of effective prevention and control strategies and the implementation of the Toxoplasmosis Surveillance Program in the Angolan NHS network.

3.3. Material and methods

3.3.1. Ethical Considerations

The present study has been approved by the Research Ethics Committee of of Lucrécia Paim Maternity Hospital (LPMH) through the National Institute of Public Health of the Republic of Angola (nº 301019; S1 File). Participating individuals provided a written signed informed consent prior to sample collection and for participants younger than 18 years, informed consent was provided by parents or guardians after a detailed explanation of the objectives of the work.

3.3.2. Study Area

The Republic of Angola is located on the west coast of sub-Saharan Africa (Figure 4). It is one of the largest countries of the continent, with a surface of 1,246,700km^2 and near 25.8 million inhabitants (INE, 2014). Angola, like most developing countries, has a fairly young population (INE, 2014). The population aged 0–14 years is 12,196,496, representing 47% of the total resident population (INE, 2014). It is estimated that there are 48% of men

and 52% of women and that children under 5 years old constitute 15% of the total population (INE, 2014). The economically active population is less than 50%, with a large number of state and family dependents. Women of reproductive age (15–49 years) make up about 44% and the estimated fertility rate is 6.2 children per woman (INE, 2015). Luanda is the capital and largest city in Angola, located on the coast with the Atlantic Ocean (Figure 4), is also the primary port and economic center of the country. According to the last census conducted in 2014, Luanda has a population of 6,945,386 inhabitants and is composed of 7 municipalities: Belas, Cacuaco, Cazenga, I´colo and Bengo, Luanda, Quissama and Viana (INE, 2014).

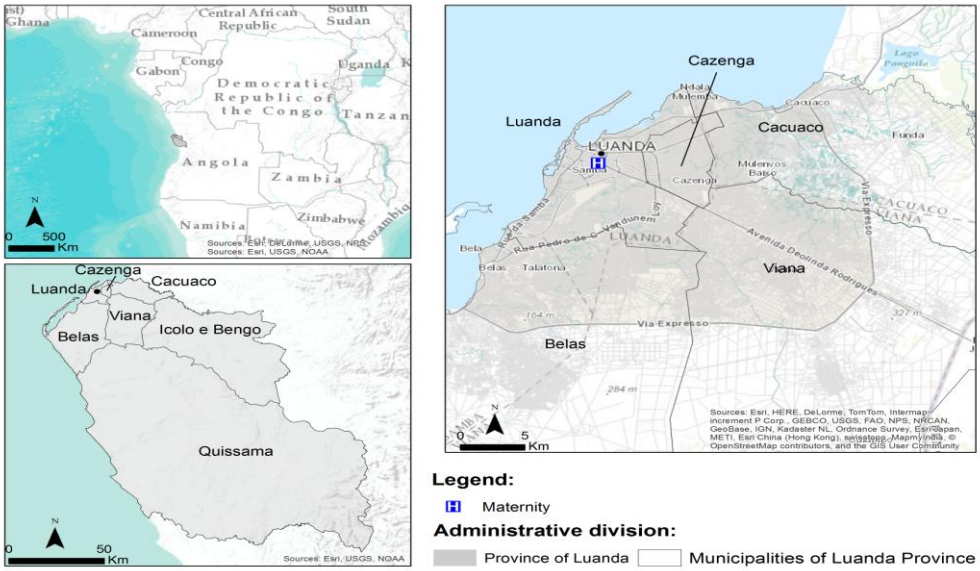

Figure 4: Localization of Luanda province; Angola, and their municipalities: Luanda, Cacuaco, Cazenga, Ícolo and Bengo, Quissama and Viana.

3.3.3. Study Population

Pregnant women monitored for routine prenatal assessment at LPMH, a reference maternity in Angola, located in Luanda, were included in the study. The referred maternity is a public health institution specializing in maternal and child health care, teaching and research. The health institution offers outpatient and inpatient services, has 400 beds for hospitalization. The study included women aged from 15 to 47 years, who had a pregnancy confirmed by ultrasonography and laboratory tests. For the obstetric follow-up we counted on the collaboration of the medical and nursing team of the department of obstetrics of the LPMH.

3.3.4. Sociodemographic, clinical, behavioral and housing characteristics of the pregnant women

An individual survey questionnaire was used to collect epidemiological variables (age, educational level, occupation, marital status and residing area), clinical information (gestational age and number of births), risk factors for *T. gondii* infection such as pets' species at home, especially cats, where they defecate and the type of food consumed, frequent contact with animals other than their own, presence of rodents in or near the house, contact with soil/ gardening, if recently had a blood transfusion, if recently had some sting with needle or sharp objects and past medical history (history of miscarriage, prematurity, presence of any under lying disease as hepatitis B and HIV) (S2 File). Information's about basic sanitation (availability of drinking water, toilets at home and garbage disposal) and alimentary habits (breeding animals for home consumption; consumption of raw/undercooked meat, game, raw eggs, and unwashed raw/vegetables/fruit) were asked. Some questions about the knowledge of toxoplasmosis, if pre-natal consultation is performed in all pregnancies and history of abortion were also included in the questionnaire (S2 File).

3.3.5. Blood sample collection and laboratory procedures

A cross-sectional and a longitudinal survey were carried out from August 2016 to May 2017. The longitudinal study included pregnant women in the first and second trimesters of pregnancy (n = 653) and the cross-sectional study included pregnant women in the third trimester (n = 225) (Figure 5).

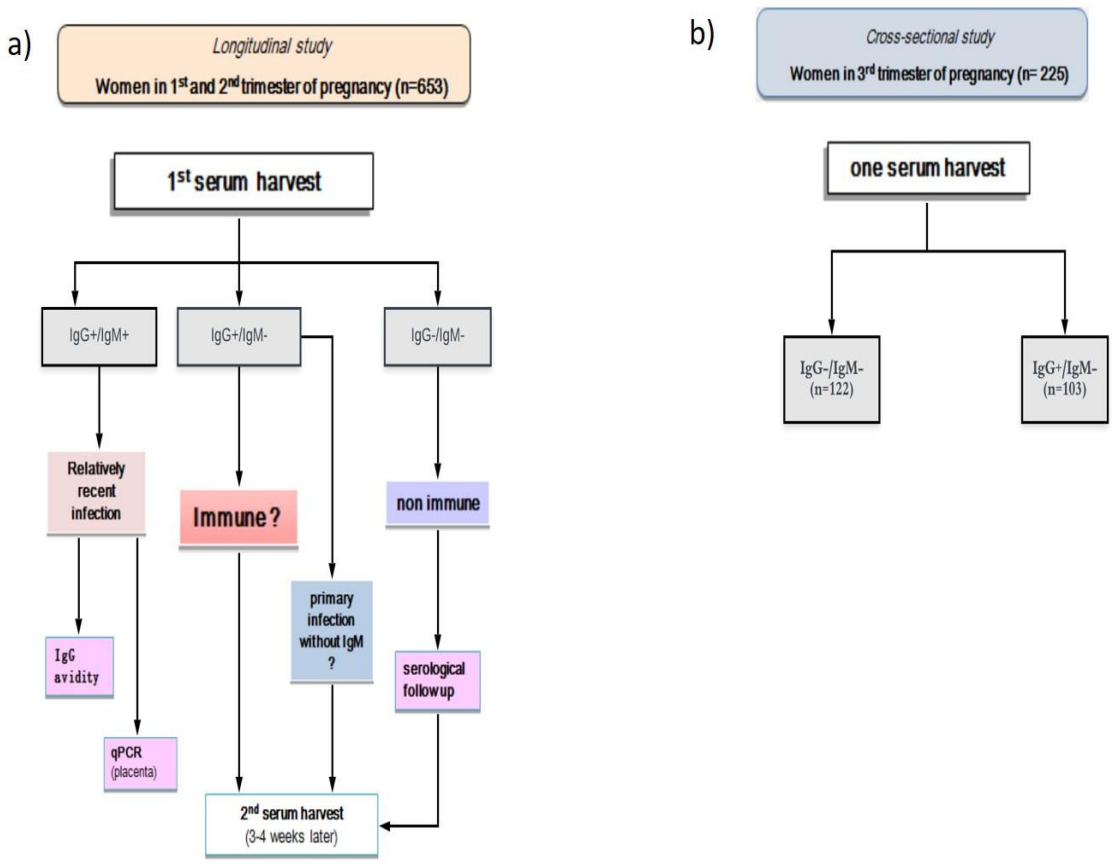

Figure 5: Flow chart of study sample and analyses for toxoplasmosis survey: (a) flow chart focuses on the longitudinal study, including only women in the 1st and 2nd trimester of pregnancy; (b) flow chart focuses on the cross-sectorial study, including only women in the 3rd trimester of pregnancy.

From each pregnant woman venous blood was collected and serum samples were obtained after centrifugation. These serum samples were immediately transferred (properly packaged in dry ice) to the Clinical Pathology Service of Clínica Sagrada Esperança (Luanda) and kept at -80˚C until serological analysis. Quantification of anti-*T. gondii* IgG and IgM antibodies was performed by Electrochemiluminescence (ECL) using commercially available kits for COBAS e411 (Roche Diagnostics, Germany). The results of anti-T gondii IgG and IgM were expressed as IU/mL and (Cut-off index) COI, respectively. Values <1 IU / mL of anti-T gondii IgG antibodies were considered negative and values > 3 IU / mL were considered positive. Values <0.8 COI of IgM anti-*T gondii* antibody were considered negative and values ≥1.0 COI were considered positive. Serum follow-up samples were collected in the second and third trimesters for seronegative women included in the longitudinal study (Figure 5). All these sera were tested concurrently with the initial sample. Also a second

sample was collected at least 3–4 weeks after the initial sample of all women suspected of having active primary infection by *T. gondii*, i.e. IgG-/IgM+. On samples that were IgG+/IgM+, a IgG avidity test was performed (Elecsys1 Toxo IgG Avidity, Roche Diagnostics, Germany). Values <70% were considered as low avidity (<70 Avi% = low avidity) and values ≥80% were considered as high avidity (≥80 Avi% = high avidity.) Values 70% ≤ value <80% are considered gray zone. Detection of anti-HIV antibodies (total anti-HIV-1, anti-HIV-2 and HIV-1 p24 antigen) was performed by ELFA using commercial kits for VIDAS (HIV DUO Ultra, HIV5 and HIV P24 II) (Biomerieux, Portugal). The hepatitis B infection was characterized by the detection of antigens (anti-HBs Ag, HBe Ag) and antibodies Anti-HBc Total II, HBc IgM, Anti-HBsT II) using ELFA commercial kits for VIDAS (Biomerieux, Portugal).

3.3.6. Molecular study

In IgM and IgG seropositive women, placental samples were collected by the medical team on the day of the babies' birth and immediately transported to Clínica Sagrada Esperança. DNA was extracted from placental fragment using the NZY Tissue gDNA Isolation kit (Nzytech, Portugal) according to the manufacturer's instructions and kept at -20˚C. Extracted DNA was analyzed by nested-PCR using primers for B1 gene locus (Table 3) (Burg et al., 1989). Amplification of the B1 gene was performed with primers B1F1 and B1R1 in the primary PCR, and with B1F2 and B1R2 primers in the secondary reaction, generating a 213bp fragment. All reactions contained 12.5µL of DyNAzymeII PCR Master Mix (Finnzymes, Finland), 1µL of each primer (10pmol/µL), 2µL of extracted DNA and 8.5µL of sterile water, performing a final volume of 25µL. PCR was carried out on the MJ Mini™ Thermal Cycler (BioRad). After an initial denaturation of 94˚C for 5min, a set of 35 cycles was run, each consisting of 30s at 94˚C, 30s of annealing (54˚C for the primary reaction, 60˚C for the second), and 60s at 72˚C, followed by a final extension step of 5min at 72˚C. Toxoplasma DNA sample and nuclease free distilled water were used as positive and negative controls, respectively. The PCR products were analyzed on 1.5% agarose gels stained with ethidium bromide and visualized using a gel documentation system (Uvitec, UK).

Table 3: List and sequence of primers for *T. gondii B1* gene locus.

Primer	Sequence (5´-3´)	Target
B1F1	CCGTTGGTTCCGCCTCCTTC	*T. gondii B1* gene (GenBank accession no. AF179871)
B1R1	GCAAAACAGCGGCAGCGTCT	
B1F2	CCGCCTCCTTCGTCCGTCGT	
B1R2	GTGGGGGCGGACCTCTCTTG	

3.3.7. Geospatial analysis

The address of pregnant women was collected during the interview allowing the identification of the residence place. This information was converted into geographic coordinates (latitude and longitude) through the www.google.pt/maps/. The spatial distribution of pregnant women was assessed through a Kernel Density Function that allowed the estimation of the intensity of events across a surface (S3 File).

3.3.8. Statistical Analysis

The data entry was carried out using Excel software and analyzed by Statistical Package for the Social Sciences (SPSS) version 20. Percentages were used to perform exploratory analysis of the categorical variables and quantitative variables are presented as mean ± standard deviation (±SD).

The statistical analysis was developed in three steps: 1) Fisher's exact test was used to compare the number of events of seropositivity between the risk factors identified; 2) Bivariate binomial logistic regression were developed to assess the effect of different risk factors on toxoplasmosis seroprevalence; 3) Multivariate binomial logistic regression models were applied to account for the the impact of age on the effect of different risk factors on toxoplasmosis seroprevalence. The level of statistical significance was set as $p<0.05$, and Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were computed.

3.4. Results

Between August 2016 and May 2017, a total of 878 pregnant women from Luanda (Angola) were tested for anti-*T. gondii* antibodies. No clinical complaint related to disease was made at the time of the serosurvey. The study has revealed 348 (39.6%) positive samples for anti-*T. gondii* antibodies (Table 4). Of these, 346 (39.4%) samples were positive for the IgG antibody and 2 (0.2%) for both IgG and IgM antibodies. In total, 530 (60.4%) of women were seronegative for *T. gondii* antibodies. In the two positive samples for IgM and IgG

antibodies, the IgG avidity test results (high avidity) showed that these women had a chronic Toxoplasma infection.

Table 4: Distribution of IgG and/or IgM antibodies to *T. gondii* of seropositive and seronegative pregnant women from Luanda (Angola).

Seroprevalence	**Positive (%)**	**Negative (%)**
Overall prevalence	348 (39.6)	530 (60.4)
Anti-*T.gondii* IgG	346 (39.4)	532 (60.6)
Anti-*T.gondii* IgM	0 (0)	878 (100)
Anti-*T.gondii* IgM/IgG	2 (0.2)	876 (99.8)

In the longitudinal study, a total of 653 pregnant women were followed during gestation: 408 (62.5%) were seronegative and of these 178 (33.6%) were in their first trimester of pregnancy and 230 (43.4%) in the second trimester (Table 5). These women were followed throughout the pregnancy and remained seronegative until the end of the pregnancy. Of the 245 (37.5%) seropositive pregnant women, 97 (27.9%) were in the first trimester of pregnancy, and 148 (42.5%) in the second trimester (Table 5).

In the cross-sectional study, 225 pregnant women in the third trimester were included, with 103 seropositive for *T. gondii* and 122 seronegative (Table 5). Once these women were in the last trimester of gestation no more samples were collected. The ages of the pregnant women ranged from 15 to 47 years, with an average of 29.7±5.8 (Mean±SD; median = 30) (Table 5). The majority of participants were between 26 and 35 years of age (n = 504). Ninety-nine percent of participants (n = 869) were educated above the primary grade level, and a lesser number had no occupation outside home (22.9%). In relation to marital status, 596 pregnant women reported being single (67.9%) and 282 (32.1%) married (Table 5). In relation to gestational age, 275 (31.2%) were in the first trimester of pregnancy, 378 (43.1%) in the second trimester and 225 (25.6%) in the third trimester. The mean gestational age was 19.8±9.4 weeks (Mean±SD; median = 16). The majority of the pregnant women had more than three children (414; 47.1%), with a mean birth rate of 1.2±0.8 (Mean±SD; median = 1.0).

No children were born prematurely although 45 (5.1%) women suffered spontaneous abortion while the present study was under way (Table 5). Additionally, 854 (97.3%) women stated that they were monitored for routine prenatal assessment in all their pregnancies and 478 (54.4%) reported history of miscarriage. Regarding other infectious diseases, 226 (25.7%)

were seropositive for hepatitis B and 118 (13.4%) for HIV virus. We also observed a high number of pregnant women (812; 92.5%) who were unaware of *T. gondii* disease. In relation to pets, 222 (25.3%) pregnant women had animals at home: 183 (20.85%) with dogs, 35 (4%) with cats and 4 (0.45%) with both. The majority of cats defecated outside home and were fed with food scraps (cooked) (30; 3.4%). The majority of the participants (602; 68.6%) stated that they only had contact with their own pets and not with those of others. Reports of the presence of rodents in or nearby the house were high (715, 81.4%) and a lesser number of pregnant women had contact with soil or were engaged in gardening (157, 17.9%) (Table 5).

Table 5: Characteristics of seropositive and seronegative pregnant women to *T. gondii*, according to the independent categorical variables evaluated.

Characteristics	**Positive n (%)**	**Seroprevalence (%)**	**Negative n (%)**	**Seroprevalence (%)**	**Total**
Age group (years)					
≤ 19	7(2.0)	17.5	33 (6.2)	82.5	40 (4.6)
20-25	53 (15.2)	27.5	140 (26.4)	72.2	193 (21.9)
26-35	222(63.8)	44.0	282 (53.2)	56.0	504 (57.4)
36-47	66 (19.0)	46.8	75 (14.2)	53.2	141 (16.1)
Education					
Illiterate	5 (1.4)	55.6	4 (0.7)	44.4	9 (1.0)
Elementary School	137 (39.4)	40.1	205 (38.7)	59.9	341 (38.9)
High school	172 (49.4)	39.3	266 (50.2)	60.7	439 (50.0)
Higher education	34 (9.8)	38.2	55 (10.4)	61.8	89 (10.1)
Occupation					
Homemakers	86 (24.7)	42.8	115 (21.7)	57.2	201 (22.9)
Public function	103 (29.6)	36.7	178(33.6)	63.3	281 (32.0)
Student	77 (22.1)	40.3	114 (21.5)	59.7	191(21.8)
Restoration	50 (14.4)	45.0	61(11.5)	55.0	111(12.6)
Street vendor	20 (5.7)	37.0	34 (6.4)	63.0	54 (6.1)
Store clerk	12 (3.5)	30.0	28 (5.3)	70.0	40 (4.6)
Marital status					
Married	108 (31.0)	38.3	174(32.8)	61.7	282 (67.9)
Single	240 (69.0)	40.3	356 (67.2)	59.7	596 (32.1)
Gestational age					
1st Trimester	97(27.9)	35.3	178 (33.6)	64.7	275 (31.3)
2nd Trimester	148 (42.5)	39.2	230 (43.4)	60.8	378 (43.1)
3rd Trimester	103 (29.6)	45.8	122 (23.0)	54.2	225 (25.6)
Number of births					
0	79 (22.7)	35.6	143(27.0)	64.4	222 (25.3)
1	79(22.7)	32.6	163 (30.8)	67.4	242 (27.6)
≥2	190 (54.6)	45.9	224 (42.2)	54.1	414 (47.1)
Spontaneous abortion					
Yes	27 (7.8)	60.0	18 (3.4)	40.0	45 (5.1)
No	321 (92.2)	38.5	512 (96.6)	61.5	833 (94.9)
Hepatitis B infection					
Negative	245 (70.4)	37.6	407 (76.8)	62.4	652 (74.3)
Positive	103 (29.6)	45.6	123 (23.2)	54.4	226 (25.7)
HIV status					
Negative	287 (82.5)	37.8	473 (89.2)	62.2	760 (86.6)

Positive	61 (17.5)	51.7	57 (10.8)	48.3	118 (13.4)
Awareness of toxoplasmosis					
Does not know anything about the disease	318 (91.4)	39.2	494 (93.2)	60.8	812 (92.5)
Heard speak, but do not know anything about it	26(7.5)	47.3	29 (5.5)	52.7	55 (6.3)
Know anything about the disease	4(1.1)	36.4	7 (1.3)	63.6	11 (1.2)
Pre-natal consultation was performed in all pregnancies					
Yes	335 (96.3)	39.2	519 (97.9)	60.8	854 (97.3)
No	13 (3.7)	54.2	11 (2.1)	45.8	24 (2.7)
History of abortions					
Yes	217 (62.4)	45.4	261 (49.2)	54.6	478 (54.4)
No	131 (37.6)	32.8	269 (50.8)	67.2	400 (45.6)
Pet at home					
Cats	91 (26.1)	49.7	92 (17.4)	50.3	183 (20.8)
Dogs	11 (3.2)	31.4	24 (4.5)	68.6	35 (4.0)
Dogs and cats	4 (1.2)	100.0	0 (0.0	000.0	4 (0.5)
No	242 (69.5)	36.9	414 (78.1)	63.1	656 (74.7)
Cats´defecating habits					
Inside home	1 (0.3)	16.7	5 (0.9)	83.3	6 (0.7)
In the vicinity of the house	10 (2,9)	47.6	11 (2.1)	52.4	21 (2.4)
Far from the house	6 (1.7)	50.0	6 (1.1)	50.0	12 (1.4)
No	331 (95.1)	39.5	508 (95.9)	60.5	839 (95.5)
Cats´ feeding habits					
Raw / uncooked meat	1 (0.3)	33.3	2 (0.4)	66.7	3 (0.3)
Cooked meat	15 (4.3)	50.0	15 (2.8)	50.0	30 (3.4)
Cat food	1 (0.3)	16.7	5 (0.9)	83.3	6 (0.7)
No	331 (95.1)	39.5	508 (95.9)	60.5	839 (95.6)
Contact with other animals					
Yes	101 (29.0)	36.6	175 (33.0)	63.4	276 (31.4)
No	247 (71.0)	41.0	355 (67.0)	59.0	602 (68.6)
Rats near the house					
Yes	278 (79.9)	38.9	437(82.5)	61.1	715 (81.4)
No	70 (20.1)	42.9	93 (17.5)	57.1	163 (18.6)
Sand/soil contact					
Yes	69 (19.8)	43.9	88 (16.6)	56.1	157 (17.9)
No	279 (80.2)	38.7	442 (83.4)	61.3	721 (82.1)
Recent blood transfusion					
Yes	3 (0.9)	42.9	4 (0.8)	57.1	7 (0.8)
No	345 (99.1)	39.6	526 (99.2)	60.4	871 (99.2)
Recently had some sting with needle or sharp objects					
Yes	9 (2.6)	36.0	16 (3.0)	64.0	25 (2.8)
No	339 (97.4)	39.7	514 (97.0)	60.3	853 (97.2)
Consumption of washed fruit/vegetables					
Always	259 (74.4)	40.7	377 (71.1)	59.3	636 (72.4)
Sometimes	89 (25.6)	36.8	153 (28. 9)	63.2	242 (27.6)

Raw/uncooked meat consumption					
Yes	81 (23.3)	37.5	135 (25.5)	62.5	216 (24.6)
No	267 (76.7)	40.3	395 (74.5)	59.7	662 (75.4)
Consumes raw or undercooked egg					
Yes	122 (35.1)	36.5	212(40.0)	63.5	334 (38.0)
No	226 (64.9)	41.5	318 (60.0)	58.5	544 (62.0)
Consumption of meat from hunting sources					
Yes	99 (28.4)	40.4	146 (27.5)	59.6	245 (27.9)
No	249 (71.6)	39.3	384 (72.5)	60.7	633(72.1)
Creation animals for own consumption					
Yes	29 (8.3)	43.9	37 (7.0)	56.1	66 (7.5)
No	319 (91.7)	39.3	493 (93.0)	60.7	812 (92.5)
Access to basic sanitation					
Yes	227 (65.2)	40.7	331 (62.5)	59.3	558 (63.6)
No	121 (34.8)	37.8	199 (37.5)	62.2	320 (36.4)
Total	**348**	**-**	**530**	**-**	**878**

In relation to blood transfusions, only 7 (0.8%) pregnant women reported having recently had a blood transfusion and 25 (2.8%) reported having recently been subjected to a needle/syringe prick or intervention (Table 5). The type of food consumed, as well as the conditions under which it was consumed, was also of interest in the present study: 636 (72.6%) wash fruits and vegetables before consumption; 216 (24.6%) consume raw or undercooked meat; 334 (38.0%) consume raw or undercooked eggs, and 245 (27.9%) reported having eaten game meat. Only 66 (7.5%) raise animals for individual consumption. Over half of the women (558; 63.6%) answered that they have proper garbage disposal, drinking water and toilets at home.

As expected, Luanda had a greater number of *T. gondii* seropositive pregnant women (179 of 348 positive cases; 51.4%) since it has the larger population surveyed (435 of 878; 49.5%) (Table 6). The other municipalities had 168 positive cases (48.6%) and we did not have participants from Quissama. However, the prevalence of toxoplasmosis was similar in almost over municipalities: 43.8% in Cazenga (28 of 64); 42.5% in Viana (88 of 207); 42.3% in Cacuaco (22 of 52); and 41.1% in Luanda (179 of 435). In contrast, the seroprevalence in municipality of Belas was lower when compared to the other municipalities (25.8%; 31 of 120) (Table 6).

Table 6: Number of seropositive and seronegative pregnant women to *T. gondii* by municipalities of the province of Luanda, Angola.

Municipality	Positive n (%)	Seroprevalence (%)	Negative n (%)	Seroprevalence (%)	Total
Belas	31 (9.0)	25.8	89 (16.8)	74.2	120 (13.7)
Cazenga	28 (8.0)	43.8	36 (6.8)	56.2	64 (7.3)
Cacuaco	22 (6.3)	42.3	30 (5.7)	57.7	52 (5.9)
Luanda	179 (51.4)	41.1	256 (48.3)	58.9	435 (49.5)
Viana	88 (25.3)	42.5	119 (22.4)	57.5	207 (23.6)
Icole and Bengo	-	-	-	-	-
Quissama	-	-	-	-	-
Total	**348**	**-**	**530**	**-**	**878**

In the univariate analysis, the risk factors associated with toxoplasmosis seroprevalence included the maternal age ($p < 0.0001$), gestational age (3rd trimester, $p = 0.0328$), have children ($p = 0.026$), spontaneous abortion ($p = 0.0048$), hepatitis B ($p = 0.040$) and HIV seropositive ($p = 0.005$), history of miscarriage ($p = 0.0001$), and owning pets (dog, cat or both) ($p = 0.032$) (Table 7). Other analyzed factors such as education, employment, contact with sand/soil, consumption of fruit/vegetables and meat ingestion were not associated with seropositivity of the surveyed population.

Table 7: Univariate analysis of associated risk factors for seropositivity of IgG anti-*T. gondii* antibodies in 878 pregnant women, from 2016 to 2017, in Luanda province, Angola.

Variables	Positive n (%)	Total n (%)	OR (95%CI)	*p*-value
Age range				
≤ 25 years old	60 (25.8)	233(26,5)	0.4299 (0.3084; 0.5993)	< 0.0001*
> 25 years old	288 (44.7)	645 (73.5)		
Education				
Low (up to elementary school)	142 (40.6)	350 (39.9)	1.067 (0.809; 1.406)	0.6726
High (high school or higher education)	206 (39.0)	528 (60.1)		
Employment				
No (homemakers)	86 (42.8)	201 (22.9)	1.185 (0.8606; 1.630)	0.3246
Yes	262(38.7)	677(77.1)		
Marital status				
Married	108 (38.3)	282 (67.9)	0.9207 (0.6885; 1.231)	0.6053
Single	240 (40.3)	596 (32.1)		
Gestational age				
Pregnant woman (1st-2nd trimester)	245 (37.5)	653 (74.4)	0.7113 (0.5235; 0.9663)	0.0328*
Pregnant woman (3rd trimester)	103 (45.8)	225 (25.6)		

Children				
Yes	269(41.0)	656(74.7)	1,445 (1,051 ; 1,986)	0,0260*
No	79 (35.6)	222 (25.3)		
Spontaneous abortion				
Yes	27 (60.0)	45 (5.1)	2.393 (1.297;4.415)	0.0048*
No	321 (38.5)	833 (94.9)		
Hepatitis B infection				
Yes	103 (45 .6)	226 (25.7)	1. 391 (1.024; 1.889)	0.0400*
No	245 (37. 6)	652 (74.3)		
HIV status				
Yes	61 (51.7)	118 (13.4)	1.764 (1.194; 2.605)	0.0050*
No	287 (37.8)	760 (86.6)		
History of abortion				
Yes	217 (45. 4)	478 (54.4)	1.707 (1.296; 2.249)	0.0001*
No	131 (32.8)	400 (45.6)		
Pet at home				
Yes	106 (47.7)	222 (25.3)	1.417 (1.041; 1.927)	0.0320*
No	242 (36.9)	656 (74.7)		
Own cats				
Yes	18 (46.2)	39 (4.4)	1.322 (0.694; 2.519)	0.4070
No	330 (39.3)	839 (95.6)		
Contact with animals other than their own				
Yes	101 (36.6)	276 (31.4)	0.830 (0.618; 1.113)	0.2350
No	247 (41.0)	602(68.6)		
Rats near the house				
Yes	278 (38.9)	715(81.4)	0.845 (0.599; 1.193)	0.3750
No	70 (43.0)	163 (18.6)		
Sand/soil contact				
Yes	69 (43.9)	157 (17.9)	1. 242 (0.876; 1.761)	0.2420
No	279 (38.7)	721 (82.1)		
Recent blood transfusion				
Yes	3 (42.9)	7 (0.8)	1.143 (0.254; 5.143)	1.0000
No	345 (39.6)	871 (99.2)		
Recently had some sting with needle or sharp objects				
Yes	9 (36.0)	25 (2.8)	0.8529 (0.3725; 1.953)	0.8366
No	339 (39.7)	853 (97.2)		
Consumption of washed fruit/vegetables				
Always	259 (40.7)	636 (72.4)	1.199 (0.883; 1. 629)	0.2790
Sometimes	89 (36.8)	242 (27.6)		
Raw/uncooked meat consumption				
Yes	81(37.5)	216 (24.6)	0.888 (0.647; 1.218)	0.4720
No	267 (40.3)	662 (75.4)		
Consumes raw or undercooked egg				
Yes	122 (36.5)	334 (38.0)	0.793 (0.599; 1.143)	0.1180
No	226 (41.5)	544(62.0)		
Consumption of meat from				

hunting sources				
Yes	99 (40.4)	245 (27.9)	1.046 (0.774; 1.413)	0.8180
No	249 (39.3)	633 (72.1)		
Creation animals for own consumption				
Yes	29 (43.9)	66 (7.5)	1.211 (0.730; 2.010)	0.5130
No	319 (39.3)	812 (92.5)		
Access to basic sanitation				
Yes	227 (40.7)	558 (63.6)	1.128 (0.851; 1.496)	0.4310
No	121 (37.8)	320 (36.4)		
Total	**348**	**878**		

* Statiscally significant ($p < 0.05$)

The bivariate logistic regression analysis has shown a significant increased risk associated to toxoplasmosis seroprevalence with pregnant women in the last trimester (OR = 1.521; p = 0.0225), suffering spontaneous abortion (OR = 2.404; p = 0.00499) and having pets (OR = 1.573; p = 0.00386) (Table 8). Regarding the presence of co-infections, pregnant women who tested seropositive for hepatitis B (OR = 1.400; p = 0.0311) or HIV (OR = 1.773; p = 0.00396) were also associated with an increased risk for toxoplasmosis. Also showing an increased risk for toxoplasmosis were pregnant women from 26–35 years of age (OR = 2.063; p = 0.000027) and from 36–47 years of age (OR = 2.258; p = 0.00029), and those having three or more children (OR = 1.631; p = 0.0245).

The multivariate logistic regression analysis (adjusted by age) confirm a significant increased risk for toxoplasmosis in women in the last trimester of pregnancy (OR = 1.457; p = 0.0435), suffering spontaneous abortion (OR = 1.863; p = 0.0457), having pets at home (OR = 1.658; p = 0.0015), testing seropositive for hepatitis B (OR = 1.375; p = 0.0437) or HIV (OR = 1.833; p = 0.0027) (Table 8). Potential associations between the area of residence and toxoplasmosis were also examined (Table 8). Pregnant women residing in the municipality of Belas were less likely to be seropositive for *T. gondii* by bivariate (OR 0.479; p = 0.00104) and by multivariate analysis (OR 0.471; p = 0.0009) compared with other municipalities.

Table 8: Binomial logistic regression models for the final analysis of risk factors associated with seropositivity of IgG anti-*T. gondii* antibodies in 878 pregnant women samples, from 2016 to 2017, in Luanda province, Angola.

Variables	**OR**	**CI**	***p*-value**	**OR**	**CI**	***p*-value**
	Unadjusted			***Adjusted by age***		
Maternal age (Ref= <26years)						
26- 35	2.063	1.476 - 2.909	0.000027 ***	-		
36-47	2.258	1.455 - 3.518	0.00029 ***	-		
Gestacional age (Ref= 1st trimester)						
2nd	1.180	0.856 - 1.632	0.312	1.165	0.841- 1.616	0.3582
3rd	1.521	1.061 - 2.185	0.0225*	1.457	1.011- 2.102	0.0435*
Number of births (Ref= 0)						
1 and 2	1.190	0.859 - 1.656	0.2987	1.005	0.715 -1.417	0.9753
≥ 3	1.525	0.996 - 2.337	0.0054**	1.127	0.682 -1.858	0.6390
Spontaneous abortion (Ref= No)						
Yes	1.984	1.086 – 3.667	0.0263*	1.863	1.014 - 3.465	0.0457*
Hepatitis B infection (Ref= Negative)						
Positive	1.400	1.030 - 1.901	0.0311*	1.375	1.008 -1.874	0.0437*
HIV status (Ref= Negative)						
Positive	1.773	1.201 - 2.623	0.0039**	1.833	1.233 - 2.730	0.0027*
History of abortions (Ref= 0)						
≥1	1.726	1.311 - 2.278	0.00010***	1.320	0.945- 1.849	0.1038
Pet at home (Ref= 0)						
≥1	1.573	1.156 - 2.140	0.00386**	1.658	1.212 - 2.269	0.0015*
Residence (Ref= Luanda)						
Belas	0.479	0.305 -0.737	0.00104**	0.471	0.299- 0.728	0.0009*
Cazenga	1.135	0.667 -1.917	0.559	1.149	0.671 -1.956	0.6079
Cacuaco	0.836	0.452 -1.510	0.636	0.796	0.427 -1.447	0.4616
Viana	1.074	0.767- 1.502	0.673	1.084	0.770 -1.522	0.6418

* Statiscally significant ($p < 0.05$)

Of the 878 pregnant women, the DNA was extracted from the placenta fragments of only two participants, who were positive for IgM and IgG antibodies. Corroborating with the results of the IgG avidity test (high values), indicating a chronic infection, both samples were negative for the *T. gondii* B1 locus gene (S4 File).

The geospatial distribution of pregnant women seropositive for *T. gondii*, HIV and hepatitis B antibodies in Luanda can be observed in Figure 6. Based on participants' place of residence we observed a marked geographical pattern, with a high incidence density near LPMH. The geographical distribution of pregnant women with and without antibodies to *T. gondii*, HIV and hepatitis B was similar, and we observed a statistically significant spatial dependency.

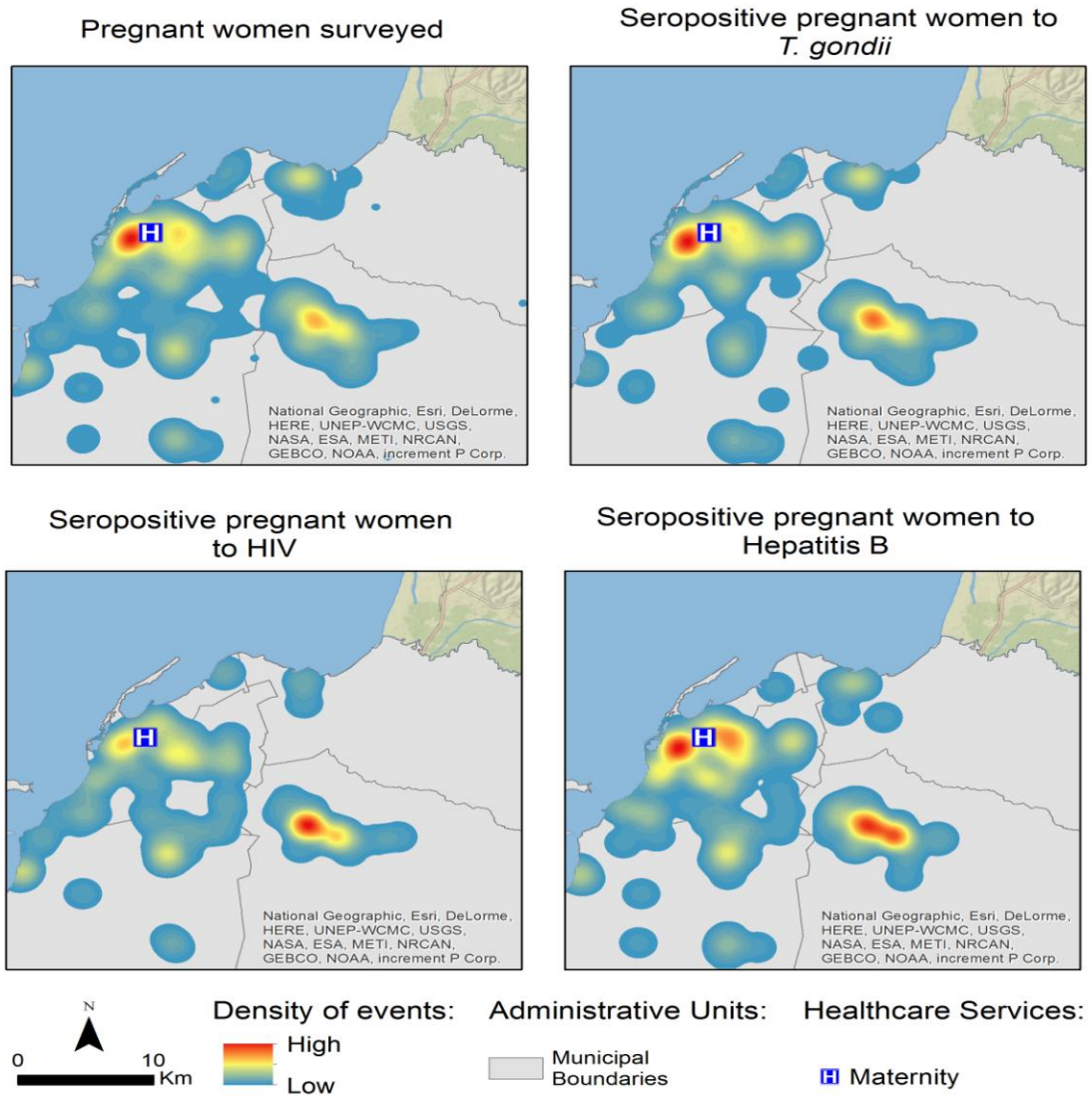

Figure 6: Geographical distribution and Gaussian kernel density surface map of pregnant women (A) with IgG anti-*T. gondii* antibodies (B), IgG anti-HIV antibodies (C) and IgG anti-HBV antibodies (D) in Luanda, Angola.

3.5 Discussion

The current study estimated the prevalence of *T. gondii* infection among pregnant women from Luanda province (Angola), evaluating some epidemiological aspects and identifying the risk factors for toxoplasmosis in this population. Spatial analysis was applied for the first time to the case of Luanda to describe the geographical distribution of toxoplasmosis, HIV and Hepatitis B in pregnant women. To measure seroprevalence of toxoplasmosis, anti-*T gondii* IgM and IgG quantification were performed on pregnant women attending on LPMH from August 2016 to May 2017.

The seroprevalence of toxoplasmosis in our study was 39.6%, and this relatively high value has been consistently reported by several studies conducted in similar populations (Bamba et al., 2017; Ibrahim et al., 2017; Kamal et al., 2015; Paul et al., 2018), even though the prevalence is higher when compared to a previous study performed in Luanda (27.3%) (Lobo et al., 2017). This difference might be related to the method used in the quantification of anti-*T. gondii* IgG and IgM antibodies (Electrochemiluminescence) which has a high sensitivity and specificity.

It is well documented that the prevalence of toxoplasmosis varies across countries, and indeed contrasting data are observed: 6.2% in México (Alvarado-Esquivel et al., 2016); 4.5% to 5.8% in Vietnam (Smit et al., 2019); 44.5% in Tanzania (Paul et al., 2018); 80.3% in the DRC (Yobi et al., 2014); and 85.3% in Ethiopia (Abamecha & Awel, 2016). The differences in prevalence rates in the worldwide population may be associated with various factors related to each region/country and specific characteristics of the population as well with the diagnostic methods used. Other examples include sanitary practices and eating/drinking habits, presence of household felines, poor or lower socioeconomic status, contact with soil, lack of knowledge about the disease, and the overall climate conditions that allow for the protracted environmental survival of the parasite's oocysts. In addition, felids infected with *T. gondii* are largely asymptomatic and can spread the infection, contributing to high infection rates (de Wit et al., 2019; Mareze et al., 2019; Pappas et al., 2009).

Previous studies report the relationship between maternal infection with congenital toxoplasmosis, which is relatively low (<20%) during the first trimester and increases to up to 90% at the end of gestation (Jones et al., 2003; Ortiz-Alegría et al., 2010). Recently, the global prevalence of acute *Toxoplasma* infection in pregnant women was estimated at about 1.1% (Rostami et al., 2019). This prevalence rate represents a significant burden of infection in pregnant women, and suggests that a large number of newborns are at risk of acquiring congenital toxoplasmosis in utero. In the present study, the seronegative pregnant women,

which represented 60.4% of the participants, had follow-up during prenatal care and none of them acquired toxoplasmosis. Initially, there was a suspicion of active toxoplasmosis in two participants who tested positive for IgM and IgG; however, the IgG avidity test allowed the exclusion of recent infection in these two women's. The IgG avidity test, that evaluates the strength of binding of the IgG antibodies to *T. gondii* antigens, allowing the exclusion of a chronic infection (those of high avidity), without risk to the fetus. The use of IgM and IgG avidity tests, together with the analysis of gestational age, has beneficial results in terms of determining the risk of vertical transmission throughout the gestation, thus becoming a model for decision making that is inexpensive and that avoids research and unnecessary treatments, in some cases (Varella et al., 2009).

The characteristics of the individual pregnant women and details on home conditions and food habits were collected using a structured questionnaire. The prevalence of *T. gondii* infection increases with age and the bivariate logistic regression analysis showed that there was a statistical association between the age groups and the positivity for IgG anti-*T. gondii*, with a higher probability of prior exposure for those aged 25 and over. These results were similar to the findings of most surveys that included not only pregnant women, postpartum and of reproductive age, but also those in the general population (Varella et al., 2003; Oliveira-Bahia et al., 2001) and could be explained by the increase in exposure to sources of infection throughout life (Avelar et al., 2018; Laboudi, 2017).

The multivariate logistic regression analysis (adjusted to age) revealed that gestational age, actual spontaneous abortion and the presence of household pets increased the risk of *T. gondii* seropositivity. However, contact with sand/soil, the type of food consumed and the conditions under which food is consumed did not have any significant statistical correlation with *T. gondii* seropositivity in pregnant women. The percentage of pregnant women with prior exposure in the third trimester was high compared with the other trimesters, showing that women in the last trimester of pregnancy had an increased likelihood of *T. gondii* seropositivity.

Moreover, spontaneous abortion was the most common form of pregnancy loss in our study, and is in line with other studies (Chintapalli & Padmaja, 2013; Kamal et al., 2015; Sarkar et al., 2012). We observed a significant association between maternal seropositivity of anti-*T. gondii* antibodies and spontaneous abortion. The high percentage of miscarriages can be explained by the fact that when primary infection in pregnant woman or reactivation in immunocompromised pregnant women occurs, tachyzoites can colonize the placenta tissues during the dissemination process and from there obtain access to the fetus (Pappas et al.,

2009; Robert-Gangneux & Dardé, 2012). In early pregnancy, transplacental passage of tachyzoites is a rare event, but the consequences are serious and one of them is miscarriage. The immunological control of placental infection is probably a key event in the occurrence of congenital infection and miscarriages (Pfaff et al., 2007), but advances in understanding the pathophysiological process still need to be achieved (Robert-Gangneux & Dardé, 2012). Unfortunately, we do not have fetus material or placenta from those cases of spontaneous abortion to confirm the presence of *T. gondii*. In the future, the implementation of the diagnosis of maternal primary infection as well the appropriate diagnosis of congenital toxoplasmosis will be crucial.

Angola is among the countries of the Southern Africa region with the lowest HIV prevalence rate, estimated at 2.35% in the adult population aged between 15–49, with 2.6% in women and 1.3% in pregnant women aged 15–49 (MINSA, 2012; MINSA, 2015). HIV transmission in Angola is predominantly heterosexual, in 79.2% of reported cases, and the vertical transmission accounts for about 6% and blood transfusion for 0.5% (via transfusion and use of contaminated objects).

In the present study, the seroprevalence of *T. gondii* in HIV-positive pregnant women was higher than those who tested HIV-negative, with the difference being statistically significant. Similar results were reported in Ghana (Ayi et al., 2016) and in Lusaka, Zambia (Frimpong et al., 2017), where the prevalence of *T. gondii* infection among pregnant HIV-positive women was also high. In contrast, however, a study carried out in northern Nigeria (Ogoina et al., 2013) found that the relationship between these two groups was not statistically significant.

The severity and clinical significance of human toxoplasmosis varies with the host's immune status (Ayi et al., 2016). Therefore, HIV-infected patients may be at higher risk of developing toxoplasmosis disease, especially when the TCD4+ cell count falls below 100 cells/L of blood (Laboudi, 2017). Toxoplasmosis is the most common opportunistic infection in HIV-seropositive immunocompromised hosts, where it predominantly occurs as a reactivation of endogenous infection (Ayi et al., 2016).

A higher prevalence of *T. gondii* infection was also observed among hepatitis B seropositive pregnant women than in seronegative women, and the statistical analysis showed that Hepatitis B-positive women are at high risk to have toxoplasmosis. It was reported that hepatitis B infection in pregnant women also leads to increased risk of chronicity of cirrhosis and / or hepatocellular carcinoma (Xiao et al., 2007). Because of the prevalence of infection among the population, it is extremely important to perform serological screening in the

prenatal period in order to initiate early treatment, or even avoid vertical transmission (Piazza et al., 2010). To have HIV and/or hepatitis B can lead to a reactivation of toxoplasmosis and, in some cases, trigger the most serious clinical manifestations of these diseases (Xiao et al., 2007).

It is believed that cats are the main carriers and transmitters of *T. gondii* infection to humans (Weiss & Kim, 2013). Several studies have observed high IgG seropositivity related to the presence of cats (de Wit et al., 2019; Jiang et al., 2018). In the present study, the seroprevalence of *T. gondii* among pregnant women who had cats was higher than in pregnant women who did not have cats. However, in our study no significant association was found between the presences of cats in the household and *T. gondii* seropositivity. Similar results were shown by studies conducted in Burkina Faso (Bamba et al., 2017), the UK (Nash et al., 2005), Brazil (Cademartori et al., 2008), Turkey (Ghoneim et al., 2009) and Nigeria (Ishaku et al., 2009). In contrast, surveys performed in the USA (Dubey & Jones, 2008), Ethiopia (Abamecha et al., 2016; Agmas et al., 2015), and Taiwan (Lin et al., 2008) showed that pregnant women in contact with infected cats will naturally be at greater risk of acquiring the infection. It is important to mention that the risk of contracting *T. gondii* infection does not result simply from contact with domestic cats only (Nissapatorn et al., 2011). Occasional contact with cats or owning them may not necessarily be a risk factor, while frequent exposure to feline stools (Dubey, 2000; Mwambe et al., 2013) and/or neglect of preventive measures, i.e. not washing hands or wearing gloves, may increase the risk of infection to an appreciable level (Cong et al., 2015). On the other hand, having cats as household pets in Angola is not a common practice. However, street cats are usually seen, which can pose a serious risk to toxoplasmosis since they may be responsible for much of the environmental contamination with oocysts in the water or soil that may remain viable for years (Torrey &, Yolken, 2013).

We found that having pets at home, not specifically the cat, is a potential risk factor for *T. gondii* seropositivity in pregnant women. The logistic regression analysis showed that contact with pets at home and/or in family member's/friends' homes is associated with *T. gondii*–seropositive women. Cats can disseminate oocysts in the environment, while dogs can act as mechanical vectors assisting in the transmission of infectious forms of *T. gondii* (Avelino et al., 2004).

Basic sanitary conditions can contribute to the transmission of several infectious diseases. In Angola, according to data from Census 2014, only 47% of households have access to appropriate sources of drinking water: 46% using tankers and 28.9% using public

water (INE, 2014; INE, 2015). There is a contrast in water supply levels between urban and rural areas of the country. The urban population with access to drinking water is around 67%, while the rural population is only 32%. Between provinces differences in access to adequate drinking water are also seen. Moreover, about one third of households (32%) have some kind of appropriate and unshared sanitation facility, with the proportion higher in urban areas (46%) than in rural areas (11%). Although our data do not show an association between the basic sanitation conditions (specifically source of drinking water) and *T. gondii* seropositivity, we emphasized that most pregnant women were from urban areas with better sanitary conditions.

The distribution of *T. gondii* seropositive pregnant women varied among the municipalities that comprise Luanda province, with the highest number of cases in municipalities with a larger population: Luanda, Viana, Belas, Cazenga and Cacuaco. The location of the LPMH is determinant, given its location in the center of Luanda and its easy access from the other municipalities, reasons for the population to commonly resort to the referred maternity hospital. However, the seroprevalence and the risk to acquire toxoplasmosis in the municipality of Belas was lower when compared to the other municipalities.

Previous studies have shown that living in rural or suburban regions with soil exposure is a risk factor for toxoplasmosis in pregnant women (Cong et al., 2015; Fromont et al., 2009). Our results point that the prevalence of *T. gondii* among pregnant women is higher in the group in frequent contact with the soil; however, no statistically significant association was observed between *T. gondii* seropositivity and activities and practices that promote contact with soil (gardening). This result may have been influenced by the fact that most of the pregnant women live in urban area and do not have contact with soil.

One of the risk factors often associated with acute toxoplasmosis in pregnant women is eating raw or undercooked meat (Al-Harthi et al., 2006). Our results showed that *T. gondii* seropositivity in pregnant women was not associated with the consumption of raw / undercooked meat or the consumption of raw vegetables and fruits. These findings are in agreement with the study carried out in China (Cong et al., 2015) and Ethiopia (Abamecha & Awel, 2016) but in contrast with other reported studies (Andiappan et al., 2014; Belluco et al., 2016; Ferreira et al., 2018; Gebremedhin et al., 2013). These differences may be due to frequency of consumption, type of consumed meat and prevalence of the parasite in the animals (Cook et al., 2000; Weiss & Dubey, 2009). Despite our data, *T. gondii* infection in animals (i.e. pigs, cattle, sheep, chickens, and goats) in the province of Luanda may contribute

to toxoplasmosis transmission. Large-scale studies should be performed to estimate the association between this potential risk factor for *T. gondii* infection in Angola, including toxoplasmosis surveys in animals and the water supply.

The study of toxoplasmosis is important in the planning of educational programs aimed at reducing the incidence of toxoplasmosis during pregnancy (Avelar et al., 2018). The best measures to prevent toxoplasmosis are those directed at primary prevention, basically characterized by public health and education programs recommended through campaigns, lectures, pamphlets and the guidance provided by health teams informing pregnant women to avoid contact with animals, potentially harmful contaminated materials, and avoid the consumption of raw or undercooked meat. The use of gloves when handling the soil is also emphasized. A reduction of 63% in the first gestational infection can be obtained following these recommendations during pregnancy (Varella et al., 2009). Unfortunately, routine screening for *T. gondii* infection during pregnancy is not performed for most Angolan women, as the test is not mandatory in prenatal care and the associated costs are high cost. In addition, poor access to higher education and the lack of promotion of public health in general explain how a very high percentage of the population lacks knowledge of toxoplasmosis as seen in the present study.

3.6. Conclusions

There is a significant number of pregnant women in Luanda who are not immunized for toxoplasmosis and thus at risk of acquiring the primary infection during pregnancy and consequently infecting the fetus (congenital toxoplasmosis). Gestational age, the presence of pets at home, HIV-seropositivity and hepatitis B-seropositivity were identified as risk factors for *T. gondii* infection. However, contact with cats and sand/soil, the type of food consumed and the conditions under which food consumption occurs were not statistically significant and may play a secondary role in these particular female populations.

Therefore, it is crucial to include education on toxoplasmosis prevention in prenatal counseling, to enable the diagnosis of maternal primary infection as well the appropriate diagnosis of congenital toxoplasmosis. Our results also underscore the need for diagnostic and clinical follow-up of other infectious diseases, including HIV and hepatitis B during pregnancy. More studies should be performed to identify the risk factors for toxoplasmosis in Angola, including *T. gondii* surveys carried out in other groups, in animals and the water supply.

CHAPTER 4

Seroepidemiology study of Cytomegalovirus and Rubella in Pregnant Women in Luanda, Angola: Geospatial distribution and its association with socio-demographic and clinical-obstetric determinants

This chapter correspond to the article:

Amélia N Vueba, Clarissa Faria, Ricardo Almendra, Paula Santana and Maria C Sousa (2020), "Seroepidemiology study of Cytomegalovirus and Rubella in Pregnant Women in Luanda, Angola: Geospatial distribution and its association with socio-demographic and clinical-obstetric determinants", submitted to: *BMC Infectious Diseases*

4.1. Abstract

The main objective was to study the seroprevalence of anti-CMV and anti-Rubella antibodies in pregnant women of Luanda (Angola), identify the primary maternal infection during gestation and to evaluate the socio-demographic risk factors associated with CMV and Rubella virus infections. A prospective cross-sectional study was conducted from August 2016 to May 2017. Blood samples were collected and specific anti-CMV and anti-Rubella antibodies (IgG and IgM) were quantified by electrochemiluminescence (COBAS e411). Demographic and clinical data were collected by standardized questionnaire. Bivariate and multivariate logistic regression analysis was used to quantify the effect of clinical and obstetric risk factors on virus seroprevalence. The level of statistical significance was set as $p<0.05$, and Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were computed.

The 396 pregnant women participated in the study aged from 15 to 47. Of the participants, 382 (96.5%) had anti-CMV IgG antibodies, 8 (2.0%) had anti-CMV IgG and IgM antibodies and 6 (1.5%) were seronegative. For Rubella virus, 347 (87.6%) were positive for anti-IgG, 4 (1.0%) positive for anti-IgG and IgM, and 45 (11.4%) were seronegative. The mean age of CMV positivity was 28.4 (SD ± 6.2) and for Rubella virus was 28.6 (SD ± 6.1). The multivariate logistic regression analysis has shown a significant association between Rubella virus infection and pregnant women without child (OR 2.673; CI: 1.026 - 7.007) and suffering spontaneous abortion (OR 3.232; CI: 1.192 - 7.952). In contrast, the level of schooling, residence, occupation, marital status, number of children in the household, basic sanitation, gestational age, history of miscarriages and hepatitis B were not significantly associated with the Rubella virus infection.

Overall, this study showed that there is a high seroprevalence of anti- CMV and anti-Rubella antibodies in pregnant women in Luanda. Therefore, it is important a rapid and accurate diagnosis of CMV and Rubella infection in pregnant women to prevent congenital infections. Rubella vaccination should be offered to women non-immune to Rubella. Overall, it would be important to implement national screening for CMV, Rubella and other diseases linked to maternal and child health.

4.2. Introduction

Several infections caused by microorganisms can be transmitted to the fetus during pregnancy, causing fetal death or sequelae to the newborn: Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, CMV and Herpes infections as known by TORCH infections (Numan et al., 2015). The agents of the TORCH infections, lead to the development of a maternal infection and can pass in the uterine circulation at any gestational age (Numan et al., 2015). On the other hand, the infection can lead to spontaneous abortions, sterility, congenital malformations and intrauterine fetal loss. Prenatal surveillance is important for the prevention of vertical transmission. The Angolan National Health Development Plan (2012-2025) does not include the control of diseases such as Rubella and CMV. The absence of early diagnosis and the consequent lack of treatment of maternal infections can considerably increase the rates of perinatal morbidity and mortality.

CMV or Herpesvirus 5 is a member of the Herpesviridae family, being the largest virus (Tavares et al., 2011). Man is the only reservoir for CMV and the virus transmission occurs after direct or indirect contact with saliva, oropharyngeal secretions, endocervical secretions, urine, sperm, breast milk, tears, blood products or organs, and by vertical transmission (Schottstedt et al., 2010). However, CMV infection is less contagious than that of other members of the same family of Varicella Zoster Virus (VZV) (Tavares et al., 2011). The main infected cells are the endothelial cells and the leukocytes, and then the hematogenous propagation is carried out in the organism. Like the other members of the Herpesviridae family, CMV arrest in body (latent phase) after a primary infection. The acquired immunity is not completely protective and secondary infections may occur during the latent phase (Benoist et al., 2008). The pregnant women are contaminated by patients who excrete large numbers of viruses, including children, immunosuppressed patients and transplanted patients. Vertical transmission is transplacental hematogenous and occurs during maternal viraemia. The transmission rate varies with the type of maternal infection: between 30 and 50% on primary infections and 0.1 and 3% on secondary infections (re-infections or reactivations of a latent infection) (Benoist et al., 2008; Tavares et al., 2011). The gestational age may also play an important role in the vertical transmission rate, increasing with gestational age: 36% in the first trimester, 44% in the second and 77.6% in the last trimester. However, the consequences of CMV infection are more severe when maternal infection occurs before 20 weeks (Benoist et al., 2008; Tavares et al., 2011).

Also, CMV is considered a common opportunistic infection among individuals infected with the HIV, a major source of serious viral complications among organ transplant recipients and a major cause of hearing loss, vision loss and mental retardation in children with congenital infection (Gumbo et al., 2014).

Rubella is caused by a virus of the Togaviridae family and genus Rubivirus. The most common form of virus transmission is through direct contact with respiratory secretions droplets of infected persons (Griffin et al., 2013). It is a benign disease characterized by a macular rash accompanied by low fever, joint pain, pharyngitis and cervical adenopathies (Griffin et al., 2013), however, it becomes severe to fetus when it occurs during pregnancy (Banatvala and Brown, 2004).

The teratogenic properties of the rubella virus were first discovered in Australia in 1941 by Gregg who associated the occurrence of rubella during pregnancy with the presence of congenital cataracts (Gregg, 1941). Maternal infection with the rubella virus during the first trimester is often associated with fetal death, miscarriage or adverse neonatal outcome, including heart problems, cataracts and deafness, known as CRS, which has a higher neonatal morbidity (Ojala et al., 1973). This severity of fetal infection is related to the period of organogenesis, due to the high virus tropism for fetal tissues (Best, 2007). Some defects were later reported as consequence of maternal infections in the second trimester (Ojala et al., 1973). In order to avoid vertical transmission, seronegative women should be detected during the preconception period and their pregnancies be planned after immunization (Bouthry et al., 2014).

In Angola, there are no reports of CMV and Rubella studies. Therefore, the main objective of this work was to study the seroprevalence of anti-CMV and anti-Rubella antibodies in pregnant women attending at LPMH of Luanda and to provide a detailed analysis of the geographical distribution. In addition, we aimed to identify the maternal infection and to evaluate the risk factors associated with CMV and Rubella infection.

4.3. Materials and methods

4.3.1. Ethical Considerations

The present study has been approved by the Research Ethics Committee of LPMH through the National Institute of Public Health of the Republic of Angola (nº 301019; S1 File). Participant individuals provided a written signed informed consent prior to sample collection and for participants younger than 18 years, informed consent was provided by parents or guardians after a detailed explanation of the objectives of the work.

4.3.2. Study Population

The study population was constituted by pregnant women monitored for routine prenatal assessment at LPMH, a reference maternity in Angola, located in Luanda. The referred maternity is a tertiary-level public health institution specialized in maternal and child health care, teaching and research. The health institution offers outpatient and inpatient services, has 400 beds for hospitalization video laparoscopy, hysteroscopy, milk bank, pathological anatomy, assisted reproductive services, genetics and mammography. Consequently, the women attended in the LPMH come from all over the country, however mainly from Luanda, the capital of Angola. The study included women aged from 15 to 47 years, who had a pregnancy proven by ultrasonography and laboratory tests. For the obstetric follow-up we counted on the collaboration of the medical and nursing team of the department of obstetrics of the LPMH.

4.3.3. Sociodemographic, clinical and housing characteristics of the pregnant women

Standardized questionnaires in face-to-face interviews were used to obtain the socio-demographic, clinical, and housing characteristics from the pregnant women (S2 File). Socio-demographic items included age, residence, occupation, schooling level, and socio-economic status. Clinical characteristics included gestation age, number of pregnancies, history of abortion, the frequency of miscarriages, child born premature, number of children with disabilities and with mental retardation, presence any underlying disease, long-term fever, history of hepatitis B and HIV. The housing characteristics like type of flooring, destination of faeces, the number of children in the household and crowding were also inclued. The questionnaire was written in Portuguese, the official language in the Republic of Angola, developed and revised accordingly.

4.3.4. Blood Sample Collection and Laboratory Procedures

A cross-sectional study was conducted from August 2016 to May 2017 and a total of 396 pregnant women were included in the survey. Blood samples were collected and serum samples were obtained after centrifugation. These samples were immediately transferred (properly packaged in dry ice) to the Clinical Pathology Service of Clínica Sagrada Esperança (Luanda) and kept at -80°C until serological analysis. The quantification of anti-CMV and anti-Rubella IgG and IgM antibodies was done by ECL using commercially available kits for

COBAS e411 (Roche, Sistemas de Diagnósticos Lda), according to the guidelines of the manufacturer.

The anti-CMV IgG and IgM levels were expressed as IU/mL and COI (Cut-off index), respectively: values <0.5 IU/mL of anti-CMV IgG antibody are considered negative and values ≥1.0 IU/mL are considered positive; values <0.7 COI of anti-CMV IgM antibody are considered negative and values ≥1.0 are considered positive.

The anti-Rubella IgG and IgM levels were expressed as IU/mL and COI, respectively: values <10.0 IU / mL of anti-Rubella IgG antibody are considered negative and values ≥10.0 IU / mL are considered positive; values <0,8 COI of anti- Rubella IgM antibody are considered negative and values ≥1.0 are considered positive.

4.3.5. Geospatial analysis

The address of pregnant women was collected during the interview allowing the identification of the residence place. This information was converted into geographic coordinates (latitude and longitude) through the www.google.pt/maps/. The spatial distribution of pregnant women was assessed through a Kernel Density Function that allowed the estimation of the intensity of events across a surface.

4.3.6. Stastical Analysis

The data entry was carried out using Excel software and analysed using SPSS version 20. The exploratory analysis of the categorical variables and quantitative variables are presented as percentages (mean ± SD). Bivariate and multivariate logistic regression was developed to assess the effect of different risk factors on rubella virus seroprevalence. The level of statistical significance was set as $p<0.05$, and Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were computed.

4.4. Results

Between August 2016 and May 2017, a total of 396 pregnant women were tested for anti-CMV and anti-Rubella antibodies. The distribution of IgG and/or IgM antibodies to CMV and rubella of seropositive and seronegative pregnant women is summarized in Table 9. The majority of women had previous exposure to the CMV virus: 382 (96.5%) were positive for anti-CMV IgG; 8 (2.0%) were positive for anti-CMV IgM and IgG; and 6 (1.5%) were seronegative for anti-CMV antibodies. For Rubella virus, 347 (87.6%) were positive for anti-Rubella IgG, 4 (1.0%) were positive for anti- Rubella IgM and IgG and 45 (11.4%) were

seronegative (Table 9). Although the majority of women are immune to rubella virus, a significant number are susceptible to the primary infection.

Table 9: Profile of IgG and IgM antibodies anti-CMV and anti-Rubella virus on pregnant women from Luanda (Angola).

Immune response	Number	Percentage (%)	Interpretation
Anti-CMV			
IgG(+) IgM (-)	382	96.5	Previous Exposure
IgG(+) IgM (+)	8	2.0	Active (Primary/Latent) infection
IgG(-) IgM (-)	6	1.6	Susceptible to primary infection
IgG(-) IgM (+)	0-	0-	Recent primary infection
Total positive	390	98.5	Overall prevalence
Total negative	6	1.5	Overall prevalence
Anti-Rubella			
IgG(+) IgM (-)	347	87.6	Immune
IgG(+) IgM (+)	4	1.0	Active (Primary/Latent) infection
IgG(-) IgM (-)	45	11.4	Susceptible to primary infection
IgG(-) IgM (+)	0	0	Recent primary infection
Total positive	351	88.6	Overall prevalence
Total negative	45	11.4	Overall prevalence

The characteristics of the individual pregnant women and details on home conditions and food habits were collected using a structured questionnaire (S5 File). The ages of the pregnant women ranged from 15 to 47 years with an average of 28.4±6.2 (Mean±SD); pregnant women between 26 and 35 years were the majority of participants (n = 207) (Table 10). Regarding education level, 3 (0.8%) are illiterate, 149 (37.6) have basic education, 200 (50.5%) have a high school education and 44 (11.1%) have higher education. In relation to residence, 180 (45.5%) live in the municipality of Luanda, 87 (22.0%) in the municipality of Belas, 86 (21.7%) in the municipality of Viana, 29 (7.3%) in the municipality of Cazenga and 14 (3.5%) in the municipality of Cacuaco. Regarding occupancy,150 (37.9%) women work on public administration services, 64 (16.2%) are students, 63 (15.9%) are domestic, 49 (12,4%) are street vendors, 39 (9.8%) are store employees and 31 (7.8%) work in the catering area. The majority of pregnant women (270; 68.2%), are single and 126 (31.8%) are married. The

mean of gestational age was 14.5± 6.8 (Mean±SD) weeks and 63.9% of the pregnant women had more than one child (with mean birth rate of 1.8±0.7 (Mean±SD).

More than half of the participants (64.4%) reported having basic sanitation at home and 141 (35.6%) did not have (Table 10). Most of the pregnant women, 355 (89.6%) reported lack of knowledge of the virus diseases under study, 36 (9.1%) had hear of CMV and Rubella but they do not know anything about it and 5 (1.3%) pregnant women reported knowing something about the diseases under study.

Table 10: Sociodemographic characteristics of seropositive and seronegative pregnant women to CMV and Rubella virus and respective prevalences.

	CMV				Rubella				
Characteristics	**Positive n (%)**	**Seroprevalence (%)**	**Negative n (%)**	**Seroprevalence (%)**	**Positive n (%)**	**Seroprevalence (%)**	**Negative n (%)**	**Seroprevalence (%)**	**Total**
Age group (years)									
≤ 19	23 (5.9)	100.0	0 (00.0)	0 (00.0)	18 (5.1)	78.3	5 (11.1)	21.7	23 (5.8)
20-25	110 (28.2)	99.1	1 (16.7)	0.9	97 (27.6)	87.4	14 (31.1)	12.6	111(28.0)
26-35	202 (51.8)	97.6	5 (83.3)	2.4	185 (52.7)	89.4	22 (48.9)	10.6	207(52.3)
36-47	55 (14.1)	100.0	0 (00.0)	0 (00.0)	51 (14.6)	92.7	4 (8.9)	7.3	55 (13.9)
Education									
Illiterate	3 (0.8)	100.0	0 (00.0)		3 (0.9)	100.0	0 (00.0)	0 (00.0)	3 (0.8)
Elementary School	148 (37.9)	99.3	1 (16.6)	0.7	136 (38.7)	91.3	13 (28.9)	8.7	149 (37.6)
High school	196 (50.3)	98.0	4 (66.7)	2.0	172 (49.0)	86.0	28 (62.2)	14.0	200 (50.5)
Higher education	43 (11.0)	97.3	1 (16.7)	2.7	40 (11.4)	90.9	4 (8.9)	9.1	44 (11.1)
Residence									
Belas	85 (21.8)	97.7	2 (33.3)	2.3	76 (21.7)	87.4	11 (24.2)	12.6	87 (22.0)
Cacuaco	13 (3.3)	92.9	1 (16.7)	7.1	13 (3.7)	92.9	1 (2.2)	7.1	14 (3.5)
Viana	85 (21.8)	98.8	1 (16.7)	1.2	76 (21.7)	88.4	10 (22.2)	11.6	86 (21.7)
Cazenga	29 (7.5)	100.0	0 (00.0)	0.0	25 (7.1)	86.2	4 (9.0)	13.8	29 (7.3)
Launda	178 (45.6)	98.9	2 (33.3)	1.1	161 (45.8)	89.4	19 (42.2)	10.6	180 (45.5)
Occupation									
Homemakers	62 (15.9)	98.4	1 (16.7)	1.6	57 (16.2)	90.5	6 (13.3)	9.5	63 (15.9)
Public function	147 (37.7)	98.0	3 (50.0)	2.0	131 (37.3)	87.3	19 (42.2)	12.7	150 (37.9)
Student	62 (15.9)	96.9	2 (33.3)	3.1	56 (16.0)	87.5	8 (17.8)	12.5	64 (16.2)
Restoration	31 (7.9)	100.0	0 (00.0)	0 (00.0)	26 (7.4)	83.9	5 (11.1)	16.1	31 (7.8)
Street vendor	49 (12.6)	100.0	0 (00.0)	0 (00.0)	45 (12.8)	91.8	4 (8.9)	8.2	49 (12.4)
shop assistants	39 (10.0)	100.0	0 (00.0)	0 (00.0)	36 (10.3)	92.3	3 (6.7)	7.7	39 (9.8)
Marital status									
Married	126 (32.3)	100.0	0 (00.0)	0 (00.0)	110 (31.3)	87.3	16 (35.6)	12.7	126 (31.8)
Single	264 (67.7)	97.8	6 (100.0)	2.2	241 (68.7)	89.3	29 (64.4)	10.7	270 (68.2)
Gestational age									
1st Trimester	199 (51.0)	98.5	3 (50.0)	1.5	181 (51.6)	89.6	21 (46.7)	10.4	202 (51.0)
2nd Trimester	152 (39.0)	98.1	3 (50.0)	1.9	135 (38.4)	87.1	20 (44.4)	12.9	155 (39.1)

3rd Trimester	39 (10.0)	100.0	0 (00.0)	0 (00.0)	35 (10.0)	89.7	4 (8.9)	10.3	39 (9.9)
Number of births									
0	84 (21.5)	97.7	2 (33.3)	2.3	71 (20.2)	82.6	15 (33.3)	17.4	86 (21.7)
1	118 (30.3)	99.2	1 (16.7)	0.8	103 (29.3)	86.6	16 (35.5)	13.4	119 (30.0)
2	66 (16.9)	97.1	2 (33.3)	2.9	61 (17.4)	89.7	7 (15.6)	10.3	68 (17.2)
≥ 3	122 (31.3)	99.2	1 (16.7)	0.8	116 (33.1)	94.3	7 (15.6)	5.7	123 (31.1)
Spontaneous abortion									
Yes	27 (6.9)	100.0	0 (00.0)	0 (00.0)	20 (5.7)	74.1	7 (15.6)	25.9	27 (6.8)
No	363 (93.1)	98.4	6 (100.0)	1.6	331 (94.3)	89.7	38 (84.4)	10.3	369 (93.2)
History of abortion									
No	219 (56.2)	98.2	4 (66.7)	1.8	190 (54.1)	73.2	33 (73.3)	26.8	223 (56.3)
Yes	171 (43.8)	98.8	2 (33.3)	1.2	161 (45.9)	93.1	12 (26.7)	6.9	173 (43.7)
children at home									
Yes	386 (99.0)	98.5	6 (100)	1.5	348 (99.1)	88.8	44 (97.8)	11.2	392 (99.0)
No	4 (1.0)	100.0	0 (00.0)	0 (00.0)	3 (0.9)	75.0	1 (2.2)	25.0	4 (1.0)
Number of children in the household									
0	4 (1.0)	100.0	0 (00.0)	0 (00.0)	3 (0.9)	75.0	1 (2.2)	25.0	4 (1.0)
1	137 (35.1)	98.6	2 (33.3)	1.4	118 (33.6)	84.9	21 (46.7)	15.1	139 (35.1)
2	152 (39.0)	98.1	3 (50.0)	1.9	141 (40.2)	91.0	14 (31.1)	9.0	155 (39.1)
≥3	97 (24.9)	99.0	1 (16.7)	1.0	89 (25.3)	90.8	9 (20.0)	9.2	98 (24.8)
Hepatitis B infection									
No	265 (67.9)	98.5	4 (66.7)	1.5	237 (67.5)	88.1	32 (71.1)	11.9	269 (67.9)
Yes	125 (32.1)	98.4	2 (33.3)	1.6	114 (32.5)	89.8	13 (28.9)	10.2	127 (32.1)
HIV status									
No	332 (85.1)	98.5	5 (83.3)	1.5	294 (83.8)	87.2	43 (95.6)	12.8	337 (85.1)
Yes	58 (14.9)	98.3	1 (16.7)	1.7	57 (16.2)	96.6	2 (4.4)	3.4	59 (14.9)
Awareness of CMV and Rubella									
Does not know anything about the disease	351 (90.0)	98.9	4 (66.7)	1.1	314 (89.5)	88.5	41 (91.1)	11.5	355 (89.6)
Heard speak, but do not know anything	34 (8.7)	94.4	2 (33.3)	5.6	33 (9.4)	91.7	3 (6.7)	8.3	36 (9.1)

about it									
Know anything about the disease	5 (1.3)	100.0	0 (00.0)	0 (00.0)	4 (1.1)	80.0	1 (2.2)	20.0	5 (1.3)
Pre-natal consultation was performed in all pregnancies									
Yes	381 (97.7)	98.4	6 (100.0)	1.6	342 (97.4)	88.4	45 (100.0)	11.6	387 (97.7)
No	9 (2.3)	100	0 (00.0)	0 (00.0)	9 (2.6)	100.0	0 (00.0)	0 (00.0)	9 (2.3)
Access to basic sanitation									
Yes	251 (64.4)	98.4	4 (66.7)	1.6	224 (63.8)	87.8	31 (68.9)	12.2	255 (64.4)
No	139 (35.6)	98.6	2 (33.3)	1.4	127 (36.2)	90.1	14 (31.1)	9.9	141 (35.6)
Total	**390**		**6**		**351**		**45**		**396**

The frequency of CMV infection was higher among pregnant women in the first trimester (199; 51.0%), followed by pregnant women in the second (152; 39.0%) and in the third trimester (39; 10.0%). The frequency of CMV infection in relation to the pregnant women age was 5.9% (n=23) in the group of ≤ 19 years, 28.2% (n=110) in the group of 20-25 years, 51.2% (n=202) in the group of 26-35, and 14.1% (n=55) in the group of 36-47 years (Table 10). The mean age of CMV positivity was 28.0±7.1 (Mean±SD). Regarding the obstetric history, the frequency of CMV infection was similar in pregnant women regardless of the month of gestation and parity (number of births or miscarriages). Among the pregnant women, 173 (43.7%) had a history of abortion and 223 (56.3 %) never had a miscarriage and there was no child death in the postpartum period (Table 10).

The frequency for Rubella virus was also higher among pregnant women in the first trimester (181; 51.6%) followed by pregnant women in the second trimester (135; 38.4%) and in the third trimester (35; 10.0%) (Table 10). The frequence of infection in relation to the age of pregnant women was 5.1% (n=18) in the group of ≤ 19 years, 27.6% (n=97) in the group of 20-25 years, 52.7% (n=185) in the group of 26-35 years and 14.6% (n=51) in the group of 36-47 years (Table 10). The mean age of rubella virus positivity was 28.6±6.2 (Mean±SD).

We also study the frequence of hepatitis B and Human Immunodeficiency Virus (HIV) in pregnant women and their association with Rubella and CMV virus infections. (Table 10). In relation to Hepatitis B, 269 pregnant women (67.9%) presented a negative result and 127 (32.1%) a positive result, of which 125 (98.4%) had a positive result to CMV infection (122 with previous exposure and 3 an active primary infection), and 2 (1.6%) presented susceptibility to primary CMV infection (Table 10). Also of the 127 pregnant women positive for hepatitis B, 114 (89.8%) presented a positive result to Rubella virus (112 had prior exposure to the virus and 2 presented active primary infection), and 13 (28.9 %) were susceptible to primary infection. In relation to HIV, 337 (85.1%) pregnant women had negative serology, and 59 (14.9%) had positive results, of which 58 (98.3%) with previous exposure to CMV, and 1 (6.7 %) with susceptibility to CMV infection (Table 10). On the other hand, of the 59 pregnant women with HIV positive serology, 57 (96.6%) had a positive result to rubella virus (56 with previous exposure to the virus and 1 with active virus infection), and 2 (4.4%) with susceptibility to the infection.

The seroprevalence of Rubella infection in Angolan pregnant women according to independent categorical variables evaluated in this study are summarized in Table 11. In the bivariate logistic regression analysis, the variables of number of births (OR 2.478; CI: 1.144 - 5.374), history of miscarriages (OR 2.062; CI: 1.069 - 4.194), and spontaneous abortions

occurred during the study (OR 3.048; CI: 1.135 - 7.394), were considered an independent predictor of IgG seropositivity against rubella among pregnant women (Table 11). Other analyzed factors such as maternal age, gestacional age, residence, occupation, educational status, awareness of Rubella, access to basic sanitation, hepatitis B, were not associated with seropositivity on surveyed population.

The multivariate logistic regression analysis (adjusted to age) confirm a significant increased risk for rubella in women without children (OR 2.673; CI: 1.026 - 7.007) and suffering spontaneous abortion (OR 3.232; CI: 1.192 - 7.952).

Table 11: Binomial logistic regression models for the final analysis of risk factors associate for seropositivity of IgG anti-rubella antibodies in 396 pregnant women in Luanda province, Angola.

Variable	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
	Unadjusted		***Adjusted by age***	
Age				
≤ 25 years old (ref)				
25 – 29 years old	0.962 (0.439 - 2.059)	0.923		
≥30 years old	0.609 (0.286 - 1.272)	0.190		
Schooling				
Low (up to elementary school)	0.619 (0.304 - 1.196)	0.167	0.615 (0.300 - 1.197)	0.166
High (high school or higher education) (ref)				
Employment				
Public administration service	1.510 (0.704 - 3.395)	0.299	1.761 (0.792 - 4.121)	0.175
Homemakers	1.033 (0.340 - 2.863)	0.951	1.102 (0.361 - 3.079)	0.856
Student	1.402 (0.516 - 3.664)	0.492	1.064 (0.368 - 3.001)	0.905
Street vendor, saleslady and restaurant waitress (ref)				
Marital status				
Single (ref)				
Married	1.347 (0.696 - 2.543)	0.363	1.390 (0.715 - 2.639)	0.319
Gestational age				
1st trimester (ref)				
2nd and 3rdtrimestre	1.346 (0.722 - 2.536)	0.351	1.377 (0.737 - 2.604)	0.317
Number of births				
0	2.478 (1.144 - 5.374)	0.0203*	2.673 (1.026 - 7.007)	0.0439*
1	1.692 (0.789 - 3.627)	0.1724	1.694 (0.748 - 3.825)	0.2020

2 or 3 (ref)				
children at home				
0 or 1	1.818 (0.969 - 3.404)	0.0606	1.689 (0.840 - 3.394)	0.139
2 or more (ref)				
Spontaneous abortions				
Yes	3.048 (1.135 - 7.394)	0.018*	3.232 (1.192 - 7.952)	0.0139*
No (ref)				
History of miscarriages				
Yes (ref)				
No	2.062 (1.069 - 4.194)	0.0364*	2.048 (0.957 - 4.508)	0.0676
Hepatitis B				
Positive	0.844 (0.413 - 1.636)	0.627	0.839 (0.410 - 1.629)	0.617
Negative (ref)				
Access to basic sanitation				
Yes (ref)				
No	0.892 (0.452 - 1.697)	0.735	0.867 (0.437 - 1.653)	0.672

OR: Odds Ratio, CI: Confidence Interval * Statistically significant ($p<0.05$)

The geospatial distribution of pregnant women seropositive for CMV and Rubella antibodies in Luanda can be observed in Figure 7. Based on participants' place of residence we observed a marked geographical pattern, with a high incidence density near Lucrécia Paim Maternity. The geographical distribution of pregnant women with and without antibodies to CMV and Rubella was similar, and we observed a statistically significant spatial dependency.

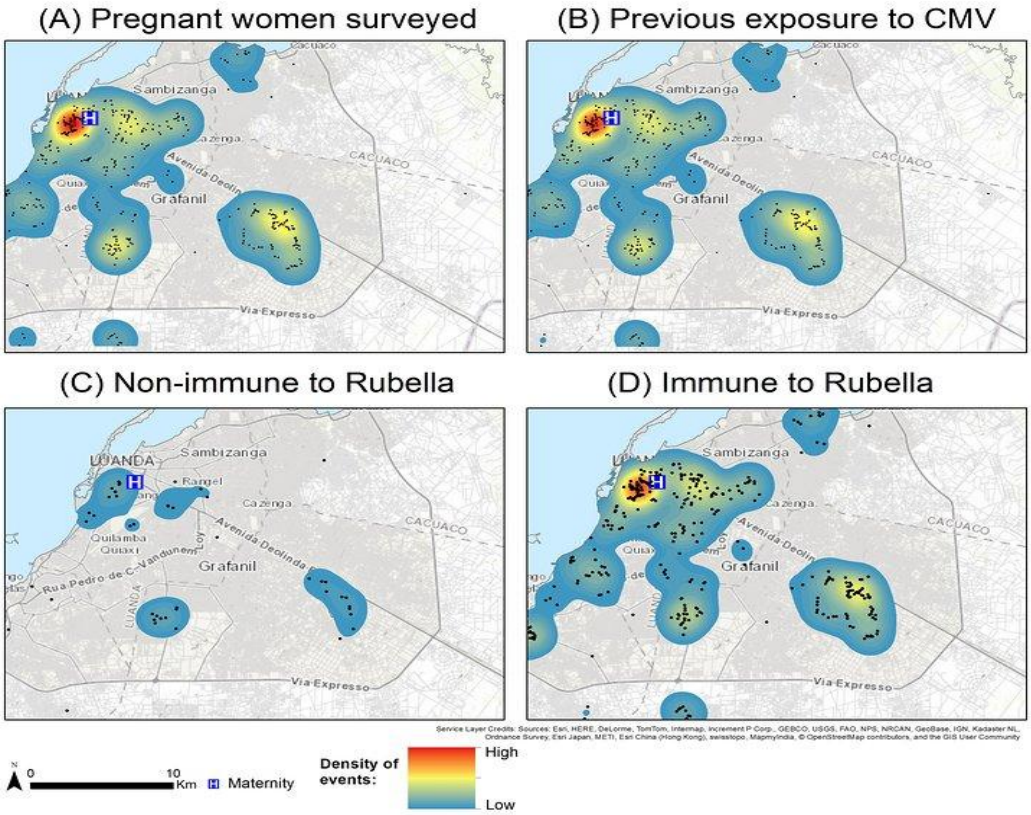

Figure 7: Geographical distribution and Gaussian kernel density surface map of pregnant women (A) with anti-CMV antibodies (B), non-immune to Rubella virus (C) and immune to Rubella virus (D) in Luanda, Angola.

4.5. Discussion

The present study was performed to investigate the seroprevalence of CMV and rubella infection in pregnant women in the northern Angola city of Luanda. We found an overall seroprevalence rate for CMV infection of 98.5% and for Rubella infection about 88.6%. This is the first study conducted in Angola to evaluate the rates of CMV and Rubella infection in pregnant women. In a general way, there is a lack of knowledge about the epidemiology of CMV and Rubella infection in Angola. In this context, we cannot compare our seroprevalence results with others in pregnant women in Angola.

CMV infection is endemic in almost all the world, occurring throughout the year without seasonal variations. The rates of seropositivity in the population vary greatly according to geographical, ethnic and socioeconomic factors. The prevalence of CMV-specific antibodies increases with age and in the less favored socioeconomic groups of developed and developing countries (Cannon et al., 2010; Wujcicka et al., 2014). Other known contributing factors for CMV infection including education, sexual promiscuity, and

blood transfusion (Foxworth et al., 2014; Ziemann & Hennig, 2014). Also the contact with children is considered a risk factor, once the young children stands out as sources of CMV infection in pregnant women (Ephraim et al., 2013).

Our results of the seroprevalence of CMV antibodies in pregnant women were similar to previous studies in other African countries: 92% in Nigeria (Ephraim et al., 2013), 97.5% in Sudan (Khairi et al., 2013), 96.3% in Tunisia (Hannachi et al., 2011), 87% in Gambia (Bello et al., 1991), 88.5% in Ethiopia (Yeshwondm et al., 2016), and 86% in Kenya (Nisbet et al., 2018). Moreover, our data were also similar with results report in other world countries with values between 92.6% and 100%: Iran (Erfanianahmadpoor et al., 2014), Palestine (Neirukh et al., 2013), China (Zhang et al., 2014), Brazil (Yamamoto et al., 2013), Turkey (Uysal et al., 2012), Nigeria (Akinbami et al., 2011) and Cuba (Festary et al., 2015). In contrast, our prevalence was higher than that reported in developed countries: 42.3% in Germany (Enders et al., 2012), 46.8% in France, 49% in the United Kingdom (Pembrey et al. 2013), 54% in Norway (Barlinn et al., 2018), 56.3% in Finland (Alanen et al., 2005), 58.7% in France (Leruez-Ville et al., 2013), 65.9% in Italy (Picone et al., 2009), 66% in Japan (Ikuta et al., 2013), 62.4% in Poland (Wujcicka et al., 2014) and 70.0% in the United States (Wang et al., 2016).

In the present study, all women who were CMV-IgM positive were also seropositive for IgG. In most of these cases there is a need to perform IgG avidity test as an alternative to provide the status of acute infection (Lazzarotto et al., 2000; Seo et al., 2009). However, in our study it was not possible define what proportion of these cases represented primary infection or reactivation because we we didn't have acess to IgG avidity test. In USA a recent analysis of CMV IgM seroprevalence in women at reproductive age also found that 97.5 % of IgM seropositive women were CMV IgG seropositive (Wang et al., 2016). Other study performed in Pakistan, showed that 95.3 % of individuals who were IgM seropositive were equally seropositive for IgG (Ibrahim et al., 2016). In countries with a high prevalence of CMV infection, such as Korea and Turkey, IgG avidity testing have shown that none of the women with a proflile of IgM and IgG positive had evidence of a primary infection (Seo et al., 2009; Uysal et al., 2012). As such, we could suggest that the great majority of seropositive cases in our study represent viral recurrent reinfection or reactivation rather than primary infection. The high IgG seropositivity is alarming which calls for the need to screen these women for potential active infections. Further studies on the impact of CMV on poor pregnancy outcomes are highly recommended in the developing countries (Chibwe et al., 2017).

The profile of CMV-IgM negative/CMV-IgG positive in pregnant women (96.5%) indicates that the great majority of infections probably occurred during childhood or adolescence. Our results showed that all age groups are equally likely to be infected with CMV, being the prevalence high at all ages (15-47 years old).

Several studies showed that low socioeconomic status was found to predict CMV IgG seropositivity (Wujcicka et al., 2014). The majority Angolan population is of low socioeconomic level. Although we did not have data about the socioeconomic level (through the material deprivation index; MDI) of the studied population, it should be pointed out that this study was carried out in a public maternity hospital where the majority of the attendants are women of low family income. Moreover, the majority of population in the city of Luanda reside in highly populated squatters with close contacts which favors transmission of airborne diseases. The womens participating in the present study resides in the urban area and there were no differences in CMV prevalence in relation to a specific area of residence. The high prevalence also could be explained by poor hygienic conditions that can to perpetuate the cycle of CMV transmission in the developing countries (Chibwe et al., 2017). However, in the present study we not found stastically association between basic sanitation and CMV seropositivity.

In the present study, HIV infected women were more likely to be CMV IgG seropositive than HIV negative women. WHO recommends that all the pregnant women should be advice and tested for HIV at the first prenatal visit. There is a need to improve prenatal services in our setting to ensure that all women are counseled and tested for HIV. CMV is a virus that has a great potential to proliferate in humans for several reasons. The infection is usually subclinical allowing that infected individuals remain active and thus maintain the possibility of transmission to other susceptible individuals.

Moreover, the CMV is not eradicated from the host after the primary infection, remaining in the body for the rest of its life (Cannon et al., 2010), and occasionally may be reactivated (endogenous infection). In addition, the host although infected do not acquire immunity and can undergo further infection by different strains of the virus (exogenous reinfection) (Yamamoto et al., 2010). Another explanation for the easy spread of CMV is that its excretion may persist for an extended period of time. For example, it is known that in the case of congenital infections, viral excretion by children may occur for years, increasing the likelihood of transmission to other individuals (CDC, 1985; Strauss & Strauss, 2008). On the other hand, some studies have observed that closed environments with many children are sites that facilitate the spread of the virus. In all these cases the transmission occurs mainly through

contact with urine or saliva of infected children (Hantz & Alain, 2019). We analyzed the influence of the contact of pregnat women with children through the number of births and children at home. The women with children at home had a greater seroprevalence of CMV infection than those without children at home.

An additional finding of our study was that the majority of pregnant women with IgG and IgM positive were in the first trimester of gestation, the period of highest risk to the fetus in case of virus transmission (Davis et al., 2017). It is reasonable to hypothesize that some infections would have been avoided had these women been informed at an earlier stage of pregnancy. Ideally, all women should be tested for CMV antibody and informed before pregnancy. Indeed, in a population of women receiving fertility treatment, preconception screening and counseling, seems to have a risk reduced to CMV infection in pregnancy (Reichman et al., 2014). Moreover, preconception testing would also reduce problems arising from the detection and interpretation of CMV-specific IgM antibody in pregnant woman (Guerra et al., 2007; Revello & Gerna, 2002).

CMV stands out as the major cause of congenital infection, reaching rates between 0.2 and 2.6% of the total number of births worldwide, being responsible for cases of neonatal mortality and morbidity (Korndewal et al., 2017). Fetal CMV infection occurs in approximately 40% of cases of maternal primary infection (Carlson et al., 2010). Therefore, it would be beneficial to inform pregnant women about the need for follow-up to detect prenatal infection and to plan appropriate intervention such as the use of drugs to control infection and / or prevent fetus infection (Yeshwondm et al., 2016).

The epidemiological importance of Rubella virus is related to the CRS that affects the fetus or the newborn due to the infection contracted by the mother during pregnancy. The overall seroprevalence of rubella among pregnant women in the present study was 88.6%. Similar results have been reported from other African countries such as Ethiopia (89%) (Wondimeneh et al., 2018), Senegal (90.1%) (Dromigny et al., 2003), Namibia (85.0%) (Jonas et al., 2016), Burkina Faso (95%) (Tahita et al., 2013) and Zimbabwe (92%) (Mamvura et al., 2015). Also in other countries of the world the prevalence is high: 88.1% and 93.5% in Turkey (Aksakal et al., 2007; Jahromi et al., 2011); and 87% in United States of America (USA), 98% in Spain, and 96.3% Iran (Karabulut et al., 2011; Remington et al., 2011). In contrast, the seroprevalence in this study is higher than reports from Democratic Republic of Congo (58.97%) (Zanga et al., 2017), Sudan (65%) (Hamdan et al., 2011) and Nigeria (68%) (Bamgboye et al., 2004). These variations might be due to the difference in the endemicity of the virus, the sample size of the studies, and the laboratory methods used.

The profile of IgM and IgG immunoglobulins is important to characterizing infection in a given area (Olajide et al., 2015). The presence of only IgM or both IgM and IgG antibodies at the same time indicates an acute/recent rubella virus infection. However, the presence of IgG antibody in the absence of IgM is a seromarker of immunity against rubella virus (Mirambo et al., 2016). The absence of both IgM and IgG antibodies indicates susceptibility to acquiring rubella infection. In the present study, both rubella-specific IgM and IgG antibodies were analyzed among pregnant women to determine acute/recent infections and the levels of immunity against rubella virus infection in Luanda, Angola. Despite the general very high seroprevalence of rubella infection, 11.4 % of the pregnant women were seronegative. The susceptibility rate in among adult women could result in outbreaks of CRS (WHO, 2011; Wondimeneh et al., 2018). Therefore, attention must be paid to the susceptible group of women in this study in order to reduce the risk of CRS in their future pregnancies.

Based on the previous recommendations of the US National Committee for Clinical Laboratory Standards (NCCLS) (Skendzel, 1996) and international agreements and guidelines (Dimech et al., 2008), in the absence of IgM, the pregnant women who had rubella IgG levels ≥10 IU/ml were classified as immune and those with IgG levels <10 IU/ml were classified as susceptible. In the present study, 87.6% of the pregnant women had IgG levels of >10 IU/ml (Table 9). None of these pregnant women had a previous history of rubella vaccination and they were immune from rubella infections. This might be due to the endemicity of the virus in the study area that sustained previous infections of the participants before conception or during their childhood, as rubella infection is common among children and teenagers in some countries (Shiferaw et al., 2016; Wondimeneh et al., 2018).

The prevalence of rubella IgG (87.6%) in this study was greater than reported in Burkina Faso (77%) (Tahita et al., 2013), in Niger (53%) (Onakewhor and Chiwuzie, 2011) and southern India (65%) (Padmaja et al., 2010). However, the IgG positivity rate was lower than that found in studies conducted in other countries like Nigeria (97.9%) (Mohammed et al., 2010), Cameroon (88.6%) (Fokunang et al., 2010), Turkey (96.1%) (Tamer et al., 2009), Italy (85.8%) (Calimeri et al., 2012) and Mexico (97.1%) (Alvarado-Esquivel et al., 2016). These variations in rubella IgG positivity in different countries might be due to the difference in the endemicity of the rubella virus and the presence or absence of rubella vaccination in their immunization programs.

In the present study, 1.0 % of the total pregnant women had both rubella IgM and IgG antibodies. The rubella virus re-infection following natural immunity is very rare

(Wondimeneh et al., 2018). Therefore, the pregnant women might be in stages of primary rubella infection. Since these pregnant women were in the first and second trimester of pregnancy, they might have acquired the infection during the pregnancy and subsequently developed IgG antibodies within 30 days of infection (Navigator, 2013). This indicates that these pregnant women might not be immune before pregnancies and the foetuses can not be excluded from rubella-associated risks. Although there are no data of CRS in Angola, the newborns from women infected with Rubella during early pregnancy might acquire a congenital rubella infection and be born with rubella-associated congenital anomalies or CRS. Therefore, the screening of women of child-bearing age before conception or during pregnancy might be crucial to reduce the consequences of acute rubella infection during pregnancy (Wondimeneh et al., 2018).

In the multivariate logistic regression analysis, a statistically significant association was found between rubella IgG positivity with spontaneous abortions during the study; all pregnant women who had a spontaneus abortion were seropositive for IgG anti-Rubella. The rubella was considered as an etiologic agent for miscarriages in many countries (Abdolreza, et al., 2011; Jahromi et al., 2011). Therefore, more attention should place on those pregnant women who had recent or acute infections due to the teratogenic nature of the virus (Lee & Bowden, 2000). Moreover, pregnant women who have a previous bad obstetric history may be more vulnerable to acquiring acute rubella infections (Priyanka et al., 2017). Although the mechanism is not clear and further studies are needed, a similar finding has also been reported in other studies (Abdolreza, et al., 2011; Noor et al., 2015).

No statistically significant difference was found between anti-Rubella antibodies positivity and socio-demographic characteristics of the pregnant women. A similar finding was also reported in Namibia (Jonas et al., 2016), Southern Ethiopia (Tamirat et al., 2017), and Nigeria (Pennap & Egwa, 2016). All the pregnant participants in the present study live in urban settings. The high population density in urban areas might increase the contact rate and pregnant women without protective levels of rubella immunity might acquire the infections (Wondimeneh et al., 2018). A finding was reported in the pre-vaccine era in other countries (Assaad et al., 1985; Goodson et al., 201; Hinman et al., 1998).

To reduce the circulation of the Rubella virus, vaccination is essential and is the only way to prevent the disease (Avila Moura et al., 2016; Jahromi et al., 2011). Rubella is commonly mistaken for other diseases because symptoms such as sore throats and headaches are common to other infections, making it difficult to diagnose (Jahromi et al., 2011). Although not serious, rubella is particularly dangerous in the congenital form. In this case, it

may leave irreversible sequels in the fetus as glaucoma, cataract, cardiac malformation, delayed growth, deafness and others. Therefore, prevention should be focused (Avila Moura et al., 2016).

The WHO (2011) suggests the following strategies for the prevention of Rubella: (i) Provide right to protection to school-age women and / or girls (ii) Vaccinate to provide indirect protection by reducing the transmission of rubella virus infection (iii) a combination of these approaches. The rubella vaccine was included in the Angolan national vaccination plan in April 2018 an initial stage only covered children up to 14 years of age (WHO, 2012).

The prevalence of CMV and Rubella infection can be attributed to low socioeconomic status and poor hygienic. Currently, about 36% of the population lives below the poverty line and with limited access to basic public services (water, sanitation, energy, health, education and housing). In the education sector, Angola is considered by UNESCO as a low educational development index country, ranking 111th out of 120 countries in the UNESCO Education for All 2012, with a value of 0.685 and a gender parity index of 0.734 (WHO, 2015).

There is no way to know how many cases of CMV and Rubella are identified each year in Angola. Thus, there aren´t effective intervention to control CMV and Rubella infection in the country. In the case of CMV, preventive measures including changes in hygiene behavior of seronegative pregnant women should be implemmented as well as routine maternal screening for primary infection.

Moreover, treatment with hyperimmune human immunoglobulin and the administration of acyclovir or its derivative valaciclovir should be considered once do not have teratogenic side effects when administered in the early stages of pregnancy (Adler et al., 2007; Kimberlin et al., 2003).

4.6. Conclusion

Overall, this study showed that there is a high seroprevalence of anti-CMV and anti - Rubella antibodies in pregnant women in Luanda. Therefore, it is important improve rapid and accurate diagnosis of CMV and Rubella infection in pregnant women to prevent major complications such as congenital infections. It would be also important to implement national screening on CMV, rubella, and other diseases linked to maternal and child health. This study showed that although most pregnant women were immune to rubella in Luanda, a considerable number are still not immune. Therefore, rubella vaccination should be offered to women with rubella-specific susceptibility to prevent further complications such as congenital infections, reduce the incidence of birth defects, and preserve maternal and child health.

CHAPTER 5

General Discussion and Concluding Remarks

5.1 General Discussion

Angola is a country with vast natural resources, such as large reserves of minerals and oil, and since 1990 its economy has shown growth rates that are among the highest in the world, especially after the end of the civil war in 2002. However, the country continues to face enormous development challenges, which include reducing dependence on oil and diversifying the economy, rebuilding infrastructures, increasing institutional capacity and improving governance and management systems public finances, human development indicators and better living conditions of the population (World Bank, 2019). Angolans' living standards remain low; the poverty rate is very highs (52%). One in two Angolans lives in multidimensional poverty (UNPAF, 2015). The country's life expectancy and infant mortality rates remain among the worst in the world, in addition to the prominent presence of economic inequality, since the majority of the country's wealth is concentrated in a disproportionately small sector of the population. Therefore, the country is also considered one of the least developed countries on the planet by the United Nations (World Bank, 2018; UN-OHRLLS, 2014).

Despite a relative improvement in economic growth, the Angola´s population still lives with serious health, education, water supply and sanitation problems. Unfortunately, infectious diseases continue to represent a public health problem (IIMS, 2017). The great social inequality is a common feature in Angola, and the province of Luanda is an example of a city with economic and social contrasts. The value of the Human Development Index (HDI) of Angola in 2017 was 0.393, typical of underdeveloped countries (UNPAF, 2015).

Infectious diseases are a great burden on many societies, including the countries in development. To reduce that burden an integrated approach is required, combining health promotion, disease prevention and patient treatment (WHO, 2001). The decrease in morbidity and mortality due to infectious diseases in the demographic structure of some countries is essentially characterized by an increase in life expectancy, a decrease in fertility and an aging population (Frenk et al, 1989; Jamison, 2006; Nii-Trebi 2017). The Angola's epidemiological profile is characterized by the predominance of communicable diseases as causes of morbidity and mortality in the population: malaria, hepatitis, ADD, tuberculosis and HIV, represent about 96.3% of the total diseases notified in 2011 (PNDS, 2012). Although toxoplasmosis, rubella and cytomegalovirus (CMV) infection are diseases that have been widely studied worldwide, there are few studies carried out in Luanda, Angola. Therefore, this work aimed to study the seroprevalence of antibodies anti-*T. gondii*, anti-Rubella and anti-CMV in pregnant women attended at the LPMH in Luanda, identify and dating maternal primary infection

through the antibodies profiles and avidity tests, perform the diagnosis of congenital infection using qPCR, studying the geospatial distribution of infections and assess the socio-demographic and clinical risk factors associated with infections.

Our work shows a high seroprevalence of *T. gondii* (39.6%) and very high prevalence of Rubella (87.6%) and CMV (98.5%) in the province of Luanda. These results corroborate those found in other African developing countries: toxoplasmosis (80.3%) in the Democratic Republic of Congo (DRC) (Yobi et al., 2014), rubella (89%) in Ethiopia (Wondimeneh et al., 2018) and CMV (86%) in Kenya (Nisbet et al., 2018).

It is well documented that the prevalence of toxoplasmosis varies across countries, and indeed contrasting data are observed: from 6.2% in México (Alvarado-Esquivel et al., 2016) to 85.3% in Ethiopia (Abamecha & Awel, 2016). These differences may be associated with various factors related to each region/country and specific characteristics of the population as well with the diagnostic methods used. Other examples include sanitary practices and eating/drinking habits, presence of household felines, poor or lower socioeconomic status, contact with soil, lack of knowledge about the disease, and the overall climate conditions that allow for the protracted environmental survival of the parasite's oocysts.

Previous studies report the relationship between maternal infection with congenital toxoplasmosis, which is relatively low (< 20%) during the first trimester and increases to up to 90% at the end of gestation (Jones et al., 2003; Ortiz-Alegría et al., 2010). Recently, the global prevalence of acute *Toxoplasma* infection in pregnant women was estimated at about 1.1%, suggesting that a large number of newborns are at risk of acquiring congenital toxoplasmosis in utero (Rostami et al., 2019). In our study, there was a suspicion of active toxoplasmosis in two participants who tested positive for IgM and IgG; however, the IgG avidity test and the qPCR allowed the exclusion of recent infection in these two women's. Moreover, the seronegative pregnant women (60.4%) had follow-up during prenatal care and none of them presented seroconversion i.e. none acquired toxoplasmosis. However, the multivariate analysis has shown that gestation age and suffering spontaneous abortion are significantly associated with *T. gondii* seropositivity. The percentage of pregnant women with prior exposure in the third trimester was high compared with the other trimesters, showing that women in the last trimester of pregnancy had an increased likelihood of *T. gondii* seropositivity. Adittionaly, spontaneous abortion was the most common form of pregnancy loss in our study, and is in line with other studies (Chintapalli & Padmaja, 2013; Kamal et al., 2015; Sarkar et al., 2012). The immunological control of placental infection is probably a key event in the occurrence of congenital infection and miscarriages (Pfaff et al., 2007), but

advances in understanding the pathophysiological process still need to be achieved (Robert-Gangneux & Dardé, 2012). Unfortunately, we do not have fetus material or placenta from those cases of spontaneous abortion to confirm the presence of *T. gondii*. In the future, the implementation of the diagnosis of maternal primary infection as well the appropriate diagnosis of congenital toxoplasmosis will be crucial.

The primary prevention measures in *Toxoplasma*-seronegative pregnant women helped to reduce seroconversion rates in many countries (Avelino et al., 2014). Unfortunately, routine screening for *T. gondii* infection during pregnancy is not performed for most Angolan women, as the test is not mandatory in prenatal care and the associated costs are high cost. In addition, poor access to higher education and the lack of promotion of public health in general explain how a very high percentage of the pregnant women (93.3%) lacks knowledge of toxoplasmosis as seen in the present study. Our data are similar to those found in several worldwide studies that report a generally low level of knowledge about toxoplasmosis, risk factors, prevention and consequences of diseases among pregnant women (Laboudi, 2017; Elsafi et al., 2015). However, they contrast with the studies carried out in Poland (Smereka et al., 2018), Geneva, Switzerland (Willame et al., 2015) and Holland (Pereboom et al., 2013), which reflects well that the policies that are adopted by each state influence the disease progress.

Regarding the risk factors for toxoplasmosis in pregnant women, the multivariate analysis has shown that tested positive for HIV, tested positive for HIV hepatitis B, and the presence of household pets are significantly associated with *T. gondii* seropositivity.

According to the literature, the severity and clinical significance of human toxoplasmosis varies with the host's immune status, therefore HIV-infected patients may be at higher risk of developing toxoplasmosis disease (Ayi et al., 2016; Laboudi, 2017). Toxoplasmosis is the most common opportunistic infection in HIV-seropositive immunocompromised hosts, where it predominantly occurs as a reactivation of endogenous infection (Ayi et al., 2016). Furthermore, latent infection by *T. gondii* in 30 to 50% of HIV patients has a high risk of progressing to toxoplasmosis encephalitis (Alfonso et al., 2009). It was also reported that hepatitis B infection in pregnant women also leads to increased risk of chronicity of cirrhosis and / or hepatocellular carcinoma (Xiao et al., 2007). In our study, the seroprevalence of *T. gondii* in HIV-positive pregnant women was higher than those who tested HIV-negative. A higher prevalence of *T. gondii* infection was also observed among hepatitis B seropositive pregnant women than in seronegative women. To have HIV and/or hepatitis B can lead to a reactivation of toxoplasmosis and, in some cases, trigger the most

serious clinical manifestations of these diseases (Xiao et al., 2007). Because of the prevalence of infection among the population, it is extremely important to perform serological screening in the prenatal period in order to initiate early treatment, or even avoid vertical transmission (UNAIDS, 2016; Piazza et al., 2010).

It is believed that cats are the main carriers and transmitters of *T. gondii* infection to humans (Weiss & Kim, 2013; de Wit et al., 2019; Jiang et al., 2018). In the present study, the seroprevalence of *T. gondii* among pregnant women who had cats was higher than in pregnant women who did not have cats. However, no significant association was found between the presences of cats in the household and *T. gondii* seropositivity. In contrast, we found that having pets at home, not specifically the cat, is a potential risk factor for *T. gondii* seropositivity in pregnant women. The logistic regression analysis showed that contact with pets at home and/or in family member's/friends' homes is associated with *T. gondii*–seropositive women. Cats can disseminate oocysts in the environment, while dogs can act as mechanical vectors assisting in the transmission of infectious forms of *T. gondii* (Avelino et al., 2004).

The investigation of the geospatial distribution of *T. gondii* seropositive pregnant women allowed us to identify the municipality of Belas as the area with lower risk to acquire toxoplasmosis. Previous studies have shown that living in rural or suburban regions with soil exposure is a risk factor for toxoplasmosis in pregnant women (Cong et al., 2015; Fromont et al., 2009). Our results point that the prevalence of *T. gondii* among pregnant women is higher in the group in frequent contact with the soil; however, no statistically significant association was observed between *T. gondii* seropositivity and activities and practices that promote contact with soil (gardening). This result may have been influenced by the fact that most of the pregnant women live in urban area.

Overall, the study of toxoplasmosis is important in the planning of educational programs aimed at reducing the incidence of toxoplasmosis during pregnancy. The best measures to prevent toxoplasmosis are those directed at primary prevention, basically characterized by public health and education programs recommended through campaigns, lectures, pamphlets and the guidance provided by health teams informing pregnant women to avoid contact with animals, potentially harmful contaminated materials, and avoid the consumption of raw or undercooked meat.

Regarding Rubella and CMV, our study shown that the seroprevalence rate for rubella infection was 88.6% and for CMV infection was 98.5%. This is the first study carried out in Luanda/Angola to assess the rates of CMV and rubella infection in pregnant women. The

profile of CMV-IgM negative/CMV-IgG positive in pregnant women (96.5%) indicates that the great majority of infections probably occurred during childhood or adolescence Several studies showed that low socioeconomic status was found to predict CMV IgG seropositivity (Wujcicka et al., 2014). Although we did not have data about the socioeconomic level (through the material deprivation index; MDI) of the studied population, it should be pointed out that this study was carried out in a public maternity hospital where the majority of the attendants are women of low family income. CMV stands out as the major cause of congenital infection, reaching rates between 0.2 and 2.6% of the total number of births worldwide, being responsible for cases of neonatal mortality and morbidity (Korndewal et al., 2017). Our study showed that the majority of pregnant women with IgG and IgM positive were in the first trimester of gestation, the period of highest risk to the fetus in case of virus transmission (Davis et al., 2017). Therefore, it would be beneficial to inform pregnant women about the need for follow-up to detect prenatal infection and to plan appropriate intervention such as the use of drugs to control infection and / or prevent fetus infection (Yeshwondm et al., 2016).

The epidemiological importance of rubella is related to congenital rubella syndrome (CRS) that affects the fetus or the newborn due to the infection contracted by the mother during pregnancy. Despite the high seroprevalence of rubella infection, 11.4% of pregnant women were seronegative. This susceptibility rate in among adult women could result in outbreaks of CRS (WHO, 2011; Wondimeneh et al., 2018). Moreover, 1.0 % of the total pregnant women had both rubella IgM and IgG antibodies. The rubella virus reinfection following natural immunity is very rare (Wondimeneh et al., 2018) and so the pregnant women in our study might be in stages of primary rubella infection. Since these pregnant women were in the first and second trimester of pregnancy, they might have acquired the infection during the pregnancy and subsequently developed IgG antibodies within 30 days of infection (Navigator, 2013). This indicates that these pregnant women might not be immune before pregnancies and the foetuses cannot be excluded from rubella-associated risks. Although there are no data of CRS in Angola, the newborns from women infected with Rubella during early pregnancy might acquire a congenital rubella infection and be born with rubella-associated congenital anomalies or CRS. Moreover, the rubella was considered as an etiologic agent for miscarriages in many countries (Abdolreza, et al., 2011; Jahromi et al., 2011). In the multivariate logistic regression analysis, a statistically significant association was found between rubella IgG positivity with spontaneous abortions during the study; all pregnant women who had a spontaneus abortion were seropositive for IgG anti-Rubella. Therefore, the screening of women of child-bearing age before conception or during

pregnancy might be crucial to reduce the consequences of acute rubella infection during pregnancy (Wondimeneh et al., 2018). To decrease the circulation of the Rubella virus, vaccination is essential and is the only way to prevent the disease (Avila Moura et al., 2016; Jahromi et al., 2011).

5.2. Concluding Remarks

Overall, this study estimated the prevalence and serological characterization of Toxoplasmosis, Rubella and CMV in pregnant women attended at the Lucrécia Paím Maternity Hospital in Luanda, as well as identifies vulnerable areas and socio-demographic and clinical risk factors for these pathologies. Also reports epidemiological data of HIV and hepatitis B and its association with these pathologies. It is the first time that the molecular diagnosis by qPCR of *T. gondii* was performed on fragments of placenta in the pregnant population of Luanda, allowing confirmation of the results of the serological tests. It was also the first time that took place study on the prevalence of rubella and CMV in pregnant women in Luanda/Angola was carried out.

The results showed that there is a high seroprevalence of anti-*T. gondii*, anti-CMV and anti-Rubella antibodies in pregnant women in Luanda. Therefore, it is important improve rapid and accurate diagnosis of toxoplasmosis, CMV and Rubella infection in pregnant women to prevent major complications such as congenital infections. It would be also important to implement rubella vaccination in women with rubella-specific susceptibility. Our results also underscore the need for diagnostic and clinical follow-up of other infectious diseases, including HIV and hepatitis B during pregnancy.

6. REFERENCES

Abamecha F, Awel H. Seroprevalence and risk factors of *Toxoplasma gondii* infection in pregnant women following antenatal care at Mizan Aman General Hospital, Bench Maji Zone (BMZ), Ethiopia. BMC Infect Dis. **2016**; 16: 460.

Abboud P, Harika G, Saniez D, Gabriel R, Bednarczyk L, Chemla C, et al. Ultrasonic signs of fetal toxoplasmosis. Review of the literature. Journal de Gynecologie Obstetrique et Biologie de la Reproduction (Paris). **1995**; 24(7):733-8.

Abdolreza SJ, Akbar K, Gita M, Abdolhossien M, Seyed-Hamid M, Bita, S. Seroprevalence of rubella virus in women with spontaneous abortion. Am J Infect Dis. **2011**; 7 (1): 16-19.

Abdulkadir A, Gebrehiwot TT. Risk Factors for Rubella Transmission in Kuyu District, Ethiopia, 2018: A Case-Control Study. Interdisciplinary Perspectives on Infectious Diseases. 4719636. 2019. Available at: https://www.hindawi.com/journals/ipid/2019/4719636/

Adler SP, Nigro G. Prevention of maternal-fetal transmission of Cytomegalovirus. Clinical Infect. Dis. **2013**; 57 Suppl 4:S189-92.

Agbede, O.O. Sero-prevalence of antenatal rubella in UITH. Open Public Health J. **2011**; 5: 10-11.

AghaKouchak, A., L. Cheng, O. Mazdiyasni, A. Farahmand. Global warming and changes in risk of concurrent climate extremes: insights from the 2014 California drought. Geophys Res Lett. **2014**; 41(28) :8847-8852

Agmas B, Reta Tesfaye R, Koye DN. Seroprevalence of *Toxoplasma gondii* infection and associated risk factors among pregnant women in Debre Tabor, Northwest Ethiopia. BMC Res Notes. **2015**; 8: 107.

Aguirre AA, Longcore T, Barbieri M, Dabritz H, Hill D, Klein PN, et al. The One Health Approach to Toxoplasmosis: Epidemiology, Control, and Prevention Strategies. Ecohealth. **2019**; 16(2):378-390.

Ahnquist J, Wamala SP, Lindstrom M. Social determinants of health-A question of social or economic capital? Interaction effects of socioeconomic factors on health outcomes. Soc Sci Med. **2012**; 74 (6): 930-939.

Ajzenberg D, Collinet F, Mercier A, Vignoles P, Darde ML. Genotyping of *Toxoplasma gondii* isolates with 15 microsatellite markers in a single multiplex PCR assay. Journal of Clinical Microbiology. **2010** 48(12):4641-5.

Ajzenberg D, Lamaury I, Demar M, Vautrin C, Cabié A, Simon S, et al. Performance testing of PCR assay in blood samples for the diagnosis of toxoplasmic encephalitis in AIDS patients from the French departments of America and genetic diversity of *Toxoplasma gondii*: A prospective and multicentric study. PLoS Negl Trop Dis. **2016** Jun 29; 10(6):e0004790.

Akinbami AA, Rabiu KA, Adewunmi AA, Wright KO, Dosunmu AO, Adeyemo TA, et al. Seroprevalence of cytomegalovirus antibodies amongst normal pregnant women in Nigeria. Int J Womens Health. **2011**; 3:423-428.

Aksakal FN, Maral I, Cirak MY, Aygun R. Rubella seroprevalence among women of childbearing age residing in a rural region: is there a need for rubella vaccination in Turkey Jpn. J. Infect. Dis. **2007**; 60:157-160.

Alanen A, Kahala K, Vahlberg T, Koskela P, Vainionpää R. Seroprevalence, incidence of prenatal infections and reliability of maternal history of varicella zoster virus, cytomegalovirus, herpes simplex virus and parvovirus B19 infection in South-Western Finland. BJOG. **2005**; 112(1):50-6.

Alfonso Y, Fraga J, Fonseca C, Jiménez N, Pinillos T, Dorta-Contreras AJ, et al. Molecular diagnosis of *Toxoplasma gondii* infection in cerebrospinal fluid from AIDS patients. Cerebrospinal Fluid Research. **2009**; 6:2.

Al-Harthi SA, Jamjoom MB, Ghaz HO. Sero-prevalence of *Toxoplasma gondii* among pregnant women in Makkah, Saudi Arabia. Umm Al-qura univ. J Sci Med Eng. **2006**; 18(2): 217-227.

Alvarado-Esquivel C, Hernández-Tinoco J, Sánchez-Anguiano LF, Ramos-Nevárez A, Cerrillo-Soto SM, Estrada-Martínez S, et al. Seroepidemiology of cytomegalovirus infection in pregnant women in Durango City, Mexico. BMC Infect Dis. **2014**; 14: 484.

Alvarado-Esquivel C, Rascón-Careaga A, Hernández-Tinoco J, Corella-Madueño MA, Sánchez-Anguiano LF, Aldana-Madrid ML, et al. Seroprevalence and correlates of *Toxoplasma gondii* infection in Yoremes (Mayos) in Mexico: a cross-sectional study. BMJ Open. **2016** 12; 6(5): e010218.

Alvarado-Esquivel C, Sánchez-Anguiano LF, Hernández-Tinoco J, Ramos-Nevarez A, Estrada-Martínez S, Cerrillo-Soto SM et al. Association between *Toxoplasma gondii*

infection and history of blood transfusion: a case-control seroprevalence study. J Int Med Res. **2018**; 46(4):1626-1633.

Alvarado-Esquivel C, Hernandez-Tinoco J, Sanchez-Anguiano LF, Ramos-Nevarez A, Cerrillo-Soto SM, Salas-Pacheco JM, et al. Rubella immune status in pregnant women in a Northern Mexican City. J Clin Med Res. **2016**; 8(9):656-61.

Anderholm KM, Bierle CJ, Schleiss MR. Cytomegalovirus vaccines: current status and future prospects. Drugs. **2016**; 76(17):1625-1645.

Andiappan H, Nissapatorn V, Sawangjaroen N, Chemoh W, Lau YL, Kumar T, et al. Toxoplasma infection in pregnant women: a current status in Songklanagarind hospital, southern Thailand. Parasit Vectors. **2014**; 7: 239.

Andrus JK, Cochi SL, Cooper LZ, Klein JD. Combining global elimination of measles and Rubella with strengthening of health systems in developing countries. Health Affairs (Millwood). **2016**; 35: 327-33.

Ashburn D, Joss AW, Pennington TH, Ho-Yen DO. Do IgA, IgE, and IgG avidity tests have any value in the diagnosis of Toxoplasma infection in pregnancy? Journal of Clinical Pathology. **1998**; 51 (4): 312-315.

Auerbach MR, Yan D, Vij R, Hongo JA, Nakamura G, Vernes JM, et al. A neutralizing anti-Gh/Gl monoclonal antibody is protective in the Guinea pig model of congenital CMV infection. PLoS Pathogens. **2014**; 0(4):e1004060.

Augusto GF. Use of services for prevention of mother-to-child transmission in Angola: a retrospective analysis. J Public Health (Oxf). **2016**; 38(2):371-377.

Avelar JB, Da Silva MG, Rezende HHA, Storchilo HR, Do Amaral WN, Xavier IR, et al. Epidemiological factors associated with *Toxoplasma gondii* infection in postpartum women treated in the public healthcare system of Goiânia, State of Goiás, Brazil. Rev Soc Bras Med Trop **2018**; 51: 57-62.

Avelino MM, Campos D Jr, Parada JB, Castro AM. Risk factors for *Toxoplasma gondii* infection in women of childbearing age. Braz J Infect Dis. **2004**; 8(2): 164-174.

Avelino MM, Amaral WN, Rodrigues IMX, Rassi AR, Gomes MBF, Costa TL, et al. Congenital toxoplasmosis and prenatal care state programs. BMC Infectious Diseases. **2014**; 14,33.

Avila Moura A, Mello MJG, Correia JB. Serological statuses of pregnant women in an urban Brazilian population before and after the 2008 rubella immunization campaign. Vaccine. **2016**; 34(4):445-450.

Ayi I, Sowah AO, Blay EA, Suzuki T, Ohta N, Ayeh-Kumi PF. *Toxoplasma gondii* infections among pregnant women, children and HIV-seropositive persons in Accra, Ghana. Tropical Medicine and Health. **2016**; 44, 17.

Bamba S, Cissé M, Sangaré I, Zida A, Ouattara S, Guiguemdé RT. Seroprevalence and risk factors of Toxoplasma gondii infection in pregnant women from Bobo Dioulasso, Burkina Faso. BMC Infect Dis. **2017**; 17(1): 482.

Bamgboye AE, Afolabi KA, Esumeh FI, Enweani IB. Prevalence of rubella antibody in pregnant women in Ibadan, Nigeria. West Afr J Med. **2004**; 23(3):245-8.

Banatvala JE, Brown DW. Rubella. Lancet. **2004**; 363(9415): 1127-37

Baraniak I, Kropff B, McLean GR, Pichon S, Piras-Douce F, Milne RSB, et al. Epitope-specific humoral responses to human cytomegalovirus glycoprotein-B vaccine with MF59: anti-AD2 levels correlate with protection from viremia. The Journal of infectious diseases. **2018**; 217(12):1907-17.

Barlinn R, Vainio K, Samdal HH, Nordbø SA, Nøkleby H, Dudman SG. Susceptibility to cytomegalovirus, parvovirus B19 and age-dependent differences in levels of rubella antibodies among pregnant women. J Med Virol. **2014**; 86:820-826.

Bastien P, Procop GW, Reischl U. Quantitative real-time PCR is not more sensitive than "conventional" PCR. J Clin Microbiol. **2008**; 46(6):1897-900.

Bastien P. Molecular diagnosis of toxoplasmosis. Transations of the Royal Society of Tropical Medicine and Hygiene. **2002**; 96, S205-S215.

Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004. Clin Infect Dis. **2010**; 50(11):1439-47.

Begeman IJ, Lykins J, Zhou Y, Lai BS, Levigne P, Bissati KE, et al. Point-of-care testing for *Toxoplasma gondii* IgG/IgM using Toxoplasma ICT IgG-IgM test with sera from the United States and implications for developing countries. PLoS Neglected Tropical Diseases. **2017**; 11(6):e0005670.

Bello C, Whittle H. Cytomegalovirus infection in Gambian mothers and their babies. J Clin Pathol, **1991**; 44(5): 366-9.

Belluco S, Mancin M, Conficoni D, Simonato G, Pietrobelli M, Ricci A. Investigating the determinants of *Toxoplasma gondii* prevalence in meat: a systematic review and meta-regression. PLoS One. **2016**; 11(4): e0153856.

Benoist G, Jacquemard F, Leruez -Ville M, Ville Y: Cytomegalovirus (CMV) congenital infection. Gynecol Obstet Fertil **2008**; 36 (3): 248-260.

Bernstein DI, Munoz FM, Callahan ST, Rupp R, Wootton SH, Edwards KM, et al. Safety and efficacy of a cytomegalovirus glycoprotein B (gB) vaccine in adolescent girls: A randomized clinical trial. Vaccine. **2016**; 34(3):313-9.

Best JM. Rubella. Semin Fetal Neonatal Med. **2007**; 12(3):182-92.

Bialas KM, Westreich D, Cisneros de la Rosa E, Nelson CS, Kauvar LM, Fu TM, et al. Maternal antibody responses and nonprimary congenital cytomegalovirus infection of HIV-1-Exposed infants. The Journal of Infectious Diseases. **2016**; 214(12):1916-1923.

Bitnun A, Samson L, Chun T-W, Kakkar F, Brophy J, Murray D, et al. Early initiation of combination antiretroviral therapy in HIV-1-infected newborns can achieve sustained virologic suppression with low frequency of CD4+ T cells carrying HIV in peripheral blood. Clin Infect Dis. **2014**; 59 (7): 1012-1019.

Blaakaer J. Ultrasonic diagnosis of fetal ascites and toxoplasmosis. Acta Obstetricia et Gynecologica Scandinavica **1986**; 65(6):653-4.

Bobadilla JL; Frenk J, Lozano R, Frejka, T, Stern, C. "The epidemiologic transition and health priorities", apud Jamison, D.T., in disease control priorities in developing countries. Oxford Medical Publications, Oxford University Press, **1993**; 161-187.

Bonyadi MR, Bastani P. Modification and evaluation of avidity IgG testing for differentiating of *Toxoplasma gondii* infection in early stage of pregnancy. Cell Journal. **2013**; 15(3): 238-43.

Bosma TJ, Corbett KM, Eckstein MB, O'shea S, Vijayalakshmi P, Banatvala JE, et al. Use of PCR for prenatal and postnatal diagnosis of congenital rubella. Journal of Clinical Microbiology. **1995**; 33: 2881-2887.

Bou G, Figueroa MS, Martí-Belda P, Navas E, Guerrero A. Value of PCR for detection of *Toxoplasma gondii* in aqueous humor and blood samples from immunocompetent patients with ocular toxoplasmosis. Journal of Clinical Microbiology. **1999**; 37(11):3465-8.

Boucoiran I, Castillo E. No. 368-Rubella in Pregnancy. Journal of Obstetrics and Gynaecology Canada. **2018**; 40: 1646-1656.

Bouthry E, Picone O, Hamdi G, Grangeot-Keros L, Ayoubi JM, Vauloup-Fellouset C. Rubella and pregnancy: diagnosis, management and outcomes. Prenatal Diagnosis. **2014**; 34: 1246-53.

Braveman P, Gottlieb L. The social determinants of health: it's time to consider the causes of the causes. Public Health Rep. **2014**; 129 Suppl 2(Suppl 2):19-31.

Braveman P, Egerter S, Williams DR. The social determinants of health: coming of age. Annu Rev Public Health. **2011**; 32:381-398.

Bretagne S, Costa JM. Towards a nucleic acid-based diagnosis in clinical parasitology and mycology. Clinica Chimica Acta. **2006**; 363(1-2):221-8.

Bukasa A, Campbell H, Brown K, Bedford H, Ramsay M, Amirthalingam G, et al. Rubella infection in pregnancy and congenital rubella in United Kingdom, 2003 to 2016. Euro Surveill. **2018**; 23: 17-00381.

Burg JL, Grover CM, Pouletty P, Boothroyd JC. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. Journal of Clinical Microbiology. **1989**; 27(8):1787-92.

Cademartori BG, Farias NAR, Brod CS. Seroprevalence and risk factors to *Toxoplasma gondii* infection in pregnant women of pelotas, south of Brazil. Rev Panam Infectol. **2008**; 10: 30-35.

Calimeri, CA, Fauci VLA, Squeri R, Grillo OC, Lo Giudice, D. Prevalence of serum anti-rubella virus antibodies among pregnant women in southern Italy. Int J Gynecol Obstet. **2012**; 116: 211-213.

Cannon MJ, Westbrook K, Levis D, Schleiss MR, Thackeray R, Pass RF. Awareness of and behaviors related to child-to-mother transmission of cytomegalovirus. Prev Med. **2012**; 54(5): 351-7.

Cannon MJ: Congenital cytomegalovirus (CMV) epidemiology and awareness. J Clin Virol. **2009**; 46 Suppl 4: S6-10.

Cannon MJ, Hyde TB, Schmid DS. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. Rev Med Virol. **2011**; 21(4):240-55.

Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol. **2010**; 20(4):202-13.

Cardona N, Basto N, Parra B, Zea AF, Pardo CA, Bonelo A, et al. Detection of Toxoplasma DNA in the peripheral blood of HIV-positive patients with neuro-opportunistic infections by a real-time PCR assay. J Neuroparasitology. **2011**; 2:1-6.

Cardoso ES, Jesus BL, Gomes LG, Sousa SM, Gadelha SR, Marin LJ. The use of saliva as a practical and feasible alternative to urine in large-scale screening for congenital cytomegalovirus infection increases inclusion and detection rates. Revista da Sociedade Brasileira de Medicina Tropical. **2015**; 48(2):206-207.

Carellos, EV, Andrade GMQ, Vasconcelos-Santos DV, Januário JN, Romanelli RMC, Abreu MNS, et al. Adverse socioeconomic conditions and oocyst-related factors are associated with congenital toxoplasmosis in a population-based study in Minas Gerais, Brazil. PLoS One. **2014**; 9(2), e88588.

Cassaing S, Bessières MH, Berry A, Berrebi A, Fabre R, Magnaval JF. Comparison between two amplification sets for molecular diagnosis of toxoplasmosis by real-time PCR. J Clin Microbiol. **2006**; 44(3):720-4.

Centers for Disease Control and Prevention (CDC). Preventing Congenital CMV Infection. **2019**. Available from: https://www.cdc.gov/cmv/index.html.

Codaccioni C, Vauloup-Fellous C, Letamendia E, Saada J, Benachi A, Vivanti AJ. Case report on early treatment with valaciclovir after maternal primary cytomegalovirus infection. Journal of Gynecology Obstetrics and Human Reprodution. **2019**; S2468-7847 (18) 30419-7.

Coll O, Benoist G, Ville Y, Weisman LE, Botet F, Anceschi MM, et al. Guidelines on CMV Congenital Infection. Journal of Perinatal Medicine. **2009**; 37(5):433-45.

Collins AT, Cromwell LD. Computed tomography in the evaluation of congenital cerebral toxoplasmosis. Journal of Computer Assisted Tomography. **1980**; 4(3):326-9.

Commission on Social Determinants of Health. CSDH Final Report: Closing the Gap in a Generation: Health Equity Through Action on the Social Determinant of Health. Geneva, Switzerland: World Health Organization. **2008**. https://apps.who.int/iris/bitstream/handle/10665/43943/9789241563703_eng.pdf;jsess

Cong W, Dong XY, Meng QF, Zhou N, Wang XY, Huang SY, et al. *Toxoplasma gondii* infection in pregnant women: A Seroprevalence and case-control study in eastern China. Biomed Res Int. **2015**; 2015: 170278.

Contini C, Seraceni S, Cultrera R, Incorvaia C, Sebastiani A, Picot S. Evaluation of a real-time PCR-based assay using the Lightcycler system for detection of *Toxoplasma gondii* bradyzoite genes in blood specimens from patients with toxoplasmic retinochoroiditis. International Journal for Parasitology. **2005**; 35(3):275-83.

Cook AJ, Gilbert RE, Buffolano W. Sources of Toxoplasma infection in pregnant women: a european multicenter case–control study. Br Med J. **2000**; 15 321(7254): 142-147.

Coonrod DV, Jack BW, Stubblefield PG, Hollier LM, Boggess KA, Cefalo R, et al. The clinical content of preconception care: infectious diseases in preconception care. Am J Obstet Gynecol. **2008**; 199 (6 Suppl 2): S296-309.

Curti SP, Figueiredo CA, de Oliveira MI, Andrade JQ, Zugaib M, Pedreira DAL, et al. Prenatal diagnosis of congenital rubella infection in São Paulo. Revista da Associação Medica Brasileira **2014**; 60: 451-456.

Cutts FT, Vynnycky E. Modelling the incidence of congenital rubella syndrome in developing countries. Indian J Epidemiol. **1999**; 28: 1176-1184.

Chaabna K, Cheema S, Abraham A, Alrouh H, Lowenfels AB, Maisonneuve P, et al. Systematic overview of hepatitis C infection in the middle east and north Africa. World Journal of Gastroenterology. **2018**; 24(27): 3038-3054.

Chalandon Y, Degermann S, Villard J, Arlettaz L Kaiser L, Vischer S, et al. Pretransplantation CMV-specific T cells protect recipients of T-cell-depleted grafts against CMV-related complications. Blood. **2006**; 107(1):389-396.

Chapey E, Wallon M, Peyron F. Evaluation of the LDBIO point of care test for the combined detection of toxoplasmic IgG and IgM. Clinica Chimica Acta. **2017**; 464:200-201.

Cheng WY, Wang HC, Liu MT, Wu HS. Molecular surveillance of rubella viruses in Taiwan from 2005 to 2011. Journal of Medical Virology. **2013**; 85: 745-753.

Chibwe E, Mirambo MM, Kihunrwa A, Mshana SE. Magnitude of the cytomegalovirus infection among pregnant women attending antenatal clinics in the city of Mwanza, Tanzania. BMC Res Notes. **2017**; 10(1):489.

Chintapalli S, Padmaja IJ. Seroprevalence of toxoplasmosis in antenatal women with bad obstetric history. Trop Parasitol. **2013**; 3(1): 62-66.

Da Costa TL, Silva MG, Rodrigues IMX, Barbaresco AA, Avelino MM, Castro AM. Clinical and laboratory diagnosis of toxoplasmosis. NewsLab. **2007**; 85: 88-104.

Daffos F, Forestier F, Grangeot-Keros L, Capella Pavlovsky M, Lebon P, Chartier M, et al. Prenatal diagnosis of congenital rubella. Lancet. **1984**; 2: 1-3.

Dahlgren G, and Whitehead M. Policies and strategies to promote social equity in health. Stockholm: Institute for Future Studies. **1991**. https://core.ac.uk/download/pdf/6472456.pdf

Dard C, Fricker-Hidalgo H, Brenier-Pinchart MP, Pelloux H. Relevance of and new developments in serology for toxoplasmosis. Trends Parasitol. **2016**; 32(6):492-506.

Das JK, Salam RA, Bhutta ZA. Global burden of childhood diarrhea and interventions. Current Opinion in Infectiuos Diseases. **2014**; 27:451-458.

Das JK, Salam RA, Bhutta ZA. Global burden of childhood diarrhea and interventions. Curr Opin Infect Dis. **2014**; 27(5):451-8.

Dasari V, Smith C, Khanna R. Recent Advances in designing an effective vaccine to prevent cytomegalovirus-associated clinical diseases. Expert Review of Vaccines. **2013**; 12(6): 661-76.

Davi-Neto E, Triboni AH, Paula FJ, Vilas Boas LS, Machado CM, Agena F, et al. A double-blinded, prospective study to define antigenemia and quantitative real-time polymerase chain reaction cutoffs to start preemptive therapy in low-risk, seropositive, renal transplanted recipients. Transplantation. **2014**; 98(10): 1077-81.

Davis NL, King CC, Kourtis AP. Cytomegalovirus infection in pregnancy. Birth Defects Research. **2017**; 109 (5), 336-346.

De Carolis S, Santucci S, Botta A, Garofalo S, Martino C, Perrelli A, et al. False-positive Igm for CMV in pregnant women with autoimmune disease: A novel prognostic factor for poor pregnancy outcome. Lupus. **2010**; 19(7):844-9.

De Jong MD, Galasso GJ, Gazzard B, Griffiths PD, Jabs DA, Kern ER, et al. Summary of the II international symposium on cytomegalovirus. Antiviral Research. **1998**; 39(3), 141-162.

de Keyzer K, van Laecke S, Peeters P, Vanholder R. Human cytomegalovirus and kidney transplantation: a clinician's update. American Journal of Kidney Diseases. **2011**; 58:118-26.

de Oliveira Azevedo CT, do Brasil PE, Guida L, Lopes Moreira ME. Performance of Polymerase Chain Reaction Analysis of the Amniotic Fluid of Pregnant Women for Diagnosis of Congenital Toxoplasmosis: A Systematic Review and Meta-Analysis. PLoS One. **2016**; 11(4): e0149938.

de Vries JJ, van Zwet EW, Dekker FW, Kroes AC, Verkerk PH, Vossen AC. The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: a population-based prediction model. Reviews in Medical Virology. **2013**; 23(4):241-9.

de Wit LA, Croll DA, Tershy B, Correa D, Luna - Pasten H, Quadri P, et al. Potential public health benefits from cat eradications on islands. PLoS Negl Trop Dis. **2019**; 13(2): e0007040.

Decoster A, Gontier P, Dehecq E, Demory JL, Duhamel M. Detection of anti-toxoplasma immunoglobulin A antibodies by Platelia-Toxo IgA directed against P30 and by IMx Toxo IgA for diagnosis of acquired and congenital toxoplasmosis. Journal of Clinical Microbiology. **1995**; 33(8): 2206-2208.

Derouin F, Pelloux H. Prevention of toxoplasmosis in transplant patients. Clinical Microbiology and Infection. **2008**; 14, 1089-1101.

Díaz J, Henao J, Rodelo J, García A, Arbeláez M, Jaimes F. Incidence and risk factors for cytomegalovirus disease in a colombian cohort of kidney transplant recipients. Transplantation Proceedings. **2014**; 46(1):160-6.

Dimech, W., Panagiotopoulos, L., Francis, B., Laven, N., Marler, J., Dickeson, D. et al. Evaluation of eight anti-rubella virus immunoglobulin g immunoassays that report results in international units per millilitre. J Clin Microbiol. **2008**; 46: 1955-1960.

Dioverti MV, Razonable RR. Cytomegalovirus. Microbiology Spectrum. **2016**; 4(4).

Dodd RY. Transmission of parasites by blood transfusion. Vox Sang. **1998**; 74 Suppl 2:161-3.

Drew RJ, Stapleton P, Abu H, Healy E, Ferguson W, De Gascun C, et al. Pregnancy outcomes of mothers with detectable CMV-specifc IgM antibodies: a three-year review in a large irish tertiary referral maternity hospital. Infect Dis Obstet Gynecol. **2015**; 2015:5

Dromigny JA, Nabeth P, Perrier Gros Claude JD.Evaluation of the seroprevalence of rubella in the region of Dakar (Senegal). Trop Med Int Health. **2003**; 8(8):740-3.

Dubey JP. Toxoplasmoses of animals and humans. 2 ed. Boca Raton: CRC Press. **2010**. 336.

Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol. **2008**; 38: 1257-1278.

Dubey JP, Lindsay DS, Speer CA. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev. **1998**; 11(2): 267-299.

Dubey JP, Tiao N, Gebreyes WA, Jones JL. A review of toxoplasmosis in humans and animals in Ethiopia. Epidemiology and Infection. **2012**; 140(11):1935-8.

Dubey JP. History of the discovery of the life cycle of *Toxoplasma gondii*. International Journal for Parasitology. **2009**; 39(8):877-82.

Dubey JP. Sources of *Toxoplasma gondii* infection in pregnancy. Until rates of congenital toxoplasmosis fall, control measures are essential. BMJ. **2000**; 321: 127-128.

Dubey JP. Toxoplasmosis. Journal of the American Veterinary Medical Association. **1994**; 205:1593-8.

Dupouy-Camet J, de Souza SL, Maslo C, Paugam A, Saimot AG, Benarous R, et al. Detection of *Toxoplasma gondii* in venous blood from AIDS patients by polymerase chain reaction. Journal of Clinical Microbiology. **1993**; 31(7):1866-9.

Duque TLA, Souto XM, Andrade-Neto VV, Ennes-Vidal V, Menna-Barreto RFS. Autophagic balance between mammals and protozoa. A molecular, biochemical and morphological

review of apicomplexa and trypanosomatidae infections. Autophagy-A Double-Edged Sword. Chapter. **2013**; 14: 290-319.

Eapen M, Mathew CF, Aravindan KP. Evidence based criteria for the histopathological diagnosis of toxoplasmic lymphadenopathy. Journal of Clinical Pathology. **2005**; 58(11):1143-6.

Economist Intelligence Unit (EIU). **2019**a. Available at: https://country.eiu.com/angola

Economist Intelligence Unit (EIU). Economist prevê recessão económica em Angola neste e no próximo ano. Jornal o Observador; 14 Ago. **2019**b. Available at: https://observador.pt/2019/08/14/economist-preve-recessao-economica-em-angola-neste-e-no-proximo-ano/

Echániz-Avilés G, Tamayo-Legorreta E, Cruz-Valdez A, Rangel-Flores H, Hernández-Nevárez P, Gatica-Marquina R, et al. Prevalence of antibodies against cytomegalovirus in women of reproductive age. Salud Pública Mex. **1993**; 35(1):20-6.

Egorov AI, Converse R, Griffin SM, Styles J, Klein E, Sams E, et al. Environmental risk factors for *Toxoplasma gondii* infections and the impact of latent infections on allostatic load in residents of Central North Carolina. BMC Infectious Diseases. **2018**; 18(1), 421.

Eguchi H, Horita N, Ushio R, Kato I, Nakajima Y, Ota E, et al. Diagnostic test accuracy of antigenaemia assay for PCR-proven cytomegalovirus infection-systematic review and meta-analysis. Clinical Microbiology and Infection. **2017**; 23(12):907-915.

Einsele H, Roosnek E, Rufer N, Sinzger C, Riegler S, Löffler J, et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. Blood. **2002**; 99(11):3916-3922.

Elsafi SH, Al-Mutairi WF, Al-Jubran KM, Hassan MMA, Zahrani. Toxoplasmosis seroprevalence in relation to knowledge and practice among pregnant women in Dhahran, Saudi Arabia. Pathogens and Global Health. **2015**; 109(8), 377-382.

Elwell K. Facilitators and barriers to treatment adherence within PMTCT programs in Malawi. AIDS Care. **2016**; 28 (8), 971-975.

Ellington SR, Clarke KE, Kourtis AP. Cytomegalovirus infection in human immunodeficiency virus (HIV)-exposed and HIV-infected infants: A systematic review. The Journal of Infectious Diseases. **2016**; 213(6):891-900.

Ellis JT. Polymerase chain reaction approaches for the detection of neospora caninum and *Toxoplasma gondii*. International Journal for Parasitology. **1998**; 28(7):1053-60.

Emery VC, Lazzarotto T. Cytomegalovirus in pregnancy and the neonate. F1000Research. **2017**; 14; 6:138.

Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet. **2000**; 355:2032-2036.

Enders G, Daiminger A, Lindemann L, Knotek F, Bäder U, Exler S, et al. Cytomegalovirus (CMV) seroprevalence in pregnant women, bone marrow donors and adolescents in Germany, 1996–2010. Med Microbiol Immunol. **2012**; 201:303-309.

Enders G., Daiminger A., Bäder U., Exler S., Enders M. Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational age. J. Clin. Virol. **2011**; 52:244-246.

Enders M, Daiminger A, Exler S, Enders G. Amniocentesis for prenatal diagnosis of cytomegalovirus infection: challenging the 21 weeks' threshold. Prenatal Diagnosis. **2017**; 37(9):940-942.

Ephraim OE, Oyinlola O, Patrick VL, Joseph UO, Charles JE. Seroprevalence and risk factors for cytomegalovirus infection among pregnant women in southern Nigeria. JMID, **2013**; 3 (3): 123-127.

Epidemiology and prevention of Vaccine- Preventable Diseases. Rubella **2019**. available in: https://www.cdc.gov/vaccines/pubs/pinkbook/rubella.html#congenital

Erfanianahmadpoor M, Nasiri R, Vakili R, Hassannia T. Seroprevalence, transmission, and associated factors of specific antibodies against cytomegalovirus among pregnant women and their infants in a regional study. Saudi Med J. **2014**; 35(4):360-364.

Fadda M, Depping MK, Schulz PJ. Addressing issues of vaccination literacy and psychological empowerment in the measles-mumps-rubella (MMR) vaccination decision-making: a qualitative study. BMC Public Health. **2015**; 15:836.

Fallahi Sh, Kazemi B, Seyyed tabaei SJ, Bandehpour M, Lasjerdi Z, Taghipour N, et al. Comparison of the RE and B1 gene for detection of *Toxoplasma gondii* infection in children with cancer. Parasitology International. 2014; 63(1):37-41.

Ferreira FP, Caldart ET, Freire RL, Freitas FM De, Miura AC, Mareze M, et al. The effect of water source and soil supplementation on parasite contamination in organic vegetable gardens. Braz J Vet Parasitol. **2018**; 27(3): 327-337.

Festary A, Kourí V, Correa CB, Verdasquera D, Roig T, Couret MP. Cytomegalovirus and herpes simplex infections in mothers and newborns in a Havana maternity hospital. MEDICC Rev. **2015**; 17(1):29-34.

Filice GA, Hitt JA, Mitchell CD, Blackstad M, Sorensen SW. Diagnosis of toxoplasma parasitemia in patients with AIDS by gene detection after amplification with polymerase chain reaction. Journal of Clinical Microbiology.**1993**; 31(9):2327-31.

Fokunang, CN, Chia, J, Ndumbe P, Mbu P, Atashili J. Clinical studies on seroprevalence of rubella virus in pregnant women of Cameroon regions. Afr J Clin Exp Microbiol. **2010**; 11: 79-94.

Fowler KB, McCollister FP, Sabo DL, Shoup AG, Owen KE, Woodruff J5, et al. A Targeted Approach for congenital cytomegalovirus screening within newborn hearing screening. Pediatrics. **2017**; 139(2):e2016-2128.

Franck J, Garin YJ, Dumon H. LDBio-Toxo II immunoglobulin G western blot confirmatory test for anti-toxoplasma antibody detection. Journal of Clinical Microbiology. **2008**; 46(7):2334-8.

Frenk J, Bobadilla JL, Sepulveda J. Cervantes ML. "Health transition in middle-income countries: new challenges for health care", Health Policy and Planning, **1989**, 4(1):29-39.

Frimpong C, Makasa M, Sitali L, Michelo C. Seroprevalence and determinants of toxoplasmosis in pregnant women attending antenatal clinic at the university teaching hospital, Lusaka, Zambia. BMC Infect Dis. **2017**; 17(1): 10.

Fromont EG, Riche B, Rabilloud M. Toxoplasma seroprevalence in a rural population in France: detection of a household effect. BMC Infect Dis. **2009**; 9: 76.

Fuentes I, Rodriguez M, Domingo CJ, del Castillo F, Juncosa T, Alvar J. Urine sample used for congenital toxoplasmosis diagnosis by PCR. Journal of Clinical Microbiology. **1996**; 34(10):2368-71.

Gadelha C. "Development and Health: Looking for a New Utopia." **2009**. https://digitalrepository.unm.edu/lasm_cucs_en/58

Galván-Ramírez ML, Sánchez-Orozco LV, Gutiérrez-Maldonado AF, Rodriguez Pérez LR. Does *Toxoplasma gondii* infection impact liver transplantation outcomes? A systematic review. J Med Microbiol. **2018**; 67(4): 499-506.

Galvan-Ramirez ML, Troyo R, Roman S, Calvillo-Sanchez C, Bernal-Redondo R. A systematic review and meta-analysis of Toxoplasma gondii infection among the Mexican population. Parasit Vectors. **2012**; 5: 271.

Garry DJ, Elimian A, Wiencek V, Baker DA. Commercial laboratory IgM testing for *Toxoplasma gondii* in pregnancy; a 20-year experience. Infectious Diseases in Obstetrics and Gynecology. **2005**; 13(3):151-3.

Gashout A, Amro A, Erhuma M, Al-Dwibe H, Elmaihub E, Babba H, et al. Molecular Diagnosis of *Toxoplasma gondii* infection in Libya. BMC Infectious Diseases. **2016**; 16, 157.

Gasparinho C, Mirante MC, Centeno-Lima S, Istrate C, Mayer AC, Tavira L, et al. Etiology of diarrhea in children younger than 5 years attending the Bengo General Hospital in Angola. The Pediatric Infectious Disease Journal. **2016**; 35:e28-e34.

Gebremedhin EZ, Abebe AH, Tessema TS, Tullu KD, Medhin G, Vitale M, et al. Seroepidemiology of *Toxoplasma gondii* infection in women of child-bearing age in central Ethiopia. BMC Infectious Diseases. **2013**; 13: 101.

Gentile I, Zappulo E, Bonavolta R, Maresca R, Messana T, Buonomo AR, et al. Prevalence and titre of antibodies to cytomegalovirus and Epstein-Barr virus in patients with autism spectrum disorder. In Vivo. **2014**; 28(4):621-626.

Gentile I, Zappulo E, Riccio MP, Binda S, Bubba L, Pellegrinelli L, et al. Prevalence of congenital cytomegalovirus infection assessed through viral genome detection in dried blood spots in children with autism spectrum disorders. In Vivo. **2017**; 31(3):467-473.

George S, Viswanathan R, Sapkal GN. Molecular aspects of the teratogenesis of rubella virus. Biological Research. **2019**; 52: 47.

Getahun M, Beyene B, Ademe A, Teshome B, Tefera M, Afework A, et al. Epidemiology of laboratory confirmed measles virus cases in the southern nations of Ethiopia, 2007 – 2014. BMC Infectious Diseases. **2017**; 17(1):87.

Getahun M, Beyene B, Gallagher K, Ademe A, Teshome B, Tefera M, et al. Epidemiology of rubella virus cases in the pre-vaccination era of Ethiopia, 2009-2015. BMC Public Health. **2016**; 18; 16(1):1168.

Ghoneim NSS, Hassanain N, Zeedan G, Soliman Y, Abdalhamed A. Detection of genomic Toxoplasma gondii DNA and anti-Toxoplasma antibodies in high risk women and contact animals. Glob Vet. **2009**; 3: 395-400.

Goga AE, Dinh TH, Jackson DJ, Lombard C, Puren A, Sherman G, et al. Population-level effectiveness of PMTCT Option A on early mother-to-child (MTCT) transmission of HIV in South Africa: implications for eliminating MTCT. J Glob Health. **2016**; 6(2): 020405.

Gold R, Bunce A, Cottrell E, Marino M, Middendorf M, Cowburn S, et al. Study protocol: a pragmatic, stepped-wedge trial of tailored support for implementing social determinants of health documentation/action in community health centers, with realist evaluation. Implement Sci. **2019**; 14(1):9.

Gomez CA, Budvytyte LN, Press C, Zhou L, McLeod R, Maldonado Y, et al. Evaluation of three point-of-care tests for detection of toxoplasma immunoglobulin IgG and IgM in the United States: proof of concept and challenges. Open Forum Infectious Diseases. **2018**; 5(10):ofy215.

Grangeot-Keros L, Bouthry E, Vauloup-Fellous C. [Rubella: a current issue?]. Presse Medicale. **2014**; 43: 698-705.

Grangeot-Keros L, Mayaux MJ, Lebon P, Freymuth F, Eugene G, Stricker R, et al. Value of cytomegalovirus (CMV) IgG avidity index for the diagnosis of primary CMV infection in pregnant women. J Infect Dis. **1997**; 175(4):944-6.

Grangeot-Keros L, Simon B, Audibert F, Vial M. Should we routinely screen for cytomegalovirus antibody during pregnancy? Intervirology. **1998**; 41(4-5):158-62.

Grant GB, Desai S, Dumolard L, Kretsinger K, Reef SE. Progress toward rubella and congenital rubella syndrome control and elimination - Worldwide, 2000-2018. MMWR Morbidity and Mortality Weekly Report. **2019**; 68: 855-859.

Grant GB, Reef SE, Dabbagh A, Gacic-Dobo M, Strebel PM. Global progress toward rubella and congenital rubella syndrome control and elimination - 2000-2014. MMWR Morbidity and Mortality Weekly Report. **2015**; 64: 1052-1055.

Gras L, Gilbert RE, Wallon M, Peyron F, Cortina-Borja M. Duration of the IgM response in women acquiring *Toxoplasma gondii* during pregnancy: implications for clinical practice and cross-sectional incidence studies. Epidemiology and Infection. **2004**; 132(3):541-8.

Gratama JW, Boeckh M, Nakamura R, Cornelissen JJ, Brooimans RA, Zaia JA, et al. Immune monitoring with iTAg MHC Tetramers for prediction of recurrent or persistent cytomegalovirus infection or disease in allogeneic hematopoietic stem cell transplant recipients: a prospective multicenter study. Blood. **2010**; 116(10):1655-1662.

Griffiths P, Baraniak I, Reeves M. The pathogenesis of human cytomegalovirus. The Journal of Pathology. **2015**; 235(2):288-97.

Griffiths PD. Cytomegalovirus. In Arie Zuckerman, JanguBanatvala, Barry Schoub, Paul Griffiths, Philip Mortimer (ed.), Principles and Practice of Clinical Virology, 6ª ed. Wiley Blackwell, Oxford. **2009**; 161-198.

Griffiths P. Diagnosis of cytomegalovirus pneumonitis following stem cell transplantation: addressing the Yin and Yang of molecular methods. The Journal of Infectious Diseases. **2017**; 215(10):1493-1495.

Grønborg HL, Jespersen S, Hønge BL, Jensen-Fangel S, Wejse C. Review of cytomegalovirus coinfection in HIV-infected individuals in Africa. Reviews in Medical Virology. **2017**; 27(1).

Grover CM, Thulliez P, Remington JS, Boothroyd JC. Rapid prenatal diagnosis of congenital Toxoplasma infection by using polymerase chain reaction and amniotic fluid. Journal of Clinical Microbiology. **1990**; 28(10):2297-301.

Guerra B., Simonazzi G., Lazzarotto T. Impact of diagnostic and confirmatory tests and prenatal counseling on the rate of pregnancy termination among women with positive cytomegalovirus immunoglobulin M antibody titers. Am. J. Obstet. Gynecol. **2007**; 196(3) (221.e1-221.e6).

Guy EC, Pelloux H, Lappalainen M, Aspöck H, Hassl A, Melby KK, et al. Interlaboratory comparison of polymerase chain reaction for the detection of Toxoplasma gondii DNA added to samples of amniotic fluid. European Journal of Clinical Microbiology & Infectious Diseases. **1996**; 15 (10):836-39.

Halonen SK, Weiss LM. Toxoplasmosis. Handb Clin Neurol. **2013**; 114(8): 125-145.

Hamdan H, Abdelbagi IE, Nasser NM, Adam I. Seroprevalence of cytomegalovirus and rubella among pregnant women in western Sudan. Virology Journal. **2011**; 8:217.

Hamilton ST, van Zuylen W, Shand A, Scott GM, Naing Z, Hall B, et al. Prevention of congenital cytomegalovirus complications by maternal and neonatal treatments: a systematic review. Reviews in Medical Virology. **2014**; 24(6):420-33.

Hampton MM.Congenital toxoplasmosis: A Review. Neonatal Network. **2015**; 34(5), 274-278.

Hannachi N, Marzouk M, Harrabi I, Ferjani A, Ksouri Z, Ghannem H, et al. [Seroprevalence of rubella virus, varicella zoster virus, cytomegalovirus and parvovirus B19 among pregnant women in the Sousse region, Tunisia]. Bull Soc Pathol Exot. **2011**; 104(1):62-7.

Harrison GJ. Current controversies in diagnosis, management, and prevention of congenital cytomegalovirus: updates for the pediatric practitioner. Pediatric Annals. **2015**; 44(5):e115-25.

Hartmann K, Addie D, Belák S, Boucraut-Baralon C, Egberink H, Frymus T, et al. *Toxoplasma gondii* infection in cats: ABCD guidelines on prevention and management. J Feline Med Surg. **2013**;15(7): 631-7.

Harvey J, Dennis CL. Hygiene interventions for prevention of cytomegalovirus infection among childbearing women: systematic review. J Adv Nurs. **2008**; 63(5): 440-50.

Helieh S, Oz. Fetomaternal and Pediatric Toxoplasmosis. Journal of Pediatric Infectious Diseases. 2017;12(4): 202-208.

Hernández-Cortazar I, Acosta-Viana KY, Ortega-Pacheco A, Guzman-Marin Edel S, Aguilar-Caballero AJ, Jiménez-Coello M. Review Toxoplasmosis in Mexico: epidemiological situation in humans and animals. Rev Inst Med Trop Sao Paulo. **2015**; 57(2): 93-103.

Hill D, Dubey JP. *Toxoplasma gondii*: Transmission, diagnosis, and prevention. Clinical Microbiology and Infection. **2002**; 8(10): 634-640.

Hill DE, Chirukandoth S, Dubey JP. Biology and epidemiology of *Toxoplasma gondii* in man and animals. Animal Health Research Reviews. **2005**; 6(1):41-61.

Hitt JA, Filice GA. Detection of *Toxoplasma gondii* parasitemia by gene amplification, cell culture, and mouse inoculation. Journal of Clinical Microbiology. **1992**; 30(12): 3181-4.

Hofflin JM, Remington JS. Tissue culture isolation of toxoplasma from blood of a patient with AIDS. Archives Internal Medicine.**1985**; 145(5): 925-6.

Hofgärtner WT, Swanzy SR, Bacina RM, Condon J, Gupta M, Matlock PE, et al. Detection of immunoglobulin G (IgG) and IgM antibodies to *Toxoplasma gondii*: evaluation of four commercial immunoassay systems. Journal of Clinical Microbiology. **1997**; 35(12):3313-5.

Homan WL, Vercammen M, De Braekeleer J, Verschueren H. Identification of a 200- to 300-fold repetitive 529bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. International Journal for Parasitology. **2000**; 30(1):69-75.

Howe DK, Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. The Journal of Infectious Diseases. **1995**; 172(6):1561-6.

Ho-Yen DO, Joss AW, Balfour AH, Smyth ET, Baird D, Chatterton JM. Use of the polymerase chain reaction to detect *Toxoplasma gondii* in human blood samples. Journal of Clinical Pathology. **1992**; 45(10):910-3.

Hübschen JM, Bork SM, Brown KE, Mankertz A, Santibanez S, Mamou MBen, et al. Challenges of measles and rubella laboratory diagnostic in the era of elimination. Clinical Microbiology and Infection. **2017**; 23: 511-515.

Hubschen JM, Kremer JR, De Landtsheer S, Muller CP. A multiplex TaqMan PCR assay for the detection of measles and rubella virus. Journal of Virological Methods. **2008**; 149: 246-250.

Hughes BL, Gyamfi-Bannerman C. Society for Maternal-Fetal Medicine (SMFM) Diagnosis and antenatal management of congenital cytomegalovirus infection. American Journal of Obstetric and Gynecology. **2016**; 214(6): B5-B11.

Hui SYA, Sahota DS, Lao TT. Impact of the two-dose rubella vaccination regimen on incidence of rubella seronegativity in gravidae aged 25 years and younger. PLoS One. **2017**; 12: e0183630.

Human Development Reports (UNDP). Inequalities in Human Development in the 21st Century: Angola **2019**. Available at: http://hdr.undp.org/sites/all/themes/hdr_theme/country-notes/AGO.pdf

Ibrahim HM, Mohamed AH, El-Sharaawy AA, El-Shqanqery HE. Molecular and serological prevalence of *Toxoplasma gondii* in pregnant women and sheep in Egypt. Asian Pac J Trop Med. **2017**; 10(10): 996-1001.

Ibrahim S, Siddiqui AA, Siddiqui AR, Ahmed W, Moss PA, Lalani EM. Sociodemographic factors associated with IgG and IgM seroprevalence for human cytomegalovirus infection in adult populations of Pakistan: a seroprevalence survey. BMC Public Health. **2016**; 16: 1112.

Ikuta K, Minematsu T, Inoue N, Kubo T, Asano K, Ishibashi K, et al. Cytomegalovirus (CMV) glycoprotein H-based serological analysis in Japanese healthy pregnant women, and in neonates with congenital CMV infection and their mothers. J Clin Virol. **2013**; 58(2):474-8.

Inquérito de Indicadores Múltiplos e de Saúde (IIMS) em Angola 2015-2016. Instituto Nacional de Estatística. Ministério da Saúde. Ministério do Planeamento e do Desenvolvimento Territorial. ICF e Rockville, Maryland, EUA: INE, MINSA, MINPLAN e ICF. **2017**. Available at: https://dhsprogram.com/pubs/pdf/FR327/FR327.pdf

Inquérito Integrado Sobre o Bem-Estar da População (IIBEP) 2008-2009: Principais Resultados Grelha de Indicadores, Angola. Ministério do Planeamento, Instituto Nacional de Estatística **2010**. Available at: https://www.ine.gov.ao/images/IBEP_Relatorio_Analitico_Vol.I.pdf

Instituto Nacional de Estatística (INE). Projecções sobre população estimada em Angola.**2018**. Available at: https://www.ine.gov.ao

Instituto Nacional de Estatística (INE). Resultados definitivos do recenseamento geral da população e da habitação de Angola **2014**. Available: http://www.embajadadeangola.com/pdf/Publicacao%20Resultados%20Definitivos%20Ce

nso%20Geral%202014_Versao%2022032016_DEFINITIVA%2018H17.pdf. Accessed 15 Sept 2015.

Ishaku BS, Ajogi I, Umoh JU, Lawal I, Randawa AJ. Seroprevalence and risk factors for Toxoplasma gondii infection among antenatal women in Zaria, Nigeria. Res J Med Med Sci. **2009**; 4: 483-488.

Ishibashi K, Tokumoto T, Shirakawa H, Hashimoto K, Ikuta K, Kushida N, et al. Lack of antibodies against the antigen domain 2 epitope of cytomegalovirus (CMV) glycoprotein B is associated with CMV disease after renal transplantation in recipients having the same glycoprotein H serotypes as their donors. Transplant infectious disease: an official journal of the Transplantation Society. **2011**; 13(3):318-23.

Jahromi AS, Kazemi A, Manshoori G, Madani A, Moosavy SH, Seddigh B. Seroprevalence of Rubella virüs in women with spontaneus abortion. Am J Inf Dis. **2011**; 7:16-9.

James, GS, Sintchenko VG, Dickeson DJ, Gilbert GL. Comparison of cell culture, mouse inoculation, and PCR for detection of Toxoplasma gondii: effects of storage conditions on sensitivity. Journal of Clinical Microbiology. **1996**; 34(6):1572-5.

Jiang RL, Ma LH, Ma ZR, Hou G, Zhao Q, Wu X. Seroprevalence and associated risk factors of Toxoplasma gondii among Manchu pregnant women in northeastern China. Microb Pathog. **2018**; 123: 398-401.

Jin Q, Su J, Wu S. Cytomegalovirus infection among pregnant women in Beijing: seroepidemiological survey and intrauterine transmissions. J Microbiol Biotechnol. **2017**; 27(5):1005-1009.

Jindal N, Aggarwal A. A pilot seroepidemiological study of cytomegalovirus infection in women of child bearing age. Indian J Med Microbiol. **2005**; 23(1):34.

Jobe, A. H. CMV transmission in human milk. J. Pediatr. **2009**.154: A1.

Johnson J, Anderson B, Pass RF. Prevention of maternal and congenital cytomegalovirus infection. Clin Obstet Gynecol. **2012**; 55(2):521-30.

Johnson J, Anderson B. Screening, prevention, and treatment of congenital cytomegalovirus. Obstetrics Gynecology Clinics of North America. **2014**; 41(4):593-599.

Joint United Nations Programme on HIV/AIDS (UNAIDS) **2011**. How to get to zero: Faster. Smarter. Better Available: https://www.unaids.org/sites/default/files/media_asset/JC2216_WorldAIDSday_report_2011_en_1.pdf. XXXX

Jonas A, Cardemil CV, Beukes A, Anderson R, Rota PA, Bankamp B, et al. Rubella immunity among pregnant women aged 15-44 years, Namibia, 2010. Int J Infect Dis. **2016**; 49:196-201.

Jones J, Lopez A, Wilson M. Congenital toxoplasmosis. Am Fam Physician. **2003**; 67(10): 2131-2138.

Jones JL, Dubey JP. Foodborne toxoplasmosis. Clinical Infectious Diseases. **2012**; 55: 845-851.

Jones JL, Dubey JP. Waterborne Toxoplasmosis-Recent Developments. Experimental Parasitology. **2010**; 124(1):10-25.

Jones JL, Parise ME, Fiore AE. Neglected Parasitic Infections in the United States: Toxoplasmosis. The American Journal of Tropical Medicine Hygiene. **2014**; 90(5): 794-799.

Joseph P, Calderón M M, Gilman RH, Quispe ML, Cok J, Ticona E, et al. Optimization and evaluation of a PCR assay for detecting toxoplasmic encephalitis in patients with AIDS. Journal of Clinical Microbiology. **2002**; 40(12): 4499-4503.

Jückstock J, Rothenburger M, Friese K, Traunmüller F. Passive Immunization against Congenital Cytomegalovirus Infection: Current State of Knowledge. Pharmacology. **2015**; 95(5-6):209-217.

Kamal AM, Ahmed AK, Abdellatif MZ, Tawfik M, Hassan EE. Seropositivity of toxoplasmosis in pregnant women by ELISA at Minia University Hospital, Egypt. Korean J Parasitol. **2015**; 53(5): 605-610.

Karabulut A, Polat Y, Türk M, Isik Balci Y. Evaluation of rubella, toxoplasma gondii and cytomegalo virus seroprevalemces among pregnant women in Denizli province. Turk J Med Sci. **2011**; 41:159-64.

Karacan M, Batukan M, Cebi Z, Berberoglugil M, Levent S, Kır M, et al. Screening cytomegalovirus, rubella and toxoplasma infections in pregnant women with unknown pre-pregnancy serological status. Archives of Gynecology and Obstetrics. **2014**; 290: 1115-1120.

Karmakar SD, Breslin FC. The role of educational level and job characteristics on the health of young adults. Soc Sci Med. **2008**; 66(9): 2011-2022.

Kasper DC, Sadeghi K, Prusa AR, Reischer GH, Kratochwill K, Forster-Waldl E, et al. Quantitative real-time polymerase chain reaction for the accurate detection of *Toxoplasma gondii* in amniotic fluid. Diagnostic Microbioloy and Infectious Disease. **2009**; 63(1): 10-5.

Koczula KM, Gallotta Andrea. Lateral flow assays. Essays in Biochemistry. **2016**; 60: 111-120.

Kaushik A, Verma S, Kumar P. Congenital rubella syndrome: A brief review of public health perspectives. Indian Journal of Public Health. **2018**; 62: 52-54.

Kawada J, Torii Y, Kawano Y, Suzuki M, Kamiya Y, Kotani T, et al. Viral load in children with congenital cytomegalovirus infection identified on newborn hearing screening. Journal of Clinical Virology. **2015**; 65:41-45.

Kendall C, Kerr LR, Mota RM, Cavalcante S, Macena RH, Chen S, et al. Population size, HIV, and behavior among MSM in Luanda, Angola: challenges and findings in the first ever HIV and syphilis biological and behavioral survey. Journal of Acquired Immune Deficiecy Syndromes (1999). **2014**; 66(5): 544-551.

Kenneson A, Cannon MJ: Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007; 17(4): 253- 276.

Khairi SI, Intisar KS, Enan KH, Ishag MY, Baraa AM, Ali YH. Seroprevalence of cytomegalovirus infection among pregnant women at Omdurman Maternity Hospital, Sudan. J. Med. Lab. Diagn. **2013**; 4(4): 45-49.

Khalil A, Jones C, Ville Y. Congenital cytomegalovirus infection: management update. Current Opinion Infectious Diseases. **2017**; 30(3):274-280.

Khan S, Shahnaz M, Jehan N, Rehman S, Shah MT, Din I. Drinking water quality and human health risk in Charsadda district, Pakistan. J Clean Prod. **2013**; 60, 93-101.

Kieffer F, Wallon M. Congenital toxoplasmosis. Handbook of Clinical Neurology. **2013**; 112:1099-101.

Kirchmeier M, Fluckiger AC, Soare C, Bozic J, Ontsouka B, Ahmed T, et al. Enveloped virus-like particle expression of human cytomegalovirus glycoprotein B antigen induces antibodies with potent and broad neutralizing activity. Clinical and Vaccine Immunology. **2014**; 21(2):174-180.

Koczula KM, Gallotta A. Lateral flow assays. Essays Biochem. **2016**; 60(1):111-120.

Kolawole OM, Anjorin EO, Adekanle DA, Kolawole CF, Durowade KA. Seroprevalence of Rubella IgG Antibody in Pregnant Women in Osogbo, Nigeria, Int J Prev Med. **2014**; 5(3): 287-292.

Kompalic-Cristo A, Frotta C, Suarez-Mutis M, Fernandes O, Britto C. Evaluation of a real-time PCR assay based on the repetitive B1 gene for the detection of Toxoplasma gondii in human peripheral blood. Parasitology Research. **2007**; 101(3):619-25.

Kompalic-Cristo, A. Nogueira SA, Guedes AL, Frota C, González LF, Brandão A, Amendoeira MR, et al. Lack of technical specificity in the molecular diagnosis of toxoplasmosis. Transations of the Royal Society of Tropical Medicine and Hygiene. **2004**; 98(2):92-5.

Korndewal MJ, Oudesluys-Murphy AM, Kroes ACM, Vossen ACTM, de Melker HE. Congenital cytomegalovirus infection: child development, quality of life and impact on daily life. Pediatr Infect Dis J. **2017**; 36(12): 1141-7.

Kuessel L, Husslein H, Marschalek J, Brunner J, Ristl R, Popow-Kraupp T, et al. Prediction of maternal cytomegalovirus serostatus in early pregnancy: A retrospective analysis in Western Europe. PLoS One. **2015**; 10 (12): e0145470.

Kupferschmidt O, Krüger D, Held TK, Ellerbrok H, Siegert W, Janitschke K. Quantitative detection of *Toxoplasma gondii* DNA in human body fluids by TaqMan polymerase chain reaction. Clinical Microbiology and Infection. **2001**; 7(3):120-4.

La Rosa C, Longmate J, Lacey SF, Kaltcheva T, Sharan R, Marsano D, et al. Clinical evaluation of safety and immunogenicity of PADRE-cytomegalovirus (CMV) and tetanus-CMV fusion peptide vaccines with or without PF03512676 adjuvant. The Journal of Infectious Diseases. **2012**; 205(8):1294-1304.

Laboudi M. Review of toxoplasmosis in Morocco: seroprevalence and risk factors for toxoplasma infection among pregnant women and HIV- infected patients. The Pan African Medical Journal. **2017**; 27, 269.

Lambert N, Strebel P, Orenstein W, Icenogle J, Poland GA. Rubella. Lancet. **2015**; 385(9984), 2297-2307.

Lamoril J, Molina JM, de Gouvello A, Garin YJ, Deybach JC, Modai J, et al. Detection by PCR of Toxoplasma gondii in blood in the diagnosis of cerebral toxoplasmosis in patients with AIDS. Journal of Clinical Pathology. **1996**; 49(1):89-92.

Lanzieri TM, Dollard SC, Bialek SR, Grosse SD. Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries. International Journal of Infectious Diseases. **2014**; 22:44-8.

Lazzarotto T, Gabrielli L, Guerra B, Cervi F, Piccirilli G, Simonazzi G, et al. Diagnosis and prognosis of congenital CMV infection: a case report and review of the literature. Scandinavian Journal of Clinical and Laboratory Investigation Supplementum. **2014**; 244:34-40.

Lazzarotto T, Guerra B, Lanari M, Gabrielli L, Landini MP. New Advances in the Diagnosis of Congenital Cytomegalovirus Infection. Journal of Clinical Virology. **2008**; 41(3):192-197.

Lazzarotto T, Ripalti A, Bergamini G, Battista M, Spezzacatena P, Campanini F, et al. Development of a new cytomegalovirus (CMV) immunoglobulin M (IgM) immunoblot for detection of CMV-specifc IgM. J Clin Microbiol. **1998**; 36(11):3337-41.

Lazzarotto T, Spezzacatena P, Pradelli P, Abate DA, Varani S, Landini MP. Avidity of immunoglobulin G directed against human cytomegalovirus during primary and secondary infections in immunocompetent and immunocompromised subjects. Clin Diagn Lab Immunol. **1997**; 4(4):469-73.

Lazzarotto T, Varani S, Spezzacatena P, Gabrielli L, Pradelli P, Guerra B, et al. Maternal IgG avidity and IgM detected by blot as diagnostic tools to identify pregnant women at risk of transmitting cytomegalovirus. Viral Immunol. **2000**; (1):137-41.

Lee JY, Bowde DS. Rubella virus replication and links to teratogenicity. Clinical Microbiology Reviews. **2000**; 13(4), 571-587.

Leeper C, Lutzkanin A 3rd. Infections during pregnancy. Prim Care. **2018**; 45(3): 567-586.

Leruez-Ville M, Magny J-F, Couderc S, Pichon C, Parodi M, Bussières L, et al. Risk factors for congenital cytomegalovirus infection following primary and nonprimary maternal infection: a prospective neonatal screening study using polymerase chain reaction in saliva. Clin Infect Dis. **2017**; 65(3): 398-404.

Leruez-Ville M, Sellier Y, Salomon LJ, Stirnemann JJ, Jacquemard F, Ville Y. Prediction of fetal infection in cases with cytomegalovirus immunoglobulin M in the first trimester of pregnancy: a retrospective cohort. Clin Infect Dis. **2013**; 56(10):1428-35.

Leruez-Ville M, Stirnemann J, Sellier Y, Guilleminot T, Dejean A, Magny JF, et al. Feasibility of predicting the outcome of fetal infection with cytomegalovirus at the time of prenatal diagnosis. American Journal of Obstetric and Gynecology. **2016**; 215(3):342.e1-9.

Levin A, Yaari S, Stoff R, Caplan O, Wolf DG, Israeli E. Diagnosis of Cytomegalovirus Infection during Exacerbation of Ulcerative Colitis. Digestion. **2017**; 96(3):142-148.

Li M, Mo XW, Wang L, Chen H, Luo QL, Wen HQ, et al. Phylogeny and virulence divergency analyses of *Toxoplasma gondii* isolates from China. Parasites & Vectors. **2014**; 7, 133.

Liesenfeld O, Press C, Press JG, Montoya JG, Gill R, Isaac-Renton JL, et al. Falsepositive results in immunoglobulin M (IgM) toxoplasma antibody tests and importance of

confirmatory testing: the Platelia Toxo IgM test. Journal of Clinical Microbiology. **1997**; 35(1): 174-8.

Lin MH, Chen TC, Kuo TT, Tseng CC, Tseng CP. Real time PCR for quantitative detection of Toxoplasma gondii. Journal of Clinical Microbiology. **2000**; 38(11):4121-5.

Lin YL, Liao YS, Liao LR, Chen FN, Kuo HM, He S. Seroprevalence and sources of Toxoplasma infection among indigenous and immigrant pregnant women in Taiwan. Parasitol Res. **2008**; 103: 67-74.

Lino TN, Carvalho FR, Cosendey RI, Souza CF, Klumb EM, Silva AA, et al. pp65 antigenemia and cytomegalovirus diagnosis in patients with lupus nephritis: report of a series. Jornal Brasileiro de Nefrologia. **2018**; 40(1):44-52.

Liu L, Liu LN, Wang P, Lv TT, Fan YG, Pan HF. Elevated seroprevalence of *Toxoplasma gondii* in AIDS/HIV patients: A meta-analysis. Meta-Analysis Acta Trop. **2017**; 176:162-167.

Liu Q, Wang ZD, Huang SY, Zhu XQ. Diagnosis of Toxoplasmosis and Typing of *Toxoplasma Gondii*. Parasites & Vectors. **2015**; 8: 292.

Lobo ML, Patrocinio G, Sevivas T, DE Sousa B, Matos O. Portugal and Angola: similarities and differences in *Toxoplasma gondii* seroprevalence and risk factors in pregnant women. Epidemiol Infect. **2017**; 145(1): 30-40.

López R, Contreras R, Font L, Vega O. Presence of antibodies against *Toxoplasma gondii* in adolescents from the African continent. Rev Latinoam Microbiol. **1992**; 34: 49-52.

Lussiana C, Clemente SVL, Ghelardi A, Lonardi M, Tarquino IAP, Floridia M. Effectiveness of a prevention of mother-to-child HIV transmission programme in an urban hospital in Angola. PLoS One. **2012**; 7(4): e36381

Macé M, Cointe D, Six C, Levy-Bruhl D, Parent du Châtelet I, Ingrand D, et al. Diagnostic value of reverse transcription-PCR of amniotic fluid for prenatal diagnosis of congenital rubella infection in pregnant women with confirmed primary rubella infection. Journal of Clinical Microbiology. **2004**; 42: 4818-4820.

Mackenbach JP, Stronks K, Kunst, AE. The contribution of medical care to inequalities in health: differences between socio-economic groups in decline of mortality from conditions amenable to medical intervention. Soc Sci Med. **1989**; 29(3):369-76.

Mackenbach JP. The contribution of medical care to mortality decline: McKeown revisited. J Clin Epidemiol. **1996**; 49(11):1207-1213.

Maingi Z, Nyamache AK. Seroprevalence of Cytomegalo Virus (CMV) among pregnant women in Thika, Kenya. BMC Res Notes. **2014** Nov 12;7:794.

Maldonado YA, Read JS. AAP Committee on infectious diseases. Diagnosis, Treatment, and Prevention of Congenital Toxoplasmosis in the United States. Pediatrics. **2017**;139:e20163860.

Malm G, Engman ML. Congenital cytomegalovirus infections. Semin Fetal Neonatal Med **2007**; 12 (3): 154-9.

Mamvura TS, Chin'ombe N, Ruhanya V, Nziramasanga P. Seroprevalence of rubella virus IgG in pregnant women in Harare, Zimbabwe. Germs. **2015**; 5(2):50-2.

Mandelbrot L. [Prevention of mother-to-child transmission of toxoplasmosis: perspectives]. Gynecol Obstet Fertil. **2012**; 40(10):591-8.

Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. Clinical Microbiology Reviews. **2013**; 26(1):86-102.

Mareze M, Benitez ADN, Brandão APD, Pinto-Ferreira F, Miura AC, Martins FDC, et al. Socioeconomic vulnerability associated to *Toxoplasma gondii* exposure in southern Brazil. PLoS One. **2019**; 14(2): e0212375.

Marsh K. Malaria Disaster in Africa. Lancet. **1998**; 352(9132):924.

Marsico C, Kimberlin DW. Congenital cytomegalovirus infection: advances and challenges in diagnosis, prevention and treatment. Italian Journal of Pediatric. **2017**; 43(1):38.

Marston M, Becquet R, Zaba B, Moulton LH, Gray G, Coovadia H, et al. Net survival of perinatally and postnatally HIV-infected children: a pooled analysis of individual data from sub-Saharan Africa. Int J Epidemiol. **2011**; 40(2): 385– 396

Martin F, Palladino C, Mateus R, Bolzan A, Gomes, P, Brito J, et al. Early infant diagnosis of HIV-1 infection in Luanda, Angola, using a new DNA PCR assay and dried blood spots. PLoS One. **2017**; 12(7): e0181352.

Martínez-Quintana E, Castillo-Solórzano C, Torner N, Rodríguez González F. Congenital rubella syndrome: a matter of concern. Rev Panam Salud Pública. **2015**; 37: 179-186.

Martins J, Abranches P. Toxoplasmosis infection in subjects of Huambo, Angola. An Inst Hig Med Trop (Lisb). **1976**; 4(1-4): 111-112.

Massa NM, Duncan E, Jovanovic T, Kerley K, Weng L, Gensler L, et al. Relationship between *Toxoplasma gondii* seropositivity and acoustic startle response in an inner-city population. Brain Behav Immun. **2017**; 61:176-183.

Matin S, Shahbazi GH. Study on abortion associated with *Toxoplasma gondii* in women based on PCR detection of aborted placenta and maternal serology in Ardabil, Iran. Journal of Medical Microbiology & Diagnosis. **2017**; 6:2.

Maudry A, Chene G, Chatelain R, Patural H, Bellete B, Tisseur B, et al. Bicentric evaluation of six Anti-Toxoplasma immunoglobulin G (IgG) automated immunoassays and comparison to the Toxo II IgG Western blot. Clinical and Vaccine Immunology. **2009**; 16(9): 1322-6.

Maxwell AE, Young S, Crespi CM, Vega RR, Cayetano RT, Bastini R. Social determinants of health in the Mixtec and Zapotec. Int J Equity Health. **2015**; 14, 16. Available at: http://www.equityhealthj.com/content/14/1/16.

McVoy MM, Tenorio E, Kauvar LM. A Native Human Monoclonal Antibody Targeting HCMV gB (AD-2 Site I). International journal of molecular sciences. **2018**; 19(12).

Mestas E. Congenital Cytomegalovirus. Advances in Neonatal Care. **2016**; 16(1): 60-65.

Meyer H, Sundqvist VA, Pereira L, Mach M. Glycoprotein gp116 of human cytomegalovirus contains epitopes for strain-common and strain-specific antibodies. The Journal of general virology. **1992**; 73 (Pt 9):2375-83.

Ministério da Saúde de Angola (MINSA). V Plano Estratégico Nacional de Resposta às ITS/VIH-SIDA e Hepatites Virais - Angola 2015 – 2018, Luanda, Angola. **2014**. Available at: https://angola.nacoesunidas.org/wp-content/uploads/2018/01/PEN-V-ITS-VIH-SIDA-2015-2018-FINAL.pdf.

Ministério da Saúde. (MINSA). Vacina da rubéola na rede de saúde. Jornal de Angola. 28 de Abril, **2017**. Ref: http://jornaldeangola.

Ministério de Planeamento de Angola. (MPA). Relatório Sobre os Objectivos de Desenvolvimento do Milénio. 2010. Available at: https://www.undp.org/content/dam/undp/library/MDG/english/MDG%20Country%20Reports/Angola/angola_**2010**.pdf

Minsart AF, Smiljkovic M, Renaud C, Gagné MP, Lamarre V, Kakkar F, et al. Use of Cytomegalovirus-Specific Hyperimmunoglobulins in Pregnancy: A Retrospective Cohort. Journal of Obstetrics and Gynaecology Canada. **2018**; 40(11): 1409-1416.

Mohammed DA, Shittu O, Sadauki H, Olayinka A, Kolawole B, Adejo D. Prevalence of rubella IgG antibodies among pregnant women in Zaria, Nigeria. Int Health. **2010**; 2: 156-159.

Moncada PA, Montoya JG. Toxoplasmosis in the fetus and newborn: an update on prevalence, diagnosis and treatment. Expert Review of Anti- Infective Therapy. **2012**; 10:815-28.

Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. **2004**; 363(9425):1965-76.

Montoya JG. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis.The Journal Infectious Diseases. **2002**; 185: 73-82.

Morgan-Capner P, Rodeck CH, Nicolaides KH, Cradock-Watson JE. Prenatal detection of rubella-specific IgM in fetal sera. Prenatal Diagnosis. **1985**; 5: 21-26.

Morozov VA, Morozov AV, Denner J. New PCR diagnostic systems for the detection and quantification of porcine cytomegalovirus (PCMV). Archives Virology. **2016**; 161(5):1159-1168.

Mosquera Mdel M, de Ory F, Moreno M, Echevarria, JE. Simultaneous detection of measles virus, rubella virus, and parvovirus B19 by using multiplex. Journal of Clinical Microbiology. **2002**; 40: 111-116.

Mousavi M, Saravani R, Modrek MJ, Shahrakipour M, Sina S. Detection of *Toxoplasma gondii* in Diabetic Patients Using the Nested PCR Assay via RE and B1 Genes. Jundishapur Journal of Microbiology. **2016**; 9(2): e29493.

Mozzatto L, Procianoy RS. Incidence of congenital toxoplasmosis in Southern Brazil: a prospective study. Rev Inst Med Trop Sao Paulo. **2003**; 45(3):147-51.

Muldoon KM. Armstrong-Heimsoth A, Thomas J. Knowledge of congenital cytomegalovirus (cCMV) among physical and occupational therapists in the United States. PLoS One. **2017**; 12(10): e0185635.

Murat, JB, Hidalgo HF, Brenier-Pinchart MP, Pelloux H. Human toxoplasmosis: which biological diagnostic tests are best suited to which clinical situations? Expert Review of Anti - Infective Therapy. **2013**; 11(9): 943-956.

Mustapha M, Musiime V, Bakeera-Kitaka S, Rujumba J, Nabukeera-Barungi N. Utilization of "prevention of mother-to-child transmission" of HIV services by adolescent and young mothers in Mulago Hospital, Uganda. BMC Infect Dis. **2018**; 18 (1): 566.

Mwambe B, Stephen EM, Benson RK, Anthony NM, Humphrey DM, Denna M, et al. Sero-prevalence and factors associated with *Toxoplasma gondii* infection among pregnant women attending antenatal care in Mwanza, Tanzania. Parasit Vectors. **2013**; 6: 222.

Naing ZW, Scott GM, Shand A, Hamilton ST, van Zuylen WJ, Basha J, et al. Congenital cytomegalovirus infection in pregnancy: a review of prevalence, clinical features, diagnosis and prevention. The Australian & New Zealand Journal Obstetric & Gynaecology. **2016**; 56(1):9-18.

Nakamura R, La Rosa C, Longmate J, Drake J, Slape C, Zhou Q, et al. Viraemia, immunogenicity, and survival outcomes of cytomegalovirus chimeric epitope vaccine

supplemented with PF03512676 (CMVPepVax) in allogeneic haemopoietic stem-cell transplantation: randomised phase 1b trial. The Lancet Haematology. **2016**; 3(2):e87-98.

Namuwulya P, Abernathy E, Bukenya H, Bwogi J, Tushabe P, Birungi M, et al. Phylogenetic analysis of rubella viruses identified in Uganda, 2003-2012. Journal of Medical Virology. **2014**; 86: 2107-2113.

Nankabirwa J, Brooker SJ, Clarke SE, Fernando D, Gitonga CW, Schellenberg, D, et al. Malaria in School-Age Children in Africa: An Increasingly Important Challenge. Trop Med Int Health. **2014**; 19(11):1294-1309.

Nash JQ, Chissel S, Jones J, Warburton F, Verlander NQ. Risk factors for toxoplasmosis in pregnant women in Kent, United Kingdom. Epidemiol Infect. **2005**; 133: 475-483.

National malaria control programme (NMCP). National malaria strategy 2016 –2020. Angola: Ministry of Health. **2015**. Available at: https://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy16/fy-2016-angola-malaria-operational-plan.pdf?sfvrsn=6

Navigator ClinLab. Rubella.; **2013** (Available from: http://www.clinlabnavigator.com/rubella.html. [cited October 18/207]).

Nayeri T, Sarvi S, Moosazadeh M, Amouei A, Hosseininejad Z, Daryani A. The global seroprevalence of anti-Toxoplasma gondii antibodies in women who had spontaneous abortion: A systematic review and meta-analysis. PLoS Negl Trop Dis. **2020**; 14(3): e0008103.

Neirukh T, Qaisi A, Saleh N, Rmaileh AA, Zahriyeh EA, Qurei L, et al. Seroprevalence of cytomegalovirus among pregnant women and hospitalized children in Palestine. BMC Infect Dis. **2013**; 13:528.

Nelson CS, Huffman T, Jenks JA, Cisneros de la Rosa E, Xie G, et al. HCMV glycoprotein B subunit vaccine efficacy mediated by nonneutralizing antibody effector functions. Proceedings of the National Academy of Scisence of the United States of America. **2018**; 115(24):6267-6272.

Nene B, Jayant K, Arrossi S, Shastri S, Budukh A, Hingmire S, et al. Determinants of women's participation in cervical cancer screening trial, Maharashtra, India. Bull World Health Organ. **2007**; 85:264-72.

Neu N, Duchon J, Zachariah P. TORCH Infections. Clinics in Perinatology. **2015**; 42 (1): 77-103.

Ng SH, Azizi A, Edamura K, Malott RJ, Charlebois RL, Logvinoff C, et al. Preliminary Evaluation of Next-Generation Sequencing Performance Relative to qPCR and In Vitro

Cell Culture Tests for Human Cytomegalovirus. PDA Journal of Pharmaceutical Science and Technology. **2014**; 68(6):563-571.

Nicolle C, Manceaux L. Sur une infection a corps de Leishman (ou organismes voisins) du gondi. C R Acad Sci (Paris), **1908**; 147: 763-6.

Nichols LM, Taylor LA. Social determinants of health: The authors reply. Health Affairs (Millwood). **2018**; 37(8):1223-1230.

Nigro G. Hyperimmune globulin in pregnancy for the prevention of congenital cytomegalovirus disease. Expert Review of Anti-Infective Therapy. **2017**; 15(11): 977-986.

Nisbet AI, Omuse G, Revathi G, Adam RD. Seroprevalence data at a private teaching hospital in Kenya: An examination of *Toxoplasma gondii*, cytomegalovirus, rubella, hepatitis A, and Entamoeba histolytica. PLoS One. **2018** Oct 16;13(10):e0204867.

Nissapatorn V, Suwanrath C, Sawangjaroen N, Ling LY, Chandeying V. Toxoplasmosis-serological evidence and associated risk factors among pregnant women in southern Thailand. Am J Trop Med Hyg. **2011**; 85(2): 243-247.

Numan O, Vural F, Aka N, Alpay M, Coskun AD. TORCH seroprevalence among patients attending obstetric care clinic of haydarpasa training and research hospital affiliated to association of Istanbul northern anatolia public hospitals. North Clin Istanb. **2015**; 2(3): 203-209.

O'Farrell B. Evolution in Lateral Flow Immunoassay Systems. In; Wong RC, Tse HY, editors. Lateral Flow Immunoassay.Springer, New York: **2009**; 1-34.

O'Connor C, Le Blanc D, Drew RJ. Epidemiological changes in rubella IgG antibody levels detected in antenatal women from a retrospective rubella seroprevalence study. Irish Journal of Medical Science. **2018**; 187: 689-692.

Ogoina D, Onyemelukwe GC, Musa BO, Obiako RO. Seroprevalence of IgM and IgG antibodies to Toxoplasma infection in healthy and HIV-positive adults from Northern Nigeria. Journal of Infection in Developing Countries. **2013**; 7(5), 398-403.

Ojala P, Vesikari T, Elo O. Rubella during pregnancy as a cause of congenital hearing loss. Am. J. Epidemiol. **1973**; 98:395-401.

Olajide OM, Aminu M, Randawa AJ, Adejo DS. Seroprevalence of rubella-specific IgM and IgG antibodies among pregnant women seen in a tertiary hospital in Nigeria. Int J Womens Health. **2015**; 7:75-83.

Olariu, TR, Remington JS, Montoya JG. Polymerase chain reaction in cerebrospinal fluid for the diagnosis of congenital toxoplasmosis. The Pediatrics Infectious Disease Journal. **2014**; 33(6): 566-570.

Oliveira-Bahia L, Abreu A, Azevedo-Silva J, Oréfice F. Toxoplasmosis in southeastern Brazil: an alarming situation of highly endemic acquired and congenital infection. Int J Parasitol. **2001**; 31: 115-144.

Onakewhor JU, Chiwuzie J. Seroprevalence survey of rubella infection in pregnancy at the University of Benin Teaching Hospital, Benin City, Nigeria. Niger J Clin Pract. **2011**; 14: 140–145.

Orenstein WA, Hinman A, Nkowane B, Olivé JM, Reingold AL. Measles and Rubella Global Strategic Plan 2012-2020 midterm review. Vaccine. **2018**; 36 Suppl 1: A1-A34.

Ortiz-Alegría LB, Caballero-Ortega H, Cañedo-Solares I, Rico-Torres CP, Sahagún-Ruiz A, Medina-Escutia ME, et al. Congenital toxoplasmosis: candidate host immune genes relevant for vertical transmission and pathogenesis. Genes Immun. **2010**; 11(5): 363-373

Ozgu-Erdinc AS, Sen C. Diagnosis of congenital cytomegalovirus antigenemia by immunohistochemical detection of immediate early antigen. Fetal and Pediatric Pathology. **2014**; 33(1):64-70.

Padmaja M, Radhakrishna PM, Varghese, SJ Seroprevalence of immunity to rubella in pregnant women. Natl Med J India. **2010**; 23: 248-249.

Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. International Journal for Parasitology. **2009**; 39(12):1385-94.

Pass RF, Anderson B. Mother-to-child transmission of cytomegalovirus and prevention of congenital infection. J Pediatric Infect Dis Soc. **2014**; 3(Suppl 1): S2 6.

Pass RF, Arav-Boger R. Maternal and fetal cytomegalovirus infection: diagnosis, management, and prevention. F1000Res. **2018**; 7: 255.

Patel HD, Nikitin P, Gesner T, Lin JJ, Barkan DT, Ciferri C, et al. In vitro characterization of human cytomegalovirus-targeting therapeutic monoclonal antibodies LJP538 and LJP539. antimicrobial agents and chemotherapy. **2016**; 60(8):4961-71.

Paul E, Kiwelu I, Mmbaga B, Nazareth R, Sabuni E, Maro A, et al. *Toxoplasma gondii* seroprevalence among pregnant women attending antenatal clinic in Northern Tanzania. Trop Med Health. **2018**; 46: 39.

Paya CV, Wold AD, Smith TF. Detection of cytomegalovirus infections in specimens other than urine by the shell vial assay and conventional tube cell cultures. Journal of Clinical Microbiology. **1987**; 25(5):755-757.

Pei R, Grund S, Verheyen J, Esser S, Chen X, Lu M. Spontaneous reactivation of hepatitis B virus replication in an HIV coinfected patient with isolated anti-Hepatitis B core antibodies. Virology Journal. **2014**; 11, 9.

Pelkonen T, Santos MD, Roine I, Anjos E, Freitas C, Peltola H, et al. Potential diarrheal pathogens common also in healthy children in Angola. The Pediatric Infectious Disease Journal. **2018**; 37(5): 424-428.

Pembrey L, Raynor P, Griffiths P, Chaytor S, Wright J, Hall AJ. Seroprevalence of cytomegalovirus, Epstein Barr virus and varicella zoster virus among pregnant women in Bradford: a cohort study. PLoS One. **2013**; 8:e81881.

Pennap, G.R. and Egwa, M.A. Prevalence of rubella virus infection among pregnant women accessing antenatal clinic at federal medical centre, Keffi, Nigeria. Int J Curr Microb. **2016**; 5: 171-178.

Perdoncini G, Pasquali AKS, Mariani F, Cembranel DJ, Escopelli KS. Prevalência de Toxoplasma gondii em aves e suínos: um problema para a saúde pública. UNOESC e Ciência – ACBS 1(1): 57-64. Available at: https://portalperiodicos.unoesc.edu.br/acbs/article/view/175/0, **2010**.

Pereboom MT, Manniën J, Spelten ER, Schellevis FG, Hutton EK. Observational study to assess pregnant women's knowledge and behavior to prevent toxoplasmosis, listeriosis and cytomegalovirus. BMC Pregnancy and Childbirth. **2013**; 13; 98.

Peretti LE, Gonzalez VDG, Marcipar IS, Gugliotta LM. Diagnosis of toxoplasmosis in pregnancy. Evaluation of latex-protein complexes by immnunoagglutination. Parasitology. **2017**; 144(8):1073-1078.

Permar SR, Schleiss MR, Plotkin SA. Advancing our uderstanding of protective maternal immunity as a guide for development of vaccines to reduce congenital cytomegalovirus infections. Journal of Virology. **2018**; 92(7). pii: e00030-18.

Peter, Lombardo. Dermatological manifestations of rubella work up: laboratory Studies. MedScape; **2015** Available from: http://emedicine.medscape.com/article/1133108-workup.

Petersen E, Borobio MV, Guy E, Liesenfeld O, Meroni V, Naessens A, et al. European multicenter study of the LIAISON automated diagnostic system for determination of

Toxoplasma gondii-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. Journal of Clinical Microbiology. **2005**; 43(4):1570-4.

Pfaff AW, Abou-Bacar A, Letscher-Bru V, Villard O, Senegas A, Mousli M, et al. Cellular and molecular physiopathology of congenital toxoplasmosis: the dual role of IFN-gamma. Parasitology. **2007**, 134: 1895-1902.

Piazza MJ, Urbanetz AA, Carvalho NS, Nascimento DJ. Hepatites virais e gestação. Diagn Tratamento. **2010**; 15(1): 12-18.

Picone OC, Vauloup F, Cordier AGI, Parent DuC, Senat MV, Frydman R, et al. A 2-year study on cytomegalovirus infection during pregnancy in a French hospital. Br. J. Obstet. Gynecol, **2009**; 116(6): 818-823.

Piergili FD. Problems and limitations of conventional and innovative methods for the diagnosis of Toxoplasmosis in humans and animals. Parassitologia. **2004**; 46(1-2):177-81.

Pittman KJ, Knoll LJ. Long-term relationships: the complicated interplay between the host and the developmental stages of *Toxoplasma gondii* during acute and chronic infections. Microbiology and Molecular Biology Review. **2015**; 79(4):387-401.

Plano Nacional de Desenvolvimento Sanitário (PNDS) do Ministério da Saúde. Vol. I; 2012-2025. **2012**. Available at: https://www.mindbank.info/item/3460

Plano Nacional de Desenvolvimento Sanitário (PNDS) do Ministério da Saúde. Vol. II; 2012-2025. **2014**. Available at: https://www.mindbank.info/item/3460

Plans P, de Ory F, Campins M, Álvarez E, Payà T, Guisasola E, et al. Prevalence of anti-rubella, anti-measles and anti-mumps IgG antibodies in neonates and pregnant women in Catalonia (Spain) in 2013: susceptibility to measles increased from 2003 to 2013. European Journal of Clinical Microbiology & Infectious Diseases. **2015**; 34: 1161-1171.

Pleyer U, Ness T, Garweg J. [Prevention of Recurrence of Ocular Toxoplasmosis - When? How? For Whom?]. Klin Monbl Augenheilkd. **2020** May;237(5):599-604.

Plotkin S. History of vaccination. Proc Natl Acad Sci U S A. **2014**; 111: 12283-12287.

Plucinski MM, Ferreira M, Ferreira CM, Burns J, Gaparayi P, João L, et al. Evaluating Malaria Case Management at Public Health Facilities in Two Provinces in Angola. Malaria Journal. **2017**; 16 (1), 186.

Pomares C, Estran R, Press CJ, Bera A, Ramirez R, Montoya JG, et al. Is real-time PCR targeting Rep 529 suitable for diagnosis of toxoplasmosis in patients infected with non-type II strains in North America? J Clin Microbiol. **2020**; 58(2): e01223-19.

Pomares C, Montoya JG. Laboratory Diagnosis of Congenital Toxoplasmosis. Journal of Clinical Microbiology. **2016**; 54(10), 2448-2454.

Population Reference Bureau. World Population Data sheet. **2006**. Washington, DC: Population Reference Bureau. Available at: https://www.prb.org/2006worldpopulationdatasheet/.

Porter SB, Sande MA. Toxoplasmosis of the central nervous system in the acquired immunodeficiency syndrome. The New England Journal of Medicine. **1992**; 327(23):1643-8.

Potzsch S, Spindler N, Wiegers AK, Fisch T, Rucker P, Sticht H, et al. B cell repertoire analysis identifies new antigenic domains on glycoprotein B of human cytomegalovirus which are target of neutralizing antibodies. PLoS Pathogens. **2011**; 7(8):e1002172.

Previato M, Frederico FB, Murata FHA, Siqueira RC, Barbosa AP, Silveira-Carvalho AP, et al. .A Brazilian report using serological and molecular diagnosis to monitoring acute ocular toxoplasmosis. BMC Research Notes. **2015**; 8:746.

Priyanka D, Vallab G, Anupriya A, Uma A, Kalamani, S.M. Seroprevalence of rubella among asymptomatic pregnant women in a rural teaching hospital. Int J Med Microbiol Res. **2017**; 1: 7–12.

Programa das Nações Unidas para o Desenvolvimento (PNUD). Relatório sobre o Índice de Desenvolvimento Humano em Angola. **2018**. Available at: https://www.ao.undp.org/content/angola/pt/home/imprensa/IDH.html

Programa Nacional de Controlo da Tuberculose (PNCT) Relatório, **2015**. Angola; 2016 http://gardcplp.ihmt.unl.pt/Documentos/Paises/Angola/Relatorio_Programa_Nacional_Controlo_TB_2015.pdf

Pukuta E, Waku-Kouomou D, Abernathy E, Illunga BK, Obama R, Mondonge V, et al. Genotypes of rubella virus and the epidemiology of rubella infections in the Democratic Republic of the Congo, 2004-2013. Journal of Medical Virology. **2016**; 88: 1677-1684.

Rajasekariah H, Scott G, Robertson PW, Rawlinson WD. Improving diagnosis of primary cytomegalovirus infection in pregnant women using immunoblots. Journal of Medical Virology. **2013**; 85(2):315-319.

Rando-Segura A, Aznar ML, Moreno MM, Espasa M, Sulleiro E, Bocanegra C, et al. Drug resistance of *Mycobacterium Tuberculosis* complex in a rural setting, Angola. Emerging Infectious Diseases. **2018**; 24(3): 569-572.

Rasti S, Ghasemi FS, Abdoli A, Piroozmand A, Mousavi SG, Fakhrie-Kashan Z. ToRCH "co-infections" are associated with increased risk of abortion in pregnant women. Congenital Anomalies (Kyoto). **2016**; 56: 73-78.

Raynor BD. Cytomegalovirus infection in pregnancy. Semin Perinatol. **1993**; 17(6):394-402.

Reichman O, Miskin I, Sharoni L. Preconception screening for cytomegalovirus: an effective preventive approach. BioMed Res. Int. **2014**:135416.

Reina J, Weber I, Riera E, Busquets M, Morales C. [Usefulness of a real-time quantitative polymerase-chain reaction (PCR) assay for the diagnosis of congenital and postnatal cytomegalovirus infection]. Anales de Pediatria (Barc). **2014**; 80(5):299-303.

Reischl U, Bretagne S, Kruger D, Ernault P, Costa JM. Comparison of two DNA targets for the diagnosis of Toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. BMC Infectious Diseases. **2003**; 2,3:7.

Remington JS, McLeod R, Wilson CB, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, Wilson CB, Nizet V, Maldonado YA, editors. Infectious Diseases of the Fetus and Newborn Infant. 7th ed. Philadelphia, PA: Elsevier-Saunders; **2011**; 918-1041.

Remington JS, Thulliez P, Montoya JG. Recent developments for diagnosisn of toxoplasmosis. Journal of Clinical Microbiology. **2004**; 42(3):941-5.

Remington JS, Miller MJ, Brownlee I. IgM antibodies in acute toxoplasmosis. I. Diagnostic significance in congenital cases and a method for their rapid demonstration. Pediatrics. **1968**a; 41(6), 1082-1091.

Remington JS, Miller MJ, Brownlee I. IgM antibodies in acute toxoplasmosis. II. Prevalence and significance in acquired cases. The Journal of Laboratory and Clinical Medicine. **1968**b; 71(5), 855-866.

Requião-Moura LR, Matos AC, Pacheco-Silva A. Infection by cytomegalovirus in kidney transplantation: clinical aspects, management and perspectives. Einstein (Sao Paulo). **2015**; 13(1):142-148.

Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin. Microbiol. Rev. **2002**; 15:680-715.

Revello MG, Lazzarotto T, Guerra B, Spinillo A, Ferrazzi E, Kustermann A, et al. CHIP study group. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. The New England Journal of Medicine. **2014**; 370(14):1316-1326.

Revello MG, Tibaldi C, Masuelli G, Frisina V, Sacchi A, Furione M, et al. CCPE study group. prevention of primary cytomegalovirus infection in pregnancy. EBioMedicine. **2015**; 2(9):1205-10.

Rigsby P, Rijpkema S, Guy EC, Francis J, Gaines Das R. Evaluation of a candidate international standard preparation for human anti-toxoplasma IgG. Journal of Clinical Microbiology. **2004**; 42(11):5133-8.

Robert -Gangneux F, Dardé ML. Epidemiology and diagnostic strategies for toxoplasmosis. Clinical Microbiology Reviews. **2012**; 25, 264-296.

Robert-Gangneux F, Belaz S. Molecular diagnosis of toxoplasmosis in immunocompromised patients. Current Opinion in Infectious Diseases. **2016**; 29(4):330-9.

Robert-Gangneux F, Brenier-Pinchart MP, Yera H, Belaz S, Varlet-Marie E, Bastien P. Evaluation of Toxoplasma ELITe MGB Real-Time PCR Assay for Diagnosis of Toxoplasmosis. Journal of Clinical Microbiology. **2017**; 55(5):1369-1376.

Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. **2012**; 25(2): 264-296.

Roberts A, Hedman K, Luyasu V, Zufferey J, Bessières MH, Blatz RM, et al. Multicenter evaluation of strategies for serodiagnosis of primary infection with *Toxoplasma gondii*. European Journal of Clinical Microbiology & Infectious Diseases. **2001**; 20(7):467-74.

Romand S, Chosson M, Franck J, Wallon M, Kieffer F, Kaiser K. Usefulness of quantitative polymerase chain reaction in amniotic fluid as early prognostic marker of fetal infection with *Toxoplasma gondii*. American Journal of Obstetrics and Gynecology. **2004**; 190(3):797-802.

Ross SA, Pati NS, Boppana SB. Overview of the diagnosis of cytomegalovirus infection. Infectious Disorders Drug Targets. **2011**; 11(5): 466-474.

Rossi CL. A simple, rapid enzyme-linked immunosorbent assay for evaluating immunoglobulin G antibody avidity in toxoplasmosis. Diagnostic Microbiology and Infectious Diseases.**1998**; 30(1):25-30.

Rostami A, Riahi SM, Contopoulos-Ioannidis DG, Gamble HR, Fakhri Y, Shiadeh MN, et al. Acute Toxoplasma infection in pregnant women worldwide: A systematic review and meta-analysis. PLoS Negl Trop Dis. **2019**; 13(10): e0007807.

Rota PA, Brown KE, Hübschen JM, Muller CP, Icenogle J, Chen M-S, et al. Improving global virologic surveillance for measles and rubella. J Infect Dis. **2011**; 204: S506-S513.

Roth A, Roth B, Höffken G, Steuber S, Khalifa KI, Janitschke K. Application of the polymerase chain reaction in the diagnosis of pulmonary toxoplasmosis in immunocompromised patients. European Journal of Clinical Microbiology & Infectious Diseases. **1992**; 11(12):1177-81.

Rouatbi M, Amairia S, Amdouni Y, Boussaadoun MA, Ayadi O, Al-Hosary AAT, et al. *Toxoplasma gondii* infection and toxoplasmosis in North Africa: a review. Parasite. **2019**; 26:6.

Saadatnia G, Golkar M. A review on human toxoplasmosis. Scandinavian Journal of Infectious Diseases. **2012**; 44(11):805-14.

Sabbaj S, Pass RF, Goepfert PA, Pichon S. Glycoprotein B vaccine is capable of boosting both antibody and CD4 T-cell responses to cytomegalovirus in chronically infected women. The Journal of Infectious Diseases. **2011**; 203(11):1534-41.

Saeed NAAH, Ibraheem LJ, Zabbon AA, Shakir EW. Seroprevalence study of rubella virus infection among bad obstetric history and primigravida pregnant women in Iraq. World Journal of Pharmaceutical Research **2015**; 4(8): 378-384.

Santos E, Felgueiras Ó, Oliveira O, Duarte R. Diagnosis Delay of Tuberculosis in the Huambo Province, Angola. Pulmonology. **2018**; 24(5); 294-299.

Santos SDRD, Bafi AT, Freitas FGR, Azevedo LCP, Machado FR. Prevalence of cytomegalovirus disease in kidney transplant patients in an intensive care unit. Revista Brasileira de Terapia Intensiva. **2017**; 29 (4):436-443.

Sarkar MD, Anuradha B, Sharma N, Roy RN. Seropositivity of toxoplasmosis in antenatal women with bad obstetric history in a tertiary-care hospital of Andhra Pradesh, India. J Health Popul Nutr. **2012**; 30(1): 87-92.

Schleiss MR. Cytomegalovirus vaccine development. Current topics in microbiology and immunology. **2008**; 325: 361-382.

Schleiss MR. Does public perception of exposure risks and transmission mechanisms drive antiviral vaccine awareness? What if cytomegalovirus was transmitted by mosquitoes? Current Opinion in Virology. **2016**; 17:126-9.

Schoppel K, Kropff B, Schmidt C, Vornhagen R, Mach M. The humoral immune response against human cytomegalovirus is characterized by a delayed synthesis of glycoprotein-specific antibodies. The Journal of infectious diseases. **1997**; 175(3):533-44.

Selvey LA, Lim WH, Boan P, Swaminathan R, Slimings C, Harrison AE, et al. A. cytomegalovirus viraemia and mortality in renal transplant recipients in the era of antiviral prophylaxis. Lessons from the western Australian experience. BMC Infectious Diseases. **2017**; 17; 17(1).

Sensini A. *Toxoplasma gondii* infection in pregnancy: opportunities and pitfalls of serological diagnosis. Clinical Microbiology and Infection. **2006**; 12(6):504-12.

Seo S, Cho Y, Park J. Serologic screening of pregnant Korean women for primary human cytomegalovirus infection using IgG avidity test. Korean J Lab Med. **2009**; 29(6):557-62.

Seppälä EM, López-Perea N, Torres de Mier MV, Echevarría JE, Fernández-García A, Masa-Calles J. Last cases of rubella and congenital rubella syndrome in Spain, 1997-2016: The success of a vaccination program. Vaccine. **2019**; 37: 169-175.

Shao Z, Tibi MA, Wakim-Fleming J. Update on Viral Hepatitis in Pregnancy. Cleveland Clinic Journal of Medicine. **2017**; 84(3): 202-206.

Sharghi M, Musavi H, Mansurkhani SM, Kooti W, Behzadifar M, Ashrafi-Zadeh H, et al. Seroprevalence of cytomegalovirus among women of reproductive age in Iran: A systematic review and meta-analysis. Iranian Journal of Public Health. **2019**; 48(2):206-216.

Shiferaw D, Birke T, Esete T, Ayenachew A, Ketema BH, Beyene B. The epidemiology of laboratory confirmed rubella cases in Oromia Region, Ethiopia, 2010–2015: data from the measles case-based surveillance system. Am J Clin Pathol. **2016**; 146: 227.

Skendzel, LP Rubella immunity: defining the level of protective antibody. Am J Clin Pathol. **1996**; 106: 170-174.

Skidmore S, Boxall E, Lord S. Is the MMR vaccination programme failing to protect women against rubella infection? Epidemiology and Infection. **2014**; 142: 1114-1117.

Slawska H, Czuba B, Gola J, Mazurek U, Włoch A, Wilczok T, et al. Diagnostic difficulties of Toxoplasma gondii infection in pregnant women. Is it possible to explain doubts by polymerase chain reaction? Ginekologia Polska. **2005**; 76(7), 536-542.

Smereka J, Szarpak L, Ruetzler K, Schacham Y, Smereka A, Dabrowski M, et al. A multicenter survey on toxoplasmosis knowledge among pregnant women in Poland (the TOWER study). BMC Pregnancy and Childbirth. **2018**; 18: 389.

Smit GSA, Vu BTL, Do DT, Do QH, Pham HQ, Speybroeck N, et al. Sero-epidemiological status and risk factors of toxoplasmosis in pregnant women in Northern Vietnam. BMC Infect Dis. **2019**; 19(1): 329.

Smith LR, Wloch MK, Chaplin JA, Gerber M, Rolland AP. Clinical development of a cytomegalovirus DNA vaccine: from product concept to pivotal phase 3 trial. Vaccines (Basel). **2013**; 1(4):398-414.

Solano C, Muñoz I, Gutiérrez A, Farga A, Prósper F, García-Conde J, et al. Qualitative plasma PCR assay (AMPLICOR CMV Test) versus pp65 antigenemia assay for monitoring cytomegalovirus viremia and guiding preemptive ganciclovir therapy in

allogeneic stem cell transplantation. Journal of Clinical Microbiology. **2001**; 39(11):3938-3941.

Splendore, A. Um nuovo protozoa parassita dei conigli incontrato nelle lesioni anatomiche d'una malattia che ricorda in molti ponti il kala-azar dell'uomo. Rev Soc Sci São Paulo.**1908**; 3,109-12.

Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis. **2006**; 43(9):1143-51.

Storch GA, Buller RS, Bailey TC, Ettinger NA, Langlois T, Gaudreault-Keener M, et al. Comparison of PCR and pp65 antigenemia assay with quantitative shell vial culture for detection of cytomegalovirus in blood leukocytes from solid-organ transplant recipients. Journal of Clinical Microbiology. **1994**; 32(4):997-1003.

Strauss, J. H., E. G. Strauss. Viruses and Human Disease. Second Edition. Elsevier Academic Press, New York. **2008**.

Su C, Shwab EK, Zhou P, Zhu XQ, Dubey JP. Moving towards an integrated approach to molecular detection and identification of *Toxoplasma gondii*. Parasitology. **2010**; 137(1):1-11.

Su Q, Ma C, Wen N, Fan C, Yang H, Wang H, et al. Epidemiological profi le and progress toward rubella elimination in China. 10 years after nationwide introduction of rubella vaccine. Vaccine. **2018**; 36: 2079-2085.

Sumeeta K, Batra N. Toxoplasmosis in organ transplant recipients: Evaluation, implication, and prevention. Tropical Parasitology. **2016**; 6(2):123-128.

Sun Q, Miao C, Hanel M, Borthwick AGL, Duan Q, Ji D, et al. Global Heat Stress on Health, Wildfires, and Agricultural Crops Under Different Levels of Climate Warming. Environ Int. **2019**; 128: 125-136

Sun X, Lu H, Jia B, Chang Z, Peng S, Yin J, et al. A comparative study of *Toxoplasma gondii* seroprevalence in three healthy chinese populations detected using native and recombinant antigens. Parasite & Vectors. **2013**; 6(1):241.

SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group, Thiébaut R, Leproust S, Cheˆne G, Gilbert R. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. Lancet. **2007**; 369(9556):115-122. PubMed: PubMed: https://pubmed.ncbi.nlm.nih.gov/17223474/

Tahita MC, Hübschen JM, Tarnagda Z, Ernest D, Charpentier E, Kremer JR, et al. Rubella seroprevalence among pregnant women in Burkina Faso. BMC Infect Dis. **2013**; 13:164.

Takao S, Shigemoto N, Shimazu Y, Tanizawa Y, Fukuda S, Matsuo T. Detection of exanthematic viruses using a TaqMan real-time PCR assay panel in patients with clinically diagnosed or suspected measles. Japanese Journal of Infectious Diseases. **2012**; 65: 444-448.

Tamer GS, Dundar D, Caliskan E. Seroprevalence of *Toxoplasma gondii*, rubella and cytomegalovirus among pregnant women in western region of Turkey. Clin Invest Med. 2009; Clin Invest Med. **2009**; 32(1):E43-7.

Tamirat B, Hussen S, Shimelis T. Rubella virus infection and associated factors among pregnant women attending the antenatal care clinics of public hospitals in Hawassa City, Southern Ethiopia: a cross-sectional study. BMJ Open. BMJ Open. **2017**; 7(10): e016824.

Taneja DK, Sharma P. Targeting rubella for elimination. Indian J Public Health. **2012**; 56(4):269-72.

Tanimura K, Yamada H. Potential biomarkers for predicting congenital cytomegalovirus infection. International Journal of Molecular Science. **2018**; 19(12): 3760.

Tavares MV, Domingues AP, Tavares M, Malheiro E, Tavares F, Moura P. Citomegalovírus. There is a place to trace during pregnancy? Acta Med Port. **2011**; 24(S4): 1003-1008.

Taylor LA, Tan AX, Coyle CE, Ndumele C, Rogan E, Canavan M, et al. Leveraging the social determinants of health: what works? PLoS One. **2016**; 11(8):e0160217.

Teimouri A, Modarressi MH, Shojaee S, Mohebali M, Zouei N, Rezaian M, Keshavarz H. Detection of toxoplasma-specific immunoglobulin G in human sera: performance comparison of in house Dot-ELISA with ECLIA and ELISA. Eur J Clin Microbiol Infect Dis. **2018**; 37(8):1421-1429.

Teixeira LE, Kanunfre KA, Shimokawa PT, Targa LS, Rodrigues JC, Domingues W, et al. The performance of four molecular methods for the laboratory diagnosis of congenital toxoplasmosis in amniotic fluid samples. Revista da Sociedade Brasileira de Medicina Tropical. **2013**; 46(5):584-8.

Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. The Internatinal Journal of Parasitology **2000**; 30: 1217-1258.

Thackeray R, Magnusson BM, Christensen EM. Effectiveness of message framing on women's intention to perform cytomegalovirus prevention behaviors: a cross-sectional study. BMC Womens Health. **2017**; 17(1):134.

Thackeray R, Wright A, Chipman K. Congenital cytomegalovirus reference material: a content analysis of coverage and accuracy. Maternal the Child Health Journal. **2014**; 18(3):584-591.

Thompson KM, Badizadegan ND. Modeling the Transmission of Measles and Rubella to Support Global Management Policy Analyses and Eradication Investment Cases. Risk Analysis. **2017**; 37: 1109-1131.

Thornton RLJ, Glover CM, Cené CW, Glik DC, Henderson JA, Williams DR. Evaluating Strategies For Reducing Health Disparities By Addressing The Social Determinants Of Health. Health Aff (Millwood). **2016**; 35 (8): 1416-1423.

Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz Storek KJ, et al. Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective. Preface. Bone Marrow Transplantation. **2009**; 44(8):453-455.

Torii Y, Yoshida S, Yanase Y, Mitsui T, Horiba K, Okumura T, et al. Serological screening of immunoglobulin M and immunoglobulin G during pregnancy for predicting congenital cytomegalovirus infection. BMC Pregnancy and Childbirth. **2019**; 19(1):205.

Torrey EF, Yolken RH. Toxoplasma oocysts as a public health problem. Trends in Parasitol. **2013**; 29(8): 380-384.

UNICEF and WHO. Progress on Sanitation and Drinking Water. **2015** Available at: https://www.unicef.pt/progressos-saneamento-agua-potavel/files/progress-onsanitation-drinking-water2015.pdf

UNICEF. Tire as suas dúvidas sobre vacinas. **2018**. Ref: https://www.unicef.org/angola/duvidas-sobre-vacinas

United Nations (UN). The Millennium Development goals Report. **2015**. New York: 75p.https://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20(July%201).pdf

United Nations Development Assistance Framework for Angola (UNPAF). Partnership Framework Between the Government of Angola and the United Nations system, 2015 – 2019. **2015**. Available at: https://www.ao.undp.org/content/dam/angola/docs/legalframework/UNDP_AO_UNPAF 2015-2019_EN.pdf

United Nations Office of the High Representative for the Least Developed Countries, Landlocked Developing Countries and Small Island Developing States. (UN-OHRLLS) **2014**. Available at: http://unohrlls.org/.

United Nations Programme on HIV/AIDS (UNAIDS) (2016). AIDS by the numbers **2016**. Available at: http://www.unaids.org/sites/default/files/media_asset/AIDS-by-the-numbers-2016_en.pdf

United Nations Programme on HIV/AIDS (UNAIDS). Preventing mother to child transmission. 27 September **2019**. Available at: https://www.unaids.org/en/keywords/preventing-mother-child-transmission

United Nations. Political Declaration on HIV and AIDS. On the Fast Track to Accelerating the Fight against HIV and to Ending the AIDS Epidemic by 2030. 17020: 1 – 26. United Nations Joint Programme on HIV/AIDS (UNAIDS). **2016**; Available at: https://www.unaids.org/sites/default/files/media_asset/2016-political-declaration-HIV-AIDS_en.pdf

Uysal A, Taner CE, Cüce M, Atalay S, Göl B, Köse S, et al. Cytomegalovirus and rubella seroprevalence in pregnant women in Izmir/Turkey: follow-up and results of pregnancy outcome. Arch Gynecol Obstet. **2012**; 286:605-608.

Van Zuylen WJ, Hamilton ST, Naing Z, Hall B, Shand A, Rawlinson WD. Congenital cytomegalovirus infection: Clinical presentation, epidemiology, diagnosis and prevention. Obstetric Medicine. **2014**; 7(4): 140-146.

Varani S Landini MP. Cytomegalovirus-induced immunopathology and it´s clinical consequences. Herpesviridae. **2011**; 2: 1-14.

Varella IS, Canti IC, Santos BR, Coppini AZ, Argondizzo LC, Tonin C, et al. Prevalence of acute toxoplasmosis infection among 41,112 pregnant women and the mother-to-child transmission rate in a public hospital in South Brazil. Mem Inst Oswaldo Cruz. **2009**; 104(2): 383-388.

Varella IS, Wagner MB, Darela AC, Nunes LM, Müller RW. Seroprevalence of toxoplasmosis in pregnant women. J Pediatr. **2003**; 79(1): 69-74.

Vide Tavares MV, Domingues AP, Tavares M, Malheiro E, Tavares F, Moura P. [Cytomegalovirus: is there a place for screening during pregnancy?]. Acta Medica Portuguesa. **2011**; 24 Suppl 4:1003-108.

Villard O, Cimon B, L'Ollivier C, Fricker-Hidalgo H, Godineau N, Houze S, et al. Serological diagnosis of *Toxoplasma gondii* infection: recommendations from the French National reference center for toxoplasmosis. Diagnostic Microbiology and Infectious Diseases. **2016**; 84(1):22-33.

Virkola K, Lappalainen M, Valanne L, Koskiniemi M. Radiological signs in newborns exposed to primary Toxoplasma infection in utero. Pediatr Radiol. Pediatric Radiology. **1997**; 27(2):133-8.

Vries JJC, Eijk AAV, Wolthers KC, Rusman LG, Pas SD, Molenkamp R, et al. Real-time PCR versus viral culture on urine as a gold standard in the diagnosis of congenital cytomegalovirus infection. Journal of Clinical Virology. **2012**; 53 (2): 167-70.

Vueba AN Sousa MC. Serological epidemiology of cytomegalovirus in pregnant women attending at Maternity Lucrécia Paim, Luanda, Angola. The 2018 PharmSci 360, M1430-04-028. **2018**. ttps://eventscribe.net/2018/PharmSci360/fsPopup.asp?efp=UUFSQlZZVFM1OTQ2&PosterID=165819&rnd=0.9247395&mode=posterinfo.

Vueba ML. evolução do ensino farmacêutico em Angola. Património histórico do espaço lusófono. Ciência, arte e cultura. XXVIII encontro da associação das universidades de língua portuguesa, Lubango, Angola. 2018; 235-241. http://id.bnportugal.gov.pt/bib/bibnacional/2016374

Vueba NA, Sousa MC. seroprevalence of rubella virus in pregnant women attending at Maternity Lucrécia Paim, Luanda, Angola. The 2018 PharmSci 360, T1230-04-027. Ref: https://www.eventscribe.net/2018/PharmSci360/fsPopup.asp?efp=UUFSQlZZVFM1OTQ2&PosterID=165821&rnd=0.8183299&mode=posterinfo

Vynnycky E, Yoshida LM, Huyen DT, Trung ND, Toda K, Cuong NV, et al. Modeling the impact of rubella vaccination in Vietnam. Human Vaccines & Immunotherapeutics. **2016**; 12: 150-158.

Wallon M, Peyron F. Congenital toxoplasmosis: a plea for a neglected disease. Pathogens. **2018**; 7:E25.

Wang C, Dollard SC, Amin MM, Bialek SR. Cytomegalovirus IgM Seroprevalence among Women of Reproductive Age in the United States. PLoS One. **2016**; 11(3): e0151996.

Wang C, Zhang X, Bialek S, Cannon MJ. Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. Clin Infect Dis. **2011**; 52(2):e11-3.

Wang D, Fu TM. Progress on human cytomegalovirus vaccines for prevention of congenital infection and disease. Current Opinion in Virology. **2014**; 6:13-23.

Wang, Y, Yin, H. Research progress on surface antigen 1 (SAG1) of *T. gondii*. Parasites & Vectors. **2014**; 7: 180.

Weiss JB. DNA probes and PCR for diagnosis of parasitic infections. Clinical Microbiology Review. **1995**; 8(1):113-30.

Weiss LM, Dubey JP. Toxoplasmosis: a history of clinical observations. Int J Parasitol. **2009**; 39(8): 895-901.

Weiss LM, Kim K. *Toxoplasma gondii*: The Model Apicomplexan - Perspectives and Methods: Second Edition. Elsevier Ltd. **2013**.

WHO. Rubella vaccines WHO position paper. Wkly Epidemiol Rec. **2011**; 86: 301-316.

Willame, A. Blanchard-Rohner G, Combescure C, Irion O, Posfay-Barbe K, Tejada BM. Awareness of cytomegalovirus infection among pregnant women in Geneva, Switzerland: a cross-sectional study. International Journal of Environmental Research and Public Health. **2015**; 12(12): 15285-15297.

Winter AK, Pramanik S, Lessler J, Ferrari M, Grenfell BT, Metcalf CJE. Rubella vaccination in India: identifying broad consequences of vaccine introduction and key knowledge gaps. Epidemiology and Infectious. **2018**; 146: 65-77.

Wondimeneh, Y, Tiruneh M, Ferede G, Abera B, Workineh M, Birhanie M, et al. Rubella virus infections and immune status among pregnant women before the introduction of rubella vaccine in Amhara Regional State, Ethiopia. International Journal of Infectious Diseases. **2018**; 76, 14-22.

Wong SY, Remington JS. Biology of *Toxoplasma gondii*. AIDS. **1993**; 7(3):299-316.

Working Group for Monitoring Action on the Social Determinants of Health. Towards a global monitoring system for implementing the Rio Political Declaration on Social Determinants of Health: developing a core set of indicators for government action on the social determinants of health to improve health equity. Int J Equity Health. **2018**; 17:136.

World Bank In Angola. The World Bank Group supports Angola's efforts to reduce poverty and promote economic growth by working with the government, development partners and civil society. **2018**. Available at: https://www.worldbank.org/pt/country/angola/overview.

World Bank. Global Economic Prospects: Managing growth in a volatile world. Washington, DC. 107p. 2019. Available at: https://openknowledge.worldbank.org/handle/10986/31066

World Bank. World development Final report **2008**. External Evaluation of the Partnership for Maternal, Newborn and Child Health. Available at: https://www.who.int/pmnch/members/meetings/doc10.pdf

World Health Organization (WHO), Department of HIV/AIDS. Global prevalence and incidence of selected curable sexually transmitted infections: overview and estimates.**2001**.https://www.who.int/reproductivehealth/publications/rtis/HIV_AIDS_2001_2/en/

World Health Organization (WHO). Dual protection against unwanted pregnancy and sexually transmitted infections, including HIV. Joint WHO/UNAIDS/UNFPA policy

statement. Geneva, Switzerland. **2015**. Available at: https://www.who.int/hiv/mediacentre/news/condoms-joint-positionpaper/en/.

World Health Organization (WHO). Global Hepatitis Programme. Global hepatitis report. **2017**. Available at: http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/

World Health Organization (WHO). Global tuberculosis report. **2019**. Available at: https://www.who.int/tb/publications/global_report/en/

World Health Organization (WHO). Guidelines for Drinking Water Quality, 4th edition, incorporating the 1st addendum. World Health Organization, Geneva, Switzerland. **2017**. Available at: https://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/

World Health Organization (WHO). Guidelines on the Management of Latent Tuberculosis Infection. Geneva, World Health Organization. **2015**. Available at: https://www.who.int/tb/publications/latent-tuberculosis-infection/en/

World Health Organization (WHO). Hepatitis C. Fact sheet No. 164. **2013**. Available at: http://www.who.int/mediacentre/factsheets/fs164/en/

World Health Organization (WHO). Hepatitis D. Fact sheet No. 164. **2019**. Available at: https://www.who.int/news-room/fact-sheets/detail/hepatitis-d

World Health Organization (WHO). HIV Surveillance Report for Africa 2000 Joint WHO/UNAIDS/UNFPA policy statement. Harara, Zimbabwe, November, **2001**. Available at: https://www.who.int/hiv/strategic/pubafr_rep00/en/

World Health Organization (WHO). Malawi Malaria Indicator Survey. **2017**. Available at: https://dhsprogram.com/what-we-do/survey/survey-display-512.cfm

World Health Organization (WHO). Measles and Rubella Surveillance Data 10 January **2020** 17:54 CET.

World Health Organization (WHO). Partnership for Maternal, Newborn & Child HealthMedia centreThe Partnership publications. **2020**. Available at: https://www.who.int/pmnch/media/publications/africanewborns_por/en/

World Health Organization (WHO). Regional Office for Africa. Health systems in Africa: community perceptions and perspectives: the report of a multi-country study. World Health Organization. Regional Office for Africa. **2012**. https://apps.who.int/iris/handle/10665/79711

World Health Organization (WHO). Rio Political Declaration on Social Determinants of Health. Rio de Janeiro: World Health Organization. **2011**. Available at: https://www.who.int/sdhconference/en/.

World Health Organization (WHO). Rubella vaccines WHO position paper. Wkly Epidemiol Rec. 2011; 86: 301–316. **2011**. Available at: https://www.who.int/immunization/policy/position_papers/rubella/en/

World Health Organization. (WHO). Cooperation Strategy 2015-2019. Angola. **2016**. Available at: https://www.afro.who.int/sites/default/files/2017-06/ccs-angola-2015-2019-p.pdf

World Health Organization. Infections and infectious diseases: a manual for nurses and midwives in the WHO European Region. **2001**. http://www.euro.who.int/__data/assets/pdf_file/0013/102316/e79822.pdf.

Wu Y, Wood J, Khandaker G, Waddington C, Snelling T. Informing rubella vaccination strategies in East Java, Indonesia through transmission modelling. Vaccine. **2016**; 34: 5636-5642.

Wujcicka W, Gaj Z, Wilczyński J, Sobala W, Spiewak E, Nowakowska D. Impact of socioeconomic risk factors on the seroprevalence of cytomegalovirus infections in a cohort of pregnant Polish women between 2010 and 2011. Eur J Clin Microbiol Infect Dis. **2014**; 33(11):1951-8.

Xavier GA, Cademartori BG, Cunha Filho NA, Farias NA. Evaluation of seroepidemiological toxoplasmosis in HIV/Aids patients in the south of Brazil. Rev Inst Med Trop Sao Paulo. 2013; 55(1):25-30.

Xiao J, Buka SL, Cannon TD, Suzuki Y, Viscidi RP, Torrey EF, et al. Serological pattern consistent with infection with type I *Toxoplasma gondii* in mothers and risk of psychosis among adult offspring. Microbes and Infection. **2009**; 11(13), 1011-1018.

Xiao XM, Li AZ, Chen X, Zhu YK, Miao J. Prevention of vertical hepatitis B transmission by hepatitis B immunoglobulin in the third trimester of pregnancy. Int J Gynaecol Obstet. **2007**; 96(3): 167-170.

Yamamoto AY, Castellucci RA, Aragon DC, Mussi-Pinhata MM. Early high CMV seroprevalence in pregnant women from a population with a high rate of congenital infection. Epidemiol Infect. **2013**; 141:2187-2191.

Yamamoto AY; Mussi-Pinhata MM; Figueiredo LTM. Congenital and perinatal cytomegalovirus infections: clinical aspects, epidemiology, diagnosis and treatment. Medicina (Ribeirão Preto). **1999**; 32(1): 49-56.

Yamamoto, AY, et al. Human cytomegalovirus reinfection is associated with intrauterine transmission in a highly cytomegalovirus-immune maternal population. American Journal of Obstetrics and Gynecology 2010; 202: 297.

Ye X, Su H, Wrapp D, Freed DC, Li F, Yuan Z, et al. Recognition of a highly conserved glycoprotein B epitope by a bivalent antibody neutralizing HCMV at a post-attachment step. PLoS Pathogens. **2020**; 16(8):e1008736.

Yeshwondm M, Balkachew N, Delayehu B, Mekonen G. Seroepidemiology Study of Cytomegalovirus and Rubella among Pregnant Women at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Ethiopian Journal of Health Sciencies. **2016**; 26: 427-438.

Yobi D, Piarroux R, L'Ollivier C, Franck J, Situakibanza H, Muhindo H, et al. Toxoplasmosis among pregnant women: High seroprevalence and risk factors in Kinshasa, Democratic Republic of Congo. Asian Pac J Trop Biomed. **2014**; 4(1): 69-74.

You D, Hug L, Ejdemyr S, Idele P, Hogan D, Mathers GP. et al. United Nations Inter-agency Group for Child Mortality Estimation (UN IGME). Global, regional, and national levels and trends in under-5 mortality between 1990 and 2015, with scenario-based projections to 2030: a systematic analysis by the UN Inter-agency Group for Child Mortality Estimation. Lancet. **2015**; 386:2275-2286.

Zanga J, Mbanzulu MK, Kabasele AF, Ngatu NR, Wumba DR. Rubella Seroprevalence and real-time PCR detection of RUBV among Congolese pregnant women. BMC Infect Dis. **2017**; 5; 17(1):250.

Zeytinoğlu A, Terek D, Arslan A, Erensoy S, Altun Köroğlu Ö, Bozdemir T, et al. Investigation of congenital CMV infection with the presence of CMV DNA in saliva samples of new born babies. Mikrobiyoly Bulteni. **2019**; 53(1):53-60.

Zhang K, Lin G, Han Y, Li J. Serological diagnosis of toxoplasmosis and standardization. Clinica Chimica Acta. **2016**; 461:83-9.

Zhang S, Hu L, Chen J, Xu B, Zhou Yi-Hua, Hu Y. Cytomegalovirus seroprevalence in pregnant women and association with adverse pregnancy/neonatal outcomes in Jiangsu Province, China. PLoS One. **2014**; 9(9): e107645.

Zheng Q, Abernathy ES, Sun H, Zhu Z, de Filippis A, Koffi CA, et al. Genotyping of rubella virus RNA in sera and dried blood spots collected during routine surveillance and in archival sera. Journal of Virological Methods. **2013**; 187: 284-287.

Zhou W, Yang H, Ling Q, Luo Y. Histologic detection of cytomegalovirus and its application in gastrointestinal cytomegalovirus infection. Zhonghua Bing Li Xue Za Zhi (Chinese Journal of Pathology). **2016**; 45(3):17

7. SUPPLEMENTARY DATA

S1. File: Ethics Committee, República de Angola, Ministério da Saúde

S2. File: Questionnaire/Questionário de Recrutamento

S3. File: Programs and datasets used to create the maps

S4. File: Amplification of *Toxoplasma gondii* B1 gene locus by nested-PCR