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Syphilis, HIV infection, hepatitis B and C in parturients from
Irene Neto Maternity in Lubango, Angola, and their association
with maternal, obstetric and newborn complications

Dinamene Oliveira

**DISSERTAÇÃO PARA A OBTENÇÃO DO GRAU DE DOUTOR EM SAÚDE
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maternal, obstetric and newborn complications

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- Oliveira D, Martins MR, Castro R, Cordeiro L, Barroso MR, Nazaré MA, Pereira F. HIV, HBV and syphilis screening in antenatal care in Lubango, Angola. *Sex Transm Infect.* 2020 Dec;96(8):621-2. doi:10.1136/sextrans-2019-054407.
- Oliveira D, Pereira F, Martins MR, Castro R, Cordeiro L, Fronteira I. A systematic review of the maternal and neonatal complications in hepatitis B infection. *J Clin Virol.* 2020 Dec;133(104680). doi:10.1016/j.jcv.2020.104680.
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“May your choices reflect your hopes, not your fears.”

Nelson Mandela

To Zulmira Eliotte, affectionately known as “aunt Zú”

An Angolan nurse whose kindness, affection, joy and smile will always inspire me

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RESUMO

Introdução: Em Angola, infecções por *Treponema pallidum* e pelos vírus da imunodeficiência humana (VIH), hepatite B (VHB) e hepatite C (VHC) estão pouco documentadas, especialmente durante a gravidez.

Objetivos: Parturientes da cidade do Lubango, província da Huíla, foram incluídas. Objetivos: 1) determinar a prevalência das infecções por *T. pallidum*, VIH, VHB e VHC; 2) identificar fatores sociodemográficos associados; 3) caracterizar a abordagem clínica pré-natal e durante o parto quanto às infecções; 4) relacionar a transmissão vertical (TV) com a abordagem das mulheres infetadas e comparar com as normas nacionais e internacionais; 5) identificar complicações maternas, obstétricas e do recém-nascido associadas às infecções; 6) descrever sinais e sintomas das parturientes e dos recém-nascidos associados às infecções; 7) recomendar procedimentos para diminuir as complicações das infecções em grávidas e recém-nascidos, atendendo às condições locais.

Métodos: Estudo observacional, transversal e analítico de base hospitalar (Maternidade Irene Neto). Entre outubro de 2016 e setembro de 2017, 500 parturientes foram acompanhadas durante o trabalho de parto, tendo sido efetuado rastreio das infecções em estudo e avaliada a TV. O questionário incluiu características maternas, cuidados pré-natais (CPN) e desfechos maternos, obstétricos e do recém-nascido. A análise estatística englobou análise bivariada e regressão logística múltipla. Uma revisão sistemática foi efetuada com base nas diretrizes PRISMA.

Resultados: Pelo menos uma infecção foi diagnosticada em 11,8% das parturientes: hepatite B em 8,6%, VIH em 3,0% e sífilis em 1,0%. Nenhum caso de hepatite C foi diagnosticado. Cada ano adicional de escolaridade reduziu em 10,0% a chance de infecção pelo VHB. O rastreio pré-natal de VIH, *T. pallidum* e VHB foi realizado em 66,5%, 60,2% e 44,6%, respetivamente, sendo as taxas significativamente menores em mulheres das zonas rurais. Os CPN tiveram início no primeiro trimestre de gravidez em 9,1%. Pelo menos quatro e oito consultas foram observadas em 62,7% e 3,4%, respetivamente. O ADN do VHB (genótipo E) foi identificado em 7,3% dos recém-nascidos de parturientes HBsAg-positivas, havendo associação entre o HBeAg materno e a positividade dos recém-nascidos para ADN do VHB. Um número considerável de casos de gravidez na adolescência, gravidez gemelar, iliteracia materna, baixa estatura materna, subdiagnóstico pré-natal de hipertensão, cesariana sem indicação médica identificada, asfíxia e baixo peso ao nascer foi descrito. Três estudos de boa qualidade incluídos na nossa revisão sistemática revelaram que a infecção materna pelo VHB aumentou o risco de aborto, nascimento pré-termo, hipertensão gestacional, sofrimento fetal e macrossomia. Na amostra do estudo não existiu uma chance maior de hipertensão, anemia, líquido amniótico com mecónio, cesariana, asfíxia e baixo peso ao nascer em parturientes com hepatite B.

Conclusão: A hepatite B é um importante problema de saúde pública. Os CPN não alcançaram os patamares adequados, com disparidade rural-urbana, pelo que se torna necessário estudar a situação e agir com vista à sua melhoria. O HBeAg é um marcador materno de maior chance de TV. Na África Subsariana, ainda não existe evidência clara sobre a associação entre a hepatite B e as complicações da gravidez, sendo que mais estudos sobre este tema deverão ser efetuados.

Palavras-chave: infecções sexualmente transmissíveis, cuidados pré-natais, transmissão vertical, complicações da gravidez, Angola

ABSTRACT

Background: In Angola, infections by *Treponema pallidum*, human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are poorly documented, especially during pregnancy.

Aims: In this study, parturients from Lubango city, Huíla province, were included. Objectives: 1) to determine *T. pallidum*, HIV, HBV and HCV infections prevalence; 2) to identify associated sociodemographic factors; 3) to characterize the clinical approach during the antenatal period and delivery with respect to those infections; 4) to relate the clinical approach towards infected women during the antenatal period and delivery with vertical transmission (VT) and compare it with the national and international guidelines; 5) to identify maternal, obstetric and newborn complications associated to the infections; 6) to describe parturients and newborns signs and symptoms associated with the infections; 7) to recommend procedures to reduce infections' complications in pregnant women and newborns, taking into account local conditions.

Methods: This is an observational, cross-sectional and analytical facility-based survey (Irene Neto Maternity). From October 2016 to September 2017, 500 parturients were followed during labour. Screening of the studied infections was performed and their VT was evaluated. Questionnaire included maternal characteristics, antenatal care (ANC) and maternal, obstetric and newborn outcomes. Statistical analysis encompassed bivariate analysis and multivariable logistic regression. A systematic review was carried out based on the PRISMA guidelines.

Results: At least one infection was diagnosed in 11.8% of the parturients: hepatitis B in 8.6%, HIV in 3.0% and syphilis in 1.0%. No cases of hepatitis C were diagnosed. Each additional year of formal education represented a 10.0% lower chance of HBV infection. HIV, *T. pallidum* and HBV antenatal screening was performed in 66.5%, 60.2% and 44.6%, respectively, rates being significantly lower in women from rural areas. ANC was initiated in the first trimester of pregnancy by 9.1%. At least four and eight visits were observed in 62.7% and 3.4%, respectively. HBV DNA (genotype E) was identified in 7.3% of newborns from HBsAg-positive parturients. An association between maternal HBeAg and HBV DNA positivity of newborns was found. A considerable number of cases of adolescent pregnancy, twin pregnancy, maternal illiteracy, maternal short stature, antenatal subdiagnosis of hypertension, caesarean without identified medical indication, birth asphyxia and low birth weight was found. Three studies of good quality included in the systematic review performed for this thesis revealed that maternal HBV infection increased risk of miscarriage, preterm birth, gestational hypertension, fetal distress and macrosomia. In our sample, a higher chance of hypertension, anaemia, meconium-stained amniotic fluid, caesarean, birth asphyxia and low birth weight was not found in parturients with hepatitis B.

Conclusion: Hepatitis B is an important public health problem. ANC did not reach adequate standards, with rural-urban disparity, and attention should be given towards its improvement. HBeAg is a maternal marker of higher chance of VT. In Sub-Saharan Africa, association between hepatitis B and pregnancy complications remains unclear and should be studied further.

Keywords: sexually transmitted infections, antenatal care, vertical transmission, pregnancy complications, Angola

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LIST OF ABBREVIATIONS

AIDS - acquired immune deficiency syndrome

ALT - alanine aminotransferase

ANC - antenatal care

Anti-HBs - hepatitis B surface antibody

ART - antiretroviral therapy

AVT - antiviral therapy

AZT - zidovudine

BA - birth asphyxia

cccDNA - covalently closed circular DNA

CDC - Centers for Disease Control and Prevention

CHB - chronic hepatitis B

CS - congenital syphilis

C-section - caesarean section

DAA - direct acting antiviral

DALY - disability-adjusted life year

DNA - deoxyribonucleic acid

EASL - European Association for the Study of the Liver

ETV - entecavir

GD - gestational diabetes

HBeAg - hepatitis B e antigen

HBIG - hepatitis B immunoglobulin

HBsAg - hepatitis B surface antigen

HBV - hepatitis B virus

HCC - hepatocellular carcinoma

HCV - hepatitis C virus
HCVcAg - HCV core antigen
HIV - human immunodeficiency virus
HSV - herpes simplex virus
ICP - intrahepatic cholestasis of pregnancy
IF - immunoprophylaxis failure
IgG - immunoglobulin G
IgM - immunoglobulin M
INM - Irene Neto Maternity
IP - intrapartum
LBW - low birth weight
LMIC - low and middle-income countries
NA - nucleos(t)ide analog
NAT - nucleic acid test
NHS - National Health System
NTT - non-treponemal test
NVP - nevirapine
OBI - occult HBV infection
PB - preterm birth
PCR - polymerase chain reaction
PHI - primary HIV infection
PP - post-partum
PVST - post-vaccination serological testing
RDT - rapid diagnostic test
RNA - ribonucleic acid

RT-PCR - reverse transcription-polymerase chain reaction

SDG - sustainable development goals

SSA - Sub-Saharan Africa

STI - sexually transmitted infection

TDF - tenofovir disoproxil fumarate

TT - treponemal test

VDB - vaccine dose at birth

VDRL - venereal disease research laboratory

VL - viral load

VT - vertical transmission

WHO - World Health Organization

1. INTRODUCTION

1.1. Infection and Pregnancy

Infections continue to be a major cause of maternal and fetal morbidity and mortality worldwide¹. Therefore, screening for infections is part of antenatal care (ANC) evidence-based interventions². It is estimated that detection and management of serious maternal and neonatal infections could avert 4.9% of all maternal and child deaths³.

Physiologic changes of pregnancy, including alterations in the cardiopulmonary system, acid-base physiology and immunologic response, impose an increased risk of some infections^{4,5}. On the other hand, evidence suggests that systemic maternal infection and consequent inflammation can disrupt placental vasculogenesis and angiogenesis, impacting on fetal growth and contributing to poor birth outcomes⁶.

The consequences of infection during pregnancy are affected by maternal serological status, gestational age at the time of infection and mother/fetus immunologic status¹. Particular aspects need to be considered, including the possible vertical transmission (VT) and treatment potential adverse effects on the fetus and pregnancy⁴.

Routine and risk-based antenatal screening is essential in managing infections, as well as to prevent VT⁷, which can be prenatal/intrauterine (via the transplacental route), perinatal (shortly before onset of labour or during delivery from contact with blood and vaginal secretions) or postnatal (breastfeeding)^{8,9}.

The classic group of perinatal infections, designated as the TORCH infections, comprises toxoplasmosis, other infections (including syphilis), rubella, cytomegalovirus and herpes simplex virus (HSV)⁴. Other microorganisms that may be added to the group are hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), varicella-zoster virus and parvovirus B19⁹. More recently was argued that Zika virus should be considered a TORCH pathogen¹⁰.

Sexually transmitted infections (STIs) are however among the most frequent infections affecting pregnant women, standing out syphilis, gonorrhoea, trichomoniasis, chlamydiosis, hepatitis B, infection by HIV, HSV-1/2 and human papillomavirus¹.

INTRODUCTION

1.1.1. Sexually Transmitted Infections

STIs impose major health and economic burdens worldwide¹¹. It is estimated that every day more than 1 million people acquire an STI, with women being disproportionately affected, especially in resource-poor settings¹². In many developing countries, due to the lack of adequate diagnostic and treatment facilities, STIs complications are commonly seen, particularly among women and children¹³.

Despite their common route of transmission, STIs are a diverse group of infections with more than 35 bacterial, viral and parasitic causative agents^{11,14}, besides other described in table 1. The potential clinical manifestations are varied as a result of that diversity in the number and type of pathogens¹⁵. Additionally, being asymptomatic, a large proportion of STIs keep undiagnosed^{16,17}.

Table 1. Causative agents of sexually transmitted infections (adapted from Richens et al, 2014¹³)

STIs producing genital lesions	
Bacteria	<i>Treponema pallidum</i> , <i>Chlamydia trachomatis</i> , <i>Neisseria gonorrhoeae</i> , <i>Ureaplasma urealyticum</i> , <i>Mycoplasma genitalium</i> , <i>Haemophilus ducreyi</i> , <i>Klebsiella granulomatis</i> , <i>Gardnerella vaginalis</i> and anaerobes
Viruses	Human papillomavirus, HSV, Molluscum contagiosum virus
Protozoa	<i>Trichomonas vaginalis</i>
Fungi	<i>Candida albicans</i> *
Arthropods	<i>Sarcoptes scabiei</i> , <i>Phthirus pubis</i>
STIs which do not generally produce genital lesions	
Bacteria	<i>Shigella</i> spp., <i>Salmonella</i> spp., <i>Campylobacter</i> spp.
Viruses	HIV, Hepatitis viruses, Cytomegalovirus, Human T-cell lymphotropic virus type 1/2
Protozoa	<i>Cryptosporidium</i> spp., <i>Giardia lamblia</i> , <i>Entamoeba histolytica</i>
Helminths	<i>Strongyloides stercoralis</i> , <i>Enterobius vermicularis</i> , <i>Trichuris trichiura</i>

*Not anymore considered an STI by many authors

Discussing STIs in the context of women's health is appropriate for a number of reasons: these infections often cause disease differently in women than in men; women are at much higher risk of adverse consequences of untreated infection such as pelvic inflammatory

disease and infertility; STIs present risks for adverse pregnancy outcomes and of VT; these continue to be highly stigmatized infections in many cultures where women are particularly vulnerable to negative social outcomes when diagnosed with an STI¹⁵.

1.1.1.1. Syphilis

Aetiology: Syphilis was the first recognized STI¹⁸ and is the only human treponemal disease that is venereally transmitted¹⁹. It is caused by the spirochaete *Treponema pallidum* subspecies *pallidum* (order Spirochaetales), being often described as a Gram-negative bacterium due to its double-membrane structure²⁰. *T. pallidum* is a slow-growing and motile spirochaete with a long spiral shape that cannot be cultured *in vitro* and humans are its only natural host²¹.

Epidemiology: Syphilis is part of the four curable STIs group, together with chlamydiosis, gonorrhoea and trichomoniasis²². It is an ancient infection that, despite an available cure for more than 70 years and no known resistance to penicillin, remains a significant global health concern²³. Globally, there were 6.3 million new syphilis cases in individuals aged 15-49 years in 2016²⁴. In that year, the World Health Organization (WHO) African Region had the highest syphilis prevalence in women (1.6%)²⁴, being estimated to be 2.9% (95% CI: 2.4-3.4) among pregnant women from Sub-Saharan Africa (SSA)²⁵.

Natural History and Clinical Manifestations: Because its clinical manifestations are highly variable and can mimic other diseases, syphilis has been called the Great Imitator or Great Masquerader^{21,26}. If left untreated, infected individuals typically follow a disease course divided into different stages: primary, secondary, latent and tertiary²⁰ (figure 1). Syphilis presentation and course during pregnancy are similar to that in the non-pregnant population²⁸.

INTRODUCTION

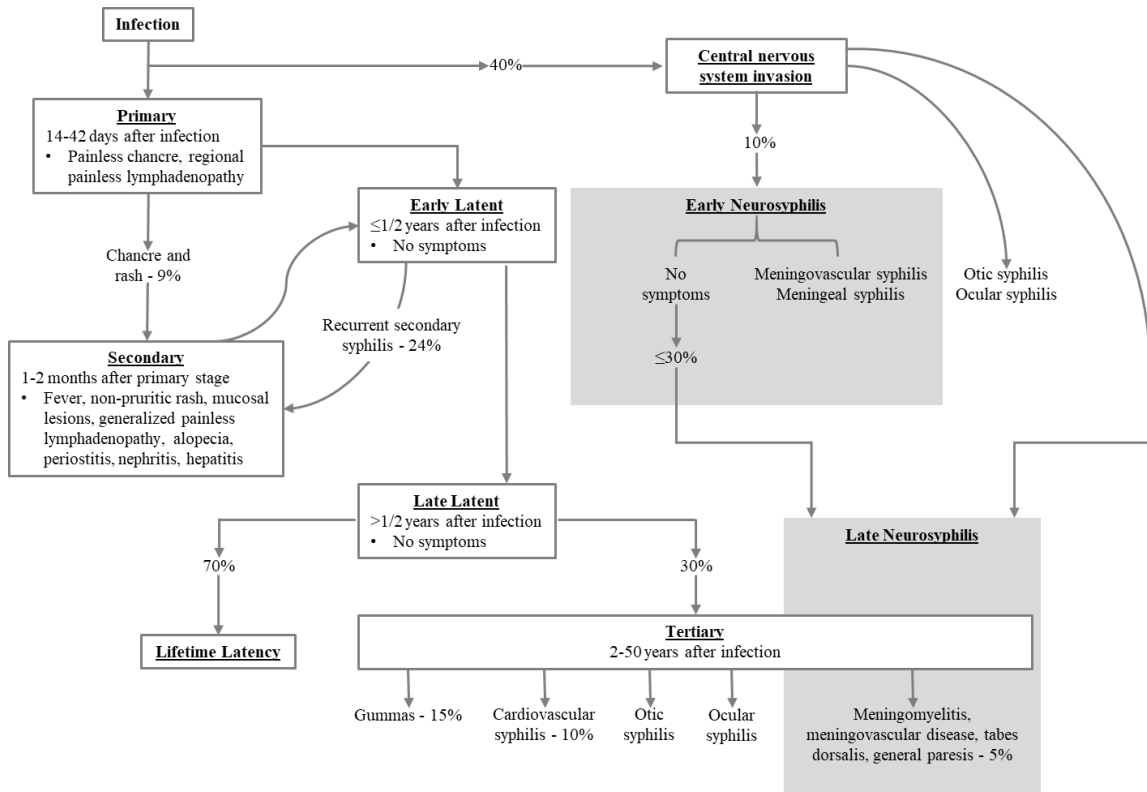


Figure 1. Course of untreated syphilis (adapted from Ghanem et al, 2020²⁷)

Transmission: Sexual acquisition of syphilis occurs when an infectious lesion contacts the injured skin or mucous membrane of an uninfected person, often during oral, vaginal, or anal sex²¹. The sexually transmissible stages of infection are primary and secondary syphilis²⁷. Bloodborne and VT also occur²¹. Although the last type of transmission can occur at the time of delivery, the overwhelming majority of cases results from in utero transmission²⁰. Risk of congenital syphilis (CS) is directly related to the stage of maternal infection⁹. The risk of VT is highest in primary and secondary stages, followed by early latent syphilis^{20,27}. It occurs in approximately 50-80% of women with untreated primary, secondary or early latent syphilis, compared with approximately 10% of women with late latent syphilis²⁶. The risk is reported to be 2% after 4 years of maternal infection²⁶.

Diagnosis: The WHO recommends syphilis screening at the first ANC visit²⁹. Retesting during pregnancy should always be done, with special emphasis if risk factors are present³⁰. In a study that included data from 12 Sub-Saharan African countries was estimated that only one in 12 pregnant women received ANC early (<4 months) at a

facility ready to provide syphilis detection and treatment³¹. Varied and often subtle manifestations of syphilis make clinical diagnosis difficult²⁰. Laboratory diagnosis of syphilis comprises direct detection of *T. pallidum* and serology testing³² (table 2).

Table 2. Diagnostic tests for syphilis (adapted from Adhikari, 2020²⁶)

Direct detection methods	Serological tests	
	Non-treponemal	Treponemal
Animal inoculation (rabbit infectivity testing)	Venereal disease research laboratory	Microhemagglutination assay <i>T. pallidum</i> particle agglutination assay
Direct fluorescent antibody	Rapid plasma reagin	Indirect fluorescent antibody
Darkfield microscopy		Chemiluminescence immunoassay
Immunohistochemistry		Enzyme immunoassay
Polymerase chain reaction		

In most clinical settings, syphilis is diagnosed by serological tests along with clinical history and physical examination²⁶. These tests are the only means for screening asymptomatic individuals and can be categorized into non-treponemal tests (NTTs) and treponemal tests (TTs)²⁰. NTTs measure tissue damage caused by *T. pallidum* through detection of antibodies to cardiolipin, cholesterol and lecithin, while TTs detect IgM and IgG antibodies specific to *T. pallidum* proteins³². Diagnostic algorithms combining NTTs and TTs are used to screen *T. pallidum* infection and to confirm its diagnosis³³. The standard screening algorithm begins with a NTT, whereas in the reverse screening algorithm a TT is used initially²⁷. Although some specific tests are typically more sensitive than the non-specific during very early infection, the first cannot differentiate individuals who have been treated from those with an active disease^{23,32}. NTTs titers increase with active disease and decrease following adequate therapy²³. Therefore, response to treatment can only be assessed by monitoring NTTs titers over time³². An inability to distinguish between infection with *T. pallidum* subsp. *pallidum* and the non-venereal *T. pallidum* subspecies that cause yaws, pinta or bejel, is the limitation of all syphilis serological tests²⁰, especially in countries where endemic treponematoses are prevalent. Point-of-care rapid serology tests for syphilis have greatly increased access to

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prenatal screening and diagnosis, being particularly useful in low and middle resourced and remote settings²⁰. The diagnosis of CS is notoriously difficult³⁴. The possible transfer of maternal non-treponemal and treponemal IgG antibodies through the placenta to the fetus complicates the interpretation of reactive serological tests for syphilis in neonates³⁵. The presence of IgM is presumed to be the result of congenital infection as it does not cross the placenta, but diagnostic sensitivity is limited in asymptomatic cases²⁸. The mainstay of diagnosis relies on reviewing maternal medical history to understand when treatment was performed, whether it was adequate for the syphilis stage and on the comparison between maternal and neonatal serological non-treponemal titers²⁸. When treatment was received less than four weeks before delivery, a possible CS case must be considered³⁵. Although their absence does not exclude the diagnosis, infant serological non-treponemal titers four or more times higher than those of the mother are suggestive of CS^{20,34,35}. Evidence of clinical, laboratory (reactive cerebrospinal fluid venereal disease research laboratory [VDRL] test, abnormal complete blood count or liver function tests) or radiographic (suggestive long-bone radiographs) syphilis manifestations in the neonate should also be taken into account^{20,35}. For neonates born to women who have reactive serology tests for syphilis during pregnancy, a positive darkfield test or PCR from lesions or body fluids indicates that CS is proven or highly probable³⁵. Despite the discussed challenges, treatment should be offered to any infant in whom there is a clinical suspicion of syphilis, given the low risk of that approach and the high risk of long-term morbidities related to untreated disease³⁴.

Pregnancy Outcomes: Systematic reviews and meta-analyses showed that delayed or lack of adequate treatment of maternal syphilis results in adverse pregnancy outcomes, including miscarriage, prematurity, low birth weight (LBW), stillbirth and neonatal death^{36,37}. It was estimated that globally, in 2016, there were 355,000 adverse birth outcomes resulting from maternal syphilis³⁸. In neonates, two-thirds of syphilis infection cases can be asymptomatic²⁸. Clinical presentation of CS is diverse and often non-specific³⁴, being divided into two syndromes, known as early CS (≤ 2 years of life) and late CS (> 2 years of life)²⁶. Mucocutaneous lesions, oedema, lymphadenopathy, hepatosplenomegaly, osteochondritis, pseudoparalysis, thrombocytopenia, anaemia or rhinitis are among the possible clinical manifestations of early CS²⁸.

Prevention and Treatment: Ensuring quality ANC that includes education and testing for syphilis early in pregnancy and providing prompt treatment of women and their partners is a fundamental public health strategy to prevent VT of syphilis and adverse pregnancy outcomes^{20,30}. Penicillin G is the treatment for syphilis in pregnant women²⁹. The regimen should be appropriate for the stage of infection and desensitization should be performed in cases of allergy history³⁹. Penicillin treatment is effective in curing maternal disease, preventing VT and treating any established fetal disease³³. CS treatments of choice are aqueous crystalline penicillin or procaine penicillin²⁸.

1.1.1.2. Human Immunodeficiency Virus Infection

Aetiology: HIV is a RNA retrovirus that was first identified in 1981¹. Viral enzyme reverse transcriptase transcribes viral RNA into DNA that is incorporated into the host genome (proviral DNA)⁴⁰. Activated CD4 T lymphocytes are the main target of HIV⁴¹. There are two types of HIV: HIV-1 and HIV-2⁴⁰, which present different epidemiological and clinical characteristics (table 3). HIV-1 is genetically diverse, comprising distinct groups, subtypes and intersubtype recombinant forms^{40,43-45}. HIV-2 is inherently resistant to non-nucleoside reverse transcriptase inhibitors⁴⁶.

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Table 3. Comparing HIV-1 and HIV-2 infection (adapted from Vijayan et al, 2017⁴²)

	HIV-1	HIV-2
World distribution	Global	West Africa, portuguese colonies (Angola, Mozambique, Brazil) and India
Heterosexual transmission	Higher	5-fold lower rate than HIV-1
VT	Higher	20 to 30-fold lower rate than HIV-1
Time to develop acquired immune deficiency syndrome	Variable, estimated median time 9.8 years	Longer, 10-25 years
Percentage of people to develop acquired immune deficiency syndrome if untreated	50% within 10 years	86-95% long-term non-progressors
Plasma RNA load	Higher	Lower
Viral replication kinetics	Higher replication, 100-fold more fit	Transient replication, less fit
Transmission fitness	100-fold more fit	Less fit

Epidemiology: HIV remains a major pandemic and its distribution is uneven within and between regions⁴⁷. In 2019, there were about 38 million people living with HIV globally⁴⁸. In this year, the number of acquired immune deficiency syndrome (AIDS) related deaths was 690,000 and 1.7 million new HIV infections were diagnosed, including 150,000 in children (0-14 years)⁴⁸. Over 90% of those children acquire the infection during pregnancy, delivery or through breastfeeding and the majority are born in SSA⁴⁹. This region is home of a disproportionate 70% of the HIV global burden⁴⁷ and between 2009 and 2012 an HIV prevalence of 5.3% (95% CI: 4.2-6.6) was estimated among pregnant women⁵⁰. More recently, a systematic review and meta-analysis estimated that HIV incidence rate among pregnant and breastfeeding women in SSA was 3.6 per 100 persons per year (95% prediction interval: 1.2-11.1)⁵¹.

Natural History and Clinical Manifestations: In 50 to 70% of the individuals, primary HIV infection (PHI) presents as an acute retroviral syndrome that typically occurs between 4-6 weeks post-exposure⁵². Fever, night sweats, headache, fatigue, rash,

lymphadenopathy, pharyngitis, arthralgias, myalgias, nausea, vomiting and diarrhoea are common symptoms¹. After acute infection, without effective antiretroviral therapy (ART), HIV will induce immune suppression⁵³. HIV-related morbidity and mortality are due to infections and neoplasms that became more frequent as immune suppression progresses⁵⁴ (figure 2).

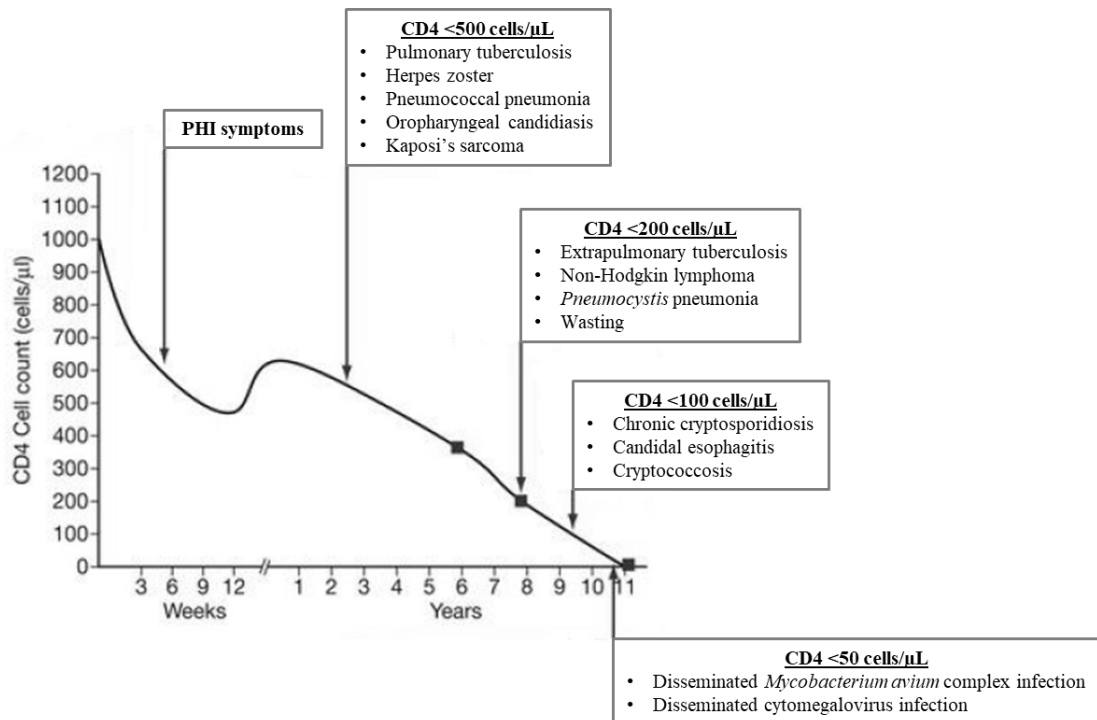


Figure 2. HIV disease progression according to CD4 count (adapted from Peters et al, 2013⁵³)

Disease progression is influenced by a multitude of interacting factors⁵⁵, such as the characteristics of the infecting virus and the host immune response, being generally more rapid in children and older adults⁵³. The utility of CD4+ T-cell count and HIV RNA quantification for disease stage and progression assessment is clearly recognized⁵⁵. AIDS usually develops 8 to 10 years after initial infection⁵⁶ and corresponds to the most advanced stage of HIV infection, characterized by severe immunodeficiency (CD4 cell count <200 cells/μL) and development of opportunistic illnesses^{53,56,57}. A wide range of opportunistic infections and cancers are included in AIDS-defining illnesses⁵⁸, some of which are already described in figure 2. An accurate disease staging is a challenge in resource-limited settings, where inadequate laboratory capacity limits the ability to measure CD4 cell counts or diagnose HIV-associated illnesses⁵³. At all stages of

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infection, HIV transmission and HIV-related morbimortality are reduced by ART, changing HIV infection from a terminal diagnosis to a chronic manageable condition⁵². In this era of potent ART, the new challenge is the impact of comorbidities complicating long-term HIV infection, specifically secondary epidemics of cancer, reproductive ill health and non-communicable diseases, including cardiovascular disease, diabetes, renal dysfunction and liver damage⁵⁹. The estimates of a systematic review and meta-analysis were consistent with pregnancy increasing the risk of progression to HIV/AIDS-defining illnesses and HIV-related or all-cause mortality in settings without ART availability, but there were too few studies to draw meaningful conclusions⁶⁰.

Transmission: HIV can be transmitted via the exchange of certain body fluids from an infected individual, namely blood, semen, pre-seminal fluid, rectal fluids, vaginal fluids and breast milk^{61,62}. For transmission to occur these fluids must be directly injected into the bloodstream or come in contact with damaged tissue or mucous membrane (rectum, vagina, penis, mouth, eyes)⁶². Globally, the predominant mode of HIV transmission is unprotected sexual contact⁴⁰. VT could occur during pregnancy, labour, delivery and during the post-partum (PP) period through breastfeeding^{53,63,64}. The risk is higher at the end of pregnancy⁴⁹ and the majority of infants are infected during delivery^{1,49}. Although precise mechanisms are still unknown, transmission could happen by transplacental maternal-fetal microtransfusion of blood contaminated with the virus (during uterine contractions) or by exposure to the virus in maternal cervicovaginal secretions and blood at delivery⁶⁵. HIV VT rate ranges from 15-45% without preventive measures and can be reduced to below 5% with effective interventions during pregnancy, labour, delivery and breastfeeding⁶⁴. VT rate can drop to 1% or less if the following is done: ART is administered throughout pregnancy and childbirth, ART is given to the infant for 4-6 weeks after birth and no breastfeed allowed⁶⁶. VT of HIV is multifactorial and includes a multitude of risk factors⁶⁷. The strongest proportional risk factor for VT is maternal plasma viral load (VL)^{65,68,69} and the other risk factors must be weighted based on it. A study by Garcia et al⁶⁹ helped to establish a desired threshold level for maternal VL of 1,000 copies/mL or less to reduce significantly the risk of VT. Timing of initiation and adherence to ART are other factors to consider. Regardless of VL, it was estimated that in women on highly active ART prior to conception that did not breastfeed the VT rate is

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approximately 0.2% compared with first-trimester initiation at 0.4%, second-trimester initiation at 0.9% and third-trimester initiation at 2.2%⁷⁰. Throughout the few weeks after exposure HIV replicates exponentially, therefore subjects with PHI are highly infectious regarding HIV transmission⁷¹. During pregnancy and lactation, incident HIV infection increases the risk of VT⁷²⁻⁷⁵. In respect of STIs coinfections, studies have shown that maternal syphilis is associated with an increased risk of in utero/intrapartum (IP)/PP HIV VT^{76,77} and HSV-2 maternal infection is associated with an increased risk of IP HIV VT⁷⁸. Concerning obstetric factors, vaginal delivery and preterm delivery (<34 weeks of pregnancy) increase the risk of HIV VT⁷⁹. Twenty years ago a meta-analysis from 15 prospective cohort studies also described that a longer duration of ruptured membranes was associated with an increased risk of HIV VT⁸⁰. However, with the use of ART, some studies have questioned whether the timing of ruptured membranes would matter in women on ART during pregnancy and/or with a low VL⁸¹⁻⁸³. The most recent one found that rupture of membranes of more than four hours was not associated with VT of HIV in women who were taking effective ART and had a full-term pregnancy delivery⁸³.

Diagnosis: A precondition to prevent HIV VT is that the HIV infection of the pregnant woman is recognized⁴⁹. The WHO recommends HIV screening for all pregnant women at least once and as early as possible during pregnancy, ideally in first trimester⁸⁴. Besides that, efforts to move towards elimination of HIV VT must include strategies to identify and treat HIV infections among women who miss ANC testing or have an initial negative HIV test but acquire the virus later in pregnancy or while breastfeeding⁸⁵. In countries with a high burden of this infection, WHO advises to undertake a third trimester retest for all pregnant women with unknown or HIV-negative status and to consider an additional PP test in specific districts or provinces with high HIV burden or incidence and among women from a key population or who have partners with HIV not virally suppressed⁸⁴. On the other hand, in low HIV burden countries, WHO advises to undertake a third trimester retest for pregnant women with unknown or HIV-negative status who are in serodiscordant relationships, when the partner is not virally suppressed on ART, or have other known ongoing HIV risk in late pregnancy⁸⁴. A second HIV test during the third trimester is a proven cost-effective strategy in populations with HIV incidence of 1.2 per 1,000 persons per year or higher⁸⁶. Additionally, according to the American College of

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Obstetricians and Gynecologists, rapid screening during labour, delivery or the immediate PP period should be done for women whose HIV status is unknown, using the opt-out approach⁸⁷. A study by Bulterys et al⁸⁸ showed that rapid HIV testing is feasible and delivers accurate and timely test results in women in labour, providing HIV-positive women prompt access to IP and neonatal antiretroviral prophylaxis, thus reducing perinatal HIV transmission. A systematic review found the following to be the most common barriers to routine antenatal HIV testing from the perspective of pregnant women in SSA: communication with male partners, patient convenience and accessibility to the testing facilities, health system and health-care provider issues, fear of disclosure, HIV-related stigma, the burden of other responsibilities at home and the perception of ANC as a ‘‘woman’s job’’⁸⁹. Regarding diagnostic tests, understanding the timeline of HIV markers is essential for identifying their limitations across clinical and research settings⁹⁰. After an exposure that leads to infection, HIV-1-specific markers will appear in the blood in the subsequent chronologic order: RNA, p24 antigen, IgM antibody and IgG antibody⁹¹ (figure 3).

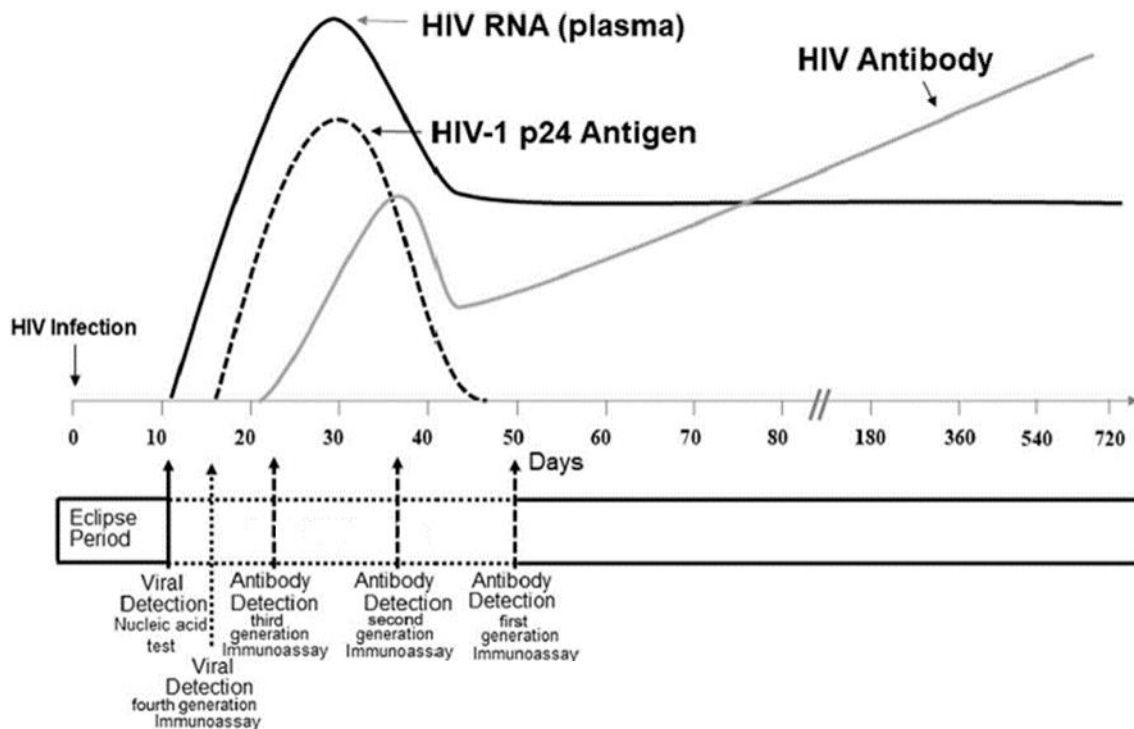


Figure 3. Timeline of laboratory markers for HIV-1 infection (adapted from Branson, 2019⁹¹)

Eclipse period is the amount of time in which no existing diagnostic test is capable of detecting HIV, while window period is the time from infection to the first reactive result on a given test⁹⁰. The p24 antigen is detectable approximately 5-7 days after HIV-1 RNA⁹². Its short-lived detectability is helpful in determining recency of infection, but makes its utility time-limited⁹⁰. Progress in testing technology continue to shorten the detection window period⁹³. The most significant methodologies for HIV testing are immunoassay, nucleic acid test (NAT), simple assay, rapid diagnostic test (RDT) and Western blot (immunoblot)⁹⁴. Three types of HIV tests are available: NATs, antigen/antibody combination tests (detects p24 antigen as well IgM/IgG antibodies) and antibody tests (detects HIV IgM and/or IgG antibodies)⁹³. NATs can be either RNA or DNA based (DNA comes from integrated provirus)⁹⁵. HIV serological tests have been grouped into generations based on the test's principle, being that each subsequent generation led to a shorter detection window period⁹¹ (table 4).

Table 4. Categorization of HIV serological tests by generation numbers (adapted from WHO, 2015⁹⁶)

Generation	Attributes
1st	Relative sensitivity and specificity, detecting IgG antibodies
2nd	Better sensitivity and specificity, detecting IgG antibodies
3rd	Improved sensitivity and specificity, detecting IgM/IgG antibodies
4th	Better sensitivity in early infection, detecting IgM/IgG antibodies and p24 antigen

More recently, there is movement toward describing the analytes an assay detects, instead of using generation numbers^{97,98}. For example, as they are capable of detecting only IgG, first and second generation assays are now designated “IgG sensitive”⁹⁰. WHO recommends the confirmation of any reactive HIV test with a second assay in settings where prevalence is $\geq 5\%$ or with both a second and third assay in settings where prevalence is $< 5\%$ ⁹⁶. A common principle should guide the choice of tests sequence to include in an algorithm: screen with a highly sensitive initial test and confirm reactive results with a different test that is both sensitive and highly specific⁹⁰. When seroconversion sensitivity is preferred, fourth generation serological tests are a suitable choice for a first-line assay⁹⁶, as they detect antibodies to both HIV-1 and HIV-2 as well

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as the p24 antigen, enhancing our ability to diagnose acute HIV-1 infection⁵². WHO states that HIV testing services can use combinations of RDTs or combinations of RDTs/enzyme immunoassays/supplemental assays, instead of enzyme immunoassay/Western blot combinations⁹⁶. This approach is particularly useful and cost-effective in resource-limited settings. Mortality is very high in the first year of life among untreated HIV-infected infants⁹⁶, making early diagnosis and management crucial. Serological tests of the HIV-exposed neonate will be positive as the fetus receives maternal IgG by transplacental passage⁵². Therefore, HIV infection can be diagnosed only by molecular techniques to detect viral nucleic acid^{99,100}.

Pregnancy Outcomes: A systematic review and meta-analysis estimated a high prevalence of gestational diabetes (GD) of 4.42% (95% CI: 3.48-5.35) among HIV-infected pregnant women¹⁰¹. Another one, that looked at whether HIV-infected women are at increased risk of direct obstetric complications, found a three times higher risk of puerperal sepsis¹⁰². According to a systematic review and meta-analysis, 12% of all deaths during pregnancy and up to 1 year PP are attributable to HIV/AIDS in regions with a prevalence of 2% among pregnant women, rising to 50% in regions with a prevalence of 15%¹⁰³. Regarding perinatal outcomes, a systematic review and meta-analysis showed that maternal HIV infection in women who have not received ART is associated with preterm birth (PB), LBW, small for gestational age and stillbirth, especially in SSA¹⁰⁴. Although their benefits in prevention of HIV VT are well known, a systematic review indicated that ART regimens during pregnancy vary substantially in their association with LBW and PB in low and middle-income countries (LMIC)¹⁰⁵.

Prevention and Treatment: Similarly to syphilis, elimination of HIV VT can only be achieved with a robust infrastructure of comprehensive sexual, reproductive and maternal-child health services¹⁰⁶. Four fundamental pillars must be taken into account for the management of HIV during pregnancy and prevention of VT: early diagnosis, determination of the disease stage (VL and CD4 count), determination of the route/moment of delivery and ART management¹⁰⁷. Besides that, WHO considers oral pre-exposure prophylaxis as an additional preventive strategy for pregnant or breastfeeding

HIV-negative women living in settings with high HIV incidence and having a substantial risk of HIV acquisition¹⁰⁸. Diagnosis and disease stage were already discussed. Regarding the route/moment of delivery, the last recommendations of the American College of Obstetricians and Gynecologists state that: a) vaginal delivery is suitable for HIV-infected women maintained on combination ART with VLs of 1,000 copies/mL or less at or near delivery; b) an elective caesarean section (C-section) at 38 weeks of gestation should be offered to women with VLs above 1,000 copies/mL (untreated or suboptimally suppressed), in conjunction with intravenous zidovudine (AZT) administration beginning 3 hours preoperatively⁶⁵. However, the WHO does not recommend a C-section in resource-limited settings specifically due to HIV infection, without another obstetric or medical indication¹⁰⁹. This organization highlights the following principles to minimize the risk of HIV VT during labour and delivery: instrumentation should be avoided unless indispensable, newborns should be washed of any blood and be cared using non-invasive techniques as much as possible¹¹⁰. Concerning ART management, since 2013, WHO recommends a triple drug ART regimen for all pregnant and breastfeeding women living with HIV, regardless of CD4 count and WHO clinical stage¹⁰⁹. In respect to IP intravenous AZT, there is insufficient data to determine whether its administration provides any additional protection against perinatal HIV transmission to women with HIV RNA levels between 50 and 999 copies/mL¹¹¹. In addition to mother's ART, ART is used in neonates of HIV-infected women for both prevention and treatment of HIV infection¹¹². WHO recommends that breastfeeding infants should receive 6 weeks of daily nevirapine (NVP), while for non-breastfeeding infants either daily NVP or twice-daily AZT for 4-6 weeks are recommended¹¹⁰. For infants at high risk of acquiring HIV, a dual prophylaxis with AZT and NVP for the first 6 weeks of life is recommended and, if breastfed, prophylaxis should continue for an additional 6 weeks¹¹⁰. Lastly, breastfeeding is a PP way of HIV VT¹⁰⁷. Although the guidance is beginning to be questioned by some¹¹³, in high-income countries HIV-infected mothers are advised to refrain from breastfeeding¹¹³⁻¹¹⁵. In resource-poor settings, breastfeeding is a key component of the wellbeing and survival of children¹¹⁶. Balancing risks and benefits, last guidelines from WHO (intended mainly for settings in which diarrhoea, pneumonia and undernutrition are common causes of children mortality) advise that HIV-infected mothers should breastfeed for at least 12 months and may extend breastfeeding for up to 24 months or

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more while being fully supported for ART adherence¹¹⁷. Several studies emphasized a lower risk of VT associated with exclusive breastfeeding when compared to mixed breastfeeding¹¹⁸⁻¹²².

1.1.1.3. Hepatitis B

Aetiology: Hepatitis viruses are a diverse pathogenic group that primarily infect hepatocytes. Only HBV and HCV cause acute and chronic infection, with similar pathologies and clinical outcomes¹²³. HBV, a partially double-stranded circular DNA virus¹²⁴, belongs to the family Hepadnaviridae¹²⁵ and was discovered in 1965¹²⁶. It is 100 times more infectious than HIV¹²⁷. The covalently closed circular DNA (cccDNA) is HBV nuclear transcription template¹²³. Currently, there are ten HBV genotypes (A-J), with distinct geographical distributions¹²⁸ (table 5). Identification of HBV genotypes is important because they influence the dominant mode of transmission, natural history of infection, clinical outcomes and treatment response^{125,129,130}.

Table 5. Geographical distribution of HBV genotypes (adapted from Nguyen et al, 2020¹²⁸)

Genotype	Location
A	SSA, Western Africa, Nigeria, Gambia, Northern Europe, India
B	Northern Canada, Greenland, Alaska, East Asia, China, Japan, Taiwan, Vietnam, Philippines, Indonesia
C	Australia, Southeast Asia, China, Japan, South Korea, Taiwan, Vietnam, Philippines, Indonesia
D	Africa, Europe, Mediterranean countries, Australia, Indonesia, India
E	Central and Western Africa, Saudi Arabia
F	South and Central America
G	Germany, France, United States
H	Central America
I	Laos, Vietnam
J	Japan

Epidemiology: Despite the existence of an effective and safe vaccine, HBV infection remains a major public health burden¹²³ and is the most common chronic viral infection worldwide¹³¹. It is estimated that two billion people (1 out of 3) have been infected with HBV and 30 million will become newly infected each year¹²⁷. In accordance with the WHO, 257 million people were chronically infected with HBV and the associated death burden was 887,000 in 2015¹³². The global prevalence of HBsAg (hepatitis B surface antigen) in 2016 was estimated to be 3.9% (95% CI: 3.4-4.6), being the highest in the WHO African Region, 7.2% (95% CI: 6.2-8.2)¹³³. The geographical prevalence of HBsAg varies widely and can be classified as high, moderate (intermediate) or low¹²⁸ (table 6). Asia Pacific and SSA are regions of high-HBV prevalence¹³⁴.

Table 6. Classification of HBsAg prevalence (adapted from Nguyen et al, 2020¹²⁸)

Prevalence	Location
Low (<2%)	Western Europe, North America
Moderate (2-8%)	Eastern Europe, Mediterranean Region
High (>8%)	Africa, East Asia

Natural History and Clinical Manifestations: HBV infection can be acute or chronic¹³⁵. It is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC)¹³⁶. About 54% of all HCC are HBV-associated¹²³. The outcome of HBV infection is determined by age at infection, HBV replication level, host immune status, coinfection with other viruses and comorbidities¹³⁷. The risk of progression from acute to chronic HBV infection is inversely related to the age at which infection occurs, namely 95% during the perinatal period, 20%-30% in children aged 1-5 years and less than 5% in adults¹³¹. The incubation period of HBV infection ranges from 1-4 months¹³⁷. In adults, acute HBV infection is usually clinically inapparent¹³⁸, with 70% of patients having subclinical/anicteric hepatitis, 30% developing icteric hepatitis and 0.1-0.5% progressing to fulminant liver failure¹³⁷. Most patients recover spontaneously with HBsAg to hepatitis B surface antibody (anti-HBs) seroconversion¹³⁸. Treatment may be required only in cases of fulminant hepatitis¹³⁹. The persistence of HBsAg for at least 6 months, concurrently with or without the e antigen (HBeAg), characterizes chronic HBV

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infection¹³². Its natural history is highly variable, reflecting the dynamic and complex interplay between HBV replication and host immune response^{135,138,140}. Inactive carrier state, chronic hepatitis, cirrhosis and HCC are included in this disease spectrum¹²⁴. The European Association for the Study of the Liver (EASL) adopted five phases of chronic HBV infection, which are not necessarily sequential: HBeAg-positive chronic HBV infection, HBeAg-positive chronic hepatitis B (CHB), HBeAg-negative chronic HBV infection, HBeAg-negative CHB and HBsAg-negative phase, also known as occult HBV infection (OBI)¹⁴⁰ (figure 4). They are based on the description of the two main characteristics of chronicity, infection vs hepatitis, and take into account the presence of HBeAg, HBV DNA levels, alanine aminotransferase (ALT) values and eventually the presence or absence of liver inflammation. The OBI is characterized by the presence of replication-competent HBV DNA (cccDNA) in the liver and/or HBV DNA in the blood among HBsAg-negative individuals¹⁴¹.

	HBeAg positive		HBeAg negative	
	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis
HBsAg	High	High/intermediate	Low	Intermediate
HBeAg	Positive	Positive	Negative	Negative
HBV DNA	>10 ⁷ IU/ml	10 ⁴ -10 ⁷ IU/ml	<2,000 IU/ml	>2,000 IU/ml
ALT	Normal	Elevated	Normal	Elevated
Liver disease	None/minimal	Moderate/severe	None	Moderate/severe
Old terminology	Immune tolerant	Immune reactive HBeAg positive	Inactive carrier	HBeAg negative chronic hepatitis

Figure 4. Phases of chronic HBV infection (adapted from EASL, 2017¹⁴⁰)

HBV is a non-cytopathic virus^{123,131,142}. Although the precise mechanisms remain poorly understood, HBV-induced liver damage is mediated by abnormal immune attack¹⁴², being that persistent liver inflammation is a determinant factor in the progression of chronic HBV infection to cirrhosis and HCC¹³¹. In 20-30% of adults who are chronically infected with HBV, the disease evolves to cirrhosis and/or liver cancer¹³². Evidence showed that multiple factors (host, viral/disease and environmental) are associated with an increased risk of cirrhosis and/or HCC (table 7).

Table 7. Factors associated with cirrhosis and/or hepatocellular carcinoma (adapted from Terrault et al, 2016¹⁴³)

	Cirrhosis	HCC
Common	>40 years of age, male sex, immune compromised, metabolic syndrome (obesity and diabetes), heavy alcohol use, high serum HBV DNA (>2,000 IU/mL), elevated ALT levels, prolonged time to HBeAg seroconversion, development of HBeAg-negative CHB, genotype C, viral coinfections (HCV, HIV and hepatitis D virus)	
Specific		Family history, born in SSA, smoking, aflatoxin, cirrhosis

Pregnancy immunologic alterations may affect the course of HBV infection¹⁴⁴⁻¹⁴⁶. Being pregnant might be a risk factor for chronicity following acute infection¹⁴⁷. During pregnancy and PP, a HBV reactivation may take place¹³⁶. HBV DNA flares and ALT flares (hepatitis flares) can occur^{136,146,148-150}, with few cases progressing to hepatic decompensation^{144,146,150}. It is recommended a frequent monitoring during pregnancy and for at least 6 months after delivery¹³⁶, especially for HBeAg-positive mothers or those who have stopped antiviral therapy (AVT) recently¹⁴⁸.

Transmission: HBV is transmitted through activities that involve percutaneous or mucosal contact with blood or other infectious body fluids¹⁵¹ and can survive outside the body for several days (at least seven)¹³². Its predominant mode of transmission varies in different geographical areas¹³⁷. In high-prevalence settings, HBV is most commonly spread through VT or early-life horizontal transmission¹³². Among adults, the majority of infections occur by sexual intercourse, unsafe injection practices and transfusion of unscreened blood¹⁵². In healthcare settings, HBV is the commonest bloodborne transmitted virus¹³⁷. Possible mechanisms of HBV VT are presented in figure 5.

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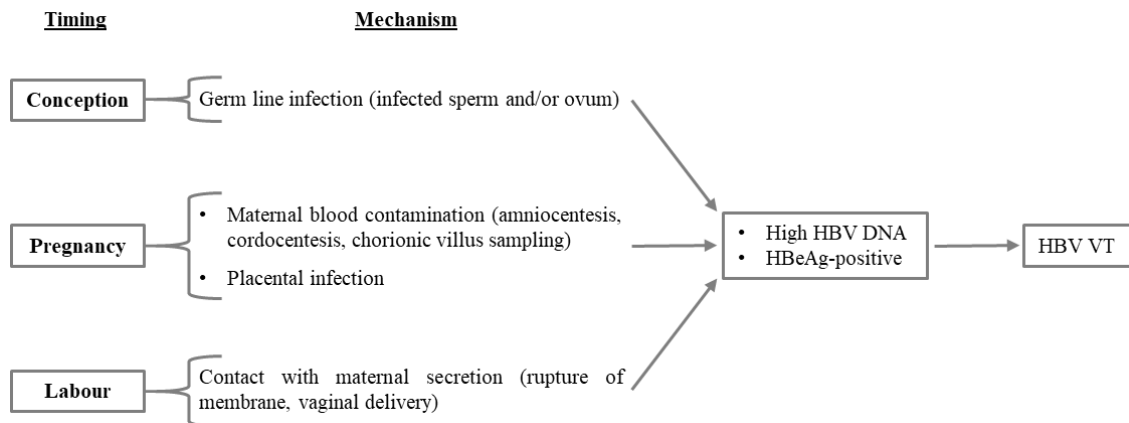


Figure 5. Possible mechanisms of HBV vertical transmission (adapted from Cheung et al, 2019¹⁵³)

Evidence suggests that HBV VT occurs predominantly during the peripartum period¹⁵⁴, especially through infant exposure to blood and vaginal secretions at the time of delivery¹⁴⁴. The risk of HBV VT is associated with maternal HBeAg status and HBV DNA level^{137,149,155,156}. It is estimated to be higher than 90% when maternal VL is greater than 10^5 copies/mL, 70-90% in HBeAg-positive and 10-40% in HBeAg-negative mothers¹⁵⁵. Postnatal active and passive immunoprophylaxis, respectively, hepatitis B vaccine and hepatitis B immunoglobulin (HBIG), reduces VT rate to approximately 5%¹⁵⁷. The predominant risk factors for immunoprophylaxis failure (IF) are high maternal HBV VL and HBeAg-positivity^{158,159}. In SSA, despite a timely vaccine dose at birth (VDB), the risk of VT was estimated to be 32.4% among infants of HBeAg-positive mothers compared with 0% among infants of HBeAg-negative mothers¹⁶⁰. There is unclear and conflicting evidence regarding the effect of the mode of delivery on the risk of HBV VT^{154,161-168}. Therefore, C-section is not recommended solely for prevention of HBV VT^{153,154,161,169-171}. Many studies also indicated that, with proper neonatal immunoprophylaxis, breastfeeding does not impose an additional risk for HBV VT¹⁷²⁻¹⁷⁶. As such, international guidelines consider that maternal HBV infection is not a contraindication to breastfeed^{140,170,177,178}. However, nipples cracking and bleeding should be avoided¹⁵⁴. A recent meta-analysis found that C-section and non-breastfeeding reduce the risk of HBV VT in infants of mothers positive for both HBsAg and HBeAg who did not receive AVT during pregnancy¹⁷⁹.

Diagnosis: Universal HBV screening in pregnant women is a major factor in decreasing the risk of VT by identifying HBV-infected mothers whose babies should be given hepatitis B vaccine in combination with HBIG¹⁵⁴. Therefore, all clinical practice guidelines recommend prenatal HBsAg screening¹⁴⁹. Even if they have been vaccinated or tested previously, all pregnant women should be tested during an early prenatal visit in each pregnancy¹⁷⁸. WHO recommends routine HBsAg testing of all pregnant women, especially in settings with an intermediate or high seroprevalence¹⁸⁰. The diagnosis of hepatitis B is commonly carried out through serological and molecular detection using serum or plasma samples¹⁸¹. Several HBV diagnostic markers have been utilized to detect current infection, as opposed to past infection or immunization¹⁸². The most important HBV diagnostic markers are highlighted in table 8.

Table 8. Essential biomarkers of HBV infection (adapted from Jackson et al, 2018¹⁸³)

Marker	Description	Role	Utility
HBsAg	Surface/envelope protein	Infectivity	Diagnosis of current HBV infection: transient (acute) or persistent ≥ 6 months (chronic) Loss/seroconversion is a criterion of functional cure Quantification allows monitoring therapeutic outcomes
HBV DNA	Found in infectious virions	Replication	Therapy monitoring Assessment of perinatal transmission risk Prediction of HCC risk
HBeAg	Secreted non-structural protein	Tolerogenicity, immune regulation, persistence	Definition of chronic infection phase Seroconversion is a main therapeutic endpoint Quantification predicts seroconversion and therapeutic response

The detection of HBsAg, the serological hallmark of HBV infection¹⁸⁴, indicates current HBV infection, either acute or chronic¹⁷⁸. A range of markers other than HBsAg can be used to further characterize HBV infection¹⁸⁰. All HBsAg-positive persons are infectious, but those with elevated HBV DNA or those HBeAg-positive are most infectious¹⁷⁸. HBsAg is the first serological marker of HBV infection to appear with a window period of around 38 days and is followed by HBeAg¹⁸⁰. HBeAg may be detected in the serum soon after HBsAg becomes detectable and correlates with high levels of infectivity¹⁸⁵, as

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is a marker of high levels of viral replication¹⁸⁰. HBV DNA is detectable prior to the detection of HBsAg¹⁷⁸ about one month after HBV infection¹⁸⁴. At present, HBV VL (levels of serum HBV DNA) is the most relevant marker of HBV replication, being also a strong predictor of liver disease progression towards cirrhosis and HCC¹⁸⁶. Additionally, it is used to monitor the efficacy of AVT¹⁸⁷. In order to guide maternal AVT during pregnancy for the prevention of perinatal HBV transmission, all HBsAg-positive pregnant women should be tested for HBV DNA level¹⁷⁸. It is important to determine if infection of the infants born to HBV-infected mothers occurred with or without the neonatal post-exposure prophylaxis¹⁸⁸. Like in adults, the presence of HBsAg diagnoses an infected infant¹⁸⁹, unless transient HBsAg-positivity after hepatitis B vaccine is occurring¹⁹⁰. Therefore, HBsAg screening should be performed prior to or postponed at least four weeks post-immunization¹⁹⁰. According to the Centers for Disease Control and Prevention (CDC), laboratory evidence of HBV infection in an infant consists of one or more of the following criteria: positive HBsAg test (only if at least four weeks after last dose of hepatitis B vaccine), positive HBeAg test and detectable HBV DNA¹⁸⁸. On the other hand, WHO advises that exposed infants should be tested for HBsAg between six and 12 months of age as, within the first six months of life, HBsAg and HBV DNA may be inconsistently detectable in infected infants¹⁸⁰. Some studies found cases of transient HBsAg and/or HBV DNA positivity at birth in infants who received HBV prophylaxis with hepatitis B vaccine and HBIG^{158,191-193}, compromising a possible definitive diagnosis at that time. The results from the study by Zhang et al¹⁹³ suggest that neither positivity of HBV markers at birth can universally be used to define VT nor their negativity can be used to exclude it. As seropositivity for HBsAg or HBV DNA at birth could be cleared by host immunity and HBV vaccination, this finding alone does not equate to IF¹⁷¹. Infants that have received HBV prophylaxis, including hepatitis B vaccination and/or HBIG (depending on local possibilities), should be re-evaluated at 9-12 months for the presence of anti-HBs and absence of HBsAg¹⁸⁹. These tests are denominated post-vaccination serological testing (PVST)^{185,194} and allow identifying infants who do not have an adequate immune response to an initial hepatitis B vaccine series (vaccine non-responders) and the HBV-infected infants despite neonatal immunoprophylaxis (IF)^{171,194}.

Pregnancy Outcomes: The impact of infection by HBV on pregnancy outcomes remains uncertain^{144,154,195,196}. Published studies have reported conflicting and inconsistent findings on the matter. Pregnancy outcomes will be affected by multiple factors, including racial disparity, viral genotypes, prevalence, phase of chronic infection, co-existing hepatic disorders, coinfections and synergism with pregnancy complications¹⁵⁰. Insufficient sample sizes, heterogeneous patient populations, diversity of clinical characteristics, differences in study designs and lack of or incomplete adjustment for important confounders may explain the conflict and inconsistency of published studies results regarding association between maternal HBV infection and adverse pregnancy outcomes^{197,198}. Additionally, most of the studies on the matter had only evaluated the effects of maternal HBsAg carrier status on pregnancy outcomes and did not examine maternal HBeAg and HBV DNA status¹⁹⁶. According to the Society for Maternal-Fetal Medicine, there is insufficient evidence to conclude that HBV infection (acute or chronic) is associated with adverse pregnancy outcomes¹⁷⁰.

Prevention and Treatment: Hepatitis B management during pregnancy comprises screening for the presence of infection, evaluating disease activity and performing measures to prevent VT¹⁹⁹. The maternal disease status could be evaluated through a baseline HBV DNA quantification in conjunction with both HBeAg and liver function tests¹⁷¹. Taking into account that the risk of acquiring chronic HBV infection is highest during the perinatal period, the prevention of VT during pregnancy and delivery is a critical step and provides the optimal opportunity to begin HBV elimination¹⁷¹. Prevention of HBV VT includes universal antenatal screening, maternal AVT if high VL is present and immunoprophylaxis (hepatitis B vaccination and HBIG) administered to infants born to infected mothers¹⁷⁰. Despite treatment, HBV cccDNA, the key molecule responsible for intrahepatic viral persistence, remains as a stable episome in the nucleus of infected hepatocytes^{123,138,186,200-203}. Therefore, there is no cure for chronic HBV infection, but suppression of viral replication and prevention of disease progression can be reached with effective antiviral treatments²⁰⁴. As the elimination of HBV replication results in the normalization of liver enzyme levels and the resolution of histologic necro-inflammatory activity, sustained viral suppression prevents fibrosis progression and leads to its regression¹²⁸. Current available antiviral treatments for CHB include nucleos(t)ide

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analogs (NAs) and pegylated interferon alpha^{128,138,186,202,205}, being that NAs are the most widely chosen option globally¹²⁸. In contrast, poor efficacy and tolerability of peginterferon limit its use as primary therapy in patients with CHB¹²⁸. The following markers are used to assess the efficacy of treatment: HBV DNA suppression, ALT normalization, HBeAg clearance and HBsAg clearance¹³⁸. A meta-analysis showed that NAs are all effective regarding viral response (HBeAg seroconversion, HBeAg loss, undetectable HBV DNA) and the majority are effective for biochemical response (ALT normalization)²⁰⁶. Most of the patients under NAs treatment achieve undetectable VL with a lower risk of developing cirrhosis and HCC¹⁸⁶, but rarely reach the goal of HBsAg clearance^{138,202}. NAs used for treatment of adults with CHB include nucleosides (lamivudine, telbivudine, entecavir [ETV]) and nucleotides (adefovir dipivoxil, tenofovir disoproxil fumarate [TDF], tenofovir alafenamide)^{202,207}. Although they have few side effects, long-term and often lifelong treatment is needed to maintain viral suppression¹³⁸. Tenofovir and ETV are recommended as first-line oral therapy^{136,208}, while lamivudine, telbivudine and adefovir have limited clinical use owing to the development of drug resistance with prolonged use¹³⁶. The course of chronic HBV infection is complex, highly variable, dynamic and fluctuating. As treatment is not recommended for all patients, decisions have to be made on who benefits the most from treatment and when it should be initiated²⁰⁸, based on patient's VL, ALT level, HBeAg status and the extent of liver fibrosis²⁰⁹. Treatment is usually recommended for patients at higher risk of disease progression^{208,209}. Risk assessment for each patient should regard the presence of clinical and laboratory characteristics associated with the development of cirrhosis and HCC²⁰⁸ that have been previously enumerated in table 7. In pregnant women, weighing benefits and risks for both the mother and the fetus, the severity of the liver disease and the risk of perinatal transmission are the determining factors in deciding whether to initiate or continue AVT¹³⁶. Safety in pregnancy and breastfeeding, therapy efficacy and duration as also barrier to resistance are factors to take into account²¹⁰. A multidisciplinary approach involving hepatologists/gastroenterologists and obstetricians is crucial¹⁵³. For the benefit of both mother and fetus, treatment should be initiated or continued if the mother has advanced liver disease (advanced fibrosis, cirrhosis or hepatic decompensation) while in another clinical situations the decision should be made after a thorough risk-benefit analysis¹⁵⁴. To guide the necessity in initiating antiviral treatment

if maternal indication or to prevent IF, baseline liver function test and HBV DNA quantification should be performed in the first trimester of pregnancy¹⁵³. As previously mentioned, the major risk factors for IF are high maternal HBV VL and HBeAg-positivity^{158,159}. Despite neonatal immunoprophylaxis, around 10-30% of the infants born to mothers with high viraemia are still at risk of acquiring HBV infection¹³⁶. As so, there is growing evidence to support both the safety and efficacy of AVT initiated in late pregnancy for reduction of VT among women with a high level of HBV DNA²¹¹. Table 9 presents a resume of the latest international guidelines regarding the use of AVT for hepatitis B management during pregnancy.

Table 9. Antiviral therapy for hepatitis B management during pregnancy (adapted from Yim et al, 2020²¹²)

Hepatitis B in pregnancy	EASL	American Association for the Study of Liver Diseases	Asian-Pacific Association for the Study of the Liver
Treatment	TDF continued, while ETV or other NA switched to TDF	TDF preferred	TDF drug of choice
Prevention of VT	If HBV DNA >200,000 IU/mL or HBsAg >4 log ₁₀ IU/mL, TDF at 24-28 weeks of gestation, continued for up to 12 weeks PP	If HBV DNA >200,000 IU/mL at 28-32 weeks of gestation, AVT recommended	If HBV DNA >6-7 log ₁₀ IU/mL, short-term maternal NA therapy recommended (tenofovir or telbivudine), beginning at 28-32 weeks of gestation
Lactation	Breastfeeding not contraindicated in HBsAg-positive untreated women or on TDF treatment or prophylaxis	Breastfeeding not contraindicated	Breastfeeding discouraged during maternal NA treatment

Recently, the WHO issued a new recommendation regarding prevention of HBV VT: HBsAg-positive pregnant women with a HBV DNA $\geq 5.3 \log_{10}$ IU/mL (equivalent to $\geq 200,000$ IU/mL) should receive tenofovir prophylaxis from the 28th week of pregnancy

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at least until the time of birth²¹³. A recent meta-analysis reinforced that threshold and, in addition, showed that HBeAg is accurate to identify women with HBV DNA levels above it and has high sensitivity to predict cases of IF²¹⁴. This result about HBeAg is particularly relevant for resource-limited settings, where HBV DNA assays are not available. The WHO recommends that, in those settings, HBeAg testing can be used as an alternative to determine eligibility for tenofovir prophylaxis²¹³. The three antivirals considered safe in pregnancy are lamivudine, telbivudine and TDF^{144,148,149,211,215}. A meta-analysis has proved that these antivirals are safe in pregnancy and effective in reducing VT in HBV chronically infected women with high VL compared to the use of passive-active immunoprophylaxis alone²¹⁶. A more recent meta-analysis reinforce these findings and supports specifically the use of TDF²¹⁷. Another one has showed specifically the safety and efficacy of this drug²¹⁸. TDF is therefore the treatment of choice for prevention of VT^{136,149,170,171,211-213}, owing to its antiviral potency and high genetic barrier to resistance^{171,212,213}. TDF low resistance profile enables long-term continuation of anti-HBV therapy for mothers if medically indicated¹⁴⁸. Newborns from HBV-infected mothers should receive a timely hepatitis B VDB in combination with HBIG and posteriorly complete the whole course of HBV vaccination^{161,171}. HBV VDB and HBIG should be provided within 12 to 24 hours after birth¹⁴⁵. The WHO recommends that all infants, including LBW and premature, receive their first dose of hepatitis B vaccine as soon as possible after birth, preferably within 24 hours²¹⁹. A study estimated that a routine infant hepatitis B vaccination with 90% coverage, including a VDB, could prevent 84% of the global HBV-related deaths²²⁰. Hepatitis B vaccination is the foundation on which other interventions to prevent VT can be built (figure 6).

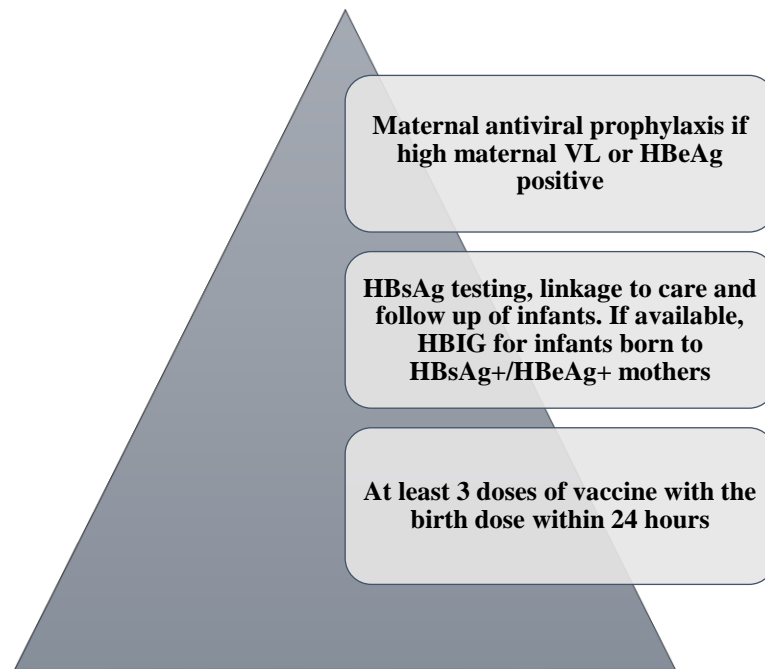


Figure 6. Approach to prevention of HBV vertical transmission (adapted from WHO, 2020²¹³)

Although the chance of preventing VT increases from 70% with VDB alone to 90% when in combination with HBIG, this is not a universal WHO recommendation due to its high cost (>100 USD in some settings), need for cold chain supply and safety issues (blood product)¹⁵⁶. In certain settings might be preferable to target HBIG to neonates of higher-risk HBeAg-positive mothers¹⁵⁴. WHO states that HBIG should be administered as an adjunct to hepatitis B vaccine and may be particularly beneficial for newborns whose mothers are both HBsAg and HBeAg positive²¹⁹. A meta-analysis found that vaccine alone seems to be as effective as the combination with HBIG for neonates of HBsAg+/HBeAg- mothers²²¹.

1.1.1.4. Hepatitis C

Aetiology: Hepatitis C is caused by infection with HCV²²², a virus that was discovered in 1989 as a cause of non-A, non-B hepatitis²²³. HCV is a positive-stranded RNA member of the Flaviviridae family without a persistent genomic form, allowing efficient targeting by antivirals¹²³. HCV has a greater genetic diversity than HBV or HIV²²⁴, with eight known genotypes²²⁵ and at least 80 subtypes²²⁶. Genotypes show different geographical distribution, pathogenicity, rate of progression to liver fibrosis and response to

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treatments^{224,227,228}. Similarities and differences of HBV and HCV infections, with special emphasis on pregnancy, are presented in table 10.

Table 10. Comparison between HBV and HCV infection (adapted from Espinosa & Jhaveri, 2018¹⁸⁹)

	HBV	HCV
Pathogen	Double-stranded DNA Hepadnaviridae	Single-stranded RNA Flaviviridae
Transmission	Bloodborne, sexual and VT	Bloodborne, sexual (specially in men who have sex with men) and VT
Risk of progression to chronic infection	Low in adults High in infants	High
VT rate	High (~90% if high VL and HBeAg positive)	Low (~6%)
Antenatal screening	Universal	Risk-based
Screening of exposed infants	PVST 1-2 months after vaccine completion	RNA PCR at 2-6 months Anti-HCV test at 18 months
Prevention of VT	Pregnant women	None available
	Neonate	

Epidemiology: Viral hepatitis caused 1.34 million deaths in 2015, overtaking those attributed to HIV (1.06 million deaths) or malaria (0.44 million deaths), with 96% being the result of complications due to both chronic HBV (66%) and HCV (30%) infections²²⁹. Most of that mortality is due to HCC and cirrhosis²³⁰. Globally, in 2017, the second, third and fourth infection/non-communicable diseases pairs with the largest burden are cirrhosis and other chronic liver diseases due to HBV (12.2 million DALYs), cirrhosis and other chronic liver diseases due to HCV (10.4 million) and liver cancer due to HBV (9.4 million)²³¹. HCV is a major global health burden²³². Worldwide, 71 million people were chronically infected with HCV and there were 1.75 million new HCV infections in 2015²²⁹. In that year, the global prevalence of viraemic HCV infection was estimated to be 1.0% (95% uncertainty interval: 0.8-1.1)²³³. In 2018, the global prevalence of viraemic

HCV infection in the paediatric population aged 0-18 years was estimated to be 0.13% (95% uncertainty interval: 0.08-0.16)²³⁴. Worldwide HCV prevalence varies widely²²⁴, with major differences among and within countries²³⁵. Data from much of Africa is limited²²⁴. Egypt has the world's highest estimated HCV prevalence^{224,226} (viraemic HCV infection prevalence of 6.3% in 2015²³³). A systematic review and meta-analysis found a HCV seroprevalence of 2.98% for SSA²³⁶. Another one estimated that the overall adult seroprevalence of HCV in SSA ranges from 3.82 to 3.94%, being the lowest in Southern Africa (0.72%)²³⁷. In this study, the HCV seroprevalence in pregnant women from SSA was 2.51% (95 % CI: 1.46-4.28)²³⁷.

Natural History and Clinical Manifestations: As HBV, HCV is a hepatotropic virus that can cause acute or chronic infection²³⁸. Acute infection corresponds to the first six months of infection after exposure²³⁹⁻²⁴¹, while a persistence of HCV RNA within the blood for more than six months is defined as a chronic infection²⁴². Acute hepatitis C is usually asymptomatic (80%)²⁴³ and very rarely is associated with life-threatening disease¹⁸⁰. If symptoms do occur, they can comprise fever, fatigue, joint pain, dark urine, clay-colored stool, loss of appetite, abdominal pain, nausea, vomiting and jaundice²⁴⁴. The complex interplay virus-host (adaptive and innate immune system) determines the outcome of acute HCV infection (viral clearance or progression to chronic infection)^{232,245}. Overall, acute HCV infection becomes chronic in 70% of cases (55-85%)²⁴³. Specifically for persons younger than 20 years, the rate of progression to chronic infection is lower (one-third)²²⁴. The progression to chronicity is influenced by several factors, including age at exposure, sex, ethnicity, liver synthetic dysfunction during initial infection, development of jaundice during the acute stage, HIV coinfection and interleukin 28B genotype^{224,239}. The natural history of chronic hepatitis C is highly variable²⁴⁶, being generally characterized by persistent hepatic inflammation²⁴². In the long-term, it may lead to mild illness²⁴⁶ or can cause liver cirrhosis, liver failure and HCC^{180,247}. Hepatic injury ranges from minimal necro-inflammatory changes to extensive fibrosis and cirrhosis with or without HCC²⁴⁶. Chronic HCV infection is a major cause of cirrhosis and HCC²²⁴. According to WHO, in chronically HCV-infected individuals, the risk of cirrhosis ranges from 15 to 30% within 20 years²⁴³. Many factors can increase an individual's risk of developing significant fibrosis or cirrhosis, such as age older than 40

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years at the time of being infected, male gender, alcohol use, non-alcoholic steatohepatitis, obesity, insulin resistance, type 2 diabetes, viral coinfection (HBV or HIV) and immunosuppression^{224,242}. Most of HCV-associated HCCs develop in cirrhotic livers²⁴⁸, since the risk of HCC development is greatly increased by cirrhosis²²⁴. Cirrhotic patients have a 1-5% annual risk of HCC and a 3-6% annual risk of hepatic decompensation²⁴². Untreated HCV viraemia can lead also to a host of extrahepatic disorders, ranging from lymphoproliferative, dermatologic to metabolic²⁴⁹, including diabetes mellitus, essential mixed cryoglobulinemia, glomerulonephritis, porphyria cutanea tarda and non-Hodgkin's lymphoma²⁴⁴. Natural history of HCV infection seems to differ between women and men, with a higher rate of spontaneous clearance among women²⁵⁰. Pregnancy does not appear to negatively affect the clinical course of chronic HCV infection²¹⁵. Perhaps due to the effect of immunosuppressive cytokines synthesized during pregnancy^{215,250}, evidence showed that, in the second and third trimesters of pregnancy, ALT serum levels tend to decrease and VL may increase²⁵¹⁻²⁵⁴, typically returning to prepregnancy levels after delivery²¹⁵. Although additional research data are needed, pregnancy is possibly associated with a decrease in HCV-mediated hepatic injury^{240,241}.

Transmission: HCV is a bloodborne virus²⁴³, being primarily transmitted through percutaneous exposure to blood²⁴⁰. Its four main routes of transmission are healthcare-associated transmission, injecting drug use, VT and sexual transmission¹⁸⁰. The leading causes of new HCV infections are unsafe healthcare procedures (iatrogenic infections) and injection drug use²³⁵. Less common are VT and sexual transmission²⁵⁵. The last is less frequent (5%) than in HBV patients (30%)²⁵⁶. Its rates are higher in HIV-infected men who have sex with men^{228,257}, which is probably explained by an interplay between several biological and behavioural factors²⁵⁸. A meta-analysis estimated an overall rate of HCV VT of 5.8% in viraemic mothers, which is significantly higher in HIV-coinfected ones (10.8%)²⁵⁹. Whether the level of HCV viraemia correlates with the risk of VT remains unclear²⁴¹. The mode of delivery (vaginal vs C-section) and breastfeeding have not been associated with an increased risk of HCV VT^{241,260,261}. Prolonged rupture of membranes, internal fetal monitoring and episiotomy may be associated with an increased

risk of HCV VT and should be avoided^{241,262}. Due to a higher potential risk for exposure, breastfeeding should be avoided when there are cracked nipples or skin breakdown²⁶³.

Diagnosis: Early diagnosis of hepatitis C allows timely treatment and prevention of complications of chronic infection, as well as disruption of transmission²⁶⁴. Since WHO advises HCV testing for who may be at increased risk of infection²⁴³, does not recommend routine testing of pregnant women¹⁸⁰. Like WHO, Society for Maternal-Fetal Medicine recommends screening women who are at increased risk for HCV at their first ANC visit²⁴¹. CDC now recommends universal hepatitis C screening for all pregnant women during every pregnancy in settings where the prevalence of viraemic HCV infection is $\geq 0.1\%$ ²⁴⁴. Antenatal diagnosis of hepatitis C has a dual benefit for mother and child, enabling them to be included in assessment and/or treatment programs²⁶⁵. Diagnosis of HCV infection consists on the detection of anti-HCV antibodies and HCV RNA^{187,240,241,255}. Anti-HCV antibodies tests identify people who have been infected with the virus²⁴³, being that antibodies may be detected approximately 8-11 weeks after exposure²⁶⁶ and persist in patients who have achieved viral clearance (spontaneously or after antiviral treatment)^{257,264}. Therefore, a reactive antibody test reflects exposure to HCV and does not distinguish between current and past infection^{257,264}, being necessary a test to detect HCV viraemia to confirm active infection²³⁹. A positive anti-HCV antibody finding should be followed by a NAT for HCV RNA^{215,240,241,262}, which is detectable 1-2 weeks after exposure¹⁸⁰. A positive result of both tests indicates an active infection²⁴⁰. A diagnosis of chronic HCV infection is made by two HCV RNA positive tests at least 6 months apart²⁶⁷. However, NATs are expensive and time consuming²⁶⁴, with a limited access in many LMIC²⁶⁸. The HCV core antigen (HCVcAg) has been proposed as an alternative marker to HCV RNA^{269,270}, because it is a marker of HCV replication^{246,270} and becomes detectable a few days after HCV RNA during acute HCV infection²⁴⁶. The approach to HCV diagnosis can be simplified by the use of the rapid oral HCV antibody test, reflex NAT and HCVcAg test²⁴⁷. Diagnosis of active HCV infection during pregnancy leads to follow-up and treatment of the mother after delivery, screening of exposed infants and treatment of infected children²⁷¹. Due to passive placental transfer, maternal HCV IgG antibodies may persist in the infants born to HCV-positive mothers for up to 18 months²⁷². Therefore, an antibody-based test should only be carried out at or

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after 18 months of age^{239,271}. Nevertheless, as waiting until 18 months of age is frequently unfeasible for both parents and physicians²⁷³, the CDC states that testing for HCV RNA can be performed at 1-2 months of age²⁴⁴. The results of the study by Gowda et al²⁷⁴ highlight that modern HCV-RNA RT-PCR assays have excellent sensitivity for the diagnosis of perinatally acquired infection at age 2-6 months.

Pregnancy Outcomes: Studies have suggested an association between maternal HCV infection and adverse pregnancy outcomes²⁴⁰. Higher rates of PB, intrauterine fetal death, small for gestational age, antepartum/PP haemorrhage, GD, premature rupture of membranes and intrahepatic cholestasis of pregnancy (ICP) have been reported²⁴⁶. It is challenging to know whether the increased risk of adverse outcomes is due to the viral effect of HCV or to potential confounders in the studied population²⁴¹. The presence of concurrent risk factors of poor outcomes need to be considered, including poor perinatal care and drug or alcohol use²⁵⁰. Meta-analyses suggested that maternal HCV infection is significantly associated with an increased risk of PB²⁷⁵, impaired intrauterine fetal growth²⁷⁶ and ICP²⁷⁷. Currently, a new systematic review is underway to synthesise the literature on the pregnancy outcomes of women living with HCV²⁷⁸.

Prevention and Treatment: Currently, there are no available immunoprophylaxis or other preventive strategies for HCV-infected pregnant women or their infants for the elimination of VT²⁶². In table 11, this scenario is compared with that of the prevention of HIV and HBV VT.

Table 11. Different scenarios of vertical transmission prevention for HIV, HBV and HCV (adapted from Barritt & Jhaveri, 2018²⁷⁹)

	HIV	HBV	HCV
Methods of prevention	Maternal ART before, during and after delivery Elective C-section if high VL (not consensual) Infant ART for 4-6 weeks after delivery	TDF for high-risk pregnant women from 24-32 weeks of gestation to 12 weeks PP Vaccine and HBIG for exposed infants	None currently Possibly maternal direct acting antivirals treatment in the near future
Benefits	Improvement of maternal health Prevention of infant infections Reduction of infant mortality	Prevention of breakthrough VT after vaccine and HBIG (TDF) Prevention of infant infections	Potential benefits: Maternal cure Elimination of VT No need for exposed infants screening

There is a lack of a vaccine against HCV²⁸⁰. Over the past decade, therapeutic treatment of HCV had a major improvement with the development of direct acting antivirals (DAAs)²³², whose regimens are simple (once-daily oral dosing), tolerable, of short duration (8-12 weeks) and highly efficacious (cure rates >95%)²⁸⁰. They act directly on viral proteins that are crucial for virus replication²²⁷. A sustained virological response, undetectable HCV RNA for 12-24 weeks after treatment completion, is considered the cure criterion^{255,281}. Genotyping is not necessary when using pangenotypic regimens²⁸², that are particularly useful in LMIC²²⁷. The current costs of DAAs treatment are very high¹⁵² with insufficient data on its safety and efficacy during pregnancy^{241,246,250,282} and currently, DAAs treatment is not recommended during pregnancy^{241,246,255,271,282}. Studies are under development to confirm the safety and efficacy of DAA therapy during pregnancy²⁸³. In the near future, it is conceivable that DAAs treatment of HCV-infected pregnant women becomes available, both to limit disease progression and to prevent VT²²².

1.2. Research Justification

Women of reproductive age are often faced with the reality of the association between STIs and pregnancy adverse outcomes, with consequences on morbimortality of mothers

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and newborns. This reality is more frequent and more severe in developing countries, since STIs are more prevalent and the possibility of preventing their complications are generally not regularly available¹³.

In Angola, *T. pallidum*, HIV, HBV and HCV epidemiology is still poorly documented together with lack of identification of associated factors. Studies about those infections during pregnancy, namely their prevalence, antenatal screening, VT and consequences for women and newborns are scarce.

There are research studies carried out in other countries with significant results on the association between STIs and adverse pregnancy outcomes. However, those on African countries are scarce and results diverge, especially with respect to HBV infection^{143,153,169,194-197}. Therefore, there is a need for a better understanding, namely in developing countries, to better adapt STIs prevention and control programs.

Lubango is the capital of Huíla province, the second most populous province of Angola, and Irene Neto Maternity (INM) is the provincial reference maternity, where most childbirths occur.

We intend to characterize infections by *T. pallidum*, HIV, HBV and HCV in parturients admitted to INM. This characterization will be directed to prevalence, association with sociodemographic factors, clinical approach during the antenatal period and delivery, VT, and association with maternal, obstetric and newborn complications.

1.3. Thesis Objectives

The general aim of this thesis is to characterize infections by *T. pallidum*, HIV, HBV and HCV in parturients admitted to INM and their complications. The specific objectives and their corresponding articles are described in table 12.

Table 12. Thesis specific objectives

Specific Objectives	Papers
1. To determine <i>T. pallidum</i> , HIV, HBV and HCV infections prevalence in the parturients	I - Seropositivity rate and sociodemographic factors associated to HIV, HBV, HCV and syphilis among parturients from Irene Neto Maternity of Lubango city, Angola
2. To identify sociodemographic factors associated to the studied infections	
3. To characterize the clinical approach towards pregnant women during the antenatal period and delivery with respect to the studied infections	II - HIV, HBV and syphilis screening in antenatal care in Lubango, Angola III - Evaluating vertical transmission of sexually transmitted infections to newborns
4. To relate the clinical approach towards infected women during the antenatal period and delivery with VT of the studied infections and compare it with the national and international established guidelines	III - Evaluating vertical transmission of sexually transmitted infections to newborns
5. To identify maternal, obstetric and newborn complications associated to the studied infections	IV - Maternal profiles and pregnancy outcomes: A descriptive cross-sectional study in a maternity of Angola
6. To describe parturients and newborns signs and symptoms which could be associated with the studied infections	V - A systematic review of the maternal and neonatal complications in hepatitis B infection VI - Maternal infection with hepatitis B virus and pregnancy outcomes in a maternity of Angola: A cross-sectional study
7. To recommend procedures to reduce complications of the studied infections in both pregnant women and newborns, taking into account local conditions	- Every paper, with the exception of the systematic review, has specific recommendations - Recommendations will also be present in the thesis discussion and conclusions - A paper in portuguese will be written in the future, which will be handed to Angolan health authorities. If found useful by these, a presentation will be performed in a meeting organized to address infections in pregnant women and neonates - A research project will be written and submitted for financing regarding “a cohort study of HBV-infected pregnant women in Lubango, Angola: vertical transmission and association with maternal, obstetric and newborn complications”

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1.4. Introduction References

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2. RESULTS

2.1. Paper I: Seropositivity rate and sociodemographic factors associated to HIV, HBV, HCV and syphilis among parturients from Irene Neto Maternity of Lubango city, Angola

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SHORT REPORT

Seropositivity rate and sociodemographic factors associated to HIV, HBV, HCV and syphilis among parturients from Irene Neto Maternity of Lubango city, Angola

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ABSTRACT

Objectives To characterise infections by HIV, *Treponema pallidum*, hepatitis B (HBV) and C virus (HCV) in parturients admitted to Irene Neto Maternity, Lubango city, Huíla province, Angola, namely its seropositivity rate and its association with sociodemographic factors.

Methods An observational, cross-sectional and analytical facility-based survey was conducted among 500 parturients at Irene Neto Maternity, from October 2016 to September 2017. Women in labour were screened for antibodies against HIV-1/2, *T. pallidum* and HCV. Antigen detection was used to diagnose HBV infections. Sociodemographic data were also collected. The seropositivity rate and respective CIs were estimated at a level of 95%. Multivariable logistic regression models were performed to explore the association between the studied infections and sociodemographic factors.

Results In 11.8% of the parturients (95% CI 9.3 to 14.9), at least one infection was detected. HBV infection was the most common (8.6%), followed by HIV infection (3.0%) and syphilis (1.0%). Coinfection with HBV and HIV was observed in two parturients (0.4%) and HBV, HIV and *T. pallidum* were all detected in one parturient (0.2%). No HCV infection was detected. For each additional year of formal education, pregnant women had a 10.0% lower chance of being infected with HBV (adjusted OR=0.900, 95% CI 0.816 to 0.992).

Conclusions This study is one of the few reports contributing for the knowledge of some sexually transmitted infections epidemiology in Angola. The seropositivity rate of the studied infections is of concern, especially the high endemicity of HBV. There is a need for a stronger commitment and further research to design cost-effective public health and clinical interventions to improve the situation.

INTRODUCTION

Sexually transmitted infections (STI) have a major impact on sexual and reproductive health worldwide, with more than 1 million new cases every day.¹ Determinants of STI are rather complex. In Angola, HIV, *Treponema pallidum*, hepatitis B (HBV) and C virus (HCV) epidemiology is still poorly documented together with lack of identification of

associated factors. Studies about those infections during pregnancy are scarce. The Angola's Multiple and Health Indicators Survey (AMHIS) 2015–2016 described an HIV prevalence of 2.6% in women between 15 and 49 years, namely 0.9% in Huíla province.² In another study, the HBsAg positivity rate in female staff, visitors and adult patients of a public hospital in Luanda was 11.6%.³

We intend to characterise infections by HIV, HBV, HCV and *T. pallidum* in parturients admitted to Irene Neto Maternity (INM), Lubango city, Huíla province, Angola, namely its seropositivity rate and its association with sociodemographic factors.

METHODS

An observational, cross-sectional, analytical facility-based survey was conducted among 500 parturients at INM from October 2016 to September 2017. Lubango is the capital of Huíla province, the second most populous province of Angola, and INM is the provincial reference maternity. Study population was estimated on basis of INM statistics for 2014, total population of 15 126 childbirths (N). Sample size was calculated using $n \geq NZ^2P(1-P)/d^2(N-1) + Z^2P(1-P)$: n is the required sample size; Z (0.975 quantile of a normal distribution for a CI of 95%)=1.96; P the prevalence of the studied infections in the population which, based on estimates of previous studies in Angola, was considered 0.3; precision (margin error) d is equal to 0.04. Estimated minimum size of the sample was 488 parturients. Considering 12 surrogate, final sample size was 500 parturients distributed proportionally per month, according to population stratification. Consecutive sampling was used to select the parturients to be observed each month. Study questionnaire included age, marital status, education level, job status, area of residence, house conditions, alcohol and tobacco use.

A blood sample was collected and plasma separated. An algorithm with three rapid tests was used for HIV-1/2 diagnosis. First-line assay was a fourth-generation test detecting HIV-1/2 antibodies and HIV-1 p24 antigen (Determine HIV-1/2 Ag/Ab Combo, Alere, UK). The other assays were third-generation tests detecting HIV-1/2 antibodies (Hexagon HIV, Human, Germany and

Info Anti-HIV 1/2, Türklab, Turkey). Inconclusive results were confirmed by western blot test (Genscreen HIV-1/2 V.2, Bio-Rad, France). HBV infection was determined by a rapid test detecting hepatitis B surface antigen (Laboquick HBsAg Test, Koroglu Medical Devices, Turkey). Two rapid tests were used to detect HCV antibodies (Hexagon HCV, Human, Germany in 313 parturients and Laboquick Anti-HCV Test, Koroglu Medical Devices, Turkey in 187 parturients). The use of different tests was due to supplier tests shortage. *T. pallidum* antibodies were screened with a treponemal rapid test (Laboquick Anti-Syphilis Test, Koroglu Medical Devices, Turkey). When reactive, the result was confirmed with RPR test (Syphilis RPR Test, Human, Germany). High seropositivity rate for HIV and syphilis when above 5% and low below 5%; HBV cut-off 8%.

Seropositivity rate of studied infections and respective CIs were estimated at a confidence level of 95% (Wilson method by EpiTools); multivariable logistic regression was performed to explore the association between HBV and HIV infection and sociodemographic factors. An OR was computed with respective 95% CI. For syphilis and HCV infection, this model was not explored due to the low seropositivity rate found. Selection of sociodemographic variables included in the regression model was guided by the literature review. Variable 'alcohol use' was not included in the model, since it was not possible to quantify it. We used a significance level of 5%. Analyses were performed with the Statistical Package for Social Science (SPSS) V.25.

The study was approved by Angola Ethics Committee (reference 35/2017), the Provincial Direction of Health and INM Direction. To be included, parturients had to sign an informed consent form. Whenever they could not read or write, pregnant woman fingerprint and witness signature were required, after informed consent was read to her. If Portuguese was not spoken, a local nurse acted as an interpreter and if younger than 18 years of age, parents/guardians consent was also requested.

RESULTS

From 500 parturients included in the study, 491 knew their correct age: between 13 and 48 years (mean age of 26.1 and SD of 6.6), 5.9% under 18 years. Regarding marital status, 243/500 (48.6%) were living with a partner, 169/500 (33.8%) were single, 87/500 (17.4%) married and one divorced (0.2%). Mean of 8

years of formal education and 8.2% of illiteracy (41/499). Half of the parturients (50.5%, 252/499) were working, the majority (86.6%, 433/500) residing in an urban area. House conditions: 136/500 (27.2%) had access to piped water, 364/500 (72.8%) public electricity, 416/500 (83.2%) latrine or toilet, 432/500 (86.4%) television and 381/500 (76.2%) radio. Tobacco use was not referred and 9.4% (47/499) assumed alcohol use.

In 11.8% (59/500, 95% CI 9.3 to 14.9) at least one infection was detected. HBV was detected in 8.6% (43/500, 95% CI 6.5 to 11.4), HIV 3.0% (15/500, 95% CI 1.8 to 4.9) and syphilis 1.0% (5/500, 95% CI 0.4 to 2.3). None was infected with HCV. HBV and HIV coinfection was observed in two (0.4%) and HBV, HIV and *T. pallidum* were simultaneously detected in one (0.2%). HIV-2 infection was diagnosed in one coinfecting with HIV-1.

According to logistic regression results, for each additional year of formal education, parturients had a 10.0% lower chance of having HBV (adjusted OR=0.900, 95% CI 0.816 to 0.992). None of the sociodemographic variables were associated with HIV infection at 5% level of significance (table 1).

DISCUSSION

In this study, hepatitis B was the most common infection (8.6%), followed by HIV (3.0%) and syphilis (1.0%). HCV infection was not detected. The HIV seropositivity rate found is three times higher than that reported in AMHIS 2015–2016 (0.9%).² The HBV seropositivity rate was high and similar to that found in a previous study done in Angola (11.6%).³

The Sustainable Development Goal 3.3 aims to combat hepatitis by 2030.⁴ Hepatitis B seropositivity rate in Lubango is of concern. Study participants carrying the HBsAg are infectious and may transmit their infection to the neonate. It is estimated that the risk of HBV vertical transmission is at least 10%–40%.⁵ Furthermore, chronic hepatitis B infection will develop in up to 90% of exposed neonates who do not receive appropriate immunoprophylaxis.⁶ Since in the present study 43 women were infected, 4–17 newborns may have HBV transmitted to them and around 3–15 will develop a chronic infection if appropriate immunoprophylaxis is not performed.

Prevention of HBV vertical transmission includes universal antenatal screening, maternal antiviral therapy if high viral load is present and immunoprophylaxis administered to infants born

Table 1 Multivariable logistic regression models for HBV and HIV (n=489)

Sociodemographic factors	HBV		HIV	
	P value	aOR (95% CI)	P value	aOR (95% CI)
Age, years	0.432	0.979 (0.928 to 1.032)	0.734	1.014 (0.934 to 1.102)
Formal education, years	0.035	0.900 (0.816 to 0.992)	0.866	1.012 (0.876 to 1.170)
Job status				
Not working		1		1
Working	0.381	0.727 (0.356 to 1.483)	0.061	3.713 (0.940 to 14.666)
Area of residence				
Rural		1		1
Urban	0.717	1.233 (0.397 to 3.831)	0.380	2.761 (0.285 to 26.731)
Television at home				
No		1		1
Yes	0.199	2.263 (0.651 to 7.864)	0.244	0.399 (0.085 to 1.872)

aOR, adjusted odds ratio; Bold values, statistically significant; 95% CI, 95% confidence intervals; HBV, hepatitis B virus; HIV, human immunodeficiency virus.

to infected mothers.⁶ Universal HBV vaccination at birth is implemented in Angola. However, there is low coverage of antenatal screening, maternal viral load is not evaluated, antiviral therapy is not offered in cases of high viral load and hepatitis B immunoglobulin is not available (personal communication).

In this study, higher formal education decreased the chance of infection with HBV. Other studies with pregnant women in African countries showed a statistically significant association between education and HBV infection.^{7,8} Education is a structural determinant of health, with specific interpretations that explain its association with health outcomes: education reflects material, intellectual and other resources of the family of origin and is a strong determinant of future employment and income. On the other hand, knowledge and skills attained through education probably make people more receptive to health education messages or better enable them to communicate with and access health services.

The association between sociodemographic factors and HIV infection was not statistically significant. Other studies in Africa, whose participants were pregnant and non-pregnant, showed significant results. For example, it was found that among pregnant women in Cameroon, HIV infection was associated with age, marital status, parity and place of residence.⁹ Possibly, we did not find a significant association between HIV infection and sociodemographic factors due to a selection bias, as in Huíla province only 30% of births occur in a health facility.²

Limitations of this study were: parturients giving birth at home or in other health institutions were not included; only serological assays were used; therefore, the lack of molecular testing did not allow detection of occult HBV, acute HIV and HCV infections; the use of two different assays for HCV weakens its antibody testing results; several factors known to be potentially associated with the risk of STI (behaviours and STI prevention knowledge) were not included in the logistic regression models for HIV and HBV.

In conclusion, our work highlights the importance of the studied STI, namely the high seropositivity rate of HBV. There is a need for stronger commitment and further research to design cost-effective public health and clinical interventions to improve the situation. Health authorities from Sub-Saharan African countries must develop national strategies to combat HBV that should be well resourced, sustainable, supported by the community and linked to other antenatal measures.¹⁰

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Angola Ethics Committee (Number 35/2017) and previously by the Provincial Direction of Health and Irene Neto Maternity Direction in 2016 before the beginning of the study, since the first was not active when the study started.

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2.2. Paper II: HIV, HBV and syphilis screening in antenatal care in Lubango, Angola

Reference: Oliveira D, Martins MR, Castro R, Cordeiro L, Barroso MR, Nazaré MA, Pereira F. HIV, HBV and syphilis screening in antenatal care in Lubango, Angola. *Sex Transm Infect.* 2020 Dec;96(8):621-2. doi:10.1136/sextrans-2019-054407.

RESEARCH LETTER

HIV, HBV and syphilis screening in antenatal care in Lubango, Angola

Screening for infections is part of antenatal care (ANC) evidence-based interventions.¹ The Angola's Health Development Plan 2012–2025² includes screening of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and syphilis in the essential package of interventions for maternal and newborn health, which is done using an opt-out approach. There are only a few reports about ANC in Angola.^{3–5} To our knowledge, this is the first one about ANC infection screening. We characterised ANC screening rates for HIV, HBV and syphilis among 500 parturient women admitted between October 2016 to September 2017 to the Irene Neto Maternity service in Lubango, Huíla, the second most populous province of Angola (Oliveira *et al*, 2020, accepted to *Sex Transm Infect*). One additional aim was to explore rural versus urban disparities in screening rates, according to parturients place of residence, and identify opportunities for improvement. Since no demographic surveillance system was available to us, rural or urban residence was determined by parturient description of her place of residence. Every parturient residing in Lubango or in its suburbs was considered to live in an urban area. On the contrary, those living outside this perimeter, whose source of livelihood was

mostly agriculture, were considered to be from a rural area. Whenever they could not read or write, pregnant woman's fingerprint and witness signature were required, after informed consent was read to her (Oliveira *et al*, 2020, accepted to *Sex Transm Infect*). Timing and number of ANC visits and results of ANC infection screening were retrieved from each participant's pregnancy card. When a positive screening result was found, the woman was asked about the treatment received. At admission in labour, 6.6% (33/500) of the women did not present a pregnancy card. Of the remaining 467 women, some presented pregnancy cards that were only partially legible (reflected in the denominators below). One way of assessing the quality of ANC is to estimate the proportion of pregnant women initiating ANC during the first trimester of pregnancy.³ Overall, 9.1% (42/462) of the women had initiated ANC in the first trimester of pregnancy (table 1), without difference based on rural or urban residence. In 2009, WHO recommended a minimum of four ANC visits and, in 2017, updated to a minimum of eight contacts to reduce perinatal mortality and improve women's experience of care.^{6 7} The Angola's Health Development Plan 2012–2025 recommends that a pregnant woman should be observed in, at least, four ANC visits.² In this study, at least four ANC visits during pregnancy were observed in 62.7% (293/467). HIV, HBV and syphilis screening was performed in 66.5% (309/465), 44.6% (208/466) and 60.2% (281/467) respectively, and all infections screening rates were

significantly lower in women from rural areas compared with those from urban areas. Only 2.6% (12/465), 0.6% (3/466) and 0.6% (3/467) of the parturients were tested two times during pregnancy against HIV, HBV and syphilis, respectively. Of the five women who had tested HIV positive, one referred that had not initiated antiretroviral therapy. Penicillin G treatment was reported by five of the seven women who tested positive for syphilis. Among the five treated with penicillin G, three recalled that had received the recommended three doses. None of those who tested positive for HBV referred having initiated treatment. In this study, the timing and number of ANC visits fell below the expectations set by the WHO^{6 7} as also, although not so low, found in previous studies conducted in other Angola provinces.^{3 5} In line with findings of previous studies,^{8–10} rural women presented significantly lower rates of HIV, HBV and syphilis screening. This could be explained by the fact that in Angolan rural areas, the offer of health services in general is lower, particularly laboratory tests. Additionally, women from rural areas have a lower educational level, which probably makes them less able to communicate with and access healthcare services. Policies are required to improve the quality of ANC in Lubango and to address rural–urban disparities in ANC infection screening rates.

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Table 1 ANC characteristics: general and according with area of residence

ANC characteristics	Total		Area of residence		P value
	N (%)	95% CI	Urban n (%)	Rural n (%)	
Timing and number of visits					
First trimester (n=462)	42 (9.1)	6.8 to 12.1	35 (8.7)	7 (11.9)	0.428*
≥4 visits (n=467)	293 (62.7)	58.3 to 67.0	262 (64.2)	31 (52.5)	0.083*
≥8 visits (n=467)	16 (3.4)	2.1 to 5.5	14 (3.4)	2 (3.4)	1.000†
Infection screening documented (at least once)					
HIV (n=465)	309 (66.5)	62.0 to 70.6	285 (70.2)	24 (40.7)	<0.001*
HBsAg (n=466)	208 (44.6)	40.2 to 49.2	201 (49.4)	7 (11.9)	<0.001*
RPR (n=467)	281 (60.2)	55.7 to 64.5	269 (65.9)	12 (20.3)	<0.001*
Infection treatment reported by women					
HIV positive (n=5)	4 (80.0)	37.6 to 96.4	4 (80.0)	0 (0.0)	NA
HBsAg positive (n=10)	0 (0.0)	0.0 to 27.8	NA	NA	NA
RPR positive (n=7)	5 (71.4)	35.9 to 91.8	4 (80.0)	1 (50)	NA

*Pearson χ^2 test.

†Fisher's exact test.

ANC, antenatal care; n, absolute frequencies; %, relative frequencies; 95% CI, 95% confidence intervals (estimated with the Wilson method using EpiTools); HBsAg, hepatitis B surface antigen; RPR, rapid plasma reagin test; NA, not applicable.

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2.3. Paper III: Evaluating vertical transmission of sexually transmitted infections to newborns

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Brief Original Article

Evaluating vertical transmission of sexually transmitted infections to newborns

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Abstract

Introduction: Sexually transmitted infections are among the most frequent infections affecting pregnant women. We assessed the transmission of hepatitis B virus, human immunodeficiency virus type 1 and *Treponema pallidum* to newborns from infected parturients.

Methodology: An observational, cross-sectional, analytical facility-based survey was conducted among 57 newborns in Irene Neto Maternity, Lubango city, Huíla province, Angola. Hepatitis B virus DNA molecular identification was done through nested PCR. Human immunodeficiency virus type 1 proviral DNA detection was carried out by two successive nested PCRs. Real-time PCR was performed to examine the presence of *T. pallidum* DNA. Amplicons from PCR positive samples were sequenced for identity search and genotype assignment.

Results: Hepatitis B virus DNA genotype E was detected in 3/41 (7.3%) newborns from HBsAg (hepatitis B surface antigen) positive mothers. To analyse the association between mothers HBeAg (hepatitis B e antigen) positivity and hepatitis B virus vertical transmission to newborns, a Fisher's exact test was performed, showing a highly significant association ($p = 0.006$). Human immunodeficiency virus type 1 provirus or *T. pallidum* DNA was not detected in any newborn.

Conclusions: To prevent hepatitis B virus vertical transmission in Angola it is important to promote universal antenatal screening, expanding hepatitis B virus markers (viral load and/or HBeAg), risk-based infected mothers' antiviral therapy and newborn passive immunoprophylaxis.

Key words: vertical transmission; HBV; HIV-1; *Treponema pallidum*; newborns; Angola.

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Introduction

Infections remain a major cause of maternal and fetal morbidity and mortality worldwide. Antenatal screening is essential in managing infections, as well as to prevent pathogen vertical transmission (VT). Sexually transmitted infections (STI) are among the most frequent infections affecting pregnant women [1]. The risk of hepatitis B virus (HBV) VT is estimated to be higher than 90% when maternal viral load is greater than 10^5 copies/mL, 70-90% in HBeAg (hepatitis B e antigen) positive and 10-40% in HBeAg-negative mothers [2]. Human immunodeficiency virus (HIV) VT rates range from 20-45% without intervention [3]. Untreated women have a 70% chance of transmitting syphilis to their fetus during the first four years of infection [4].

In Angola, studies on STI during pregnancy and VT are scarce. We assessed the transmission of HBV, HIV-1 and *Treponema pallidum* to newborns from infected

parturients admitted to Irene Neto Maternity (INM), Lubango city, Huíla province, Angola.

Methodology

Study Design

An observational, cross-sectional, analytical facility-based survey was conducted among 500 parturients at INM, from October 2016 to September 2017 [5]. INM is the reference maternity in Huíla, the second most populous province in Angola.

Sample Characterization

Blood samples were obtained from 57 newborns of 36, 12, four and two mothers infected with HBV, HIV-1, *T. pallidum* and HBV/HIV-1, respectively [5]. There were three twin pregnancies among the former, totalizing 41 newborns from HBV-infected mothers. Maternal HBV infection was defined by HBsAg (hepatitis B surface antigen) positivity in a rapid test (Laboquick HBsAg Test, Koroglu Medical Devices

Ltd., Turkey). An algorithm with three rapid tests was used for maternal HIV-1 diagnosis: first-line assay was a fourth-generation test (Determine™ HIV-1/2 Ag/Ab Combo, Alere Ltd., UK) and the other assays were third-generation tests (Hexagon HIV, Human, Germany and Info Anti-HIV 1/2, Türklab, Turkey). Inconclusive results were confirmed by Western blot (Genscreen™ HIV-1/2 Version 2, Bio-Rad, France). Maternal *T. pallidum* antibodies were screened with a treponemal rapid test (Laboquick Anti-Syphilis Test, Koroglu Medical Devices Ltd., Turkey) and, when reactive, the result was confirmed with RPR (Syphilis RPR Test, Human, Germany) [5]. We also performed HBeAg detection in 33/38 HBsAg-positive mothers (HBe Ag&Ab, DIA.PRO, Italy).

Molecular Identification of HBV, HIV-1 and T. pallidum in Newborns

Shortly after birth, a single capillary blood sample was collected from each newborn through foot heel puncture and conserved in filter paper (Grade 2, Whatman, UK). A QIAamp DNA Mini Kit (Qiagen, Germany) was used for DNA extraction and purification, according to manufacturer's instructions.

Molecular identification of HBV DNA was conducted through nested PCR targeting a 342 bp highly conserved fragment of the genome *S/P* region, as described by Oluyinka *et al.* [6]. HIV-1 proviral DNA detection was carried out by two successive nested PCRs for amplification of protease and reverse transcriptase coding regions (460 and 650 bp, respectively) [7]. Real-time PCR was performed to examine the presence of *T. pallidum* DNA, using primers targeting DNA polymerase I (*polA*) gene and a TaqMan probe [8], both designed by TIB MOLBIOL (Germany). A commercial reaction mix (NZYSpeedy qPCR Probe Master Mix, NZYTech, Portugal) was used and amplification was carried out in a Rotor-Gene 3000 (Corbett, Australia) thermal cycler.

Amplicons from PCR positive samples were sent to STAB VIDA (Portugal) to be sequenced (Sanger) in both directions (forward and reverse). The complementary sequences were edited in BioEdit Sequence Alignment Editor (v.7.0.9.0.) and submitted to NCBI BLASTn (Basic Local Alignment Search Tool) for identity search and genotype assignment.

Statistical Analysis

To analyse the association between mothers HBeAg positivity and HBV VT to newborns, a Fisher's exact test was performed, at the 1% level of significance,

using SPSS (Statistical Package for the Social Sciences) software v.26.

Ethical Considerations

Parturients signed an informed consent form to be included in this study, which was approved by the Angola Ethics Committee (No. 35/2017).

Results

HBV DNA genotype E was detected in 3/41 (7.3%) newborns from HBsAg-positive mothers. None of the corresponding three parturients was coinfecting with HIV-1 and only one had been aware of her HBV infection, but neither her viral load was evaluated, nor did she receive antiviral therapy (AVT).

HBeAg was detected in 2/33 HBsAg-positive mothers (6.1%). These transmitted the infection to their newborns (100%), while of the 31 HBeAg-negative, only one transmitted the infection (3.2%). There was an association between mothers HBeAg positivity and HBV VT to newborns ($p=0.006$).

HIV-1 provirus or *T. pallidum* DNA was not detected in any newborn from infected mothers. Regarding the 14 mothers infected with HIV-1, the majority (71.4%) received antiretroviral therapy (ART) before and/or during pregnancy and/or intrapartum (data on specific antiretrovirals largely incomplete), but four (28.6%) did not receive any ART. Among the four mothers infected with *T. pallidum* (RPR titers 1:2-1:8), one did not present her pregnancy card, two had a negative antenatal screening and one was treated with one dose of benzathine penicillin G at 14 weeks of pregnancy along with her partner.

Discussion

Prevention of VT is a major issue to consider in the management of STI during pregnancy. VT is related to adverse pregnancy outcomes, particularly in resource-limited countries, where access to adequate antenatal care is scarce. To our knowledge, this is one of the few reports on VT in Angola, the first on hepatitis B and syphilis.

Regarding HBV VT, the risk is higher in HBeAg-positive mothers (70-90%) [2]. In our study, despite the small numbers involved, a HBV VT rate of 100% in HBeAg-positive versus 3.2% in HBeAg-negative mothers was found, with an association between mothers' HBeAg and HBV VT to newborns ($p = 0.006$), as described before. For example, for Africa, a study previously conducted in Cameroon reported HBeAg as a serum marker associated with HBV VT [9].

Prevention of HBV VT includes universal antenatal screening, maternal AVT if high viral load is present and immunoprophylaxis in infants born to infected mothers. Evidence on the impact of HBeAg positivity must be taken into account when designing policies concerning prevention of HBV VT, particularly in low and middle-income countries. For instance, Lu *et al.* [10] suggested that vaccine alone may be enough for preventing HBV transmission in neonates of HBsAg-positive/HBeAg-negative mothers. Furthermore, Ségéral *et al.* [11] found that an algorithm of HBsAg and HBeAg rapid diagnostic tests could be a low-cost strategy to identify HBV-infected pregnant women at risk of VT when DNA quantification is not routinely available. Although universal HBV vaccination at birth is implemented in INM, other measures to prevent HBV VT in Lubango are missing. Only one of the three parturients with HBV DNA positive newborns had been aware of her HBV infection, but viral load had not been evaluated and AVT had not been administered. It is important to highlight that HBV VT remains a major source of perpetuation of chronically infected individuals' reservoir, having a huge impact in countries with a high burden of disease, as Angola, a country with a reported high HBV carrier rate of 15.1% [12].

There are several HBV genotypes, with distinct geographical distributions. They are important epidemiological markers, but they can also influence transmission patterns and clinical outcomes. In our study, the three HBV DNA positive newborns were infected with genotype E. In Angolan patients, this genotype is highly predominant [12,13], but genotypes A and D have also been reported [12].

HIV-1 provirus or *T. pallidum* DNA was not detected in any newborn from infected mothers. Regarding HIV-1, VT rate can be reduced to well below 5% with effective interventions [3]. We evaluated HIV-1 VT in 14 infected mothers with no data on viral load and absence of any preventive intervention was observed in four of them. High HIV-1 genetic diversity in Angola is also a challenge for molecular diagnosis [14]. Concerning *T. pallidum* VT among four infected mothers, there was almost no disease history available. They presented low nontreponemal titers (1:2-1:8), which could suggest the possibility of a serofast syphilis or recent treated syphilis, among others, and could explain the absence of congenital syphilis cases.

Among major limitations of this study, one should refer that it did not include women giving birth at home, which is very frequent in Angola, and maternal occult HBV infection was not evaluated.

Conclusions

Our study identified three cases of HBV genotype E VT, from mothers who were not properly managed during pregnancy regarding its prevention. In addition, a highly significant association between mothers HBeAg positivity and HBV VT to newborns was found. To prevent HBV VT in Angola it is important to promote universal antenatal HBV screening, expanding HBV markers (viral load and/or HBeAg), risk-based infected mothers' AVT and newborn passive immunoprophylaxis. A stronger integrated multisectoral commitment and further research are clearly needed in this field in Angola.

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2.4. Paper IV: Maternal profiles and pregnancy outcomes: A descriptive cross-sectional study in a maternity of Angola

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Maternal profiles and pregnancy outcomes: A descriptive cross-sectional study in a Maternity of Angola --Manuscript Draft--

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Abstract:	<p>Objectives: To characterize parturients admitted to Irene Neto Maternity, Lubango city, Huíla province, Angola, and to describe their pregnancy outcomes.</p> <p>Methods: We conducted a descriptive cross-sectional facility-based survey between October 2016 and September 2017, involving 500 parturients aged 13-48 years, followed from admission in labor until the end of delivery.</p> <p>Results: Regarding age groups, 18.3% of the parturients were adolescents and 14.5% had advanced maternal age. Illiteracy was reported by 8.2%. One in three (33.6%) had a short stature. Malaria was the most frequent infection during pregnancy (16.3%). At admission in labor, 18.1% of the parturients were anemic and hypertension was frequent (36.0%), contrasting with the few cases reported of chronic hypertension and pregnancy-induced hypertension. There were 15 twin pregnancies among the studied parturients. In 23.0% of the cesarean section cases, a medical indication for it was not identified. Among live births from singleton pregnancies, birth asphyxia was observed in 22.7% and one in every ten (10.3%) had low birth weight.</p> <p>Conclusions for Practice: There are very few reports contributing for the knowledge of pregnancy outcomes in Angolan women. This is the first one related to Huíla province, the second most populous province of Angola. We identified parturients characteristics that could put them in a higher risk of adverse pregnancy outcomes: adolescence, illiteracy and short stature. An antenatal subdiagnosis of hypertensive disorders was revealed. Among newborn outcomes, birth asphyxia and low birth weight demand special attention. Further research is needed to explore cesarean section non-medical</p>

indications and twinning rate in Lubango.

Maternal profiles and pregnancy outcomes: A descriptive cross-sectional study in a Maternity of Angola

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Declarations

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Ethics approval: The Angola Ethics Committee (reference 35/2017) approved this study.

Consent to participate: Parturients had to sign an informed consent form to be included in this study.

Consent for publication: Not applicable.

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Code availability: Not applicable.

Authors' contributions: DO, FP, MRM, RC and MRB designed the research; DO

collected the data; DO, MRM and JMO analysed the data; DO and FP wrote the paper; all authors commented on previous versions of the manuscript; all authors approved the final version.

Maternal profiles and pregnancy outcomes: A descriptive cross-sectional study in a Maternity of Angola

Abstract

Objectives: To characterize parturients admitted to Irene Neto Maternity, Lubango city, Huíla province, Angola, and to describe their pregnancy outcomes.

Methods: We conducted a descriptive cross-sectional facility-based survey between October 2016 and September 2017, involving 500 parturients aged 13-48 years, followed from admission in labor until the end of delivery.

Results: Regarding age groups, 18.3% of the parturients were adolescents and 14.5% had advanced maternal age. Illiteracy was reported by 8.2%. One in three (33.6%) had a short stature. Malaria was the most frequent infection during pregnancy (16.3%). At admission in labor, 18.1% of the parturients were anemic and hypertension was frequent (36.0%), contrasting with the few cases reported of chronic hypertension and pregnancy-induced hypertension. There were 15 twin pregnancies among the studied parturients. In 23.0% of the cesarean section cases, a medical indication for it was not identified. Among live births from singleton pregnancies, birth asphyxia was observed in 22.7% and one in every ten (10.3%) had low birth weight.

Conclusions for Practice: There are very few reports contributing for the knowledge of pregnancy outcomes in Angolan women. This is the first one related to Huíla province, the second most populous province of Angola. We identified parturients characteristics that could put them in a higher risk of adverse pregnancy outcomes: adolescence, illiteracy and short stature. An antenatal subdiagnosis of hypertensive disorders was revealed. Among newborn outcomes, birth asphyxia and low birth weight demand special attention. Further research is needed to explore cesarean section non-medical indications and twinning rate in Lubango.

Keywords: Angola, maternal characteristics, pregnancy outcomes

Significance

The significance of this paper is that it is one of the very few reports contributing for the knowledge of detailed maternal, obstetric and newborn outcomes in Angolan women. Although only addresses the outcomes descriptively, it can be used as a first step in monitoring and improving outcomes of women assisted in Irene Neto Maternity. Maternal and child health is a key issue to address sustainable development in Angola.

Introduction

Maternal health is a key issue to sustainable development (Graham et al. 2016). Maternal and infant mortality are indicators of a country social and economic development, reflecting how women and children are valued and human rights respected (Nimi et al. 2016). Monitoring the reduction of maternal mortality, stillbirths and neonatal mortality is part of the global health and development agenda and, as a consequence, data on that subject is at a certain point available, originating some actions to overcome mortality related causes. However, monitorization of maternal and neonatal morbidity profile is scarcer (Moller et al. 2019). Angola's maternal and child mortality rates are among the highest in the world (U.S. Agency for International Development, 2019). In 2015, maternal mortality ratio and stillbirth and neonatal mortality rate were estimated to be 514.3 per 100,000 live births, 26.91 per 1,000 live births and 23.48 per 1,000 live births, respectively (GBD 2015 Maternal Mortality Collaborators, 2016; GBD 2015 Child Mortality Collaborators, 2016). These numbers referring to mortality, one may guess that higher numbers may exist for morbidity.

There are very few recent studies addressing detailed maternal, obstetric and newborn outcomes of Angolan women (Nimi et al. 2016; Umar and Kabamba 2016; Nimi et al. 2019; Rosário et al. 2019). Monitoring and evaluation data at country level, subnationally, and at district and facility levels are essential to track progress and to support decentralized planning (Moller et al. 2018). The aim of the present study is to characterize parturients admitted to Irene Neto Maternity (INM), Lubango city, Huíla province, Angola, and to describe their pregnancy outcomes.

Methods

Study design, setting and participants

An observational, descriptive, cross-sectional facility-based survey was conducted among 500 parturients admitted to INM, from October 2016 to September 2017 (*). INM is the reference

1 provincial maternity in Huíla, the second most populous province of Angola. Sample size was
2 calculated on basis of INM statistics for 2014, as previously described (*). In Huíla, there are
3 another maternity and several health centres, where only vaginal births can take place. In that
4 province, only 30% of births occur in a health facility (INE, MINSA, MINPLAN and ICF 2017).
5 Antenatal care (ANC) of the studied parturients was previously characterized (**). A
6 questionnaire was developed for this study, including maternal characteristics and maternal,
7 obstetric and newborn outcomes (see supplementary material).
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19 *Data collection*

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21 Pregnancy cards were consulted whenever available and parturients were interviewed to collect
22 data on sociodemographic characteristics (age, education, area of residence), alcohol and tobacco
23 use, obstetric and pathological history, consanguinity, and outcomes during current pregnancy.
24 Parturients with 19 or less years of age were considered adolescents, while when 35 years or more
25 were considered as having advanced maternal age (WHO 2020; Lean et al. 2017). Rural or urban
26 residence was determined as previously described (**). Regarding obstetric history, miscarriages,
27 stillbirths and preterm births were not reported due to missing data on gestational age that would
28 make it possible to classify the occurrence of these outcomes.
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39 At admission in labor, parturients' height (Microtoise Heightmeter Rollmessband,
40 Comed, France), blood pressure (Blood Pressure Monitor BU 535, Medisana GmbH,
41 Deutschland), capillary glycemia (MediTouch, Medisana AG, Germany), hemoglobin level
42 (HemoCue® Hb 301 System, HemoCue AB, Sweden) and temperature (Infrared Thermometer
43 TM 750, Medisana AG, Deutschland) were evaluated. To our knowledge, cut-offs for Angolan
44 women height are not defined. We reduced to three, the six cut-offs previously defined in a study
45 that used the most recent Demographic and Health Survey (DHS) for 34 countries from Sub-
46 Saharan Africa (SSA) (Arendt et al. 2018). Hypertension was defined as a systolic blood pressure
47 (BP) \geq 140 mmHg and/or a diastolic BP \geq 90 mmHg (Berhe et al. 2020). According to
48 manufacturer's instructions for interpretation of capillary glycemia of adults that had not fasted,
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1 a value ≥ 140 mg/dL was considered hyperglycemia. Women were considered anemic
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3 (hemoglobin level < 11 g/dl) or non-anemic (≥ 11 g/dl), the first ones being classified as having
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5 mild (10.0-10.9g/dl), moderate (7.0-9.9g/dl) or severe anemia (< 7.0 g/dl), according to the WHO
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7 reference values for pregnant women adjusted for the altitude (WHO 2011a), as Lubango is 1786
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9 meters above sea level. It is important to highlight the absence of information on existing
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11 haemoglobinopathies and that a screening for malaria was not performed at admission in labor.
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13 Fever was defined as a temperature $\geq 38^{\circ}\text{C}$ (Towers et al. 2017).
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17 Data on obstetric and newborn outcomes of each parturient were collected through the
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19 follow-up from admission in labor until the end of delivery, in collaboration with the midwife and
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21 the doctor that were assisting her. As in Angola there is not a norm to classify medical indications
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23 for cesarean section (CS), we decided to use the Portuguese norm for registration of CS
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25 indications (DGS 2015). Regarding newborn data, birth asphyxia (BA) was defined as an Apgar
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27 score < 7 at 5 minutes (Wosenu et al. 2018). Resuscitation included at least one of the following
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29 procedures: oxygen administration, insufflations by self-inflating bag-mask device and chest
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31 compressions. For newborn weight evaluation, a Digital Baby Scale Seca 354 (Seca gmbh & co.
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33 kg, Germany) was used. A birth weight < 2.5 kg and ≥ 4 kg was considered low birth weight
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35 (LBW) and macrosomia, respectively (WHO 2014; ACOG 2020). As the result of an ultrasound
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37 scan at the end of the first trimester of pregnancy, a reliable menstrual history or data on serial
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39 fundal height measurements during ANC was not available for every parturient, the occurrence
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41 of preterm birth (PB) was not possible to be determined.
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48 *Data analysis*

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50 Mean (standard deviation) was computed to characterize quantitative variables, while relative and
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52 absolute frequencies were determined for categorical variables. Analyses were performed with
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54 the Statistical Package for Social Science version 26 (IBM, Armonk, NY, USA). Confidence
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56 intervals were estimated at a level of 95% (Wilson method by EpiTools).
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Ethical considerations

Parturients had to sign an informed consent form to be included in this study, which was approved by the Angola Ethics Committee (reference 35/2017). Whenever they could not read or write, pregnant woman's fingerprint and witness signature were required, after informed consent was read to her. If Portuguese was not understood and/or spoken, a local nurse acted as an interpreter and if younger than 18 years of age, parents/guardians consent was also requested.

Results

The mean (SD) age of the parturients was 26.1 (6.6), with 13 and 48 as minimum and maximum, respectively (table 1). Regarding age groups, 18.3% (90/491) were adolescents and 14.5% (71/491) had advanced maternal age. Illiteracy was reported by 8.2% (41/499) of the women and the majority (86.6%, 433/500) was residing in an urban area. A short stature was observed in 33.6% (108/321). None reported tobacco use and 9.4% (47/499) assumed alcohol use. Almost 74% of the parturients (74.3%, 370/498) had been pregnant between two to 12 times and among women with a previous pregnancy, approximately two in every ten had a previous CS (19.9%, 74/371). Consanguinity was observed in 6.6% (33/498) of the parturients, including half-brother, first cousin, uncle and great uncle. Chronic hypertension (CH) was referred by six women and one reported pregestational diabetes.

Malaria was the most frequent infection during pregnancy (16.3%, 81/498, table 2), followed by urinary tract infection (11.4%, 57/498) and typhoid fever (3.2%, 16/498). Pregnancy-induced hypertension (PIH) was detected in six parturients, while none was diagnosed with gestational diabetes. At admission in labor, 36.0% (178/494) of the pregnant women were hypertensive and 18.1% (90/497) anemic, mostly mild (10.3%, 51/497) and moderate (7.4%, 37/497).

There were 15 twin pregnancies among the 500 parturients (3.0%, table 3). Meconium-stained amniotic fluid was detected in 25.8% (129/500), with a vaginal breech birth in 1.4%. Vaginal/perineal laceration was the most frequent complication of vaginal birth (13.4%, 50/374).

1 CS was performed in 25.2% (126/500) of the women, the most frequent medical indication being
2
3 two or more previous CS (23.0%, 29/126), followed by fetal distress (19.8%, 25/126), abnormal
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5 fetal lie or malpresentation (10.3%, 13/126), multiple pregnancy (9.5%, 12/126) and failed
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7 induction of labor (9.5%, 12/126). According to the norm that we used to classify its medical
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9 indications, in 23.0% (29/126) of the parturients a medical indication was not identified, including
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11 12.7% (16/126) that had one previous CS. Two maternal deaths occurred in our sample. The
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13 suspected causes of death were acute pulmonary edema during surgery and amniotic fluid
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15 embolism after vaginal birth.
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19 Stillbirth occurred in 2.9% (14/485, table 4) of the singleton pregnancies. Among live
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21 births, BA was observed in 22.7 (107/471) and resuscitation was performed in 8.5% (40/471).
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23 One in every ten newborns (10.3%, 50/485) and 4.1% (20/485) had LBW and macrosomia,
24
25 respectively.
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28 29 30 **Discussion**

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32 Maternal and child morbidity and mortality remain high in Angola, with very few recent studies
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34 addressing detailed maternal, obstetric and newborn outcomes in Angolan women (Nimi et al.
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36 2016; Umar and Kabamba 2016; Nimi et al. 2019; Rosário et al. 2019). This evidence should be
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38 used to drive decision making and accountability nationwide, from the lower to the highest level
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40 with respect to health.
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44 In this study, 18.3% of the parturients were adolescents. Low and middle-income
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46 countries host the majority of adolescent births (WHO 2019), with a systematic review and meta-
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48 analysis estimating an adolescent pregnancy prevalence of 19.3% in SSA (Kassa et al. 2018). A
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50 study conducted in 29 countries from Africa, Latin America, Asia and Middle East showed higher
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52 risks of adverse pregnancy outcomes in adolescent mothers, namely eclampsia, puerperal
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54 endometritis, systemic infections, LBW, PB and severe neonatal conditions (Ganchimeg et al.
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56 2014).
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1 Illiteracy was reported by 8.2% of the studied parturients, which could lead to a higher
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3 number of adverse pregnancy outcomes. Education is a structural determinant of health, with
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5 specific interpretations that explain its association with health outcomes: education reflects
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7 material, intellectual and other resources of the family of origin and is also a strong determinant
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9 of future employment and income (WHO 2010). On the other hand, knowledge and skills attained
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11 through education make people more receptive to health education messages or better enable them
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13 to communicate with and to access appropriate health services (WHO 2010). In previous studies
14
15 conducted in Angola, women with lower education were less likely to use maternal health care
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17 (Rosário et al. 2019; Shibre et al. 2020) and their children experienced a higher risk of neonatal
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19 and infant death (Shibre 2020; Yaya 2020).
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23 One in three (33.6%) parturients had a short stature (< 1.55 m). Maternal stature is a
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25 cumulative outcome of environmental exposures from fetal to adult life, involving nutritional,
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27 infectious, sociocultural and economic influences (Christian 2010). In a study that used data of
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29 the most recent DHS for 34 countries from SSA, short stature was associated with an increased
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31 prevalence of CS and neonatal mortality (Arendt et al. 2018).
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34 Malaria was the most frequent infection during pregnancy (16.3%). This is in accordance
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36 with previous studies conducted in Luanda province, Angola, that highlighted the high prevalence
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38 of malaria in pregnant women (Valente et al. 2011; Campos et al. 2012). It is known that maternal
39
40 malaria is associated with adverse birth outcomes, including LBW and PB (Tshotetsi et al. 2019).
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42 A study that took place in a regional maternity in the Kuando Kubango province, Angola, revealed
43
44 that malaria accounted for 14% of all maternal deaths (Umar and Kabamba 2016).
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47 At admission in labor, hypertension was frequent among studied parturients (36.0%), in
48
49 contrast with the reported few cases of CH and PIH, which may indicate an antenatal subdiagnosis
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51 of hypertension. Hypertensive disorders are major cause of severe morbidity, long-term disability
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53 and death among both mothers and their babies, being responsible for nearly one tenth of all
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55 maternal deaths in Africa (WHO 2011b). Additionally, the development of a hypertensive
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57 pregnancy, especially preeclampsia, increases future risks for cardiovascular and renal diseases
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1 (Reddy and Jim 2019). In a study from Ethiopia, women with PIH gave birth to babies with a
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3 higher risk of LBW, BA, small for gestational age (SGA), PB, stillbirth, admission to neonatal
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5 intensive care unit and perinatal death, compared to normotensive pregnant women (Berhe et al.
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7 2020).
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10 There were 15 twin pregnancies among the 500 studied parturients, which may indicate
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12 a very high twinning rate in Lubango. Very high twinning rates of above 18 per 1,000 are found
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14 in most Central-African countries (Smits and Monden 2011). If the high twinning rate found in
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16 this study would prove to be a reflex of pregnant women from Lubango city, it would imply an
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18 additional maternal and newborn health challenge, as the risk of complications and adverse
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20 pregnancy outcomes is higher in women and fetuses of a multiple pregnancy when compared with
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22 a singleton pregnancy (Dodd and Crowther 2005).
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25 CS can effectively prevent maternal and newborn mortality when used for medically
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27 indicated reasons, but is associated with short and long-term risks that affect women and child
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29 health and future pregnancies, beyond substantial costs (WHO 2018). CS was performed in 25.2%
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31 of our sample, a value below the 44.0% reported in a study that took place in Lucrecia Paím
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33 Maternity Hospital, the national reference centre for maternal health care located in Luanda (Nimi
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35 et al. 2019). Both prevalences are overrated, as women giving birth at home were not included,
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37 which is very frequent in Angola. It is known that to establish if CS prevalence in a population is
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39 appropriate, one should focus on the life-threatening causes for the mother and child (Belizán et
40
41 al. 2018). The challenge is to reduce unnecessary CSs and retain those that are needed to save
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43 lives and decrease morbidity (Lundgren et al. 2015). In 23.0% of the studied parturients submitted
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45 to CS, a medical indication for it was not identified, namely the 12.7% that only had one previous
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47 CS. In the 1970s, the following dogma used to be said: “once a cesarean always a cesarean”
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49 (Christmann-Schmid et al. 2016). Nowadays it is recognized that, after one previous CS, pregnant
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51 women can deliver by elective repeat CS or have a labor trial which can end in a vaginal birth or
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53 an unplanned CS (Vankan et al. 2019). Therefore, one pre-existent CS is by itself no reason to be
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55 repeated.
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Table 1 Maternal characteristics

Variable	Value*
Age (years), n=491	26.1 (6.6)
≤ 19	18.3 (90)
20-34	67.2 (330)
≥ 35	14.5 (71)
Formal education (years), n=499	8.0 (4.0)
Illiteracy	8.2 (41)
Area of residence, n=500	
Urban	86.6 (433)
Rural	13.4 (67)
Height (m), n=321	
Short stature (< 1.55 m)	33.6 (108)
Average height (1.55-1.59 m)	31.5 (101)
Tall stature (≥ 1.60 m)	34.9 (112)
Alcohol use, n=499	9.4 (47)
Tobacco use, n=499	0.0 (0)
Gravidity, n=498	
Nulliparous primigravida	25.7 (128)
2 nd - 4 th pregnancy	48.0 (239)
≥ 5 th pregnancy	26.3 (131)
History of ectopic pregnancy, n=370	1.1 (4)
Previous cesarean section (at least once), n=371	19.9 (74)
History of neonatal death, n=359	2.5 (9)
Consanguinity, n=498	6.6 (33)
Chronic hypertension, n=498	1.2 (6)
Pregestational diabetes, n=498	0.2 (1)

*Values are given as mean (SD) or percentage (number)

Table 2 Maternal outcomes

Outcomes	% (n)	95% CI*
During pregnancy, n=498		
Malaria	16.3 (81)	13.3-19.8
Urinary tract infection	11.4 (57)	9.0-14.5
Typhoid fever	3.2 (16)	NA
Pregnancy-induced hypertension	1.2 (6)	NA
Anemia	1.2 (6)	NA
Antepartum hemorrhage	0.6 (3)	NA
Gestational diabetes	0.0 (0)	NA
Hospitalization	3.8 (19)	NA
At admission in labor		
Hypertension, n=494	36.0 (178)	31.9-40.4
Hyperglycemia, n=498	2.2 (11)	NA
Anemia, n=497	18.1 (90)	15.0-21.7
Mild	10.3 (51)	7.9-13.2
Moderate	7.4 (37)	5.5-10.1
Severe	0.4 (2)	NA
Fever, n=498	0.0 (0)	NA

*Confidence intervals were estimated only for relative frequencies ≥ 30

Table 3 Obstetric outcomes

Outcomes	% (n)	95% CI*
Twin pregnancies, n=500	3.0 (15)	NA
Amniotic fluid, n=500		
Meconium-stained	25.0 (125)	21.4-29.0
Foul-smelling	1.0 (5)	NA
Meconium-stained and foul-smelling	0.8 (4)	NA
Vaginal birth complications, n=374		
Vaginal/perineal laceration	13.4 (50)	10.3-17.2
Syncope	1.1 (4)	NA
Intrapartum hemorrhage	1.1 (4)	NA
Fever	0.5 (2)	NA
Retained placenta	0.5 (2)	NA
Premature rupture of membranes	0.5 (2)	NA
Hypotension	0.3 (1)	NA
Cesarean section (CS), n=500	25.2 (126)	21.6-29.2
Medical indication, n=126	77.0 (97)	69.0-83.5
Previous uterine surgery (two or more previous CS)	23.0 (29)	NA
Fetal distress	19.8 (25)	NA
Abnormal fetal lie or malpresentation	10.3 (13)	NA
Multiple pregnancy	9.5 (12)	NA
Failed induction of labor	9.5 (12)	NA
Suspected cephalopelvic disproportion	1.6 (2)	NA
Prolonged labor	1.6 (2)	NA
Maternal disease	0.8 (1)	NA
Maternal disorder related to pregnancy	0.8 (1)	NA
Non-medical indication, n=126	23.0 (29)	NA
One previous CS	12.7 (16)	NA
Other	10.3 (13)	NA
Maternal Death, n=500	0.4 (2)	NA

*Confidence intervals were estimated only for relative frequencies ≥ 30

Table 4 Newborn outcomes (singleton pregnancies)

Outcomes	% (n)	95% CI*
Sex, n=484		
Female	48.1 (233)	43.7-52.6
Male	51.9 (251)	47.4-56.3
Stillbirth, n=485	2.9 (14)	NA
Birth asphyxia, n=471	22.7 (107)	19.2-26.7
Resuscitation, n=471	8.5 (40)	6.3-11.4
Low birth weight, n=485	10.3 (50)	7.9-13.3
Macrosomia, n=485	4.1 (20)	NA
Signs and symptoms, n=485		
Pallor	1.0 (5)	NA
Hepatosplenomegaly	0.6 (3)	NA
Suffusions	0.6 (3)	NA
Jaundice	0.4 (2)	NA
Anasarca	0.2 (1)	NA
Congenital malformations, n=485		
Genital birth defect (underdeveloped male genitals)	0.2 (1)	NA
Cleft lip	0.2 (1)	NA
Clubfoot	0.2 (1)	NA

*Confidence intervals were estimated only for relative frequencies ≥ 30

Questionnaire

SYPHILIS, HIV, HEPATITIS B AND C IN PARTURIENTS FROM IRENE NETO MATERNITY IN LUBANGO, ANGOLA, AND ITS RELATIONSHIP WITH MATERNAL, OBSTETRIC AND NEWBORN COMPLICATIONS

Parturient Identification Number _____ Date ____/____/____

1 - Sociodemographic Characteristics

1.1. Area of residence: Rural ___ Urban ___

1.2. House conditions:

1.2.1. Public electricity: Yes ___ No ___

1.2.2. Tap water: Yes ___ No ___

1.2.3. Latrine or toilet: Yes ___ No ___

1.2.4. Radio: Yes ___ No ___

1.2.5. Television: Yes ___ No ___

1.3. Age _____

1.4. Marital status _____

1.5. Formal education (years) _____

1.6. Job status: Employed ___ Unemployed ___ Student ___ Housewife ___

If employed, profession _____

1.6.1. Partner job status: Employed ___ Unemployed ___ Student ___

If employed, profession _____

1.7. Tobacco use: Yes ___ No ___ If yes, how many cigarettes per day, week or month _____

1.8. Alcohol use: Yes ___ No ___

If yes, which _____

If yes, how many cups per day, week or month _____

1.9. Illicit drug use: Yes ___ No ___ If yes, which _____

2 - Obstetric History

2.1. Number of previous pregnancies _____

2.1.1. Term _____

2.1.2. Preterm _____

2.1.3. Spontaneous miscarriage _____

2.1.4. Induced miscarriage _____

2.1.5. Ectopic pregnancy _____

2.1.6. Stillbirths _____

2.1.7. Live births _____

2.1.7.1. If live births, neonatal deaths _____

2.1.7.2. If live births, children alive _____

2.2. Number of previous births _____

2.2.1. Vaginal delivery _____

2.2.2. Assisted vaginal delivery _____

2.2.3. Caesarean section _____

2.2.4. Previous newborns weight _____

3 - Antenatal Period

3.1. Antenatal care (ANC): Yes ___ No ___

3.1.1. If yes, timing of first ANC visit:

1° Trimester ___ 2° Trimester ___ 3° Trimester ___

3.1.2. If yes, number of ANC visits _____

3.2. HIV screening: Yes ___ No ___

3.2.1. If yes, gestational age and result?

1° Trimester ___ Result _____

2° Trimester___ Result_____

3° Trimester___ Result_____

3.2.2. If positive, ART: Yes___ No___ If yes, which_____

3.3. HBV screening: Yes___ No___

3.3.1. If yes, gestational age and result?

1° Trimester___ Result_____

2° Trimester___ Result_____

3° Trimester___ Result_____

3.3.2. If positive, antiviral therapy: Yes___ No___ If yes, which_____

3.4. Syphilis screening: Yes___ No___

3.4.1. If yes, gestational age and result?

1° Trimester___ Result_____

2° Trimester___ Result_____

3° Trimester___ Result_____

3.4.2. If positive, penicillin G: Yes___ No___

3.4.2.1. If yes, how many doses?_____

3.4.2.2. If yes, gestational age? 1° Trimester___ 2° Trimester___ 3° Trimester___

3.4.2.3. If yes, partner treatment: Yes___ No___

3.5. Pathologies during pregnancy: Yes___ No___ If yes, which:

Arterial hypertension___ Preeclampsia___ Eclampsia___ Gestational diabetes___

Trauma_____ Infectious diseases_____

Other(s)_____

3.6. Hospitalization during pregnancy: Yes___ No___

3.6.1. If yes, diagnosis:_____

3.6.2. If yes, duration:_____

4 - Clinical Data at Admission in Labour

4.1. Consanguinity: Yes___ No___ If yes, degree_____

4.2. Health problems: Yes___ No___ If yes, which_____

4.3. Infections in the last year: Yes ___ No ___

4.3.1. If yes, which and when _____

4.4. Weight _____ Height _____ Temperature _____ Blood Pressure _____

Capillary Glycemia _____ Haemoglobin Level _____

5 - STI Screening

5.1. Syphilis

Rapid test : Reactive ___ Non-reactive ___

RPR: Reactive ___ Non-reactive ___ Not applicable (NA) ___

RPR titer: _____ NA ___

5.2. Hepatitis B

Rapid test: Reactive ___ Non-reactive ___

5.3. Hepatitis C

Rapid test: Reactive ___ Non-reactive ___

5.4. HIV

First-line rapid test: Reactive ___ Non-reactive ___

Second-line rapid test: Reactive ___ Non-reactive ___ NA ___

Repeat first-line rapid test: Reactive ___ Non-reactive ___ NA ___

Repeat second-line rapid test: Reactive ___ Non-reactive ___ NA ___

Third-line rapid test: Reactive ___ Non-reactive ___ NA ___

6 - Labour and Delivery

6.1. Rupture of membranes: Date _____ Time _____

6.2. Amniotic fluid: Clear ___ Meconium-stained ___ Foul-smell ___

6.3. Birth: Date _____ Time _____

6.4. Type of delivery: Vaginal ___ Assisted vaginal delivery ___ Caesarean section ___

6.5. Type of pregnancy: Singleton ___ Twin ___

6.6. Fetal presentation/lie _____

6.7. Antibiotic therapy: Yes ___ No ___ If yes, which _____

6.8. Other(s) intrapartum therapy: Yes ___ No ___ If yes, which _____

6.9. Labour complications: Yes ___ No ___ If yes, which:

Hemorrhage ___ Fever ___ Uterine hypertonia ___ Seizures ___ Uterine rupture ___

Other(s) _____

7 - Clinical Evaluation of the Newborn

7.1. Apgar score: 1 minute ___ 5 minutes ___

7.2. Resuscitation: Yes ___ No ___ If yes, procedures _____

7.3. Weight _____

7.4. Length _____

7.5. Cephalic perimeter _____

7.6. Signs and symptoms:

Anasarca: Yes ___ No ___

Palmoplantar blisters: Yes ___ No ___

Splenomegaly: Yes ___ No ___

Hepatomegaly: Yes ___ No ___

Jaundice: Yes ___ No ___

Congenital malformations: Yes ___ No ___ If yes, which _____

Pallor: Yes ___ No ___

Petechiae: Yes ___ Não ___ If yes, location _____

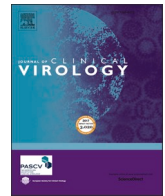
Suffusions: Yes ___ No ___ If yes, location _____

Other(s) _____

7.7. Therapy/Vaccines _____

2.5. Paper V: A systematic review of the maternal and neonatal complications in hepatitis B infection

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A systematic review of the maternal and neonatal complications in hepatitis B infection

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ABSTRACT

The association between hepatitis B virus (HBV) infection and maternal, obstetric and newborn outcomes remains controversial, as previous studies have reported conflicting and inconsistent results on the matter. The aim was to investigate whether HBV infection increases the risk of maternal, obstetric and newborn complications. We conducted a systematic literature review, according to PRISMA statement guidelines. Studies were eligible for inclusion if they were observational cohort, case-control or cross-sectional studies, comparing maternal, obstetric or newborn complications in HBV-infected and uninfected pregnant women. PubMed was searched for published literature in English, with no date restrictions, using combinations of keywords. The titles and abstracts were independently screened for eligibility by three authors. Two authors assessed the quality of each included study and no meta-analysis was performed. We retrieved 275 records and included 15 papers. The methodological and statistical heterogeneity as well as a great variation on the types of maternal, obstetric and newborn complications studied did not allow quantitative analysis of results and conclusions about the level of evidence. Seven studies are of good quality, which makes their results more reliable. Three of them revealed that maternal HBV infection increased the risk of miscarriage, preterm birth, pregnancy-induced hypertension, fetal distress and macrosomia. These three studies were performed in China and the one with the largest number of participants only included women from rural areas. Larger, more robust, well-designed prospective cohort studies are needed. These must include adjusted estimates for confounding factors, such as other possible complications determinants, like the antenatal care quality.

1. Introduction

Globally, it is estimated that 257 million people are chronically infected with hepatitis B virus (HBV) [1]. The associated burden of death is 887000 per year [1] and 30 million people will become newly infected each year [2]. In 20–30 % of adults who are chronically infected, the disease evolves to cirrhosis and/or liver cancer [1], the major cause of chronic infection being perinatal transmission [3].

For pregnant women with HBV infection, several particular aspects need to be considered, including the effect of HBV infection on pregnancy and vice versa, the potential viral transmission from mother to newborn, its possible prevention and the potential teratogenic effect of antiviral drugs [4]. Society for Maternal-Fetal Medicine states that “data are insufficient to suggest that acute or chronic HBV infection is

associated with adverse pregnancy outcomes” [5].

The aim of this study was to investigate whether HBV infection increases the risk of maternal, obstetric and newborn complications, by using a systematic approach to review the literature on observational studies (cohort, case-control and cross-sectional), comparing the risk of complications in HBV-infected and uninfected women. Based on PICOS format, we intended to answer to the research question: what is the evidence from cohort, case-control and cross-sectional observational studies (S) on the risk of maternal, obstetric and newborn complications (O) in pregnant women (P) infected with HBV when compared to uninfected women (C)? Our research question did not include interventions, as we did not include experimental studies.

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2. Materials and methods

We conducted a systematic literature review, according to PRISMA statement guidelines [6] on observational studies (cohort, case-control and cross-sectional) on maternal, obstetric and newborn complications associated with HBV infection. The protocol for the systematic review was not registered.

3. Eligibility criteria

Our objective was to explore the possible maternal, obstetric and newborn complications associated with infection by HBV. Studies were eligible for inclusion in the systematic review if they were either cohort, case-control or cross-sectional studies, comparing maternal, obstetric or newborn complications in HBV-infected and HBV-uninfected pregnant women. Articles not written in English, which did not have an available abstract or did not include humans, were excluded. No time limits were defined besides those of the database where the search was conducted in order to allow the inclusion of as many articles as possible.

4. Systematic search strategy

A single author (DO) searched PubMed for published literature in English, with no date restrictions, to identify studies assessing maternal, obstetric and newborn complications in HBV-infected and uninfected women. The search was conducted in January 24th 2020 using the following combinations of terms: hepatitis B AND pregnancy outcomes, hepatitis B AND pregnancy complications, hepatitis B AND perinatal outcomes, hepatitis B AND perinatal complications, hepatitis B AND maternal outcomes, hepatitis B AND maternal complications, hepatitis B AND obstetric outcomes, hepatitis B AND obstetric complications, hepatitis B AND newborn outcomes, hepatitis B AND newborn complications, hepatitis B AND pregnancy. PubMed database was chosen since it is a free and widely used international recognized resource of peer-

reviewed biomedical and life sciences literature. The titles and abstracts of all retrieved documents were independently screened for eligibility by three authors (DO, FP, IF). Disagreements were solved through reanalysis and consensus.

5. Data extraction and management

Data on authors; year of data collection; geographical location (country); type of study; participants; sampling methodology; diagnostic test used; outcomes; and main results from the comparison between HBV-infected and uninfected pregnant women were extracted from included studies by a single author (DO). We considered the complications terminology used in the original articles. Results were summarized in a table. The included studies were classified as of good, fair or poor quality by two authors (DO and IF or DO and MRM), using the Study Quality Assessment Tools from the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health & Human Services [7]. Disagreements were solved through reanalysis and consensus. No meta-analysis was performed due to methodologic and statistic heterogeneity of the included studies.

6. Results

We retrieved 275 records from PubMed. After screening and applying the eligibility criteria, a total of 15 papers [8–22] were included in our review (Fig. 1). Among the 101 papers that were excluded because were not observational cohort, case-control or cross-sectional studies, 12 were systematic reviews and meta-analyses, 6 systematic reviews, 41 literature reviews, 19 pharmacological interventional studies, 13 case reports, 5 cost-effectiveness studies, 4 organizational/scientific guidelines and 1 an erratum. Data extracted from the included articles are presented in Table 1. A table with sampling methodology and diagnostic tests used, as well as three tables with detailed final consensual quality assessment by study type follow as

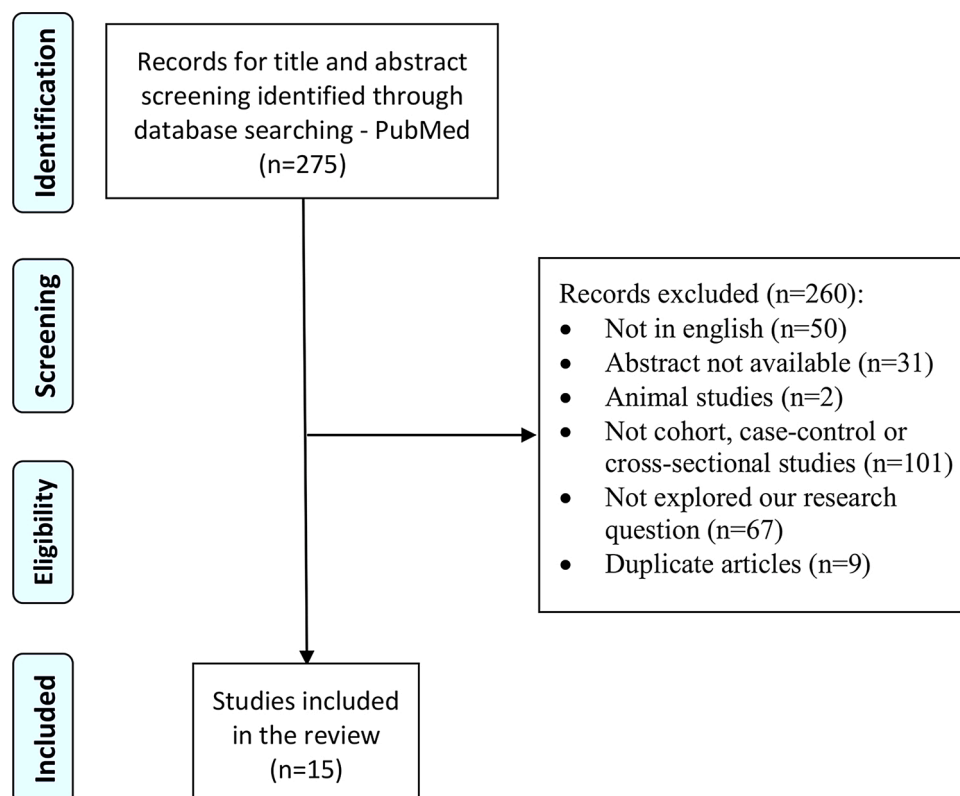


Fig. 1. PRISMA flow diagram.

Table 1

Methodological characteristics, quality, outcomes and main results of the studies included in the review (*type of study not specified by authors, classification by the review authors).

Authors	Year of data collection	Country	Type of study	Study quality	Participants: HBV infected vs uninfected pregnant women	Outcomes	Main results
Pastorek II et al [8]	1983–1985	United States	Case-control study* n = 120	Fair	60 vs 60: same race, ages within 3 years and delivery in the same calendar month for cases and controls	Pregnancy weight gain, hemoglobin at delivery, gestational age (GA) at delivery, birth weight (BW), Apgar score (1 and 5 min), toxemia, maternal morbidity and perinatal morbidity	By bivariate analysis, no statistically significant differences were noted between the two groups [t test, χ^2 and Fisher exact test]
Wong et al [9]	1996–1998	China (Hong Kong)	Cross-sectional study* n = 7,105	Fair	824 vs 6,281: delivery \geq 24 weeks of gestation and babies' birth weight \geq 500 g (inclusion criteria). Excluded women with acute hepatitis, twin pregnancy, acute pelvic inflammatory disease, active sexually transmitted disease or syphilis infection complicating pregnancy, and smoking or illicit drug users women (exclusion criteria).	Antepartum hemorrhage (APH) of unknown origin, placenta previa (PP), placenta abruption, meconium and blood-stained liquor, gestational hypertension (GH), proteinuria, preeclampsia (PE), eclampsia, anemia, iron deficiency, gestational diabetes (GD), prelabor rupture of membranes (PROM) required induction of labor, preterm PROM (PPROM), maternal death (MD), GA, BW, preterm delivery (PD), small for gestational age (SGA), fetal distress (FD), asphyxia, neonatal jaundice (NJ), congenital anomaly (CA), gastrointestinal tract anomaly, stillbirth, neonatal death (ND) and perinatal death	By bivariate analysis, no statistically significant differences were noted between the two groups [t test, Mann-Whitney test, χ^2 and Fisher exact test]
Connell et al [10]	1998–2007	United States	Population-based retrospective cohort study n = 1,670,369	Good	1,458 vs 1,668,911: singleton, live viable births \geq 20 weeks of gestation (inclusion criteria). Excluded women who were not of NH-black, NH-white, Hispanic or Asian race/ethnicity (exclusion criteria). Cases of alcohol abuse and HIV carriers were excluded from statistical modelling.	Premature rupture of membrane, placental abruption (PA), PP, GD, GH, preeclampsia, eclampsia, anaemia, low birth weight (LBW), SGA, preterm birth (PB), NJ, FD and CA	Bivariate analysis showed that women with HBV infection had higher rates of premature rupture of membrane (1.78 vs 1.10 %, $p = 0.01$), GD (7.20 vs 4.42 %, $p < 0.0001$) and anaemia (10.43 vs 5.93 %, $p < 0.0001$). Regarding neonatal outcomes, compared with uninfected women, women with HBV infection also had higher frequencies of infants born LBW (8.64 vs 6.37 %, $p < 0.0001$) and preterm (10.78 vs 8.84 %, $p < 0.0001$). [χ^2 test] Adjusting for sociodemographic variables (maternal age, marital status, educational level, maternal race/ethnicity), parity, obstetric complications, tobacco use and drug abuse during pregnancy, a slightly decreased risk of SGA among mothers who were HBV carriers was found (aOR = 0.79; 95 %CI = 0.66–0.95). [multivariable logistic regression, OR and 95 %CI]
Lobstein et al [11]	2001–2006	Germany	Cross-sectional study* n = 8,193	Fair	39 vs 8,154: only singleton pregnancy (inclusion criteria)	Anemia, fetal death syndrome, oligohydramnios, polyhydramnios, pathological cardiocogram, PP, PA, APH of unknown origin, PE, eclampsia, HELLP syndrome, threatened preterm labor, intrauterine growth restriction, PROM/premature PROM, GD, secondary cesarean section, green amniotic fluid, tocolysis, eclolysis, duration of delivery, impaired separation of placenta, aggravated	By bivariate analysis, no statistically significant differences were noted between the two groups [t test, Mann-Whitney test, χ^2 test and a Bonferroni correction]

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Table 1 (continued)

Authors	Year of data collection	Country	Type of study	Study quality	Participants: HBV infected vs uninfected pregnant women	Outcomes	Main results
						intrapart bleeding, blood loss, hysterectomy, laparotomy, uterine rupture, sepsis, fever, impaired wound healing, lie abnormality, synclitism abnormality, posture abnormality, shoulder dystocia, umbilical cord abnormality, BW, birth length, head circumference, PB, prolonged gestation, stillbirth, Apgar score (1, 5 and 10 min), pH value of umbilical cord, base excess, FD, reanimation, congenital abnormality and macrosomia	
Hu et al [12]	2011–2013	China	Prospective case-control study* n = 100	Poor	50 vs 50: no statistically significant differences in weight, age and gestational age for cases and controls	Premature rupture of membranes, meconium-stained amniotic fluid (MSAF), FD, neonatal asphyxia (NA) and birth defects	Bivariate analysis showed that, when compared to the healthy pregnancy group, increased rates of premature rupture of membranes/MSAF (22.0 vs 6.0 %, $p < 0.05$) and FD (14.0 vs 6.0 %, $p < 0.05$) were observed in cases of HBV infection [χ^2 and Fisher exact test]
Sirilert et al [13]	2002–2012	Thailand	Retrospective cohort study n = 23,803	Fair	1,472 vs 22,331. From HBsAg positive and, after excluding 26 aborted pregnancies, 301 tested positive for HBeAg, 474 negative, 671 not screened. Singleton pregnancy; absence of medical or surgical disease such as liver disease, pregestational diabetes mellitus, chronic hypertension, or heart disease (inclusion criteria). Excluded smoking or drug users women (exclusion criteria).	With respect to HBsAg status: abortion, pre-eclampsia, GD, PP, cesarean delivery due to FD, GA, PB, macrosomia, LBW, SGA, Apgar score <7 (1 and 5 min) and stillbirth. With respect to HBeAg status: pre-eclampsia, GD, PP, GA, PB, macrosomia, LBW, SGA, Apgar score <7 (1 and 5 min) and stillbirth.	Bivariate analysis showed that the proportion of PB was slightly higher among pregnant women with positive HBsAg status compared with women with negative HBsAg (11.8 vs 10.0 %, $p = 0.026$, RR = 1.013). The proportion of GD (17.8 vs 9.2 %, $p = 0.022$, RR = 1.434), PB (13.6 vs 8.6 %, $p = 0.028$, RR = 1.250) and LBW (21.9 vs 14.1 %, $p = 0.005$, RR = 1.258) was higher among women with positive HBsAg and HBeAg status compared with women with positive HBsAg but negative HBeAg.
Ye et al [14]	2007–2008	China	Cohort study* n = 64	Poor	64 couples who received assisted reproductive technology treatment: in 14 only the wife was chronically infected with HBV (subgroup A1); 50 with negative serum HBV markers for both husband and wife (group B). Couples were tested to exclude hepatitis A, C, D and HIV infection.	Early abortion (EA) and neonatal malformation	[t test, χ^2 test, RR and 95 %CI] In bivariate analysis the EA rate of subgroup A1 was significantly higher than that of group B (60.0 vs 9.1 %, $p = 0.030$) [Fisher exact test]
Chen et al [15]	2002–2010	China	Case-control study* n = 808	Good	380 vs 428: singleton pregnancies > 28 weeks' gestation (inclusion criteria). Excluded cases of spontaneous abortion and iatrogenic preterm (exclusion criteria). Every women had no HCV or HIV co-infection, did not receive anti-HBV therapy. Hepatitis exacerbation or liver decompensation were not reported.	GA, birth height, Apgar score (1 and 5 min), LBW, macrosomia, PB, stillbirth, ND and congenital malformations	By bivariate analysis, no statistically significant differences were noted between the two groups [t test, χ^2 and Fisher exact test] Adjusting for maternal age, parity, educational level and history of abnormal pregnancies, maternal HBsAg positivity did not add risk for complications [multivariable logistic regression, OR and 95 %CI]
Cui et al [16]	2012–2015	China	Hospital-based cohort study n = 21,004	Good	513 asymptomatic chronic HBV carriers (HBsAg positivity > 6 months) vs 20,491. Normal alanine transaminase at study entry; no HCV, HIV or active syphilis infection; no IgM antibodies against toxoplasma, rubella virus,	All pregnancies: completed weeks' gestation, PB, stillbirth, miscarriage, PE, GD, intrahepatic cholestasis of pregnancy, PP and preterm premature rupture of the membrane. Singleton pregnancies: PB,	By bivariate analysis, for all pregnancies, the proportion of miscarriage was higher among the HBV carriers than the controls (9.36 vs 5.70 %, $p < 0.001$). For singleton pregnancies, the proportion of miscarriage was also higher

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Table 1 (continued)

Authors	Year of data collection	Country	Type of study	Study quality	Participants: HBV infected vs uninfected pregnant women	Outcomes	Main results
					cytomegalovirus and herpes simplex virus (inclusion criteria). Excluded other liver diseases (alcoholic, nonalcoholic fatty or autoimmune), preexisting chronic diseases (diabetes mellitus, hypertension or heart diseases), and HBV carriers if they had received antiviral treatment within the previous year (exclusion criteria).	stillbirth, miscarriage, weight of neonates, LBW, SGA and Apgar at 5 min.	among the HBV carriers than the controls (9.66 vs 5.81 %, $p < 0.001$). [χ^2 and Fisher exact test] Adjusted estimates for maternal age, nulliparity, educational level, pre-maternal BMI, previous abortion and in vitro fertilization, showed that HBV carrier status is a factor independently associated with miscarriage (aOR = 1.71; 95 % CI = 1.23–2.38) [multivariable logistic regression, OR and 95 % CI]
Liu et al [17]	2010–2012	China	Population-based retrospective cohort study n = 489,965	Good	Rural women aged 21–49 years with singleton livebirths (inclusion criteria), divided into three groups according to their pre-pregnancy status regarding HBsAg/HBeAg: 469,138 uninfected with HBV (control group), 14,979 HBsAg positive and HBeAg negative (exposure group 1), and 5,848 both HBsAg and HBeAg positive (exposure group 2). Excluded chronic diseases (hypertension, diabetes, chronic nephritis, thyroid disease, heart disease, anaemia, cancer), genital tract infectious disease, abortion, miscarriage or stillbirth, missing data on gestational weeks and post-term pregnancies (exclusion criteria).	PB and early PB	Adjusting for sociodemographic characteristics of women (age, educational level, ethnic origin, occupation, region), history of pregnancy, history of adverse pregnancy outcomes, pre-pregnancy BMI, pre-pregnancy elevated alanine aminotransferase, smoking status of husbands during pregnancy, smoking and alcohol drinking status of women during pregnancy, compared with women from control group, women from exposure group 1 had a 26 % ($p < 0.0001$, aRR = 1.26) higher risk of PB and a 18 % ($p = 0.0095$, aRR = 1.18) higher risk of early PB. Compared with women from control group, women from exposure group 2 had a 20 % ($p = 0.0006$, aRR = 1.20) higher risk of PB and a 34 % ($p = 0.0025$, aRR = 1.34) higher risk of early PB. [multivariable log-binomial regression model, RR and 95 % CI]
Wan et al [18]	2008–2015	China	Hospital-based case-control study n = 3,225	Good	1,728 vs 1,497: excluded multiple births and infection with HCV and/or HIV (exclusion criteria). From HBsAg-positive 1078 women with any HBV DNA level included and women with HBV DNA negative selected as the reference group.	Premature rupture of membrane, pregnancy-induced hypertension (PIH), FD, PB, LBW, macrosomia, SGA and congenital abnormality	Adjusted estimates for maternal age, employment, parity and history of abortion, showed that: - maternal HBsAg carriage was associated with increased risk of PIH ($p = 0.001$, aOR = 2.20), FD ($p = 0.015$, aOR = 1.40) and macrosomia ($p = 0.014$, aOR = 1.68) - maternal HBV DNA positivity during pregnancy was associated with increased risk of PB ($p = 0.011$, aOR = 2.10) [multivariable logistic regression, OR and 95 % CI]
Bajema et al [19]	1992–2014	United States	Population-based retrospective cohort study n = 26,801	Good	4,391 vs 22,410: matched by birth year in a 1:5 ratio. Singleton pregnancies (inclusion criteria).	GD, pre-eclampsia, eclampsia, PP, PD, LBW, SGA and large for gestational age (LGA)	Adjusted estimates for maternal age, race, country of birth, pre-pregnancy BMI, tobacco consumption and hepatitis C (that altered the effect by 10% or more), showed that HBV-infected pregnant women did not experience a higher risk of adverse outcomes. Women with HBV had a lower risk of delivering SGA infants (aRR = 0.79; 95 % CI = 0.67–0.93) [RR and 95 % CI]
	2012–2016			Fair	Refugee and migrant women with a singleton fetus who	Anemia, PIH, PE/eclampsia, diabetes in pregnancy, MD,	By bivariate analysis, no statistically significant

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Table 1 (continued)

Authors	Year of data collection	Country	Type of study	Study quality	Participants: HBV infected vs uninfected pregnant women	Outcomes	Main results
Bierhoff et al [20]		Myanmar-Thailand border	Retrospective cohort study n = 11,025		screened negative for HIV and syphilis (inclusion criteria): 476 HBsAg+/HBeAg- vs 211 HBsAg+/HBeAg+ vs 10,338 HBsAg-/HBeAg-	miscarriage, GA, PB, stillbirth, congenital abnormality, birthweight, SGA, LGA, delivery by caesarean section (CS) and ND	differences were noted between the three groups [t test, Mann-Whitney test, χ^2 test, Bonferroni correction, OR and CI95%]
Wang et al [21]	2010–2018	China	Retrospective cohort study n = 8,459	Good	Women undergoing their first IVF treatments: 714 HBsAg+/HBeAg- vs 180 HBsAg+/HBeAg+ vs 7,565 HBsAg-. Excluded other viral infection (HCV, HIV), syphilis, age above 38 years, treatment with antagonist protocols, chromosomal abnormality, intrauterine death, medical abortion, stillbirth and ectopic pregnancy (exclusion criteria).	Miscarriage (early and late), PD, delivery by cesarean, GD and PIH	By bivariate analysis, no statistically significant differences were noted between the three groups [χ^2 and Fisher exact test] Adjusted estimates for age, duration of infertility, BMI, total dose of gonadotropin used, serum estradiol (E2) level, endometrial thickness on the day of ovulatory dose of hCG, number of fertilization, number of embryos transferred, embryo type and quality, showed that HBV serostatus was not associated with miscarriage [multivariable logistic regression, OR and 95 %CI]
Xiao et al [22]	2016–2018	China	Case-control study* n = 120	Poor	Lying-in women for the first time of pregnancy and single pregnancy (inclusion criteria): 60 vs 60. Excluded other general fundamental diseases, like cardiac or pulmonary diseases, and hepatitis, caused by infection of other viruses or drug-induced (exclusion criteria).	Pregnancy weeks, premature rupture of fetal membrane, premature delivery, postpartum hemorrhage (PPH), pregnancy hypertension (PH), CS, FD, ND, NA and neonatal deformity	In bivariate analysis, incidence rates of premature rupture of fetal membrane (18.3 vs 6.7 %, $p = 0.001$), premature delivery (11.7 vs 1.7 %, $p < 0.001$), PPH (20.0 vs 8.3 %, $p < 0.001$), PH (18.3 vs 6.7 %, $p = 0.001$), FD (8.3 vs 1.7 %, $p < 0.001$) and NA (11.7 vs 1.7 %, $p < 0.001$) were higher among the HBV carriers than the controls (application of χ^2 test is questionable in some cases) [t test and χ^2 test]

supplementary data.

The included studies were conducted between 1983 and 2018. The majority of them were from China (9), followed by the United States (3), Germany (1), Thailand (1) and Myanmar-Thailand border (1). In seven of the 15 studies, the authors did not specify the type of study. The remaining were seven cohort studies and one a case-control study. The methodological and statistical heterogeneity as well as a great variation on the types of maternal, obstetric and newborn complications studied in the included studies did not allow quantitative analysis of results and conclusions about the level of evidence. Overall, more than 60 maternal, obstetric and newborn complications were assessed in the included studies. Regarding quality of the 15 included studies, seven were classified as of good quality [10,15–19,21], five of fair quality [8,9,11,13, 20] and three of poor quality [12,14,22]. Four studies with good quality did not found a significant association between HBV maternal infection and assessed complications [10,15,19,21]. On the other hand, three good quality studies revealed that maternal HBV infection increased the risk of miscarriage, preterm birth, early preterm birth, pregnancy-induced hypertension, fetal distress and macrosomia [16–18].

7. Discussion

During pregnancy, the diagnosis, management and treatment of HBV infection are essential to prevent perinatal transmission, as well as to reduce the risk of maternal and neonatal complications [10]. The causes of maternal, obstetric and newborn complications are complex and multifactorial. Studies exploring the association with HBV infection have reported conflicting and inconsistent results. Therefore, in this systematic review we investigated whether HBV infection increases the

risk of maternal, obstetric and newborn complications, comparing the risk of these complications in HBV-infected and uninfected women.

The majority of studies included in this review were from China. This fact can be probably explained by the high-HBV prevalence (> 8 %) in the Asia Pacific region [23]. Sub-Saharan Africa is another region with high-HBV prevalence [23], but research studies are scarce and were not found when publications were searched for this review, which can be explained by language restrictions during the search or even by low publication ratio in this region.

It should be noted that there are at least ten HBV genotypes (A–J) with distinct geographical distributions [24]. Genotypes influence the dominant mode of transmission, the natural history of infection, the tendency for chronicity, the clinical outcomes and disease progression [24,25]. As so, and given the predominance of studies conducted in the Asia Pacific region included in this systematic review, results should be interpreted bearing this in mind.

Six of the included studies explored HBV maternal infection only through HBsAg (hepatitis B surface antigen) presence. The use of other markers, namely HBeAg (hepatitis B e antigen) and HBV DNA load, could add further evidence on the relationship between maternal HBV infection and maternal, obstetric and newborn complications. It is well-known that the presence of HBsAg indicates infection, HBeAg is an index of viral replication, infectivity, inflammation, disease severity and response to antiviral therapy and HBV DNA is useful to determine the level of HBV virus particles [25,26]. In one included study, identification of HBeAg imposed a higher risk of early preterm birth than of the HBsAg alone (34 vs 18 % compared with control group) [17]. In another study, detection of HBV DNA was associated with a significantly increased risk of preterm birth [18].

The 15 studies included in this review are heterogeneous in respect

to the maternal, obstetric and newborn complications that were assessed. Additionally, other methodologic and statistical aspects differ, making it difficult to compare them. However, seven studies are of good quality, which makes their results more reliable. Four of them (three retrospective cohort studies and one case-control study) did not find a significant association between HBV maternal infection and complications [10,15,19,21]. The other three studies (one hospital-based prospective cohort study, one population-based retrospective cohort study and one hospital-based case-control study) revealed that maternal HBV infection increased the risk of miscarriage, preterm birth, early preterm birth, pregnancy-induced hypertension, fetal distress and macrosomia [16–18]. It should be pointed out that these three studies were performed in China and the one with the largest number of participants [17] only included women from rural areas. Evidence from studies conducted in Asia and Africa, comparing urban to rural areas regarding antenatal care (ANC), showed worse indicators for women from rural areas [27,28]. Furthermore, it is well-known that the quality of ANC is a major factor in the occurrence of maternal, obstetric and newborn complications [29–36]. The studies included in this review did not adjust their estimates for the quality of ANC.

The complications found to be associated with HBV infection in this systematic review (miscarriage, preterm birth, early preterm birth, pregnancy-induced hypertension, fetal distress and macrosomia) are known to cause maternal, fetal and neonatal morbidity and mortality worldwide. Early and late miscarriage are attributed to reproductive tract infections in 15 % and 66 % of cases, respectively [37]. With its multifactorial etiology, preterm birth is the major cause of neonatal mortality and morbidity worldwide, totalizing 13 million births annually [38]. Further research is needed on the mechanisms of spontaneous preterm birth [39], namely the contribution of HBV infection, so that it can be better predicted and prevented. Hypertension can complicate up to 10 % of pregnancies and it is an important risk factor for perinatal mortality of both mothers and fetuses, increasing the risk of placental abruption, cerebral and cardiac incidents, multiorgan failure and disseminated intravascular coagulation [40,41]. Studies are needed on the pathophysiological mechanisms that may explain the association between HBV infection and hypertension. Fetal distress is a topic not widely and deeply investigated worldwide, having limited epidemiological data and great potential for misclassification [42]. These aspects should be taken into consideration when analysing studies results regarding association with HBV infection. Macrosomia is associated with serious maternal and neonatal adverse outcomes, namely emergency cesarean section, postpartum hemorrhage, obstetric anal sphincter injury, shoulder dystocia, obstetric brachial plexus injury and birth fractures [43]. The possible pathophysiological mechanisms involved in the association between HBV infection and macrosomia needs to be investigated.

We searched for systematic reviews that specifically investigated whether HBV infection increases the risk for one of the following: miscarriage, preterm birth, pregnancy-induced hypertension, fetal distress and macrosomia. We found two that revealed an increased risk of preterm birth in pregnant women with chronic hepatitis B infection [44,45].

Limitations of this systematic review must be acknowledged. Publications were searched only in one database and articles not written in English were excluded. We acknowledge that the use of more specific search terms (example miscarriage, fetal distress, etc), instead of general ones, such as maternal, obstetric and newborn complications, could have allowed the inclusion of more studies.

In conclusion, our research question is still without a clear answer as the studies included in this review present discrepant results about the association between HBV infection and maternal, obstetric and newborn complications. These are probably related to different characteristics of the study populations, regional differences, methodologic and statistical heterogeneity. However, three studies of good quality demonstrated that HBV infection can cause poor maternal, obstetric and newborn

outcomes. Healthcare providers and policy makers should not neglect the risk of these complications. In the future, larger, more robust, well-designed prospective cohort studies, specifically addressing the complications identified as being associated with HBV in this systematic review (miscarriage, preterm birth, pregnancy-induced hypertension, fetal distress and macrosomia), are needed, regardless of other complications also worthy of research. These must include adjusted estimates for confounding factors, such as other possible complications determinants, like the ANC quality.

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CRediT authorship contribution statement

Dinamene Oliveira: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Project administration. **Filomena Pereira:** Methodology, Validation, Formal analysis, Writing - review & editing, Supervision. **Maria do Rosário Martins:** Validation, Formal analysis, Writing - review & editing. **Rita Castro:** Writing - review & editing. **Lemuel Cordeiro:** Writing - review & editing. **Inês Fronteira:** Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors have no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jcv.2020.104680>.

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2.6. Paper VI: Maternal infection with hepatitis B virus and pregnancy outcomes in a maternity of Angola: A cross-sectional study

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Abstract

Hepatitis B virus (HBV) infection continue to be a major public health burden, especially in Asia Pacific and Sub-Saharan Africa (SSA) regions. There is conflicting and inconsistent evidence about the impact of this infection on pregnancy outcomes with a scarcity of studies from SSA. Our aim was to investigate whether HBV infection increases the risk of hypertension, anemia, meconium-stained amniotic fluid (MSAF), cesarean section (CS), birth asphyxia (BA) and low birth weight (LBW) in parturients admitted to Irene Neto Maternity (INM), Lubango city, Huíla province, Angola. An observational, cross-sectional and analytical facility-based survey was conducted among 500 parturients at INM, from October 2016 to September 2017. A multivariable logistic regression model was performed to analyze the association with HBV infection for each of the six studied maternal, obstetric and newborn outcomes. Mothers with hepatitis B did not have a higher chance of hypertension, anemia, MSAF, CS, BA and LBW when compared with the uninfected ones. HBV-infected women had a lower chance of MSAF (16.3% vs 26.7%; aOR, 0.336; 95% CI, 0.126-0.898). The issue of the association between hepatitis B and adverse pregnancy outcomes remains unclear, especially in SSA. Due to the evident limitations of the present study, larger, more robust, well-designed prospective cohort studies are needed, which should be based on a better characterization of the maternal HBV infection and taking into account all possible confounding factors.

Keywords: HBV; Pregnancy outcomes; Angola

Significance Statement

Association between hepatitis B and adverse pregnancy outcomes remains unclear. There is a scarcity of studies from Sub-Saharan Africa. An observational, cross-sectional and analytical facility-based survey was conducted among parturients admitted to Irene Neto Maternity, Lubango city, Huíla province, Angola. A higher chance of hypertension, anemia, meconium-stained amniotic fluid, cesarean section, birth asphyxia and low birth weight was not found.

INTRODUCTION

Viral hepatitis caused 1.34 million deaths in 2015, overtaking those attributed to human immunodeficiency virus, with 1.06 million, or malaria, with 0.44 million.¹ Despite the existence of an effective and safe vaccine, hepatitis B virus (HBV) infection remains a major public health burden,² being the most common chronic viral infection worldwide.³ It is estimated that 2 billion people (1 out of each 3) have been infected with HBV and 30 million will become newly infected each year.⁴ Chronic HBV infection is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma.⁵ The global prevalence of HBsAg (hepatitis B surface antigen) in 2016 was estimated to be 3.9% (95% confidence interval [CI], 3.4-4.6).⁶ Asia Pacific and Sub-Saharan Africa (SSA) are regions of high-HBV prevalence (> 8%).⁷ A recent published study conducted in Southern Angola found a HBsAg seropositivity rate of 8.6% in pregnant women.⁸

For pregnant women infected with HBV, several particular aspects need to be considered, as the effect of HBV infection on pregnancy and vice versa.⁹ The impact of infection by HBV on pregnancy outcomes remains uncertain.¹⁰⁻¹³ Studies have reported conflicting and inconsistent results on the matter.¹⁴ According to the Society for Maternal-Fetal Medicine, there is insufficient evidence to conclude that HBV infection (acute or chronic) is associated with adverse pregnancy outcomes.¹⁵ However, results from systematic reviews and meta-analyses are a good help and should be the starting point for clarifying this issue. Two systematic reviews and meta-analyses found an increased risk of preterm birth in pregnant women with chronic HBV infection.^{16,17} Another one showed that pregnant women with positive HBsAg had a higher risk of gestational diabetes mellitus (GDM).¹⁸ Three good quality studies included in a recent systematic literature review revealed that maternal HBV infection increased the risk of miscarriage, pregnancy-induced hypertension, fetal distress, preterm birth and macrosomia.¹⁴ A more recent meta-analysis revealed that HBV infection had a positive correlation to GDM and preterm birth.¹⁹ It is important to highlight that none of the studies included in these systematic reviews and meta-analyses were conducted in SSA and more than half were from Asia Pacific region.

Hence, the aim of this study was to investigate whether HBV infection increases the risk of hypertension, anemia, meconium-stained amniotic fluid (MSAF), cesarean section (CS), birth asphyxia (BA) and low birth weight (LBW) in parturients admitted to Irene Neto Maternity, Lubango city, Huíla province, Angola.

MATERIALS AND METHODS

Study design, setting and population

An observational, cross-sectional, analytical facility-based survey was conducted among 500 parturients admitted to Irene Neto Maternity, from October 2016 to September 2017.⁸ Irene Neto Maternity is the reference provincial maternity in Huíla, the second most populous province of Angola. Sample size was calculated on basis of Irene Neto Maternity statistics for 2014, as previously described.⁸ A questionnaire was developed for this study, including maternal characteristics and maternal, obstetric and newborn outcomes. Pregnancy cards were consulted whenever available and parturients were interviewed to collect data on sociodemographic characteristics, alcohol and tobacco use, obstetric and pathological history, consanguinity, timing and number of antenatal care visits, and outcomes during current pregnancy. Data on obstetric and newborn outcomes for each parturient were collected through the follow-up from admission in labor until the end of delivery, in collaboration with the midwife and the doctor that were assisting her. The two groups of interest are the parturients with HBV infection (HBsAg positive) and the ones without HBV infection.⁸

Maternal, obstetric and newborn outcomes

The outcomes analyzed in this study as dependent variables were hypertension, anemia, MSAF, CS, BA and LBW. The newborn ones referred only to singleton pregnancies. At admission in labor, parturients' blood pressure (Blood Pressure Monitor BU 535, Medisana GmbH, Deutschland) and hemoglobin level (HemoCue® Hb 301 System, HemoCue AB, Sweden) were evaluated. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg²⁰, being parturients with chronic hypertension excluded from the analysis. Classification of anemic (hemoglobin level < 11 g/dl) or non-anemic (≥ 11 g/dl) parturients was performed according to the WHO reference values for pregnant women adjusted for the altitude²¹, as Lubango is 1786 meters above sea level. It is important to highlight the absence of information on existing hemoglobinopathies. Parturients who had a vaginal breech delivery were excluded from MSAF analysis.²² As in Angola there is not a norm to classify medical indications for CS, we decided to use the Portuguese norm.²³ The CS cases due to fetal distress, failed induction of labor, prolonged labor, maternal disorder related to pregnancy and suspected cephalopelvic disproportion were considered in the analysis. Hyperglycemic parturients (capillary glycemia ≥ 140 mg/dL by MediTouch,

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4 Medisana AG, Germany, according to manufacturer's instructions) were excluded from the analysis, as
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6 maternal hyperglycemia is a cause of macrosomia^{24,25} that, in turn, can led to cephalopelvic disproportion.²⁶
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8 Regarding newborn data, BA was defined as an Apgar score < 7 at 5 minutes.²⁷ Newborn weight evaluation
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10 was performed with a Digital Baby Scale Seca 354 (Seca gmbh & co. kg, Germany). A birth weight < 2.5
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12 kg was considered LBW.²⁸ Hyperglycemia, stillbirths and macrosomia were not analyzed as dependent
13
14 variables because of the low number of observations that preclude robust statistical models. As the result
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16 of an ultrasound scan at the end of the first trimester of pregnancy, a reliable menstrual history or data on
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18 serial fundal height measurements during antenatal care was not available for every parturient, the
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20 occurrence of preterm birth was not possible to be determined.

21 22 23 **Statistical analysis**

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25 Pearson's chi-square test (categorical variables) or Mann-Whitney U test (continuous variable) was used to
26
27 compare baseline characteristics between mothers with and without hepatitis B. A multivariable logistic
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29 regression model was performed to analyze the association with HBV infection for each of the six studied
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31 maternal, obstetric and newborn outcomes. Crude and adjusted odds ratio were computed with respective
32
33 95% confidence intervals. We considered maternal age (≤ 19 years, 20-34, ≥ 35)^{29,30}, formal education³¹,
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35 gravidity (nulliparous primigravida, 2nd-4th pregnancy, ≥ 5 th pregnancy)³² and number of antenatal care
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37 visits (< 4 , ≥ 4)³³ as potential confounders for all outcomes. Alcohol use was not included, since it was not
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39 possible to quantify it and tobacco use was not reported by any parturient. Selection of other confounders
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41 was guided by the literature review.^{20,27,34-43} Infection by *Treponema pallidum* was not included in BA and
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43 LBW models as a confounder, due to the low number of cases. In relation to the anemia model, only malaria
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45 diagnosis during pregnancy was included, since its screening was not performed at admission. Data on the
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47 use of traditional medicine could not been collected.

48 49 50 **Ethical considerations**

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52 Every women signed an informed consent before being included in the study, which was approved by the
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54 Angola Ethics Committee (reference 35/2017). Informed consent was read to those who could not read or
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56 write and a fingerprint and witness signature were required. A local nurse served as an interpreter when
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Portuguese was not understood and/or spoken. Parents/guardians informed consent was asked for parturients younger than 18 years of age.

RESULTS

The percentage of parturients aged 19 or younger, nulliparous primigravidas or those who had \geq five pregnancies, and with less than four antenatal care visits was higher in the HBV infected group. (Table 1).

However, the differences were not statistically significant.

Mothers with hepatitis B did not have a higher chance of hypertension, anemia, MSAF, CS, BA and LBW when compared with uninfected ones (Table 2). On the other hand, HBV-infected women had a lower chance of MSAF (16.3% vs 26.7%; aOR, 0.336; 95% CI, 0.126-0.898).

DISCUSSION

Hepatitis B management during pregnancy comprises screening for the presence of infection, evaluating disease activity and performing measures to prevent vertical transmission.⁴⁴ To our knowledge, this study is the first report exploring the association between maternal HBV infection and pregnancy outcomes in Angola and maybe in SSA in general.

Although studies are contradictory, a higher risk of miscarriage, pregnancy-induced hypertension, GDM, fetal distress, preterm birth and macrosomia among HBV-infected pregnant women was revealed in previous published systematic reviews and meta-analyses.^{14,16-19} In our study, a higher chance of hypertension, anemia, MSAF, CS, BA and LBW was not found.

Findings across the literature regarding the association between hepatitis B and adverse pregnancy outcomes remain controversial and inconsistent.⁴⁵ Several aspects may explain this fact, namely different characteristics of the study populations, regional differences, as well as methodologic and statistical heterogeneity.¹⁴ Pregnancy outcomes will be affected by racial disparity, viral genotypes, prevalence, phase of chronic infection, co-existing hepatic disorders, coinfections and synergism with pregnancy complications.⁴⁶ HBV genotypes have distinct geographical distributions and influence the dominant mode of transmission, natural history of infection, clinical outcomes and treatment response.⁴⁷⁻⁵⁰ Insufficient sample sizes, heterogeneous patient populations, diversity of clinical characteristics, differences in study designs and lack of or incomplete adjustment for important confounders may explain the conflict and

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4 inconsistency of published studies' results.^{18,45} In addition, most of the studies had only evaluated the effects
5 of maternal HBsAg carrier status on pregnancy outcomes and did not examine maternal HBeAg (hepatitis
6 B e antigen) and HBV DNA status¹³, or have not differentiated between acute or chronic infection. This is
7 important, since the presence of HBeAg indicates high levels of viral replication.⁵¹ On the other hand, HBV
8 viral load (levels of serum HBV DNA) is the most relevant marker of HBV replication. Being so, it is a
9 strong predictor of liver disease progression towards cirrhosis and hepatocellular carcinoma.⁵² However, in
10 most studies, as in the present one, information on those hepatitis biomarkers are not available.

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12 A decreased chance of MSAF among mothers who were HBV carriers was found in our study (aOR, 0.336;
13 95% CI, 0.126-0.898). The clinical significance of this finding is uncertain. It is important to highlight that
14 MSAF was not classified in different grades and secondary MSAF was not distinguish from primary, which
15 could help to clarify its meaning. We did not found another study with a similar result. The exact etiologic
16 mechanisms underlying MSAF are not clear³⁵, being controversial to differentiate if it is physiologic or
17 pathologic.³⁶ Maturation, hypoxia and infection are the three major causes for meconium passage *in utero*.⁵³
18 Regarding the first, gestational age was not included in MSAF regression model as a confounder since it
19 was not possible to determine it. Monen et al.⁵³ argued that MSAF should be regarded as a symptom rather
20 than a syndrome and be interpreted taking into account the presence of other symptoms.

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22 Our study has the merit of lifting the veil over characterization of the association between maternal HBV
23 infection and pregnancy outcomes in Angola and maybe in SSA in general. However, it has clear limitations
24 that must be acknowledged. We were not able to ascertain maternal HBeAg status and HBV viral load or
25 measure abnormalities in liver function tests, and acute and chronic infection were not differentiated.
26 Furthermore, some pregnancy outcomes identified in previous studies as being associated with hepatitis B
27 have not been analyzed, namely miscarriage, GDM, fetal distress, preterm birth and macrosomia.

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29 The issue of the association between hepatitis B and pregnancy outcomes remains unclear and to our
30 knowledge no such study has been conducted in SSA. In our study, HBV-infected pregnant women did not
31 face a higher chance of adverse pregnancy outcomes. In the future, larger, more robust, well-designed
32 prospective cohort studies are needed, which should be based on a better characterization of the maternal
33 HBV infection and taking into account all possible confounding factors. The carrying out of these studies
34 in SSA should be prioritized, given the high HBV prevalence and the possibility of different clinical
35 outcomes related to each genotype.

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Table 1. Demographic, obstetric history and antenatal care characteristics of women with and without hepatitis B

	Hepatitis B Positive (n = 43) [†]	Hepatitis B Negative (n = 457) [†]	<i>p</i> Value
Age, y			0.417*
≤ 19	25.6 (11) [‡]	17.6 (79)	
20-34	62.8 (27)	67.6 (303)	
≥ 35	11.6 (5)	14.7 (66)	
Formal education, y	7 ± 5 [§]	8 ± 6	0.163**
Gravidity			0.320*
Nulliparous primigravida	32.6 (14)	25.1 (114)	
2nd-4th pregnancy	37.2 (16)	49.0 (223)	
≥ 5th pregnancy	30.2 (13)	25.9 (118)	
Number of ANC visits			0.473*
< 4	42.5 (17)	36.8 (157)	
≥ 4	57.5 (23)	63.2 (270)	

Abbreviations: ANC, antenatal care.

[†]Total numbers of infected and uninfected parturients vary by outcome due to different patterns of missing data.

[‡]Percentage (No.) for categorical variables.

[§]Median ± interquartile range for continuous variable.

*Pearson's chi-square test.

**Mann-Whitney U test.

Table 2. Maternal, obstetric and newborn outcomes of women with hepatitis B compared to those without hepatitis B

	Hepatitis B		Hepatitis B		Crude OR (95% CI)	Adjusted OR (95% CI)
	Positive		Negative			
	(n = 43) ^a		(n = 457) ^a			
	No.	%	No.	%		
Maternal outcomes						
Hypertension ^b	10	23.3	164	37.0	0.516 (0.248-1.073)	0.564 (0.264-1.206)
Anemia ^c	7	16.3	83	18.3	0.869 (0.374-2.021)	0.807 (0.319-2.042)
Obstetric outcomes						
MSAF ^d	7	16.3	120	26.7	0.535 (0.232-1.234)	0.336 (0.126-0.898)
CS ^b	2	4.7	40	9.0	0.493 (0.115-2.113)	0.537 (0.122-2.368)
Newborn outcomes						
BA ^e	10	27.0	97	22.4	1.287 (0.602-2.751)	1.869 (0.723-4.830)
LBW ^f	7	17.5	43	9.7	1.983 (0.827-4.753)	1.642 (0.614-4.388)

Abbreviations: BA, birth asphyxia; CI, confidence interval; CS, cesarean section; LBW, low birth weight; MSAF, meconium-stained amniotic fluid; OR, odds ratio.

^aTotal numbers of infected and uninfected parturients vary by outcome due to different patterns of missing data and excluded cases.

^bAdjusted for age, formal education, gravidity and number of ANC visits.

^cAdjusted for age, formal education, gravidity, number of ANC visits, malaria during pregnancy and human immunodeficiency virus infection.

^dAdjusted for age, formal education, gravidity, number of ANC visits, hypertension at admission in labor, labor induction and CS.

^eAdjusted for age, formal education, gravidity, number of ANC visits, hypertension at admission in labor, hyperglycemia at admission in labor, anemia at admission in labor, human immunodeficiency virus infection, MSAF, CS and LBW.

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^fAdjusted for age, formal education, gravidity, number of ANC visits, consanguinity, malaria during pregnancy, hypertension at admission in labor, anemia at admission in labor and human immunodeficiency virus infection.

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3. DISCUSSION AND CONCLUSIONS

The aim of this thesis is to characterize infections by *T. pallidum*, HIV, HBV and HCV in parturients admitted to INM, Lubango city, Huíla province, Angola, and their complications. Lubango is the capital of Huíla province, the second most populous province of Angola¹. INM is the reference provincial maternity. Data collection took place between October 2016 and September 2017. The thesis comprises six articles. In this chapter, the main results of these papers will be discussed from a public health perspective, taking into account the sustainable development goals (SDG) agenda, as well as the national and international health policies and guidelines. Thesis strengths and limitations will be acknowledged and recommendations will be made.

3.1. Discussion

In this study, at least one screened infection was detected in 11.8% of the parturients. Hepatitis B was the most common (8.6%), followed by HIV infection (3.0%) and syphilis (1.0%). None hepatitis C case was diagnosed. *T. pallidum* and HIV seropositivity rates found can be classified as low (<5%)^{2,3}, while for HBV (HBsAg) is considered high (>8%)⁴. The syphilis rate is below the value estimated for pregnant women from Southern Africa (3.6%)⁵. The HIV rate is three times higher than that reported in Angola's Multiple and Health Indicators Survey 2015-2016 for women from Huíla with 15-49 years of age (0.9%)⁶, but is in line with the one revealed in a recently published study that included pregnant women attending ANC in Luanda, the capital (2.6%)⁷. The HIV rate described in our work is far below the estimated HIV prevalence for pregnant women from Southern Africa (16.1%)⁸. Regarding HBV, a prevalence of 3.8% was estimated among pregnant women from this region⁹. The high HBV rate found in our study is above the mentioned value and reinforces the results of few previous studies regarding hepatitis B in Angola¹⁰⁻¹². It is known that high-HBV prevalence is common in much of the SSA¹³. Hepatitis B is the major cause of cirrhosis and HCC in the region¹⁴, where four to 13% of infected individuals have cirrhosis and need immediate AVT¹⁵. HCC is the second and the third most common cancer in African men and women, respectively, being related to chronic HBV infection in 75% of the cases¹⁶.

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Until recent years, viral hepatitis has been neglected as a health and development priority¹⁷. In Africa, WHO efforts against infectious diseases have been focused on HIV, malaria and tuberculosis¹⁸. Morbimortality data related to viral hepatitis demands attention and the SDG target 3.3 calls for action to combat it¹⁹. In 2016, WHO launched the first global health sector strategy on viral hepatitis for its elimination as a major public health threat by 2030, with a particular focus on hepatitis B and C¹⁷. In order to achieve the goals proposed by WHO, viral hepatitis strategic plans and management guidelines are being developed in many SSA countries²⁰. On the one hand, in those countries, the lack of awareness, resources and political commitment affects all the steps of the HBV continuum of care¹⁴. On the other hand, there is very limited knowledge regarding determinants of hepatitis B in those countries¹⁴. As so, in SSA, the first step on the battle against this infection should be gaining accurate epidemiological data to allow proper understanding of the dimension of the problem and engaging appropriate resources²¹. However, research in the field of HBV has been characterized by underfunding²². The Gastroenterology and Hepatology Association of SSA was established in the end of 2017²³. The following priority actions have been recommended for the elimination of hepatitis B in SSA: prevent VT; identify high-risk groups for screening, vaccination or linkage to care; screen and vaccinate health-care workers; establish pathways of linkage to care and ensure sustainable access to tenofovir for individuals who are HBV monoinfected²⁰. An effective multisectoral and multiprofessional collaborative action is needed, involving affected communities, health professionals, researchers, professional and community organisations, national departments of health, governments and pharmaceutical companies^{20,21}.

In Angola, the National Institute for the Fight against AIDS is also responsible for coordinating the national response to viral hepatitis. In 2015, the HBV vaccine was introduced in the Angola's Expanded Programme of Immunization²⁴, including a monovalent VDB (within 24 hours), followed by three pentavalent vaccine doses (diphtheria, pertussis, tetanus, hepatitis B and *Haemophilus influenzae* type b) at two, four and six months of age²⁵. However, several challenges remain in order to achieve an adequate coverage. First, it is indispensable to guarantee a sustainable availability of the vaccine. Second, it is necessary to ensure adequate follow-up so that, after the VDB, the remaining three doses are administered in the recommended schedule. Third, it is

DISCUSSION AND CONCLUSIONS

imperative to reach children born at home in a country where home births are very frequent. In 2016, a multisectoral and multiprofessional technical group was formed to work on the first National Protocol for the Prevention, Diagnosis, Treatment and Care of Viral Hepatitis that was issued in 2018, with a particular focus on hepatitis B²⁶. In January 2021, hepatitis B treatment was implemented in the Angolan National Health System (NHS), but only in Luanda province, the major challenge for clinicians being the unavailability of VL tests for the therapeutic decision making.

ANC provides the opportunity to carry out vital interventions on both women and children health. Regarding the coverage and quality of ANC services, SSA is still at the bottom of the pyramid²⁷. As for HIV infection and syphilis, Angolan and international health guidelines recommend hepatitis B screening during ANC^{2,28-30}. In our study, HIV, *T. pallidum* and HBV ANC testing (at least once) was only performed in 66.5%, 60.2% and 44.6% of the parturients, respectively, the rates being significantly lower in women from rural areas when compared with those from urban areas. We predict that these rates are even lower in women who deliver at home. After a diagnosis has been made, some women referred not having received treatment for either HIV infection or syphilis and none of those who were positive for hepatitis B referred having initiated treatment. Those facts show losses along the continuum of care for the first two infections and for the last, as we previously discussed, treatment is still unavailable from NHS in Huíla province and related national guidelines were only launched in 2018.

To our knowledge, this is the first report about infection screening during ANC in Angola. The rate of HIV testing is slightly higher than the one described in a study that included data from four countries of different geographical regions of SSA (60.7%)³¹. A study with data from 12 countries from this region showed an uneven availability of *T. pallidum* testing in ANC facilities, ranging from three to 92%³². We did not find published studies concerning tests availability or HBV screening rates during ANC in SSA. In Angola, there is a discrepancy in the health services available to rural communities in comparison with urban settings³³. The statistical significant rural-urban disparities in ANC screening rates reported by us are in line with the results of previous studies from Angola^{6,34,35}, SSA^{27,36-38} and LMIC in general³⁹, showing that women living in rural settings were less likely to receive maternal health services. These disparities and the result about the 10% lower chance of infection with HBV for each additional year of formal education

DISCUSSION AND CONCLUSIONS

emphasize the importance of social determinants of health inequalities in general and of maternal health inequalities in particular, namely the place of residence and education. WHO defines social determinants of health as “the conditions in which people are born, grow, work, live, and age, and the wider set of forces and systems shaping the conditions of daily life” and argues that for addressing it is indispensable a multisectoral action and the involvement of the civil society⁴⁰. It is important to highlight that one of the 17 SDG aims at reducing inequality within and among countries⁴¹. Based on data of 36 LMIC, Leventhal et al⁴² argue that maternal health interventions delivered at community level are usually less inequitably distributed than those delivered in fixed facilities. Particularly in rural areas with limited access to health services, WHO recommends interventions that include household and community mobilization and ANC home visits⁴³.

Timely initiation of ANC has the benefit of allowing early identification and management of pregnancy complications and prevention of their possible adverse outcomes⁴⁴. In our study, the majority of the parturients attended ANC late in pregnancy, with only 9.1% initiating it in the first trimester of pregnancy. This value is well below the estimates for the country (40.1%) and for SSA in general (40.8%)^{6,39}. WHO used to recommend a minimum of four ANC visits⁴⁵, having recently updated to eight in order to reduce perinatal mortality and improve women’s experience of care⁴³. At least four and eight ANC visits were observed in 62.7% and 3.4% of the studied women, respectively. This coverage of four or more ANC visits is similar to the one estimated for the country (61.4%) and above the one estimated for SSA (53.0%)^{6,37}. The WHO updated recommendation of a minimum of eight ANC visits is far away from being fulfilled in our sample. In a study conducted in Angola, lower than four ANC visits was significantly associated with LBW and PB⁴⁶.

Maternal and newborn morbidity and mortality remain high in Angola. It is known that to track progress and to manage decentralized planning, there is a need to monitor and evaluate data from country to facility level, without forgetting subnational and district levels⁴⁷. In this study, we identified several issues that must be taken into account in the design and planning of policies regarding the improvement of maternal and newborn health in Huíla province and probably in Angola as a whole: adolescent pregnancy, maternal illiteracy, maternal short stature, antenatal subdiagnosis of hypertensive disorders, birth asphyxia (BA), LBW, non-medical indications for C-section and twinning

DISCUSSION AND CONCLUSIONS

rate. In the corresponding article, we have already specifically discussed the relevance of each issue, but it is important to highlight that some of them can be related. A study conducted in 29 countries from Africa, Latin America, Asia and Middle East showed a higher risk of LBW in babies of adolescent mothers⁴⁸. A systematic review and meta-analysis found that, in LMIC, a higher maternal education is associated with a decreased risk of delivering a LBW infant⁴⁹. Furthermore, and although not from Africa or LMIC in general, a retrospective cohort study of 1,775,984 births revealed that newborns from taller women were less likely to have LBW⁵⁰. Finally, in accordance with a systematic review of the literature, maternal young age (<20 years), limited literacy and hypertension are among risk factors for BA in low-resource communities⁵¹.

When we talk about pregnant women infected with HBV, one of the particular aspects that need to be considered is the effect of this infection on pregnancy⁵². This thesis includes a systematic literature review exploring maternal, obstetric and newborn complications associated with infection by HBV. Although the included studies present discrepant results, three from those with good quality revealed that maternal HBV infection increased the risk of miscarriage, PB, pregnancy-induced hypertension, fetal distress and macrosomia. Other three systematic reviews and meta-analyses that targeted specific adverse pregnancy outcomes have found a higher risk of PB in mothers chronically infected with HBV^{53,54} and of GD in the HBsAg-positive ones⁵⁵. More than half of the included studies in those four systematic reviews were conducted in Asia Pacific region and none was from SSA. In our sample, we did not find a higher chance of hypertension, anaemia, meconium-stained amniotic fluid, C-section, BA and LBW in parturients with hepatitis B when compared with the uninfected ones. However, we have not analyzed some pregnancy outcomes identified in previous studies as being associated with hepatitis B, namely miscarriage, GD, fetal distress, PB and macrosomia. We were also not able to identify maternal HBeAg status and HBV VL or measure abnormalities in liver function tests, and acute and chronic infection were not differentiated. We have done a new search on PubMed to verify if new articles have been published since the search date of our systematic review and we found two new papers that respond to the research question. These articles were from China and the one that used adjusted estimates for confounding factors revealed an increased risk of GD, PB, ICP and neonatal asphyxia for HBsAg-positive women⁵⁶. Among these women, higher risks of the last two

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complications were found in those HBeAg-positive or with a high HBV-DNA load. The carrying out of studies in SSA exploring adverse pregnancy outcomes associated with infection by HBV should be then prioritized, given the high HBV prevalence and the possibility of different clinical outcomes related to each genotype.

Other aspect that needs to be considered when a pregnant woman is infected with HBV is its possible VT⁵². At birth, we identified HBV DNA in 7.3% of the newborns from HBsAg-positive parturients, corresponding to genotype E. The rate was 100.0% for HBeAg-positive mothers, in contrast with 3.2% for the HBeAg-negative ones. Despite the small numbers involved, we found a highly significant association between mothers HBeAg positivity and HBV DNA positivity of the newborns. It is important to highlight that WHO warns that, within the first six months of life, HBV DNA may be inconsistently detectable in infected infants³⁰. On the other hand, HBV DNA detected at birth can be cleared by host immunity and HBV vaccination⁵⁷. The studied newborns received the HBV VDB and supposedly the following three pentavalent vaccine doses, so they should have been re-evaluated at 9-12 months for the presence of anti-HBs and absence of HBsAg (PVST)⁵⁸⁻⁶⁰. It is well known that the risk of HBV VT is associated with maternal HBeAg status and HBV DNA level⁶¹⁻⁶⁴. A systematic review and meta-analysis estimated that, without prophylaxis, the risk of HBV VT in SSA is 38.3% among HBeAg-positive women, while for the HBeAg-negative ones is 4.8%⁶⁵. The study was based on data from 11 countries, but Angola was not included in the group. There are several HBV genotypes, with distinct geographical distribution. They influence the dominant mode of transmission, natural history of infection, clinical outcomes and treatment response^{4,66-68}. In Africa, the most prevalent are genotypes A, D and E⁶⁹. In Angolan patients, genotype E is highly predominant, but genotypes A and D have also been reported^{11,70}. It was described that high frequency of HBeAg-positivity and high VLs are found in individuals infected with genotype E⁷¹, which, as previously discussed, implies a greater risk of VT. Prevention of HBV VT is an underestimated and underresearched public health issue in Africa⁶⁴. It includes the following strategies: antenatal screening, third-trimester AVT if HBV DNA >200,000 IU/mL and/or HBeAg-positive, VDB, HBIG and full vaccine coverage²². These strategies are recommended in Angolan guidelines, although the use of HBeAg as an alternative to VL to guide the necessity in initiating AVT for prevention of VT is not mentioned²⁶. The utility of HBeAg status for that purpose was demonstrated by

evidence and was incorporated in recent international guidelines^{72,73}. For the majority of HBV-positive pregnant women in SSA, due to the expense and expertise required for VL testing, HBeAg is the only available marker of infectiousness⁷⁴. The HBIG is mentioned in Angolan guidelines, but it is unavailable from NHS. The cost and logistics of its administration are prohibitive for most women in SSA countries¹⁶. A systematic review and meta-analysis found that vaccine alone seems to be as effective as the combination with HBIG for neonates of HBsAg+/HBeAg- mothers⁷⁵. This result can be used by LMIC to lower costs by targeting HBIG for newborns who need it most. In our study, among the three HBV infected babies' mothers, only one had been aware of her HBV infection, but neither her VL or HBeAg status was evaluated, nor did she receive AVT. However, the babies have received VDB. As previously pointed, hepatitis B ANC testing (at least once) was only performed in 44.6% of the studied parturients. These results about the coverage of ANC preventive interventions for HBV VT are of concern, but is important to highlight that national guidelines about it were launched after the end of our fieldwork.

HIV infection, syphilis and hepatitis B, infections studied in this thesis, share several common features, which is why a linked agenda for these infections is advocated, namely a triple elimination of VT⁷⁶. Synergies should be capitalized and a horizontal, integrated, multidisciplinary and person-centred approach within maternal and newborn health services is needed. The highest burden of the three infections is found in the WHO African Region⁷⁶. In Angola, there is already a triple elimination plan for 2019-2022, where community-based interventions were pointed among the issues to be improved⁷⁷.

3.2. Strengths and Limitations

This study is one of the few reports contributing for the knowledge of some STIs epidemiology in Angola, a group of infections with an undeniable burden in that country. To our knowledge, this is the first report about STIs screening rates during ANC, hepatitis B and syphilis VT, and association between maternal HBV infection and pregnancy outcomes in Angola. This thesis has also the merit of having been able to identify several issues that must be addressed with multisectoral, multidisciplinary and integrated strategies in order to improve maternal and newborn health in Angola, with a particular

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focus on HIV, *T. pallidum* and HBV infections. During the year of the fieldwork, the PhD student of this thesis has followed *in loco* the studied parturients from admission in labour until the end of delivery. It was a very challenging experience in a context of lack of asepsis, scarcity of medical supplies, few qualified healthcare workers and very deficient surveillance system, but it allowed her to do the work herself and as a consequence to collect all data that was needed.

Limitations of this thesis were already mentioned in detail in each one of the papers presented in the results chapter, with the exception of article two due to the journal length limitations. Here it only will be highlighted the broader and more relevant ones, as also those from article two:

- The study only included parturients giving birth at INM, being that the ones delivering in other health institutions or at home were not studied, which limits the representativeness of the data;
- Maternal OBI was not investigated;
- For syphilis and HCV infection, multivariable logistic regression models exploring the association with sociodemographic factors were not performed due to the low seropositivity rates found;
- Regarding ANC, we evaluated rates of timely initiation and attendance of a minimum of four and eight visits, but we did not characterize those visits or evaluated their quality, which could add important insights;
- VT of HCV infection was not investigated because there were no maternal cases;
- The occurrence of PB was not assessed in the studied parturients;
- Association of HIV infection and syphilis with pregnancy complications was not analyzed due the low number of infection cases that preclude robust statistical models;
- When analysing the association between HBV infection and pregnancy complications, we were not able to do a complete characterization of the maternal infection. Additionally, some pregnancy complications identified in previous studies as being associated with hepatitis B and previously mentioned have not been analyzed.

3.3. Conclusions

This thesis reinforces that hepatitis B is a highly endemic public health problem in Angola, with a scarce research about its determinants. There are already national guidelines for its prevention, diagnosis, treatment and care from 2018, whose implementation is not completed, having only taken the first steps. ANC indicators of the studied parturients are yet to reach adequate standards, especially timely initiation and attendance of a minimum of eight visits. Rural-urban disparities on ANC screening rates for HIV infection, syphilis and hepatitis B were revealed. In Angola, the improvement of maternal and newborn health still have a long way to go. Adolescent pregnancy, maternal illiteracy, maternal short stature, antenatal subdiagnosis of hypertensive disorders, BA, LBW, non-medical indications for C-section and twinning rate are issues to take into account in Huíla province and probably in Angola as a whole. The issue of the association between hepatitis B and adverse pregnancy outcomes remains unclear, especially in SSA, where it is understudied. We found a highly significant association between mothers HBsAg positivity and HBV DNA positivity of the newborns at birth. This result should be incorporated in national strategies for the prevention of HBV VT.

3.4. Recommendations

The findings of this thesis have already been shared with the provincial and national Angolan health authorities. The following recommendations should be considered in Huíla province and probably in the rest of the country:

- Hepatitis B importance within the infectious diseases landscape should be recognised;
- Implementation of the national guidelines for hepatitis B must continue and additional research that will allow local adequacy of the interventions and assessing their impact should be conducted;
- Efforts must be carry out to improve access and linkage to ANC. A particular focus on rural areas is needed. The efforts should include community-based interventions, involving community health agents, community stakeholders, non-governmental organizations and mobile clinics. Community health agents should

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link communities to ANC and home ANC visits must be implemented. Mobile clinics could use a same-day test and treat approach for preventing losses along the continuum of care for HIV infection, syphilis and hepatitis B. The midwives and clinicians of these clinics and the community health agents should be able to refer for specialized care in a health facility whenever necessary and means must be provided to women to facilitate the process. Health education interventions through radio, television and mobile phones must not be neglected;

- In INM and other maternal healthcare facilities, rapid screening for HIV, *T. pallidum* and HBV should be implemented during labour, delivery or in the immediate PP period for women whose status is unknown;
- Efforts must continue towards increasing the proportion of educated women, what would positively affect maternal and newborn health;
- Maternal characteristics and maternal, obstetric and newborn outcomes highlighted in this thesis should be taken into account in view of redesigning and planning interventions to improve maternal and newborn health;
- A larger, more robust, well-designed prospective cohort study exploring pregnancy complications associated with infection by HBV, as well as VT rates and efficacy of interventions to prevent it should be performed, based on a complete characterization of the maternal HBV infection;
- Coverage of all interventions for HBV VT prevention must increase and HBeAg status should be incorporated in the therapeutic decision making. Particular focus on women more prone to give birth at home and their children is needed.

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