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Co-infections associated with human immunodeficiency virus type 1 in women during pregnancy in rural Gabon: a cross-sectional study

Dissertation

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Table of Content

List of tables

List of figures

1 Introduction

1.1 General introduction

According to the latest UNAIDS factsheet, 37.7 million people in the world are living with the human immunodeficiency virus (HIV), of whom 25.4 million are living in sub-Saharan Africa. More than half (57%) of the 37.7 million people that are living with HIV are girls and women (UNAIDS, 2021; UNWomen, 2018).

In Western and Central Africa, 48% of HIV-positive pregnant women receive antiretroviral treatment (ART) to prevent mother-to-child transmission (MTCT) of HIV infection (UNWomen, 2018). The immunodeficiency caused by chronic HIV infection increases the risk to acquire several co-infections such as infection with *Plasmodium* spp., hepatitis B virus (HBV) and hepatitis C virus (HCV) (Chang et al., 2013). Chronic HIV infection may lead to the recurrence of infections that may result in diseases with congenital anomalies caused by the so-called TORCH pathogens. TORCH is an acronym and includes **T**oxoplasmosis, **O**ther (syphilis, human parvovirus B19, varicella-zoster, HBV) etc., **R**ubella, **C**ytomegalovirus, and **H**erpes infections (Singh et al., 2015, Stegmann and Carey 2002). Co-infections can increase the risk of MTCT of HIV by weakening the child's natural defences to MTCT (King et al., 2015). Particularly co-infections targeting the placenta, genital tract, foetal membranes, and systemic maternal infections, are known to influence the infant's organogenesis (Singh et al., 2015) as well as the maturation of the pre- and perinatal immune system (Gollwitzer and Marsland, 2015). Maternal infections can directly influence the pre- and perinatal immune system through the transplacental transfer of maternal immune factors including maternal antibodies, which provide protective effects on the newborns. These factors might be able to modulate the vulnerability to homologous or heterologous infectious pathogens of the neonate (Gollwitzer and Marsland, 2015). The limited immunological memory in neonates and regulatory responses requires adaptation of the immune system from a sterile to a non-sterile environment (Abu-Raya et al., 2016).

Therefore, by increasing the susceptibility to co-infections during pregnancy HIV-infection may not only negatively affect the health of the mother, but also the offspring.

1.2 Rationale for the study

As pregnant women are a vulnerable population, particularly those living with HIV, research to improve mother and child health is crucial and is of high public health priority. There are only few data that characterise the most frequent co-infections among HIV-positive women during pregnancy in comparison to HIV-negative pregnant women in Gabon – a country in central Africa with a high burden of infectious diseases. There is currently a knowledge gap concerning the effect of HIV status on the risk of acquiring co-infections during pregnancy. We aimed to bridge this gap by conducting two separate studies within the framework of this doctoral thesis: First, the HIV prevalence study, in which the prevalence of HIV was determined among pregnant women who presented to antenatal care (ANC) units in Gabon. The prevalence study provided data on HIV prevalence during pregnancy for 21 ANC units during the year 2018 in seven towns in Gabon. The HIV prevalence study was the preparation for the second part of this doctoral thesis: the co-infection study. The co-infection study aimed at detecting various infectious pathogens in HIV-positive and HIV-negative women and comparing the prevalence of co-infections between HIV-positive and HIV-negative pregnant women. A list of all pathogens of assessed co-infections is provided on page 17.

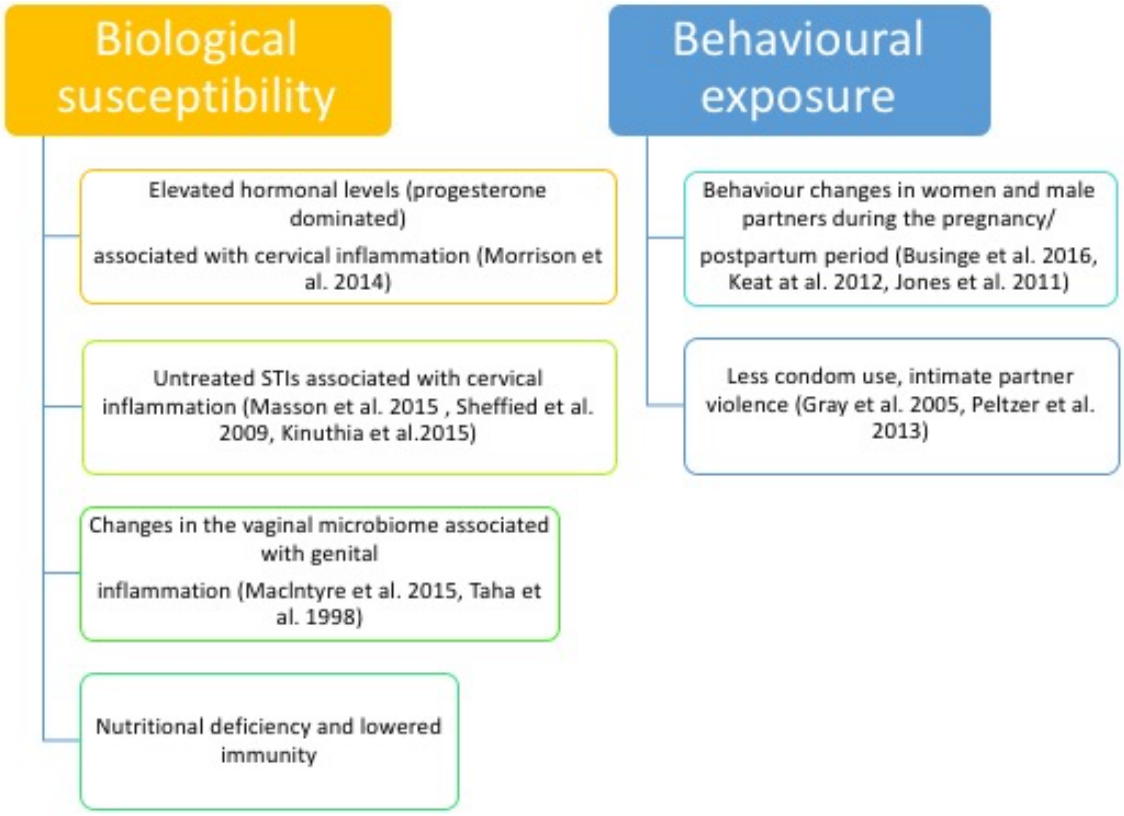
Furthermore, we also identified deficiencies in antenatal care in Gabon. These data may be used to provide feedback to the national programme for mother and child health to discuss relevant outcomes with concerned stakeholders on a regional and national level, and to improve mother and child health in Gabon on a regional and national level.

1.3 Susceptibility to infections and infectious diseases during pregnancy

Generally, the pathogens that cause infections and infectious diseases are bacteria, viruses, parasites, or fungi. Usually, pathogens can be cleared by the immune system or if necessary and indicated, the pathogens are cleared using antibacterial, antifungal, and antiviral treatments in an immunocompetent individual. Pregnant women undergo immunological changes to develop a diminished inflammatory response in order to ensure tolerance of the foetus. Therefore, pregnant women are more susceptible to several pathogens and their severity (Sappenfield et al., 2013). Figure 1 shows different aspects of women that may put women at greater risk for HIV infection during pregnancy.

Figure 1

Perinatal prevention of HIV transmission



Reference: Moodley, 2017

During pregnancy, the embryo or foetus can be infected at three different points in time: before delivery (prepartum) and during delivery (intrapartum); and after delivery (post-partum) through breastfeeding. During pregnancy, MTCT transmission of pathogens to the foetus can occur via different pathways (Teasdale et al., 2011): ascension from the vagina or the cervix, hematogenic spreading through the placenta (transplacental), retro-grade dissemination from the abdominal cavity through the salpinx, or iatrogenic through e.g. amniocentesis; the most frequent being the ascension pathway (Teasdale et al., 2011, Olthoff, 2019). Some pathogens like *Plasmodium (P.) falciparum* infiltrate the intervil-lous space of the placenta through erythrocytes infected with *P. falciparum* but do not usually cross the placental barrier (Sharma and Shukla, 2017).

Pathogens that may cause miscarriages, malformations, long-term disability, prematurity, and stillbirths include *Chlamydia (C.) trachomatis*, *Listeria monocytogenes*, *Ureaplasma urealyticum*, *Mycoplasma genitalium*, *Treponema (T.) pallidum*, *Streptococcus*

agalactiae, viruses such as viral hepatitis and protozoa like *Toxoplasma gondii,* and *P. falciparum* (Olthoff, 2019; Romero et al., 2002; Sharma and Shukla, 2017).

1.4 Sexually transmitted infections

Sexually transmitted infections (STIs) are a group of various infections. During sexual intercourse, the pathogens that cause STIs can be acquired by crossing the vagina, or the oral or anal mucosa during oral or anal sex, respectively. *T. pallidum*, *Neisseria* (N.) *gonorrhoea,* and *Haemophilus ducreyi* belong to the group of "classic" STIs. Apart from such classic STIs, there are several bacterial, viral, and parasitic pathogens that are also transmitted sexually such as *Trichomonas* (T.) *vaginalis*, HBV, *Herpes simplex*, *C. trachomatis*, HIV and *Human papillomavirus* (Olthoff, 2019). In this study and due to their potential route of transmission, HBV and HCV are assigned to the group of STIs.

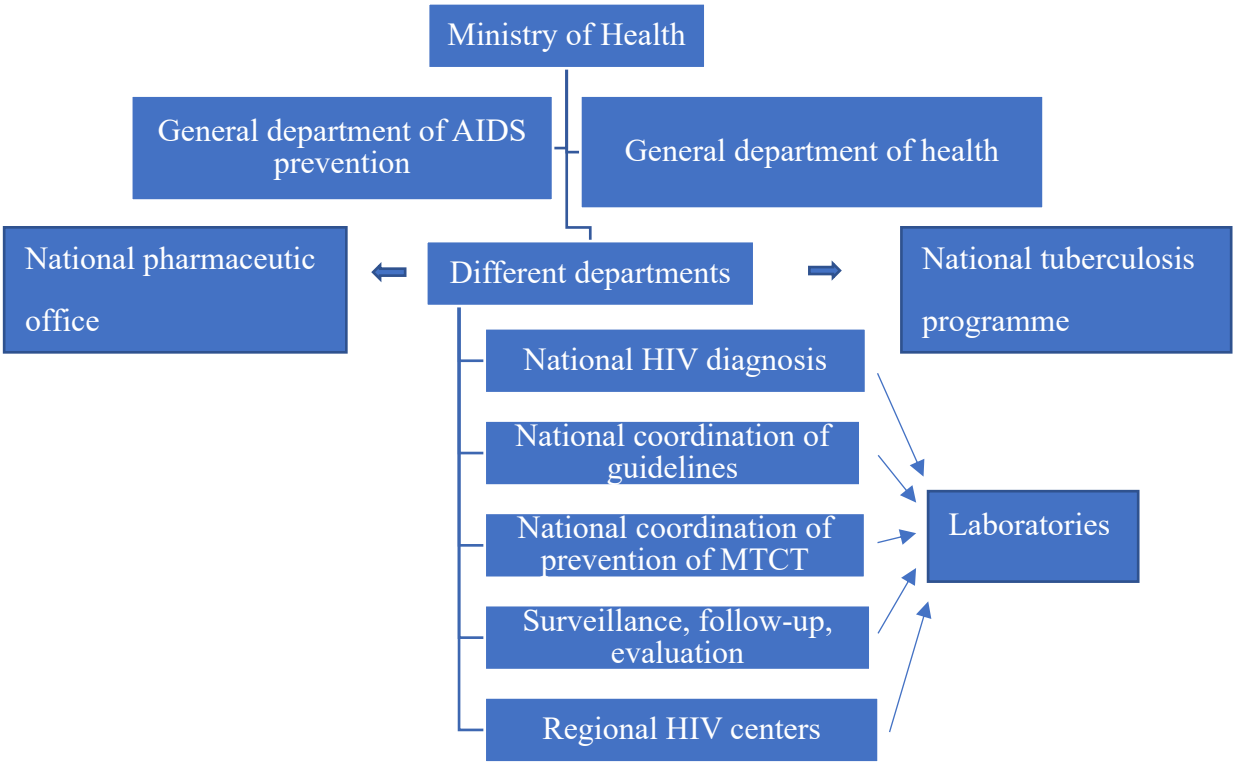
1.5 National perinatal HIV/AIDS surveillance programme in Gabon

Gabon has a national HIV/AIDS control programme, which is coordinated and organised by two entities of the Ministry of Health (MoH): The general department of AIDS (Acquired Immunodeficiency Syndrome) prevention and the general department of health conducted by the national control programme in the fight against STIs and HIV/AIDS (PNLIST, 2021).

The national control programme in the fight against STIs and HIV/AIDS establishes and coordinates the national guidelines of HIV/AIDS prevention, treatment, diagnosis, and public health interventions with different governmental and international organisations like UNAIDS, WHO, and UNICEF (PNLIST, 2021). They also train, supervise, and control the implementation of national guidelines by health care personnel across the country. They purchase and distribute all consumables related to STIs and HIV/AIDS. This includes e.g., the distribution of antiretroviral drugs, and reagents for the diagnosis of HIV according to the national guidelines (PNLIST, 2021). Details about different departments involved in the fight against HIV/AIDS are shown in Figure 2.

Figure 2

Chart of different departments involved the national HIV/AIDS programme in Gabon



Reference: PNLIST, 2017

The National HIV/AIDS Control programme of Gabon has established the screening for HIV among ANC unit attendees in Gabon as one of the methods for collecting data on HIV prevalence for pregnant women to strengthen the prevention of MTCT of HIV. During the first antenatal care visit, the pregnant woman is systematically screened for HIV using rapid diagnostic tests according to the national guidelines. If a pregnant woman tests negative, it is recommended that the pregnant woman is screened again after three months to exclude HIV. If a pregnant woman is tested positive during the first screening test, two confirmatory tests are done with other rapid diagnostic test assays. (PNLIST, 2021). There is a list of different rapid diagnostic tests provided by the National HIV/AIDS Control programme, which clarifies which tests are used for the first testing (screening), the second and the third test (confirmation). A pregnant woman is classified as positive if all three tests are positive. A pregnant woman has an undetermined result when the first test and either the second or third test are positive with one of the latter

being negative. In this case, the sample is sent to a higher level laboratory approved by the National HIV/AIDS Control programme for final diagnostic classification.

When the pregnant woman is tested positive, the pregnant woman is counselled and immediately put under ART irrespective of CDC stage or CD4 counts. A pregnant woman can deliver vaginally if the treatment was started before the 33rd week of gestation or if the viral load is equal to or less than 1 000 copies/ml at the 33rd week of gestation, otherwise an elective caesarean section is recommended (PNLIST, 2021). If a pregnant woman is in labour in the ANC unit and the HIV status is unknown, the pregnant woman is tested during labour. The first-line treatment in Gabon at the beginning of the study was efavirenz + tenofovir + emtricitabine but in September 2019 it became the second-line treatment with a switch to the the first-line treatment tenofovir + lamivudine (or emtricitabine) + dolutegravir (PNLIST, 2021).

1.6 Presentation of individual pathogens

Throughout the course of the co-infection study, the prevalence of different co-infections was evaluated in pregnant Gabonese women. The following co-infections were assessed:

- *Treponema pallidum*
- *Plasmodium* spp.
- *Loa loa* microfilariae
- *Mansonella perstans* microfilariae
- Group B *Streptococcus*
- Human papillomavirus (and subtypes),
- *Trichomonas vaginalis*
- Human T-lymphotropic virus type 1 and 2
- Hepatitis B virus
- *Schistosoma* spp.
- Hepatitis E virus
- Hepatitis C virus
- *Neisseria gonorrhoea*
- *Chlamydia trachomatis*
- Epstein-Barr virus

The subsequent parts of this doctoral thesis are dedicated to a review of these pathogens that have been investigated in the co-infection study.

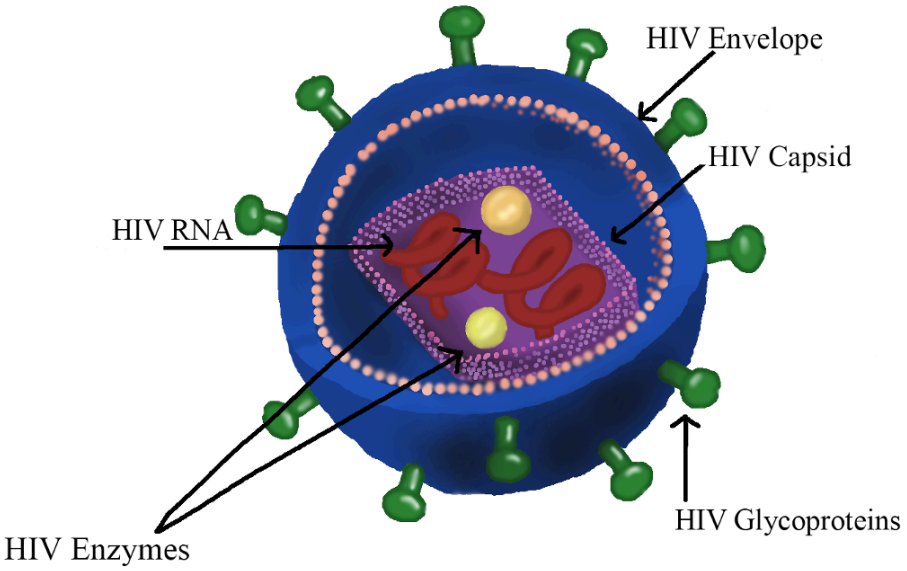
1.6.1 HIV

1.6.1.1 Biology

HIV is a retrovirus that was first discovered in 1984. It belongs to the lentivirus family and leads to acquired immunodeficiency syndrome (AIDS) if untreated (Aiamkitsumrit et al., 2014a). It is an enveloped RNA virus (Figure 3), with a size of 100 nm (German Advisory Committee Blood (Arbeitskreis Blut), Subgroup 'Assessment of Pathogens Transmissible by Blood', 2016). There are two types of HIV, HIV-1 and HIV-2. Infectious diseases caused by lentiviruses are usually chronic diseases with a long period of clinical latency, a persistent viremia and a spread through other organs including members of the nervous system, and immune system (Aiamkitsumrit et al., 2014a). It mainly infects cells with a CD4 receptor, which is expressed on the surface of T-cells. However, the monocyte-macrophage lineage and dendritic cells also have CD4 receptors on their surface. Dendritic cells, endothelial cells of the brain, perivascular macrophages, astrocytes and microglial cells also have CD4 receptors. (Aiamkitsumrit et al., 2014a).

Figure 3

HIV structure



Reference: Leandre Marvin Mawuli Kordts 2022

The life and replication cycle of HIV consists of 7 steps as follows:

1. **Attachment/binding**: The following structures are important to allow the entry of the virus: The principal receptor for HIV is the CD4 receptor. It serves as a binding molecule and simultaneously induces conformational changes to glycoprotein 120 (gp120). This enables gp120 to interact with a co-receptor, such as CXCR4 and CCR5, in addition to the CD4 receptor.
2. **Fusion:** Consequently HIV gp41 is transformed from a nonfusogenic to a fusogenic state, so that the membranes of the host and the virus are easily merged (Aiamkitsumrit et al., 2014b).
3. **Reverse transcription:** HIV releases viral reverse transcriptase into the cytoplasm of the host cell. The reverse transcriptase and two copies of genomic viral RNA are transported to the cell nucleus. During the transport, the viral RNA converts into double-stranded proviral HIV DNA, a crucial step as it allows HIV to invade the nucleus of the host cell. Despite viral DNA being read, viral proteins are produced.
4. **Integration:** After having entered the host nucleus, HIV DNA can be inserted into the DNA of the host cell by using an HIV enzyme called integrase.
5. **Replication:** The so-called provirus, now integrated into the host cell, uses the host's machinery for viral replication.
6. **Assembly:** New HIV proteins and HIV RNA get to the surface of the host cell and assemble into virions. The virions are not yet infectious due to their immaturity.
7. **Budding:** The immature virions pinch themselves off the CD4 cell. Once outside the host cell, the immature virions release protease and HIV enzymes. The protease cuts off the long protein chains in the immature virions. This step serves to create the mature and infectious virus (Aiamkitsumrit et al., 2014a).

The virus infects active cells. However, HIV is able to remain latent within mainly memory $CD4^+$-T-cells but also in other cell types, where HIV persists in a silent state. This latent reservoir allows HIV to evade both the immune system of the host and antiretroviral treatment (ART) for several years (Palermo et al., 2019). Infected cells in the latent reservoir can activate at any time and can replicate. Contact with antigens during a common infection or during an opportunistic infection can activate these cells. Instead of

fighting against the given pathogen, they starts producing new HIVs (Aiamkitsumrit et al., 2014a).

1.6.1.2 Epidemiology and transmission

Epidemiological data about the geographical distribution and prevalence of HIV world-wide was mentioned earlier in the general introduction section. HIV is transmitted from human to human and accumulates in different body fluids. Table 1 shows the transmission probability based on organ and body fluid e.g., semen, blood, where HIV occurs. Table 1 outlines the estimated contribution to HIV cases worldwide in relation to the transmission route. Unprotected genital and anal sexual intercourse constitute the highest risk of HIV transmission, followed by bloodstream and MTCT (Robert-Koch-Institut, 2018a).

Table 1

HIV transmission

HIV invasion site	Anatomical sublocation	Body fluid	Transmission probability	Estimated contribution to HIV cases worldwide
Female genital tract	Vagina	Semen; blood	1 in 200 – 1 in 2 000	12.6 million
	Ectocervix			
	Endocervix			
Male genital tract	Inner foreskin	Cervicovaginal and rectal secretions; blood	1 in 700 – 1 in 3 000	10.2 million
	Penile urethra			
Intestinal tract	Rectum	Semen; blood	1 in 20 – 1 in 300	3.9 million
	Upper GI tract	Semen; blood	1 in 2 500	1.5 million
		Breast milk	1 in 5 – 1 in 10	960 000[c]
Placenta	Chorionic villi	Maternal blood (intrauterine)	1 in 10 – 1 in 20	480 000[c]
Bloodstream	/	Blood products, sharps	95 in 100 – 1 in 150	2.6 million

Reference: Shaw et al. 2012

1.6.1.3 Mother-to-child-transmission of HIV

If a HIV-positive pregnant woman is not under ART, the risk of MTCT of HIV ranges between 25 – 30% during pregnancy or delivery. Several factors can increase the risk of MTCT. Advanced clinical progression usually correlates with a higher viral load and a low CD4 count. The higher the viral load, the higher the risk of MTCT. The risk of MTCT may be reduced by an elective caesarean section at 38 weeks of gestation. An indication for a caesarean section in HIV-positive pregnant women is a high viral load and when the initiation of ART is too late to achieve full viral repression during vaginal delivery. Other known risk factors that increase MTCT of HIV are chorioamnionitis, ascending genital infection, placentitis, and systemic coinfections. The baby can be infected at three different stages: Prepartum (in utero), intrapartum and postpartum.

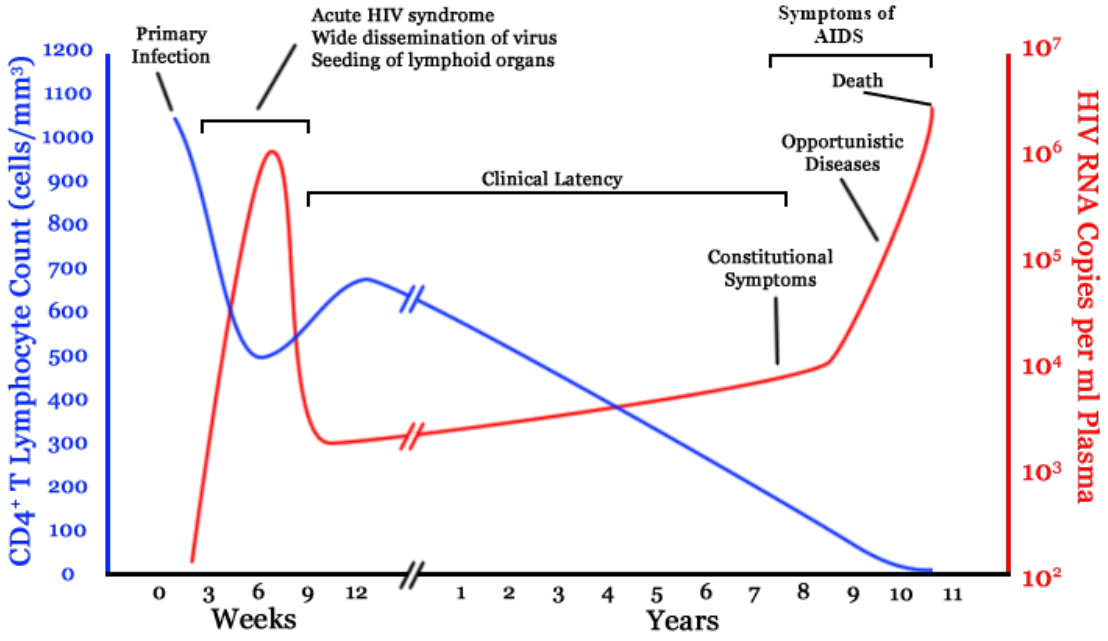
It is estimated that transmission of HIV in utero is 7.7 %. The risk of MTCT of HIV in utero and during intrapartum is estimated to be 17.6%. Late postnatal transmission is estimated to be 4.9%. Thus, the perinatal transmission rate is 22.5%. Transmission by breastfeeding is estimated to be 14% (Volmink and Marais, 2008).

1.6.1.4 Clinical aspects

The incubation period usually lasts 2 – 3 weeks and is characterised by unspecific symptoms. The time course and progression from the primary infection until the onset of AIDS, the decrease in CD4 counts, and the increase in viral load are shown in Figure 4.

Figure 4

HIV time course



Another categorisation of HIV is based on the presence of clinical symptoms. Table 2 shows the clinical staging of HIV according to the Centre for Disease Control (CDC), which is commonly used in combination with the CD4 count (Center for Disease Control, 1993). In addition, the CD4 count is stratified into three groups. The numbers (1, 2, 3) and the CD4 count indicate the severity of the acute stage, the latent and the AIDS stage

Table 2

CDC stages of HIV

Stage	CD-4+-T-cell-count per µl	HIV Stage A (acute)	HIV Stage B (latency)	HIV Stage C (AIDS)
1	≥500	A1	B1	C1
2	200-499	A2	B2	C2
3	<200	A3	B3	C3

Reference: CDC, 2021

HIV stage A is defined as an asymptomatic HIV infection (CDC, 2021). In this thesis, the American classification of CDC has been used. HIV stage A is characterised by a high viral load (>50 000 – 100 000 copies/ml) and high contagiousness (Robert-Koch-Institut, 2018). Some individuals in stage A may have a primary infection with unspecific symptoms that are described as flu-like symptoms, such as fever, lymphadenopathy, myalgias, exanthema, diarrhoea, and throat pain (CDC, 2014). Some individuals may be asymptomatic.

HIV stage B is characterized by some diseases that are not the AIDS-defining diseases but that are referred to an infection with HIV and are an indicator for a dysfunction of the cellular immune defense. In stage B HIV is still active but reproduces at a low level. The duration of stage B can last months to years and is influenced by several factors such as the age at transmission and being or not being on ART (CDC, 2014). At the end of stage B the viral load increases, the CD4 cell count decreases as shown in Figure 4, and the individual may develop symptoms. Unspecific symptoms that are not AIDS-defining symptoms like reduced general health, weight loss, subfebrile temperatures for more than one month, chronic diarrhoea for more than one month, and generalised lymphadenopathy can occur. If the individual is not placed on ART, then the infection will progress to stage C.

HIV stage C is also defined as acquired immunodeficiency syndrome (AIDS) characterized by AIDS defining diseases listed in table 3. AIDS is the most severe phase of HIV infection due to a damaged immune system. AIDS occurs much earlier in babies and children than in adults. Cohort studies have shown that without ART, 50% of individuals develop AIDS after ten years. Thus, AIDS is not only a consequence of untreated but also

insufficiently treated HIV infection. According to the CDC classification, one is diagnosed with AIDS when the CD4 cell count drops below 200 cells/mm^3 or if AIDS-defining diseases occur. Individuals diagnosed with AIDS usually have a high viral load and can be very infectious. Without treatment, individuals with AIDS typically survive about three years (CDC, 2021). Opportunistic infections such as thrush, herpes zoster, and the disseminated distribution with molluscum contagiosum can occur. Table 3 shows the AIDS-defining diseases, of which, to date there are 26 (Selik et al., 2014).

1.6.1.5 Diagnosis

There are different HIV testing algorithms. It is recommended that HIV testing is performed at two time points with a screening test and with a confirmatory test. Usually, enzyme-linked immunosorbent assay (ELISA) is used as a screening test; however, in some cases, an unspecific antibody binding can occur. Therefore, a second test (Western Blot), which is highly specific, is performed to confirm the specificity of the antibody binding to HIV viral antigens. If the result of the immunoblot is indeterminate, a third test, usually a Nucleic Acid Amplification test (NAAT), must be performed. If a result still cannot be confirmed, a second blood sample from the individual needs to be collected after one to three weeks.

When HIV testing is performed, the diagnostic window period needs to be considered (Figure 4). This period ranges from the timepoint from exposure until HIV can be detected in blood, depending on the test used, meaning tests performed during this period can be false-negative. The window period depends on the sensitivity of the applied diagnostic tool and can therefore differ from a few days up to three months after exposure. Specific antibodies occur on average 22 days after infection. The viral p24-antigen is already detectable on days 16 to 18 after infection. Viral RNA in the blood can already be detected 11 days after infection. These varying time points relative to the window period and testing sensitivities and specificities mean that exclusion of HIV transmission should take into account these factors.

Table 3

Stage 3 defining opportunistic diseases in HIV infection CDC

Infections	Bacterial infections
	Bacterial infections, multiple or recurrent*
	Mycobacterium avium complex or *Mycobacterium kansasii*, disseminated or extrapulmonary
	Mycobacterium tuberculosis of any organ, pulmonary§, disseminated or extrapulmonary
	Mycobacterium, other species, or unidentified species disseminated or extrapulmonary
	Salmonella septicaemia, recurrent
	Mycotic infections
	Candidiasis of bronchi, trachea, or lungs
	Candidiasis of oesophagus
	Coccidioidomycosis, disseminated or extrapulmonary
	Cryptococcosis, extrapulmonary
	Pneumocystis jirovecii pneumonia
	Histoplasmosis, disseminated or extrapulmonary
	Parasitic infections
	Cryptosporidiosis, chronic intestinal (> 1 month's duration)
	Toxoplasmosis of the brain, onset at age > 1 month
	Isosporiasis, chronic intestinal (> 1 month's duration)
	Viral infections
	Cytomegalovirus disease (other than liver, spleen, or nodes), > onset at age 1 month
	Cytomegalovirus retinitis with blindness
	Herpes simplex: chronic ulcers (>1 month's duration) bronchitis, pneumonitis, esophagitis, > onset at age 1 month
	Progressive multifocal leukoencephalopathy (Jacob-Creutzfeldt infection)
Cancer	Cervical cancer, invasive
	Kaposi sarcoma
	Lymphoma, Burkitt
	Lymphoma, primary, of brain
	Lymphoma immunoblastic
Other	Encephalopathy attributed to HIV
	HIV-associated wasting-syndrome
	Pneumonia, recurrent§

Reference: Selik et al., 2014

Note. * Only among children that are younger than six years old

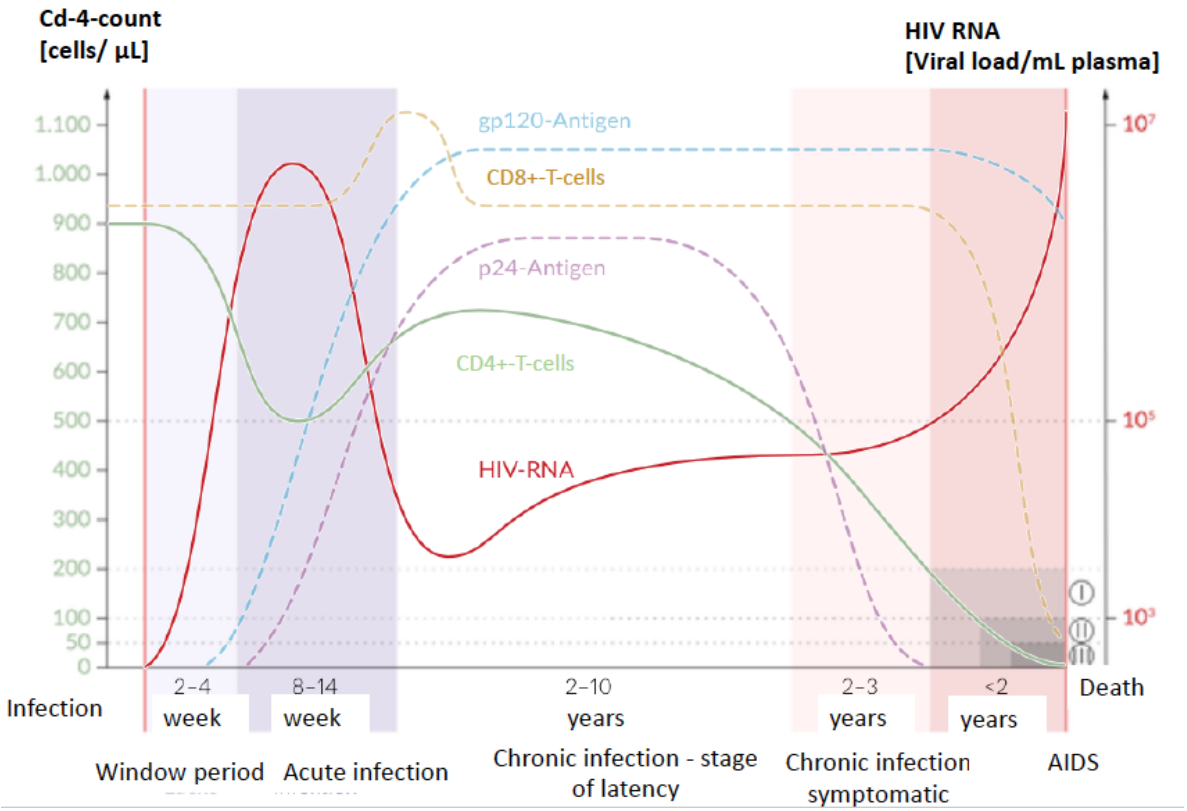
§ Only among, adults, adolescents, and children ≥ 6 years old

When HIV is diagnosed based on antibodies (3rd Generation ELISA), the window period usually takes 12 weeks from the transmission to diagnose or exclude an infection with HIV. Therefore, if an individual was exposed, and has been tested during the window period, a second test after the window period needs to be performed to exclude an infection with HIV. The CDC and the European guidelines recommend combined antigen-antibody assays for HIV testing. When using 4th generation tests that detect p24-antigens in addition to antibodies, the window period is considered to be six weeks (Robert-Koch-Institut, 2018). To differentiate between HIV-1 and HIV-2, HIV type-specific immunoblots or NAAT assays are used, as immunoblots are not specific to a type of HIV and therefore might provide false positive results due to cross-reactivity. However, these mentioned tests require a more sophisticated laboratory and qualified staff. HIV can also be diagnosed by rapid-diagnostic tests (RDTs), which can detect antibodies against viral HIV antigens. They are used in resource-limited settings or as self-tests at home in high-resource settings (Robert-Koch-Institut, 2018).

For the diagnosis of HIV-exposed babies younger than 15 months, NAAT is considered the diagnostic gold standard, as serological tests might be false-positive due to the maternal antibody transfer. Figure 5 shows the most important laboratory parameters during the different clinical stages of an untreated HIV infection. The viral load, defined as virus copies/ml plasma, is an important indicator of contagiousness, risk for transmission, development of viral resistance, and consecutive ART treatment failure (Robert-Koch-Institut, 2018). HIV positivity can be staged based on individual CD4 cell count. The lower the CD4 cell count, the higher the risk of getting AIDS-defining diseases. I, II, and III show the counts where AIDS-defining diseases occur. I (<200 cells/µl), *Pneumocystis jirovecii*, pneumonia; II (<100 cells/µl), cryptococcus meningitis, candidiasis, cerebral toxoplasmosis; III (<50 cells/µl), CMV-infection, atypical mycobacteriosis.

Figure 5

Diagnostic parameter and clinical stages of HIV/AIDS



Reference: Adapted from Amboss GmbH, 2022a

1.6.1.6 Antiretroviral treatment

The aim of ART is to achieve viral suppression and reduce HIV progression and transmission. ART is a lifelong treatment and must be taken every day. The WHO recommends ART initiation immediately after diagnosis, irrespective of CD4 cell count. Antiretroviral drugs have different targets based on viral replication and life cycle. Figure 6 shows the viral replication and life cycle of HIV and the target of different ART (World Health Organization, 2021).

- **Nucleoside reverse transcriptase inhibitor (NRTIs):** e.g Emtricitabine, lamivudine, abacavir, zidovudine, didanosin, stavudin.

  These treatments inhibit the reverse transcription of HIV-RNA by breaking off the nucleic strands after insertion in a newly synthesized DNA strand. DNA synthesis is stopped as a result.
- **Nucleotide reverse-transcriptase-inhibitor (NtRTI):** e.g Tenofovir

These have the same mechanism of action as NRTIs.

- **Non-nucleoside reverse-transcriptase-inhibitor (NNRTI**): e.g Rilpivirin, efavirenz, nevirapin, etravirin.

  Their aim is to stop viral replication by non-competitive inhibition of reverse transcriptase.

- **Protease-inhibitor:** e.g Darunavir, atazanavir, lopinavir, fosamprenavir, saquinavir, indinavir, ritonavir

  They inhibit the activity of catalytic protease, which is necessary for viral maturation.

- **Integrase-inhibitor:** e.g. Elvitegravir, raltegravir, dolutegravir.

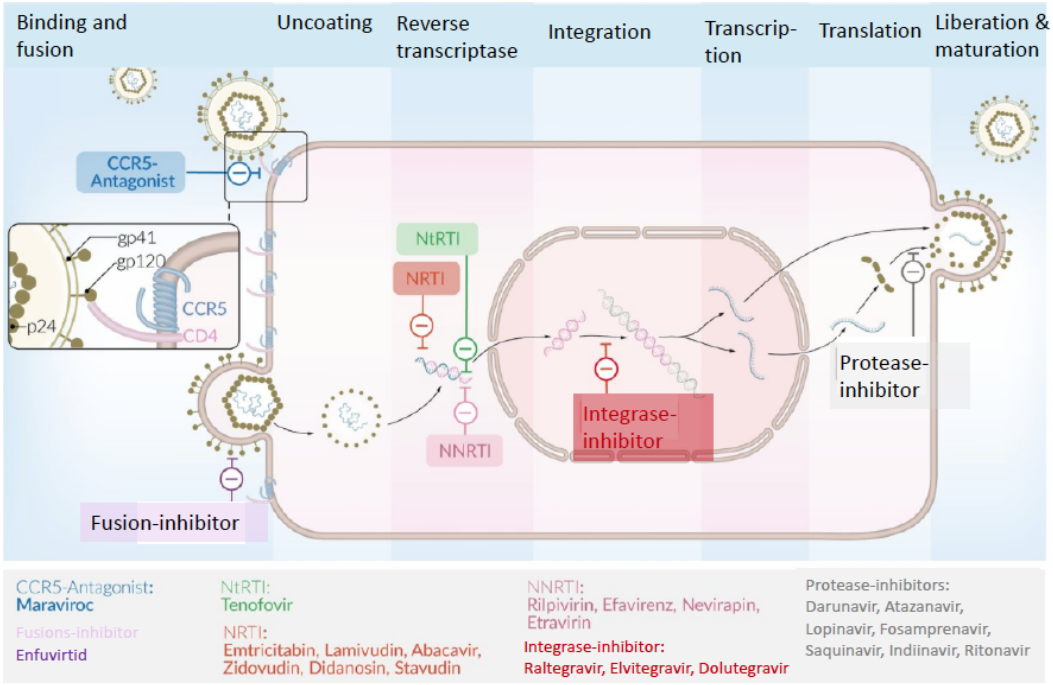
  Their function is to inhibit the integration of viral HIV-DNA in the genomic DNA of the host cell.

- **CCR5-inhibitor**: Maraviroc. CCR5-inhibitors inhibit fusion through the inhibition of the CCR5 co-receptor.

- **Fusion-inhibitor:** Enfuvirtide, fusion-inhibitor inhibits fusion through the inhibition of the glycoprotein gp41 (Herold, 2020).

Figure 6

Current HIV treatments



Reference: Amboss GmbH, 2022b

Dolutegravir is recommended by the WHO and currently remains the antiretroviral drug of choice. It is also a second-line ART among adults and adolescents as well as in individuals in whom a non-dolutegravir-based first-line regimen has failed. The WHO recommends that a boosted PI-containing regimen shall be used if the first-line treatment that failed contained dolutegravir (Thoden, 2014).

In addition to ART, cotrimoxazole (CTX), an antibiotic that prevents most HIV-associated opportunistic infections, is recommended in adults (including pregnant women). It should be initiated when the HIV infection is severe/advanced, defined as WHO clinical stage 3 or 4, and/or with a CD4 cell count ≤350 cells/mm^3. The CTX treatment can be stopped when individuals remain stable and have an immune recovery defined as virally suppressed on ART for at least six months and CD4 >350 cells/mm^3.

1.6.2 Human T-lymphotropic virus type-1

1.6.2.1 Biology

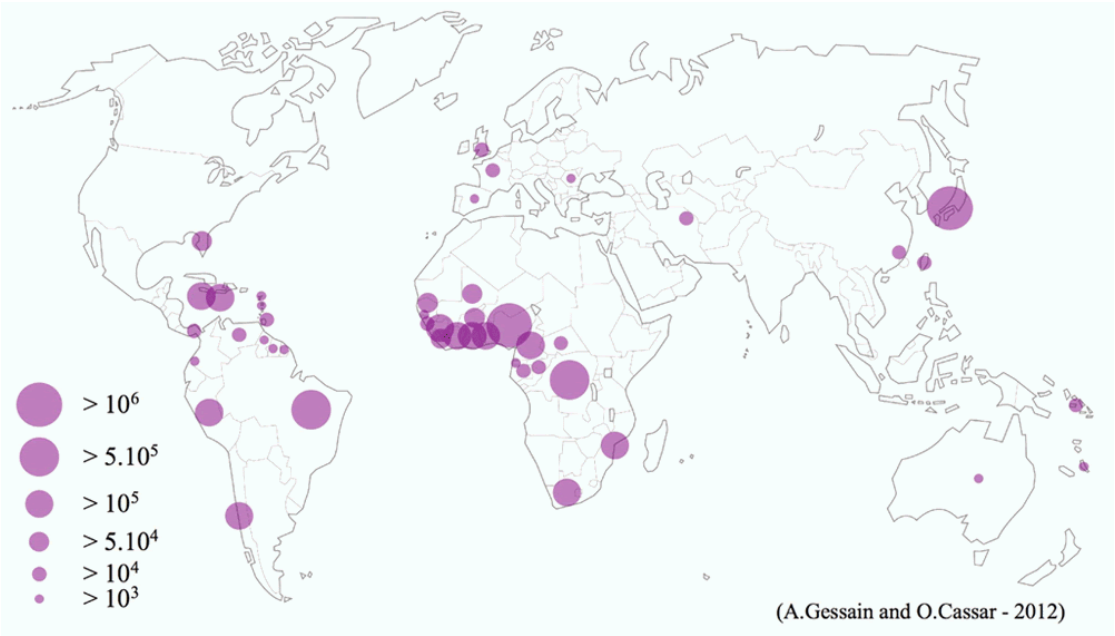
The human T-lymphotropic virus type-1 (HTLV-1), an oncogenic retrovirus, belongs to the genus of the *Deltaretrovirus* from the family *Retroviridae*. HTLV-1 is the first human retrovirus (Quaresma et al., 2015) to be described. It was discovered between 1980 and 1981 (Tagaya et al., 2019). HTLV-1 is a two-stranded RNA virus with reverse transcriptase and an integrase enzyme. These enzymes enable the virus to insert into the host genome and subsequently produce a provirus (Quaresma et al., 2015). The retroviral genes gag, pro/pol and env are inside the virus genome. Furthermore, the virus includes various other regulatory proteins involved in viral infection, transcription, proliferation and cell behaviour transformation (Quaresma et al., 2015). Target cells are principally CD4$^+$ T cells (Tagaya et al., 2019). To be infected with HTLV-1, a cell-to-cell contact is necessary, which means that a virion without having infected a cell cannot cause an infection.

1.6.2.2 Epidemiology

The estimated prevalence of HTLV-1 infection all over the world is shown in Figure 7 and affects about 5 000 000 – 10 000 000 people (Institut Pasteur, 2021).

Figure 7

Global distribution of endemic foci of HTLV-1 infection



Reference: Gessain and Cassar, 2012

The infection with HTLV-1 occurs especially in South-West Japan, the Caribbean, sub-Saharan Africa, and Central and South America (Tagaya et al., 2019). HTLV-1 can be transmitted vertically and horizontally; in endemic regions MTCT of HTLV-1 contributes significantly to prevalence of disease and perinatal infection accounts for 20 – 25% of all HTLV-1 infections. 15 – 20% of HTLV-1 infected mothers transmit HTLV-1 to their children. The risk of MTCT increases during prolonged breastfeeding (more than six months). Horizontal transmission pathways are through blood transfusions, organ transplantations and sexual intercourse. Overall, the prevalence of HTLV-1 is higher in women than in men (Tagaya et al., 2019).

1.6.2.3 Clinical aspects

HTLV-1 infection can cause several diseases, including inflammatory disorders like Sjögren's syndrome, uveitis, rheumatoid arthritis, dermatitis, and T-cell immunodeficiency that leads to bronchiectasis (Institut Pasteur, 2021; Quaresma et al., 2015). The major diseases caused by HTLV-1 are HTLV-1 associated myelopathy/ tropical spastic paraparesis and adult-T-cell-lymphoma. An infection with HTLV-1 leads in 3 – 8% of the cases to an adult-T-cell-lymphoma or HTLV-1 associated myelopathy/ tropical

spastic paraparesis and appears around 20 – 30 years after the primary infection (Institut Pasteur, 2021). Usually, individuals suffering from an aggressive type of lymphoma associated with HTLV-1, have 5% of abnormal lymphocytes, hypercalcemia, hepatosplenomegaly and enlarged lymph nodes. The tropical spastic paraparesis causes weakness, urinary, and anorectal incontinence and peripheric paraesthesia (Institut Pasteur, 2021; Quaresma et al., 2015).

1.6.2.4 Diagnosis

HTLV-1/2 is diagnosed by ELISA. If an individual has been tested positive, a confirmatory test with western blot needs to be done (Suerbaum et al., 2016).

1.6.2.5 Treatment

Currently mogamulizumab, a humanized anti-CCR4 monoclonal antibody, has been evaluated in clinical trials for the specific treatment of HTLV-1. In an uncontrolled phase 1-2a open-label trial conducted in the St. Marianna University Hospital in Kawasaki, Japan, Sato et al assessed the safety, efficacy, and pharmacokinetics of mogamulizumab. Mogamulizumab was shown to have an effect on the proviral load of HTLV-1 (Kim et al., 2018; Sato et al., 2018). Treatment of diseases caused by HTLV-1, such as adult-T-cell-lymphoma or HTLV-1 associated myelopathy/ tropical spastic paraparesis, is recommended (Institut Pasteur, 2021; Tagaya et al., 2019).

1.6.3 Hepatitis B virus

1.6.3.1 Biology

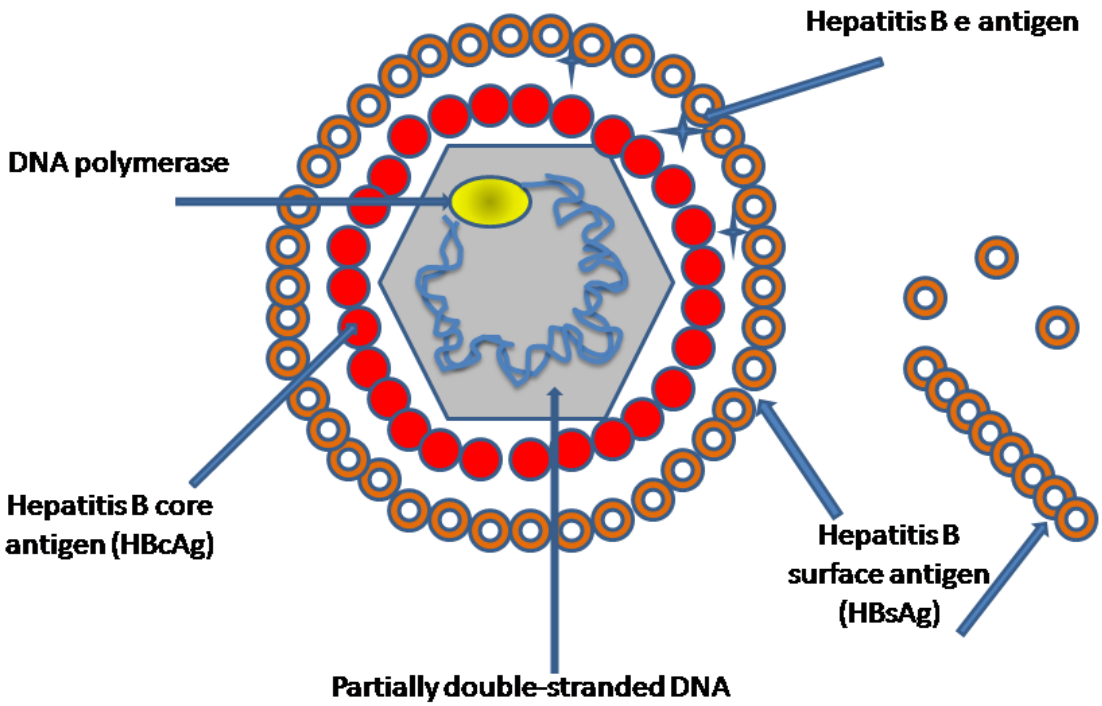
HBV is an enveloped hepatotropic and cancerogenic DNA virus belonging to the *hepadnavirus* family. There are ten different genotypes (A – J). HBV targets and replicates in hepatocytes. HBV is not a hepatotoxic virus and therefore does not injure the liver directly. The liver injury, resulting in liver inflammation, that may occur in diseased individuals is mainly caused by the host immune response.

The host immune response, that aims to clear HBV infection, is mainly composed of activated interferon-γ and tumour necrosis factor alpha, secreted from cytolytic $CD8^+$ T cells. The antigenic structures of the virus are used for diagnostic purposes. The genome of the virus encodes the outer surface protein, called HBsAg, the inner core protein, HBcAg, and the envelope protein HBeAg (Figure 8). HBsAg and HBeAg can be detected

in the serum, whereas HBcAg is resident in the nucleus of infected hepatocytes. Other direct virus markers are the presence of HBV DNA in human serum, a representation of the viral load, which is a quantitative marker to evaluate the viral replication (Yuen et al., 2018).

Figure 8

Hepatitis B virus



1.6.3.2 Epidemiology

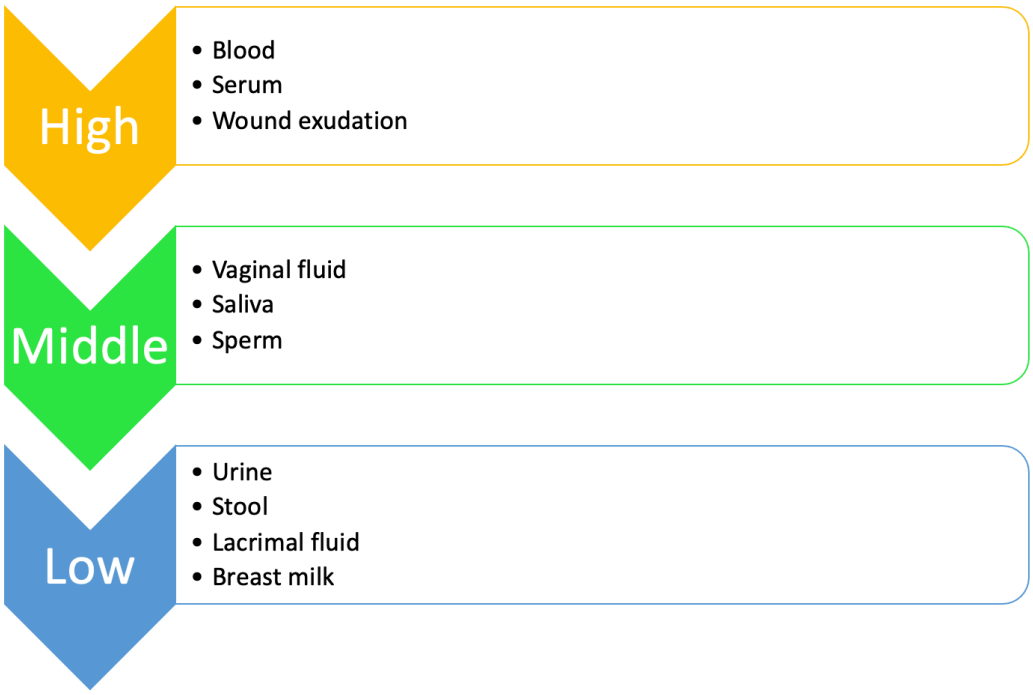
According to the WHO, 296 million individuals have chronic hepatitis B infection worldwide. The highest prevalence of hepatitis B infection is in the WHO Western Pacific Region and the WHO African Region, where 116 million and 81 million individuals have chronic hepatitis B infection (WHO, 2021a). The WHO estimates that in 2019, 820 000 individuals worldwide died from the complications of hepatitis B. The most frequent of these complications are liver cirrhosis and hepatocellular carcinoma (HCC) (WHO, 2021a).

The flow chart in Figure 9 shows the concentration of HBV in different body fluids. The highest concentration of HBV is detected in blood, serum, and wound secretion. Medium concentration is found in sperm, vaginal fluid and saliva (RKI, 2016). Most of the

transmissions of HBV occur through percutaneous or mucosal exposure to infected body fluids. MTCT of HBV plays an important role in sub-Saharan Africa. Transmission of HBV in utero is possible but remains scarce (Kim et al., 2021; Yuen et al., 2018). Children born to mothers with a high viral load and who are HBeAg seropositive are at great risk of perinatal infection, with progression to chronic disease in 90% of cases, if the child does not receive post-exposure prophylaxis (RKI, 2016). The risk of developing chronic hepatitis B infection is strongly associated with age. Children under five years infected with HBV are at high risk (20 – 60%) of developing chronic hepatitis B infection.

Figure 9

Flowchart for the concentration of HBV in different body fluids



Reference: based on the publication from Kidd-Ljunggren et al., 2006

1.6.3.3 Clinical aspects

The incubation period of HBV infection ranges from one to six months and is classified in an acute and a chronic course. An acute infection with HBV may lead to an acute hepatitis B with jaundice in one third of the infected individuals. Another third of the infected individuals experience an acute hepatitis without jaundice. One third remain infected and

asymptomatic (RKI, 2016). Symptoms during acute HBV infection may include weight loss, nausea, vomiting, concentration deficits, headache, arthralgia, respiratory tract infections, fever, and bradycardia. Individuals can be icteric, and in complicated cases, an acute HBV infection can lead to a fulminant hepatitis B, which is rare but life-threatening (Herold, 2020). Usually, acute HBV infection is self-limiting after three to six weeks with 90% of those infected achieving a total cure of the disease; however, the remaining 10% do progress to the chronic phase of infection. In immunocompromised individuals the risk of a chronic HBV infection increases from <10% to 30.9% (RKI, 2016). Chronic HBV infection is diagnosed when HBsAg is detectable in the serum for more than six months, irrespective of ongoing or arrested viral replication. Chronic HBV infection is an important driver for liver cirrhosis and HCC. Exogenic factors such as the genotype of the virus, life style (steatohepatitis), the consumption of alcohol and concomitant infection with HIV, hepatitis C virus and hepatitis D virus can increase the likelihood of disease progression and also the risk of developing HCC (Yuen et al., 2018). Among individuals with a high viral load, 10 – 20% describe different extrahepatic manifestations that are caused by immune complexes produced by viral antigens, antibodies to the virus, and complement factors. Neuritis, and peripheric polyneuropathy may occur. Vascular alterations such as polyarteritis nodosa, Raynaud syndrome, Sicca syndrome, uveitis, membranous glomerulonephritis can also occur (RKI, 2016).

1.6.3.4 Diagnosis and staging

In order to diagnose HBV infection, serology remains the first step for screening (Cornberg et al., 2021). Serological markers that can be used to diagnose acute or chronic HBV infection have been partially mentioned above, as these consist of components of the virus and the host response to the virus. These markers are HBsAg and HBeAg, and the antibodies against these markers include antibodies against HBcAg. Table 4 shows the different serological markers that are common in an acutely infected individual, chronically infected individual, vaccinated persons and other scenarios. The principal marker of both acute and chronic HBV infection is HBsAg. In addition, liver transaminases are elevated during acute and chronic infection.

Table 4

Overview of the different serological markers according to the infection phase of the individual

HBV markers	HBsAg	Anti-HBs	Anti-HBc-IgM	Anti-HBc-(total)	HBeAg	Anti-HBe	HBV-DNA
Acute infection	↑	/	↑↑	↑	↑	/	↑
Recovered	/	↑	/	↑	/	↑	/
HBV-vaccinated	/	↑	/	/	/	/	/
Chronic infection	↑	/	/	↑	(↑)	(↑)	↑
Low viremia HBsAg carrier	↑	/	/	↑	/	↑	↑
High viremia HBsAg carrier	↑	/	/	↑	↑	/	↑

Clinical and diagnostic meaning of different viral antigens:

- HBsAg = surface antigen, acute or chronic infection, carrier,
- HBeAg = envelope antigen, high viral replication,
- HBcAg = core antigen, only detectable in liver parenchyma (biopsy),
- HBV-DNA = viral replication,
- Anti-HBc = cured infection,
- Anti-HBc IgM = acute infection,
- Anti-HBs =cured infection or vaccinated,
- Anti-HBe = viral replication has ended

HBeAg/Anti-HBe: HBeAg shows high viral replication and thus high contagiousness. The simultaneous presence (seroconversion) of HBeAg and Anti-HBe means that viral replication is decreased, corresponding to a decreased viral load and therefore a better clinical outcome. PCR is used for virological evaluation and determination of contagiousness. HBV DNA concentration correlates with disease progression (Cornberg et al., 2021). Furthermore, HBV DNA concentration allows differentiating active HBeAg-negative infection from inactive chronic infection. This allows for the evaluation of treatment options and treatment response and continuous monitoring of disease evolution (Cornberg

et al., 2021). Clinical evaluation is crucial in order to fully evaluate the severity of infection, clinical outcomes, and complications such as liver cirrhosis and decompensation (Cornberg et al., 2021).

1.6.3.5 Treatment

Treatment for acute infection with HBV is usually not indicated as it is generally self-limiting: however, an acute fulminant hepatitis B infection needs to be treated early. The best way to prevent HBV infection is via vaccination, which has high efficacy and good tolerability (RKI, 2016). An individual that is an asymptomatic HBV carrier may not be treated but kept under surveillance for chronic HBV infection. Treatment for chronic hepatitis B, is indicated based on the levels of HBV DNA and alanine-aminotransferase, and the severity of liver disease. The level of HBV DNA that indicates treatment varies from region to region. Treatment aims are to reduce morbidity and mortality. An individual can be treated with regimens that include seven antiviral agents: lamivudine, adefovir, entecavir, telbivudine, tenofovir, emtricitabine belonging to the nucleotides and nucleosides analogues; and pegylated (PEG) interferon (IFN) alpha. PEG-INF alpha is highly potent against HBV as it does not cause viral resistance and achieves higher rates of HBeAg and HBsAg clearance. PEG-INF alpha is not accessible in every country due to its high costs. The disadvantage of PEG-INF alpha apart from the high costs is that it has several contraindications, and side effects. PEG-INF alpha is contraindicated in including pregnant women, in individuals with decompensated liver cirrhosis, various diseases that target the immune system, the haematological system, psychiatric illness, retinopathy, concomitant use of certain drugs. Nucleotides and nucleosides analogues target the viral polymerase and inhibit viral replication. They are highly effective but rarely lead to full cure, which indicates the necessity for lifelong treatment. Tenofovir is a nucleotide analogue with high potency against HBV, low susceptibility to resistance, and cheap, and is also known and used in the first-line treatments of HIV (Cornberg et al., 2021). Furthermore, it can also be used in pregnant women (Kim et al., 2021).

1.6.4 Hepatitis C virus

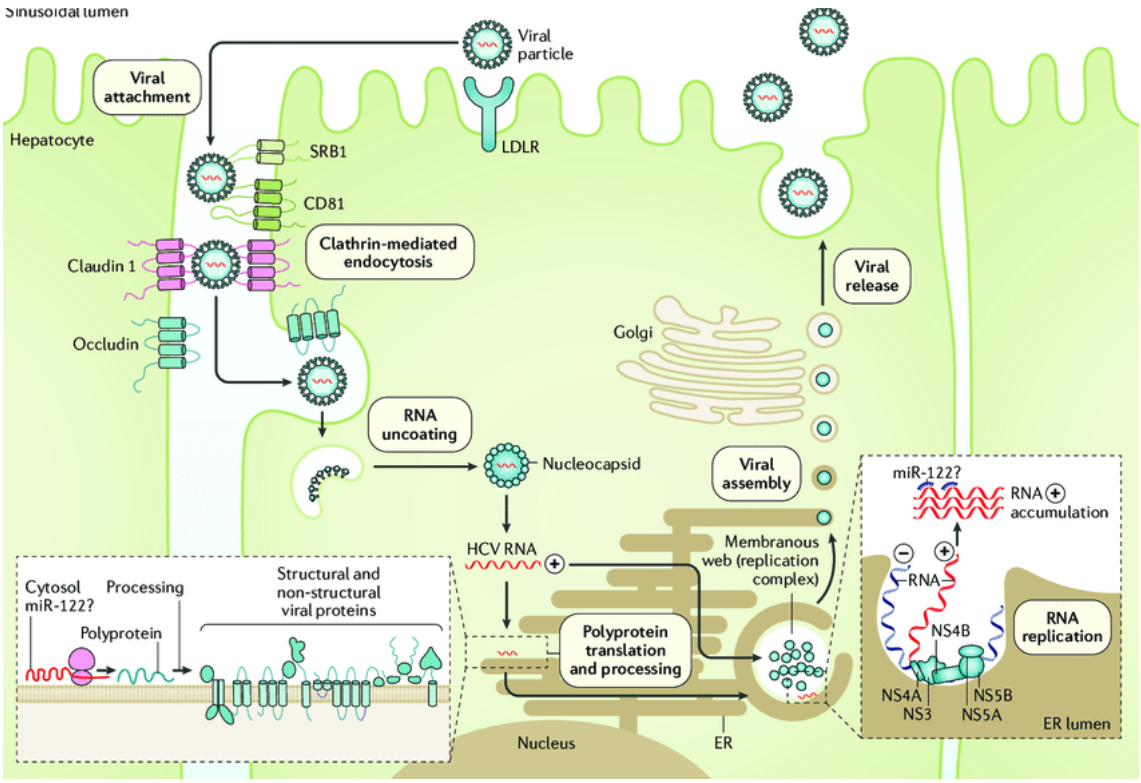
1.6.4.1 Biology

HCV is a hepatotropic positive single-stranded RNA virus with a lipid bilayer and belongs to the genus *Flaviviridae* (Manns et al., 2017). There are seven different genotypes

of HCV (Manns et al., 2017). The nucleotide sequences of the different genotypes differ by more than 30% from each other, having more than 100 subtypes. HCV is known for high variability with different viral quasi-species in the same individual. The high variability is generated during replication by the viral RNA-dependent RNA polymerase, which has no tool for correction during replication. It is thought that the high genetic variability of HCV enhances the rate of chronic infection and the failure of a vaccine and treatment. A high replication rate is also the reason why the adaptive immune systems of the host struggle to produce an effective immune response (Manns et al., 2017).

Figure 10

HCV life cycle and different steps of viral replication



Reference: Manns et al., 2017, endoplasmic reticulum; LDLR: Low-density lipoprotein receptor; miR: microRNA

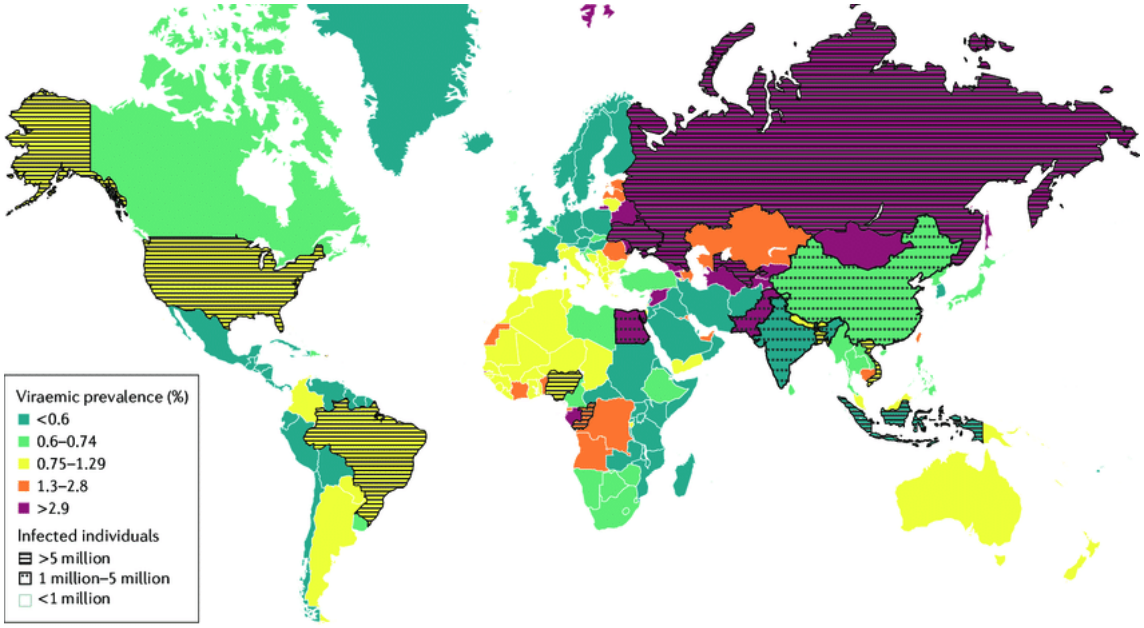
The viral replication of HCV is shown in Figure 10. It begins with the attachment of the virus to the host cell before its invasion. The envelope glycoproteins E1 and E2 apolipoproteins, which are on the surface of the lipoviro particles and various cell surface structures, are necessary for the viral attachment. The viral glycoproteins E1 and E2 interact with CD81 and scavenger receptor class B member 1 of the host cell (Manns et al., 2017).

After entering the host cell by endocytosis, viral and endosomal membranes merge and liberate the nucleocapsid into the cytoplasm, followed by an uncoating of the viral RNA. Consequently, the viral RNA is released into the cytosol. The virus uses the organelles (ribosomes) of the host cell for viral replication (Manns et al., 2017). After viral RNA replication, different viral particles are built together, followed by viral maturation and then the liberation of the virus (Manns et al., 2017). The virus circulates in the blood of infected persons in different forms, either as a free virus particle or bound to different proteins, such as low-density lipoproteins, very low-density lipoproteins, and immunoglobulins. Outside the organism, HCV cannot survive for a long time because of its fragility and susceptibility to an ambient temperature of 37°C. Besides its hepatotropic properties, HCV can also invade lymphocytes, kidney cells, glial cells, and dendritic cells (Manns et al., 2017).

1.6.4.2 Epidemiology

Figure 11

HCV prevalence: Viraemic prevalence in 2017 of HCV per country



Reference: Manns et al., 2017

Ninety-two to one-hundred-fifteen million individuals, 1.3 – 2.1% of the global population, have anti-HCV antibodies present in their blood. However, of these individuals, not all have HCV infection; some may have cleared the virus spontaneously or due to treatment. The global prevalence of HCV infection is estimated to range between 0.8 – 11% (62 –79 million individuals). The estimation is based on studies that have been conducted in 100 countries. The prevalence of HCV infection is particularly high in countries that had or still have a high rate of iatrogenic infections. The following countries have a high iatrogenic infection rate: Cameroon, Egypt, Gabon, Georgia, Mongolia, Nigeria, and Uzbekistan. In these countries, the antibody prevalence is with >5% in adults high. In Egypt, HCV was significantly transmitted during the intravenous treatment for schistosomiasis in the sixties and seventies. In high-income countries, the prevalence of HCV infection remains small in comparison to China, Pakistan, India, Egypt, and Russia, where approximately half of the total viraemic HCV infections occur. The age distribution among individuals infected with HCV depends on the mode of transmission in a country. Horizontal transmission is related to drug abuse and occurs most frequently in Australia, the

Czech Republic, Finland, Luxembourg, Portugal, Russia, and the UK, where the average age of the infected population corresponds to the mid-thirties. The average age in countries where iatrogenic transmissions are the major source of transmission currently ranges between 50 to 60 years. Therefore, the peak in terms of age in countries with iatrogenic infections, is higher (Manns et al., 2017).

1.6.4.3 Clinical aspects

The incubation period of the virus is about eight weeks. An acute infection with HCV is mostly asymptomatic; however, a few individuals develop jaundice, fatigue, nausea, vomiting, abdominal pain on the right upper side, or discomfort or arthralgia and myalgia (Herold, 2020). Fulminant courses with liver failure are scarce (Herold, 2020). The risk of progression to chronic hepatitis C infection in individuals who are symptomatic is lower than in individuals who have an acute HCV infection and are asymptomatic. Seventy-five to eighty-five percent of infected individuals with acute HCV infection progress to the chronic stage of infection. Among these individuals, a subgroup will progress to liver cirrhosis, among whom some will further progress to HCC. Before the onset of the long-term complications caused by chronic infection with HCV, these individuals may suffer from fatigue, right upper abdominal pain, weight loss, muscle and joint pain, discomfort, or itching. Risk factors for a rapid progression of chronic hepatitis C infection include coinfections with other viruses such as HBV, HIV or other pathogens such as an infection with *Schistosoma* spp.; and demographic aspects such as age and male sex, and alcohol consumption (Manns et al., 2017). In addition, the outcome of symptomatic infection depends on the HCV genotype. Infection with HCV genotype three is thought to have a worse clinical outcome due to its increased virulence and the increased progression to hepatocellular carcinoma in comparison to other genotypes (Manns et al., 2017).

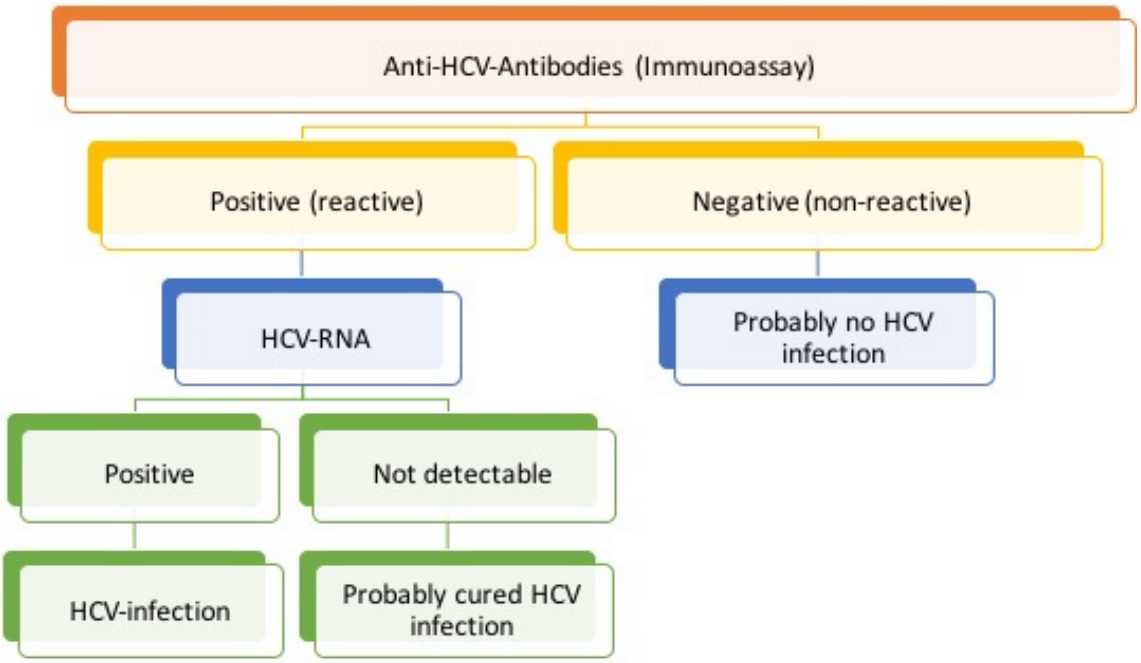
1.6.4.4 Diagnosis

Infection with HCV should be suspected when individuals have elevated levels of liver enzymes such as alanine aminotransferase and aspartate aminotransferase. Increased levels of liver enzymes are an indicator of increased liver cell death and damage. Figure 12 shows the algorithm for screening for HCV infection based on anti-HCV-antibodies. When anti-HCV antibodies occur in a blood sample, confirmation should be provided by

HCV RNA with NAAT or the determination of HCV core antigen to distinguish an active infection from a past infection.

Figure 12

HCV screening: Algorithm of HCV screening based on immunoassay or rapid-diagnostic tests



When antibodies of HCV are present, but HCV-RNA is negative, the individual has either been cured or the antibody result is a false positive one. It is also possible that HCV RNA is positive and HCV-antibodies are negative because seroconversion has not yet occurred. In this case, a second test should be done a few weeks later. With this diagnostic approach, a chronic infection cannot be distinguished from an acute infection based on initial testing. If HCV RNA persists for more than six months and HCV antibodies are also persistent, then an individual is considered to have a chronic HCV infection. It is preferable to determine the genotype or subtype for an appropriate treatment regimen ("EASL Recommendations on Treatment of Hepatitis C 2015," 2015).

1.6.4.5 Treatment

The aim of the treatment is to ensure the permanent absence of HCV-RNA in the serum. Individuals with confirmed HCV infection are eligible for antiviral therapy with direct-acting antiviral agents (DAA). The treatment regimens based on DAA combine different

agents and exclude INF alpha and ribavirin due to their side effects. DAA, like sofosbuvir and ledipasvir, are the antiviral drugs of choice in all HCV infected individuals. In more than 90% of cases, individuals can be cured with DAAs. When an individual is infected with HCV genotypes 1, 4, 5, and 6, sofosbuvir plus ledipasvir should be given for 8, 12, or 24 weeks. If the individual is infected with genotype 2 and/or 3, sofosbuvir plus velpatasvir should be given for 12 to 24 weeks. Individuals with genotype 3 are very difficult to treat. If the individual experiences decompensated liver cirrhosis or has obtained a liver transplantation, treatment is given irrespectively of genotype with sofosbuvir plus velpatasvir or sofosbuvir plus daclatasvir for 12 or 24 weeks. As an alternative for individuals with compensated liver cirrhosis and treatment failure, pegylated (PEG)-interferon (INF) can be administered subcutaneously and ribavirin should be given for 16 to 24 weeks. Individuals with genotype 1 are more difficult to treat with PEG-INF (Manns et al., 2017).

The absence of HCV-RNA three months after the end of the treatment is defined as a sustained virologic response.

1.6.5 Hepatitis E virus

1.6.5.1 Biology

Hepatitis E is a liver disease caused by HEV. HEV is a single plus-stranded RNA virus without a core, belonging to the species *Orthohepevirus A*. The viral genome can be infectious (Koch-Institut, 2015). Four genotypes of the humane pathogen HEV exist (HEV-1, HEV-2, HEV-3, HEV-4) (Koch-Institut, 2015).

1.6.5.2 Epidemiology

According to the WHO, one-third of the world's population (> 2 billion) live in endemic regions and are at risk of infection with HEV. Young adults are mostly affected. The geographical presence of genotypes varies. HEV genotype 1 and HEV genotype 2 occur mainly in developing and emerging countries in Asia and Africa. In these regions, the seroprevalence of HEV-IgG can be higher than 50% in the adult population. HEV is prevalent in countries with limited resources and low standards of hygiene, like in Southeast Asia, the Middle East, India, Central Asia, and Central and South America. It is transmitted through contaminated water via the faecal-oral transmission pathway. HEV-3 and

HEV-4 are present in various industrialised countries. HEV-4 sometimes occurs in Asia and can be transmitted by animals. HEV-1- and HEV-2, HEV-3, and HEV-4 are transmitted by humans. They are found in humans but also in animal products like pork meat and game (Koch-Institut, 2015). Table 5 summarizes the different genotypes and their prevalence in different parts of the world (Koch-Institut, 2015).

Table 5

Genotypes of HEV in the world

Characteristics	HEV in Africa and Asia	HEV in industrialised countries
HEV genotypes	HEV-1 and HEV-2	HEV-3 and HEV-3
Spread	epidemic and sporadic	sporadic (autochthonous)
Source of infection	water	zoonotic transmission (especially pork meat and blood products)
Fulminant hepatitis among pregnant women	frequent	scarce
Chronic hepatitis	not described	usually among immunosuppressed individuals

Reference: translated from Koch-Institut, 2015

1.6.5.3 Clinical aspects

The incubation period of an infection with HEV ranges between 15 – 64 days and is typically clinically indistinguishable from an infection with Hepatitis A virus. An acute infection with HEV varies clinically and can be subclinical, asymptomatic, and in some rare cases, fulminant (0.5% – 4%). Of those who are infected with HEV, 5% – 20% are symptomatic and are mostly aged between 14 – 40 years. An infection with HEV can be controlled by the immune system if the individual is immunocompetent without any sequelae. Typical symptoms of symptomatic infection with HEV are mild fever, loss of appetite, abdominal pain, itching, skin rash, jaundice, dark urine, pale stool and hepatomegaly. Chronic infection with HEV is defined as the presence of HEV for more than six months in the blood (Koch-Institut, 2015). Chronic infection with HEV usually occurs when an individual is immunosuppressed, such as individuals who have received an

organ- or bone marrow transplantation (Koch-Institut, 2015). Individuals with HIV are also at increased risk of developing chronic infection with HEV. However, to date, only HEV-3 and HEV-4 are described to cause chronic infection with HEV (Koch-Institut, 2015).

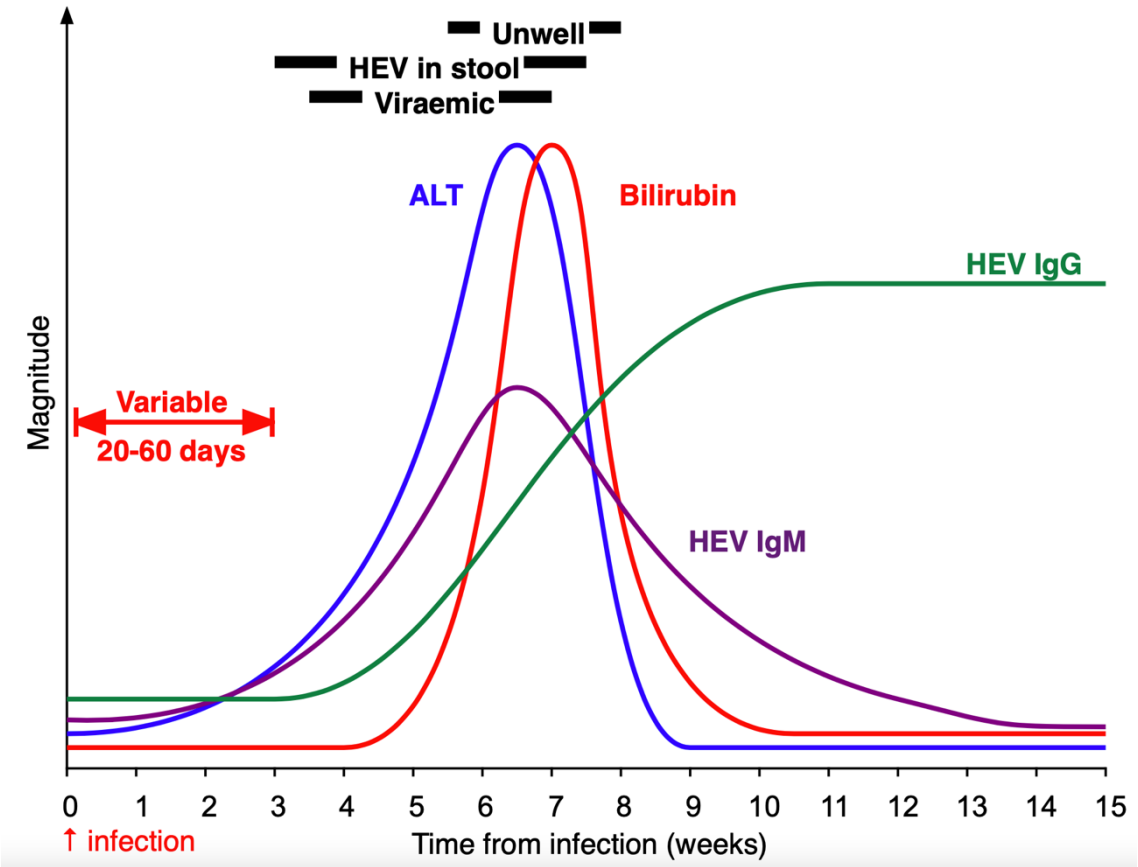
Individuals with liver disease and pregnant women are more likely to die of hepatitis E. The mortality rate of acute symptomatic infection with HEV during pregnancy is between 25 – 30% (Koch-Institut, 2015). Studies have shown that acute symptomatic infection with HEV during pregnancy does not only lead to higher mortality but also leads to more severe disease outcomes, adverse birth outcomes like miscarriages, premature births, low birth weight, stillbirth, and perinatal mortality (Koch-Institut, 2015). Mostly HEV-1 and HEV-2 have been observed to result in adverse birth outcomes. There is evidence that HEV can replicate in human placenta tissue, which was correlated with an increased mortality rate of mother and child. Reasons for a more severe acute symptomatic infection with HEV in pregnant women seem to be multifactorial: Environmental factors, different pathogenicity of the different genotypes, social factors, etc. Also, the direct effect of hormones on the viral replication of HEV or the direct impact on immune cells have been discussed but data are scarce (Koch-Institut, 2015).

1.6.5.4 Diagnosis

During infection, liver transaminases (alanine aminotransferase and aspartate aminotransferase), bilirubin, alkaline phosphatase, and gamma-GT are elevated. The liver enzymes normalise two to four weeks after recovery. For serological diagnosis, ELISA is commonly used, and a molecular test (RT-PCR) should be added. HEV RNA can be detected until two to four weeks in blood and until six to eight weeks in stool after the onset of clinical symptoms. The screening with PCR for HEV allows the detection of viral RNA before the onset of clinical symptoms in the early phase in stool and serum. Furthermore, HEV can also be determined serologically by the detection of IgG-antibodies and IgM, which has a sensitivity of up to 99%. Figure 13 shows the evolution of transaminases, the presence of antibodies and HEV RNA at different time points in different samples during the course of infection (Koch-Institut, 2015).

Figure 13

Presence of liver enzymes and HEV infection time course



Currently, there is no specific treatment recommended, that has a relevant impact on the course of acute symptomatic infection with HEV. Hospitalisation is not necessary, as acute infection with HEV is self-limiting. Hepatotoxic medication should be avoided or used sparsely if necessary. However, individuals with chronic infection with HEV, who are immunosuppressed, profit from treatment and can be treated with nucleoside-analogue, such as ribavirin. However, some individuals remain HEV-RNA-positive after antiviral treatment (Koch-Institut, 2015).

1.6.6 Human papillomavirus

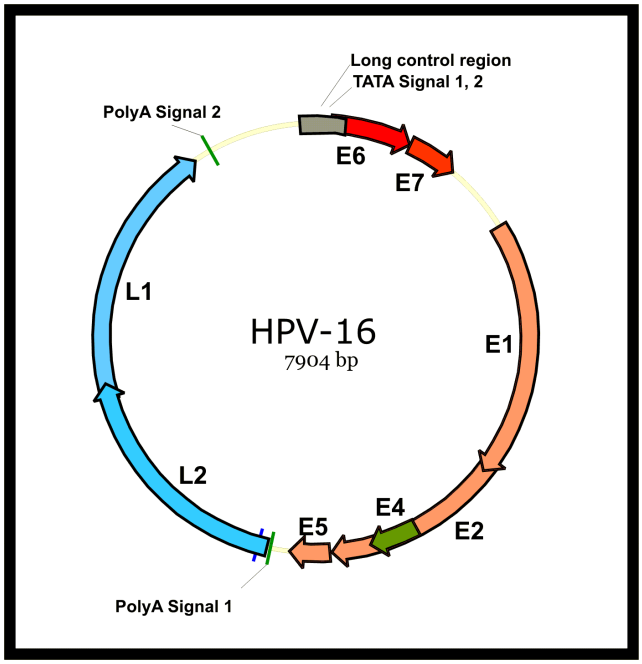
1.6.6.1 Biology

HPV is a small, double-stranded DNA-virus belonging to the family of *Papillomaviridae.* More than 200 viral subtypes have been described to date. As the virus is oncogenic, it is usually divided into high-risk subtypes and low-risk types. HPV subtype 6, 11, 42, 43,

54, 57, 70, 72, and 90 are low-risk subtypes. They are known to cause benign genital lesions, which are usually genital warts (Moody and Laimins, 2010; Robert-Koch-Institut, 2018b). According to the WHO, 12 HPV types are known to be high-risk: Subtype *16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59* (Koch-Institut, 2018). HPV subtypes 16 and 18 are the subtypes most frequently found in up to 70% of all HPV-related malignancies (Moody and Laimins, 2010; Robert-Koch-Institut, 2018b). An infection with high-risk HPV subtypes is a significant but insufficient cause of malignancy. Further determinants that result in genomic instability caused by mutations in cellular genes and chromosomal rearrangements are crucial. As low-risk HPV subtypes can also cause intraepithelial neoplasia, the strong differentiation between low and high-risk HPV subtypes is nowadays obsolete. E5, E6 and E7 are the principal viral oncoproteins of HPV and are shown in Figure 14. These oncoproteins are crucial for oncogenesis. HPV targets keratinocytes in the basal layer of the epithelium (Moody and Laimins, 2010; Robert-Koch-Institut, 2018b).

Figure 14

HPV-16 genome organization



Note. Early genes: E1 – Replicaton, E2 – Replication and transcription, E4- Viral release, E5 – Immune evasion, E6 – Binds p53, E7 – Binds pRB

Late genes: L1 – Major capsid protein, L2 – Minor capsid protein

URR: Promoter and enhancer elements

1.6.6.2 Epidemiology

Humans are the only reservoir for the virus (Koch-Institut, 2018). HPV infection occurs worldwide in women and men. It is estimated that HPV infection is one of the most frequent STIs. HPV infection is usually transmitted by vaginal, oral, or anal sex but can also be transmitted from mother to child during birth. Most individuals who are sexually active get infected at least once in life, usually after the first sexual intercourse.

It is possible that individuals get infected by more than one HPV subtype (Koch-Institut, 2018). Common risk factors for infection with HPV are a high number of sexual partners during life, homosexuality, oral and anal sex, consumption of cigarettes, genital infectious diseases, and immunosuppression caused by HIV and chemotherapies (DGGG, 2010). The most common cancer induced by HPV is cervical cancer, which is the fourth most common cancer in women worldwide. The WHO estimates that 342 000 deaths out of 604 000 new cases of cervical cancer occurred in 2020. The WHO estimates that 95% were linked to HPV in 2020. According to the WHO, 90% of the global burden of cervical cancer is in developing regions (WHO, 2022). In addition, infection with high-risk HPV subtypes is linked to penile, vulvar, vaginal, and anal carcinoma and oral cancer (Moody and Laimins, 2010).

1.6.6.3 Clinical aspects

Individuals with infection with HPV may be asymptomatic. In this paragraph clinical aspects of symptomatic HPV infection are described.

1.6.6.3.1 Low-risk HPV subtypes

The estimated incubation period until genital warts may appear is around two to three months but can also appear between two weeks and eight months. Individuals may have benign exophytic skin lesions in the anogenital region. First, they appear as single lesions and then in disseminated groups. Initially, they can be accompanied by pruritus but pain is scarce. Oral papilloma can occur in babies and people having oral sex. Low-grade intraepithelial neoplasia grade 1, can be caused by high and low-risk subtypes. They are flat, white, and brownish, lightly exophytic, and disseminated skin lesions in the vulva and anal region. They have a little risk of becoming an epithelial carcinoma and usually a high rate of spontaneous remission (Suerbaum et al., 2016).

1.6.6.3.2 High-risk HPV subtypes

A persistent infection with high-risk HPV subtypes is asymptomatic. The time it takes for an infected individual with high-risk HPV subtypes to develop high-grade cervical dysplasia ranges between three and six years. The period between the presence of a high-grade cervical dysplasia and an invasive carcinoma is about 10 to 30 years (Koch-Institut, 2018). HPV associated carcinomas are listed below:

- Cervical intraepithelial neoplasia (CIN) → cervical carcinoma
- Vulvar intraepithelial neoplasia (VIN) → vulvar carcinoma
- Vaginal intraepithelial neoplasia (VAIN) → vaginal carcinoma
- Anal intraepithelial neoplasia (AIN) → anal carcinoma
- Penile intraepithelial neoplasia → penile carcinoma
- Oral carcinoma in the mouth, throat, and larynx area.

A rare complication is the giant condyloma acuminatum, also called Buschke-Löwenstein tumor, which is usually caused by HPV subtypes 6 and 11. It is a strong, exophytic, aggressively growing wart that is a verrucous carcinoma (Gross G.E., 2018).

1.6.6.4 Diagnosis

Early diagnosis of HPV infection is crucial. To better determine HPV infection, current tests have mostly been based on the PCR method, which allows precise detection of HPV, including the determination of HPV subtypes. Further methods are clinical diagnosis by inspection and palpation of the genital region. In women, a gynaecological examination, including the Papanicolaou smear, also called a PAP test, is used in routine screening and is the method of choice for the early detection of cervical cancer (DGGG, 2010). The PAP test is based on the presence of cell differentiation. A suspicious PAP smear, however, requires further diagnostic testing for HPV DNA by PCR. A limitation of the PAP test is the fact that the false positive and false negative rates are high (Suerbaum et al., 2016).

1.6.6.5 Treatment

Depending on their spread, size, and localisation, genital warts can be self-treated with a cream, solution, or a salve, containing the agents' podophyllotoxin 0.5%, imiquimod 5%,

or sinecatechine 10% by adults. Alternatively, genital warts can be treated by electrocauterization, curettage, laser therapy, surgical interventions, 80 to 90% trichloracetic acid, or cryotherapy (Koch-Institut, 2018). There is no treatment for infections with high-risk HPV subtypes. When an individual has a precancerous lesion, conisation of the cervix can be done. HPV-induced cancers are treated according to national guidelines.

The use of condoms does reduce but does not completely protect from an infection with HPV (Moody and Laimins, 2010; Robert-Koch-Institut, 2018b). To prevent HPV infection, especially HPV-induced cancers, a vaccine has been introduced and is available for girls and boys. There are three vaccines. The vaccines lead to a high level of neutralising, type-specific antibodies by producing a strong B cell-mediated immune response. All vaccines must be given in three injections in a time frame of six months to gain a stronger immune response than a natural HPV infection. This leads to long-term immunity (Koch-Institut, 2018).

1.6.7 Plasmodium spp.

1.6.7.1 Biology

Plasmodium (P.) spp. is the pathogen that causes malaria in humans and is transmitted by an infected *Anopheles* spp. mosquito (CDC, 2019). Sexual reproduction of the parasite occurs whilst it is in the female *Anopheles* mosquito. The transmission of *Plasmodium* spp. occurs when the mosquito bites its host during a blood meal (Hafalla et al., 2011; White et al., 2014). The parasite undergoes several maturation steps. The sporozoites infect hepatocytes, where they mature into schizonts. The liver schizonts rupture and release merozoites into the blood, where the merozoites then infect erythrocytes, thereby commencing the erythrocytic stage of the disease. The parasites multiply via asexual reproduction within the erythrocytes. Some parasites undergo further differentiation and become gametocytes, which is the sexual stage of the parasite. The blood stage parasites cause the typical malaria symptoms (White et al. 2014). When the infected individual gets bitten by an *Anopheles* mosquito, the mosquito ingests both female and male gametocytes, resulting in the infection of the mosquito. Five species of *Plasmodium* are currently known to cause infection in humans: *P. falciparum* which causes the dangerous malaria tropica; *P. vivax*; *P. ovale wallikeri* and *P. ovale curtisi* cause malaria tertiana; *P. malariae* causes malaria quartana; and *P. knowlesi* (White et al., 2014).

1.6.7.2 Epidemiology

In this paragraph, epidemiological data about the diseased form of *Plasmodium* infection are provided. According to the WHO, 241 million cases of malaria occurred in 2020 in 87 countries, an increase in the number of cases when compared to the 227 million cases that occurred in 2019. The WHO also estimates that malaria accounted for 627 000 deaths in 2020 compared to 558 000 cases in the previous year. The disruptions caused by the COVID-19 pandemic were the principal reason for the higher number of deaths in 2020 (WHO, 2021b). The burden of disease is especially high in sub-Saharan Africa, where 93% of global malaria cases and malaria-associated deaths occur. Pregnant women are at higher risk of being infected with *Plasmodium* spp, developing disease and severe malaria. Malaria plays a major role in maternal and infantile mortality and morbidity (Jäckle et al., 2013).

1.6.7.3 Clinical aspects

Infection with *Plasmodium* spp. may be asymptomatic. *Plasmodium* spp. infection may lead to malaria, characterized by recurring fever, with the period of recurrence dependending on the *Plasmodium* species. The fever corresponds to the erythrocytic stage of infection, with each period of rupture of infected erythrocytes corresponding with a spike in temperature of the human host (White et al., 2014). Fever appears on every third day for tertian malaria, and every fourth day for quartan malaria, which corresponds to the erythrocytic parasite development cycle of *P. malariae* (White et al., 2014). In contrast, when infected with *P. falciparum,* fever appears in unsynchronized fever peaks according to current knowledge (CDC, 2019). In general, however, the occurrence of periodicity of fever is not reliable due to the diagnosis of *Plasmodium* spp. infection.

The disease caused by *Plasmodium* spp. infection does not have specific clinical symptoms. Individuals with symptomatic uncomplicated *Plasmodium* spp. infection can suffer from various clinical signs such as fever, chills/rigors, pain, headache, arthralgia, diarrhoea, cough, splenomegaly, hepatomegaly, pallor, etc.

Severe *Plasmodium* spp. infection, usually caused by *P. falciparum* , is defined as severe when the infection is complicated by serious organ failure or abnormalities in the metabolism of the individual or in the blood. The following manifestations can occur during an episode of severe malaria:

- Brain: Cerebral malaria with behavioural changes, altered consciousness, seizures, coma, or other neurological abnormalities
- Blood: Severe anaemia, abnormalities in coagulation
- Kidney: Acute kidney injury
- Lung: Acute respiratory distress syndrome, lung oedema
- Metabolism: Metabolic acidosis

Severe *Plasmodium* spp. infection must be treated urgently as it is a medical emergency (CDC, 2019a; White et al., 2014).

1.6.7.4 Diagnosis

The gold standard for *Plasmodium* spp. infection diagnosis is light microscopy, which refers to a blood smear on a microscopy glass slide. If applied by a trained assessor, light microscopy allows to determine if an individual is infected with *Plasmodium* spp. or not; if infected, with what species one is infected, and in addition also allows to determine the quantity of the parasite in one blood smear (parasitaemia). Light microscopy is not expensive and provides quick results (Wongsrichanalai C, 2007). There are two types of blood smears, namely the thick blood smear, which is prepared with approximately ten microliters of blood, and the thin blood smear, which is prepared with approximately two to five microliters of blood. To prepare a thick blood smear, Giemsa stain is commonly used. For preparing a thin blood smear, fixation with methanol is necessary before Giemsa stain is used. Other diagnostic methods include RDTs, ELISA, loop-mediated isothermal amplification, and PCR (CDC, 2019a; Wongsrichanalai et al., 2007).

1.6.7.5 Treatment

A quick antimalarial chemotherapy is crucial when an individual is infected in order to control the infection or disease. It plays a major role in control strategies recommended by the WHO. Antimalarial chemotherapy is the only way to fully clear and therefore cure an individual, as the immune system is not able to do so (WHO, 2021c). Between 1820 and 1920, quinine was the only effective agent for antimalarial chemotherapy (Muangphrom et al., 2016). After the appearance of *Plasmodium* strains resistant to quinine, the derivative of quinine was developed, namely chloroquine (Muangphrom et al., 2016). Due to a further increase in resistance to antimalarial drugs, the Chinese

government set up a national project against malaria in 1967. During this programme, 2000 traditional Chinese medicines that were used for fever treatment were investigated by Professor Youyou Tu. *Artemesia annua* was shown to have a high efficacy in clearing the infection with *Plasmodium* spp. However, the use of *Artemesia annua* as an antimalarial drug was limited due to low solubility in oil and water. Thus, in 2006 the WHO endorsed the implementation of artemisinin-based combination therapies (ACTs) for uncomplicated malaria tropica (Muangphrom et al., 2016; White et al., 2014; WHO, 2006). ACTs have been shown in various studies to have good efficacy in treating non-falciparum malaria. It is therefore also recommended for malaria infections with *P. vivax, P. ovale, P. malariae,* and *P. knowlesi*, especially in areas where *Plasmodium* spp. have become resistant to chloroquine (White et al., 2014; WHO, 2021). Because of the importance of ACTs and because they are among all relevant malaria treatments the most important ones, the study focuses on ACTs. The current recommendations for ACT regimens according to WHO are listed below (WHO, 2021c):

- Artemether + lumefantrine,
- Artesunate + mefloquine,
- Artesunate + amodiaquine,
- Dihydroartemisinin + piperaquine,
- Artesunate + sulphadoxine-pyrimethamine
- Artesunate-pyronaridine.

ACTs consist of an artemisinin derivative and another antimalarial agent. The advantage of the artemisinin derivative is its quick clearance of the parasite as it kills a large proportion of parasites (WHO, 2021c). The bioavailability of artemisinin derivatives is short so that a proportion of untreated residual parasites are left and can recrudesce easily. In consequence, an antimalarial partner drug with a longer bioavailability is co-administered with the artemisinin derivative. This co-drug ensures parasitical clearance. Under controlled study conditions, ACT can achieve efficacy of more than 90% when it is given as a 3-day regimen (White et al., 2014).

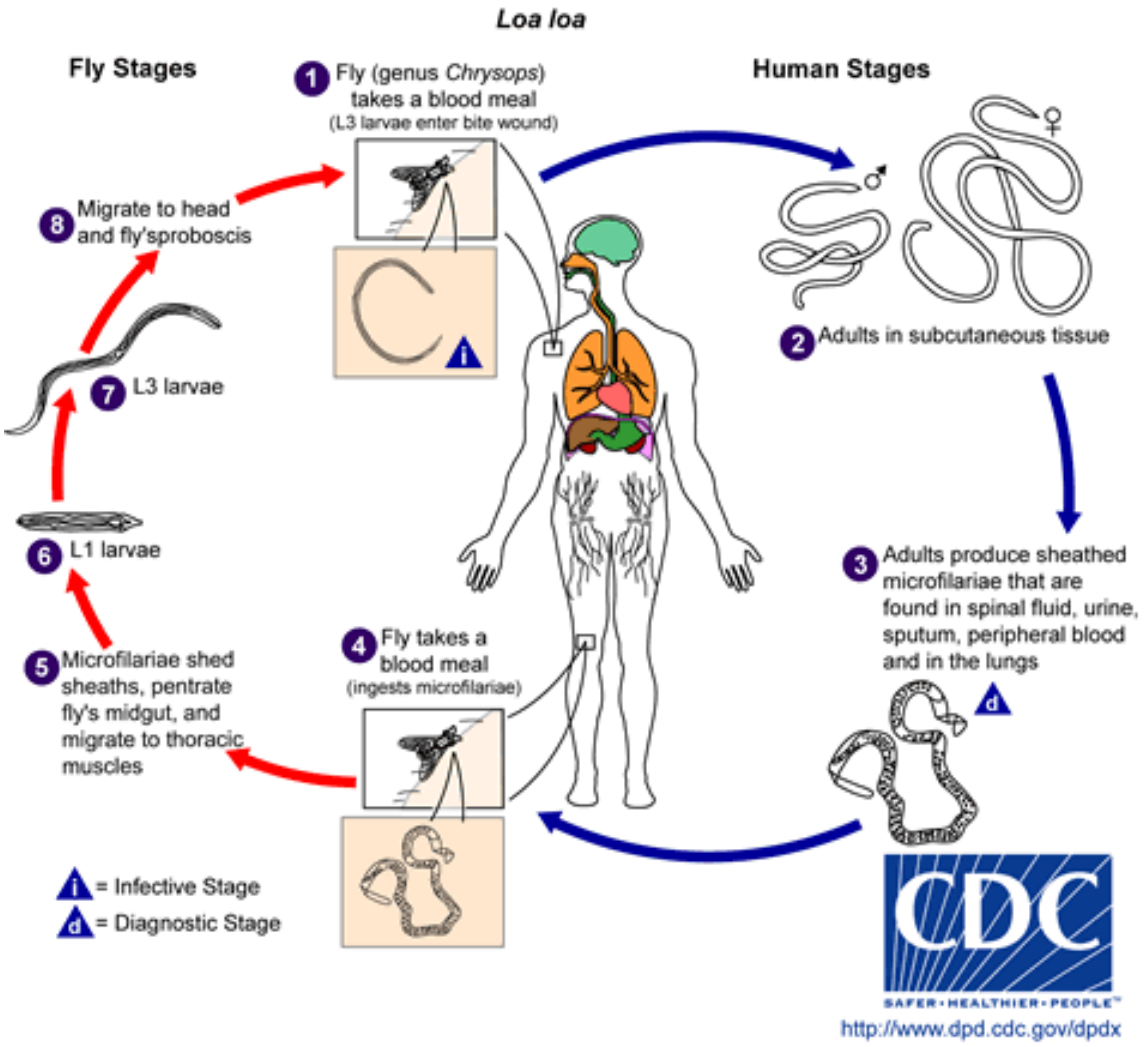
1.6.8 Loa loa microfilariae

1.6.8.1 Biology

Loa loa is a filarial nematode worm. It is a parasite that is transmitted by the tabanid fly of the genus *Chrysops* spp. *(Chrysops dimidiata* and *Chrysops silacea)*. *Loa loa* can cause the disease loiasis. Loiasis is a neglected chronic infectious disease caused by the African eye worm *Loa loa* (CDC, 2020a). The insect transmits the parasites approximately between 10:00 and 16:00 by biting the human host. The different stages of the parasite are shown in Figure 15. The release of infective third-stage filarial larvae onto the skin of the host is done during the blood meal. This allows the larvae to penetrate the bite wound. In the subcutaneous tissue, where the worm usually resides, female and male larvae become mature five months after the infection and sexually reproduce. Adult worms can live up to 17 years in the host and continue to produce new microfilariae; these microfilariae migrate into the lymph vessels of the body. From there, the microfilariae are released from time to time, commonly during midday (CDC, 2020a). They can occur in the subconjunctival tissue of the eye, which is why the name of the disease is also known as the African eye worm (Boussinesq, 2006).

Figure 15

Different life stages of Loa loa



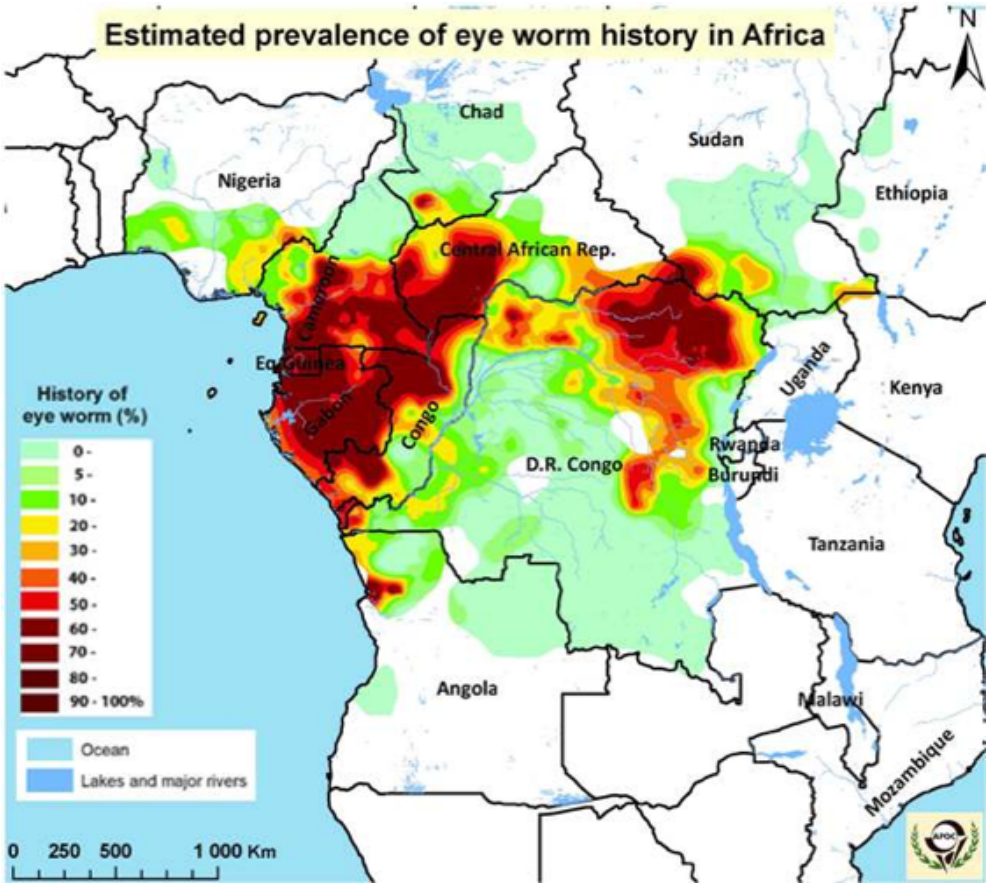
Reference: CDC, 2020b

1.6.8.2 Epidemiology

More than 14.4 million people live in high-risk disease areas. Many high-risk communities are in hard-to-reach remote areas, like in the rainforests of Central and West Africa. The infection with *Loa loa* is highly prevalent in Cameroon, Equatorial Guinea, Gabon and the Democratic Republic of Congo as shown in Figure 16 (Zouré et al., 2011a).

Figure 16

Loa loa prevalence in Africa



Reference: WHO, Zouré et al., 2011b

1.6.8.3 Clinical aspects

According to the literature, many infected individuals in endemic regions remain without symptoms (Boussinesq, 2006). However, although infection with *Loa loa* has been thought for a long time to have a rather benign disease course, this has been proven wrong recently indicating an important burden on populations in high transmission regions (Veletzky et al., 2020). The most common signs and symptoms of loiasis are localized painful subcutaneous joints (Calabar swellings) and the migration of adult worms through the subconjunctiva of the eye. The eye worm passage usually lasts less than a week, usually a few hours, and infrequently can cause eye congestion, pruritus, pain, and light sensitivity (CDC, 2020a). Calabar swellings are recurrent episodes of painless angioedema that usually appear on the extremities. Calabar swelling can last for a couple of hours to multiple days (Boussinesq, 2006). This transient event is associated with swelling,

itching, excruciating pain, and inability to perform daily chores. Chronic pruritus and general asthenia are other typical long-term consequences. Rare complications include transient vision loss, paraparesis of extremities, and neurological events (Boussinesq, 2006). It has been demonstrated that hypermicrofilaraemic infection with *Loa loa* is associated with increased mortality. The burden of disease caused by symptomatic infection with *Loa loa* is similar to other neglected tropical diseases (NTDs) (Veletzky et al., 2020).

1.6.8.4 Diagnosis

The presence of *Loa loa* infection can be determined by the presence of *Loa loa* microfilariae in peripheral blood using light microscopy (CDC, 2020a). The same blood sample can be used to determine microfilaremia. It is important that blood samples are taken at the time when *Loa loa* microfilariae are most active (Boussinesq, 2006). Its characteristic shape allows for distinguishing *Loa loa* from other filarial infections that also produce microfilaremia (Boussinesq, 2006; CDC, 2019a). In order to see the microfilariae of *Loa loa*, the samples need to be prepared with a staining solution and prepared with a Giemsa concentration of e.g. 4% (Boussinesq, 2006). Furthermore, *Loa loa* infection can be diagnosed by the detection of *Loa loa* specific DNA that circulates in the body using PCR. However, the results are not reliable during prepatency as their results often return false-positive in individuals with previous *Loa loa* infections due to the detection of parasite DNA from dead microfilariae in the blood (Boussinesq, 2006). Diagnosis by serology has a low specificity and cannot differentiate a present or past infection (Boussinesq, 2006). Diseased individuals suffering from loiasis can be diagnosed clinically, but there are no specific symptoms that confirm loiasis. If an individual has the *"African eye worm",* an infection with *Loa loa* is highly suspected. However, eye worm passage can also be caused by other pathogens (Boussinesq, 2006). Thus, light microscopy remains the standard diagnostic test for an infection with *Loa loa*. One tool to identify regions with a high prevalence of infection with *Loa loa* is the rapid assessment of loiasis (RAPLOA) questionnaire (Zouré et al., 2011a). It constitutes the following questions:

- "Have you ever noticed a worm migrating through your eye?"
- A picture of a person having an episode of *eye worm* will be shown to the individual:

  "Has it looked like in this picture?"
- Was the duration no longer than seven days?

RAPLOA constitutes three different questions that all need to be answered with “yes” in order to be “RAPLOA positive”.

1.6.8.5 Treatment

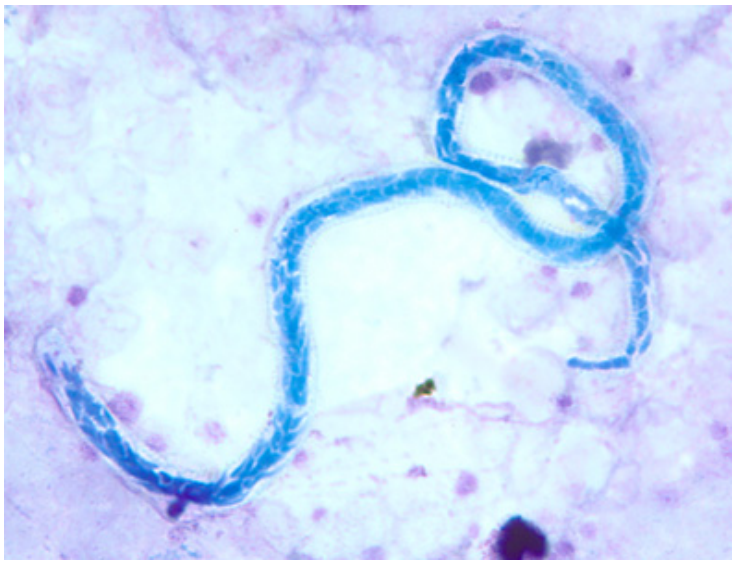
Symptomatic *Loa loa* infection can be treated with three different drugs, which are diethylcarbamazine (DEC) (CDC, 2020), ivermectin, and albendazole (Boussinesq, 2006). The advantage of DEC is that it may completely clear adult worms and microfilariae from the host (Boussinesq, 2006). The disadvantage of the treatment is the side effects, which occur among individuals with a high microfilarial density (>8000/ml blood), including meningoencephalitis and renal failure due to the massive simultaneous release of antigenic material from dying microfilariae (Boussinesq, 2006). Ivermectin only targets the microfilariae in individuals suffering from an infection with *Loa loa*. Like DEC, ivermectin should not be given to individuals with a high microfilarial density, as it leads to severe adverse events (Boussinesq, 2006). Albendazole has an impact on the adult worm and thus can decrease microfilaremia (Metzger and Mordmüller, 2014). Albendazole is given for three weeks as a dose of 200 mg to be taken two times per day for 21 days (Boussinesq, 2006).

1.6.9 Mansonella perstans microfilariae

1.6.9.1 Biology

Figure 17

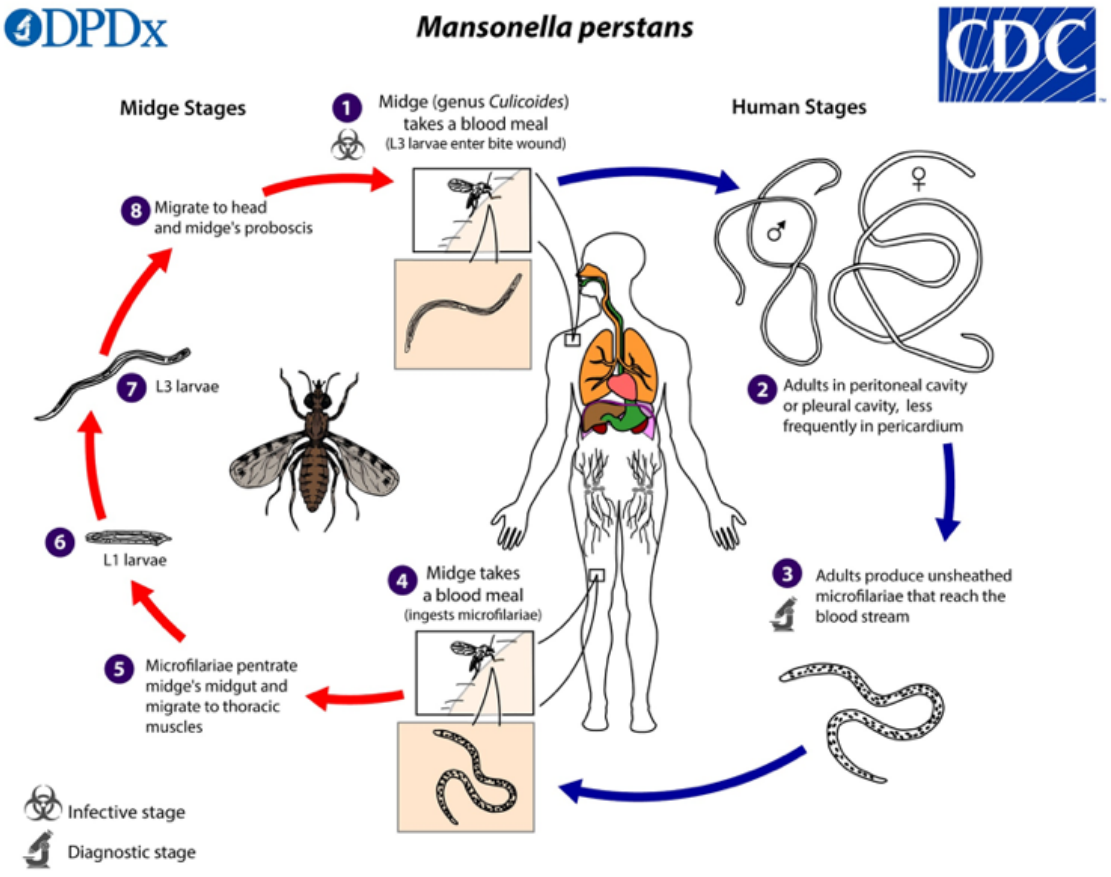
Microfilaria in Giemsa stain



Mansonella (M.) perstans (Figure 17) is a filarial nematode that uses humans as its principal host. *M. perstans* was first described by Patrick Manson in 1891 as "filaria sanguinis hominis minor" in the blood of an individual from West Africa who was brought to a London hospital suffering of haemato-chyluria (Haynes, 2000; Manson, 1891). To date, three species, *M. perstans*, *M. ozzardi,* and *M. streptocerca,* cause human mansonellosis, a vector-borne disease. The size of the adult worms differs depending on sex and subspecies. Adult male worms of *M. perstans* have measurements of 35 – 45 mm., whilst adult female worms have measurements of 50 – 80 mm (Ta-Tang et al., 2018). This study focuses on *M. perstans*.

Figure 18

Life Cycle cycle of Mansonella M. perstans from



Reference: CDC, 2019b

M. perstans is transmitted by midges of the genus *Culicoides*. However, it is known that non-*Culicoides Ceratopogonidae* biting midges and black-flies from the genus *Simulium* are also able to transmit *M. perstans* to humans. Figure 18 shows the life and transmission cycle of *M. perstans*. During a blood meal from an infected host, the female *Culicoides* spp. ingests microfilariae of *M. perstans*, which differentiate into L3 larvae in the midge (CDC, 2019). During another blood meal, the infected midge introduces the L3 larvae to the human host. Having reached the final host, L3 larvae reach their final maturation level and become adult worms (CDC, 2019). Adult worms usually live in body cavities, mainly in the pleural and peritoneal cavities. Other living areas are the retroperitoneal spaces, perirenal spaces, pericardium, retroperitoneal spaces, and mesentery. Adult worms produce microfilariae that migrate into the cerebrospinal fluid and peripheral blood (Ta-Tang et al., 2021, 2018). The periodicity of *M. perstans* is currently unclear. It was reported by Asio et. al. in their work "Analysis of the 24 h microfilarial periodicity of *M. perstans*" that *M. perstans* has a light diurnal periodicity with a peak around 06:45 a.m. in the morning; analysis was of peripheral blood samples from 32 healthy individuals in an endemic community in Uganda (Asio et al., 2009). This light periodicity means that the time at which blood is sampled has a minor impact on diagnosis (Ta-Tang et al., 2018).

1.6.9.2 Epidemiology

Infection with *M. perstans* has been reported in 33 countries around the world. It is particularly widespread in wet subtropical and tropical regions in sub-Saharan Africa and Latin America and affects poor people living in rural areas. *M. perstans* occurs in the north of the Amazonas, beginning from Brazil to the Caribbean coast of South America (Mediannikov and Ranque, 2018; Ta-Tang et al., 2018). There is an assumption that *M. perstans* was brought to America during the slave trade (Mediannikov and Ranque, 2018). *M. perstans* was frequently described from the mid-twenties until the seventies in Papua New Guinea. The prevalence of *M. perstans* is estimated to be very high in endemic areas and is thought to cause one of the most prevalent filarial infections in sub-Saharan Africa. It is estimated that in Africa, more than 100 million individuals are infected with *M. perstans* and 600 million individuals live at high risk of infection with *M. perstans* (Ta-Tang et al., 2018). Despite the burden, very few epidemiological studies regarding its health impact in endemic areas have been conducted (Ta-Tang et al., 2018).

1.6.9.3 Clinical aspects

Infection with *M. perstans* is of a mild clinical presentation. Most infections are asymptomatic, particularly for individuals living in endemic areas. Symptomatic individuals may have itching, urticaria, abdominal pain, fatigue, arthralgia, pericarditis, pleuritis, and inflammatory granulomatous nodules, which are built around the dead adult worms (Ta-Tang et al., 2018).

1.6.9.4 Diagnosis

Infection with *M. perstans* is diagnosed with light microscopy by looking for microfilariae in the peripheral blood using Giemsa-stained blood smears. Microfilariae of M streptocerca which usually reside in the skin can be diagnosed by using skin snips. The skin snips should be approximately 2 mm thick and must immediately be put into saline or distilled water, covering the specimen. Important differential diagnoses are infection with *Loa loa* and *Wuchereria bancrofti*. As *M. perstans* has a blunt rounded tail and nuclei in the end of the tail, it can easily be differentiated from *Loa loa* and *Wuchereria bancrofti* (their terminal nuclei are bigger than those of other microfilariae) (Mediannikov and Ranque, 2018; Ta-Tang et al., 2021). In research settings, molecular detection via real-time PCR or loop-mediated-isothermal amplification assays (LAMP) can be used for diagnosis and quantification in the field (Ta-Tang et al., 2018).

1.6.9.5 Treatment

It is very difficult to treat an infection with *M. perstans* due to the limited efficacy of the drugs that are available. Clinical trials have been conducted with traditional antifilarial drugs such as ivermectin, different antihelmintic benzimidazoles like albendazole, and DEC but due to study limitations, there is no consensus on treatment in the scientific community (Mediannikov and Ranque, 2018; Ta-Tang et al., 2021, 2018).

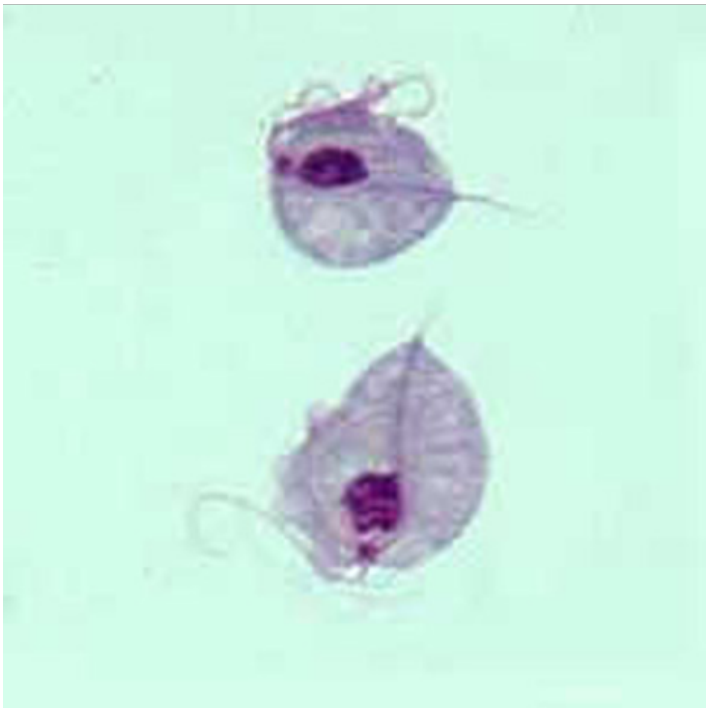
1.6.10 Trichomonas vaginalis

1.6.10.1 Biology

T. vaginalis is a flagellated pathogenic protozoan with a size of 7-25 µm and can cause trichomoniasis. *T. vaginalis* has four flagella on the front side, and the fifth flagellum is shaped on the back side of the surface of the protozoan and making it motile. *T. vaginalis* does not have mitochondria but hydrogenosomes with which it can produce H_2, CO_2, and ATP in an anaerobic environment. As *T. vaginalis* does not produce cysts, the protozoan dies in a dry environment. However, it can survive in water for several hours. Reproduction takes place by means of binary fission. Figure 20 shows the different stages of *T. vaginalis*. The only known hosts are humans (Suerbaum et al., 2016). Transmission occurs only from human to human, usually sexually, but can also be transmitted from (CDC 2017) mother-to-child during delivery. The parasite targets the female and male urogenital tract mucosa (Suerbaum et al., 2016). The following chapter focuses on women as infected or diseased individuals.

Figure 19

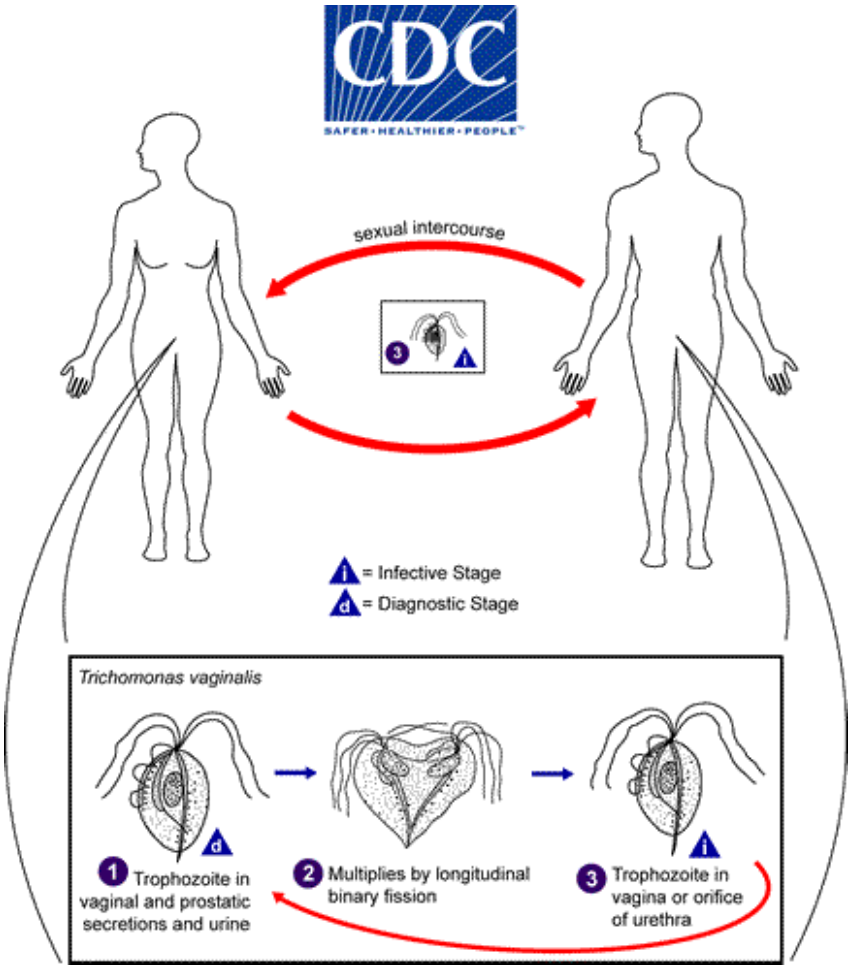
T. vaginalis Giemsa stain



Reference: CDC, 2017

Figure 20

Life cycle of T. vaginalis



Reference: CDC, 2017

1.6.10.2 Epidemiology

Infection with *T. vaginalis* is one of the most prevalent and curable STIs worldwide (CDC, 2017). According to the WHO, in 2016, infection with *T. vaginalis* affected around 156 million individuals worldwide (Van Gerwen and Muzny, 2019). It is estimated that the prevalence of *T. vaginalis* in Africa is 42.8 million. Prevalence is ten times higher in women than in men (Tchankoni et al., 2021). Women of reproductive age are at higher risk. It has been estimated that the global prevalence of an infection with *T. vaginalis* among pregnant women is up to 25 million (Silver et al., 2014). The spread of infection is strongly associated with social disadvantages and health (Silver et al., 2014). Risk factors for contracting an infection with *T. vaginalis* include promiscuity and poor hygiene (Kissinger, 2015; Rowley et al., 2019).

1.6.10.3 Clinical aspects

The incubation period ranges from five to twenty-eight days. Most women (85%) and men (77%) infected with *T. vaginalis* are asymptomatic. In the following section, the symptoms that are described are frequent for women. One-third of the individuals with an asymptomatic infection present get diseased within six months. The diseased individuals may have a colpitis with a yellow-green, sometimes frothy vaginal discharge and dysuria. The discharge typically has a foul smell and is associated with pruritus. Furthermore, they can suffer from dyspareunia, vulvar pruritus, and erythema. The strawberry cervix or *coplitis macularis* can be observed in 5% of all infected individuals worldwide and rises to almost 50% of all infected individuals worldwide when examined by colposcopy. The infection can ascend, infect the upper genital organs, and may cause pelvic inflammatory disease (PID) as well as urethritis. Half of the infections in women cause little or no symptoms (Kissinger, 2015; Suerbaum et al., 2016). During pregnancy, infection with *T. vaginalis* is associated with preterm birth, premature rupture of membranes, and low associated birth weight (Silver et al., 2014). 90% of the men infected with *T. vaginalis* are asymptomatic (Suerbaum et al., 2016). When symptoms occur in men, they suffer from urethritis, such as discharge, or dysuria. Complications of trichomoniasis in men are chronic prostatitis and epididymitis. Individuals infected with *T. vaginalis* are at higher risk for HIV acquisition. (Kissinger, 2015).

1.6.10.4 Diagnosis

The most common and rapid method to diagnose *T. vaginalis* is wet mount microscopy of vaginal fluid, which is cheap but varies in diagnostic sensitivity. Movements of *T. vaginalis* can be observed on the microscopic slide. The sensitivity of wet mount microscopy ranges from 44 – 68%. An explanation for the poor sensitivity may be the rapid loss of the motility of *T. vaginalis*, which is characteristic and crucial for the determination of *T. vaginalis* by microscopy. Samples must be taken by a cervical and urethral swab for women and a urethral swab in men and must be evaluated immediately after sample collection when wet mount microscopy is chosen as the diagnostic method. Another possibility to determine the presence of *T. vaginalis* is NAAT, which has a sensitivity and specificity of up to 90 – 95%. Another possibility is the diagnosis with direct immunofluorescent antibody staining, which is also more sensitive than microscopy, albeit a more complex procedure. Currently the gold standard is broth cultivation of trichomonads but

results are only available after two to seven days of incubation. During the cultivation microscopic examination has to be performed every day (Van Gerwen and Muzny, 2019).

1.6.10.5 Treatment

According to the WHO guidelines, an infection with *T. vaginalis* has to be treated with 2 g of metronidazole in a single oral dose and can have a high efficacy with cure rates of up to 90% under the condition that both partners are treated. Alternatively, 500 mg two times a day for one week can be given to the individual. A vulnerable group of individuals is pregnant women. Treatment with metronidazole has been shown to be safe in all stages of pregnancy (Kissinger, 2015).

1.6.11 Schistosoma spp.

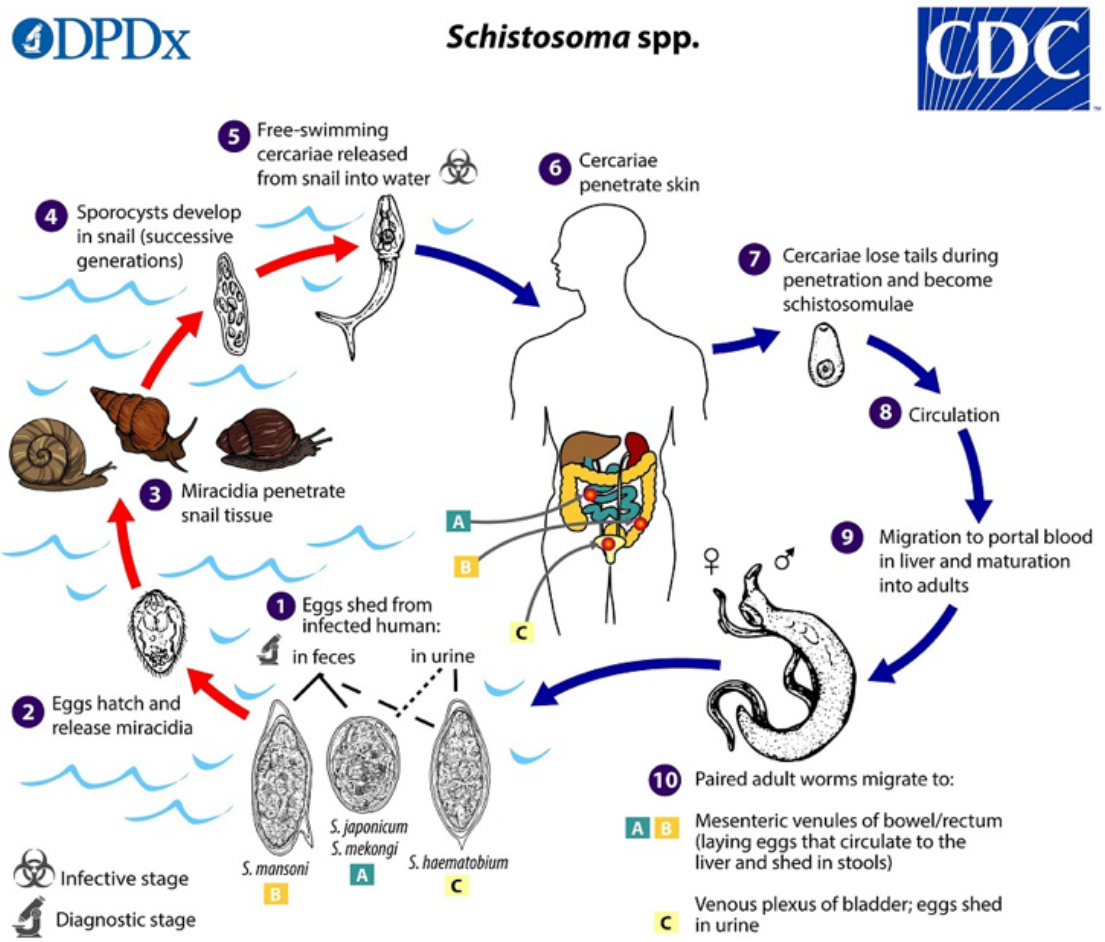
1.6.11.1 Biology

Schistosomiasis is a parasitic infectious disease caused by blood flukes *(Schistosoma* spp*.). Schistosoma* spp. belong to the trematodes. Adult *Schistosoma* have a size of 6 – 22 mm and have reproductive organs. The male has the form of a leaf. The outer edges serve as gynecophoral canals, in which the round female lives. Five different *Schistosoma* species exist, of which *Schistosoma (S.) haematobium, S. mansoni,* and *S. japonicum* are the most relevant to humans. Additionally, there is *S. guineensis*, *S. mekongi,* and *intercalatum*. Table 6 shows the different species, their geographical distribution, clinical aspects, and the incubation period (Suerbaum et al., 2016). The eggs of *Schistosoma* are released with human stool or urine. Figure 21 shows the life cycle of *Schistosoma* spp. (1) Based on the tropism of the species, eggs can be found in stool or urine; (2) the eggs hatch and develop miracidia and release them into the water, in which they swim and invade specific freshwater snails, which serve as intermediate host; (3) the stage inside their intermediate host, includes two generations of sporocysts (4) and production of cercariae (5); the infective cercariae leave the snail and can survive for 24 – 48 hours; to survive, they must invade their final host by penetrating its skin (6); during penetration they shed their forked tails and become schistosomulae (7); The first organ that schistosomulae usually reach through venous circulation (8) are the lungs, then follows the heart and afterwards the liver, where they stay for further maturation (9); the species that are known to cause intestinal schistosomiasis resides in the mesenteric venules (10); *S. mansoni* usually resides in the inferior mesenteric veins migrating to the large intestine (10b);

S. haematobium is usually found in the vesicular and pelvic venous plexus of the bladder (10c), it sometimes also resides in the rectal venules. To release its eggs, the female worm leaves the male and moves to the small venules of the portal and perivesical systems. In this way, the eggs are passively moved toward the intestinal lumen (*S. mansoni, S. japonicum, S. mekongi, S. intercalatum/guineensis*) and of the bladder and ureters and are eliminated in stool or urine (1) (CDC, 2019).

Figure 21

Life cycle of Schistoma spp.



Reference: CDC, 2019c

Various studies have shown that the eggs of *Schistosoma* and not the adult worm itself cause morbidity in the infected host (Colley et al., 2014). As many eggs are not eliminated via urine or faeces, they permanently reside in the intestine, the liver (for *S. mansoni* and *S. japonicum*), or the bladder or the urogenital system (for *S. haematobium*), where a

granuloma is formed through the host's immune response to the eggs' proteolytic enzymes that they use to prevent tissue necrosis. The immune reaction leads to chronic inflammation due to granuloma formation. This results in the clinical presentation of infection with *Schistosoma* spp. While the adult worm is capable of evading the immune response, the eggs cause a strong immune response (Colley et al., 2014).

1.6.11.2 Epidemiology

The WHO estimates that 779 million individuals are at risk of infection and over 250 million are infected worldwide, of which 81 % of the infected live in Africa. Infection with *Schistosoma* spp. is waterborne. The infection occurs in tropical and subtropical regions. The disease caused by *Schistosoma* spp. is listed as a neglected tropical disease by the WHO and is known to be a poverty-related disease (McManus et al., 2018). The population mostly affected by an infection with *Schistosoma* spp. is more likely to live in rural areas and to engage in agricultural, domestic, occupational, and recreational activities in freshwater. All these environmental factors are known to be risk factors for acquiring an infection with *Schistosoma* spp. As women usually do domestic chores involving infested water and often engage in further activities exposing them to freshwater, they are at greater risk than men to get infected with *Schistosoma* spp. Children living in remote areas are also at high risk of getting infected with *Schistosoma* spp. because they play in freshwater. The more often children are exposed to *Schistosoma* spp., the higher the risk of developing the disease within ten years. During childhood, infected individuals are exposed frequently to *Schistosoma* spp.. In consequence, the highest prevalence occurs in young adolescents (Colley et al., 2014; McManus et al., 2018).

Table 6

Different species of Schistosoma

Species	Presence	Clinic	Incubation period	Diagnosis
S. haematobium	Africa, Middle East, India, Corsica (France)	Urogenital schistosomiasis	Twelve weeks	Eggs in urine samples
S. mansoni	Africa, Middle East, Central and South America and the Caribbean	Hepato- and intestinale schistosomiasis	Seven weeks	Eggs in stool samples
S. japonicum	East Asia	Hepato- and intestinale schistosomiasis	Ten weeks	Eggs in stool samples
S. mekongi	South East Asia	Hepato and intestinale schistosomiasis	Ten weeks	Eggs in stool
S. guineensis and related S. intercalatum	Rain forest areas of Central Africa	Hepato and intestinale schistosomiasis	Seven weeks	Eggs in stool

Reference: based on the table of Thieme Endspurt Hygiene, Mikrobiologie 2013

1.6.11.3 Clinical aspects

An infection with *Schistosoma* spp. can progress in two different stages, which is influenced by how long one has been infected. One can have an acute infection or a late chronic infection. The different stages of infection differ in terms of egg excretion rate found in stool or urine as well as in symptoms and clinical manifestations (McManus et al., 2018). In the acute stage, when cercariae invade the skin, a local small maculopapular cutaneous lesion (may itch), due to a local hypersensitivity reaction, can appear. This is called cercarial dermatitis and can occur in individuals who are exposed for the first time to *Schistosoma* spp. i.e., travellers and migrants coming from non-schistosomiasis-endemic regions. After the parasite has invaded the host and maturation of the schistosomula takes place in the human, one may have a symptomatic acute stage. The symptomatic acute stage is also known as Katayama syndrome. Katayama syndrome occurs in *Schistosoma*-naïve individuals undergoing first-time exposure to the parasite and is the result of systemic hypersensitivity. Acute symptomatic infection with *Schistosoma* spp. occurs rarely

in individuals that live in disease-endemic regions. A theory on why individuals from endemic regions are much less susceptible to acute symptomatic infection with *Schistosoma* spp. may be due to prenatal desensitisation due to primed T-lymphocyte and B-lymphocyte responses, that reduce the immune response against schistosome antigens in children born to infected mothers (Colley et al., 2014; McManus et al., 2018). Symptoms occur between two weeks to three months after being infected as the parasite needs to mature, produce and release eggs (Colley et al., 2014). Individuals with the Katayama syndrome can present with symptoms like fever, cough, abdominal pain, diarrhoea, hepatosplenomegaly, and eosinophilia (Colley et al., 2014).

Chronic inflammation due to continuous exposure to *Schistosoma* spp. results in disease progression and thus may lead to the chronic disease. There are two types of chronic infection with *Schistosoma* spp., namely the chronic intestinal form and the chronic urogenital form, both characterised by different symptoms. However, symptoms associated with all species include anaemia, malnutrition, growth restriction in children, impaired iron metabolism, reduced physical fitness, and reduced cognitive function. Further complications of chronic infection with *Schistosoma* spp. are lesions in the central nervous system manifesting as encephalopathy, symptoms of spinal compression, meningoencephalitis with pyrexia, headache, and Jacksonian epilepsy (Colley et al., 2014).

Chronic symptomatic infection with *S. mansoni* manifests as non-specific intermittent abdominal pain, diarrhoea, and rectal bleeding. A complication of the intestinal disease form is the hepatosplenic disease with periportal fibrosis, (Symmer's pipe-stem fibrosis) which occurs five to fifteen years after infection. It causes ascites, hematemesis due to oesophageal varices, portal hypertension. and pulmonary hypertension and can therefore lead to death (Colley et al., 2014). The chronic infection of the urogenital form caused by *S. haematobium* leads to haematuria, dysuria, and suprapubic pain. Complications of the urogenital form result in chronic fibrosis of the urinary system and may cause a hydronephrosis, obstruction of the ureter and urethra, and thus recurrent cystitis with bacterial superinfection and acute renal failure. Bladder carcinoma has been shown to be strongly associated with chronic *S. haematobium* infection (Colley et al., 2014). Female genital schistosomiasis, which is a disease manifestation of an infection with *S. haematobium* in the urogenital tract of infected women, has been associated with a three- to four-fold increase in the risk of HIV acquisition (Colley et al., 2014).

1.6.11.4 Diagnosis

According to the medical history, clinical manifestations, and the type of *Schistosoma* infecting, there are different diagnostic approaches. First, one should know that the diagnostic approach for an acute infection with *Schistosoma* spp. differs from the diagnostic approach of an established active or latent chronic infection with *Schistosoma* spp. As the determination of schistosome eggs in light microscopy in individuals with an acute infection with *Schistosoma* spp. is not sensitive enough due to the low schistosome eggs excretion, anamnestic information about risk exposure to fresh water may give a hint. Also, eosinophilia appears to be common in individuals with a helminthic infection, such as an infection with *Schistosoma* spp. In naive individuals from low transmission areas, antibody detection can be performed when microscopy results remain negative. Antigen detection tests of *Schistosoma* spp. have a great impact because of their higher sensitivity in comparison to microscopic methods. Since 2008, a point-of-care diagnostic test (POC-CCA) has become commercially available. It detects *Schistosoma* derived circulating cathodic antigen (CCA) in urine and stool but has a weaker test performance for the urogenital form. The up-converting phosphor-based lateral flow test detecting circulating anodic antigen (CAA) detects all *Schistosoma* species in urine and has a high sensitivity and specificity. It quantifies CAA levels reflecting active, ongoing infections of less than a single *Schistosoma* worm (Colley et al., 2014; WHO, 2021d). In endemic regions, stool is the sample of choice to determine eggs of *S. mansoni* by light microscopy, and urine is the sample of choice to determine eggs of *S. haematobium*. Eggs of *S. haematobium* can also be found in stool samples, but eggs of *S. mansoni* cannot usually be found in urine. Eggs per 10 ml of urine or eggs per gram of stool indicate the level of infection. To increase the sensitivity, specimen collection should take place on three consecutive days. Table 6 indicates in which sample the different types of *Schistosoma* spp. can be found. Microscopic identification is the common method and recommended by the WHO. For microscopy 1.2 mg of faecal material must be placed on the glass slide for microscopic identification. To avoid false negative results, the examination should be repeated as eggs could have passed intermittently or in small amounts. Another method recommended by WHO is the Kato-Katz technique: It allows the quantification of eggs of the intestinal form in stool with light microscopy. The technique is based on the use of methylene blue-stained cellophane, which is soaked in glycerine or glass slides. A filtration method with nylon, paper, or polycarbonate filters is currently recommended by the WHO for the

diagnosis of the urogenital form. Urine dipstick assays are recommended for home testing, which plays an important role when diagnosing children (Colley et al., 2014; WHO, 2021d).

The determination of schistosome DNA by NAAT like PCR in stool, urine, and serum samples has higher sensitivity, but when schistosome eggs may have passed intermittently, or the amount of schistosome eggs is low, this method also has its limitations (Colley et al., 2014; WHO, 2021d).

1.6.11.5 Treatment

Praziquantel (PZQ) is highly effective, well tolerable, and has great efficacy against all *Schistosoma* species, and therefore it remains the first-line treatment. It can also be taken during pregnancy after the first trimester and during childhood. The mechanism of PZQ is not fully understood. It is known that PZQ targets adult schistosome worms but has a limited effect against immature schistosome larvae. A single standard dose of 40 mg/kg is advised (Colley et al., 2014).

1.6.12 Treponema pallidum

1.6.12.1 Biology

T. pallidum is a Gram-negative spirochete bacterium that causes the venereal disease syphilis. Humans are the only reservoir of *T. pallidum* (Suerbaum et al., 2016). *T. pallidum* can only survive outside the human body for a short time.

The infection with *T. pallidum* is transmitted through direct skin-to-skin contact with someone that has active mucocutaneous syphilitic lesions such as chancre (painless ulcer that is usually a single lesion) or condyloma lata (grey or white lesions in the mouth, perineum or on the genitalia) during sexual intercourse. Another mode of transmission is vertical transmission from mother to child during pregnancy. Transmission can occur in utero when the spirochetes cross the placenta or during delivery due to direct skin-to-skin contact (Eickhoff and Decker, 2016; Kojima and Klausner, 2018). Another way of transmission is through blood transfusion (Eickhoff and Decker, 2016), which usually does not occur in high-income countries but constitutes an issue in low- and middle-income countries (Kojima and Klausner, 2018).

1.6.12.2 Epidemiology

Infection with *T. pallidum* was first described around 1490 in Europe. It was documented that sailors accompanying Christopher Columbus had brought the disease to Europe upon their return from their first journey to the New World (Barnett, 2018). Six million new cases of syphilis are estimated to occur globally in individuals aged between 15 – 49 years (Kojima and Klausner, 2018). According to the WHO, one million pregnancies are affected every year, and in 27% of cases the child acquires congenital syphilis, in 46% of cases the mother suffers miscarriage or stillbirth, and in 27% of cases, the results are preterm birth and low birth weight. Over 300 000 foetal and neonatal deaths are accredited to syphilis worldwide. In addition, 215 000 infants have a higher mortality risk due to the consequences of congenital syphilis (Rowley et al., 2019). During pregnancy, infection with *T. pallidum* can be transmitted at any syphilitic stage, at any time, as *T. pallidum* crosses the placenta, although this frequently occurs around the 18th week of gestation. The risk of MTCT increases the closer the time of infection is to the time of pregnancy (Eickhoff and Decker, 2016). A special high-risk group is men who have sex with men due to the tendency of having multiple sex partners (Kojima and Klausner, 2018). The prevalence of an infection with *T. pallidum* is higher in the HIV-positive population. An individual that has an infection with *T. pallidum* and is HIV positive has worse clinical outcomes as the viral replication of HIV is triggered, leading to an increased viral load, decreased CD4 count, and therefore a higher risk of transmitting HIV to others (Eickhoff and Decker, 2016).

1.6.12.3 Clinical aspects

The incubation period upon exposure usually lies between 14 and 24 days but can sometimes range from 10 – 90 days. In this paragraph, the clinical aspects of the disease of an infection with *T. pallidum* are described (syphilis). Syphilis is categorised into five different stages. The allocation to the different disease stages is based on clinical manifestations, as shown in Table 7. Diseased individuals are highly contagious when having primary syphilis and less contagious when having secondary syphilis. Despite the severity of latent and quarter syphilis, individuals are not contagious anymore (Robert-Koch-Institut, 2020). Herpes simplex, chancroid granuloma inguinal (*Donovanosis*), and lymphogranoluma venereum are important differential diagnoses for primary syphilis, which is characterised by a chancre and regional swollen lymph nodes (Eickhoff and Decker,

2016). Congenital syphilis can have different clinical manifestations. The severity of congenital syphilis depends on the disease severity of the mother. There are two types of congenital syphilis: early congenital syphilis, which affects children from zero to two years, and late congenital syphilis, which affects children at two years and older (Olthoff, 2019). Fifty – sixty percent of the children infected with *T. Pallidum* are asymptomatic at delivery. Clinical manifestations may include acute respiratory distress syndrome, anaemia, hydrops, hepatosplenomegaly, and icterus. Children with late congenital syphilis get sick at three years or later during childhood. Late congenital syphilis is characterised by the Hutchinson's triad, which consists of three phenomena: interstitial keratitis, malformed teeth and eighth nerve deafness. In addition, children may have a deformed nose called saddle nose deformity (Eickhoff and Decker, 2016; Olthoff, 2019).

Table 7

Different stages of syphilis and disease progression

State	Description	Typical clinical manifestation
Primary syphilis	Local lesions that heal with scars	Primary infection: ulcus durum Local swollen lymph nodes
Secondary syphilis	Systemically spread of *T. pallidum* with immunological reaction	General symptoms: generalised lymphadenopathy with fever, tiredness, headache Specific symptoms: polymorphed maculopapular exanthema on the inner side of hands and feet usually no itch. Condyloma lata: wettened, large, papules with erosions in the intertriginous and ano-genital region Acute tonsillitis = angina specifica Alopecia areolaris Plaques opalines

		Ocular syphilis (e.g. Uveitis) Special form = lues maligna Severe form in immunosuppressed individuals multiple ulcerations
Latent syphilis	Duration: months, years up to a lifelong phase without activity	No clinical symptoms despite positive serology
Tertiary syphilis	Late inflammatory reaction due to *T. pallidum* with Granulomas	Specific symptoms: gummas: Presence of granulomatous lesions skin: subcutaneous infiltration, that releases a liquid after ulceration gummas can occur in every organ Cardiovascular syphilis: aorta aneurysm, mesaortiti
Quarternery syphilis	The central nervous system (CNS) is attacked by *T. pallidum* with an immunological contra reaction	Neurosyphilis asymptomatic neurosyphilis = no symptoms despite of the confirmed presence of *T. pallidum* in the central nervous system. Cerebral obliterating arteriitis – stroke tabes dorsalis: demyelination of the dorsal column of spinal cord and spinal ganglions syphilitic meningitis increase of the intercranial pressure focal and/or generalized cramp attacks complications neurosyphilis: progressive paralysis brain atrophy and dementia (usually frontotemporal) organic psychosis with alteration of psyche and personality typical findings: Argyll-Robertson-pupil

Reference: based on Herold 2016

1.6.12.4 Diagnosis

An infection with *T. pallidum* can be diagnosed directly and indirectly. Samples from a primary syphilitic lesion or condylomata lata can be used to determine *T. pallidum* directly. The most common methods for direct detection are PCR and dark-field-microscopy in combination with immunofluorescence (Robert-Koch-Institut, 2020). To diagnose infection with *T. pallidum,* serological tests are usually used. As false positives and false negatives are a frequent occurrence, a single test for treponemal or nontreponemal serologic status is insufficient. The first test is usually a nontreponemal test which is confirmed by a treponemal test, also known as a confirmatory test (Robert-Koch-Institut, 2020). Rapid Plasma Reagin (RPR) test and Venereal Disease Research Laboratory (VDRL) test are among these nontreponemal tests. The sensitivity and specificity of the diagnostic tests differ according to the clinical stage of symptomatic infection. Referring to the clinical stages of a symptomatic infection with *T. pallidum*, the sensitivity of RPR and VDLR ranges between 78 – 86% for the detection of primary syphilis and 100% sensitivity for secondary syphilis with a specificity lying between 85 – 99% for all syphilitic stages. Treponemal tests include Fluorescent treponemal antibody (FTA-ABS) test, *T. pallidum* passive particle agglutination (TP-PA) assay, enzyme immunoassays (EIAs), chemiluminescence immunoassay (CIA) and rapid treponemal assays (Eickhoff and Decker, 2016; Robert-Koch-Institut, 2020).

The specificity of the FTA-ABS test for primary syphilis and other stages is 97%, the sensitivity for primary syphilis is 84%, and for syphilis in other stages is 100% (Eickhoff and Decker, 2016). In many cases, individuals that were once infected with *T. pallidum* produce positive results for several years or lifelong even though they have been successfully treated. This phenomenon is known as “serofast reaction” (Eickhoff and Decker, 2016).

When neurosyphilis is suspected, further testing with a lumbar puncture and cerebrospinal fluid (CSF) analysis is necessary. Diagnostic analysis should include CSF cell count, protein, glucose, and CSF-VDRL. Evaluation by slit lamp examination is necessary for diagnosing ocular syphilis (Eickhoff and Decker, 2016).

1.6.12.5 Treatment

An individual can be empirically treated before the laboratory results are available if an infection with *T. pallidum* is suspected. Early treatment is crucial to avoid further

transmission. Infection with *T. pallidum* is treated parenterally with penicillin G (benzathine penicillin), irrespective of the clinical stage of infection.

All stages of infection with *T. pallidum* are treated with penicillin G. Repeated nontreponemal testing and the follow-up of individuals are important (Eickhoff and Decker, 2016). All individuals with tertiary syphilis must receive a CSF diagnostic to confirm or exclude neurosyphilis. If CSF analysis is abnormal (pleocytosis, increased IgG, and increased proteins), the individual should be treated for neurosyphilis, in case it is normal, a treatment for neurosyphilis is not necessary. Neurosyphilis is treated with a regimen of aqueous crystalline penicillin G 18. A continuous infusion for a total of 10 – 14 days is possible (Eickhoff and Decker, 2016).

1.6.13 Chlamydia trachomatis

1.6.13.1 Biology

Chlamydia spp. is a Gram-negative obligate intracellular bacterium. There are three important types of *Chlamydia* spp. that can infect humans: C. *trachomatis*, *C. pneumoniae,* and *C. psittaci* (Olthoff, 2019). Table 8 shows the different types of human pathogenic *Chlamydia* spp., their target organ, the way of transmission, and the diseases they cause. As *Chlamydia* spp. was first thought to be a virus, the terminology of the life cycle corresponds to the terminology of viral life and replication cycle (Cai and Tiscione, 2016). Figure 22 shows the different steps during the life cycle of *Chlamydia* spp., starting with the extracellular attachment of the elementary body (the infectious form of *Chlamydia* spp.) onto the target cell in the urogenital tract.

Table 8

Different stages of Chlamydia spp. and clinical manifestations

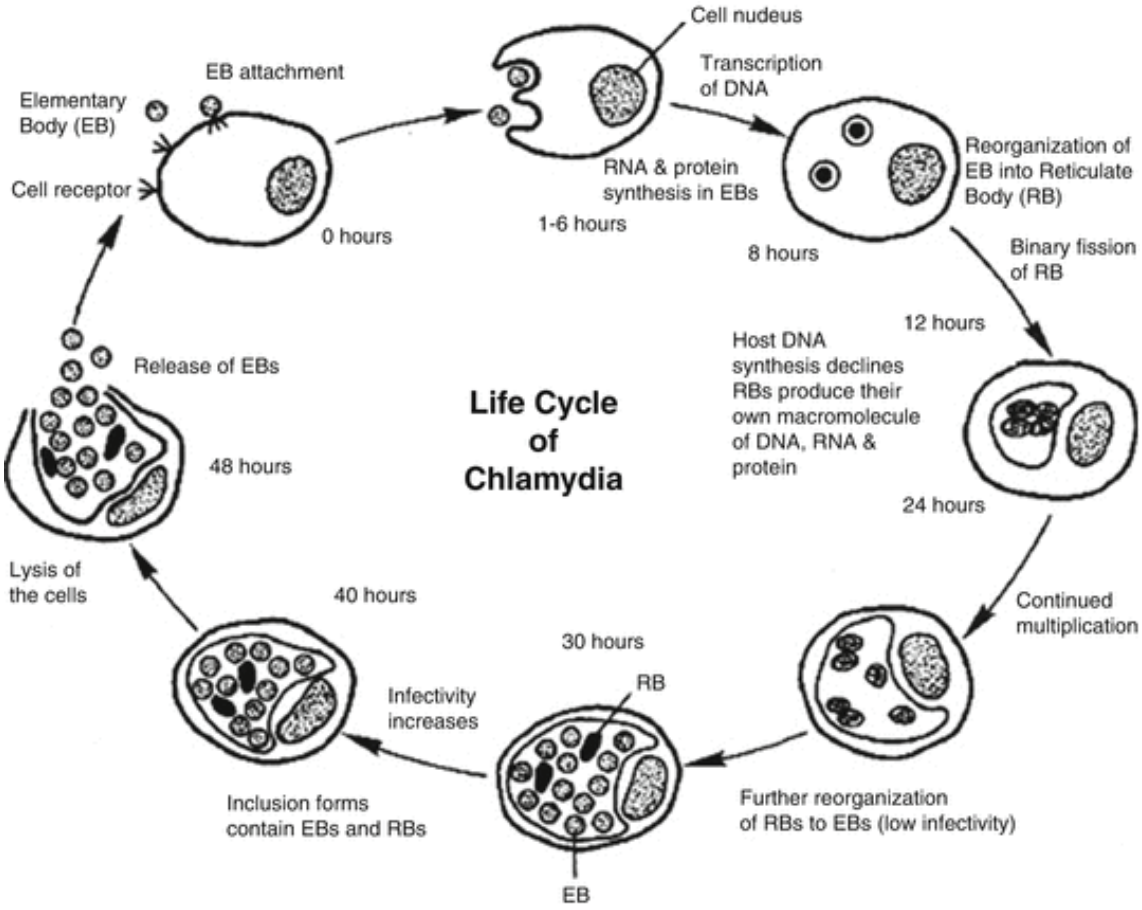
Type	Strain	Organ	Transmission mode	Disease
C. trachomatis	A-C	Eye	Direct transmission through flies from human to human	Trachoma
	D-K	Eye, urogenital tract Urogenital tract	STI Oculogenital Vertical transmission	Paratrachoma Adnexitis/ pelvic inflammatory disease urethritis arthritis conjunctivitis
	L1-L3	Urogenital tract	STI	Lymphogranuloma venereum
C. pneumoniae	None	Lungs	Aerogenic transmission	Pneumoniae
C. psittaci	None	Lungs	Aerogenic transmission	Airway infection (Ornithosis)

Reference: based on Suerbaum 2012

The elementary bodies enter the host cell within one to six hours via endocytosis, where they become reticulate bodies. The reticulate bodies are metabolically active, and they divide in the endosome of the host and can multiply. After three days, new infective elementary bodies are produced and released from the host cell and can now infect other cells in the host's body (Suerbaum, 2016). This study focuses on *C. trachomatis* as STI. Therefore, the focus on the following sections concerning epidemiological data, treatment, and clinical aspects focus on *C. trachomatis* strain D – K.

Figure 22

Life cycle of Chlamydia C. trachomatis



Reference: Cai and Tiscione, 2016

1.6.13.2 Epidemiology

C. trachomatis is the most common and most frequent bacterial STI in the world. According to the estimation of the WHO, 131 million new cases of chlamydia occurred among adults and adolescents aged 15 – 49 years worldwide in 2012. The global incidence rates are stated as 38 per 1 000 females and 33 per 1 000 males. The global prevalence is estimated to be 128 million persons infected with chlamydia. In the majority of the settings, the incidence is particularly high among girls aged 15 – 19 years and young women between 20 – 24 years (Rowley et al., 2019). The probability of contracting *C. trachomatis* per sexual intercourse is estimated to be about 10% and 20% (Rowley et al., 2019).

1.6.13.3 Clinical aspects

The infection of the urogenital tract with *C. trachomatis* strain D – K is defined as genital infection with chlamydia (Witkin et al., 2017). Through ascension, *C. trachomatis* can cause, as shown in Table 8, cervicitis, endometritis, salpingitis, and/or perihepatitis in women. The danger of an infection with *C. trachomatis* is that in 75% of the cases, infected women remain asymptomatic, leading to deleterious consequences. *C. trachomatis* is the most frequent cause of PID, ectopic pregnancy, and tubal infertility. Some studies have tried to investigate the duration of an asymptomatic infection and found that it can last up to 18 months. There is evidence that this infection can be cleared spontaneously through the adaptive and innate immune systems (Lane and Decker, 2016). Furthermore, among women having genital infection with *C. trachomatis* a vertical infection occurs in 60% of deliveries. Between 60 – 90% of these children are infected perinatally with *C. trachomatis*, showing conjunctivitis (ophthalmia neonatorum) in 40% of the cases and pneumonia in 20% of them (Petersen 2003, Hoyme 2007). Further, *C. trachomatis* during pregnancy can lead to prematurity, premature membrane rupture, low birth weight, and chorioamnionitis (Olthoff, 2019).

The perihepatitis, known as Fitz-Hugh-Curtis syndrome, is an adhesion between the capsule of the liver and the peritoneum and usually does not affect the liver parenchyma (Bremer, 2016). Clinically, individuals suffering from Fitz-Hugh-Curtis syndrome are characterised by sudden pain in the upper abdomen and the right shoulder. As the cervix is not innervated much, symptoms range from a vaginal discharge with a mucopurulent colour, to unspecific cervicitis with few or no symptoms. 70 – 80% of the women infected with *C. trachomatis* are asymptomatic. When the infection targets the Bartholin's glands or the urethra, symptoms can be painful.

1.6.13.4 Diagnosis

The best method to diagnose an infection with *C. trachomatis* directly is with NAAT, like PCR, using a sample of the infected region. NAAT remains the gold standard because of its high sensitivity and specificity. Another method is cell culture, which remains difficult since *C. trachomatis* is an obligate intracellular pathogen. The test requires four days to deliver results but has a high specificity. Serology searching for chlamydia-antibodies can be conducted with e.g. ELISA, but usually generates false negative results during the acute phase. Antibodies appear six to eight weeks after the beginning of the infection.

Antibodies can persist for months or years and should not be diagnosed as a chronic infection if asymptomatic (Lane and Decker, 2016).

1.6.13.5 Treatment

The WHO recommends 1 g azithromycin orally as a single dose or 100 mg of doxycycline orally twice a day for one week to treat uncomplicated genital chlamydia in adults and adolescents between 10 – 19 years, individuals living with HIV, and at-risk populations such as sex workers, men who have sex with men (MSM) and transgender persons. For pregnant women, WHO STI recommends 1 g of azithromycin orally as a single dose, amoxicillin 500 mg orally three times a day or as an alternative erythromycin 500 mg four times a day. When newborns suffer from ophthalmia neonatorum, therapy with azithromycin remains the first choice. Azithromycin is given as a dose of 20 mg/kg/day orally, one dose per day for three days. The second choice for treatment is erythromycin 50 mg/kg/day orally, in four divided doses daily for 14 days (Bremer, 2016).

1.6.14 Neisseria gonorrhoeae

1.6.14.1 Biology

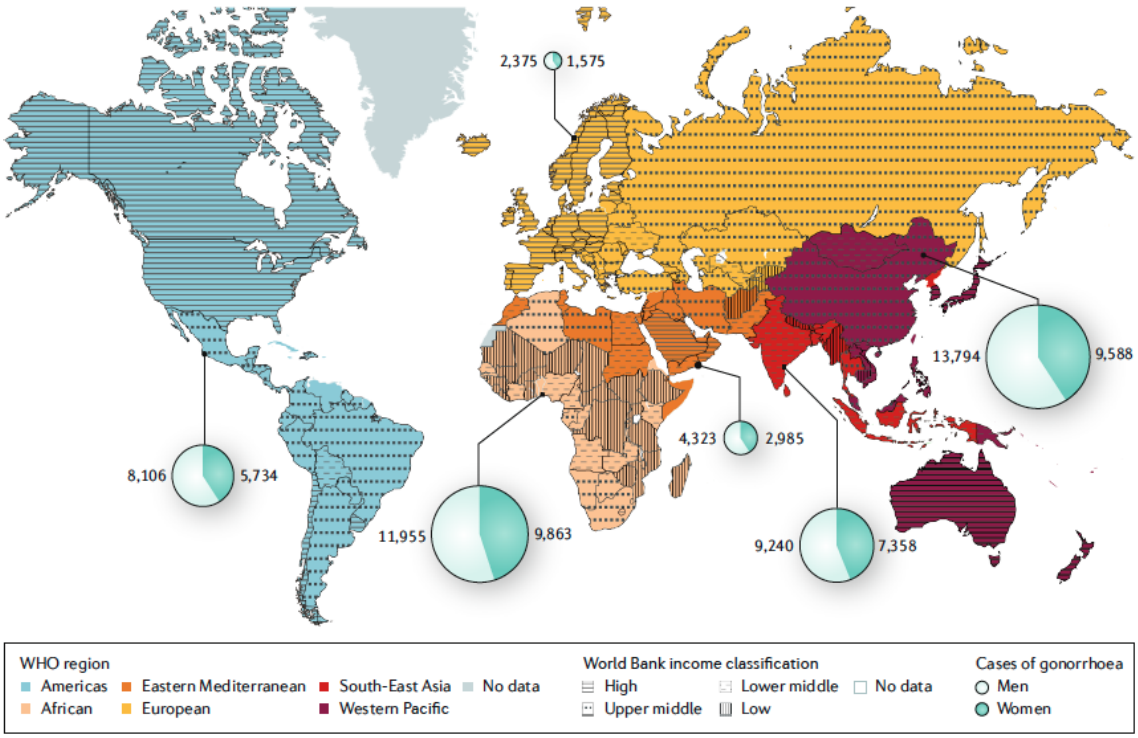
N. *gonorrhoeae* is a Gram-negative coccus and is the causative agent of gonorrhoea. Infection with N. *gonorrhoeae* is one of the most frequent STIs worldwide. *N. gonorrhoeae* is characterised to appear in pairs (diplococcus) with a size of 0.5 – 1 mm. *N. gonorrhoeae* is an obligate pathogen to humans. To date, 23 species of *Neisseria* are known, of which half infect only human beings, others infect only animals, and others both animals and humans (Tønjum and van Putten, 2017). *N. gonorrhoeae* grows best aerobically between 32° and 37°C under an atmosphere with 5 – 10% CO_2 (Tønjum and van Putten, 2017; Unemo et al., 2019). *N. gonorrhoeae* resembles other pathogenic *Neisseria* species like e.g. *Neisseria meningitidis* in terms of genome, morphology, and phenotypes (Tønjum and van Putten, 2017). The bacterium grows and multiplies in the mucosa of the urethra in men and women. It also grows in the cervix, the uterus, and the uterine tubes in women (Unemo et al., 2019).

1.6.14.2 Epidemiology

The principal way of *N. gonorrhoeae* transmission is through anal, oral, or unprotected vaginal intercourse. Another mode of transmission is during delivery, when the baby passes the birth canal. According to the WHO, in 2016, 86.9 million new cases of infection with N. *gonorrhoeae* occurred worldwide among adults between the age of 15 – 49 with a global prevalence of 0.9%. The average incidence is 20 new infections per 1 000 women and 26 per 1 000 men worldwide. Prevalence and incidence differ geographically. The WHO African region has an incidence of 41 gonococcal infections per 1 000 women and 50 per 1 000 men and is the region with the highest incidence globally. The second highest incidence is found in the WHO region of the Americas where, 23 cases per 1 000 women and 32 cases per 1 000 men occur. The WHO European region has the lowest incidence, with seven new infections per 1 000 women and 11 per 1 000 men (Unemo et al., 2019). The infection rate depends on sexual activity, sexual orientation, access to and quality of sex education, socioeconomics, demographics, geography, preventive methods, testing and diagnostics, cultural aspects, and awareness campaigns (Unemo et al., 2019). Due to STI prevention programmes, particularly in industrialised countries, a decline in the incidence of diseases was observed in the late eighties, but only for a short duration, following which the incidence increased again in the late nineties. The reasons for the global increase of gonococcal infections are strongly associated with ethnicity, economic status, and risky behaviour, especially in the era of ART for HIV. ART meant that HIV was no longer deadly, and improved electronic connectivity lead to an increased number of casual unknown partners in the frame of online dating apps and increased travel and access to many different services offered by the sex industry (Unemo et al., 2019). Men having sex with men, migrants, adolescents and young adults, and sex workers are at much higher risk of contracting gonococcal infections in comparison to the rest of the population (Unemo et al., 2019).

Figure 23

Global estimation of N. gonorrhoeae prevalence



Reference: (Unemo et al., 2019)

1.6.14.3 Clinical aspects

The incubation period for an infection with *N. gonorrhoeae* ranges between two to eight days. In women, an infection with *N. gonorrhoeae* can be categorised as an infection with *N. gonorrhoeae* of the lower genital tract or an infection with *N. gonorrhoeae* of the upper genital tract. Infection with *N. gonorrhoeae* of the lower genital tract refers to infection of the lower part of the endocervix and is usually asymptomatic or mildly symptomatic. Gonococcal cervicitis may be combined with bartholinitis, urethritis, proctitis, or cervicitis. When infection with *N. gonorrhoeae* affects the upper part of the genital tract symptoms occur more often, causing endometritis, salpingitis, and pelvic peritonitis. Women with infection with *N. gonorrhoeae* may complain of purulent discharge, dysuria, and a foul smell. *N. gonorrhoeae* is also the causative agent of PID with long-term pelvic/abdominal pain. Fever and an acute abdomen can occur. Furthermore, symptomatic infection with *N. gonorrhoeae* can also lead to a disseminate infection characterised by three

different symptoms: polyarthritis associated with haematogenic pustules and petechiae. In the worst-case scenario, infection with *N. gonorrhoeae* can disseminate and lead to sepsis with endocarditis, meningitis, osteomyelitis, and pneumonia (Unemo et al., 2019). Complications such as ectopic pregnancies, miscarriages, or tubal infertility can occur due to adhesion-forming chronic inflammation. In newborns of gonococcal infected individuals, a vaginal delivery may lead to neonatal conjunctivitis, called gonococcal ophthalmia neonatorum. Symptoms are a strong purulent secretion, red eyes, and chemosis. An untreated gonococcal ophthalmia neonatorum can cause blindness, ulcer, and even perforation of the cornea (Yeshanew and Geremew, 2018).

1.6.14.4 Diagnosis

Infection with *N. gonorrhoeae* can be diagnosed through light microscopy of Gram-stained samples, but sensitivity and specificity vary between 40 – 95% (Unemo et al., 2019), depending on specimen type and if an individual is infected or diseased. Light microscopy of urethral swabs of symptomatic men had the highest sensitivity and specificity in comparison to endocervical or urethral specimens from asymptomatic women (Unemo et al., 2019). In the past, culture was the gold standard, however this has changed since the introduction of NAATs. Culture is an inexpensive method and is the first recommendation to evaluate treatment success or failure (Unemo et al., 2019). Another benefit of culturing *N. gonorrhoeae* is the fact that antimicrobial resistance can only be detected and evaluated by culture (Unemo et al., 2019). The specificity of urogenital samples ranges between 72% to 95% and can even reach up to 95% to 100% in a laboratory with high expertise and the appropriate specimen. They are preferred because sample collection is non-invasive using urine or self-collected vaginal swabs. NAATs are highly specific and sensitive, ranging between 81.1% and 100%, even if the individual is asymptomatic. The sensitivity and specificity depend on the used test and the collected specimen. Diagnosis by NAATs leads to quick results. Another advantage is that the use of various NAATs allows the diagnosis of other STI-associated pathogens like *C. trachomatis* (Unemo et al., 2019).

1.6.14.5 Treatment

Using syndromic management of vaginal discharge usually leads to overtreatment as infection with *N. gonorrhoeae* cannot be distinguished from individuals that suffer from

symptomatic infections caused by *C. trachomatis, Mycoplasma genitalium*, *T. vaginalis,* or bacterial vaginosis (Unemo et al., 2019). The WHO recommends a calculated dual antimicrobial regimen with ceftriaxone and azithromycin for the treatment of infection caused by *N. gonorrhoeae* in the lower genital tract. Some countries recommend cefixime plus azithromycin as a second line treatment if ceftriaxone is not accessible or refused (WHO, 2016). The background for the dual therapy is the frequency of coinfection with *C. trachomatis* (10% to 40%), which can be effectively treated with azithromycin. Simultaneous treatment of partners is crucial to avoid a ping-pong effect (WHO, 2016). Infection with *N. gonorrhoeae* of the upper genital tract (salpingitis, adnexitis, PID) is treated with ceftriaxone plus azithromycin or levofloxacin and metronidazole (WHO, 2016).

1.6.15 Group B Streptococcus

1.6.15.1 Biology

Streptococcus agalactiae, also known as Group B *Streptococcus* (GBS), is a Gram-positive, catalase-negative, facultative anaerobe that is immotile and does not produce spores (Suerbaum et al., 2016). It was first described by Nocard and Mollerau in 1887 as the causative agent of bovine mastitis (Vornhagen et al., 2017). In the thirties, Rebecca Lancefield differentiated GBS from other *Streptococci*. Lancefield isolated GBS from the human vagina of asymptomatic women, but human pathogenicity was first described in 1938. In the reports that were published, adverse birth outcomes were described (Raabe and Shane, 2019). In the sixties, invasive reports of GBS disease, such as neonatal invasive disease, were increasingly published. It is now known that GBS may be the causative agent of preterm birth and some other neonatal diseases (Vornhagen et al., 2017). Thus, women that are colonised with GBS at 35 weeks of gestation until delivery are at risk for MTCT (Vornhagen et al., 2017). There are ten serotypes of GBS (Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX). The serotypes that are associated with invasive GBS disease vary from region-to-region (Raabe and Shane, 2019). GBS can be classified clinically or based on the Lancefield grouping. The Lancefield grouping considers the specific cell wall carbohydrate antigen in *streptococci*. Therefore, *streptococcus* is grouped in serogroup A – W and in a group without any serogroup. *Streptococcus agalactiae* is classified in GBS. Furthermore, *Streptococcus agalactiae* can also be classified by their haemolytic characteristics.

Streptococci grow best on blood agar, which allow for the description of three different types of haemolysis (Suerbaum et al., 2016):

- Alpha haemolysis: *Streptococci* release H_2O_2 and oxidize haemoglobin from erythrocytes. This leads to a compound that resembles biliverdin, creating a green area surrounding the streptococcal colonies.
- Beta haemolysis: Haemolytic enzymes completely lyse erythrocytes and thereby cause a colourless surrounding area.
- Gamma-haemolysis: *Streptococci* of this group do not have haemolytic characteristics (Suerbaum et al., 2016).

Streptococcus agalactiae is a beta-haemolytic coccus.

1.6.15.2 Epidemiology

The presence of GBS in the vagina is not defined as infection with GBS but as the colonisation of the affected individual and plays an important role in pregnant women. Thus, in this paragraph, epidemiological aspects of invasive GBS disease are described. It is estimated that the global incidence of systemic invasive GBS disease during pregnancy is 0.38 cases per 1 000 pregnancies, with a case fatality rate of 0.2%. Invasive GBS disease is mostly caused by serotypes Ia and III. The odds of premature delivery and neonatal sepsis are higher in women with systemic maternal GBS disease. It is estimated that five to thirty percent of pregnant women globally are colonised with GBS and are asymptomatic (Raabe and Shane, 2019). In the study by Raabe and Shane, the colonisation rate among pregnant women was highest in Caribbean women with a colonisation rate of 35%, and lowest in East Asian women with a colonisation rate of 11% (Raabe and Shane, 2019). In Gabon, 16 – 23% of pregnant women are colonised with GBS (Capan-Melser et al., 2015). As GBS plays an important role worldwide, screening between 35 to 37 weeks of gestation has been an efficacious and life-saving strategy for mother and children. Screening allows for the identification of women colonised with GBS, meaning prophylactic therapy can be given during delivery and post-partum to prevent early-onset GBS infection and disease in neonates. These strategies have diminished rates of early-onset infection and disease (Olthoff, 2019).

1.6.15.3 Clinical aspects

When a pregnant woman is colonised with GBS, GBS can get to the uterus, where foetal membranes can be infected, causing chorioamnionitis. Chorioamnionitis increases the risk of premature labour, miscarriage, or infections in utero.

Further invasive GBS disease in neonates may have an early onset, defined as a disease that occurs within the first week of life. Some consider a newer definition of early-onset disease as a disease occurring within 72 h of life, with this definition proving more popular (Olthoff, 2019). Invasive GBS disease in neonates may have a late onset, defined as a disease that occurs seven to ninety days after delivery, with a median onset of the disease at 37 days (Olthoff, 2019). Common clinical manifestations of early onset disease may include meningitis, bacteraemia, bone, joint, and soft tissue infection, and pneumonia. During delivery, GBS can invade the respiratory tract of the neonate. As GBS can destroy the alveolar epithelia in neonates through beta-haemolysis, they can cross the respiratory barrier and access the bloodstream, where they may cause neonatal sepsis in 80% of neonates with early onset disease. Bloodstream GBS can also access the CSF by invading the blood-brain barrier and therefore cause neonatal meningitis in five to ten percent of cases of neonates with early onset disease. In some neonates with early onset disease, septic arthritis has been described (Raabe and Shane, 2019; Vornhagen et al., 2017). Clinical symptoms occur in 61% – 95% of neonates one hour after delivery. They develop symptoms like grunting respirations, cyanosis, tachypnoea, apnoea, lethargy, abdominal distention, pallor, jaundice, poor feeding, hypotension, and tachycardia within the first 24 hours. Fever can also occur but is more common in term neonates and is not present in preterm infants.

The symptoms of neonates with late-onset disease resemble those of early-onset disease (Cortese et al., 2016). Meningitis occurs more frequently in late-onset disease than in early-onset disease. Other clinical manifestations include osteomyelitis and cellulitis-adenitis syndrome, which is usually unilateral, including facial or submandibular sites, and pyogenic arthritis (Cortese et al., 2016). Most of the neonates with a late-onset disease are premature infants or infants with a very low birth weight (Cortese et al., 2016).

1.6.15.4 Diagnosis

A culture-based screening is recommended for women between 35 and 37 weeks of gestation (Olthoff, 2019, Puopolo et al., 2019). Therefore, a rectovaginal swab from the

woman has to be collected for GBS culture. Pregnant women at higher risk for preterm birth can profit from a culture-based screening before 35 weeks of gestation. In addition to a GBS culture, antimicrobial resistance testing is recommended to optimise antibiotic prophylaxis or to have alternatives if the woman has a penicillin allergy (Suerbaum et al., 2016). Further screening includes the detection of GBS in urine which is recommended throughout the whole pregnancy (Puopolo et al., 2019).

When early onset or late onset disease of the neonate is suspected, microbiological diagnosis is performed by the cultivation of GBS. Samples of affected body parts including blood samples have to be collected from a sterile body site to avoid contamination. Furthermore, 30% of neonates presenting with meningitis, have a negative blood culture, which is the reason why a lumbar puncture is crucial for the diagnosis of meningitis. Other methods such as rapid antigen detection are not appropriate in the management of early or late onset disease or need further evaluation, such as real-time PCR method (Puopolo et al., 2019).

1.6.15.5 Treatment

The antibiotic agent of choice in order to prevent MTCT of GBS is the administration of penicillin G or ampicillin (Puopolo et al., 2019), and should be administered to women with a positive GBS culture during screening (Puopolo et al., 2019). Premature rupture of membranes for more than 18 hours, mothers presenting with more than 38°C fever during labour, unknown GBS status in preterm deliveries (less than 37 weeks of gestation), and women having previously given birth to a child with invasive early onset disease with GBS are absolute indications for antibiotic prophylaxis (Puopolo et al., 2019). The intravenous administration of ampicillin followed by further administration of ampicillin every four hours intrapartum until delivery is an alternative when penicillin G is not accessible. Penicillin allergy is a contraindication for ampicillin and penicillin (Puopolo et al., 2019). Clindamycin and cefazolin are alternatives. When GBS is resistant to clindamycin, vancomycin can be administered. Administration of antibiotic prophylaxis more than four hours before delivery is the best prevention of early-onset disease. Nevertheless, any antibiotic prophylaxis given less than four hours before delivery is still reasonable because it still can offer some protection (Puopolo et al., 2019).

In children with GBS neonatal sepsis, ampicillin and aminoglycosides such as gentamicin are used for clinical management. The dual therapy is highly effective as most GBS

strains and other pathogens are susceptible to one of the antibiotics. A monotherapy with penicillin G should be administered if a microbiological analysis has confirmed that GBS is the causative agent. Dose and duration depend on the severity and weight of the child (Puopolo et al., 2019); Cortese et al., 2016).

2 Materials and Methods

2.1 Study site Gabon

The Republic of Gabon is a country on the West Coast of Central Africa located on the Equator. Neighbouring countries of Gabon are Equatorial Guinea in the Northwest, Cameroon in the North, and the Republic of Congo in the East and South. In the West, Gabon borders the Gulf of Guinea (CIA, 2021; Ramharter et al., 2007). The country has a size of 267 667 km^2, with a landscape of which 85% is covered by tropical rainforest. It has a population of two million people, most recently reported in 2017. The capital city Libreville is the largest and the most populated city in Gabon (CIA, 2021). Libreville and Port-Gentil (the economic capital) account for 59% of the Gabonese population (United Nations Development Programme, 2018; Worldbank, 2021). With the seventh highest Human Development Index (Human Development Index = 0.702) in sub-Saharan-Africa, Gabon is one of the most prosperous countries in that region thanks to its crude oil and foreign private investment (United Nations Development Programme, 2018; Worldbank, 2021).

Lambaréné, located 250 kilometres in the Southeast of Libreville and 75 kilometres South of the Equator, is the capital of the province Moyen-Ogooué. Lambaréné is situated in the middle of the Central African tropical rainforest at the Ogooué River. As of 2013, 38 775 people lived in Lambaréné (Ramharter et al., 2021, 2007).

The Albert-Schweitzer hospital in Lambaréné was founded in 1913 by Dr. Albert Schweitzer. His purpose was to provide medical care to the local population. Infections with *Plasmodium* spp, HIV, or *Mycobaterium tuberculosis* and the diseased form of the infection play an important role in Lambaréné and have a negative impact on morbidity, mortality, and socioeconomic development (Ramharter et al., 2021, 2007). The Centre de Recherches Médicales de Lambaréné (CERMEL) is a research centre in Lambaréné, which was established in 1981. CERMEL is situated in the centre of the Albert Schweitzer hospital (Ramharter et al., 2007). Over the past 25 years, CERMEL has performed clinical trials for drugs and vaccines, and diagnostic studies, mainly in the field of malaria and NTDs (Ramharter et al., 2021).

2.2 Clinical trial MAMAH

The co-infection and the prevalence study are embedded in a larger umbrella study called: "*Improving **m**aternal he**a**lth by reducing **m**alaria in **A**frican **H**IV women (MAMAH)".* MAMAH is a multicentric, two-arm placebo-controlled individually randomised trial financed by the European and Developing Countries Clinical Trials Partnership (EDCTP). The MAMAH study is coordinated by IS-Global from Barcelona, Spain, and is conducted in clinical trial centres in Mozambique and Gabon (González et al., 2021).

2.3 Doctoral thesis project "Little MAMAH"

Due to the fact that this doctoral thesis project was nested in the clinical trial MAMAH, it was denominated as "Little MAMAH". Little MAMAH consisted of two sub-studies: One study was a prevalence study. The prevalence study was conducted at first in different ANC units in Central, South, and North Gabon. The second study was the so-called "co-infection study", which aimed to characterise the different co-infections in HIV-positive and HIV-negative women during pregnancy. Both studies are further described in this chapter.

2.3.1 Study setting and study design of HIV-prevalence study

The first phase of Little MAMAH was a cross-sectional study that was conducted from December 2018 until February 2019 in five out of nine provinces in Gabon: Moyen-Ogooué, Nyanga, Ngounié, Estuaire, Woleu Ntem. In each location, ANC units in which pregnant women received routine care during pregnancy were identified. Subsequently, prevalence of HIV was determined retrospectively among pregnant women who attended the wards of the selected ANC units.

Due to the ongoing activities of the National HIV/AIDS Control programme of Gabon, data on the frequency of first visits, number of women tested for HIV, and those who were tested HIV-positive were accessible in each ANC unit. Prevalence of HIV-positivity was calculated as the number of HIV-positive cases by the number of all pregnant women tested during the period evaluated.

2.3.2 Study setting, study population and study design of co-infection study

The second phase of Little MAMAH was also a cross-sectional study, this time conducted from February 2019 to February 2020 only in Lambaréné of the province of Moyen-

Ogooué, at the ANC units of both the Georges Rawiri (also called the regional hospital) and Albert Schweitzer Hospitals. The rate of several infections was determined in both HIV-positive and HIV-negative pregnant women. The pregnant women were approached by the study personnel and screened for eligibility. A pregnant woman was eligible to participate in the co-infection study if she gave written consent, if HIV status was known, and if she was pregnant. All pregnant women were seen only once. The sample of HIV-positive pregnant women was frequency-matched with a sample of HIV-negative women for age and parity in order to limit bias. The frequency matching was considered successful if the median age in the HIV-positive and HIV-negative samples was within a difference of four years or less. Similarly, median parity needed to be within one parity of difference or no difference at all.

2.3.2.1 Study procedures

Data collection was done using paper-based source documents from which data were transcribed to an electronic database. Data that was routinely available in clinical records was used and otherwise determined by the study team. The following data were ascertained:

- Baseline characteristics included age, parity, HIV status and the presence of concomitant infections, vector-borne infections, and STIs.
- HIV-related variables included CD4 counts, viral load, time since HIV diagnosis, whether a HIV-positive pregnant woman was under ART, time since ART initiation, and whether she took CTX.
- Individual behavioural variables included history of sexual partners.
- Socio-economic factors included employment status.

2.3.3 Aims of Little MAMAH

Little MAMAH (the prevalence and the co-infection study) aimed to identify shortcomings in antenatal care in rural Gabon, particularly among HIV-positive pregnant women. Below are the specific objectives for the HIV prevalence study and the co-infection study.

2.3.4 Objectives of HIV prevalence study

The primary objective was to identify ANC units with a high HIV prevalence. This meant providing HIV prevalence data for 21 ANC units in five provinces in Gabon and determining the number of HIV-positive pregnant women among all tested pregnant women attending the different ANC units.

2.3.5 Objectives of co-infection study

The primary objective was to determine the prevalence of 15 co-infections in a cross-sectional sample of HIV-positive pregnant women compared to a sample of HIV-negative pregnant women. This included the assessment and comparison of the prevalence of having any concomitant co-infection, any concomitant vector-borne infection, and any concomitant STI in a sample of HIV-positive and HIV-negative pregnant women.

2.4 Data ascertainment for the HIV prevalence study

For the purpose of the HIV prevalence study, ANC units were visited, and routine data on HIV prevalence was captured. ANC units used rapid diagnostic tests to detect the presence of HIV infection. The detailed sample processing has been described in chapter 2.7.37 at page 97.

2.5 Blood sampling for the co-infection study

For the assessment of various co-infections listed in chapter 1.6, 9 ml of venous blood was collected using EDTA tubes and serum tubes. After using some blood for the preparation of thick and thin smears to determine *Plasmodium* spp., and thick smears for the determination of *Mansonella perstans* and *Loa loa* microfilariae, blood was directly aliquoted into 2.5 ml Eppendorf Tubes and conserved at -80°C for shipment to the University Medical Care Center Hamburg-Eppendorf (UMCHE). Serum tubes were first centrifuged before aliquoting them into 2.5 ml Eppendorf tubes and conserved at -80°C prior to shipment.

Infections with HEV, HCV, and HTLV-1 were determined with ELISA at the UMCHE. The presence of anti-HTLV-1/HTLV-2 antibodies, anti-HEV-IgM, and anti-HCV antibodies was considered as positive results; the absence of IgG and IgM antibodies was considered as negative. The presence of IgM antibodies indicated a current or recent

infection, while IgG antibodies indicated a past infection. HIV-viral load and EBV infection were determined by PCR.

Infection with *S. haematobium* was determined by PCR at the Bernhard-Nocht-Institute for Tropical Medicine.

As HIV-1 and 2 testing was included in the routine diagnostic services according to the guidelines of the National HIV/AIDS Control programme of Gabon in the frame of the first visit, results were transcribed from clinical records, and no further HIV tests were performed. The same applied to infections with HBV and *Treponema pallidum*. As mentioned above, HIV viral load was determined by PCR at UMCHE. CD4 count was often available for HIV-positive women as part of routine diagnostic services. Infection with *Plasmodium* spp. was determined using a thick and a thin blood smear, and *Mansonella perstans* and *Loa loa* microfilariae were determined using thick blood smears at CERMEL.

2.6 Sampling of vaginal and cervical smears for the co-infection study

A vaginal examination was performed by first examining the vulva for any infectious signs, and then a speculum examination was performed by inspecting the cervix for signs of infection, vaginal discharge, and odour. To determine HPV, *T. vaginalis*, *C. trachomatis,* or *N. gonorrhoea* infections, ecto- and endocervical samples were taken by using nylon swabs (eNat Copan Diagnostics, Brescia, Italy) containing a one ml liquid Amies transport medium. Then the swabs were stored in a freezer at -80°C prior to shipment to the microbiology ward at the UMCHE, where sample assessment was performed.

2.7 Sample processing

2.7.1 Bacterial infections

2.7.1.1 Treponema pallidum

T. pallidum results were taken from routine hospital records. No further testing was performed. The hospitals used Alere Determine™ Syphilis TP.

2.7.1.2 Group B Streptococcus

For the determination of GBS colonization, a recto-vaginal swab (eSwab, Copan Diagnostics, Brescia, Italy) was taken. Five ml of Lim Broth (Todd–Hewitt broth, 1% yeast

extract, 15 mg/mL nalidixic acid, and 10 mg/mL colistin) (Becton Dickinson) was used to selectively cultivate GBS (Capan et al., 2010). After having incubated the medium under aerobic conditions at 36.8°C for 24 h, the medium was subcultured for another 24 h on Granada agar selective for GBS (Becton Dickinson) at 36.8° C in an anaerobic box as described by the manufacturer, where 30 ml were put before. According to the manufacturer, the sample was positive when yellow-orange-pigmented colonies appeared (Capan et al., 2010).

2.7.1.3 Neisseria gonorrhoea and Chlamydia trachomatis

The Qiasymphony (Qiagen®) system was used with the DSP Virus/Pathogen mini (Qiagen®) kit to extract DNA according to the manufacturer's instructions. To perform the PCR for *N. gonorrhoea* and *C. trachomatis* with the LightCycler 480II (Roche®), a LDT-PCR (dual target. porA, opa) was used. Primers and probes were slightly modified afterwards (Goire et al., 2012) for the determination of *N. gonorrhoea*. For the determination of *C. trachomatis,* primer and probes were slightly modified after (Chen et al., 2008). The PCR reaction contained the mastermix PerfeCTa qPCR ToughMix®. For the internal control spike/assay a Roche® control kit was used.

2.7.2 Parasitic infections

2.7.2.1 Plasmodium spp.

Thick and thin blood smears were prepared. Thick blood smears contained 10 µl and were put on glass slides. The slides were then dried. After 20 minutes the slides were stained in a 20% Giemsa solution (Giemsa R-Solution, Merck, Darmstadt; Titrisol Puffer pH 7.2, Merck, Darmstadt) and rinsed with tap water. Afterwards, the slides were dried again. The slides were observed in 100 high power fields (HPF) under a 100 x immersion oil objective (Nikon Eclipse E200, Tokyo, Japan or Olympus CH 30, Tokyo, Japan). The thick smear was used to determine if a pregnant woman was infected with *Plasmodium* spp. and to determine parasitaemia (Joanny et al., 2014). Parasitaemia was expressed as parasites per microliter determined by the Lambaréné method (Mischlinger et al., 2018). For the thin smears, five microliters of blood were used and put on the glass slides. After drying, the glass slides were fixated with methanol prior to staining. The thin smear was used in order to determine the species of *Plasmodium* (CDC, 2018).

2.7.2.2 Loa loa and Mansonella perstans microfilariae

EDTA blood samples were collected to determine if a pregnant woman had an infection with *Loa loa* or *Mansonella perstans* microfilariae or possible co-infection. Thick blood smears contained 10 µl and were put on glass slides. The slides were then dried. After 20 minutes the slides were stained in a 20% Giemsa solution (Giemsa R-Solution, Merck, Darmstadt; Titrisol Puffer pH 7.2, Merck, Darmstadt) and rinsed with tap water. Afterwards, the slides were dried again (Veletzky et al., 2020). The slides were observed in a 100 high power fields (HPF) under a 100 x immersion oil objective (Nikon Eclipse E200, Tokyo, Japan or Olympus CH 30, Tokyo, Japan).

2.7.2.3 Trichomonas vaginalis

The Qiasymphony (Qiagen®) system was used with the DSP Virus/Pathogen mini (Qiagen®) kit to extract DNA according to the manufacturer's instructions. To perform the PCR for *T. vaginalis* with the LightCycler 480II (Roche®), a LDT-PCR (single target) was used. Primer and probes were slightly modified after (Caliendo et al., 2005). The PCR reaction contained the mastermix PerfeCTa qPCR ToughMix®. For the internal control, spike/assay Roche® control kit was used.

2.7.2.4 S. haematobium

DNA was extracted from serum with the QIAamp DNA MinElute ccfDNA Mini kit (Qiagen® Benelux, Venlo, the Netherlands) according to the manufacturer's guidelines.

The Hot StarTaq mastermix kit of Qiagen® was used for the Taqman® qPCR.

2.7.3 Viral infection

2.7.3.1 Human Papillomavirus (and subtypes)

The Qiasymphony (Qiagen®) system was used with the DSP Virus/Pathogen mini (Qiagen®) kit to extract HPV DNA according to the manufacturer's instructions. To perform the semiquantitative real-time multiplex PCR assay the LightCycler 480II (Roche®) an Anyplex II HPV28 (Seegene®) was used. Thus, HPV was screened and genotyped for 28 HPV subtypes in two PCR reactions.

2.7.3.2 Epstein-Barr virus

The DNA was isolated using the extraction system of Magna Pure (Roche®) with the Viral DNA/RNA small volume kit (Roche®). The quantitative PCR was done with the LightCycler 480II (Roche®). The kit LDT-PCR (single target) was used. Primer and probes were slightly modified after Pollak 2012 (Luetgehetmann, 2022). The PCR reaction contained the mastermix PerfeCTa qPCR ToughMix®, and for the internal control of the assays, the control kit from Roche® was used.

2.7.3.3 HTLV1 and 2

Chemiluminescent ImmunoAssay (CLIA) was done in order to detect anti-HTLV-1/HTLV-2 antibodies in serum with the system Alinity I (Abott®). The assay anti-HTLV I/II (Abbott®) was performed according to the manufacturer's instructions.

2.7.3.4 Hepatitis E virus

ELISA was done with the Euroimmun analyzer I (Euroimmun®). An anti-HEV-IgG and anti-HEV IgM assay (Wantai®) was used for qualitative determination of IgG antibodies against HEV in serum. The samples were processed according to the manufacturer's instructions.

2.7.3.5 Hepatitis C virus

Same as described in chapter 2.6.3.3. Anti-HCV antibodies in serum were determined with the system Alinity I (Abott®). The assay anti-HCV (Abbott®) was performed according to the manufacturer's instructions.

2.7.3.6 Hepatitis B virus

Results of infection with HBV were taken from the routine records. No further testing was performed. The hospital used VIKIA® HBs Ag (Biomérieux). The tests detected HbsAg.

2.7.3.7 Human immunodeficiency virus

HIV 1 and HIV 2 infection was determined in full blood according to the national guidelines. Three different rapid diagnostic tests were used. The first test was Determine 1/2 which is based on antibodies. If the test was positive, Determine 1/2 Combo, which is

based on antibodies and antigens, was used. The third test was ABON 1/2/0, used to differentiate and confirm whether the pregnant woman was either HIV 1 or HIV 2 positive.

2.8 Statistical Methods

2.8.1 HIV prevalence study

The prevalence was calculated as the number of positive cases divided by the number of pregnant women tested during the period evaluated for each ANC unit. Chi square (X^2) test was used for hypothesis testing of proportions.

2.8.2 Co-infection study

The data set included baseline characteristics of pregnant women such as age, parity, HIV status, and the presence of co-infections.

Co-infections included the infection with *T. vaginalis*, HPV and subtypes, HTLV 1 and 2, *S. haematobium,* HEV, HCV, *N. gonorrhoea*, *C. trachomatis*, EBV, *T. pallidum*, HBV, *Plasmodium* spp*., Loa loa* microfilariae, *M. perstans* microfilariae, GBS. Co-infections were classified in three different sub-groups:

- Concomitant infections (all infections listed chapter 1.6),
- Vector-borne infections: (*Loa loa* microfilariae*, M. perstans* microfilariae*, Plasmodium* spp., *S. haematobium*),
- STIs (HIV-2, *C. trachomatis*, *N. gonorrhoea*, HBV, HCV, *T. vaginalis*, HPV and subtypes, *T. pallidum*).

Furthermore, variables that characterise the sample of HIV-positive pregnant women were used. These variables included time since HIV diagnosis, initiation of ART, time since ART initiation, CD4 count, viral load, and the intake of CTX. Statistical analyses were conducted using STATA 17.0 Basic Edition (StataCorp, USA). The prevalence of each co-infection was determined among all pregnant women participating in the co-infection study by calculating the number of positive cases by the number of pregnant women tested during the study period in the selected ANC units. For proportions, p-values were determined using the Chi square (χ^2) test. If a pregnant woman had at least one concomitant infection, this was termed positive for “any concomitant infection”. Similarly, this was applied for vector-borne infections and STIs. Prevalence of the number of

concomitant co-infections, vector-borne infections, and STIs was determined by calculating the number of positive cases by the number of pregnant women tested during the co-infection study period. Median and interquartile ranges were determined. For non-parametrically distributed continuous data, p-values were determined using the *Wilcoxon rank sum* test. Next, logistic regression models were used to estimate unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of any concomitant infection, vector-borne infection, and STI. In the group of any concomitant infection and any vector-borne infection, adjustment was done for the following variables: age and being employed.

In the group of any STIs, adjustment was done in addition to the variables age and being employed for the following variables: gravidity, sexual partners in life, sexual partners within the previous month, living with a partner, age, being employed, and method of contraception. The likelihood ratio test was used to determine the p-value to assess the quality of fit of the adjusted model in the unadjusted model.

2.9 Ethics

The study protocol of the HIV prevalence study and the co-infection study, and consent of Little MAMAH were approved by the institutional ethics committee of CERMEL, the national ethics committee of Gabon, and the ethics committee of the medical board in Hamburg, Germany.

Written consent of all pregnant women enrolled in the co-infection study was obtained according to the ethical principles stated in the Declaration of Helsinki, the applicable guidelines for ICH-GCP, and the applicable laws and regulations of Gabon. A copy of the signed and dated informed consent form was given to all enrolled pregnant women. The signed and dated original consent form was kept with the study records. In case a pregnant woman tested positive for an infection, the pregnant woman was informed and treatment advice was given accordingly.

3 Results

3.1 HIV prevalence among pregnant women (prevalence study)

Overall, 16 417 pregnant women were screened for HIV in 2018 at 21 selected ANC units in the provinces of Moyen Ogooué (Lambaréné), Estuaire (Libreville), Ngounié (Fougamou), Nyanga (Tchibanga and Mayoumba), and Woleu Ntem (Bitam and Oyem), with the overall prevalence being 3.93% (646/16 417). The overall prevalence in Libreville was 3.61% (450/ 12 471). There was a marked variability between ANC units within Libreville. The HIV prevalence in Libreville ranged between 1.67% (17/1017) and 5.95% (92/1546). The HIV prevalence in the northern province, Woleu-Ntem (Oyem and Bitam), was 4.16% (53/1273); while in Moyen-Ogooué (Lambaréné), in the centre of Gabon, the HIV prevalence was 5.22% (18/345); and in the southern provinces of Ngounie and Nyanga 3.8% (10/263) and 5.56% (33/594) respectively.

3.2 Study population and baseline characteristics of co-infection study

Overall, 185 pregnant women were enrolled in the co-infection study, with 63 HIV-positive and 122 HIV-negative pregnant women. The median age of all pregnant women was 27 (interquartile range (IQR): 22 – 33) years; HIV-positive pregnant women had a median age of 29 (IQR: 24 – 35) years, and HIV-negative pregnant women had a median age of 25 (IQR: 20 – 33) years. The age demographic was classified into three different groups: 10 – 19 years, 20 – 29 years, and 30 years and above. The age distribution among all pregnant women was 23 years (12.43%; 23/185). 12.43% (23/185) women were 10 – 19 years old, 49.19% (91/185) were 20 – 29 years, and 38.38% (71/185) were 30 years and older. Among HIV-positive pregnant women, 4.76% (3/63) women were 10 – 19 years old, 47.62% (30/63) were 20 – 29 years, and 47.62% (30/63) were 30 years or older. Among HIV-negative pregnant women, 16.39% (20/122) were 10 – 19 years old, 50% (61/122) were 20 – 29 years old, and 33.61% (41/122) were 30 years or older. The median parity of all pregnant women was 2 (IQR: 1 – 3) parities (details shown in Table 9). Parity was higher among HIV-positive pregnant women. Parity of 3 (IQR: 1 – 4), whilst HIV-negative pregnant women had a parity of 2 (IQR: 1 – 3). 19.46% (36/185) of the pregnant women were nulliparous. 28.92% (35/185) of all pregnant women were primiparous; 61.62% (114/185) were multiparous. Among the multiparous pregnant women, 50.27% (93/185) had a parity between two to five and 11.35% (21/185) had a parity higher than

five. Among the HIV-positive pregnant women, 14.29% (9/63) were nulliparous, 12.70% (8/63) were primiparous, 60.32% (38/63) had a parity between two to five, and 12.70% (8/63) HIV-positive pregnant women had a parity greater than five. In the sample of HIV-negative pregnant women, 22.13% (27/122) were nulliparous, 22.13% (27/122) were primiparous, 45.08% (55/122) with parity of two to five, and 10.66% (13/122) with parity of more than five.

Table 9

Baseline characteristics of the study population in the co-infection study

Characteristics	Totals (N=185)	HIV+ (n=63)	HIV- (n=122)
	n (column %)	n (column %)	n (column %)
Age			
Median age in years (IQR)	27 (22 – 33)	29 (24 – 35)	25 (20 – 33)
10 – 19 years	23 (12.43%)	3 (4.76%)	20 (16.39%)
20 –29 years	91 (49.19%)	30 (47.62%)	61 (50.00%)
30 and above	71 (38.38%)	30 (47.62%)	41 (33.61%)
Parity			
Median number of parities (IQR)	2 (1 – 3)	3 (1 – 4)	2 (1 – 3)
Nulliparous	36 (19.46%)	9 (14.29 %)	27 (22.13%)
Primiparous	35 (18.92%)	8 (12.70 %)	27 (22.13 %)
Multiparous (2 – 5)	93 (50.27%)	38 (60.32 %)	55 (45.08 %)
Multiparous (>5)	21 (11.35%)	8 (12.70 %)	13 (10.66%)
Any concomitant infection (n=183)			
Negative	53 (28.96%)	23 (37.10%)	30 (24.79%)
Positive	130 (71.04%)	39 (62.90%)	91 (75.21%)
Any vector-borne infection (n=175)			87 (71.90%)
Negative	111 (63.43%)	35 (63.64%)	76 (63.33%)
Positive	64 (36.57%)	20 (36.36%)	44 (36.67%)
Any sexually transmitted infection (n=178)			
Negative	81 (45.51%)	34 (59.65%)	47 (38.84%)
Positive	97 (54.49%)	23 (40.35%)	74 (61.16%)

3.3 Social factors (co-infection study)

3.3.1 Socio-economic factors

45.16% (28/62) of HIV-positive pregnant women were employed, and 54.84% (34/62) were unemployed. In the sample of HIV-negative pregnant women 65.55% (78/119) were employed and 34.45% (41/119) were unemployed (p = 0.008).

3.3.2 Sexual behaviour

The median number of sexual partners in a lifetime was higher in the sample of HIV-positive pregnant women in comparison to HIV-negative pregnant women: 5 (IQR: 3 – 8) vs. 4 (IQR: 2 – 6; p = 0.022), respectively. The median number of sexual partners within the previous month was equal in both: 1 (IQR: 1 – 1; p = 0.272).

3.4 HIV characteristics (co-infection study)

3.4.1 Baseline characteristics

In the co-infection study, 63 HIV-positive pregnant women were enrolled. The median age of the HIV-positive pregnant women was 29 (IQR: 24 – 35) years, and the median parity was 3 (IQR: 1 – 4). Characteristics of HIV-positive pregnant women are described in Table 10.

3.4.2 Time to HIV diagnosis

The median time since HIV diagnosis (n=35) was 0.23 (IQR: 0.02 – 3.5) years. Twenty-two out of 35 (62.86%) HIV-positive pregnant women who knew their HIV-status had been aware for less than a year. Seven out of 35 (20%) of the HIV-positive pregnant women had received their diagnosis one to four years before enrolment into this study, five out of 35 (14.29%) had known their HIV-status for five to nine years prior to study enrolment, and only one out of 35 HIV-positive pregnant women (2.86%) was diagnosed more than ten years ago.

3.4.3 Antiretroviral treatment

Data about if a pregnant woman was on ART was available for 62 pregnant women. Data about time since ART initiation was available for 35 pregnant women. Median time in years since ART was 0.23 (IQR: 0.02 – 3.5) years. The majority of the HIV-positive

pregnant women, 62.86% (22/35), had been on ART for less than one year. 20% (7/35) of the HIV-positive pregnant women had been on ART treatment for one to four years, 14.29% (5/35) HIV-positive pregnant women for five to nine years before study enrolment, and only 2.86% HIV-positive pregnant woman (1/35) had been on ART for more than ten years. However, only 61.29% (38/62) HIV-positive pregnant women initiated ART, and 38.71% (24/62) did not initiate ART at all.

3.4.4 CD4 count

Data for CD4 count was available for 31 HIV-positive pregnant women. The median CD4 count was 534 cells/mm^3 (IQR: 255 – 688). Among the 31 HIV-positive pregnant women, 3.23% HIV-positive pregnant women (1/31) had a CD4 count below 50 cells/mm^3, 3.23% (1/31) had a CD4 count between 50 – 99 cells/mm^3, 12.90% (4/31) had a CD4 count of 100 – 199 cells/mm^3, 6.45% (2/31) had a CD4 count of 200 – 299 per cells/mm^3, 12.90% (4/31) had a CD4 counts of 300 – 399 cells/mm^3, 9.68% (3/31) had a CD4 count between 400 – 499 cells/mm^3, and 51.61% (16/31) had a CD4 count of 500 cells/mm^3 and above.

3.4.5 Cotrimoxazole

Data for CTX intake was available for 49 pregnant women. The majority of pregnant women (35/49, 71.43%) did not take CTX. CTX was taken by 28.57% (14/49) pregnant women.

3.4.6 Viral load

The viral load of 37 HIV-positive pregnant women was determined. The median (range) viral load was 384 (Range: 0 – 654 000) copies/ml. The lowest viral load was 0 copies/ml, whilst the highest viral load was 654 000 copies/ml. 35.14% HIV-positive pregnant women (13/37) had a viral load of 0 copies/ml, 5.41% (2/37) had a viral load between 1 – 99 copies/ml, 21.62% (8/37) had a viral load between 100 – 999 copies/ml, 18.92% (7/37,) had a viral load of 1 000 – 9 999 copies/ml, 10.81% (4/37,) had a viral load between 10 000 – 99 999 copies/ml, and 8.11% (3/37) had a viral load above 100 000 copies/ml.

Table 10

Characteristics of HIV pregnant women

Variables	HIV+ (n=63)
	n (column %)
Time since HIV diagnosis (n=35)	
Median time in years (IQR)	0.23 (0.02 – 3.5)
< 1 year	22 (62.86%)
1 – 4 years	7 (20%)
5 – 9 years	5 (14.29%)
10 years and more	1 (2.86%)
ART initiated (n=62)	
Yes	38 (61.29%)
No	24 (38.71%)
Time since ART initiation (n=35)	
Median time in years (IQR)	0.23 (0.02 – 3.5)
< 1 year	22 (62.86%)
1 – 4 years	7 (20%)
5 – 9 years	5 (14.29%)
10 years and more	1 (2.86%)
CD4 counts (n=31) in cells/mm^3	
Median (IQR)	534 (255 – 688)
< 50	1 (3.23%)
50 – 99	1 (3.23%)
100 –199	4 (12.90%)
200 – 299	2 (6.45%)
300 –399	4 (12.90%)
400 – 499	3 (9.68%)
500 and above	16 (51.61%)

CTX taken (n=49)	
Yes	14 (28.57%)
No	35 (71.43%)
Viral load (n=37) in copies/ml	
Median (Range)	384 (0 – 654 000)
0	13 (35.14%)
1 – 99	2 (5.41%)
100 - 999	8 (21.62%)
1 000 – 9 999	7 (18.92%)
10 000 – 99 999	4 (10.81%)
> 100 000	3 (8.11%)

3.5 Prevalence of different co-infections among HIV-positive and HIV-negative pregnant women

The following section summarizes the results of all co-infections that were assessed in HIV-positive pregnant women and HIV-negative pregnant women and are shown in figure 24.

3.5.1 Bacterial infections

3.5.1.1 Treponema pallidum

Hundred-forty-five samples were assessed for *T. pallidum*, of which 43 were samples from HIV-positive pregnant women and 102 from HIV-negative pregnant women. One (1/145, 0.69%) pregnant woman with a negative HIV status was positive for *T. pallidum* (p = 0.52).

3.5.1.2 Group B Streptococcus

To assess GBS, rectovaginal swabs were obtained from 52 pregnant women, of which 15 were HIV-positive and 37 were HIV-negative. Eight pregnant women out of 52 (15.38%) had a rectovaginal swab that was positive for GBS. Among the HIV-positive pregnant women, three out of 15 (20%) had GBS, and 12 out of 15 (80%) were negative for GBS. Among the HIV-negative pregnant women, five out of 37 (13.51%) had GBS in their rectovaginal swabs and 32 out of 37 (86.49%) without (p = 0.56).

3.5.1.3 Neisseria gonorrhoea

N. gonorrhoea was assessed in 118 pregnant women, of which 27 were HIV-positive and 91 were HIV-negative. Five out of 118 (4.24%) pregnant women tested positive. In the sample of HIV-positive pregnant women, one out of 27 pregnant women (3.7%) was positive for *N. gonorrhoea*. In the sample of HIV-negative pregnant women four out of 91 (4.4%) were positive for *N. gonorrhoea* (p = 0.88).

3.5.1.4 Chlamydia trachomatis

C. trachomatis was assessed in 118 pregnant women, of which 27 were HIV-positive, and 91 were HIV-negative. Eight out of 118 (6.78%) pregnant women tested positive for *C. trachomatis*. In the sample of HIV-positive pregnant women, one out of 27 (3.70%) pregnant woman was positive for *C. trachomatis,* and 26 out of 27 (96.30%) were negative. In the sample of HIV-negative pregnant women, seven out of 91 (7.69%) were positive for *C. trachomatis,* and 84 out of 91 (92.31%) were negative (p = 0.47).

3.5.2 Parasitic infections

3.5.2.1 Plasmodium spp.

In total, a blood smear was performed for 162 pregnant women. Twenty-two out of 162 (13.58%) pregnant women had a positive blood smear with *Plasmodium* spp., and 140 out of 162 (86.42%) had a negative blood smear. In the sample of HIV-positive pregnant women, seven out of 43 (14.00%) had a positive and 43 out of 50 (86%) had a negative blood smear. In the sample of HIV-negative pregnant women, 15 out of 112 (13.39%) had a positive blood smear and 97 out of 112 (86.61%) negative (p = 0.917).

3.5.2.2 Loa loa microfilariae

In total, 174 samples were assessed for *Loa loa* microfilariae. Of these samples, there were samples from 54 HIV-positive pregnant women and 120 HIV-negative pregnant women. Thirty-five pregnant women out of 174 (20.11%) were positive for *Loa loa* microfilariae, and 139 out of 174 (79.89%) were negative. In the HIV-positive sample, 12 out of 54, (22.22%) were positive for *Loa loa* microfilariae, and 42 out of 54 (77.78%) were negative. In the HIV-negative sample, 23 out of 120 (19.17%) were positive for *Loa loa* microfilariae, and 97 out of 120 (80.83%) were negative (p = 0.64).

3.5.2.3 Mansonella perstans microfilariae

Samples from 165 pregnant women were assessed for *M. perstans*, of which 50 were HIV-positive, and 115 were HIV-negative. None (0/165, 0%) of the pregnant women was positive for *M. perstans*.

3.5.2.4 Trichomonas vaginalis

T. vaginalis diagnostics were performed for 119 pregnant women. *T. vaginalis* was detected in 29 out of 119 (24.37%) pregnant women, while in 90 out of 119 (75.63%) cases, it was not detected. *T. vaginalis* was detected in more than one-third (10/28, 35.71%) of the HIV-positive pregnant women. Among HIV-negative pregnant women, *T. vaginalis* was detected in 19 out of 91 (20.88%) pregnant women and was not detected in 72 out of 91 (79.12%) pregnant women (p = 0.11).

3.5.2.5 S. haematobium

In total, 106 samples were collected for the diagnosis of *S. haematobium* by PCR. DNA of *S. haematobium* was found in 19 out of 106, (17.92%) of these samples. Samples from 37 HIV-positive pregnant women were collected. DNA of *S. haematobium* was detected in 7/37 (18.92%) of these samples and was not detected in the remaining 30 out of 37 (81.08%) samples. In the sample of HIV-negative pregnant women, *S. haematobium* was detected in 12 out of 69 (17.39%) samples and was not detected in 57 out of 69 (82.61%) samples (p = 0.85).

3.5.3 Viral infections

3.5.3.1 Human papillomavirus (and subtypes)

In total, 119 pregnant women were assessed for HPV and subtypes. The majority (84/119, 70.59%) of them had an infection with HPV. Only 35 out of 119 (29.41%) were not infected with HPV. In the sample of HIV-positive pregnant women, 20 out of 27 (74.07%) were positive for HPV, and 7 out of 27 (25.93%) women were negative. In the sample of HIV-negative pregnant women 64 out of 92 (69.57%) were positive for HPV and 28 out of 92 (30.43%) were negative (p = 0.65).

3.5.3.2 Epstein-Barr virus

Hundred-fifty-four samples were tested for the presence of EBV. There were 42 samples from HIV-positive pregnant women and 112 samples from HIV-negative pregnant women. Two samples out of 154 (1.30%) contained detectable amounts of EBV DNA. Among the HIV-positive pregnant women, 1 out of 42 (2.38%) had an EBV infection while the other (41/42, 97.62%) did not. Hundred-eleven out of 112 (99.11%) of the HIV-negative pregnant women were not infected with EBV, and only one pregnant woman (1/112, 0.89%) tested positive for EBV ($p = 0.47$).

3.5.3.3 HTLV 1 and 2

Anti-HTLV 1 and 2 antibodies were assessed in 39 pregnant women with HIV and 113 pregnant women without HIV. In total, HTLV 1 and 2 were assessed in 152 pregnant women. Five pregnant women out of 152 (3.29%) had antibodies for HTLV 1 and 2. In the sample of HIV-positive pregnant women, antibodies were detected in 2.56% (1/39) pregnant women but not in the remaining 38 pregnant women. In the sample of HIV-negative pregnant women, antibodies for HTLV 1 and 2 were detected in 3.54% (4/113) pregnant women, while in 96.46% (109/113) pregnant women, no antibodies of either type were found 1/2 ($p = 0.78$).

3.5.3.4 Hepatitis E virus

Infection with HEV was determined in 148 pregnant women samples. Samples were collected from 39 HIV-positive pregnant women and 110 HIV-negative pregnant women. Three out of 148 pregnant women gave samples that (2.03%) contained detectable amounts of anti-HEV antibodies, while the other (145/148, 97.97%) did not. Of the HIV-positive pregnant women, anti-HEV antibodies were detected in only one (1/39, 2.56%) pregnant woman. Among HIV-negative pregnant women, anti-HEV antibodies were detected in 1.82% (2/110) pregnant women and were not detected in 98.18% (108/110) pregnant women ($p = 0.759$).

3.5.3.5 Hepatitis C virus

In total, 152 samples were assessed for HCV antibodies. Samples from 39 HIV-positive and 113 HIV-negative pregnant women were collected. In total, anti-HCV antibodies were detected in 5.26% (8/152) pregnant women and were not detected in 94.74%

(144/152). In the sample of HIV-positive pregnant women, 2.56% (1/39) had anti-HCV antibodies, and 97.44% (38/39) women did not. In the sample of HIV-negative pregnant women, anti-HCV antibodies were found in 6.19% (7/113) pregnant women, and 93.81% (106/113) did not have antibodies (p = 0.381).

3.5.3.6 Hepatitis B virus

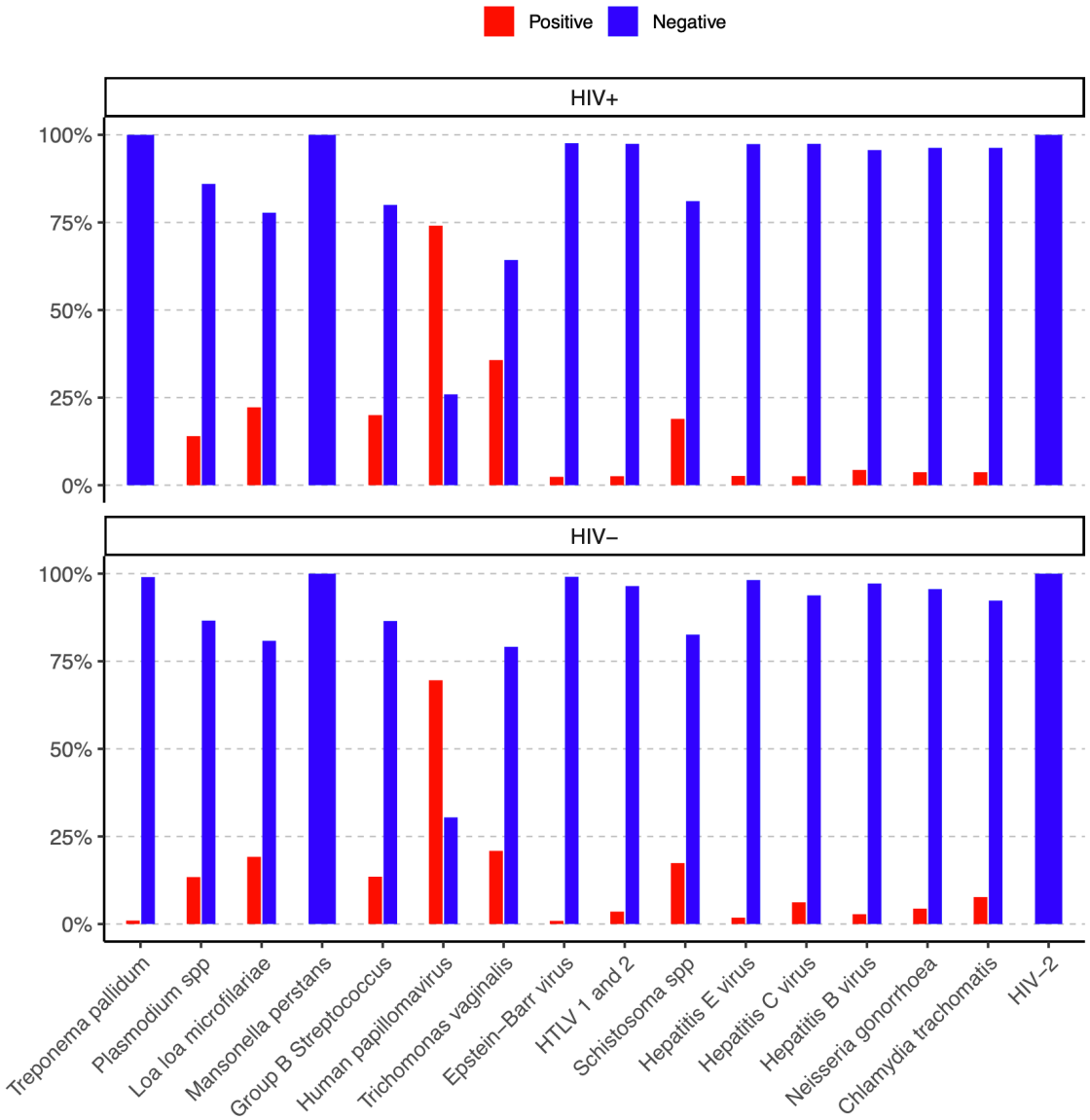
Samples were collected from 153 pregnant women for analysis for HbsAg positivity. HbsAg was present in 3.27% (5/153) pregnant women and was not present in 96.73% (148/153). Of the HIV-positive pregnant women, HbsAg was present in 4.35% (2/46) and was not present in 95.65% (44/46). Among the HIV-negative pregnant women, HbsAg was detected in 2.80% (3/107) and was not present in 104 (p = 0.622).

3.5.3.7 HIV 2 virus

HIV-2 results were obtained from 174 pregnant women. None (0/174, 0.00%) of the pregnant women were diagnosed with HIV-2.

Figure 24

Prevalence of individual co-infections in HIV-positive and HIV-negative pregnant women



3.6 HIV associated co-infections

3.6.1 Overall concomitant infections

The number of overall concomitant infections was determined in 183 pregnant women and is shown in Table 11. The median number of concomitant infections among all pregnant women was 3 (IQR: 2 – 4); 53 pregnant women out of 183 (28.96%) were negative for all assessed infections, and the majority (130/183, 71.04%) was positive for at least one infection. Among all 62 HIV-positive pregnant women, the median number of

concomitant infections was 3 (IQR: 2 – 4). In the sample of HIV-negative pregnant women, where samples from 121 were assessed, the median number of concomitant infections was 3 (IQR: 2 – 4, p = 0.0183). 37.10% (23/62) of the HIV-positive pregnant women were negative for all concomitant infections, and 62.90% (39/62) were positive for at least one infection. In the sample of HIV-negative pregnant women, 24.79% (30/121) were negative for all concomitant infections, while the majority (91/121, 75.21%) had at least one concomitant infection (p = 0.082). The odds of a concomitant infection in HIV-positive pregnant women in the unadjusted model was 0.52 times (95% CI: 0.27 –1.00) the crude odds for HIV-negative pregnant women (p = 0.052). After adjusting for age and employment status, the adjusted odds of finding a concomitant infection in HIV-positive pregnant women was 0.64 (95% CI: 0.32 – 1.28), and any association was lost (p = 0.21).

3.6.2 Vector-borne infections

The number of vector-borne infections was determined in a sample of 175 pregnant women. The median number of vector-borne infections among all pregnant women was 0 (IQR: 0 – 1). 63.43% (111/175) pregnant women tested negative for all vector-borne infections, while 36.57% (64/175) pregnant women were positive for at least one vector-borne infection. Among all 55 HIV-positive pregnant women, the median number of vector-borne infections was 0 (IQR: 0 – 1). In the sample of HIV-positive pregnant women, the median number of vector-borne infections was 0 (IQR: 0 – 1), the same as in the sample of HIV-negative pregnant women. There was no difference in the number of vector-borne infections between HIV-positive and HIV-negative pregnant women (p = 0.87). 35 out of 55 (63.64%) HIV-positive pregnant women were negative for all vector-borne infections, while 20 out of 55 (36.36%) were positive for at least one vector-borne infection. Seventy-six out of 120 (63.33%) HIV-negative pregnant women were negative for all vector-borne infections, while 44 out of 120 (36.67%) had at least one vector-borne infection (p = 0.97). The odds of a concomitant vector-borne infection in HIV-positive pregnant women in the unadjusted model was 0.99 (95% CI: 0.51 –1.92), the crude odds for HIV-negative pregnant women (p = 0.97). After adjusting for age and employment status, the odds increased to 1.40 (95% CI: 0.68 to 2.88) but the p-value remained statistical insignificant (p = 0.36). There was no association between HIV status and vector-borne infection (p = 0.59).

Table 11

Number of concomitant infections

Infection	Totals (N=185)	HIV+ (n=63)	HIV- (n=122)	p-value	Name of test
	n (column %)	n (column %)	n (column %)		
Number of concomitant infections (n=183)					
Median (IQR)	3 (2 – 4)	3 (2 – 4)	3 (2 – 4)	0.0183	Wilcoxon rank sum test
Negative for all infections	53 (28.96%)	23 (37.10%)	30 (24.79%)	0.082	Chi2 test
Positive for at least one infection	130 (71.04%)	39 (62.90%)	91 (75.21%)		
Number of vector-borne infections (n=175)					
Median (IQR)	0 (0 – 1)	0 (0 – 1)	0 (0 – 1)	0.87	Wilcoxon rank sum test
Negative for all infections	111 (63.43%)	35 (63.64%)	76 (63.33%)	0.97	Chi2 test
Positive for at least one infection	64 (36.57%)	20 (36.36%)	44 (36.67%)		
Number of sexually transmitted infections (n=178)					
Median (IQR)	2 (2 – 3)	2 (2 – 3)	3 (2 – 3)	0.056	Wilcoxon rank sum test
Negative for all infections	81 (45.51%)	34 (59.65%)	47 (38.84%)	0.009	Chi2 test
Positive for at least one infection	97 (54.49%)	23 (40.35%)	74 (61.16%)		

3.6.3 Sexually transmitted infections

Samples of 178 pregnant women were tested for STIs. Among the samples, 57 were collected from HIV-positive pregnant women, and 121 came from HIV-negative pregnant women. The median number of STIs in all pregnant women was 2 (IQR: 2 – 3). The median number of STIs in HIV-negative pregnant women was 3 (IQR: 2 – 3) and thus higher in comparison to HIV-positive pregnant women (i.e. 2 [IQR: 2 – 3]). The p-value

of the Wilcoxon rank sum test was 0.056. More than half (97/178, 54.49%) of all pregnant women were positive for at least one STI. Among the HIV-positive pregnant women, 34 out of 57 (59.65%) were negative for STIs, and 23 out of 57 (40.35%) were positive for at least one STI. However, the majority of the HIV-negative pregnant women 74 out of 121 (61.16%) were positive for at least one STI, while 47 out of 121 (38.84%) HIV-negative pregnant women were negative for all STIs (p = 0.009). Table 12 shows that HIV-positive pregnant women had 60% decreased odds of having an STI infection in comparison to HIV-negative pregnant women in the unadjusted model 0.40 (95% CI: 0.21 – 0.76). STIs occurred more often in HIV-negative pregnant women (p = 0.005). After adjusting for confounders, there was no difference between HIV-positive and HIV-negative women (p = 0.28). After adjustment, the odds of finding an STI in HIV-positive pregnant women was 0.57 (95% CI: 0.20 – 1.59) times the odds of finding an STI in HIV-negative pregnant women. Having any concomitant STI was strongly associated with HIV status (p = 0.021*).* After adjustment the p value increased, and no association was shown between having any concomitant STI and HIV status (p = 0.64).

Table 12

Presence of any concomitant infection in pregnant women

Infection	HIV+	HIV-	Crude ORs (95% CIs)	p-value	Adjusted variables	Adjusted ORs (95% CIs)	p-value	Likeli-hood ratio test (p-value)
	n (column %)	n (column %)						
Any concomitant infection								
Negative	23 (37.10%)	30 (24.79%)	1	0.052	Age, employment status	1	0.21	0.21
Positive	39 (62.90%)	91 (75.21%)	0.52 (0.27 – 1.00)			0.64 (0.32 – 1.28)		
Any vector-borne infection								
Negative	35 (63.64%)	76 (63.33%)	1	0.97	Age, employment status	1	0.36	0.36
Positive	20 (36.36%)	44 (36.67%)	0.99 (0.51 – 1.92)			1.40 (0.68 – 2.88)		
Any sexually transmitted infection								
Negative	34 (59.65%)	47 (38.84%)	1	0.005	Gravidity, #Sexual Part-ners in life, #Sexual Part-ners last month, Living with a partner, Age, em-ployment status, Method of contraception	1	0.28	0.28
Positive	23 (40.35%)	74 (61.16%)	0.40 (0.21 – 0.76)			0.57 (0.20 – 1.59)		

Note. Odds Ratio and adjusted Odds Ratio

4 Discussion

4.1 HIV prevalence study

The aim of the prevalence study was to assess HIV prevalence in 21 ANC units for the year 2018 across seven towns in Gabon. The overall prevalence of HIV amongst pregnant women living in Libreville, Lambaréné, Fougamou, Tchibanga, Mayoumba, Oyem, and Bitam was 3.93% (646/16 417). There was an observed variability in prevalence, based on data by province, as the Northern province of Woleu-Ntem (Oyem and Bitam), the more central Moyen-Ogooue province (Lambarene), and the southern provinces of Ngounie (Fougamou) and Nyanga (Tchibanga and Mayoumba) had a prevalence of 4.16% (53/1273), 5.22% (18/345), 3.8% (10/263), and 5.56% (33/594) respectively.

HIV prevalence was higher in North Gabon (Oyem and Bitam) than in South Gabon (Tchibanga and Fougamou). Furthermore, disparities in HIV prevalence within the same province, especially in Estuaire (Libreville), have been underlining high HIV variability in different regions. There was a marked variability between ANC units within Libreville. The HIV prevalence in Libreville ranged between 1.67% (17/1017) and 5.95% (92/1546). The variability of HIV prevalence between the different geographical locations should stimulate concern among public health and community stakeholders and the Gabonese MoH. These entities should look to further investigate the unequally distributed disease burden of HIV in the target region. Such investigations could consolidate evidence that HIV prevalence is truly unequally distributed among provinces or indicate that unequal access to the antenatal care national surveillance programme creates a bias that over- or under-estimates HIV prevalence across regions of Gabon. If the latter is true, then pregnant women and their unborn babies are at higher risk of developing adverse birth outcomes, where access to surveillance measures is limited, as a consequence of undiagnosed and untreated HIV. Therefore, the data collected by this prevalence study could help to monitor the implemented national HIV programme during antenatal care; revaluate whether the collaboration between national and regional stakeholders was insufficient; identify gaps in the current programme and thereby support the process of identifying priorities in healthcare, prevention and policy considering local particularities. This might help to develop specific strategies for each province, taking the given local context into account. The identification of gaps could also result in an extension of the current

programme, which might need to outsource tasks so that the regional stakeholders could have a bigger impact.

4.2 The co-infection study

In the co-infection study, 185 pregnant women were enrolled. Sixty-three pregnant women were HIV positive, and 122 pregnant women were HIV negative. Overall, HIV-positive pregnant women in the co-infection study were older than HIV-negative pregnant women. The proportion of HIV-positive pregnant women older than 30 years was higher, and the proportion of HIV-positive pregnant women between 10 – 19 years was lower in comparison to HIV-negative pregnant women. HIV-positive pregnant women were also found to have higher parities than HIV-negative pregnant women. However, this needs to be interpreted with caution as frequency matching was done based on age and parity so that HIV-negative pregnant women would be artificially similar to HIV-positive pregnant women regarding age and parity. Firstly, HIV-positive pregnant women were recruited prior to HIV-negative pregnant women. Since Gabon is a low prevalence country for HIV, we recruited all pregnant women who tested positive for HIV during their ANC unit visit as pregnant women. However, only a sub-sample of all HIV-negative pregnant women was recruited. While we are confident that age and parity are accurately reflected by our HIV-positive cohort, the same data for the HIV-negative cohort is rather much subjected to bias. A cross-sectional study by Suehiro et al., conducted in Brazil (N=254) between 2017 and 2018, found that HIV-positive pregnant women had, on average, higher parity than HIV-negative pregnant women. The results from the Brazilian study were similar to findings in this study (Suehiro et al., 2020) and might be explained by the age distribution of HIV-positive and HIV-negative pregnant women.

In this co-infection study, it was found that HIV-positive pregnant women were more often unemployed than HIV-negative pregnant women in the co-infection population, but it was found that HIV-negative pregnant women a had higher number of STIs than HIV-positive pregnant women. In some studies, it has been described that a positive HIV-status is associated with unemployment, however, so has an association between the prevalence of STIs and unemployment.

In a cross-sectional study conducted in 2002 in Kampala, Uganda, by Råssjö et al., 306 sexually active adolescents, visiting the youth health clinic in Kisenyi were recruited in order to investigate the prevalence of STIs and associated risk factors. It was found that

unemployment was one of the risk factors that was highly associated with risk for HIV and STIs acquisition (Råssjö et al., 2006). Schofield et al. conducted a longitudinal study in 1996 in Australia, where they recruited 14 762 women in order to examine associations between self-reported STIs and sociodemographic, lifestyle, health status, health service use, and quality of life factors among young Australian women. They found that one of the risk factors associated independently with the prevalence of STIs was unemployment (Schofield et al., 2000). Thus, being unemployed may be an indicator of socio-economic disadvantage and increased susceptibility in comparison to the employed (Råssjö et al., 2006; Schofield et al., 2000). This was a partial finding in this co-infection study. Further studies would be necessary to investigate whether employment status has an impact on the number of concomitant infections in HIV-positive and HIV-negative pregnant women in rural Gabon.

The majority of HIV-positive pregnant women had been on ART for less than one year and did not take CTX. These were important findings as treatment with CTX may decrease the prevalence of concomitant infections such as infection with *Plasmodium* spp., *C. trachomatis*, *N. gonorrhoea,* and colonization with GBS (Walliczek-Dworschak, 2019), (Manyando et al., 2013), among the sample of HIV-positive pregnant women and could thus have been a confounder in this study. A systematic review from Hassani et al. used MEDLINE, Embase, Global Health, CINAHL, SOCA, and African Index Medicus to identify publications that addressed the impact of CTX prophylaxis (CTXp) on the outcomes of mortality, morbidity, retention in care, quality of life, and/or prevention of ongoing HIV transmission from 1995 to 2014 (N=1418). The review found that CTXp was a cost-effective intervention that had a high impact on morbidity and mortality reduction in HIV-positive individuals (Hassani et al., 2016). This review underlined that CTXp was a protective factor. This is a conclusion that would have been a confounder in our co-infection study so that the comparison of both samples of HIV-positive and HIV-negative pregnant women would have been difficult. The median CD4 count was 534 cells/mm^3 (IQR: 255 – 688). In the medical community, it is defined that individuals living with HIV who have a CD4 count of over 500 cells/mm^3 are “healthy”. The normal CD4 count of an individual without HIV ranges between 500 – 1500 cells/mm^3 (Garcia & Guzman, 2021). In the co-infection study, we found that HIV-positive pregnant women had a rather normal CD4 count that was on the lower end of the normal range.

The enrolled pregnant women were mostly made aware of their HIV status within the previous year, and this was reflected in the median CD4 count, which suggested that our HIV-positive sample was rather healthy, based on CD4 count, at the point of study enrolment. The median (IQR) CD4 count was important to evaluate if the health status of HIV-positive pregnant women was comparable to the health status of HIV-negative pregnant women in the co-infection study sample. However, CD4 count was not available for the majority of HIV-positive pregnant women in this study (32 information available versus 31 information not available). Therefore, it was not possible to have an accurate discussion on how HIV might have influenced the health of all HIV-positive pregnant women. As the National HIV/AIDS Control programme of Gabon has established screening for HIV among ANC unit attendees as one of the methods to strengthen the prevention of MTCT of HIV, newly diagnosed pregnant women immediately received ART. This might be an indication that the health status of HIV-positive pregnant women was favourable and that their HIV infection was well controlled.

4.2.1 Prevalence of concomitant infections during pregnancy

The co-infection study highlights a high prevalence of concomitant infections in both HIV-positive and HIV-negative pregnant women attending the ANC units in Lambaréné, Gabon. More than two-thirds of pregnant women enrolled in the co-infection study tested positive for at least one concomitant infection. There was evidence for a difference in the median number of concomitant infections between HIV-positive and HIV-negative women (3 [IQR: 2 – 4] vs. 3 [IQR: 2 – 4]; p = 0.0183). Also, the odds of having a concomitant infection were lower in HIV-positive pregnant women when compared to HIV-negative pregnant women the crude OR (95% CI: 0.52; p = 0.052). Apparently, there was a correlation between being HIV-positive and the acquisition of concomitant infections, so that HIV-positive pregnant women may have less concomitant co-infections than HIV-negative pregnant women in this co-infection study. These findings counterintuitively suggest that during pregnancy, HIV-negative pregnant women in rural Gabon were at higher risk of being co-infected with other pathogens compared to HIV-positive pregnant women. However, this did not apply to GBS colonization in HIV-positive pregnant women and HIV-negative pregnant women. In the co-infection study, the prevalence of all pregnant women colonized with GBS was 15.38% (8/52). In a satellite study, embedded in a multicentre randomized controlled clinical trial comparing the safety and efficacy

of mefloquine and sulfadoxine/pyrimethamine as intermittent preventive treatment in sub-Saharan Africa, carried out at CERMEL, Gabon from April 2010 and January 2012 it was reported, that the prevalence of GBS colonization in the study region was 19% at delivery (95% CI: 16% – 23%; 106 of 549 participants). The findings of Capan-Melser et al. were comparable to the findings in our co-infection study (Capan-Melser et al., 2015). In a review, in which a total of 390 articles, 85 countries and almost 300 000 pregnant women were included, the global prevalence of GBS colonization among pregnant women was estimated to be at 18% (95% CI: 17% – 19%). In West Africa the prevalence of GBS colonization during pregnancy was 14% and in Europe, Australia, and North America the prevalence was similar (95% CI: 15% – 21%) (Raabe and Shane, 2019; Russell et al., 2017). The GBS colonization was higher in HIV-positive pregnant women who were more often colonized with GBS than HIV-negative women (20% (3/15) vs. 13.51% (5/37); p = 0.56). A correlation between the intake of CTX and the GBS colonization could not be done as the data was lacking. The findings about the GBS colonization during pregnancy were consistent with several studies (Capan-Melser et al., 2015; Raabe and Shane, 2019; Russell et al., 2017). As GBS colonization at delivery is known to be an important risk factor for adverse birth outcomes such as early neonatal meningitis, pneumonia and sepsis, the findings emphasized the need to implement a GBS prevention program among pregnant women before delivery and during delivery in Gabon as recommended in other countries (Belard et al., 2015; Olthoff, 2019; Quan et al., 2016; Raabe and Shane, 2019; Russell et al., 2017; Vornhagen et al., 2017). The prevention programme could include the collection of a recto-vaginal swab in the third trimester to determine if GBS colonization is present or not and if present the administration of antibiotics to prevent neonatal infections caused by invasive GBS.

When looking only at vector-borne infections, the prevalence of vector-borne infections did interestingly not differ between HIV-positive and HIV-negative pregnant women (63.33% (76/120) vs. 63.64% (35/55), p = 0.97). Therefore, HIV status was not at all associated with the prevalence of vector-borne infections in this co-infection study. This seems plausible, as to date no differential blood-feeding preferences of insect vectors are known that would pre-dispose vectors to disproportionately often bite HIV-positive or HIV-negative women. A description of the prevalence of *P. falciparum* infection in women at delivery was included in the study protocol for the MAMAH trial published by Gonzalez et al., concerning all study sites, including Lambaréné, Gabon. The prevalence

data were obtained from 2010 to 2012 in pregnant women receiving either two intermittent preventive treatment doses of mefloquine or sulfadoxine pyrimethamine (González et al., 2021; Tuike-Ndam, n.d.). The prevalence of *P. falciparum* infection in women at delivery in Lambaréné, was 11% between 2010 – 2012 (González et al., 2021). The prevalence of *Plasmodium* spp. infection in pregnant women observed in the co-infection study was similar to the findings published by (González et al., 2021). Other studies have described that in regions with a high prevalence of *Plasmodium* spp. infection like Gabon (González et al., 2021), HIV-infection was associated with a higher prevalence of *Plasmodium* spp. infection (Ayisi et al., 2004; Wumba et al., 2015). A reason why in the co-infection study, the prevalence of *Plasmodium* spp. infection did not differ between both HIV samples, may be due to systematic intermittent preventive treatment with sulfadoxine pyrimethamine and the distribution of insecticide treated bed nets, that has been introduced by the National Malaria programme (WHO, 2012). To the best of our knowledge, there are no studies in which *Loa loa* microfilariae and *M. perstans* microfilariae infection prevalence have been directly compared between HIV-positive and HIV-negative pregnant women.

The analysis identified that an infection with *Schistosoma* spp. occurred irrespective of the HIV status (7/37, 18.92%) for HIV-positive women vs. for HIV-negative women (12/69, 17.39%, p = 0.85). However, in a cross-sectional study in rural Tanzanian villages near Lake Victoria, in which 345 women of reproductive age were enrolled between 2010 and 2011, it was found that HIV prevalence was higher among women suffering from severe schistosomiasis. Of 185 pregnant women with an infection with *Schistosoma* spp., 17 out of 185 (9%) were HIV positive and of those who did not have an infection with *Schistosoma* spp., 4 out of 160 (3%) were HIV positive (OR 3.9 [95% CI: 1.3 – 12.0], p = 0.015). In comparison to HIV-negative pregnant women, the median intensity of an infection with *Schistosoma* spp. was higher in HIV-positive pregnant women. The findings of Changalucha et al. suggested that an infection with *S. mansoni* may be a risk factor for HIV acquisition as a result of the chronic inflammation (Changalucha et al., 2012). In a review that assessed the association of schistosomiasis with HIV based on 364 studies published between January 1973 and December 2018, a significant association of schistosomiasis with HIV was found (Patel et al., 2021). Possible explanations for these differing results between our results and those in the articles cited could be due to differences

in the study population or study area. However, it is also a possibility that our co-infection study was underpowered and unable to detect such an effect.

In the co-infection study, a high prevalence of STIs in both HIV-positive and HIV-negative pregnant women were identified. Similar findings were described in previous studies of pregnant women in sub-Saharan Africa (Joseph Davey et al., 2019). Almost two-thirds tested positive for at least one STI in our co-infection study. The association of the difference in the median number of STIs between the sample of HIV-positive 2 (IQR: 2 – 3) and HIV-negative pregnant women 3 (IQR: 2 – 3) was of a $p = 0.056$, on the borderline of significance.

Concordantly, the odds of an existing STI were lower in HIV-positive pregnant women than in HIV-negative pregnant women. As mentioned above, a positive HIV status has in the past been shown to increase the risk of STI acquisition (Joseph Davey et al., 2019). In the population of the co-infection study, the majority of the HIV-negative pregnant women (61.16%, 74/121) was positive for at least one STI, while the other HIV-negative pregnant women (47/121, 38.84%) were negative for all STIs. Although we found a strong correlation between HIV-positivity and a lower rate of concomitant STI ($p = 0.009$), which was supported by the OR of 0.40 ([95% CI: 0.21– 0.76], $p = 0.005$), indicating that the odds of STIs was lower in HIV-positive pregnant women, we did not find it as a reasonable conclusion. However, awareness of HIV-status and further behavioural factors might be a protective factor. This indicates that pregnant women in the HIV-negative sample could be at high risk of getting infected with HIV in the future, as having an STI has been known to increase the risk of acquiring HIV infection (Joseph Davey et al., 2019). In other studies, younger maternal age has been demonstrated to be a positive predictor of STIs. In our co-infection study, HIV-negative pregnant women were more likely to be infected with STIs, perhaps as they were younger (Joseph Davey et al., 2019). However, all pregnant women were at high-risk for multiple STIs. The question of why all pregnant women in this co-infection study were at high-risk of having multiple STIs should be further investigated.

STI-positive status was associated with decreased odds of HIV-positive status, and vector borne infection status was independent of HIV status. Since the category of overall concomitant infections was primarily composed of STIs and vector-borne infections, the comparatively higher median (IQR) number of concomitant infections in HIV-negative

pregnant women is explained by an overrepresentation of STIs. STIs constituted the majority of co-infections in the category of overall concomitant infections.

HIV-positive pregnant women may be conscious of their HIV status because in Gabon, to raise awareness, ART requires regular visits and counselling done by nurses, midwives, and physicians (PNLIST, 2021). Thus, HIV-positive pregnant women in Gabon may have fewer sexual risk factors than HIV-negative pregnant women based on their exposure to HIV counselling by the Gabonese health care system. Reduction in the number of primary and non-primary partners, sexual abstinence, or the use of condoms as means of reducing transmission risk are known behaviours among HIV-positive adults (Steward et al., 2009; The Voluntary HIV-1 Counseling and Testing Efficacy Study Group, 2000). Adopting such behavioural aspects would, in consequence, also lower the risk of becoming infected with other pathogens that are sexually transmissible.

Another reason might be that HIV-positive pregnant women in Gabon usually hide their HIV status due to stigma (PNLIST, 2021). The ultimate fear of stigma and resulting ostracism may reduce risk behaviour associated with the spread of HIV, which simultaneously protects from the acquisition of other STIs. However, this was not reflected by the number of sexual partners HIV-positive pregnant women had in their lifetime, as the median number of sexual partners was significantly higher than that of the HIV-negative sample by one (five partners for HIV-positive women vs. four partners for HIV-negative women, $p = 0.022$). Yet, HIV-positive pregnant women were also four years older by median which may also explain, why the median number of sexual partners in a lifetime was higher. This hypothesis was supported by the median (IQR) number of sexual partners during the last month, whereby hypothesis testing did not yield any evidence for a difference in the median numbers of sexual partners between HIV-positive pregnant women and HIV-negative pregnant women (median numbers of sexual partners 1; IQR: 1 – 1, $p = 0.272$). After adjustment of confounders, the association between HIV status and the prevalence of STIs was lost ($p = 0.28$). This is reflected by the adjusted odds ratio of 0.57 (95% CI: 0.20 – 1.59). Compared with the crude odds ratio of 0.40 (95% CI: 0.21 – 0.76), this indicated the presence of positive confounders. Interestingly, the majority of adjusted confounders constitute sexual risk factors. Therefore, the above-mentioned hypothesis that HIV-positive pregnant women might have fewer sexual risk factors than HIV-negative pregnant women is indirectly supported by the adjusted odds ratio that is closer to the null-value of one than the crude odds ratio.

The overall high prevalence of STIs highlights the need to develop an appropriate education and screening programme for STIs during antenatal care in Gabon. Such programmes would reduce maternal morbidity and consequently neonatal morbidity and mortality. High prevalence of STIs such as *N. gonorrhoea*, HPV, *C. trachomatis*, *T. vaginalis*, *T. pallidum,* and HBV in pregnant women in sub-Saharan Africa*,* particularly treatable STIs (*N. gonorrhoea*, HPV, *C. trachomatis*, *T. vaginalis, T. pallidum),* has been described elsewhere (Akarolo-Anthony et al., 2013; Joseph Davey et al., 2019; Msuya et al., 2009; Mutagoma et al., 2017). From April 2012 to August 2012, a case-control study conducted in Abuja, Nigeria, enrolled HIV-positive and HIV-negative women (N= 278) and investigated the prevalence of HPV infection. They found that HIV was associated with an increased risk of HPV infection (Akarolo-Anthony et al., 2013). These findings differed from the findings in this co-infection study, where HPV infection was high among both HIV-positive and HIV-negative pregnant women (20/27, 74.07%) for HIV-positive pregnant women and (64/92, 69.57%) for HIV-negative pregnant women, p = 0.65). Another cross-sectional study, conducted from 2012 to 2013 in the Brong-Ahofo region, Ghana, investigated HBV and HCV co-infection in HIV-positive (n=148) and HIV-negative pregnant women (n=100). They found that the prevalence of HCV and HBV infection was high in both HIV-positive and HIV-negative women during pregnancy. They also found that infection with HCV was lower in HIV-positive women than in HIV-negative women, which is an observation similar to findings in this co-infection study (Frempong et al., 2019). In a cross-sectional study conducted from 2017 to 2018 in Cape Town, South Africa, researchers found that the prevalence of the following STIs was high among pregnant women in sub-Saharan Africa: Infection with *N. gonorrhoea*, *C. trachomatis*, *T. vaginalis,* and *T. pallidum.* Among the 242 pregnant women that were enrolled, 44% (106/242) were HIV-positive pregnant women. However, the results were different from the findings of this co-infection study, as HIV-positive pregnant women were more likely to be infected with STIs and were younger than in this co-infection study's population (Joseph Davey et al., 2019).

The findings in the cited studies suggest a higher prevalence of STIs and a higher prevalence of multiple STIs in HIV-positive pregnant women (Joseph Davey et al., 2019; Msuya et al., 2009) and therefore do not reflect the findings of the co-infection study, in which HIV-positive women had fewer STIs than HIV-negative pregnant women. Studies investigating the prevalence of STIs in HIV-positive and HIV-negative women were

usually cross-sectional studies, the same as the co-infection study. Cross-sectional studies have the limitation that causality cannot be established. Therefore, the results of this co-infection study cannot be interpreted as being HIV-positive would protect from becoming infected with other STIs.

Furthermore, neither the prevalence study nor the co-infection study involved sample size calculations. Therefore, there was a possibility that the co-infection study was prone to beta error, meaning it is possible that the association between HIV-positivity and decreased odds for STIs was a false-positive finding that would not have been found in a significantly larger study population. Additionally, residual confounding might play a role in the co-infection study, even though matching methodology and regression analyses were applied to account for this as best as possible.

Apart from methodology, another explanation for the difference in results relative to other studies may have been the difference in study areas, for e.g. studies conducted in South Africa recruited in an HIV high prevalence country (Joseph Davey et al., 2019). In HIV high prevalence countries, it might be difficult to identify all HIV-positive individuals in the target region. In Gabon (a low HIV prevalence setting), all efforts were made to ensure that all HIV-positive pregnant women were enrolled in the co-infection study. Since it is generally easier to recruit all cases in a low prevalence setting than in a high prevalence setting, it is believed that the probability of (case) selection bias was minimal. However, it cannot be ruled out that selection bias may have happened in sampling the control group of HIV-negative pregnant women, in spite of the fact that frequency matching was applied. However, other studies were also conducted in HIV low prevalence countries such as Ghana (1.7%) and Nigeria (1.3%) (Akarolo-Anthony et al., 2013; CIA, 2021; Frempong et al., 2019) and reported the same results as in HIV high-prevalence countries (Akarolo-Anthony et al., 2013; Frempong et al., 2019; Joseph Davey et al., 2019). Different study areas may mean different characteristics of the study population in terms of age distribution and cultural aspects and may be an additional explanation for why the results found in this co-infection study differ from the results of other studies. Another explanation may be national and regional public health interventions in the prevention of HIV-positive women during pregnancy in Gabon and thus better surveillance. Therefore, further research should be conducted on this topic in Gabon including the sampling of a much larger group of pregnant women, preferentially in a study with a longitudinal design.

The findings of this co-infection study must be interpreted with caution. First, a cross-sectional design does not allow for any causal inferences from these findings i.e. whether HIV status was causal for subsequent acquisition of co-infections or whether the presence of co-infections was responsible for subsequent infection with HIV. Second, as Gabon is a low HIV prevalence country, the aim was to include all HIV-positive pregnant women. Indeed, we managed to recruit all HIV-positive pregnant women who presented to the ANC unit in the study area during the observation period of the study. While this is a methodological strength, there might still have been selection bias, as it cannot be ruled out that very sick HIV-positive pregnant women may have stayed at home and therefore did not present at the ANC unit. Furthermore, data collection was challenging and resulted in missing data, which in turn resulted in increased statistical imprecision. Further limitations could have been the results from some routine diagnostics like syphilis and HBV, for which rapid diagnostic tests were used; this results in decreased diagnostic sensitivity and specificity compared with the gold standard of PCR. Some diagnostic methods were more objective than others (microscopy versus PCR). However, microscopy reading at CERMEL was only performed by trained personnel, who regularly underwent external validation by independent international organizations. In addition to this, light microscopy is still regarded as the diagnostic gold standard in clinical routine for the diagnosis of *Plasmodium* infections, in spite of being dependent on the microscopist's level of expertise. Additionally, the co-infection study did not sufficiently take into account associated risk alleviating behaviour such as sexual abstinence after HIV-positive pregnant women received their diagnosis for the first time (Onoya et al., 2015). Therefore, further studies need to be conducted to assess the impact of behavioural factors on the prevalence and incidence of concomitant infections and STIs in HIV-positive and HIV-negative pregnant women in rural Gabon. Another limitation involved questions that aimed to assess the number of sexual partners in a pregnant woman's lifetime, to which interviewees may not have answered truthfully for fear of stigmatization (Onoya et al., 2015). An anonymous questionnaire could be a solution. Yet, even if this cannot be ruled out the reported numbers of sexual partners do not necessarily indicate underreporting. Furthermore, no data about intermittent preventive treatment against *Plasmodium* spp. infection during pregnancy was collected. These questions could have helped to understand, why the odds of having a *Plasmodium* spp. infection was the same in HIV-positive pregnant women as in HIV-negative pregnant women.

In summary, HIV-positive pregnant women in the co-infection study were slightly less affected by concomitant infections, particularly STIs, in comparison to HIV-negative pregnant women in crude analysis but not so after statistical correction. Reasons could be that HIV-negative pregnant women were younger and might have had higher risk behaviour in comparison to HIV-positive pregnant women, who were older and may have been less exposed to STIs due to reduced risk behaviour. However, beta-error and residual confounding may also explain these findings. Further studies with different designs, e.g. cohort-studies, would be necessary to find out more about associated risk factors and causality in HIV-positive and HIV-negative pregnant women in rural Gabon.

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6 Summary

6.1 Summary (English)

Background: There is a need for more epidemiological information about HIV infection among pregnant women in Gabon. To bridge this knowledge gap, two separate studies were conducted: The first study assessed HIV prevalence for pregnant women visiting 21 ANCs from 2018 – 2019 in seven towns in Gabon. Second, the co-infection study aimed to evaluate HIV-associated co-infections during pregnancy in rural Gabon. The co-infection study was conducted from February 2019 to February 2020 in Lambaréné, Gabon.

Methods: In the HIV prevalence study, the prevalence was calculated as the number of HIV-positive cases divided by the total number of pregnant women tested for HIV during the period. For the co-infection study, samples of 63 HIV-positive pregnant women and 122 HIV-negative pregnant women were collected. The HIV-negative sample was frequency matched to the HIV-positive sample based on age and parity. The prevalence of the following co-infections was determined: *Treponema pallidum, Plasmodium* spp.*, Loa loa, Mansonella perstans,* Group B *Streptococcus,* Human papillomavirus (and subtypes), *Trichomonas vaginalis,* Human T-lymphotropic virus type 1 and 2*,* Hepatitis B virus*, Schistosoma* spp.*,* Hepatitis E virus*,* Hepatitis C virus*, Neisseria gonorrhoea, Chlamydia trachomatis,* and Epstein-Barr virus.

Results: The prevalence of HIV among pregnant women in Gabon was 3.93% (646/16 417), with a marked variability within the study area. In the co-infection study, HIV-positive women were older than HIV-negative women (median: 29 vs. 25 years) and had higher parity (median: 3 vs. 2). Median number of concomitant infections was 3 (IQR: 2 – 4) in both samples. The median number of vector-borne infection was 0 (IQR: 0 – 1) in both samples. The prevalence of STIs was higher in HIV-negative women than in HIV-positive women (61.16%, 74/121 vs. 40.35%, 23/57, p= 0.009). Based on the results of logistic regression, having a positive HIV status was associated with lower odds 0.52 (95% CI: 0.27 – 1.00) of having any concomitant infection compared with those having a negative HIV status. A positive HIV status was associated with lower odds for any STI (OR: 0.40 (95% CI: 0.21 – 0.76)) compared with negative HIV status. In summary, HIV-positive pregnant women in this study were less affected by concomitant infections, particularly STIs, relative to HIV-negative pregnant women.

6.2 Zusammenfassung (Deutsch)

Hintergrund: Über HIV Infektionen bei Schwangeren in Gabun ist bisher wenig bekannt. Daher wurden zwei Studien durchgeführt: Zum einen wurde von 2018 – 2019 eine HIV-Prävalenz bei Schwangeren in Geburtsvorsorgeeinrichtungen in sieben Städten in Gabun durchgeführt. Zum anderen wurde eine sogenannte Koinfektionsstudie durchgeführt, mit dem Ziel, HIV-assoziierte Koinfektionen während der Schwangerschaft im ländlichen Gabun zu verstehen. Die Koinfektionsstudie wurde von 2019 – 2020 in Lambaréné, Gabun, durchgeführt.

Methoden: In der HIV-Prävalenzstudie wurde die Prävalenz als die Anzahl der HIV-positiven Fälle geteilt durch die Gesamtzahl der Schwangeren, die während der Studie auf HIV getestet wurden, berechnet. Für die Koinfektionsstudie wurden Proben von 63 HIV-positiven und 122 HIV-negativen Schwangeren gesammelt. Die HIV-negative wurde mit der HIV-positiven Stichprobe anhand des Alters und der Parität abgeglichen. Die Prävalenz der folgenden Koinfektionen wurde bestimmt: *Treponema pallidum*, *Plasmodium* spp., *Loa loa*, *Mansonella perstans*, Streptokokken der Gruppe B, Humanes Papillomavirus (und Subtypen), *Trichomonas vaginalis*, Humanes T-lymphotropes Virus Typ 1 und 2, Hepatitis-B-Virus, *Schistosoma* spp., Hepatitis-E-Virus, Hepatitis-C-Virus, *Neisseria gonorrhoea*, *Chlamydia trachomatis* und Epstein-Barr-Virus

Ergebnisse: Die HIV-Prävalenz bei Schwangeren in Gabun betrug 3.93% (646/16 417), wobei es innerhalb des Studienregion deutliche Unterschiede gab. In der Koinfektionsstudie waren HIV-positive Frauen älter als HIV-negative (Median: 29 vs. 25 Jahre) und hatten mehr Kinder (Median: 3 vs. 2 Parität). Der Median der Anzahl der Begleitinfektionen lag sowohl bei HIV-positiven als auch bei HIV-negativen Frauen bei 3 (IQR: 2 - 4). Der Median der durch Vektoren übertragenen Infektionen lag bei 0 (IQR: 0 - 1) in beiden Stichproben. Die Prävalenz von STIs war bei HIV-negativen Frauen höher als bei HIV-positiven (61.16%, 74/121 vs. 40.35%, 23/57, p = 0.009). Nach den Ergebnissen der logistischen Regression war ein positiver HIV-Status mit einer um 0,52 niedrigeren Odds (95 % CI: 0,27 - 1,00) für eine Begleitinfektion verbunden als bei Frauen mit einem negativen HIV-Status. Ein positiver HIV-Status war mit einer geringeren Odds für eine STI verbunden (OR: 0,40 (95% CI: 0,21 - 0,76)), als ein negativer HIV-Status. Zusammenfassend waren HIV-positive Schwangere in dieser Studie im Vergleich zu HIV-negativen Schwangere weniger von Begleitinfektionen, insbesondere STIs, betroffen.

7 List of abbreviation

ACT	Artemisinin Combined Therapy
AIDS	Acquired Immunodeficiency Syndrom
AIN	Anal intraepithelial Neoplasia
ANC	Antenatal Care
Anti-HBc	Antibody against the Hepatitis-B-Core protein
Anti-HTLV	Antibodies against HTLV
Anti-T.-pallidum	Antibodies against Treponema pallidum
ART	Antiretroviral treatment
ARV	Antiretroviral
ATP	Adenosine triphosphate
BNITM	Bernhard-Nocht-Institut für Tropenmedizin
CAA	Circulation Anodic Antigen
CDC	Centres for Disease Control and Prevention
CD4	Cluster of differentiation 4
CERMEL	Centre de Recherches Médicales Lambaréné
CIN	Cervical intraepithelial Neoplasia
CO2	Carbon Dioxide
CSF	Cerebrospinal fluid
CTX	Cotrimoxazole
CTXp	Cotrimoxazole prophylaxis
DEC	Diethylcarbamazine
DGGG	Deutsche Gesellschaft für Gynäkologie und Geburtshilfe
DHA-PPQ	Dyhdroartemisinin – Piperaquine
DNA	Deoxyribonucleic acid

DTG	Dolutegravir
EBV	Epstein-Barr-virus
EDCTP	European and Developing Countries Clinical Trials Partnership
E.g.	Exempli gratia/ for example
ELISA	Enzyme-Linked Immunosorbent Assay
FTA-ABS	Fluorescent treponemal antibody absorption
GBS	Group-B-Streptococcus
HBsAg	Hepatitis-B-virus surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEV	Hepatitis E virus
HIV	Human Immunodeficiency virus
HHSV	Human Herpes Simplex virus
ICH-GCP	International Conference on harmonization of Technical Requirements for registration of pharmaceuticals for human use - Good clinical Practice
ID	Unique identifier
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPTp	Intermittent preventive Treatment of pregnancy
IQR	Interquartile Range
MTCT	Mother-to-child-transmission
NAAT	Nucleic Acid Amplification Tests
NNRTI	Non-Nucleoside reverse-transcriptase-inhibitor

NRTI	Nucleoside reverse-transcriptase-inhibitor
NtRI	Nucleotide reverse-transcriptase
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Diseases
POC	Point of Care
POC-CCA	Point-of-care circulating cathodic antigen
PZQ	Praziquantel
RDT	Rapid diagnostic test
RKI	Robert-Koch-Institute
RPR	Rapid Plasma Reagin
SSA	Sub-Saharan Africa
Spp.	subspecies pluralis
STI	Sexually Transmitted infections
SToRCH	Acronym for Syphilis, Toxoplasmosis, other's, Rubella, Cytomegaly Virus, Herpes Simplex 1 and 2
UKE	Universitätsklinikum Hamburg-Eppendorf
UMCHE	University Medical Centre Hamburg-Eppendorf
UN	United Nations
UNAIDS	United Nations programme on HIV and AIDS
VAIN	Vaginal Intraepithelial Neoplasia
VDRL	Venereal Disease Research Laboratory
VIN	Vulva Intraepithelial Neoplasia
Vs	Versus
WB	Western Blot
WHO	World Health Organization

8 Acknowledgements

Writing the final lines of my thesis reminds me of the first semester in 2014 when I said to myself: I want to write a medical doctor thesis in the field of tropical medicine. *"Before a dream is realized, the Soul of the World tests everything that was learned along the way. It does this not because it is evil, but so that we can, in addition to realizing our dreams, master the lessons we've learned as we've moved toward that dream. That's the point at which most people give up. It's the point at which, as we say in the language of the desert, one 'dies of thirst just when the palm trees have appeared on the horizon."*(Paulo Coelho). Indeed, this was a long way, on which I received help and accompany the whole way. Unfortunately, I cannot mention everybody by name, but I would like to give some words of thanks in chronological order according to the steps of my thesis. I thank the *Zentrum für Mission und Ökumene* in Hamburg, particularly Julia Brockmeier, who has trained me in global disparities, international collaborations, and global health and who has introduced me to Dr. Gudrun Rieger-Ndakorerwa. I thank you, Gudrun, for all the advice that you gave me and that you orientated me towards Prof. Dr. Marylyn Addo, University of Hamburg. I thank the University of Hamburg for teaching me all the necessary medical skills. I thank Prof. Dr. Marylyn Addo. MSc, DTM&H. Thank you, Marylyn for having guided me from the first semester until the end of my studies and being a mentor and role model in several fields of life. I thank Prof. Dr. Michael Ramharter, MSc, DTM&H. Thank you, Michael for your supervision, for the freedom that you gave me at work, and thank you, that you do not only see me as one of your several medical doctor students but also as a person. I'm happy that you have asked me to join your team further. I am looking forward to staying in your team. I thank Dr. Johannes Stefan Mischlinger MSc, MSc, DTM&H, PhD for his great supervision. Thanks, Hannes, for all the energy, time, enthusiasm, patience, and many teaching lessons. You have not only been a great supervisor and teacher to me, but you have become a friend to me. I thank the German Research Centre for Infectious diseases and the Deutsche Gesellschaft für Internationale Zusammenarbeit for having funded this thesis. I thank Nina Hackbarth who has been around during my desperate moments in the laboratory and whose ears I could blend with complaints!

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9 Curriculum Vitae

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Since 02/2018	Medical doctor student at the University of Hamburg Project title: "HIV associated coinfection in pregnant women in rural Gabon: a cross-sectional study " Research activities in Gabon: Centre de Recherches Médicales de Lambaréné (CERMEL), Gabon (Africa), Germany (Europe)
04/2015 – 06/2022	Medical studies, Medical School Brandenburg
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10/2014 – 02/2015	Environmental sciences, University of Lueneburg Professional experiences
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2021 – 2022	Medical intern in gynaecology and obstetrics, tropical medicine (Gabon respectively), surgery (Brandenburg, Germany)
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