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Hepatitis B and Hepatitis E Viruses: Epidemiology, Genotypes and Co-infection Dynamics in Southwest Cameroon

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1. Introduction

Viral hepatitis remains a public health challenge worldwide, where hepatitis B virus (HBV) and hepatitis E virus (HEV) contribute substantially to morbidity and mortality, particularly in regions where these infections are highly prevalent. Despite effective HBV vaccines and antivirals being available, sub-Saharan Africa (SSA) continues to experience a high burden of HBV. Furthermore, HEV remains under-recognised in the region, and its actual burden is not known. Both these viruses continue to raise concerns regarding the safety of blood transfusions, maternal and neonatal health, and in individuals living with weakened immune systems who are at risk of severe disease. Several aspects of HBV and HEV epidemiology remain poorly understood, including the actual burden, associated risk factors, genotype distribution, and co-infection patterns. In Cameroon, data on key populations are particularly limited. To address these gaps, this thesis aims to generate region-specific evidence that can inform and guide policy decisions for national health priorities, while supporting regional efforts to eliminate viral hepatitis.

1.1. Epidemiology of HBV in sub-Saharan Africa

The burden of HBV in SSA is among the highest worldwide. As of 2022, the World Health Organization (WHO) African Region accounted for approximately a quarter of the global HBV burden, with nearly 65 million chronically infected individuals and 300,000 deaths occurring each year (WHO 2024). These estimates largely reflect the situation in SSA, where many individuals are chronic HBV carriers, and prevalence in the general population ranges between 8% to 13% (GBD 2022). HBV prevalence rates are especially pronounced among high-risk populations, including 6.2% of pregnant women and more than 28% among individuals living with human immunodeficiency virus (HIV) (Riches et al., 2025; Stabinski et al., 2015). Western SSA carries the highest burden in the region, where HBV contributes to almost half of the deaths from cirrhosis and liver cancer (Daniel and Anna, 2021). Despite universal vaccination, the HBV prevalence among children under 5 years of age remains nearly 3%, which is the highest worldwide, indicating the contribution of mother-to-child transmission in sustaining HBV's burden (Riches et al., 2025). Moreover, transmission is sustained by two key factors. Firstly, the region experiences a high prevalence of HIV, which shares common transmission routes, and secondly, it is linked to unsafe blood transfusions (Candotti et al., 2021; Villa and Iwuji, 2020). Among the ten genotypes of HBV (A-J), the main genotypes circulating in SSA are A, D, and E, but there have been increasing reports of other genotypes, such as B and C, as well as mixed genotypes (Kafeero et al., 2023). Although more countries

are developing national strategies to control HBV, screening and testing capacities remain insufficient, suggesting that the actual burden of HBV in specific populations is likely to be underestimated.

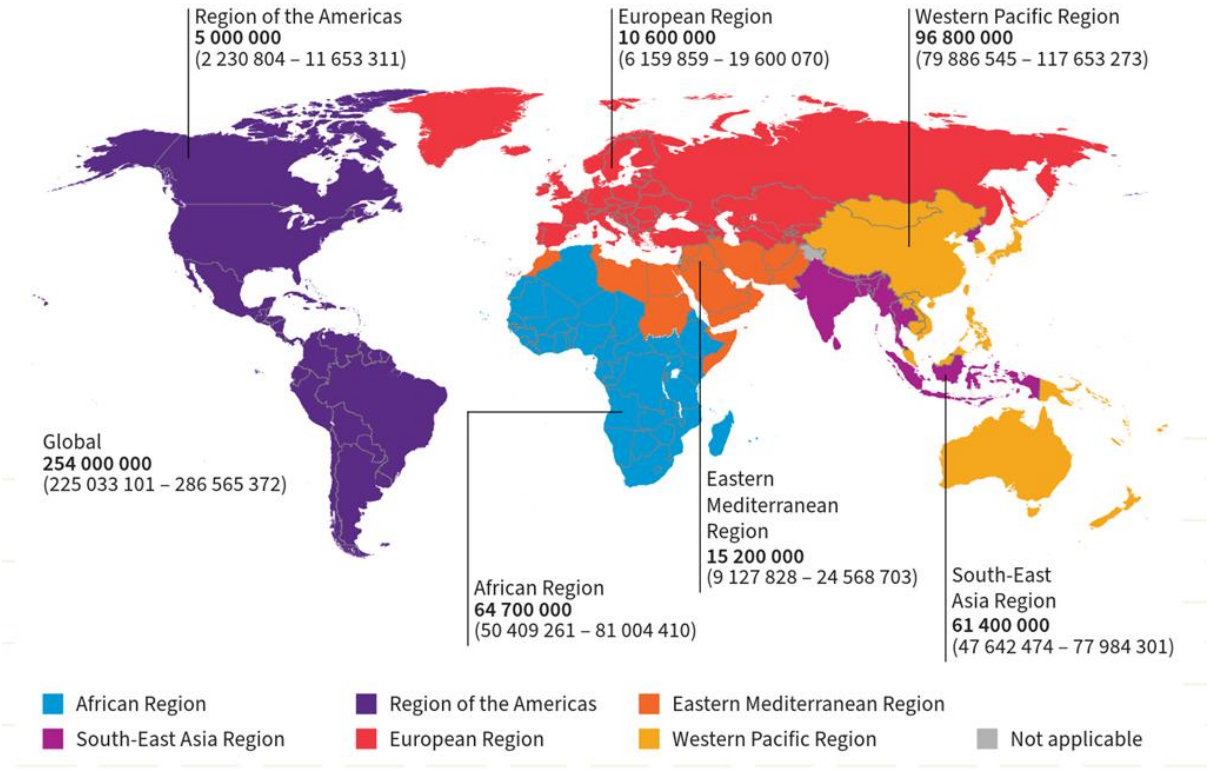

Figure 1: Global prevalence of chronic hepatitis B infection by 2022

Source: World Health Organization. *Global hepatitis report 2024: action for access in low- and middle-income countries*. Geneva: WHO; 2024 (reproduced under CC BY-NC-SA 3.0 IGO licence).

1.1.1. HBV in Cameroon

1.1.1.1. HBV endemicity and genotype diversity

Chronic HBV infection is still a challenging public health concern in Cameroon. Reports for 2023 show that nearly 15,000 new infections occurred, including 3,505 deaths (WHO, 2024). The national seroprevalence (HBsAg-positivity) of chronic HBV is estimated at over 11%, with regional variations (Bigna et al., 2017). In the Northern and Southern regions of the country, the prevalence is above 15%, while in the Centre, East, and Southwest, it ranges from 8% to 15%, and in the West, Northwest, and Littoral, it is below 8% (Bigna et al., 2017). Rural communities are the most affected. Equally, certain populations do have higher prevalence rates, including nearly 18% among blood donors and 13% among HIV patients (Bouba et al., 2024; Bigna et al., 2018). In pregnant women, prevalence is above 5% in most regions and rises to over 17% in the North (Torimiro et al., 2024; Shimakawa et al., 2022). Since the

introduction of HBV infant vaccination in Cameroon (in 2005), the prevalence among children has remained low (approximately 1%) (Dominique et al., 2020), representing a significant decrease from the nearly 20% rate observed before the implementation of the national vaccination program (Chiaramonte et al., 1991; Shimakawa et al., 2022). However, mother-to-child transmission remains a significant route of infection in Cameroon, especially when vaccination schedules are missed.

The main HBV genotypes circulating in Cameroon are A and E, which have been detected in various populations, including blood donors, pregnant women, and HIV patients (Fopa et al., 2019; Ducancelle et al., 2013; Gachara et al., 2017). Genotype D is also occasionally reported. Besides, recombinant A/E strains have been described mainly in the Southern, Eastern, and Central regions, particularly among the Pygmies and Bantus (Rodgers et al., 2017; Shimakawa et al., 2022). Sub-genotype A3 occurs almost exclusively in Cameroon and circulates within these groups (Foupouapouognigni et al., 2011).

Cameroon lacks a comprehensive national strategic plan for HBV control. Testing and treatment remain out of reach for most of the population. While routine HBsAg screening is recommended for blood donation services, during antenatal care, and as part of HIV management programs, implementation is still not consistent outside the transfusion setting. There is also no routine population-based HBV testing program, and linkage to clinical care is generally poor. As a result, only about 1.9% of chronic HBV carriers are diagnosed, and 0.2% being treated (WHO, 2024), highlighting significant gaps in HBV control in Cameroon.

1.1.1.2. Low vaccine coverage in pre-2005 birth cohorts

HBV vaccination was added to the immunization schedule for infants in Cameroon in 2005, supported by the Global Alliance for Vaccines and Immunization (GAVI) (Nguwoh et al., 2024). Infants receive vaccine doses at weeks six, ten, and fourteen, with a documented vaccination coverage rate of 99% for at least one dose, and 68% for the complete vaccination series (WHO, 2024). Although the HBV birth dose is crucial for preventing mother-to-child transmission, it was only introduced in Cameroon in February 2025, and administered only to newborns of infected mothers (Akem, 2025).

On the contrary, HBV vaccination is not free for individuals born before its introduction in 2005, and remains beyond the means of many people, as there is no national catch-up program. Consequently, the vaccination coverage among adults is only around 5% and only rises to about 11% among healthcare workers due to workplace recommendations (Biloungа Ndongo et al.,

2018). This cohort, which now consists of adolescents and adults, includes many individuals who were infected with HBV during childhood and are now chronic carriers, representing a significant reservoir of asymptomatic infection (Mbouyap et al., 2025). Without strategic and timely interventions, these unprotected individuals may continue to drive transmission through several ways, including pregnancy, blood donations, and sexual contact, thereby complicating elimination efforts.

1.2. Occult hepatitis B infection (OBI) in sub-Saharan Africa

1.2.1. OBI in blood transfusion

The high prevalence of chronic HBV infections in SSA poses serious risks to blood transfusion safety. The estimated HBV seroprevalence among blood donors in SSA is about 6.93%, ranging from 2.47% in Southern Africa to more than 10% in Western Africa (Quintas et al., 2024). Although the routine use of hepatitis B surface antigen (HBsAg) testing for donor selection has greatly improved transfusion safety, it cannot identify blood donors with occult hepatitis B infection (OBI) (Gemechu et al., 2022). OBI is characterised by the presence of HBV DNA in blood at very low levels (<200 IU/mL) despite negative HBsAg test results (Raimondo et al., 2019). This condition can arise from a resolved acute infection or a chronic infection in its late phase, when HBsAg is lost or remains minimal (Raimondo et al., 2019). Also, individuals with OBI are often asymptomatic and may or may not show any serological evidence of past HBV exposure, making it difficult to accurately identify infected blood donors (Raimondo et al., 2019; Gherlan, 2022; Makvandi, 2016).

In SSA, OBI among blood donors is largely under-recognized and insufficiently documented. Available studies show wide variation in prevalence, which generally reflects HBV endemicity. Some of the highest estimates come from West Africa, reaching 17% in Nigeria and 33% in Burkina Faso (Oluyinka et al., 2015; Somda et al., 2016). Lower but still notable rates are reported in Southern Africa, including 0.98% in Mozambique and 3% in Angola (Mabunda et al., 2020; Peliganga et al., 2021). Data from Central Africa is limited, leaving significant knowledge gaps. These trends suggest that many apparently healthy HBsAg-negative donors still carry HBV DNA, making the donated blood potentially infectious. Although such donors can be identified by nucleic acid testing (NAT), most countries in SSA rely solely on HBsAg screening (Candotti et al., 2021). This reliance means that OBI often goes undetected and represents a hidden source of transfusion-transmitted HBV in this region.

Even with minimal amounts of the virus present in the blood, OBI still poses a significant risk to those receiving transfusions, especially individuals with weakened immune systems. These include HIV patients, transplant and haemodialysis patients, patients receiving immunosuppressive treatment, and individuals with underlying liver disease (Makvandi, 2016). Such recipients face risks of acute HBV infection, which can have serious clinical consequences, especially if they lack prior immunity through vaccination or natural exposure. However, in SSA, the actual burden of transfusion-transmitted OBI is largely unknown because blood recipients are not being followed up. Nonetheless, a lookback study in England reported an OBI transmission rate of over 8%, predominantly from blood donors born in Eastern Europe, Asia, or Africa (Harvala et al., 2021). This highlights OBI as a serious yet overlooked challenge to transfusion safety, especially in endemic regions, and underscores the need to improve regional surveillance to inform national blood safety policies.

1.2.2. OBI prevalence among different population cohorts

In high-risk populations, OBI prevalence rates are highly variable. Reports from different parts of SSA show that the average prevalence rate among HIV patients is about 27.7% in Southern SSA, 9.1% in Eastern SSA, and 8.5% in Western SSA (Kajogoo et al., 2022). Country-specific estimates range from 5.3% in Kenya to 30.8% in Ghana (Salyani et al., 2021; Attiku et al., 2021). In pregnant women populations, OBI has not been extensively investigated compared to chronic HBV infection. Available data show OBI prevalence rates of about 2.5% in Nigeria, 6.6% in Botswana, and as high as 20.3% in Ethiopia (Shuaib et al., 2024; Mbangiwa et al., 2018; Meier-Stephenson et al., 2020). Other vulnerable groups also show notable variation, with a prevalence of nearly 6% in patients suffering from chronic liver disease, 15.9% in haemodialysis patients, 51.4% in kidney transplant recipients, and 18.3% in an apparently healthy adult population (Bazie et al., 2024).

The absence of standardized diagnostic assays and testing protocols for OBI contributes to these variations observed across SSA (Saitta et al., 2022). Routine HBV testing relies solely on HBsAg detection, which cannot detect OBI, whereas nucleic acid testing is rarely available outside of research settings (Mitchell et al., 2023). Also, there are no established guidelines for OBI detection in transfusion services, antenatal care, or HIV programs in most of the region. These gaps hinder accurate estimation of OBI prevalence, assessment of its clinical impact, and the development of targeted public health interventions.

1.3. Epidemiology of HEV in sub-Saharan Africa

While HBV is the focus of global health efforts, HEV continues to receive little attention in SSA. Knowledge of its epidemiology is still evolving as most countries lack sufficient surveillance data to determine its true burden. While HEV causes nearly 20 million acute infections globally and approximately 3,450 deaths annually, regional proportions remain poorly quantified (Ouyang et al., 2024). Available reports suggest that SSA could account for as much as 15% of the worldwide burden, with the younger population being most at risk (Bagulo et al., 2021). Seroprevalence studies show wide variations, with past exposure rate (anti-HEV IgG) ranging from 0.25% to over 70% in the general population and up to 34% for recent exposure (anti-HEV IgM) (Bagulo et al., 2021; Maphumulo et al., 2024; Li et al., 2020b). The burden is also notable in high-risk populations, including pregnant women (61%), HIV patients (68%), and pig farmers (76%) (Musa et al., 2016; Traoré et al., 2015; Demi Sibiro et al., 2018). Nevertheless, the true burden of HEV is probably understated since the condition is frequently unrecognised or misdiagnosed, resulting from an absence of differential diagnoses. The main HEV genotypes circulating in SSA are 1 and 2, which are transmitted by the faecal-oral route, through unclean water and poor hygiene practices (Bagulo et al., 2021). These genotypes also account for large and recurrent HEV outbreaks, which are often observed in refugee and displaced persons' camps (Desai et al., 2022). While the death rate (case fatality rate) in the general population is generally low (1–2%), it exceeds 20% in women in late pregnancy and can be as high as 65% during outbreaks, with an increased risk of severe complications (Amanya et al., 2017; Jin et al., 2016). Furthermore, although transfusion-transmitted HEV remains a concern, its true burden in SSA is not well understood due to limited surveillance.

1.3.1. HEV genotype distribution and zoonotic potential

HEV has eight genotypes (genotypes 1 to 8), of which genotypes 1 and 2 are restricted to human hosts, genotypes 3, 4, and 7 are zoonotic, while genotypes 5, 6, and 8 are currently restricted to animals, and no well-documented natural infections in humans are yet known (Velavan et al., 2021). Of these, genotypes 1 and 2 remain the predominant genotypes circulating across SSA (Bagulo et al., 2021). Genotype 1 (subtype 1e) accounts for most of the repeated outbreaks that occur in East and Central SSA, while genotype 2 (subtype 2b) is predominant in West Africa, although it also occurs in parts of the Central and Southern subregions (Orf et al., 2024). In certain countries, particularly in Western SSA, both genotypes 1 and 2 circulate simultaneously, probably influenced by population movements or contamination of water

sources that flow through neighbouring countries, although this is not fully understood (Lagare et al., 2025).

In contrast, zoonotic HEV genotypes, including genotypes 3 and 4, do not seem to contribute significantly to HEV outbreaks in SSA (Bagulo et al., 2021). Nevertheless, the presence of the virus and its antibodies in various domestic and wild animals raises concerns about possible transmission to humans (El-Duah et al., 2020). Genotype 3 is common in pigs in parts of West and Central Africa, while genotype 7 has been reported in camels in East Africa (Modiyinji et al., 2021a). Also, genotypes 3c, 3f, and 4b have been identified in pigsty waste in South Africa, while genotype 3a has been detected in wastewater in Cameroon, suggesting possible environmental exposure (Fatawou et al., 2023; Salemane et al., 2024).

The potential for zoonotic transmission is further supported by the higher prevalence of HEV antibodies among individuals who have close contact with animals, such as pig farmers, as well as hunters, when compared to the general population (Oluremi et al., 2023). However, confirmed human infections with these genotypes are somewhat rare. Genotype 3 has been detected in immunocompromised patients in South Africa, while genotypes 3f and 4b have been found in patients with jaundice in Cameroon (Andersson et al., 2015; Modiyinji et al., 2024). Due to limited reports on HEV genotyping in SSA, it is not very clear whether the rarity of these zoonotic genotypes among the human population is due to under-detection or limited circulation in the region. This raises the need for more comprehensive molecular investigations to better understand genotype distribution, transmission routes, and the role of zoonotic strains in SSA.

1.3.2. Past HEV outbreaks and regional risk factors

Since the 1980s, HEV has caused several outbreaks in SSA, reaffirming its role as an ongoing regional health problem. The first laboratory-confirmed HEV outbreak in SSA was reported in Côte D'Ivoire in 1986 (Sarthou et al., 1986). Since then, large-scale outbreaks have been increasingly reported, occurring mainly in refugee and displaced persons' camps, particularly in Eastern and Central SSA (Desai et al., 2022). Countries such as South Sudan, Sudan, Uganda, Namibia, Chad, and the Central African Republic have experienced recurrent and sometimes prolonged outbreaks, where hundreds to thousands of cases are often reported (Pallerla et al., 2020). For instance, there were more than 11,000 cases in the 2012-2013 South Sudan outbreak, while an outbreak in Namibia lasted from 2017 to 2022 (Nghikevali and Crowley, 2025; CDC 2013). In total, tens of thousands of cases and hundreds of deaths have

been reported, with the death rate (case fatality rate) among pregnant women reaching 65% in some outbreaks (Amanya et al., 2017). Since HEV surveillance and reporting are generally weak in the region, the true extent of HEV burden may even be understated.

The persistence of outbreaks in SSA may reflect common regional factors that are related to the faecal-oral route of HEV transmission, involving genotypes 1 and 2. Most outbreaks are linked to the use of unclean water sources, including rivers and wells, and are often worsened by heavy rainfall and flooding (Desai et al., 2022). Also, overcrowding in displacement camps or informal urban settlements further facilitates spread due to poor sanitation (Desai et al., 2022). Other risks include the consumption of unsafe food, poor water storage, and inadequate hygiene practices (Bagulo et al., 2021). Although genotype 3 circulates in pigs in the region, and environmental sources (Salemane et al., 2024; Modiyinji et al., 2021a), its role in major outbreaks remains uncertain. HEV transmission from person to person, which was reported from an outbreak in Uganda, has not been further observed in other outbreaks in the region (Teshale et al., 2010). Control measures remain largely based on emergency water and hygiene interventions.

Although Cameroon has not experienced large-scale outbreaks, the same risk factors may be present. Humanitarian crises have displaced thousands of people into overcrowded shelters with poor sanitation, while recurring cholera outbreaks further highlight weaknesses in the country's water infrastructure (Bangwen et al., 2024). In an HEV outbreak that occurred in Cameroon's Northern region, which is the only documented outbreak so far, 33 cases were recorded, including two deaths, of which one was a pregnant woman (Maurice et al., 2013). Subsequent investigations of the outbreak samples confirmed HEV genotypes 1 and 3 (Modiyinji et al., 2021b). However, potential risk factors that could be linked to the outbreak were not investigated, leaving important gaps in understanding HEV transmission dynamics in the country.

1.3.3. Clinical relevance of HEV in blood transfusion and high-risk populations

While the primary route of HEV transmission in endemic regions is faecal–oral, its relevance extends beyond this route. There are increasing reports of cases of HEV transmitted through transfusion in industrialized countries (Singson et al., 2024; Harvala et al., 2022; Gallian et al., 2019; Boland et al., 2019), prompting some European countries to include HEV screening in their blood safety protocols (Boland et al., 2019). However, the extent of transfusion-transmitted HEV in SSA is still unclear due to limited surveillance among blood donors.

Limited data show high HEV seroprevalence rates (anti-HEV IgG) of 39% in Burkina Faso, 43% in South Africa, and 87% in Nigeria, along with occasional reports of active viraemia, suggesting potential ongoing transmission (Traoré et al., 2016; Maponga et al., 2020; Ushie et al., 2023). Yet, no country in SSA currently screens blood donations for HEV, implying that asymptomatic donors may contribute to blood transfusions, which poses a significant risk to vulnerable recipients (Westhölter et al., 2018).

In most healthy individuals, HEV infection is self-limiting and usually resolves without problems. However, individuals with weakened immune systems, such as those infected with HIV or transplant recipients, can develop chronic infections due to ineffective virus clearance (Kupke et al., 2025). These infections can rapidly progress to cirrhosis or liver failure (Ingiliz et al., 2016). Given the high burden of HIV in SSA, HIV patients represent a particularly vulnerable population for severe HEV disease in the region. In several countries, HEV seroprevalence among HIV patients (anti-HEV IgG) is over 30%, with sporadic reports of active viraemia as well (Alexandrova et al., 2024). Although antiretroviral therapy may improve immune function, chronic HEV infection can persist (Ingiliz et al., 2016; Kuniholm et al., 2016). Moreover, genotypes 3 and 4, which are associated with chronicity, are increasingly detected in animals and environmental sources in SSA, exposing already vulnerable populations to ongoing risk (Bagulo et al., 2021; Nombot-Yazenguet et al., 2024; Salemane et al., 2024).

Furthermore, pregnant women infected with genotype 1, especially in late pregnancy, have a heightened risk for severe disease and complications (Wu et al., 2020). Maternal complications and adverse birth outcomes, including haemorrhage, eclampsia, preterm birth, miscarriage, mother-to-child transmission, and neonatal death, are common and can lead to acute liver failure and death (Li et al., 2020a; Sharma et al., 2017; Bigna et al., 2020). Such outcomes have been attributed to hormonal changes, altered immune responses, malnutrition, and enhanced viral replication (Wu et al., 2020). In SSA, where HEV outbreaks are recurrent, and the anti-HEV IgG seroprevalence in pregnant women exceeds 60%, this raises public health concerns (Musa et al., 2016). Still, HEV screening is not included in antenatal care programs, and there is no approved antiviral treatment available (Hui and Wei, 2023). Additionally, the only approved vaccine (HEV239) remains largely unavailable in SSA (Dudman et al., 2025). Therefore, to develop effective prevention measures, it is crucial to close knowledge gaps about HEV infection, particularly among high-risk populations.

In Cameroon, HEV surveillance is equally limited. Available data are not regionally representative and are largely obtained from retrospective analyses of samples from previous studies, or from testing samples from reference laboratories after various causes of jaundice, like yellow fever, have been ruled out, or from outbreak investigations (Modiyinji et al., 2024; Modiyinji et al., 2021b). Due to limited awareness of HEV among healthcare providers, misdiagnosis is common, and the true burden in high-risk populations, including pregnant women and HIV patients, remains poorly characterized. The limited data available generally show high seroprevalence rates in various populations, reaching 35% (for anti-HEV IgM) in the elderly (Modiyinji et al., 2019), but genotyping is often not done. So far, the HEV genotypes identified to be circulating in Cameroon include 1e, 3f, and 4b (Modiyinji et al., 2024). Equally, blood transfusion facilities do not routinely test for HEV, but focus only on HBV, HIV, hepatitis C virus (HCV), and syphilis (Dei-Adomakoh et al., 2021). As a result, asymptomatic infections in donors may go undetected. The prevalence of HEV among blood donors has not been investigated before in Cameroon. This oversight is particularly concerning for immunocompromised blood recipients, such as pregnant women, individuals on haemodialysis, as well as patients suffering from various liver conditions who may develop chronic HEV or complications if infected (Alexandrova et al., 2024). The limited systematic surveillance for HEV and the lack of screening in blood donors in Cameroon not only underestimates the true burden but also hinders efforts to control viral hepatitis in the region.

1.4. Risk factor associations in high-risk groups

The major risk factors linked to persistent HBV infections in SSA, such as mother-to-child transmission, early childhood exposure through contact with infected household members or playmates, unprotected sexual intercourse, co-infection with other viruses, and unsafe medical practices and procedures, are well characterized (Spearman et al., 2017). In contrast, despite high HEV seroprevalence in certain populations and repeated outbreaks, the biological, behavioural, and environmental risk factors that may predispose individuals to infection or severe disease are not well understood. During several HEV outbreaks across SSA, a higher number of deaths have consistently been reported among pregnant women (Kim et al., 2014). Similarly, data showing high seroprevalence rates of HEV among immunocompromised individuals, such as HIV patients, may also suggest increased susceptibility (Demi Sibiro et al., 2018; Alexandrova et al., 2024). However, the specific individual-level risk factors that contribute to vulnerability to HEV infection have not yet been sufficiently investigated. This

underlines significant knowledge gaps regarding HEV infection and the associated poor outcomes observed in high-risk populations.

Beyond host susceptibility, several sociodemographic, dietary, and environmental factors are believed to heighten the risk of HEV infection (Pavio et al., 2021). Previous studies have associated various factors, such as marital status, educational level, advanced age, close contact with pigs and their products, drinking water source, alcohol consumption, poor sanitation and hygiene practices, with HEV infection (Bagulo et al., 2021). However, these associations are not uniform across the SSA region, and together with the uneven distribution of outbreaks, they may suggest that local factors may contribute to shaping subregional transmission dynamics. This highlights the need for local investigations to identify relevant population-specific risk factors for HEV and other factors influencing HBV exposure and infection, to inform targeted prevention strategies.

1.5. HBV–HEV co-infection

1.5.1. HBV–HEV co-endemicity and potential for co-infection

Although HBV and HEV largely have different pathways of infection, they often co-exist in highly endemic areas, particularly in Asia and Africa (Nasir and Wu, 2020). Their geographical overlap may be influenced by shared transmission routes, including blood transfusions and mother-to-child transmission. Individuals with a compromised immune system, such as pregnant women and HIV patients, as well as patients with existing liver conditions, are particularly affected by both viruses (Kilonzo et al., 2019a). These overlaps create natural conditions for dual exposure and co-infections. Co-infections can occur either through simultaneous exposure to both viruses or through HEV superinfection in individuals with chronic HBV infection (Nasir and Wu, 2020). In SSA, where HBV is often acquired at a young age, HEV superinfection in chronic HBV carriers may aggravate the liver diseases (Kilonzo et al., 2019a).

The majority of available reports on co-infections with HBV and HEV come from the Asian region, where anti-HEV IgG rates among HBV patients reach 45% in Vietnam and 18.7% in China (Hoan et al., 2015; Shi et al., 2025), whereas the SSA region is underrepresented. Existing evidence in SSA shows anti-HEV IgG rates reaching 4.2% in HBV-positive adults in Nigeria, as well as 5% in Chad, with associated alterations in lipid profiles in the Chadian population (Adedeji et al., 2024; Djasrabe et al., 2022). Higher anti-HEV IgG rates have been documented in specific groups, including nearly 29% in HBV-infected pregnant women and

12.2% in those who handle animals (Adedeji et al., 2024; Gidado et al., 2021). Although simultaneous viral replication for HBV and HEV is rarely observed, co-exposure appears to be common in SSA. Without systematic surveillance, HBV and HEV co-infections may continue to be underreported, representing a hidden public health challenge that could slow progress toward viral hepatitis elimination in the region.

1.5.2. HBV–HEV co-infection patterns and clinical outcomes.

While the clinical significance of HBV and HEV co-infection is increasingly recognized, its epidemiological patterns and clinical characteristics in SSA are not yet well understood. This is because research has focused primarily on HBV mono-infection, coupled with the limited and inconsistent HEV surveillance in the region (Bagulo et al., 2021). As a result, the patterns of HBV, OBI, and HEV co-infection are poorly described, especially since most studies also focus on single-population investigations and rarely perform comprehensive testing for both HBV and HEV. This, therefore, limits the understanding of their joint transmission and viral interaction dynamics, as well as their combined effects on liver disease progression.

Evidence is mounting that HBV co-infection with HEV can significantly worsen clinical outcomes, particularly in patients who already suffer from liver disease (Wang et al., 2020). Longitudinal studies from Asia report that HEV superinfection in chronic HBV carriers can accelerate progression to cirrhosis, increase the likelihood of having a liver failure, and contribute to higher rates of liver-related death, which may exceed 30% in patients with cirrhosis (Tseng et al., 2020). Superinfection can also trigger reactivation of HBV replication, especially when treatment is interrupted (Obeidat et al., 2021). Additionally, individuals with co-infection may exhibit more severe clinical symptoms, such as fever, nausea, and enlargement of the liver and spleen, than HBV monoinfected individuals (Fu et al., 2016). In HIV patients who already have a higher chance of developing a chronic disease course from either virus alone, co-infections can further worsen outcomes (Ruta et al., 2023). Similarly, co-infected pregnant women are at risk of maternal complications and adverse birth outcomes than women infected with only one virus (Chen et al., 2024). There are reports of low birth weight in babies born to co-infected women, as well as evidence of HEV antibodies in umbilical cord blood, suggesting a potential risk of transmission (Huang et al., 2016).

In SSA, despite the widespread presence of both viruses, not much is known regarding the prevalence of simultaneous replication or the extent to which co-infection may worsen the clinical course in co-infected individuals. Nonetheless, patterns observed in Asia suggest that

SSA may face similar risks. Limited evidence from Côte D'Ivoire and Cameroon shows that prior HEV exposure may aggravate liver damage and promote the development of complications in individuals with chronic HBV infections or other liver diseases (Sévédé et al., 2019; Amougou Atsama et al., 2017).

In all, HBV–HEV co-infection (including OBI–HEV co-infection) may represent an underestimated health risk in SSA. It is therefore crucial to enhance integrated surveillance and testing for both viruses. These measures are essential to clarify the epidemiology of co-infection, better identify high-risk groups, and enable targeted clinical care.

1.6. Aim and objectives

The high endemicity of chronic HBV infection in Cameroon and the emerging evidence of circulating HEV create an epidemiological overlap for which little is currently known. This co-endemicity is particularly concerning, given the current regional and global targets for viral hepatitis elimination (WHO, 2024). HBV and HEV share some transmission routes, including mother-to-child transmission and transmission through infected blood, and disproportionately affect the same vulnerable populations, including pregnant women and people infected with HIV, raising significant public health concerns. Although routine HBV screening is performed in all blood banks in Cameroon and is recommended during antenatal care and in the clinical management of HIV patients, it is primarily based on the detection of HBsAg, which leaves occult infections undetected. HEV testing, on the other hand, is not performed in clinical settings. The co-endemicity of these viruses and diagnostic limitations leave a hidden viral burden that is likely underestimated, posing risks to transfusion recipients and the health of mothers and their newborns.

The simultaneous presence of both viruses can also worsen liver disease (Nasir and Wu, 2020). Although direct evidence in SSA remains limited, a pilot study from Cameroon reported a higher seroprevalence of HEV among individuals who had HBV or HCV-related liver disease than in uninfected control subjects (Amougou Atsama et al., 2017), suggesting possible viral interactions. This is particularly concerning since the burden of chronic HBV carriers in Cameroon is high. Yet, the extent of concurrent viral replication in the population and its clinical implications remains unclear. Therefore, parallel studies of HBV/OBI and HEV, as well as their co-infection patterns, are important to obtain a more comprehensive epidemiological picture and quantify the hidden disease burden in key populations. These include third-trimester pregnant women and individuals infected with HIV, who are vulnerable to chronic

infection and severe course of disease, as well as blood donors, who serve as a proxy for the general population and community-level exposure.

Most previous studies on HBV and HEV in Cameroon have relied primarily on serological testing. However, combining serological and molecular techniques would provide a more comprehensive understanding of their epidemiology, especially since occult HBV infections can be quantified, which would otherwise be missed by conventional serological HBsAg testing. In addition, molecular characterization would enable detection of concurrent viral replication and circulating genotypes, thereby improving understanding of transmission dynamics. Therefore, this thesis aimed to address critical knowledge gaps regarding the epidemiology of HBV infections, including OBI, and equally HEV in Cameroon by assessing their burden, characterizing genotype distributions, identifying associated risk factors, and investigating potential co-infection patterns.

The following objectives guided the work presented in this thesis:

1) To determine the prevalence of OBI and genotype diversity in Cameroonian blood donors and its implications for transfusion safety.
2) To determine the burden and genotypic characteristics of HBV and OBI in high-risk populations: HIV patients and women in the third trimester of pregnancy.
3) To investigate HEV epidemiology, including genotype distribution, and risk factors among blood donors and high-risk populations.
4) To investigate co-infection dynamics of HBV, including OBI, and HEV infections among HIV-positive adults, pregnant Women, and the general population.

1.7. Methodology

1.7.1. Ethics

Ethics approval for this study was obtained by submitting the study protocol, along with other required documents, to the Institutional Review Board of the University of Buea in Cameroon (Ethics approval number: 2022/1849-10/UB/SG/IRB/FHS), which is affiliated with the Faculty of Health Sciences. The University of Tübingen, Germany, equally granted ethics approval (Ethics approval number: 379/2023B02). Additionally, administrative authorization was obtained from the Southwest Regional Delegation of Public Health in Cameroon and from the directors of the hospitals and departments where study participants were recruited (Approval number: 84/MPH/SWR/RHL/DO/03/2023). All participants were provided with details about the study, after which each gave written informed consent before being enrolled.

1.7.2. Study Setting

This research was conducted in Cameroon's Southwest Region, which is one of the country's ten administrative regions and one of its two English-speaking regions. The region covers approximately 25,410 km² with an estimated population density of 63–70 inhabitants per km². It is home to two large referral hospitals: Buea Regional Hospital, at the foot of Mount Cameroon in Buea, and Limbe Regional Hospital in the coastal town of Limbe. Both Buea and Limbe are semi-urban areas. Each hospital has a blood transfusion department and a special clinic for the follow-up care of HIV patients receiving ART. Also, there are several local health facilities in the region, including an integrated health centre in Buea and another located in Mutengene, which mainly provide antenatal care.

1.7.3. Study Populations and Sampling

Participants in this cross-sectional study were recruited primarily in a hospital setting, and samples were collected from March to June 2023. It included apparently healthy adults from the blood bank department who were ready for blood donation, as well as high-risk groups, including women in the third trimester of pregnancy and HIV patients receiving ART.

Participants in the blood donor study population were enrolled at the Buea Regional Hospital blood bank, provided they met the standard criteria for blood donation, including being at least 18 and at most 65 years old, having a minimum weight of 50kg, a stable blood pressure and heart rate, and healthy haemoglobin concentrations. Individuals were not included if they reported being pregnant, had a period within the last two weeks, had a chronic disease, were

taking medication, or reported having multiple sexual partners. Eligible and consenting blood donors were then tested for HBV, HIV, HCV, and syphilis (as recommended by WHO) using rapid tests followed by an enzyme-linked immunosorbent assay for HBV and HCV. All pre-donor selection procedures and tests were performed by the blood bank staff in accordance with routine protocols. Participants provided their demographics and other data by filling out a structured questionnaire.

HIV patients were also recruited during the same time frame from the HIV clinics of the Regional Hospitals in Buea and Limbe, which mainly provide ART services. All consenting patients were enrolled consecutively during their routine appointments. None of the participants reported being pregnant. Also, HIV viral load data were obtained from patient records, and demographics were documented using a structured questionnaire. However, data on liver function were not available.

Pregnant women were recruited during antenatal care visits at two health facilities: the Integrated Health Centres of Buea and Mutengene. Only women in their third trimester who had provided written informed consent were consecutively enrolled. All participants had no documented history of HIV infection nor showed any clinical evidence of liver disease.

For enrolled participants, venous blood was collected, and, where possible, matching faecal samples were also collected. An overview of the sample sizes and sample types collected for each study population is summarized in the table below.

	Sampling sites	**Blood samples**	**Matching Faecal samples**
Blood donors (n=289)	Regional Hospital Buea	289	107
HIV patients on ART (n=233)	Regional Hospital Buea	135	133
	Regional Hospital Limbe	98	16
Third-trimester pregnant women (n=190)	Integrated health centres: Buea	64	34
	Mutengene	126	35
	Total samples	**712**	**325**

n represents the total number of participants enrolled in each of the study populations.

2. Results

Chapter 1

Prevalence of OBI and genotype diversity in Cameroonian blood donors and its implications for transfusion safety

Publication No.1

Incidence of Occult Hepatitis B Infection (OBI) and hepatitis B genotype characterization among blood donors in Cameroon

Mbencho MN*, Hafza N*, Cao LC, Mingo VN, Achidi EA, Ghogomu SM, Velavan TP.

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RESEARCH ARTICLE

Incidence of Occult Hepatitis B Infection (OBI) and hepatitis B genotype characterization among blood donors in Cameroon

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Data Availability Statement: All data generated or analysed during this study are included in this article. A total of 14 successfully sequenced HBV

Abstract

Background

Occult hepatitis B infection (OBI) is characterized by the presence of hepatitis B virus (HBV) DNA at low levels in serum (<200 IU/mL) with a negative hepatitis B surface antigen (HBsAg) test. OBI remains a major challenge to blood safety, particularly in HBV-endemic regions like Cameroon, where HBV detection relies solely on HBsAg testing. This cross-sectional study aimed to investigate the actual incidence and genotype characteristics of OBI in Cameroonian blood donors.

Methods

Between March and June 2023, samples were collected from 288 HBsAg-negative blood donors aged 18 to 55 years and analysed for antibodies against the HBV core (anti-HBc) and surface antigens (anti-HBs). Following DNA extraction from the serum samples, qualitative nested PCR and quantitative real-time PCR were used to detect HBV viral DNA and viral load respectively. For positive samples, sequencing of a fragment of the *S* gene was performed to identify the circulating HBV genotypes.

Results

The findings revealed that 58% (n = 167/288) of blood donors tested positive for anti-HBc, 29% (n = 83/288) tested positive for anti-HBs, and 26% (n = 75/288) being positive for both anti-HBc and anti-HBs. Occult hepatitis was confirmed in 4.5% of the blood donors, all of whom belonged to either HBV genotypes *A* or *E*, which are predominant in Cameroon. The amino acid substitution *sA184V* associated with HBsAg detection failure in genotype E was observed in 70% of OBI sequences, and the HBsAg immune escape variants (s*T131N* and s*S143L*) implicated in OBI were also observed. The mutation *rtN139K* in the reverse

DNA positive samples were submitted to the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers PP746847-PP746860 (n=14).

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Competing interests: The authors declare no conflict of interest.

transcriptase (RT) domain of the overlapping HBV polymerase (*P*) gene was present in 17% of OBI-positive sequences of genotype *E*, likely contributing to masking HBsAg secretion.

Conclusion

The results suggest a considerable risk of transfusion-transmitted HBV in this region. Therefore, to ensure blood safety, nucleic acid testing (NAT) is recommended, as relying solely on HBsAg assays is insufficient to eliminate this risk.

Introduction

Hepatitis B, caused by the hepatitis B virus (HBV), poses a significant health challenge, especially in Africa. Despite the World Health Organization (WHO) aiming to eliminate viral hepatitis as a public threat by 2030 [1], many African countries, including Cameroon, continue to struggle with high prevalence rates. Cameroon reports a pooled HBV prevalence of 11% [2]. The introduction of HBV vaccination in 2005 as part of the Expanded Program on Immunization (EPI) for infants (administered at 6, 10, and 14 weeks of age), aimed to address this problem. Before its implementation, the seroprevalence rates were as high as 20% among school-aged children [3], and 14% in the adult population [4]. Moreover, the vaccine is not provided free for children born before 2005, who would now be adult chronic carriers if infected.

Among other viral infections such as hepatitis C (HCV) and Human Immunodeficiency Virus (HIV), HBV is routinely screened by serology to ensure the safety of blood transfusions. However, despite testing negative for the hepatitis B surface antigen (HBsAg), there remains a residual risk of HBV transmission, wherein detectable viral DNA in the liver and/or blood, typically <200IU/mL, contributes to the risk of transfusion-transmitted infection. This phenomenon is referred to as occult hepatitis B (OBI) [5], which significantly contributes to silent HBV transmissions. The molecular basis of OBI lies in the long-term persistence of a stable replication-competent episomal HBV covalently closed circular DNA (cccDNA) within the nucleus of infected hepatocytes [5]. The mechanisms underlying OBI are multifactorial, including both host and viral factors that contribute to the suppression of viral replication, thereby keeping the virus under control. However, immunosuppressed individuals, such as patients undergoing chemotherapy, hemodialysis, or those with HIV who receive transfusions containing HBV, are at risk of reactivation of the transcriptional activity of the viral cccDNA, leading to a full-blown overt infection that often progresses to liver cirrhosis and hepatocellular carcinoma [6].

In developed countries, nucleic acid tests (NAT) for screening blood donations are increasingly used to reduce the risk of OBI [7]. In contrast, other countries utilize antibodies to the HBV core protein (anti-HBc) as a surrogate marker. However, OBI remains largely unrecognized in low-to-middle-income countries like Cameroon, where blood donor selection relies solely on serological testing of HBsAg. Data on the prevalence of OBI in Cameroon is limited. In 2019, a study (in Yaoundé, Cameroon) reported an OBI incidence of 1.1% among 522 anti-HBc reactive donor samples [8], while another study in 2021 reported an OBI residual risk of 1.6% in seropositive donors from the same region [9]. These studies have not considered seronegative donors despite hypothesis suggesting that OBI development involves stronger suppression of viral replication and HBsAg expression. For instance, mutations in the *PreS/S* regions might alter HBsAg expression, secretion, and antigenicity, thus inhibiting the production of anti-HBs [10].

This study investigated the OBI incidence, defined by the presence of hepatitis B viral DNA in the serum, associated viral factors, and circulating HBV genotypes in the blood donor population of the Southwest Region of Cameroon who tested negative for HBsAg.

Materials and methods

Ethics statement

This study was approved by the Institutional Review Board of the University of Buea, Cameroon (Ethics approval number: 2022/1849-10/UB/SG/IRB/FHS) and the University of Tübingen for the project 'Molecular surveillance of hepatitis E and Occult hepatitis B in the Cameroonian population' (Ethics approval number: 379/2023B02). In addition, administrative clearance was obtained from the Southwest Regional Delegation of Public Health (Approval number: 84/MPH/SWR/RHL/DO/03/2023). Signed informed consent was obtained from all study participants prior to enrolment.

Study cohort and sampling

Between March and June 2023, 288 consented, healthy blood donors aged 18 to 55 years were recruited from the Buea Regional Hospital blood bank in Cameroon. Demographic data, vaccination status, and blood donation history were recorded using a structured questionnaire in this cross-sectional study. After meeting the eligibility criteria, which included being HBsAg-negative, having no medical history of chronic disease, and testing negative for HIV, HCV, and syphilis, 3 mL of blood was collected from each donor. The eligibility criteria for blood donation included being aged 18 to 65 years, weighing at least 50 kg, being in good general health, free from acute illness, with normal blood pressure and pulse rates, and having adequate hemoglobin levels as determined during pre-donation screening. Individuals with chronic diseases, those on medication, and pregnant women are not eligible to donate. The HBsAg status, HIV, HCV, and Syphilis was determined using rapid test kits and ELISA (Fortress Diagnostics, Antrim, United Kingdom). The HBsAg assay used a sensitivity of 99.75% and a specificity of 99.87%, while the anti-HCV assay had a sensitivity of 99.79% and a specificity of 99.55%. Additionally, the HIV (Ag/Ab) assay had an analytical sensitivity for p24 antigen of 5 pg/mL, and the *Treponema pallidum* antibody rapid test kit showed a sensitivity of 100% and a specificity of 99.7%.

Screening of HBV serological markers

All 288 blood donor sera were screened for HBV serological markers anti-HBs (Monolisa™ anti-HBs PLUS, Bio-Rad, Hercules, CA, USA) and anti-HBc (Monolisa™ anti-HBc PLUS, Bio-Rad, Hercules, CA, USA) using ELISA procedures, following manufacturer's instructions. The absorbance was measured on a CLARIOstar microplate reader (BMG Labtech, Ortenberg, Germany). The anti-HBs positivity was defined as a titer value >10 mIU/mL and the assay had a sensitivity of 99.2% and a specificity of 99.4%. The anti-HBc assay (qualitative) had a sensitivity of 99.53% and a specificity of 99.9%.

Qualitative and quantitative detection of HBV

HBV DNA was extracted from 200μl of serum using the QIAamp Viral DNA mini kit (Qiagen GmbH, Hilden, Germany). For HBV DNA qualitative detection, HBV-specific nested-PCR targeting a highly conserved overlapping *S/P* region (332 bp) was conducted as previously described [11–13]. PCR reactions (25μL) consisted of 1xPCR buffer, 0.2 mM dNTPs, 0.4 μM primers (S1 Table), and 1U Taq DNA Polymerase (Qiagen GmbH, Hilden, Germany).

Thermal cycling parameters include initial denaturation at 94˚C for 5 mins, followed by 35 cycles of denaturation (94˚C, 30s), annealing (55˚C for outer, 54˚C for inner, 30s), and extension (72˚C, 30s), with a final extension at 72˚C for 5 mins. Controls (positive: HBV plasmid DNA; negative: master mix) were incorporated to validate PCR products. The nested PCR detection limit for HBV DNA was approximately 2.5 copies per reaction, equivalent to 30 to 40 copies/mL. Amplicons were visualized by agarose gel electrophoresis. PCR positives were purified using (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced with the Big Dye™ Terminator v.3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA) on an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Beverly, MA, USA) following the manufacturer's protocol.

For DNA quantification, real-time PCR was conducted using virus-specific primers and a Taqman probe that targeted a 90-bp fragment within the conserved region of the S gene in the HBV genome (position 182–271, GenBank number X75657), following a previously described protocol [14]. The SensiFAST™ one-step RT-PCR kit (Meridian Biosciences, Memphis, Tennessee, USA) was used on a LightCycler480-II (Roche, Mannheim, Germany). Each real-time PCR reaction was performed in 20 μL volume, containing 0.8 μL each of 10 μM forward primer (HBV-61) and reverse primer (HBV-62), 2X real-time-PCR master mix (10 μL), 0.3 μL probe (HBV TM-05) (S1 Table), with 15–20 ng (5 μL) of DNA. Cycling conditions included an initial denaturation at 95˚C for 5 mins, followed by 45 cycles of 95˚C for 10 seconds and 60˚C for 34 seconds. The limit of detection for the real-time PCR used to quantify HBV viral load was 25 IU/mL. This limit was established using a control plasmid with a concentration of 10^6 copies/μL, serially diluted tenfold. The linear equation relating Ct values to log (copies/μL) was used to calculate viral loads. The highest Ct value detected, which was 40, corresponded to 25 IU/mL.

Phylogenetic and mutation analysis of HBV sequences

The HBV-specific gene sequences obtained were trimmed in Seqman version 6.1 (DNASTAR, Lasergene, USA) and the resulting consensus sequences were manually verified. Alignment was performed using MAFFT version 7.0 using the G-INS-I model [15]. For the phylogenetic tree reconstruction, a combined set of 28 representative reference sequences was used consisting of 16 sequences specific to HBV genotypes *A-H* retrieved from the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/): Genotypes *A* (M57663, AF090842), *B* (D23678, D50522), *C* (D23680, M38636), *D* (AF151735, X02496), *E* (AB032431, X75657), *F* (X69798, AY090455), *G* (AB064313, AF160501), and *H* (AB059659, AY090454) along with an additional 12 sequences identified through an NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search for the HBV DNA positive sequences: Genotypes *A* (LC513655, MH213535, MH580639), and *E* (KY494007, MH253771, MK840530, MK174170, KU702933, DQ060826, MF772360, MN507847, KU522302). The phylogenetic tree was reconstructed using MEGA version 11 [16] employing the Maximum Likelihood method using the Kimura 2 parameter plus Gamma Distribution model (K2+G). The statistical robustness and reliability of the branching order were confirmed via bootstrapping with 1000 replicates. The resulting phylogenetic tree was annotated and visualized using the online tool iTOL v6 (https://itol.embl.de/) [17]. Mutation analysis was done on BioEdit version 7.2.6 (https://bioedit.software.informer.com/7.2/) using genotype-matched references for the identified genotypes (*A* and *E*). The representative OBI sequences obtained in this study have been deposited in the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) and can be retrieved with accession numbers PP746847-PP746860 (n = 14).

Data analysis

Statistical analysis was performed using R version 4.0. The median age of the blood donors was determined as the median value in the ordered age distribution, with variability described as interquartile range (IQR). The incidence of OBI was calculated as the proportion of HBsAg-negative blood donors who tested positive for HBV DNA and with a viral load not exceeding 200 IU/mL. Viral load in positive samples was quantified using real-time PCR, with results expressed in IU/mL based on a standard curve generated from a serially diluted control plasmid with a concentration of 10^6 copies/μL.

Results

Demographic characteristics of the study cohort

Of the 288 blood donors sampled, males dominated, accounting for 97% of the study cohort (n = 279). The median age of donors was 30 years (IQR; 24–38) and the dominant age group was 21–30 years (48%; n = 137) followed by 31–40 years old (32%; n = 91). Majority of donors had a donation history (79%; n = 227) and only 3% (n = 9) had been vaccinated for HBV.

Serological profiles

The serology results for HBV showed that 58% (n = 167/288) were positive for anti-HBc and 29% (n = 83/288) positive for anti-HBs. Of these, 26% (n = 75/288) tested positive for both antibodies and 39% (n = 113) tested negative for both anti-HBc and anti-HBs antibodies (Fig 1).

HBV DNA detection

HBV DNA was detected in 14 out of 288 samples (5%) by nested PCR. The median age of the HBV DNA-positive blood donors was 24 years (range: 18–41). Among these samples, four had detectable viral loads measured by qPCR, ranging from <25 to 2593 IU/mL (Table 1). Three of those with detectable viremia were positive for anti-HBc, while one was positive for anti-HBs but not anti-HBc. Of all samples tested positive for HBV DNA, 93% remained negative for anti-HBs (Table 1). Following the consensus definition of OBI, one HBV DNA positive sample (BD229) was considered 'false' OBI since its viral load is > 200IU/mL. Therefore, the incidence of OBI in this population is 4.5%.

OBI genotyping and genetic variation

Sequences amplified from the *S/P* region of HBV were aligned with the reference sequences from the NCBI genotyping tools and the HBV database (HBVdb). BLAST searches identified 13 sequences as genotype E and one as genotype A (Table 1). Phylogenetic analysis with 28 reference sequences corroborated these findings (Fig 2). Using reference genomes obtained from NCBI tool (https://www.ncbi.nlm.nih.gov/nuccore/; M57663 and AF090842 for genotype *A*; AB032431 and X75657 for genotype *E*), the *S* and RT domains of OBI-positive sequences were analyzed. Nonsynonymous substitutions in the amino acids were found in the S protein's "α" determinant region (aa 124–147) within the major hydrophilic region (MHR), and at the reverse transcriptase (RT) domain of the *P* gene (Table 2, S1 and S2 Figs). Notably, the *sA184V* substitution was present in 70% of the OBI sequences, and *sY206C* was also observed in 62% of sequences, both located outside the "α" determinant and MHR (aa 99–169) regions. Within the "α" determinant, substitutions *sT131N* and *sS143L* were observed, while *sK122R* and *sK160R* were found in the major hydrophilic region. The mutations *rtW153R*, *rtI163V*, *rtI122V*, *rtN139K*, and *rtF151L* were present in the RT domain. The sample with a high viral

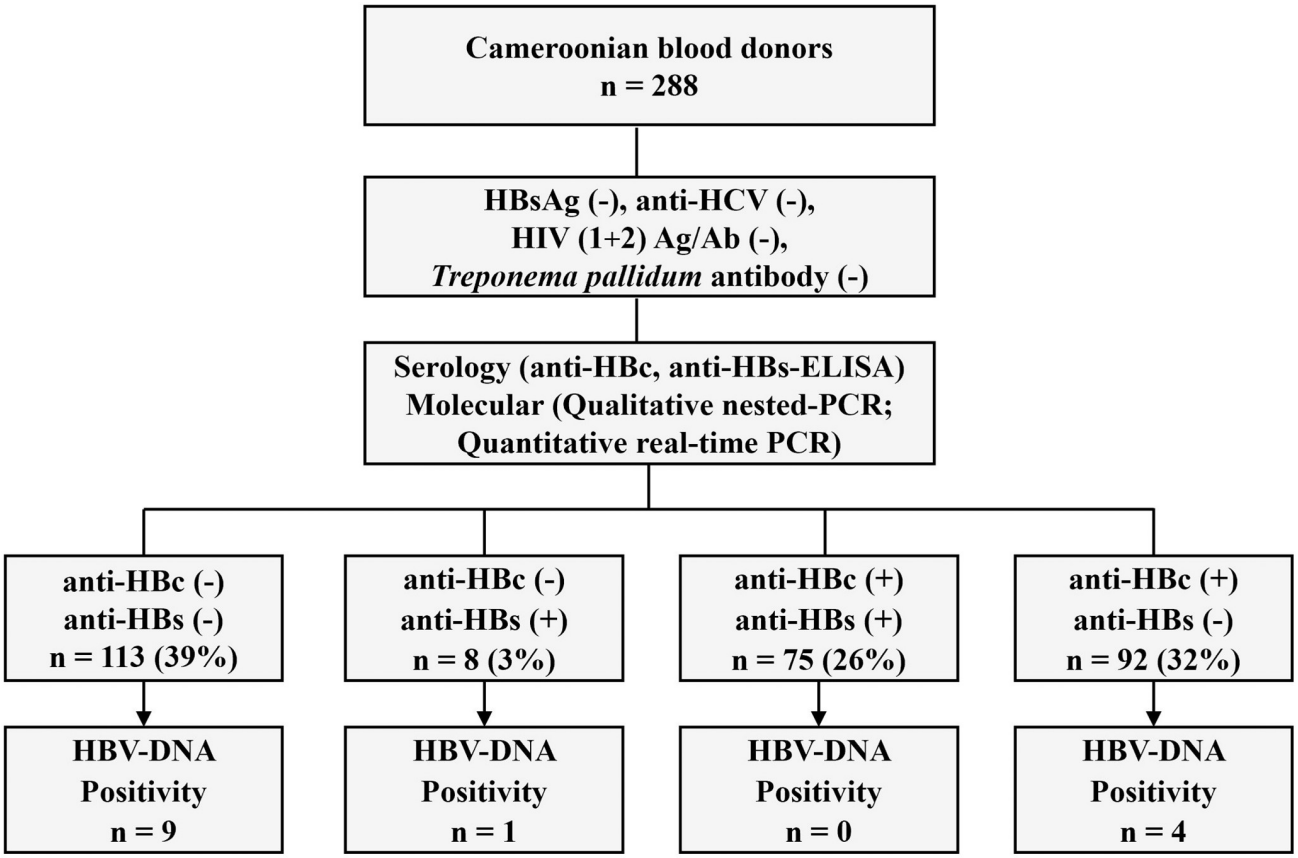

Fig 1. Study design and summary of results. A total of 288 HBsAg-negative blood donor serum were analyzed by Enzyme Linked Immunosorbent Assay (ELISA) for anti-HBc and anti-HBs, and subsequently by nested PCR for HBV DNA in the highly conserved *S/P* region of the HBV genome.

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Table 1. Characteristics of OBI blood donors in terms of seropositivity, viral load, and hepatitis B virus (HBV) genotypes.

Sample ID	anti-HBc	anti-HBs	HBV PCR	HBV qPCR	Viral load (IU/mL)	HBV Genotypes
BD072	-	-	+	-	undetectable	*E*
BD078	-	-	+	-	undetectable	*E*
BD092	-	-	+	-	undetectable	*E*
BD118	-	-	+	-	undetectable	*E*
BD147	+	-	+	+	81	*A*
BD157	-	-	+	-	undetectable	*E*
BD163	-	-	+	-	undetectable	*E*
BD172	-	-	+	-	undetectable	*E*
BD174	-	-	+	-	undetectable	*E*
BD183	-	-	+	-	undetectable	*E*
BD211	+	-	+	-	undetectable	*E*
BD225	+	-	+	+	<25	*E*
BD229	+	-	+	+	2593	*E*
BD284	-	+	+	+	31	*E*

https://doi.org/10.1371/journal.pone.0312126.t001

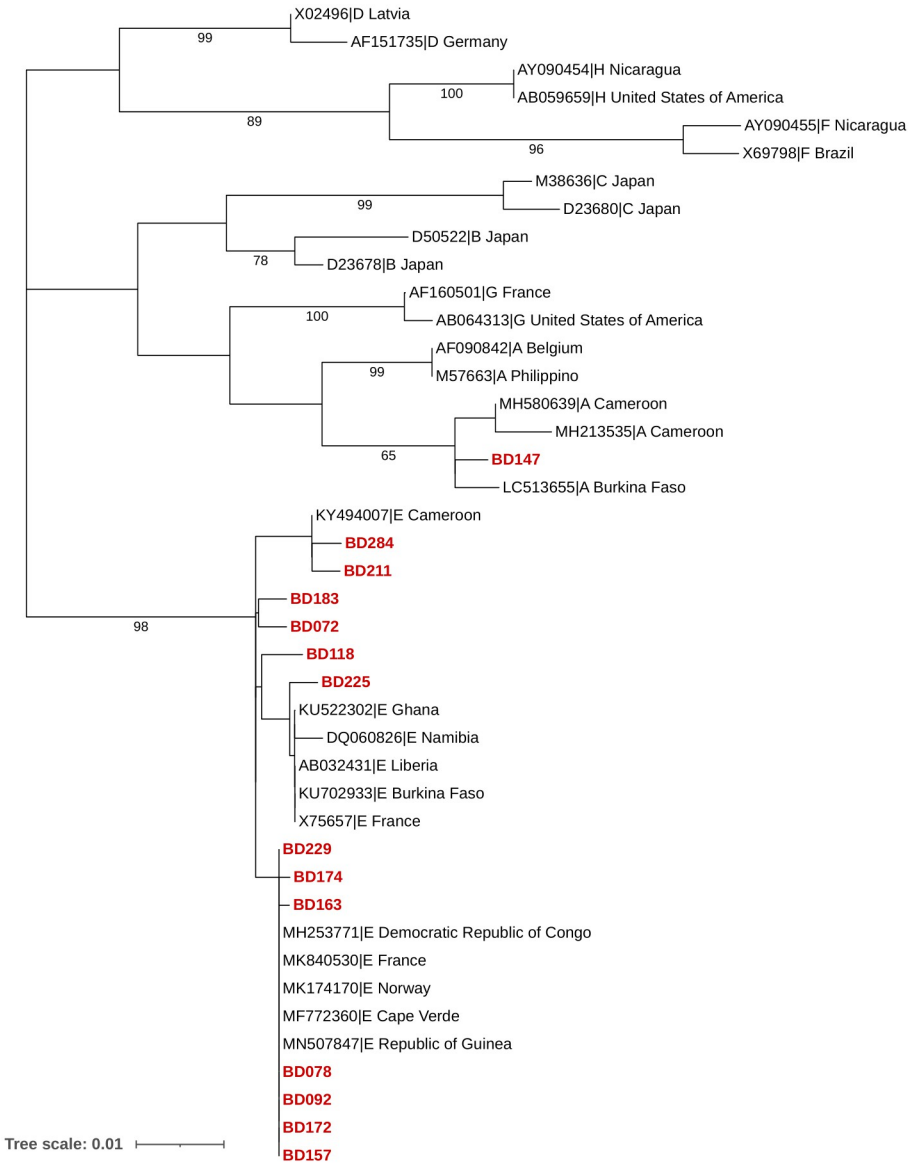

Fig 2. Reconstructed phylogenetic tree of the *S/P* gene of the HBV genome. The Maximum Likelihood method was employed for the reconstruction of the phylogenetic tree, and the evolutionary distances were computed using the Kimura 2-parameter plus Gamma Distribution (K2+G) model with 28 representative sequences (16 specific references for HBV genotypes A-H, and 12 sequences obtained through NCBI BLAST search; https://blast.ncbi.nlm.nih.gov/Blast.cgi). The OBI sequences from blood donors in this study clustered in the HBV genotypes A and E. The numbers at the nodes indicate bootstrapping values as a percentage of 1000 replicates. Only bootstrap values > 60 are shown. The OBI sequences are highlighted in red. BD—Blood donor.

https://doi.org/10.1371/journal.pone.0312126.g002

load (2593 IU/mL), exhibited mutation *sA184V* consistent with findings in 70% of all samples tested.

Discussion

Despite the availability of vaccines, occult hepatitis B infection (OBI) remains a significant challenge to HBV elimination. In this study, we found a 4.5% incidence of OBI in the population of Buea, Southwest region of Cameroon. This raises a significant concern, particularly for

Table 2. Mutations observed in the hepatitis B virus (HBV) (*S*) gene and in the reverse transcriptase (RT) domain of the polymerase (*P*) gene.

HBV genome	Amino acid substitution	HBV Genotypes
'S' gene that encodes the Hepatitis B surface antigen (HBsAg)	*sK122R*	*A*
	sA194V	
	sT131N	*E*
	sS143L	
	sK160R	
	sA184V	
	sY206C	
'P' gene that encodes the viral DNA polymerase, essential for the replication of the viral genome.	*rtW153R*	*A*
	rtI163V	
	rtI122V	*E*
	rtN139K	
	rtF151L	

https://doi.org/10.1371/journal.pone.0312126.t002

immunocompromised recipients, such as those undergoing hemodialysis, living with HIV, receiving immunosuppressive therapies, or suffering from liver disease. In these patients, there is a heightened risk for reactivation of HBV replication which could lead to acute hepatitis B infection [5].

Furthermore, there was a high seroprevalence of anti-HBc, observed at 58%. The OBI carriage rate reported in this study is notably higher compared to the prevalence of 1.1% [8] and 1.6% [9] previously reported in the Centre Region of Cameroon, where anti-HBc seroprevalence among donors was 49%. A higher OBI prevalence (17%) has been reported in neighboring Nigeria [12]. The risk of HBV transmission from OBI is high in low- and middle-income countries due to the lack of anti-HBc testing and NAT. In contrast, developed countries use NAT for blood screening, significantly reducing the residual risk of HBV transmission [7]. The prevalence of OBI among blood donors typically reflects the endemicity of HBV, ranging from 0.06% in low-endemicity countries to 0.98% in high-endemicity countries [18]. Additionally, 64% of the OBI samples in this study were seronegative (negative for any HBV antibodies). Seronegative individuals can account for up to 20% of all OBI cases [5], suggesting that selective testing only seropositive donors for HBV DNA poses a risk for HBV transmission.

Variations in OBI prevalence may arise from socioeconomic factors, regional differences, and the criteria used for detecting OBI among seropositive and seronegative donors. All identified OBI carriers in this study were males, reflecting a common gender imbalance in donor populations in developing countries, where males typically outnumber females. This disparity complicates the assessment of OBI prevalence in relation to gender [19]. Notably, 97% of our study cohort was not vaccinated against HBV, including the 4.5% of individuals with OBI. This contrasts with the high vaccination coverage rate of 99% in the infant population [20] in Cameroon and highlights the lower vaccination coverage in the general adult population. Previous studies have shown that genotypes A and E co-circulate in Cameroon, with reported mixed infections and recombination between genotypes A and E. In our study, the majority of OBI sequences (93%) were identified as genotype *E*, which is commonly found in Cameroon. Only one sequence belonged to genotype *A*. Genotype *E* predominates across Western Africa, from Mauritania to Namibia [8]. Conversely, HBV genotype *A* has been notably prevalent in specific Cameroonian populations, including Pygmies, Bantus, and HIV-infected individuals [21, 22].

The mechanisms underlying OBI are complex and include variations in the HBV genome that can result in OBI. For instance, the "α" determinant region (aa 124–147) within the major hydrophilic region (MHR, aa 99–169) of the S protein is a mutational hotspot. Mutations here affect the expression, antigenicity, and immunogenicity of HBsAg, leading to detection failures by commercial assays [23]. Notably, the amino acid residues at positions 122 and 160 of the *S* gene (arginine, R or lysine, K) determine the serotypes *d/y* and *w/r*, respectively [24]. Specifically, R at position 122 defines subtype *y*, while K defines subtype *d* [24]. Substitutions at these positions have been linked to immune escape of HBsAg [25]. In this study, the *sK122R* mutation, which indicates a serotype change from *d* to *y*, was observed in OBI genotype *A*. This mutation has been significantly associated with OBI and is known to contribute to reduced HBsAg secretion, as shown in *in vitro* studies [10, 26]. Additionally, we observed the *sK160R* mutation (change from subtype *w* to *r*) in OBI individuals with genotype *E*, which has been demonstrated to reduce extracellular HBsAg expression in OBI individuals [27].

The *sT131N* mutation in the "α" determinant region was observed in 15% of OBI individuals with genotype *E*. This mutation has also been found in OBI blood donors with genotype *C* in a Chinese population [28] and is associated with low HBsAg concentrations (<100 IU/mL) [29] and reduced HBsAg antigenicity, contributing to immune escape [30]. The *sS143L* mutation found in genotype *E* in this study is a typical HBsAg escape mutant and has been detected more frequently in Italian OBI blood donors of genotype *D* [31]. Moreover, the *sA184V* amino acid substitution (70% in genotype *E*) observed in this study was associated with impaired HBsAg detection in genotype *E* HBV/HIV-infected Nigerian population [32]. Also, *sY206C* observed in 62% of OBI sequences is located in the C-terminus of HBsAg known to be involved in virion and/or HBsAg secretion [30]. In a previous study, this variant was significantly associated with low HBsAg and serum HBV-DNA levels [33].

OBI sequences revealed several documented RT mutations. Among these, *rtN139K* (17%, genotype *E*) is common in treatment-naïve patients in Asia [34] and significantly associated with progression to HCC [35]. The primers targeted the overlapping S/P region of the HBV genome, spanning nucleotides 455 to 786, which includes the Reverse Transcriptase (RT) domain of the Polymerase gene—a region associated with resistance to nucleoside/nucleotide analogues (NAs). Upon analysis using geno2pheno and the HBV database, no well-characterized drug-resistance mutations were detected. However, some of the mutations we identified, such as rtW153R and rtI163V, have been associated with resistance to Adefovir and Lamivudine in previous studies investigating RT mutations in both treated and untreated individuals [36, 37]. The *rtI122V* mutation (8%) detected in our study was also reported in the Indonesian population as a putative drug resistance mutant [38]. While amino acid substitutions within the RT domain may affect the efficiency of viral replication and HBsAg secretion, their direct association with OBI remains unclear.

In one sample with a high viral load (>2000 IU/mL), one mutation (*sA184V*) was observed which also occurred in nine other OBI sequences. Despite the high viremia, qualitative ELISA HBsAg assay used in the blood bank, which has a detection limit of 0.5 ng/mL, failed to detect it, suggesting a potential laboratory misdiagnosis. This study has several limitations. It is cross-sectional, and all blood donors were male, indicating a gender bias in the study cohort. Additionally, individuals with clinically confirmed HBV infection from this population were not included, preventing comparative analysis.

Conclusion

Our study highlights that relying solely on routine HBsAg testing is inadequate to prevent HBV transmission through transfusions in endemic regions. We recommend supplementing

HBsAg testing with nucleic acid amplification testing (NAT) and/or anti-HBc testing as surrogates for detecting OBI.

Supporting information

S1 Table. Primers and probes used for HBV DNA detection.
(PDF)

S1 Fig. HBV surface protein (S) alignment. Surface protein alignment of OBI-positive sequences with reference sequences for genotypes *A* and *E* (reference genomes: M57663|A, AF090842|A, X75657|E, and AB032431|E written in red). The alignment shows amino acids 100 to 210 of the S protein. A dot represents homology in amino acids as observed in the reference sequence; only those with amino acid substitutions are illustrated. Bold squares indicate amino acid substitution for genotype *A* and dash lines show amino acid substitutions for genotype *E*.
(PDF)

S2 Fig. Reverse transcriptase (RT) alignment of the polymerase (*P*) gene. The alignment of the RT region of the *P* gene of OBI-positive sequences with reference sequences for genotypes *A* and *E* (reference genomes: M57663|A, AF090842|A, X75657|E, and AB032431|E, written in red). The alignment shows amino acids 110 to 220 of the RT domain. A dot represents homology in amino acids as observed in the reference sequence; only those with amino acid substitutions are illustrated. Bold squares indicate amino acid substitution for genotype *A* and dash lines show amino acid substitutions for genotype *E*.
(PDF)

S1 File. Information sheet, consent form, and questionnaire.
(PDF)

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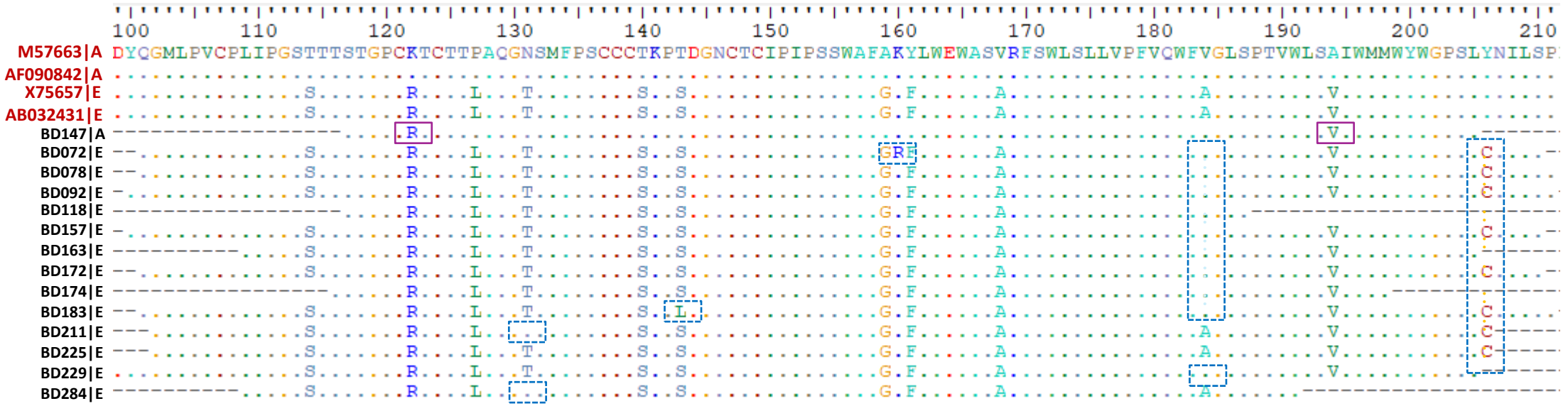
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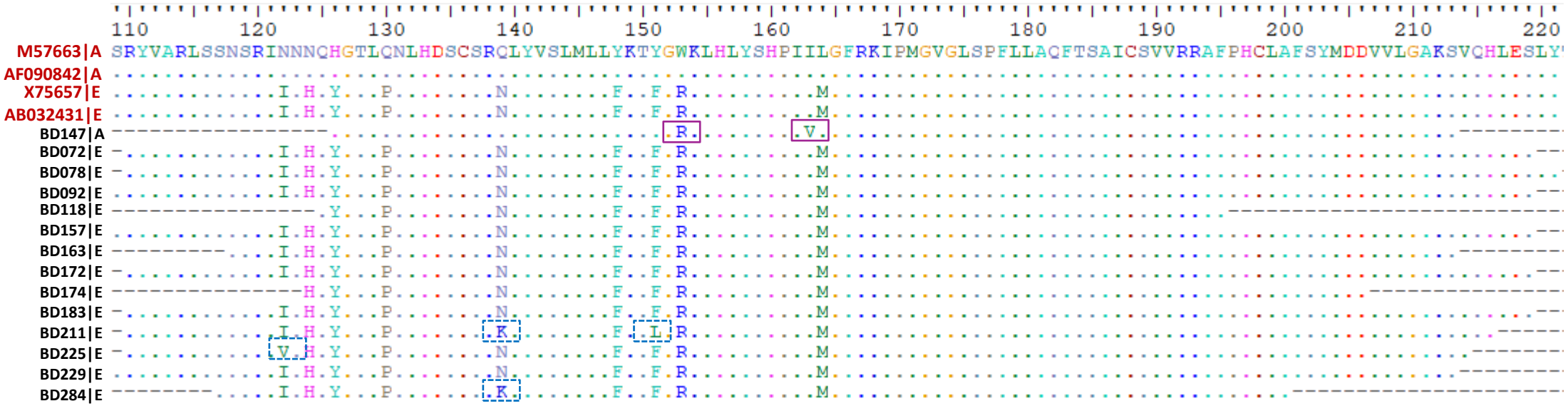
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AF090842|A
X75657|E
AB032431|E
BD147|A
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BD092|E
BD118|E
BD157|E
BD163|E
BD172|E
BD174|E
BD183|E
BD211|E
BD225|E
BD229|E
BD284|E

Qualitative nested PCR	
HBV-022	5’-TGCTGCTATGCCTCATCTTC-3’
HBV-065	5’-CACAGATAACAAAAAATTGG-3’
HBV-066	5’-CAAAGACAAAAGAAAATTGG-3’
HBV-024	5’-CAAGGTATGTTGCCCGTTTGTCCT-3’
HBV-041	5’-GGACTCAMGATGYTGCACAG-3’
HBV-064	5’-GGACTCACGATGCTGTACAG-3’
Quantitative qPCR	
HBV-61	5’-GGACCCCTGCTCGTGTTACA
HBV-62	5’-GAGAGAAGTCCACCACGAGTCTAGA
HBV-TM-5	FAM 5'-TGTTGACAARAATCCTCACAATACCRCAGA-3' DabCyl

Chapter 2

Burden and genotypic characteristics of HBV and OBI in high-risk populations: HIV patients and women in the third trimester of pregnancy

Publication No.2

High Burden of Hepatitis B Virus and Occult Infection Among HIV-Positive Adults and Pregnant Women in Southwest Cameroon

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Article

High Burden of Hepatitis B Virus and Occult Infection Among HIV-Positive Adults and Pregnant Women in Southwest Cameroon

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Abstract

Chronic hepatitis B virus (HBV) and Occult HBV infection (OBI) remain a health burden in sub-Saharan Africa. This study investigated HBV prevalence, circulating genotypes, and associated risk factors with HBV exposure among HIV-positive adults on antiretroviral therapy and pregnant women in southwestern Cameroon. A total of 233 HIV patients and 190 third-trimester pregnant women were screened for HBV DNA, viral load, serological markers (HBsAg, anti-HBc, and anti-HBs), and HBV genotypes were determined by partial sequencing of the S gene. HBV DNA was detected in 10% of HIV-positive patients and 4% of pregnant women, with an overall prevalence of 7%. OBI accounted for 9% and 3%, respectively. Anti-HBc seroprevalence was high (75% in HIV, 46% in pregnant women), while self-reported vaccination coverage was low (1% and 11%). Genotypes A, B, D, and E were identified, with genotype B reported for the first time in Cameroon. Immune escape mutations and the adefovir resistance mutation rtA181V were detected. Self-reported alcohol use was associated with HBV exposure in HIV patients (aOR = 2.08; $p = 0.028$) and inversely associated with tertiary education in pregnant women (aOR = 0.18; $p = 0.038$). This study highlights a significant burden of HBV and OBI among vulnerable populations in Cameroon.

Keywords: chronic hepatitis B; occult hepatitis; pregnant women; HIV; Cameroon

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1. Introduction

Chronic hepatitis B virus (HBV) infection is a global health concern, disproportionately affecting individuals living in sub-Saharan Africa and the Western Pacific regions [1]. Of the estimated 254 million people living with chronic HBV worldwide, over 64 million reside in Africa, including approximately 3.6 million children under five years of age [2]. Between 2019 and 2022, global HBV-related deaths rose from 820,000 to 1.1 million, primarily due to cirrhosis and hepatocellular carcinoma (HCC) [1]. Despite the availability of an effective HBV vaccine for over four decades, mother-to-child transmission continues to sustain HBV endemicity in sub-Saharan Africa [3]. Nearly one-quarter of HBV-infected individuals

in Africa are under 20 years of age [2]. Without timely intervention, 80–90% of neonates infected perinatally and 30–50% of children infected between one and five years of age progress to chronic infection compared to 5–10% in those infected as adults [4]. In 2022, only 5% of individuals with chronic HBV in Africa were diagnosed, and a mere 2% were receiving treatment [1]. Among pregnant women, treatment coverage was below 1%, while timely birth-dose vaccine coverage stood at only 14% [2].

The human immunodeficiency virus (HIV) and HBV co-infection presents an additional challenge, with sub-Saharan Africa accounting for approximately 1.9 million of the 2.7 million people co-infected [5]. The overlap in transmission routes and limited screening strategies contributes to the persistence of co-infection. Moreover, HIV infection accelerates HBV-related liver disease progression, increasing the risk of fibrosis and HCC, even among those on antiretroviral therapy (ART) [6]. Cameroon has a national HBV seroprevalence of approximately 11% for hepatitis B surface antigen (HBsAg) [7], with high rates among HIV-positive individuals (12%) and pregnant women (≥6%) [8,9]. Although infant HBV vaccination has been part of the national immunization schedule since 2005, the country lacks a coordinated strategy for adult HBV screening, treatment, and long-term care [10]. The World Health Organization (WHO) guidelines emphasize the importance of targeted screening in high-risk populations such as people living with HIV and pregnant women, who are at greater risk of end-stage liver disease when co-infected.

While HBsAg testing is commonly used for HBV screening, it may fail to detect cases of occult hepatitis B infection (OBI), a condition in which HBV DNA is present at very low levels (<200 IU/mL) in blood despite a negative HBsAg result [11]. In OBI, the episomal covalently closed circular DNA (cccDNA) within infected hepatocytes remains transcriptionally active at a low rate, resulting in reduced HBsAg expression and secretion, often falling below the detection limit of most commercial HBsAg assays with low analytical sensitivity (approximately 0.05 IU/mL). In addition, mutations within the surface gene (S gene) can alter antigenic sites, leading to diagnostic escape [11]. In our previous study among Cameroonian blood donors, we reported an OBI prevalence of 5%, underscoring the magnitude of undetected HBV transmission [12]. This study aimed to assess the prevalence of HBV infection, OBI, and distribution of genotypes, and associated risk factors among two vulnerable populations, including people living with HIV receiving ART and third-trimester pregnant women, in the Southwest region of Cameroon. Our findings aim to inform national policy on HBV control and advocate for expanded diagnostics and vaccination strategies in high-risk groups.

2. Materials and Methods

2.1. Study Design and Ethical Approval

A cross-sectional study was conducted from 21 March to 30 June 2023, in the Southwest region of Cameroon. The study was approved by the Institutional Review Board of the University of Buea, Cameroon (Reference: 2022/1849-10/UB/SG/IRB/FHS) and by the Ethics Committee of the University of Tübingen, Germany (Reference: 379/2023B02). Administrative clearance was obtained from the Southwest Regional Delegation of Public Health. Written informed consent was obtained from all participants before enrolment.

2.2. Study Population and Sampling

In this study, 233 HIV-positive adults undergoing ART were recruited consecutively after obtaining their consent during their routine follow-up appointments at the HIV treatment departments of the Regional Hospitals in Buea and Limbe. No records of liver disease were available for these patients. Also, 190 pregnant women in their third trimester who were receiving antenatal care at integrated health centers in Buea (PMI) and Mutengene

(CMA) were consecutively enrolled in the study after providing written consent. None of the HIV patients were pregnant, and none of the pregnant women had a record of HIV infection. For each participant, 3 mL of venous blood was collected, and serum was separated, preserved in DNA/RNA Shield (Zymo Research, Irvine, CA, USA), and stored at −20 °C for subsequent molecular and serological analyses. Socio-demographic and clinical information were obtained using structured questionnaires.

2.3. HBV DNA Detection and Quantification

Viral DNA was extracted from 200 µL of serum using the QIAamp Viral DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Nested polymerase chain reaction (PCR) targeting the conserved overlapping S/P region of the HBV genome was used to qualitatively detect HBV DNA, as previously described [12] (Supplementary Table S1). PCR-positive samples were subsequently analyzed by real-time PCR using a TaqMan probe-based assay targeting a conserved 90 bp segment within the S gene (nucleotide positions 182–271, GenBank accession: X75657), as previously described [12] (Supplementary Table S1). Quantification was performed using the QuantiTect Probe PCR Master Mix (Qiagen) on a LightCycler 480 II (Roche, Mannheim, Germany). The assay detection limit was 12 IU/mL, established using tenfold serial dilutions of a control plasmid (10^6 copies/µL). HBV viral load was calculated from cycle threshold (Ct) values based on a standard curve.

2.4. Serological Testing

All participants were screened for antibodies against the HBV core antigen (total anti-HBc) using the qualitative Monolisa™ anti-HBc PLUS assay (Bio-Rad, Hercules, CA, USA). HBV DNA-positive individuals were additionally tested for HBsAg using the Determine™ HBsAg 2 rapid test (Abbott Laboratories, Abbott Park, IL, USA), and for antibodies against the surface antigen (anti-HBs) using the Monolisa™ anti-HBs PLUS assay (Bio-Rad). Anti-HBs titers > 10 mIU/mL were considered positive. Absorbance readings were taken using a CLARIOstar microplate reader (BMG Labtech, Ortenberg, Germany). All assays were performed following the manufacturer's protocols.

2.5. HBV Genotyping and Phylogenetic Analysis

Amplicons from HBV DNA-positive samples were purified using the Exo-SAP-IT kit (USB, Affymetrix, Santa Clara, CA, USA) and sequenced using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Consensus sequences were assembled using SeqMan (DNASTAR Lasergene). A reference set of 47 HBV sequences representing genotypes A–I was included. Multiple sequence alignment was performed using MAFFT v7 (G-INS-i algorithm) [13], and the phylogenetic tree was reconstructed using MEGA v12 [14] with the Neighbor-joining method and the Kimura 2-parameter model with gamma distribution (K2+G). Bootstrapping (1000 replicates) assessed the robustness of phylogenetic assembly. The phylogenetic tree was annotated and visualized using the Interactive Tree of Life (iTOL) version 6 [15].

Mutation analysis of the surface (S) gene and polymerase (P) gene was performed using BioEdit v7 and the Geno2pheno [hbv] online tool (https://hbv.geno2pheno.org/) (accessed on 1 May 2025). Sequences were compared to genotype-specific references from GenBank (Genotype A: M57663, AF090842; Genotype B: AB642091; Genotype D: M32138; Genotype E: X75657, AB032431). Nonsynonymous mutations were annotated, with a focus on immune escape variants and drug resistance markers. Additionally, the mutations observed were categorized according to whether they occurred in HBV or OBI cases.

Sequences generated in this study were submitted to GenBank under accession numbers PV604144–PV604172.

2.6. Statistical Analysis

Statistical analyses were performed using R version 4.0. Continuous variables were summarized using medians and interquartile ranges (IQR), while categorical variables were expressed as frequencies and percentages. The Pearson chi-square test and Fisher's exact test were initially used to assess associations between categorical variables, with the latter applied when expected cell counts were $\leq$5. Logistic regression models were then applied to preselected variables with a *p*-value threshold of $\leq$0.2 to evaluate factors associated with HBV exposure (anti-HBc positivity). Adjusted odds ratios (aOR) with 95% confidence intervals (CI) were calculated. A p-value < 0.05 was considered statistically significant

3. Results

3.1. Participant Demographics

A total of 423 individuals were enrolled, comprising 233 HIV-positive adults and 190 third-trimester pregnant women. Among the HIV cohort, 83% were female, with a median age of 46 years (interquartile range [IQR]: 39–55). In the pregnant women cohort, the median age was 27 years (IQR: 24–31). HBV vaccination coverage was low: 1% in HIV patients and 11% in pregnant women (Table 1).

Table 1. Overall prevalence of anti-HBs and anti-HBc markers and combination.

Characteristics	Persons with HIV (n = 233) (%)	Pregnant Women (n = 190) (%)
Gender (Female)	194 (83)	190 (100)
Median Age (IQR)	46 (39–55)	27 (24–31)
Age $\leq$ 20	1 (1)	17 (9)
Age 21–40	67 (29)	169 (89)
Age 41–60	134 (58)	4 (2)
Age > 60	31 (13)	0
anti-HBc-positive	174 (75)	87 (46)
HBV DNA Positive	24 (10)	7 (4)
† HBV viral load (IU/mL) median [Range]	12.4 (12.4–76,700)	26,523 (246–52,800)
OBI Positive (HBV DNA+ve, HBsAg-ve)	21 (9)	5 (3)
HBsAg Positive	3 (1)	2 (1)
‡ anti-HBs-positive mIU/mL [Range]	6 (25) (20–384)	0 (0)
HIV viral load range (copies/mL)	Undetectable to 401,538	Not available
HBV genotypes	A n = 14 (58) B n = 7 (29) E n = 1 (4)	A n = 1 (14) D n = 1 (14) E n = 5 (71)

† detectable viral load (HIV: n = 8/24; Pregnant women: n = 2/7), ‡ anti-HBs-positive measured only in HBV DNA positives (HIV: n = 6/24; Pregnant women: n = 0/7); IQR: Inter Quartile Range, HBV: Hepatitis B virus; OBI: Occult hepatitis B infection; HBsAg: Hepatitis B Surface Antigen; anti-HBc: hepatitis B core antibody.

3.2. Prevalence of HBV Infection

HBV DNA was detected in 10% (24/233) of HIV patients and 4% (7/190) of pregnant women, corresponding to an overall prevalence of 7.3% (31/423) (Table 1). Occult hepatitis B infection, defined as HBV DNA positivity with negative HBsAg, was identified in 9% (21/233) of HIV patients and 3% (5/190) of pregnant women (Table 1). HBsAg positivity was 1% (HIV: 3/233; pregnant women 2/190) in both cohorts (Table 1).

3.3. HBV Serological Markers and Viraemia

The seroprevalence of anti-HBc, a marker of past or ongoing HBV exposure, was 75% (174/233) in HIV patients and 46% (87/190) in pregnant women, yielding an overall exposure rate of 62% (261/423) (Table 1). In OBI-positive HIV patients, 86% (18/21) were anti-HBc-positive, of whom 29% (6/21) were also anti-HBs-positive. Among OBI-positive pregnant women, 20% (1/5) were anti-HBc-positive, and none of them had detectable anti-HBs antibodies. Isolated anti-HBc seropositivity (anti-HBc$^+$/anti-HBs$^-$) was found in 57% of OBI-positive HIV patients and in 20% of OBI-positive pregnant women. In individuals with detectable anti-HBs, the mean anti-HBs titer was 167.7 mIU/mL (standard deviation: 133.9) and ranged from 20 to 384 mIU/mL (Table 1). All five HBsAg-positive individuals (three HIV patients and two pregnant women) had detectable HBV DNA levels ranging from 2.46×10^2 to 7.67×10^4 IU/mL, whereas all OBI cases (n = 26) in both cohorts had HBV DNA levels that were either undetectable or at the assay's lower limit of detection (Table 1). In the study population, 1% of HIV patients and 11% of pregnant women reported having received HBV vaccination.

3.4. HBV Genotypes and Mutations

Phylogenetic analysis of the sequenced S gene fragments identified HBV genotypes A, B, D, and E. Among HIV patients, genotype A was predominant (14/24), followed by genotype B (7/24), with one genotype E infection (Figure 1). Genotype B was detected exclusively in OBI-positive cases and represents the first report of this genotype in Cameroon. Two HIV samples could not be genotyped due to sequencing failure. In pregnant women, genotype E was most common (5/7), followed by one case each of genotypes A and D. Of the seven genotyped samples, four genotype E infections were OBI-positive (Figure 1).

Mutation analysis revealed several nonsynonymous substitutions in the major hydrophilic region (MHR) of the S gene, including sP120T and sT126A, both associated with HBsAg immune escape and OBI. The sF200Y mutation was observed in all genotype B OBI cases. In the reverse transcriptase (RT) region, the rtA181V mutation associated with adefovir resistance was found in one HIV patient. Additional non-synonymous substitutions (sY200F, rtW153R) were identified in both HBsAg-positive and negative genotype A sequences (Table 2).

Table 2. Mutations in HBV Surface (S) Gene and Polymerase (P) RT Domain.

Study Cohort	HBV Genome	Amino Acid Substitution	HBV Genotypes
HIV patients (n = 233)	'S' gene that encodes the Hepatitis B surface antigen (HBsAg)	sY200F sS207T *	A
		sF200Y * sT126A * sP120T * sK122R *	B
	'P' gene that encodes the viral DNA polymerase, essential for the replication of the viral genome.	rtW153R rtQ215H	A
		rtD131N * rtN134S * rtN134D * rtA181V * rtI187V *	B
Pregnant Women (n = 190)	'S' gene that encodes the Hepatitis B surface antigen (HBsAg)	sK122R sP135A	A
		sG130S * sL193S *	D

* Mutations found in OBI cases. No mutations were observed in genotype E in both study cohorts.

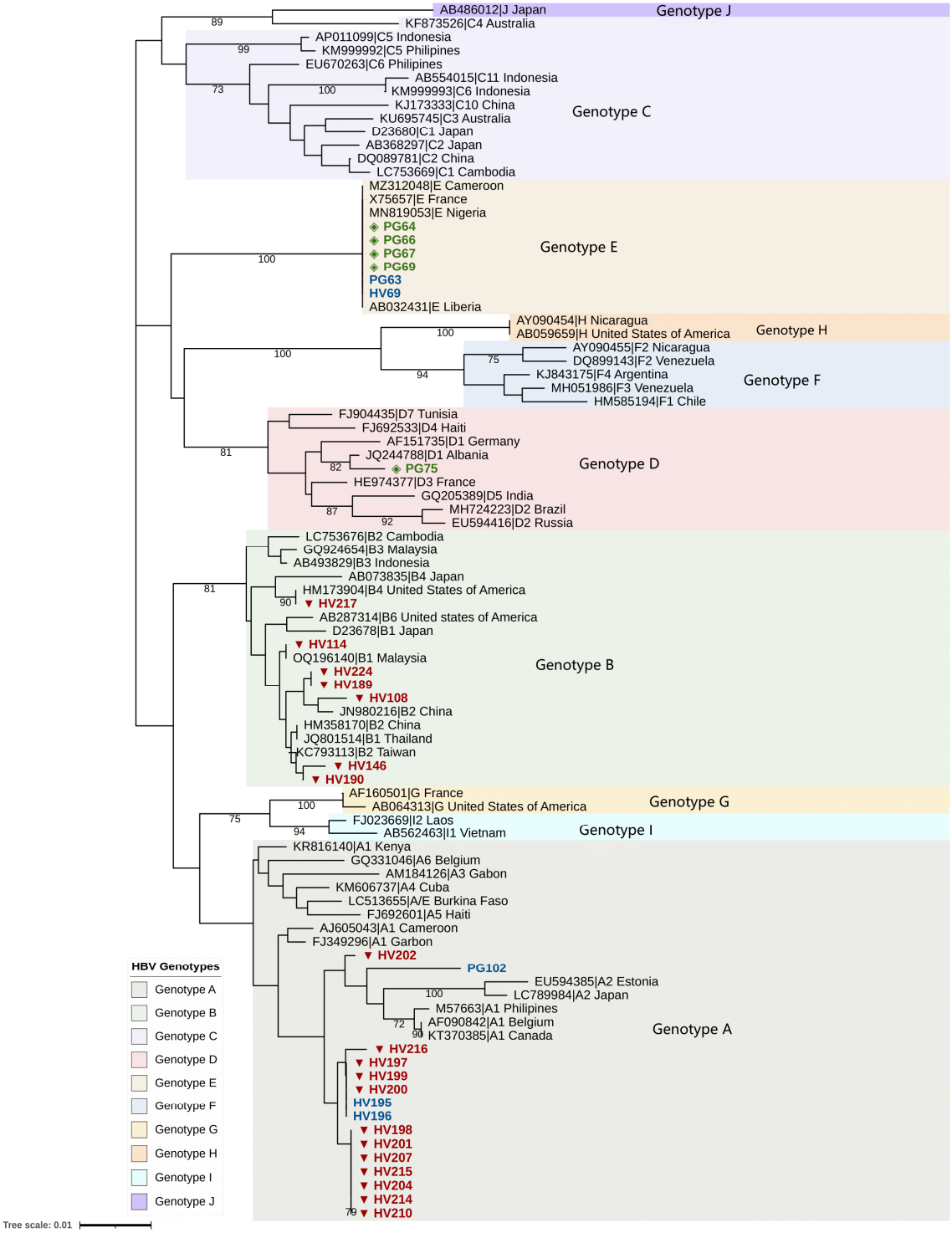

Figure 1. Phylogenetic tree of the S/P region of HBV sequences from study participants. Sample identifiers are labeled as HV (person with HIV) and PG (pregnant woman). Occult HBV infection sequences are indicated by red triangles (persons with HIV, n = 19) and green diamonds (pregnant women, n = 5). HBsAg-positive sequences are indicated in blue (HIV; n = 3; Pregnant women; n = 2). Sequences from individuals living with HIV clustered with HBV genotypes A and B, while sequences from pregnant women clustered with genotypes A, D, and E. A total of 47 reference genotypes A–I were included in the analysis. Bootstrap values are shown at the nodes and represent percentages from 1000 replicates; only values greater than 70% are displayed. Of the 332 bp fragment of the S/P region amplified, a sequencing coverage of 328 bp was achieved for the successfully sequenced samples. Two of the 24 samples from HIV-positive participants could not be genotyped due to sequencing failure, whereas all samples from pregnant women (n = 7) were successfully genotyped.

3.5. Factors Associated with HBV Exposure

In HIV patients, self-reported alcohol consumption and HIV viral load were significantly associated with anti-HBc positivity (Table 3). Alcohol consumers had a higher prevalence of HBV exposure (80%) compared to non-consumers (64%) ($p = 0.0115$). Additionally, anti-HBc positivity was more frequent in participants with undetectable HIV viral loads (79%) than those with low (53%) or high (75%) viral loads ($p = 0.0008$) (Table 3). Also, there was no association between OBI status and HIV viral load. Among pregnant women, marital status, education level, and parity were significantly associated with HBV exposure (Table 3). Married women had a higher anti-HBc positivity rate (56%) than single women (33%) ($p = 0.0013$) (Table 3). Women with only primary education showed higher exposure (82%) than those with tertiary education (38%) ($p = 0.0315$) (Table 3). Multigravida women had higher exposure (57%) compared to primigravida (38%) ($p = 0.0139$) (Table 3). Multivariate logistic regression confirmed that alcohol use was independently associated with HBV exposure in HIV patients (adjusted odds ratio [aOR] = 2.08; 95% CI: 1.08–4.00; $p = 0.028$) (Table 4). In pregnant women, tertiary education was protective (aOR = 0.18; 95% CI: 0.03–0.78; $p = 0.038$), and marital status remained borderline significant (aOR = 2.00; 95% CI: 1.01–4.00; $p = 0.048$) (Table 4).

Table 3. Association between demographic/clinical factors and anti-HBc positivity (HIV and pregnant women cohorts).

Study Cohort	Demographic and Clinical Factors *	Anti-HBc Positive (%)	*p*-Value
HIV cohort (n = 233)	Alcohol consumption	80%	0.0115
	HIV viral load (undetectable)	79%	0.0008
Pregnant women (n = 190)	Marital status	56% (married)	0.0013
	Education level	82% (primary)	0.0315
	Parity (≥2 births)	57%	0.0139

* Only variables with significant associations are shown.

Table 4. Multivariate logistic regression for factors associated with HBV exposure.

Variables *	Adjusted Odds Ratio (aOR) Confidence Interval (95% CI)	*p*-Value
Alcohol use (among persons with HIV)	2.08 (1.08–4.00)	0.028
Married (pregnant women)	2.00 (1.01–4.00)	0.048
Tertiary education (pregnant women)	0.18 (0.03–0.78)	0.038

* Only variables independently associated with HBV exposure are shown.

4. Discussion

This study provides updated insights into the prevalence, genotype distribution, and risk factors associated with HBV infection among two high-risk populations: HIV-positive adults and pregnant women in Southwestern Cameroon. Despite national vaccination efforts, the findings reveal persistent gaps in HBV prevention and underscore the importance of enhanced screening and targeted public health interventions.

We report an overall HBV DNA prevalence of 7%, with a notably higher rate among HIV patients (10%) compared to pregnant women (4%). These figures are consistent

with regional and global estimates for HIV/HBV co-infection and pregnant populations, particularly in sub-Saharan Africa [16]. Notably, the prevalence of hepatitis B surface antigen (HBsAg) was only 1% in both cohorts, emphasizing the limitations of HBsAg-based screening alone. A significant proportion of HBV infections identified in this study were occult hepatitis B infections defined as HBV DNA positivity despite negative HBsAg. OBI was observed in 9% of HIV patients and 3% of pregnant women. These rates are higher than our previously reported 5% OBI prevalence in blood donors from Cameroon [12] and are within the expected range for people living with HIV in Africa (8–26%) [17]. The high OBI burden in HIV patients may be influenced by immunosuppression and antiretroviral therapy, which can suppress HBsAg expression without clearing HBV DNA.

The seroprevalence of anti-HBc, a marker of past or current HBV exposure, was strikingly high: 75% among HIV patients and 46% in pregnant women in this study, suggesting substantial transmission within the population. While anti-HBs levels were not measured for the entire study population, the high anti-HBc prevalence may suggest low vaccine-derived immunity among adults. Indeed, from self-reported vaccination history, only about 1% of HIV patients and 11% of pregnant women reported having received HBV vaccination despite their high-risk status. In Cameroon, vaccination coverage for at least one dose reaches 99% among individuals born after the vaccine's inclusion in the national infant immunization program in 2005 [18]. However, coverage among the general adult population remains around 5% [19]. These findings highlight the lack of comprehensive vaccination strategies for adults in Cameroon and the on-going need to strengthen implementation of the WHO-recommended birth dose to prevent early-life infection and subsequent transmission.

HBV genotypes A, B, D, and E were detected in the study population. Genotype A predominated among HIV patients, whereas genotype E was more common among pregnant women, with both representing the dominant genotypes in the region. Notably, genotype B was identified exclusively in HIV patients, and all were OBI-positive, constituting the first documented detection of this genotype in Cameroon. The emergence of genotype B may be related to cross-border migration, travel, or demographic shifts. Mutations in the S gene, including sP120T and sT126A, were identified in multiple OBI cases. These mutations, located in the major hydrophilic region (MHR) of HBsAg, are associated with immune escape and diagnostic failure [20,21]. The sF200Y mutation was observed in all genotype B sequences. In the polymerase gene, we detected rtA181V, a known drug resistance mutation associated with reduced susceptibility to adefovir [22] and potential cross-resistance to tenofovir [23]. Its presence in an HIV-positive patient receiving Tenofovir Disoproxil Fumarate/Lamivudine/Dolutegravir (TDF/3TC/DTG) raises concern for antiviral resistance and calls for closer monitoring.

In the HIV cohort, self-reported alcohol use was significantly associated with HBV exposure (anti-HBc positivity), after adjusting for confounders. Alcohol use may reflect underlying behavioral risk factors, such as unprotected sex or injection drug use that expose individuals to HBV, rather than a direct virological effect [24]. However, the type, amount, or frequency of alcohol was not assessed, limiting the ability to examine a potential dose–response relationship. Similarly, in pregnant women, lower education, marital status, and multiparity were associated with higher HBV exposure. Tertiary education appeared protective, potentially due to greater awareness and health-seeking behavior.

Our findings underscore several urgent public health priorities for HBV control in Cameroon and similar settings. Routine screening must extend beyond HBsAg testing to include anti-HBc and nucleic acid testing, particularly among high-risk groups such as pregnant women and HIV patients, to ensure early detection and appropriate clinical management. Integrating nucleic acid testing into existing HIV programs would also sup-

port optimal ART regimen selection and help avoid drug combinations that are unsuitable for HBV co-infected individuals. Furthermore, it appears OBI may persist independently of HIV replication control, emphasizing the importance of continued HBV monitoring regardless of viral suppression status to prevent HBV reactivation. Expanding vaccination coverage is also critical, with emphasis on administering the birth dose promptly, implementing catch-up immunization for adolescents and adults, and prioritizing vaccination for healthcare workers, HIV-positive individuals, and expectant mothers. Continuous surveillance of circulating HBV genotypes and resistance-associated mutations is necessary to guide diagnostic strategies, assess vaccine effectiveness, and inform treatment decisions. Furthermore, health education initiatives that address underlying behavioral risk factors, including alcohol consumption and limited awareness, can significantly reduce transmission and enhance the effectiveness of prevention programs.

Several limitations must be considered. First, the cross-sectional study design limits the causal interpretation of the observed associations. Therefore, the relationships between HBV markers and factors such as alcohol use or educational level should be considered correlational rather than causal. Second, anti-HBs were only measured in HBV DNA-positive individuals, limiting assessment of population-level immunity. As such, the self-reported vaccination rates may not reflect actual protective immunity. Third, clinical parameters such as liver function tests or fibrosis scores were not included, restricting the interpretation of the clinical significance of HBV infections. Finally, while phylogenetic analysis identified genotypes and mutations, whole-genome sequencing could provide a more comprehensive picture of viral diversity.

5. Conclusions

This study highlights a considerable burden of HBV, including a high rate of occult infection and the first documentation of genotype B in Cameroon. Strengthened policies on HBV screening, adult vaccination, and education, particularly in high-risk groups, are urgently needed to meet global hepatitis elimination goals.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pathogens14111128/s1, Table S1: Primers and PCR conditions for qualitative and quantitative PCR.

Author Contributions: M.N.M.: Data Curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing—Original Draft, Writing—Review and Editing. L.C.C.: Data Curation, Formal analysis, Investigation. E.A.A.: Project administration, Supervision. S.M.G.: Supervision, Data Curation, Methodology, Project administration. T.P.V.: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing—Review and Editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the Institutional Review Board of the University of Buea, Cameroon (Reference: 2022/1849-10/UB/SG/IRB/FHS, date: 7 November 2022) and by the Ethics Committee of the University of Tübingen, Germany (Reference: 379/2023B02 date: 25 July 2023). Administrative clearance was obtained from the Southwest Regional Delegation of Public Health. Written informed consent was obtained from all participants before enrolment.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated in this study are included in this article. All obtained sequences were available in the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) (accessed on 5 May 2025) with the following accession numbers: PV604144 to PV604172.

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Table S1: Primers and PCR conditions for qualitative nested PCR

Primers and PCR conditions for qualitative nested PCR			
PCR rounds	Primer pairs	**Sequence (5′ – 3′)**	PCR conditions
Outer	HBV-022 (sense)	TGCTGCTATGCCTCATCTTC	94°C for 5 minutes; 94°C for 30 seconds; 55°C for 30 seconds; 72°C for 30 seconds; 72°C for 5 minutes. (35 cycles) Amplicon size: 408bp
	HBV-065 (antisense)	CACAGATAACAAAAAATTGG	
	HBV-066 (antisense)	CAAAGACAAAAGAAAATTGG	
Nested	HBV-024 (sense)	CAAGGTATGTTGCCCGTTTGTCCT	94°C for 5 minutes; 94°C for 30 seconds; 54°C for 30 seconds; 72°C for 30 seconds; 72°C for 5 minutes. (35 cycles) Amplicon size: 332bp
	HBV-041 (antisense)	GGACTCAMGATGYTGCACAG	
	HBV-064 (antisense)	GGACTCACGATGCTGTACAG	
Primers and PCR conditions for quantitative PCR (qPCR)			
qPCR	HBV-61	GGACCCCTGCTCGTGTTACA	95°C for 5 minutes; 95°C for 10 seconds; 60°C for 34 seconds; 40°C for 10 minutes. (45 cycles)
	HBV-62	GAGAGAAGTCCACCACGAGTCTAGA	
	HBV-TM-5	FAM5'-tgttgacaaRaatcctcacaataccRcaga-3' DabCyl	

Chapter 3

HEV epidemiology, including genotype distribution, and risk factors among blood donors and high-risk populations

Publication No.3

Prevalence, genotype distribution, and risk factors of Hepatitis E virus in blood donors, HIV patients, and pregnant women in Southwest Cameroon

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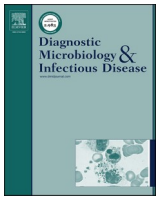

Original Article

Prevalence, genotype distribution, and risk factors of Hepatitis E virus in blood donors, HIV patients, and pregnant women in Southwest Cameroon

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ABSTRACT

Most HEV infections are self-limiting, but pregnant women in their third trimester and immunocompromised individuals, such as those with HIV, face risks, including fatal liver failure and chronic infection. This study investigates HEV prevalence and genotypes in healthy blood donors and high-risk groups, such as HIV patients and pregnant women, in Southwest Cameroon, where surveillance is limited. A cross-sectional study conducted between March and June 2023 recruited 712 participants: 289 blood donors, 233 HIV patients, and 190 pregnant women. Serum and stool samples were tested for anti-HEV IgG and IgM antibodies using ELISA, and HEV RNA was detected by nested PCR targeting the ORF1 and ORF2 regions. HEV RNA-positive samples were sequenced, and genotypes identified. Among the 712 participants, 7 % tested positive for anti-HEV IgG and 2 % for anti-HEV IgM. Blood donors had the highest anti-HEV IgG prevalence (9 %). No significant associations were found between HEV seropositivity and demographic or dietary risk factors. The overall HEV RNA positivity rate was 1 %, with the highest rates in blood donors (2 %) and pregnant women (1 %), while no cases were found in HIV patients. Phylogenetic analysis revealed that 75 % of HEV RNA-positive samples belonged to genotype 3a, and 25 % to genotype 3e. The nucleotide diversity between human and pig HEV genotype 3 suggests the involvement of environmental or other indirect transmission routes, rather than direct pig-to-human transmission. This study highlights HEV risk in Cameroon, especially among blood donors and pregnant women, underscoring the need for enhanced surveillance in HBV-endemic regions.

1. Introduction

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis, transmitted via the fecal-oral route, contaminated blood, and food-borne zoonoses [1]. It causes 20 million infections annually, with 3 million symptomatic cases, 70,000 deaths, and 3,000 stillbirths, mainly in Asia and Africa, accounting for 3.3 % of viral hepatitis-related deaths [2]. HEV is a single-stranded RNA virus of the *Paslahepevirus* genus, has a 7.2 kb genome with three primary ORFs and a fourth unique to genotype 1 [3]. It circulates in a quasi-enveloped form in blood and is excreted as infectious, non-enveloped virions [4]. HEV infects humans and mammals, with eight genotypes (HEV-1 to HEV-8) [5]. Genotypes 1 and 2 infect humans via the faecal-oral route, particularly in areas with poor sanitation, while genotypes 3, 4, and 7 are zoonotic, spread through undercooked meat, animal contact, or contaminated blood. Genotype 5 infects animals, with genotypes 6 and 8 potentially zoonotic, however this is unclear [6,7].

Acute HEV infection has a 2–9-weeks incubation period. HEV-RNA is detectable in blood 2–6 weeks post-infection, with faecal shedding lasting up to 2 weeks longer [1]. IgM antibodies appear at 3–4 weeks and

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persist for 4–6 months, while IgG antibodies last for several years [1]. HEV genotype 1 poses a significant risk to pregnant women, particularly in the third trimester, with a fatality rate up to 30% due to fulminant hepatic failure [8]. HEV is associated with vertical transmission, premature delivery, spontaneous abortion, and stillbirths [9]. Hormonal imbalances, immune dysregulation, and increased viral replication causing high viral loads and severe hepatic injury in pregnancy [9]. In immunocompromised individuals, such as HIV patients, genotypes 3 and 4 can cause chronic infections, often transfusion-transmitted, leading to rapid liver cirrhosis and a high mortality rate [10].

The true burden of HEV is likely underestimated due to limited diagnosis and low awareness, especially in sub-Saharan Africa. This is exacerbated in HBV-endemic regions, where HEV superinfection in chronic HBV carriers can worsen liver disease, contributing to about 20% of exacerbation episodes [11]. In Cameroon, with an HBV prevalence of 11% [12], HEV surveillance is limited. While neighbouring Chad and the Central African Republic continue to experience HEV outbreaks [13,14], Cameroon's only documented outbreak occurred in 2013 in the northern region, with 37 cases and two deaths, including a pregnant woman [15]. Until date, no data exist on transfusion-transmitted HEV, as it is not routinely screened in blood donors. The objective of this study is to investigate HEV prevalence, genotype distribution, and associated risk factors in blood donors, HIV patients, and third-trimester pregnant women in Southwest region of Cameroon, with a focus on assessing potential transmission routes, including transfusion-transmitted HEV, and HEV prevalence in high-risk populations. This study also aims to provide crucial data on HEV dynamics in a region with limited surveillance, contributing to improved understanding and more effective public health strategies.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Institutional Review Boards of the University of Buea (Ethics approval number: 2022/1849-10/UB/SG/IRB/FHS) and the University of Tübingen (Ethics approval number: 379/2023B02). Administrative clearance was obtained from the Southwest Regional Delegation of Public Health (Approval number: 84/MPH/SWR/RHL/DO/03/2023). Signed informed consent was obtained from all participants prior to enrolment.

2.2. Participant enrolment and data collection

A cross-sectional study was conducted between March and June 2023 in the Southwest region of Cameroon (Fig. 1), recruiting 712 consenting individuals from three cohorts: healthy blood donors (n = 289), HIV patients (n = 233), and pregnant women (n = 190) (Table 1).

Table 1
Study cohort overview: recruitment sites, blood samples, and matching rectal swab/stool collection.

Study cohort	Recruitment sites	Blood (n)	Matching Rectal swab/stool (n)
Blood donors	Buea Regional Hospital blood bank	289	107
HIV Patients	Buea Regional Hospital	135	133
	Limbe Regional Hospital	98	16
Pregnant women (Third trimester)	Integrated Health Centers in Buea (PMI)	64	34
	Mutengene (CMA)	126	35
	Total	**712**	**325**

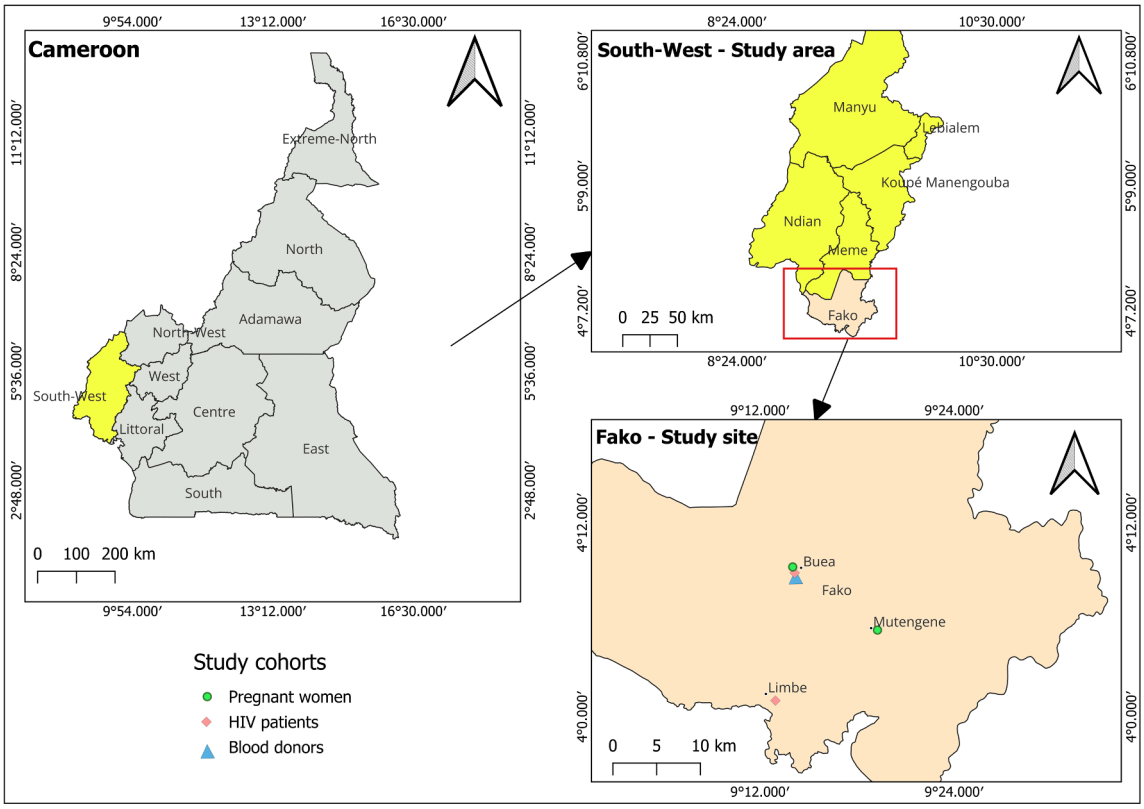

Fig. 1. A map of the study area with sampling sites for blood donors (triangles), HIV patients (diamonds), and pregnant women (spheres), created using QGIS 3.4 software.

Among the blood donors, 107 consented to provide rectal swabs. Blood donors were recruited from the Buea Regional Hospital blood bank after meeting the eligibility criteria, which included negative tests for HIV, HBV, Hepatitis C virus (HCV), and syphilis using rapid test kits and enzyme-linked immunosorbent assays (ELISA). Blood donors also had normal blood pressure, pulse rate, and hemoglobin levels, and were free of acute illnesses. HIV patients were recruited from outpatient treatment centers at Buea and Limbe Regional Hospitals. Pregnant women in their third trimester attending antenatal care at the Integrated Health Centers in Buea (PMI) and Mutengene (CMA) were also recruited.

A 3 mL venous blood sample was collected from all participants, along with matching rectal swabs or stool samples where possible (Table 1). Samples were preserved in a DNA/RNA shield (Zymoresearch, Irvine, CA, USA) and stored at -20°C for subsequent analysis. Demographic and lifestyle factors, including age, gender, marital status, educational level, alcohol consumption, and smoking habits, were assessed using a structured questionnaire. Furthermore, alimentary risk factors for HEV were documented across cohorts, encompassing pork and bushmeat consumption, pig contact, drinking water sources, and raw vegetable intake.

2.3. ELISA screening for anti-HEV IgG and IgM antibodies

Serum samples from all study cohorts (blood donors, HIV patients, and pregnant women) were tested for HEV-specific antibodies (anti-HEV IgM and anti-HEV IgG) using the Wantai HEV qualitative ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China), following the manufacturer's instructions. Sample absorbance values, measured with a CLARIOstar microplate reader (BMG Labtech, Ortenberg, Germany), were compared to the cut-off values.

2.4. RNA extraction and cDNA synthesis

Total RNA was extracted from all samples using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. RNA was eluted in 60 μL of elution buffer, and its quality and quantity were assessed using a NanoDrop™ (Thermo Fisher Scientific, Waltham, MA, USA). The isolated RNA was then stored at -20 °C until further use. The RNA was reverse transcribed into complementary DNA using the LunaScript RT SuperMix Kit (New England BioLabs, Ipswich, MA, USA).

2.5. Nested PCR for HEV detection

All samples were tested for HEV RNA using nested PCR. The nested PCR targeting both ORF-1 and ORF-2 followed the protocol as described earlier [16]. The primer pairs used in this study and their corresponding annealing temperatures for the PCR reactions for both ORF-1 and ORF-2 are provided (Supplementary Table 1). In addition, samples positive for ORF-1 but negative for ORF-2, were further subjected to ORF-2 using two sets of degenerate primers (3156N-3157N; and 3158N and 3159N) capable of detecting HEV strains with significant sequence variation, as previously described [17,18] (Supplementary Table 1). A plasmid containing HEV cDNA was used as the positive control, while a non-template control (master mix with nuclease-free water) served as the negative control. The amplicons (307 bp for ORF-1 and 489 bp or 348 bp for ORF-2) were visualized under UV light on 1.5 % agarose gels stained with SYBR Green dye. Samples positive for either ORF-1 or ORF-2 were considered positive for HEV RNA, and all positive samples were replicated for confirmation.

2.6. HEV genotyping and phylogenetic analysis

PCR products were purified using the Exo-SAP-IT kit (USB, Affymetrix, Santa Clara, CA, USA) and utilized as templates for Sanger sequencing (Bigdye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, CA, USA) with the ABI 3130XL sequencing system following the manufacturer's instructions. The HEV ORF1 and ORF2 specific sequences were manually verified and corrected using Seqman version 6.1 (DNASTAR, Lasergene, USA). For phylogenetic tree reconstruction, 49 reference sequences representing all genotypes and subtypes of the genus *Paslahepevirus* were used. This included 43 curated reference sequences for HEV genotyping proposed by Smith et al. [19], six sequences with high similarity to the samples obtained through NCBI BLAST (three for ORF1 and one for ORF2), and eight additional pig genotype 3 sequences (three for ORF1 and five for ORF2) previously reported in Cameroon, sourced from NCBI GenBank. The consensus sequences, along with the reference sequences, were aligned using MAFFT version 7.0 with the G-INS-I model [20]. Phylogenetic trees for ORF-1 and ORF-2 were reconstructed in MEGA version 11 [21] using the maximum likelihood method with the General Time Reversible Model plus gamma distribution (GTR+G) and branching reliability was assessed with 1000 bootstrap replicates. The phylogenetic tree was annotated and visualized using iTOL v6 [22]. Additionally, nucleotide sequence similarity between the human genotype 3 sequences from the study and the pig genotype 3 sequences reported in Cameroon was assessed using BioEdit v7.2.

2.7. Data analysis

Statistical analysis was performed using R software (version 4.4.1). Age was reported as the median with interquartile range (IQR) for each study cohort. The frequencies of HEV exposure markers (anti-HEV IgM and anti-HEV IgG) were presented as numbers and percentages (n, %) for each cohort and the overall population. HEV prevalence was calculated as the percentage of positive samples out of the total tested. The association between anti-HEV seropositivity and potential risk factors was evaluated using Pearson's chi-square (χ^2) test and Fisher's exact test. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Demographic and study population characteristics

A total of 712 individuals participated in the study, comprising 289 blood donors, 190 pregnant women, and 233 HIV patients, with 55 % female and 45 % male (Table 2). The overall median age was 33 years (IQR; 26–43), with the youngest participant aged 18 years in both the blood donor and pregnant women cohorts, and the oldest aged 80 years in the HIV cohort. The blood donor cohort had a median age of 30 years (IQR; 24–38), with 80 % in the 21–40-year range. In their third trimester, pregnant women had a median age of 27 years (IQR; 24–31), with 89% in the 21–40-year range, 58 % pregnant for the first time, 42 % pregnant for at least the second time, and 15 % with a history of miscarriage. HIV patients had a median age of 46 years (IQR; 39–55), with 58 % in the 41–60-year range. In the overall population, 7% had a history of blood transfusion, and <4.5% had knowledge on hepatitis E virus.

3.2. anti-HEV antibodies seroprevalence

Of the 712 individuals, 49 (7 %) tested positive for anti-HEV IgG, and 12 (2%) for anti-HEV IgM (Table 2). Among 289 blood donors, 25 (9 %) were positive for anti-HEV IgG, and 4 (1 %) for anti-HEV IgM, with all seropositive donors being male. The highest anti-HEV IgG positivity (15 %) was observed in the 41-60-year age group, and the highest IgM positivity (2%) in the 21-40-year group (Supplementary Table 2). No significant differences were observed by gender or age ($p > 0.05$). Among 233 HIV positive individuals, 17 (7 %) tested positive for anti-HEV IgG, and 6 (3 %) for anti-HEV IgM (Table 2), with no significant differences by gender or association with age or HIV viremia ($p > 0.05$) (Supplementary Table 2). Among 190 pregnant women, 7 (4 %) were

Table 2
Demographic and hev prevalence characteristics across study cohorts

Characteristics	Blood Donors (n=289)	HIV Patients (n=233)	Pregnant (n=190)	All study group (n=712)
Age (years)				
Median	30	46	27	33
Interquartile range (IQR)	[24 – 38]	[39 – 55]	[24 – 31]	[26 – 43]
Range (min–max)	18 – 59	19 – 80	18 – 53	18 – 80
Age group (years) n (%)				
≤20	11 (4)	1 (1)	17 (9)	29 (4)
21-40	230 (80)	67 (29)	169 (89)	466 (65)
41-60	48 (17)	134 (58)	4 (2)	186 (26)
>60	None	31 (13)	NA	31 (4)
Gender				
Male	280 (97)	39 (17)	NA	319 (45)
Female	9 (3)	194 (83)	190 (100)	393 (55)
HEV prevalence n (%) [95% CI]				
anti-HEV IgG (+ve)	25 (9) [5.68 – 12.5]	17 (7) [4.31 – 11.4]	7 (4) [1.49 – 7.44]	49 (7) [5.13 – 9.00]
anti-HEV IgM (+ve)	4 (1) [0.15 – 1.43]	6 (3) [0.31 – 1.83]	2 (1) [0.03 – 1.01]	12 (2) [0.87 – 2.93]
HEV RNA positivity	6 (2) [0.31 – 1.83]	0	2 (1) [0.03 – 1.01]	8 (1) [0.48 – 2.20]

HEV-hepatitis E virus; IgG-Immunoglobulin G; IgM-Immunoglobulin M; NA: not applicable; CI: confidence interval.

positive for anti-HEV IgG, and 2 (1 %) for IgM (Table 2), with no significant associations with age, gravidity, history of miscarriage, or blood transfusion ($p > 0.05$) (Supplementary Table 2).

Although not statistically significant, several trends in anti-HEV seropositivity were observed concerning alcohol consumption, education level, marital status, and cigarette smoking (Supplementary Table 1). For anti-HEV IgM seropositivity, individuals with primary education had the highest prevalence in the blood donor and HIV cohorts, whereas all seropositive pregnant women had secondary education. Additionally, married individuals exhibited the highest seropositivity rates. For anti-HEV IgG seropositivity, alcohol consumers consistently showed the highest prevalence across all cohorts. In the blood donor and HIV cohorts, seropositivity was more common among smokers. Furthermore, divorced or widowed individuals in the donor and HIV cohorts had higher seroprevalence, whereas married individuals exhibited the highest prevalence among pregnant women. Moreover, no significant association or trend was found between HEV seropositivity and alimentary risk factors, including the consumption of pork or bushmeat, contact with pigs, drinking water sources, or the consumption of raw vegetables from wetlands ($p > 0.05$) (Supplementary Table 2).

3.3. *HEV RNA positivity and phylogenetic analysis*

HEV RNA was tested by nested PCR in serum, rectal swabs, and stool samples across all study cohorts. The overall HEV RNA positivity rate for ORF1 and ORF2 was 1% (8/712) (Table 2), with three positive samples from serum (2/190 among pregnant women; and 1/289 among blood donors respectively) and five from rectal swabs (5/107 among blood donors). The blood donor cohort had the highest positivity rate at 2 % (6/289), followed by the pregnant women cohort at 1 % (2/190) (Table 2). No HEV RNA positivity was detected in the HIV patient cohort. Of the 8 HEV RNA-positive samples, 6 were ORF-1 positive, while only 2 were also ORF-2 positive, which were independently validated. Among the eight RNA-positives, two blood donors showed serological evidence of HEV exposure - one had anti-HEV IgM in serum, indicating an acute infection, while another had anti-HEV IgG.

Phylogenetic analysis of the eight HEV RNA-positive samples revealed that 75 % belonged to HEV genotype 3a and 25 % to genotype 3e (Fig. 2 and Fig. 3). All HEV RNA-positive samples in the blood donor cohort clustered with genotype 3a from Asia, while genotype 3e, detected in all positive pregnant women samples, clustered with European strains. The representative sequences have been deposited in the NCBI GenBank database with the following accession numbers: ORF1 (PQ639439 to PQ639444) and ORF2 (PQ605262 and PQ605263). The human genotype 3 sequences exhibited only 85% identity for ORF-1 and 82% for ORF-2 compared to previously reported pig genotype 3 sequences from Cameroon.

4. Discussion

This study, to our knowledge, is the first to provide detailed insights into HEV prevalence and risk factors, and genotype distribution in Southwest Cameroon, filling a critical gap in understanding the virus's burden in sub-Saharan Africa. The true burden of HEV remains underexplored in sub-Saharan Africa, particularly in HBV-endemic regions, due to limited diagnostic capabilities. Pregnant women in the third trimester face heightened risks, including fatal liver failure and stillbirths, while immunocompromised individuals, such as those living with HIV, are susceptible to chronic HEV infection and rapid progression to liver cirrhosis. This study examined the prevalence, genotype distribution, and risk factors of HEV in blood donors, HIV patients, and third-trimester pregnant women in a heterogeneous population from Southwest Cameroon. Our study revealed an anti-HEV IgG seroprevalence of 7 %, lower than the global estimate of 12.5 %, while anti-HEV IgM seroprevalence (2 %) and HEV RNA positivity (1 %) were higher than global estimates of 1.5 % and 0.2 %, respectively [23].

In the blood donor cohort, 9 % tested positive for anti-HEV IgG, and 1% for anti-HEV IgM. While no prior studies on HEV prevalence in blood donors exist for Cameroon, reports from neighboring Nigeria show lower anti-HEV IgG seropositivity (2.9–5.3 %) but similar anti-HEV IgM levels (1.3–1.9 %) compared to our study [24,25]. The highest HEV RNA positivity rate (2 %) was observed among blood donors, with HEV genotype 3a identified. Consistent with several studies in Europe and Asia, genotype 3 is the predominant genotype in blood donors [26]. Two RNA-positive blood donors also showed serological evidence of HEV exposure—one had anti-HEV IgM in serum, indicating an acute infection, while the other had anti-HEV IgG. This highlights a potential risk of HEV transmission through transfusion, especially in immunocompromised recipients, where viral reactivation could lead to acute hepatitis. HEV RNA screening has been implemented in several European countries [1], but it is not yet part of the donor screening protocol in Cameroon, a country endemic for both HIV and HBV. While age was not significantly associated with HEV seroprevalence in this study, it is noteworthy that all donors with recent exposure were in the young adult age group (21-40 years).

In this study, among HIV-infected individuals, 7% tested positive for anti-HEV IgG and 3% for anti-HEV IgM, which is higher than what is typically reported in HIV-infected individuals across sub-Saharan Africa [27]. However, the HEV RNA positivity rate was 0% in both serum and fecal samples. Adherence to antiretroviral therapy (ART) can restore immune function, potentially facilitating HEV clearance, which may explain the absence of viremia in this study, as HIV infected persons recruited were on ART. Additionally, no significant difference in HEV prevalence was observed between patients with detectable and undetectable viral loads, consistent with several other reports in the literature [28]. A study conducted in Cameroon among HIV-infected populations reported a much higher IgM seroprevalence rate of 7 % [29]. Other Central African countries report anti-HEV IgG rates as high as 53 % in HIV patients, while rates in West Africa range from 11 % to 45 %, and exceed 70% in Southern Africa [27]. Among HIV patients, the 21–40 age group exhibited higher seroprevalence rates, suggesting that HEV infection is often acquired at a younger age in this population, resulting in cumulative exposure over time.

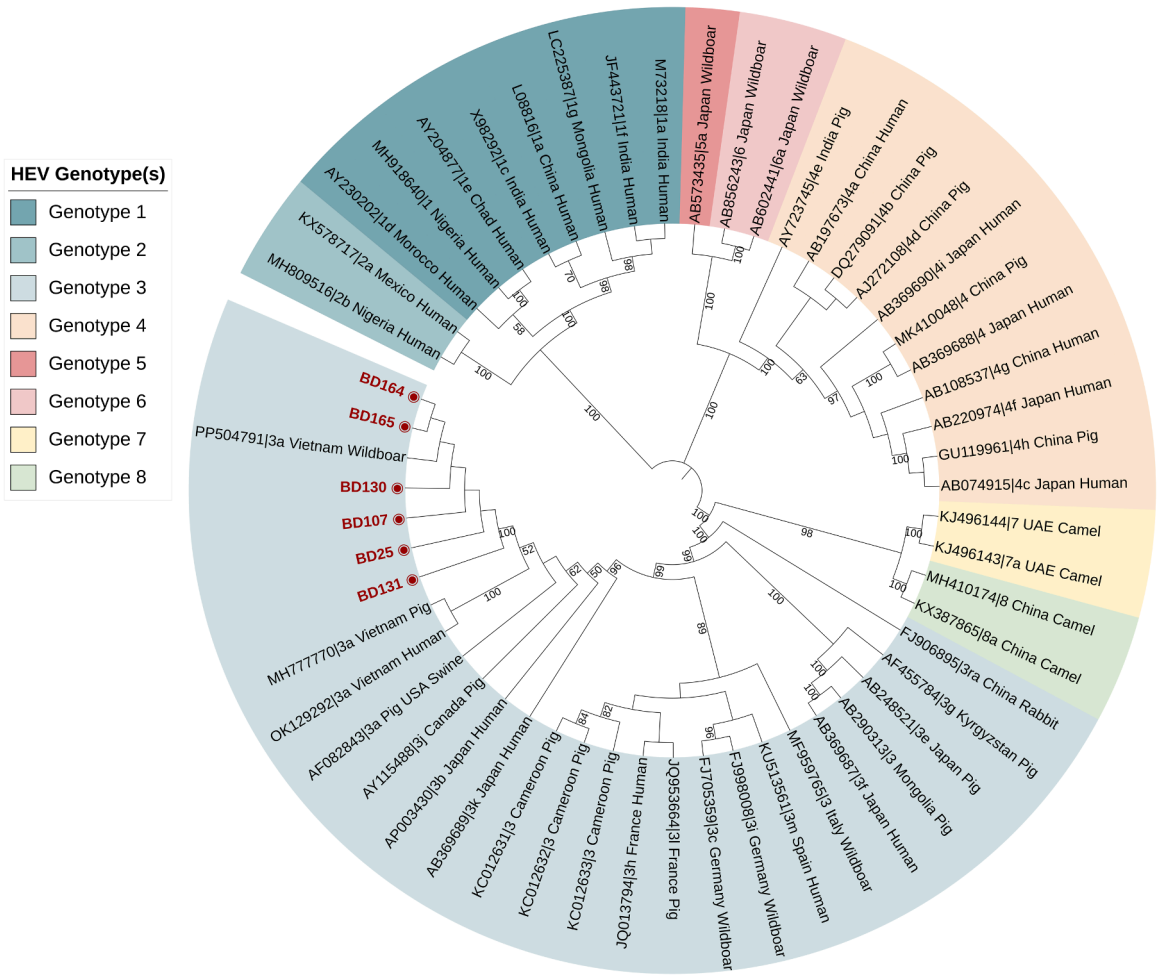

Fig. 2. Phylogenetic analysis of all HEV ORF-1 specific sequences obtained in this study from both serum and rectal swabs/stool. Reference sequences representing various HEV genotypes/subgenotypes were retrieved from the GenBank - NCBI database, along with accession numbers, genotypes, countries of origin, and hosts. Six independent positive sequences obtained from this study are highlighted in red.

The seroprevalence observed among pregnant women in our study was 4% for anti-HEV IgG and 1 % for anti-HEV IgM. The IgM seroprevalence in our study is notably lower compared to a study conducted in the South and Central regions of Cameroon, which reported 13% for anti-HEV IgM [29]. While genotype 1 is commonly associated with severe maternal and fetal complications, studies suggest that acute hepatitis caused by genotype 3 during pregnancy is typically cleared rapidly without signs of a fulminant course [30,31]. Additionally, whether transplacental anti-HEV IgG transfer occurred to confer passive immunity to infants remains unclear, as cord blood was not tested. This study used the widely adopted WANTAI HEV IgG and IgM tests, validated in diverse cohorts, including high-risk groups, and reliable for detecting infections caused by various HEV genotypes, supporting global surveillance efforts. Comparing anti-HEV antibody prevalence across studies is challenging due to differences in the sensitivity of HEV assays used [32]. Most studies employed different ELISA tests, while this study used the widely used WANTAI test, including the study on blood donors in Nigeria [33]. Thus, comparisons should be interpreted with caution.

Although HEV genotyping studies in sub-Saharan Africa (SSA) are limited, genotypes 1 and 2 are prevalent and primarily linked to large waterborne outbreaks, particularly in refugee camps [34]. Genotype 3 has been reported in both pigs and humans across SSA, while genotype 4 is detected in pigs and humans mainly in Central Africa [35]. The HEV RNA isolates from our study, specifically genotypes 3a and 3e, showed alignment with strains circulating in both animal and human populations in Asian and European countries. In Cameroon, HEV genotypes 1e, 3f, and 4b have been documented in the human population [15,35], while genotype 3 has been predominant in pig faeces, and serum [36], with genotype 3a also detected in wastewater [37]. Although zoonotic transmission is considered the primary route of HEV infection, our study found no significant association between HEV seroprevalence and alimentary risk factors such as pork consumption, bushmeat, undercooked meat, or contact with pigs across cohorts, aligning with previous reports from Cameroon [29]. Additionally, the nucleotide diversity between human and pig HEV genotype 3 suggests the involvement of environmental or other indirect transmission routes, rather than direct pig-to-human transmission, in human infections in this region.

Alcohol consumers had higher anti-HEV IgG seropositivity across cohorts, and all anti-HEV IgM-positive blood donors reported alcohol consumption. Rather than being a direct risk factor for HEV exposure, excessive alcohol intake may contribute to subclinical liver injury, increasing susceptibility to symptomatic infection [38]. A similar mechanism could explain the higher seropositivity observed among smokers. However, data on volume intake, and frequency were not assessed. Higher seropositivity among married individuals may reflect increased household transmission through shared items, toilets, and drinking sources [39]. Likewise, lower education levels may be linked to poor sanitation and limited awareness of HEV risks. Identifying the primary HEV transmission routes in Cameroon remains challenging. Future studies exploring these factors in greater detail could inform targeted prevention strategies.

In contrast, studies in West Africa have reported varying

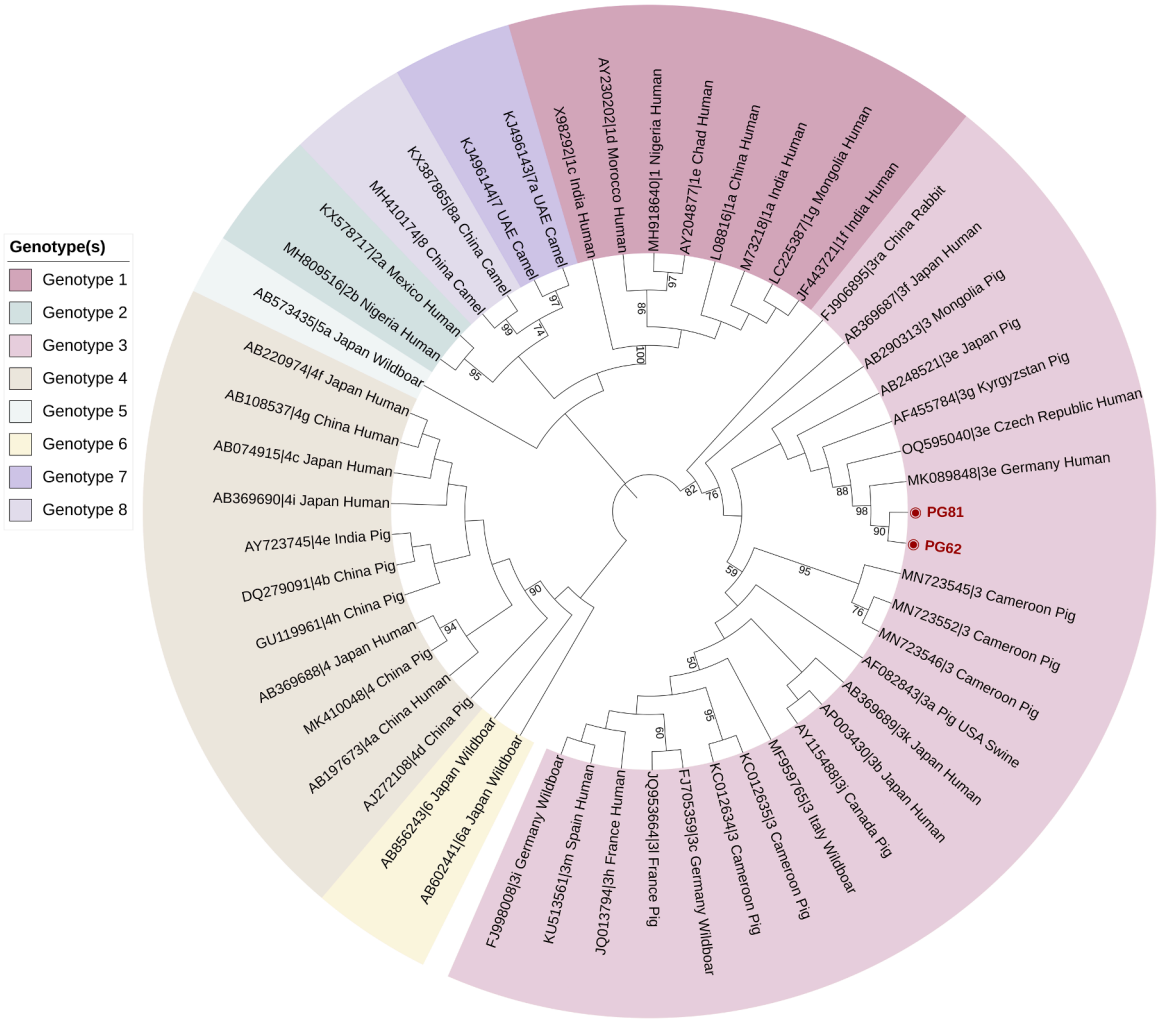

Fig. 3. Phylogenetic analysis of all HEV ORF-2 specific sequences obtained in this study from serum. Reference sequences representing various HEV genotypes/subgenotypes were retrieved from the GenBank - NCBI database, along with accession numbers, genotypes, countries of origin, and hosts positive sequences are highlighted in red.

seroprevalence rates, including 19.1% in blood donors and 11.6% in pregnant women in central Burkina Faso [40], and a notably higher 80% prevalence among pork butchers with occupational exposure [41]. Furthermore, the detection of HEV-3 in pigs in Guinea underscores the need to evaluate HEV seroprevalence in human populations [42]. To better understand HEV transmission dynamics, larger studies focusing on high-risk groups such as animal handlers, butchers, and hunters are essential.

This study has several limitations. Firstly, its focus on Southwest Cameroon may not fully represent HEV prevalence and genotypes in other regions or neighbouring countries. Expanding the study geographically would offer a more comprehensive understanding of HEV transmission. Secondly, the cross-sectional design limits the ability to establish causal relationships between risk factors and HEV infection. Lastly, the predominance of male blood donors introduces a gender bias in the cohort.

5. Conclusion

Taken together, this study underscores the HEV risk in Southwestern Cameroon, particularly among blood donors, HIV patients, and pregnant women. Routine HEV screening for blood donors and annual HEV RNA testing for HIV patients should be considered, as chronic infections can occur even without detectable antibodies. Early HEV screening during pregnancy is crucial due to the lack of treatment. Public health efforts should focus on vulnerable groups to raise awareness of HEV risk factors and prevention strategies.

Institutional review board statement

This study was approved by the Institutional Review Boards of the University of Buea (Ethics approval number: 2022/1849-10/UB/SG/IRB/FHS) and the University of Tübingen (Ethics approval number: 379/2023B02). Administrative clearance was obtained from the Southwest Regional Delegation of Public Health (Approval number: 84/MPH/SWR/RHL/DO/03/2023).

CRediT authorship contribution statement

Macqueen Ngum Mbencho: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Nourhane Hafza:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Le Chi Cao:** Writing – review & editing, Visualization, Validation, Software, Methodology. **Victorine Ndiwago Mingo:** Methodology, Investigation. **Emmanuella Nyarko-Afriyie:** Methodology, Investigation. **Eric A. Achidi:** Supervision, Project administration. **Stephen Mbigha Ghogomu:** Writing – review & editing, Supervision,

Resources, Project administration, Methodology, Data curation. **Thirumalaisamy P. Velavan:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2025.116748.

Data availability

All data generated in this study are included in this article. All eight obtained sequences were submitted to the NCBI GenBank database with accession numbers for ORF1 ranging from PQ639439 to PQ639444 and for ORF2 ranging from PQ605262 to PQ605263.

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Supplementary Table S1: Primers and Annealing temperatures used for nested PCR

Target	Primers pairs used		Sequence (5′ – 3′)	PCR conditions
ORF1	Outer	HEV-38 (sense)	GAGGCYATGGTSGAGAARG	94°C for 5 minutes; 95°C for 30 seconds; 56°C for 30 seconds; 72°C for 30 seconds; 72°C for 10 minutes; (30 cycles)
		HEV-39 (antisense)	GCCATRTTCCAGACRGTRTTCC	
	Nested	HEV-37 (sense)	GGTTYCGYGCYATTGARAARG	94°C for 5 minutes; 95°C for 30 seconds; 54°C for 30 seconds; 72°C for 30 seconds; 72°C for 10 minutes; (30 cycles); Amplicon size: 307bp
		HEV-27 (antisense)	TCRCCAGAGTGYTTCTTCC	
ORF2	Outer	HEV-34 (sense)	CCGACGTCYGTYGAYATGAA	94°C for 5 minutes; 95°C for 30 seconds; 54°C for 30 seconds; 72°C for 30 seconds; 72°C for 10 minutes; (36 cycles)
		HEV-36 (antisense)	TTRTCCTGCTGAGCRTTCTC	
	Nested	HEV-35 (sense)	AAGTGAGCGCCTACAYTAYCG	94°C for 5 minutes; 95°C for 30 seconds; 56°C for 30 seconds; 72°C for 30 seconds; 72°C for 10 minutes; (36 cycles); Amplicon size: 489 bp
		HEV-29 (antisense)	CTCGCCATTGGCTGAGAC	
	Outer	3156N (sense)	AATTATGCC(T)CAGTAC(T)CGG(A)GTTG	95°C for 10 minutes; 94°C for 1 minute ; 54°C for 1 minute; 72°C for 1 minute; 72°C for 7 minutes; (39 cycles)
		3157N (antisense)	CCCTTA(G)TCC(T)TGCTGA(C)GCATTCTC	
	Nested	3158N (sense)	GTT(A)ATGCTT(C)TGCATA(T)CATGGCT	95°C for 10 minutes; 94°C for 1 minute; 54°C for 1 minute; 72°C for 1 minute; 72°C for 7 minutes; (39 cycles); Amplicon size: 348bp
		3159N (antisense)	AGCCGACGAAATCAATTCTGTC	

Supplementary Table S2: Prevalence of anti-HEV seropositivity by demographic and alimentary risk factors within study cohorts.

Study Cohort	Characteristics	anti-HEV IgM	anti-HEV IgG
Blood Donors	*Male*	4/289	25/279
	Female	0/9	0/9
	≤20 Years	0/11	0/11
	21-40 Years	4/229	18/211
	41-60 Years	0/48	7/48
	>60 Years	0	0
	Marital status		
	Single	2/188	13/188
	Married	2/95	11/95
	Divorced/widowed	0	1/4
	Educational level		
	Primary	1/23	3/23
	Secondary	0	3/42
	High	0	8/68
	Tertiary	2/152	10/152
	Alcohol consumption		
	Yes	4/273	21/273
	No	0	4/15
	Cigarette smoking		
	Yes	0	4/34
	No	4/253	21/253
	Pork Consumption		
	Yes	3/234	18/234
	No	1/54	7/54
	Bushmeat consumption		
	Yes	4/193	15/193
	No	0/95	10/95
	Well/Borewell water		
	Yes	2/112	10/112
	No	2/176	15/176

	Tap water		
	Yes	2/171	15/171
	No	2/117	10/117
	Consumption of Raw Vegetables		
	Yes	4/278	24/278
	No	0/10	1/10
	Contact with pigs		
	Yes	0/25	5/25
	No	4/263	20/263
	Knowledge on hepatitis E		
	Yes	0/6	0/6
	No	4/282	25/282
	Characteristics	**anti-HEV IgM**	**anti-HEV IgG**
HIV patients	*Male*	0/39	4/39
	Female	6/194	13/194
	≤20 Years	0/1	0/1
	21-40 Years	4/67	4/67
	41-60 Years	2/134	9/134
	>60 Years	0/31	4/31
	Marital status		
	Single	2/102	6/102
	Married	3/86	6/86
	Divorced/widowed	1/45	5/40
	Educational level		
	Primary	3/108	8/109
	Secondary	1/103	8/103
	High	0	0
	Tertiary	2/21	1/21
	Alcohol consumption		
	Yes	3/152	12/152
	No	3/81	5/81
	Cigarette smoking		
	Yes	0	1/7

	No	6/226	16/226
	Pork consumption		
	Yes	6/199	13/199
	No	0/34	4/34
	Bushmeat consumption		
	Yes	5/202	17/202
	No	1/31	0/31
	Well/Borewell water		
	Yes	3/65	4/65
	No	3/168	13/168
	Tap water		
	Yes	3/157	12/157
	No	3/76	5/76
	Consumption of Raw Vegetables		
	Yes	6/224	16/224
	No	0/9	1/9
	Contact with pigs		
	Yes	1/31	4/31
	No	5/202	13/202
	Viral load		
	Detectable (40 – 3.1x10^5 copies/mL)	1/38	2/38
	Not detectable	5/195	15/195
	Knowledge on hepatitis E		
	Yes	1/15	2/15
	No	5/218	15/218
	Characteristics	**anti-HEV IgM**	**anti-HEV IgG**
Pregnant women	*≤20 Years*	0/11	0/11
	21-40 Years	2/153	6/153
	41-60 Years	0/3	1/3
	>60 Years	0	0
	Marital status		
	Single	0	1/69
	Married	2/98	6/98

	Divorced/widowed	0	0
	Educational Level		
	Primary	0	0
	Secondary	2/90	3/90
	High	0	0
	Tertiary	0	4/68
	Alcohol consumption		
	Yes	1/119	6/119
	No	1/48	1/48
	Cigarette smoking		
	Yes	0	0
	No	2/167	7/67
	Pork consumption		
	Yes	1/136	5/136
	No	1/31	2/131
	Bushmeat consumption		
	Yes	2/126	5/126
	No	0/ 41	2/41
	Well/borewell water		
	Yes	0/11	0/11
	No	2/156	7/156
	Tap water		
	Yes	2/138	6/138
	No	0/29	1/29
	Consumption of raw vegetables		
	Yes	2/165	7/165
	No	0/2	0/2
	Contact with pigs		
	Yes	0/35	0/35
	No	2/132	7/132
	Gravidity		

	Primigravida	1/98	3/98
	Multigravida	1/69	4/69
	History of miscarriage		
	Yes	0/24	2/24
	No	2/143	5/143
	Blood transfusion history		
	Yes	0/8	0/8
	No	2/159	7/159
	Knowledge on HEV		
	Yes	0/10	0/10
	No	2/157	7/157

HEV: hepatitis E virus; HIV: human immunodeficiency Virus; IgG: Immunoglobulin G, IgM: Immunoglobulin M;

No significant association was found between HEV seropositivity (IgG and IgM) and risk factors ($p>0.05$) across cohorts.

Chapter 4

Co-infection dynamics of HBV and HEV among HIV-positive adults, pregnant Women, and the general population

Publication No.4

Low prevalence of concurrent active hepatitis E and B virus infection in high-risk groups in Southwestern Cameroon

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Short Communication

Low prevalence of concurrent active hepatitis E and B virus infection in high-risk groups in Southwestern Cameroon

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ABSTRACT

Objectives: Coinfection with the hepatitis E virus (HEV) and hepatitis B virus (HBV) can have clinical implications, particularly in immunocompromised and high-risk populations. This study investigated HEV seroprevalence and viraemia among individuals with HBV exposure in the Southwest region of Cameroon.

Methods: We analyzed three cohorts with prior HBV exposure, defined as anti-HBc total positivity: HIV-infected individuals (n = 174), pregnant women (n = 87), and blood donors (n = 167). Participants were screened for anti-HEV immunoglobulin (Ig)G and IgM using an enzyme-linked immunosorbent assay, and HEV RNA was detected by reverse transcription-polymerase chain reaction. HBV DNA was quantified in those with hepatitis B surface antigen positivity.

Results: Among anti-hepatitis B core total-positive participants, anti-HEV IgG seroprevalence was 8.6% in HIV-infected individuals, 5.7% in pregnant women, and 12.0% in blood donors. Anti-HEV IgM was detected in 1.7%, 0%, and 1.2%, respectively. Also, HEV RNA was detected in two blood donors (genotype 3a) and two pregnant women (genotype 3e), but not in HIV-infected individuals. No cases of concurrent active HBV and HEV replication were observed.

Conclusions: Previous HEV exposure is relatively common in this region, but active HBV-HEV coinfection appears rare among HIV-infected individuals, pregnant women, and blood donors in Southwest Cameroon.

Introduction

Hepatitis E virus (HEV) is an emerging cause of acute viral hepatitis worldwide, with the highest burden in low- and middle-income countries, particularly in Africa and Asia [1]. The African region is also disproportionately affected by chronic hepatitis B virus (HBV) infection, driven largely by perinatal transmission and sustained high co-endemicity with HIV [2]. Coinfection with HEV and HBV can exacerbate liver damage, especially in immunocompromised individuals such as HIV-infected individuals and pregnant women [3,4]. In settings where HEV and HBV are co-circulating, dual infection can result in acute-on-chronic liver failure and increased mortality, especially among those with HBV-related cirrhosis [5]. Despite the potential clinical severity, the prevalence and clinical significance of concurrent HEV and HBV infection in different population groups remain insufficiently characterized in Central Africa. This study aimed to determine the prevalence of past HEV exposure, recent infection, and active HEV viremia in three distinct population cohorts from southwestern Cameroon, and to assess their co-occurrence with markers of HBV replication.

Methods

Ethical approval

The study was approved by the Institutional Review Board of the University of Buea, Cameroon (Reference: 2022/1849-10/UB/SG/IRB/FHS), and by the Ethics Committee of the University of Tübingen, Germany (Reference: 379/2023B02). Administrative clearance was obtained from the Southwest Regional Delegation of Public Health. Written informed consent was obtained from all participants before enrollment.

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Table 1
Prevalence of HEV exposure, recent infection, and active viremia in relation to HBV markers across study cohorts in Southwestern Cameroon.

Study cohort	HBV parameters	Anti-HEV IgG positivity	Anti-HEV IgM positivity	HEV-RNA Positivity
HIV patients	Anti-HBc total positivity (n = 174)	15 (8.6%)	3 (1.7%)	0
	HBV-DNA positivity (n = 24)	1 (4.2%)	0	0
Pregnant women	Anti-HBc total positivity (n = 87)	5 (5.7%)	0	2 (2.3%)
	HBV-DNA positivity (n = 7)	0	0	0
Blood donors	Anti-HBc total positivity (n = 167)	20 (12%)	2 (1.2%)	2 (1.2%)
	HBV-DNA positivity (n = 14)	0	0	0

HBc, hepatitis B core; HBV, hepatitis B virus; HEV, hepatitis E virus; Ig, immunoglobulin.

Laboratory investigations

We performed serological and molecular screening for HEV and HBV markers in three cohorts recruited in the Southwest region of Cameroon that included HIV-infected individuals (n = 233), pregnant women (n = 190), and blood donors (n = 289). Anti-hepatitis B core (anti-HBc) total, HBV deoxyribonucleic acid (DNA), and HBV viral load were assessed to determine HBV status. HEV exposure and infection were evaluated using anti-HEV immunoglobulin (Ig)G, anti-HEV IgM, and HEV ribonucleic acid (RNA) detection by reverse transcription-polymerase chain reaction, as described in our previous studies [6,7].

Results

Among participants who tested positive for anti-HBc total, anti-HEV IgG was detected in 8.6% (15/174) of HIV-infected individuals, 5.7% (5/87) of pregnant women, and in 12% (20/167) of blood donors (Table 1). Anti-HEV IgM positivity was rare and was observed in 1.7% (3/174) of HIV-infected individuals, none among the pregnant women, and 1.2% (2/167) of blood donors (Table 1). HEV RNA positivity was detected in only two blood donors (genotype 3a) and two pregnant women (genotype 3e), while no cases were detected among HIV-infected participants. Importantly, no cases of HEV RNA positivity occurred in individuals with detectable HBV DNA. Among HBV DNA-positive individuals—24 (10%) in the HIV cohort, 7 (3%) in the pregnant women cohort, and 14 (5%) in the blood donor cohort—none had detectable anti-HEV IgM or HEV RNA (Table 1), suggesting the absence of concurrent active HBV and HEV replication in the study population. Furthermore, among anti-HBc total-positive individuals across cohorts, markers of recent or active HEV infection (anti-HEV IgM and/or HEV RNA) were more common in the 21–40 years age group, whereas previous HEV exposure (anti-HEV IgG) was predominant in older adults above 40 years.

Discussion

In this HBV-endemic setting, previous HEV exposure was detected in all three cohorts, with the highest prevalence among blood donors, intermediate among HIV-infected individuals, and lowest among pregnant women.

While there is substantial evidence for exacerbated liver disease with HEV coinfection or superinfection in various populations with different underlying HBV-related liver disease stages [8], reports for those living with HIV are scarce. The absence of concurrent active HBV-HEV replication in the HIV cohort is consistent with reports from other endemic regions [8,9], potentially explained by the transient nature of HEV viremia, non-overlapping transmission routes, and possible immune-mediated viral interference [8,10]. The relatively low anti-HEV IgG prevalence in HIV-infected individuals compared to similar settings may relate to differences in exposure patterns or assay sensitivity, as importantly, no active HEV infection was observed in this group despite their higher risk of chronicity. Given the heightened risk of HIV acquisition associated with HBV in endemic regions and the potential for accelerated liver disease progression in coinfected individuals, our findings underscore the need for targeted public health strategies to prevent HEV in this and similar settings.

The age distribution of HEV markers, with recent infection in younger adults and past exposure in older adults, matches the known epidemiology in endemic regions [1], while HBV infection in this setting is typically acquired in early infancy [2]. Our data indicate a low prevalence of recent or active HEV infection among pregnant women. Studies from similar settings have reported higher HEV seropositivity in HBV-positive pregnant women compared to HBV-negative, with coinfection associated with increased obstetric complications and adverse birth outcomes [4]. HEV RNA was detected in two pregnant women of genotype 3e, with negative anti-HEV IgM, suggesting early infection with a low-titer IgM response. No severe courses were observed during the study, nor presumed, as severe outcomes have predominantly been associated with genotype 1 [4,7]. Nevertheless, close monitoring remains essential to mitigate any potential risks for both mother and child.

In HBV-endemic regions like Cameroon, with a high prevalence of anti-HBc total in the general population [6], the high rate of HEV exposure among blood donors likely reflects a similar level of community-wide exposure to HEV. This pattern suggests that HEV transmission and exposure may be driven by factors such as water contamination, poor sanitation, and food-borne exposure that affect the wider population. Notably, in our study, no blood donor exhibited concurrent HBV and HEV viraemia. This absence of dual active replication aligns with observations from other endemic settings, where co-exposure is relatively common but simultaneous replication of both viruses is rare [8,9].

Taken together, in a region with high HBV exposure, HEV co-exposure is relatively common, but active infection is rare, and simultaneous replication of both viruses was not detected. These results underscore the need for continuous HEV surveillance, particularly in pregnant women and immunocompromised individuals, and support the integration of HEV education and prevention into existing HBV and HIV programs. Further longitudinal studies are needed to investigate the temporal dynamics, risk factors, and clinical consequences of HEV-HBV coinfection in this and similar settings.

Declaration of competing interest

The authors have no competing interests to declare.

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Ethical approval

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Author contributions

Macqueen Ngum Mbencho: Data curation, formal analysis, investigation, methodology, software, validation, visualization, writing - original draft, writing - review & editing. Eric A. Achidi: Project administration, supervision. Stephen Mbigha Ghogomu: Supervision, data curation, methodology, project administration. Thirumalaisamy P. Velavan: Conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing - original draft, writing - review & editing.

Data availability statement

All data generated in this study are included in this article.

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3. Discussion

To better understand the epidemiology of HBV, OBI, and HEV in Cameroon, three populations with varying host immunological status and exposure risks in the southwestern region were studied. These included adult blood donors and those in high-risk groups, including HIV patients and women in their third trimester of pregnancy. This section of the thesis provides an integrative thematic discussion of the key findings from each of the published chapters, highlighting prevalence patterns, genotype diversity, associated OBI mutations that complicate virus detection, risk factor associations, and co-infection patterns, shedding light on their broader implications for public health in Cameroon and for the SSA region.

3.1. Prevalence and genotype diversity of HBV and occult HBV infection in blood donors and high-risk groups in Cameroon

3.1.1. Prevalence of OBI among blood donors and implications for transfusion safety

In SSA, where the demand for blood transfusions remains high, ensuring the safety of blood remains challenging due to the persistent burden of transmissible pathogens, including HBV (Dei-Adomakoh et al., 2021). This challenge is further compounded by OBI, especially since most countries still rely on serological testing for donor selection, including blood banks in Cameroon. The first chapter of my thesis examined the prevalence and serological patterns of OBI among apparently healthy blood donors in southwestern Cameroon who tested negative for HBV HBsAg, and other pathogens, and were thereby eligible to donate blood. Serological methods were used to determine the markers for HBV exposure (total anti-HBc) and the marker for immunity to HBV (anti-HBs), while a molecular approach (qualitative nested PCR and quantitative real-time PCR) detected the presence of HBV DNA and quantified the burden of OBI. Among these HBsAg-negative blood donors, an OBI prevalence of nearly 5% was observed, indicating substantial hidden reservoir of HBV infection within the donor population. This finding reinforces existing evidence that reliance on HBsAg testing alone is insufficient to fully mitigate the risk of transfusion-transmitted HBV in endemic settings. Although the OBI rate in this thesis exceeds previous reports from Cameroon (<2%) (Fopa et al., 2019), higher rates have been documented in other settings in West Africa and HBV-endemic regions of Asia (Takuissu et al., 2022). On the contrary, the OBI rate is below 1% in regions with low HBV endemicity, such as Europe (Takuissu et al., 2022). This suggests that OBI is a major challenge in regions where HBV exposure occurs early in life, and routine HBV testing is based solely on HBsAg. Furthermore, a high anti-HBc positivity rate of almost 60% was observed in this population, which aligns with regional rates reflecting the high cumulative previous exposure

in such endemic areas, and provides an important epidemiological context for the substantial OBI rate in the region. However, over 60% of OBI cases detected in this study were seronegative (anti-HBc and anti-HBs negative), while only a minority reflected the classical anti-HBc-positive and anti-HBs-negative pattern highly associated with the presence of OBI. Although less consistently reported, seronegative OBI have been documented in previous studies in Africa; however, its prevalence generally remains lower than that of seropositive OBI (Ondigui et al., 2022). The predominance of seronegative OBI in this population may suggest that other mechanisms may also be contributing to occult infections in this setting, including waning antibody responses or failure to develop detectable antibodies from infection onset (Raimondo et al., 2019). These findings suggest that although anti-HBc remains an important marker for the detection of OBI, selective detection of OBI only in anti-HBc-positive blood donors may not rule out the risk of transfusion-transmitted HBV in this population and similar settings.

The observed OBI rate is of concern, given that individuals with occult infections are often asymptomatic (Yuen, 2017). Moreover, even long after recovery from an acute infection, HBV DNA can persist in infected hepatocytes as an episomal minichromosome called the covalently closed circular DNA (cccDNA), periodically initiating viral replication (He et al., 2023). This may indicate that recipients of OBI-infected blood are at risk of HBV infection, especially if they are immunocompromised (Candotti et al., 2019). Apart from the recipient's immune status, the infectivity of transfused OBI-positive blood can also be influenced by the anti-HBs status of both the blood donor and recipient, and by the type and volume of blood component received (Candotti et al., 2019). Studies tracing blood transfusion recipients have found that the infectious dose of HBV is extremely low (approximately 3 IU/mL; 16 copies/mL) and that donors with low anti-HBs concentrations (<10 IU/mL) may pose a higher risk for infecting blood components (Candotti et al., 2019; Seed et al., 2017). In our study, the majority (92%) of OBI donors had anti-HBs levels below 10 mIU/ml, which potentially increases the risk of infection, especially if the recipients are not immunized. In addition, up to 79% of blood donors were repeat donors, suggesting that unidentified infected donors continue to put many recipients at risk. This further highlights the inadequacy of HBsAg tests for screening blood donors. The use of nucleic acid testing (NAT), as practiced in several industrialised countries (Seed et al., 2019), would significantly improve the identification of infected donors. However, to date, only South Africa and Namibia in SSA have introduced NAT for donor testing (Candotti et al., 2021).

3.1.2. Burden of HBV and OBI among HIV patients and pregnant women in Cameroon and clinical implications

Furthermore, the burden of HBV infections in SSA, including Cameroon, is perpetuated by mother-to-child transmission and the high HIV burden in the region. Although the WHO recommends systematic testing of pregnant women and HIV-infected individuals, routine testing often relies on HBsAg alone, potentially overlooking occult infections. The second chapter of this thesis examined the prevalence of HBV infection and OBI in HIV patients and pregnant women in their third trimester to understand the HBV burden among these high-risk groups. As expected, HBV DNA positivity was higher among HIV patients (10%) compared to pregnant women (4%). These findings are consistent with estimates showing that the prevalence of HBV infection among individuals infected with HIV is substantially high (Dagnaw et al., 2025), reaching an overall 11% HBsAg seroprevalence and 17% HBV DNA positivity across SSA (Kenfack-Momo et al., 2022). Similarly, reports for pregnant women show an estimated pooled HBsAg seroprevalence of about 7% across the African region (Larebo et al., 2024). However, HBsAg seroprevalence in the current study was comparably low; 1% for both the HIV patient and pregnant women populations, reflecting the huge proportion of occult infections in this population. OBI was most frequently detected in HIV patients (9%) compared to pregnant women (3%), which is consistent with reports associating HIV infection with increased risk of OBI (Mphahlele et al., 2006; Ryan et al., 2017). Globally, OBI rates are particularly high among at-risk populations for HBV infection, including HIV patients, where prevalence can be several-fold higher compared to the general population (Takuissu et al., 2022). In SSA, where both HBV and HIV are highly prevalent, OBI prevalence is also expected to be higher in HIV patients, partly because of concurrent exposure due to shared transmission routes. This aligns with our findings showing higher anti-HBc positivity (a marker for HBV exposure) among HIV patients (75%) compared to other populations (pregnant women: 46% and blood donors: 58%). Additionally, the high OBI burden in HIV patients may be attributed to impaired HBV-specific immune responses due to incomplete immune restoration under ART, which may allow low-level viral persistence and reduced HBsAg expression (Zhu et al., 2022).

The high burden of occult HBV infections in these high-risk groups raises important concerns for their clinical management. There is need to integrate NAT and/or at least anti-HBc into existing HIV control programs, as this will ensure early detection, identify individuals who need to be vaccinated, and guide suitable ART regimen selection for optimized clinical

monitoring of co-infected patients. Moreover, partial immune reconstitution under ART increases the risk of HBV reactivation in HIV patients co-infected with HBV or OBI under an unsuitable ART regimen or treatment interruption (Denyer et al., 2023). Extending routine HBsAg testing to include NAT in this risk group is therefore crucial to reduce HBV-related morbidity and improve long-term outcomes. In pregnancy, while active HBV replication and high viral loads (>2 x 10^5 IU/mL) are strongly linked with perinatal HBV transmission (Wong and Lemoine, 2025), experimental evidence using woodchuck hepatitis virus (WHV) and clinical observation suggest that OBI may also be transmitted perinatally (Coffin and Michalak, 1999; Saito et al., 1999). However, whether OBI in newborns can persist to overt HBV infections over time is not well understood. In a Taiwanese study, after following up OBI-infected newborns for one year, HBV viremia was cleared following complete timely infant vaccination series (Lai et al., 2022). Nevertheless, incorporating NAT for HBV testing in pregnant women is crucial for stratifying the risk of mother-to-child transmission and proper clinical management of mother and child, especially in Cameroon and similar settings where the HBV birth dose is only administered to newborns from infected mothers.

Taken together, the high-risk groups, including HIV patients and third-trimester pregnant women in Cameroon, carry a significant burden of HBV infections. At the same time, blood donors act as silent carriers and a potential source of transmission. Complementing routine screening with improved HBV diagnostic approaches and targeted preventive strategies is therefore crucial in this setting, especially for the high-risk groups.

3.1.3. HBV genotype diversity in blood donors and high-risk groups and its epidemiological implications.

To better understand the dynamics of HBV transmission and persistence in Cameroon, the genotype diversity of circulating strains was investigated in blood donors, HIV patients, and pregnant women. The identified HBV genotypes generally showed an expected pattern. Among HBsAg-negative blood donors, genotype E was overwhelmingly predominant (93%), whereas genotype A was less common. Among HIV patients, genotype A (56%) was predominant for both OBI and HBsAg-positive individuals, while genotype E was rarely detected. Interestingly, genotype B, which is rare in the region, was detected exclusively in OBI-positive HIV patients (32%), representing new findings for the country. Genotype E was also the most frequently detected genotype (71%) in both OBI and HBsAg-positive pregnant women, with only a single detection of genotypes A and D.

The predominance of genotypes A and E is in line with earlier reports in Cameroon (Magoro et al., 2016; Pinho-Nascimento et al., 2018) and throughout SSA (Hudu, 2022). While genotype D is most common in North Africa, it also circulates in parts of SSA, including Cameroon (Hudu, 2022). These HBV genotypes may be implicated in HBV transmission and other clinical aspects, such as disease progression and treatment outcomes (Chen et al., 2023). According to some reports, genotype A may be linked with the horizontal transmission of HBV, while genotype E is more likely to be associated with mother-to-child transmission in SSA (Kramvis, 2018; Shimakawa et al., 2022). Also, both genotypes A and E may be contributing significantly to the high burden of chronic infection in West Africa, characterized by severe liver damage (Kramvis, 2018; Wongjarupong et al., 2020). These suggest that Cameroon and other countries in the region where these genotypes are prevalent could benefit significantly from the HBV birth dose vaccine and catch-up vaccination programs for the adult population. While genotype B mostly circulates in Asia (Bello et al., 2023) and has emerged in parts of East and Southern SSA (Kafeero et al., 2023), its detection in Cameroon could be linked to migration-related introduction (Potter et al., 2024). Therefore, strengthening HBV genotype surveillance is important to monitor local genotype profiles and to quickly detect any newly introduced strains that could compromise control measures.

3.1.4. OBI-associated mutations and diagnostic implications

Beyond the epidemiological relevance of HBV genotypes, mutations in the HBV genome can influence the performance of routine tests, determining whether an infection is correctly detected or not. The S protein (226 amino acids) of HBV is encoded by the S gene and forms the hepatitis B surface antigen (HBsAg), which is the serological marker for acute and chronic HBV infection (Pondé and Amorim, 2024). It contains the highly immunogenic major hydrophilic region (MHR) (spanning amino acids 99 to 169), which contains the immunodominant "α" determinant (from amino acids 124 to 147). Mutations within this MHR can pose a challenge for HBsAg detection, as the antibodies in most routinely used immunoassays target this region. In the current study, several immune-escape single-nucleotide changes were identified within the MHR in sequences from HBsAg-negative blood donors (sT131N, sS143L, sK122R, sK160R), HIV patients (sT126A, sP120T, sK122R), and pregnant women (sG130S) that have been associated with OBI previously (Wang et al., 2022). Such non-synonymous mutations can alter the structural conformation of HBsAg and reduce its binding affinity to antibodies used in routine tests, leading to detection failure (Zhang et al., 2018a). Additionally, it's been shown that such mutations can also impair HBsAg expression

and its secretion into the blood, leading to undetectable levels (Xiang et al., 2017). Furthermore, the mutations observed in the transmembrane region outside the MHR in OBI-positive cases (such as sL193S, sA184V, sA194V, sF200Y, sY206C) may also be contributing to OBI through a similar mechanism of low HBsAg production or antigenicity (Liu et al., 2024). Several mutations were also observed in the Polymerase gene, particularly within the reverse transcriptase (RT) domain that overlaps with the S gene. Mutations in this region can reduce viral replication efficiency, resulting in decreased HBsAg production and detection failure (Liu et al., 2024). Nevertheless, the mechanisms underlying OBI are not yet well understood, but may involve a complex interplay of both host and viral factors (Samal et al., 2012). Taken together, these findings suggest that routine HBsAg testing may not be sufficient to correctly identify all HBV-infected individuals, particularly those with OBI. This poses a significant challenge for transfusion safety as well as for the clinical management of high-risk groups.

3.2. Prevalence of HEV in blood donors, HIV patients, and pregnant women in Cameroon

The third chapter investigated the prevalence and genotype distribution of HEV in Southwestern Cameroon, where surveillance is limited. Markers for prior (anti-HEV IgG) and recent (anti-HEV IgM) HEV exposure were detected across the study populations. Anti-HEV IgG was most frequently detected among blood donors (9%), followed by HIV patients (7%) and pregnant women (4%). Anti-HEV IgM seroprevalence was highest among HIV patients (3%), while active infection, indicated by HEV RNA positivity, was detected in blood donors (2%) and pregnant women (1%). However, no chronic infection was observed in HIV patients. In this baseline study in Cameroon's southwest region, these findings indicate an ongoing HEV transmission in this population, as is the case with other settings across SSA, although prevalence rates vary considerably (Modiyinji et al., 2019; Dagnew et al., 2019; Alexandrova et al., 2024). Moreover, in regions of Asia facing similar water and sanitation challenges, comparable but also highly variable prevalence rates have been reported (Mirzaev et al., 2024). In contrast, in Europe where HEV is often locally acquired through contaminated meat, HEV incidence is on the rise but remains lower than in Asia and Africa (Mrzljak et al., 2019). Variability in HEV seroprevalence across studies may be influenced by the sensitivities of different assays used to estimate seroprevalence rates (Mirzaev et al., 2025). The Wantai ELISA used in our study offers improved sensitivity for the reliable estimation of HEV seroprevalence (Mirzaev et al., 2025).

In Cameroon and most of SSA, where HEV surveillance and diagnostic capacity remain weak (Candotti et al., 2021), acute HEV infections in blood donors can compromise transfusion

safety, especially since HEV infections in healthy individuals are often asymptomatic. While reports about the susceptibility of HIV patients to HEV infection remain inconsistent, the risk of chronic infection and recurrent infections in this group is well-documented (Rivero-Juarez et al., 2019). It is also known that pregnant women who get infected with HEV, particularly during the third trimester, may face severe complications, including poor birth outcomes (Khuroo, 2021). Therefore, the detection of active infection in these women highlights the need for preventive strategies as part of antenatal care in this region. Interestingly, younger adults (21–40 years) across all populations had higher anti-HEV IgM rates, while older participants (≥41 years) had higher anti-HEV IgG levels, suggesting that, in this population, HEV is acquired at a young age, leading to cumulative exposure with increasing age, which is a typical pattern of HEV infection (Golkocheva-Markova et al., 2023).

3.2.1. HEV genotype distribution across study populations

To understand the HEV genotypes circulating in the region and their epidemiological implications, molecular characterization of HEV was performed based on sequencing of the open reading frames 1 and 2 (ORF1 and ORF2) genomic regions. The findings revealed an interesting deviation from the typical local genotype diversity (genotypes 1 and 2) in SSA. Genotype 3 (subtype 3a) was detected in blood donors and clustered with Asian strains, whereas genotype 3 (subtype 3e) was found in pregnant women and clustered with European strains rather than local strains. This clustering pattern suggests the possibility of multiple sources of introduction into the population, potentially including imported pigs (Modiyinji et al., 2024). However, genotype 3 may not be a major cause of HEV outbreaks in SSA. Nonetheless, its increasing detection raises serious public health concerns in this region, particularly given that HEV testing is not performed in transfusion services or among high-risk groups. For maternal health, although no consistent associations have been found between genotype 3 infections and the development of severe obstetric complications, chronic infection can occur under immunosuppression (Marion et al., 2024). The implications of HEV genotype distribution and RNA positivity for transfusion safety and maternal health are discussed in the following subsections.

3.2.2. Implications of HEV RNA positivity for transfusion safety

The transmission of HEV through blood transfusion was first documented in Japan in 2002 (Matsubayashi et al., 2004), after which numerous reports have emerged, particularly in Europe (Domanović et al., 2017). This is concerning, especially for immunocompromised recipients of HEV-infected blood who have a heightened risk of developing chronic infections, which can

be fatal (Gallian et al., 2019). Nonetheless, there is also evidence of transfusion-related acute and chronic infections in immunocompetent recipients (Riveiro-Barciela et al., 2017), further emphasising that the risk posed by viraemic donors is not limited to immunocompromised recipients. In our cohort of blood donors, one seropositive donor with viraemia was identified with HEV genotype 3. Over the years, this genotype has emerged as the most common HEV genotype transmitted through transfusions, particularly in Europe, and is the cause of most chronic HEV infections in immunocompromised individuals (Boland et al., 2019). Despite these clinical concerns, HEV screening has not yet been incorporated into blood safety protocols in SSA. Although there are still mixed opinions on whether every blood donation should be tested for HEV RNA or not (Denner et al., 2019), several countries in Europe have now introduced either universal or targeted testing of donations for high-risk groups (Boland et al., 2019). This demonstrates that low- and middle-income settings could equally benefit from such strategies to ensure safer transfusions. The impact of HEV viraemia extends beyond the transfusion setting and also poses a potential risk to the health of mothers and newborns.

3.2.3. Potential risks for vertical transmission of HEV in pregnancy

The third trimester of pregnancy is a high-risk period for severe disease course with HEV. HEV infections during this phase can lead to acute liver failure and death, as well as poor birth outcomes such as premature births, stillbirths, and vertical transmission (Khuroo, 2021). The severity of HEV infections during pregnancy depends largely on the infecting genotype, with genotype 1 most associated with poor outcomes (Khuroo, 2021). The virulence of genotype 1 is attributed to its ability to replicate efficiently outside the liver in placental tissue, resulting in a high viral load (Ratho et al., 2022). This replication is further enhanced by pregnancy-related immunological and hormonal changes, facilitating vertical transmission (Khuroo, 2021). On the other hand, genotype 3 (3e), which was detected in two of the pregnant women in this study, is rarely linked with serious complications for the mother or newborn (Huy et al., 2021). However, one case report described an immunosuppressed woman in late pregnancy who developed a chronic infection with genotype 3 and whose placental tissue tested positive for HEV RNA, as well as the breast milk. Still, vertical transmission did not occur, and the mother's viraemia resolved after 16 months of Ribavirin treatment (Marion et al., 2024). Nevertheless, close monitoring of viraemic pregnant women remains crucial in endemic regions, especially in late pregnancy, which is currently not provided in the antenatal care system in Cameroon. Besides, recent findings provide evidence of obstetric complications and

vertical transmission with genotype 4, which was also previously thought to be unrelated to poor outcomes (Qian et al., 2023).

In general, the detection of HEV RNA among blood donors and women in late pregnancy in Cameroon underscores a serious, yet overlooked, public health challenge in the region that warrants attention.

3.3. Risk factors for HEV and HBV exposure and public health implications

Understanding the potential risk factors for HEV and HBV infections remains crucial for strengthening current efforts towards prevention and clinical management. Given the recurrent HEV outbreaks in SSA and the limited surveillance capacities, it is important to continuously identify potential risk factors. Various sociodemographic, behavioural, biological, and nutritional factors for HEV infection were investigated across all study populations. Surprisingly, none of the risk factors assessed had a statistically significant association with HEV infection. However, notable trends were observed. Across all population groups, individuals with only primary or secondary education had the highest anti-HEV IgM levels compared to those with tertiary education. While married individuals had the highest proportion of anti-HEV IgM seropositivity, divorced or widowed participants had higher rates of previous HEV exposure, as indicated by anti-HEV IgG positivity. Similarly, alcohol consumers in all study populations had a higher proportion of previous HEV exposure, while all blood donors with recent exposure reported alcohol use.

The absence of statistically significant associations between the risk factors examined and HEV infection in this population is consistent with previous findings in Cameroon and other settings in SSA (Modiyinji et al., 2019; Obiri-Yeboah et al., 2018). Nevertheless, the observed trends suggest that sociodemographic and behavioural factors may be influencing HEV exposure and transmission within this population. Attaining a higher level of education has previously been associated with a reduced risk of HEV infection, probably because highly educated individuals are better informed about transmission routes and practice good hygiene and health-conscious behaviours (Abebe et al., 2017). Additionally, the higher seroprevalence among married individuals may be linked to the common living conditions in large households prevalent in SSA, which can compromise hygiene and facilitate HEV transmission (Junaid et al., 2014). While an association between alcohol use and HEV exposure has also been observed somewhere else in SSA (Osundare et al., 2020), alcohol use may simply reflect social behaviours accompanied by poor food hygiene, rather than causality. Nonetheless, excessive

alcohol use can also compromise the host's immune defence against infection and can lead to subclinical liver damage, increasing susceptibility to frequent and symptomatic infections (Szabo and Saha, 2015).

In all, these patterns observed in the Cameroonian population are consistent with the common vulnerabilities linked to faecal-oral transmission in endemic regions (Koyuncu et al., 2021). However, the lack of statistically significant associations or noticeable patterns between HEV exposure and risk factors, such as pork or wild meat consumption and animal contact, leaves gaps in our understanding of HEV transmission dynamics in the region. Plausibly, zoonotic transmission via contaminated meat may not yet be a predominant route of HEV infection in this region, likely due to limited consumption of organ meat (such as liver) or thorough cooking practices. Alternatively, HEV could be spreading from animals to humans through indirect routes, such as environmental contamination or water sources contaminated by animal waste.

Similarly, sociodemographic and behavioural factors appear to have an important influence on the risk of HBV exposure in Cameroon, particularly among at-risk groups. Tertiary education was significantly associated with a lower risk of HBV exposure among pregnant women (adjusted odds ratio = 0.18; p = 0.038). This protective association may be attributed to improved awareness of the transmission risks of HBV among highly educated individuals, which may influence healthier lifestyles and sexual behaviours (Ngaira et al., 2016). Conversely, no formal education significantly increases the likelihood of HBV infection (Abay et al., 2024), highlighting the need for targeted health education strategies in Cameroon and similar contexts to reduce these inequalities.

In addition, a borderline significant association was observed between marital status and HBV exposure among pregnant women (adjusted odds ratio = 2.00; p = 0.048). These findings suggest that marriage may represent a context of increased risk for HBV exposure in the region, particularly in the context of continuous unprotected sexual contact with one or more chronically infected partners. Indeed, there are reports of HBV transmission between couples in endemic settings, which are mostly attributed to a lack of awareness of the partner's infection status (Talla et al., 2021; Zhang et al., 2016). Therefore, considering the partners of pregnant women for HBV screening and vaccination during antenatal care could be an effective strategy to minimize exposure risk and lower the overall HBV burden in the population.

Among HIV patients, alcohol use was an independent risk factor for HBV exposure (adjusted odds ratio = 2.08; p = 0.028), which is similar to the findings in southern SSA (Shivakumar et

al., 2024). Although causality cannot be established, this association could reflect high-risk behaviours such as unprotected or transactional sex, which facilitate the transmission of HIV and HBV (Bello et al., 2017). Beyond transmission risks, unsafe alcohol use in HIV patients with chronic HBV may heighten risk for severe liver disease and reduce adherence to ART, thereby complicating clinical management (Jaquet et al., 2017; Velloza et al., 2020). Given the high HIV burden in SSA, integrating measures that promote healthy alcohol use into HIV control programs could significantly improve clinical outcomes, particularly for patients with HBV co-infection.

3.4. Co-infection dynamics:

3.4.1. Low rate of concurrent HBV-HEV replication despite high co-exposure

In regions where HBV and HEV co-circulate, such as SSA, exposure to both viruses is highly likely. However, whether co-exposure leads to co-infection with detectable simultaneous replication has not yet been investigated in the Cameroonian population. Chapter four of my thesis investigated the patterns of HBV and HEV co-infection across study populations. There was clear serological evidence of co-exposure in HIV patients (anti-HBc/anti-HEV IgM: 2%; anti-HBc/anti-HEV IgG: 9%), pregnant women (anti-HBc/anti-HEV IgG: 6%), and blood donors (anti-HBc/anti-HEV IgM: 1%; anti-HBc/anti-HEV IgG: 12%). However, simultaneous active replication, as determined by the detection of both HBV DNA and HEV RNA, was rare. This observation is consistent with other reports from regions of Asia where HBV and HEV co-exist, showing that although co-exposure to both viruses is relatively common, simultaneous replication is rare (Thi Hong Van et al., 2025; Zhang et al., 2018b). Even among individuals with chronic HBV infection, simultaneous viral replication is uncommon despite high HEV seroprevalence (Cao et al., 2025).

Although a higher frequency of co-exposure could, in theory, increase the likelihood of detecting both HBV and HEV, differences in their viral biology may contribute to the rarity of such events. HBV replication involves the cccDNA intermediate in the nuclei of infected hepatocytes, serving as a template for the transcription of all viral RNAs (Allweiss and Dandri, 2017). This nuclear reservoir of cccDNA is replenished during successive replication cycles, contributing to the persistence of HBV in chronic carriers. On the other hand, HEV infection is usually acute and self-limiting in most immunocompetent individuals, characterized by a short viraemic phase and the absence of a stable replication intermediate (Wang et al., 2025). During the incubation period of HEV of about two to nine weeks, HEV viraemia can typically be detected between weeks four and six, and up to two weeks longer in faecal material (Velavan

et al., 2021). The transient nature of HEV viraemia likely narrows the window for detecting concurrent active replication of both viruses, particularly given the low and fluctuating DNA levels in OBI individuals.

Beyond these factors, experimental studies have described interactions between hepatotropic viruses that are often associated with viral interference, whereby the activity of a superinfecting virus suppresses the replication of a pre-existing virus, mainly through host-mediated immune mechanisms. A typical example is HBV and hepatitis D virus (HDV) co-infection, in which HDV infection triggers a strong interferon response that suppresses HBV replication while inducing a moderate inhibitory effect on its own replication (Chida et al., 2023). Although there is limited experimental evidence regarding HBV-HEV co-infection, some clinical studies have shown that HBV viral loads are generally lower in HBV-HEV co-infected patients compared to those infected with HBV alone (Shi et al., 2025; Kilonzo et al., 2019b), suggesting that HEV may induce an antiviral environment in the host that is unfavourable for HBV replication.

While HBV persistence is characterized by a dampened interferon (mostly type I)-mediated immune response, as well as immune exhaustion of HBV-specific CD8+ and CD4+ T cells (Zhao et al., 2022), HEV infection induces a strong interferon immune response (type I and III), with upregulation of interferon-stimulated genes and antiviral effectors (Brüggemann et al., 2024) that may suppress HBV replication upon superinfection. This plausible mechanism is supported by a study in which HBV-HEV co-infected individuals were found to have significantly higher concentrations of pro-inflammatory cytokines (IL-6 and TNF-α) and the regulatory cytokine IL-10 compared to HBV-monoinfected individuals (Kilonzo et al., 2019b). On the other hand, it is thought (as shown in vitro) that when HEV infection persists as in immunocompromised individuals, it could be due to a sustained induction of several interferon-stimulating genes by type III interferons in infected hepatocytes, which leads to temporary insensitivity to subsequent interferon stimulation by immune cells (Yin et al., 2017). However, no chronic infection was observed among the HIV patients in our study, which may be explained by suboptimal recovery of the immune system due to ART adherence, which may enhance the suppression of HBV replication as well as the effective clearance of acute HEV.

Pregnancy, on the other hand, further complicates the dynamics of virus interaction. Several alterations in late pregnancy, including changes in immune response and dramatic hormonal changes (e.g., increased oestrogen and progesterone levels), may favour viral replication with high viral loads in infected women (Wu et al., 2024). However, although HBV and HEV

viraemia were detected independently in pregnant women in our study, no simultaneous viral replication was observed. This may be attributed to transient viraemia during acute HEV infection, as well as host-mediated immune-suppression mechanisms, as mentioned earlier. It is also likely that viral factors, such as HEV genotypes, play a significant role in the dynamics of co-infection, particularly as genotypes differ in their ability to replicate in different tissues and their propensity for chronic infection (Gouilly et al., 2018). Nevertheless, how viral genotypes may influence co-infection dynamics of HBV and HEV remains largely unknown.

Overall, although simultaneous viral replication appears to be rare in a highly co-exposed population, HEV superinfection (even transiently) on chronic HBV infection can worsen liver disease progression (Hoan et al., 2015). This highlights the need for an integrated control strategy for viral hepatitis in the region.

3.4.2. Need for integrated HBV–HEV control

The substantial transmission and co-circulation of HBV and HEV in the Cameroonian population requires greater public health attention, especially as both viruses continue to threaten the safety of blood transfusions, maternal health, as well as the clinical management of HIV patients. Although HBV is highly recognized as a serious public health concern in Cameroon, existing control strategies remain inadequate, especially for the population born before 2005, when universal vaccination had not been introduced. Vaccination coverage among these adults remains low at only 5%, compared to 68% (for third dose) in the cohort born after 2005 (Akem, 2025). Consequently, these adults represent a large reservoir for continued transmission. Despite the clinical relevance of HEV, it remains largely underdiagnosed in clinical settings. Therefore, there is a dire need for integrating HEV and HBV control into existing health systems, such as blood transfusion services, antenatal care, and HIV programs, to improve detection and prevention. NAT, especially for detecting occult HBV infections, is crucial for ensuring safer transfusions. Also, NAT for HEV is equally important for screening blood donations as well as symptomatic pregnant women and HIV patients. Additionally, it is critical to improve HBV vaccination coverage among adults, especially in high-risk groups, and to provide targeted health education on HEV and HBV prevention measures and risk behaviours. Building on existing public health programs, such as preventive mother-to-child transmission and HIV management programs to integrate HEV and HBV surveillance, testing, and vaccination could further improve cost-effectiveness and strengthen control efforts in the region.

In conclusion, HBV continues to represent a serious public health burden in Cameroon and across SSA, with current control strategies proving inadequate. The ongoing hidden transmission of HBV through undetected occult infections continues to add to the disease burden, complicating control efforts. Since 2016, WHO has been committed to eliminating viral hepatitis as an important public health threat, although with a primary focus on HBV and HCV. On the other hand, global projections suggest that without timely interventions, the incidence of HEV could rise sharply to jeopardize the elimination targets for viral hepatitis planned for 2030 (Wu et al., 2025). Continuous monitoring of key populations that potentially drive transmission is therefore essential to strengthening ongoing control measures.

The significant burden of HBV exposure and OBI found in blood donors and high-risk groups in chapters one and two of this thesis, thereby confirms the high endemicity of HBV in the region and highlights the limitations of HBsAg testing alone, which does not detect OBI and thus compromises blood safety and the clinical management of at-risk individuals. The introduction of NAT and/or anti-HBc screening to complement HBsAg testing, especially in blood transfusion services, is crucial for this region. NAT in pooled samples may offer a more practical, cost-effective approach in such resource-limited settings. However, further studies evaluating the economic feasibility of introducing NAT for universal HBV screening in blood transfusions could provide additional information for policymakers. Equally important is the strengthening of systematic HBV screening, including NAT, as part of antenatal and HIV care programs. Expanding HBV vaccination in the adult population through vaccine catch-up campaigns and the systematic implementation of the WHO-recommended timely birth dose would further reduce transmission significantly.

Also, in chapter three, a considerable HEV seroprevalence was found in the population, with viraemia detected among blood donors and women in the third trimester of pregnancy. As NAT remains the most reliable approach to preventing transfusion-transmitted HEV, selective screening for blood transfusions for high-risk groups could be considered in the absence of universal NAT. Furthermore, it is crucial to integrate awareness programs for HEV prevention into antenatal care and HIV programs, especially given the current unavailability of the HEV vaccine in the region. Although HEV viraemia was detected in pregnant women, this study's cross-sectional design limited the evaluation of associated obstetric complications. Future longitudinal studies should therefore assess the associated complications of HEV infection during pregnancy on maternal and foetal health to enable evidence-based interventions.

Genotype analysis revealed that HBV genotype patterns in Cameroon remain largely consistent with those in West and Central Africa, with the first-time detection of genotype B. For HEV, the zoonotic genotype 3 predominated, rather than the typical faecal-oral-transmitted genotypes that prevail in SSA, suggesting evolving transmission routes. Continuous molecular surveillance is therefore essential to track local genotypes and emerging strains. There were no statistically significant associations between sociodemographic or behavioural risk factors and HEV infection, and no link could be established between dietary risk factors and the occurrence of the zoonotic genotype. This leaves yet unanswered questions about possible transmission routes of HEV in the population. This knowledge gap underlines the need for further research on the contribution of environmental and occupational exposures, such as wastewater from slaughterhouses and individuals in close contact with animals, to provide more insights into the transmission dynamics of HEV in Cameroon.

Finally, the significant overlap in exposure to HBV and HEV, as shown in chapter four, suggests the need to integrate HEV control into existing viral hepatitis control programs. In addition, longitudinal studies examining clinical outcomes associated with HBV and HEV co-infection, particularly in high-risk groups, would provide further guidance for clinical management. Overall, the work presented in this thesis provides timely, evidence-based epidemiological insights into HBV and HEV infections in Cameroon, serving as an important basis for informing health policy and guiding future research directions.

4. Summary

This PhD thesis investigates the epidemiology of hepatitis B virus (HBV), occult hepatitis B infection (OBI), and hepatitis E virus (HEV) in Cameroon, one of Africa's persistently high HBV-endemic regions with emerging evidence of HEV transmission. Despite the introduction of infant HBV vaccination in 2005, large unvaccinated adult cohorts and the absence of HEV surveillance create a substantial hidden viral burden with implications for blood transfusion safety, antenatal care, and HIV clinical management. A cross-sectional study (March–June 2023) was conducted in the Southwest Region among three key populations: apparently healthy blood donors (n=289), HIV-positive adults on ART (n=233), and third-trimester pregnant women (n=190). Participants were recruited from regional hospitals and health centres.

Chapter 1 quantifies the burden of OBI among HBsAg-negative blood donors. Despite routine HBsAg testing, a considerable proportion of donors (5%) carried low-level HBV viremia detectable only by DNA PCR. Genotype E predominated, followed by genotype A, with several S-gene and polymerase mutations linked to immune escape and impaired HBsAg detection. These findings demonstrate that HBsAg-only screening fails to detect a substantial reservoir of infectious donations.

Chapter 2 evaluates HBV epidemiology, including OBI among HIV-positive adults and pregnant women. HBV DNA positivity and OBI prevalence were highest in HIV patients, reflecting extensive prior exposure and incomplete immune restoration under ART. Pregnant women also showed notable HBV DNA/OBI rates despite low HBsAg prevalence, indicating under-recognised maternal infection and risk of vertical transmission. Genotype A dominated in HIV patients, genotype E in pregnant women, and genotype B was detected for the first time in Cameroon.

Chapter 3 characterises HEV infection across the three populations using serology, RNA detection, and genotyping. All cohorts showed evidence of past or recent HEV exposure, with active viraemia identified among blood donors and pregnant women, indicating ongoing transmission and potential risks for transfusion and pregnancy outcomes. Zoonotic HEV genotype 3 (subtypes 3a and 3e) predominated and clustered phylogenetically with Asian and European strains, an unexpected finding that suggests evolving or previously unrecognised zoonotic or environmental transmission pathways. Although individual risk factors were not statistically significant, patterns in education, marital status, and alcohol use align with faecal–oral exposure routes.

Chapter 4 examines co-exposure and co-infection dynamics between HBV (including OBI) and HEV. Serological evidence of dual exposure was common, especially among HIV patients and blood donors, but concurrent active replication (HBV DNA plus HEV RNA) was rare. This mirrors patterns in Asian settings and reflects differences in viral biology. While simultaneous viraemia was uncommon, the findings highlight how transient HEV superinfection in chronic HBV carriers may accelerate liver disease.

Overall, this thesis provides epidemiological insights into HBV, OBI, and HEV in Cameroon and identifies several priority areas for public health action. HBsAg-only screening is insufficient in high-endemic settings and must be complemented with nucleic acid testing and/or anti-HBc screening to ensure transfusion safety. Expanding adult HBV vaccination, particularly for individuals born before 2005, is urgent. Incorporating HBV and HEV testing and surveillance into antenatal care, HIV clinics, and blood transfusion services will significantly strengthen national hepatitis control. Finally, longitudinal cohort studies are needed to better understand clinical outcomes of HEV infection and HBV–HEV co-infection in high-risk populations.

5. German summary (Zusammenfassung)

Diese Doktorarbeit untersucht die Epidemiologie des Hepatitis-B-Virus (HBV), der okkulten Hepatitis-B-Infektion (OBI) und des Hepatitis-E-Virus (HEV) in Kamerun, einer der Regionen Afrikas mit anhaltend hoher HBV-Endemizität und ersten Anzeichen für eine HEV-Übertragung. Trotz der Einführung der HBV-Impfung für Säuglinge im Jahr 2005 führen große Kohorten ungeimpfter Erwachsener und das Fehlen einer HEV-Überwachung zu einer erheblichen versteckten Viruslast mit Auswirkungen auf die Sicherheit von Bluttransfusionen, die Schwangerschaftsvorsorge und die klinische Behandlung von HIV. Eine Querschnittsstudie (März–Juni 2023) wurde in der Südwestregion unter drei Schlüsselpopulationen durchgeführt: scheinbar gesunde Blutspender (n = 289), HIV-positive Erwachsene unter ART (n = 233) und Schwangere im dritten Trimester (n = 190). Die Teilnehmer wurden aus regionalen Krankenhäusern und Gesundheitszentren rekrutiert.

Kapitel 1 quantifiziert die Belastung durch OBI bei HBsAg-negativen Blutspendern. Trotz routinemäßiger HBsAg-Tests wies ein beträchtlicher Anteil der Spender (5 %) eine geringe HBV-Virämie auf, die nur durch DNA-PCR nachweisbar war. Der Genotyp E dominierte, gefolgt vom Genotyp A, mit mehreren S-Gen- und Polymerase-Mutationen, die mit einer Immunflucht und einer beeinträchtigten HBsAg-Detektion in Verbindung stehen. Diese Ergebnisse zeigen, dass ein Screening nur auf HBsAg nicht ausreicht, um ein erhebliches Reservoir an infektiösen Spenden zu erkennen.

Kapitel 2 bewertet die HBV-Epidemiologie, einschließlich OBI bei HIV-positiven Erwachsenen und Schwangeren. Die HBV-DNA-Positivität und die OBI-Prävalenz waren bei HIV-Patienten am höchsten, was auf eine umfangreiche vorherige Exposition und eine unvollständige Immunwiederherstellung unter ART zurückzuführen ist. Schwangere Frauen wiesen trotz geringer HBsAg-Prävalenz ebenfalls bemerkenswerte HBV-DNA-/OBI-Raten auf, was auf eine unterschätzte mütterliche Infektion und das Risiko einer vertikalen Übertragung hindeutet. Der Genotyp A dominierte bei HIV-Patienten, der Genotyp E bei Schwangeren, und der Genotyp B wurde zum ersten Mal in Kamerun nachgewiesen.

Kapitel 3 charakterisiert die HEV-Infektion in den drei Populationen anhand von Serologie, RNA-Nachweis und Genotypisierung. Alle Kohorten zeigten Anzeichen einer früheren oder kürzlichen HEV-Exposition, wobei bei Blutspendern und Schwangeren eine aktive Virämie festgestellt wurde, was auf eine anhaltende Übertragung und potenzielle Risiken für Transfusionen und Schwangerschaftsausgänge hinweist. Der zoonotische HEV-Genotyp 3

(Subtypen 3a und 3e) dominierte und gruppierte sich phylogenetisch mit asiatischen und europäischen Stämmen, ein unerwartetes Ergebnis, das auf sich entwickelnde oder bisher nicht erkannte zoonotische oder umweltbedingte Übertragungswege hindeutet. Obwohl einzelne Risikofaktoren statistisch nicht signifikant waren, stimmen die Muster in Bezug auf Bildung, Familienstand und Alkoholkonsum mit fäkal-oralen Expositionswegen überein.

Kapitel 4 untersucht die Dynamik der Koexposition und Koinfektion zwischen HBV (einschließlich OBI) und HEV. Serologische Hinweise auf eine doppelte Exposition waren häufig, insbesondere bei HIV-Patienten und Blutspendern, aber eine gleichzeitige aktive Replikation (HBV-DNA plus HEV-RNA) war selten. Dies spiegelt Muster in asiatischen Umgebungen wider und zeigt Unterschiede in der Virusbiologie auf. Während eine gleichzeitige Virämie selten war, zeigen die Ergebnisse, wie eine vorübergehende HEV-Superinfektion bei chronischen HBV-Trägern die Lebererkrankung beschleunigen kann.

Insgesamt liefert diese Arbeit epidemiologische Erkenntnisse zu HBV, OBI und HEV in Kamerun und identifiziert mehrere vorrangige Bereiche für Maßnahmen im Bereich der öffentlichen Gesundheit. Ein Screening nur auf HBsAg ist in Gebieten mit hoher Endemizität unzureichend und muss durch Nukleinsäuretests und/oder Anti-HBc-Screening ergänzt werden, um die Transfusionssicherheit zu gewährleisten. Die Ausweitung der HBV-Impfung für Erwachsene, insbesondere für Personen, die vor 2005 geboren wurden, ist dringend erforderlich. Die Einbeziehung von HBV- und HEV-Tests und -Überwachung in die Schwangerschaftsvorsorge, HIV-Kliniken und Bluttransfusionsdienste wird die nationale Hepatitis-Bekämpfung erheblich stärken. Schließlich sind Längsschnitt-Kohortenstudien erforderlich, um die klinischen Ergebnisse von HEV-Infektionen und HBV-HEV-Koinfektionen in Hochrisikopopulationen besser zu verstehen.

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7. Declarations of Contribution

We hereby declare that this doctoral thesis entitled "**Hepatitis B and Hepatitis E Viruses: Epidemiology, Genotypes, and Co-infection Dynamics in Southwest Cameroon**," submitted to the members of the PhD Board of the Medical Faculty of the University of Tübingen, is an original work by Macqueen Ngum Mbencho and co-authors at the Institute of Tropical Medicine of the University of Tübingen under the supervision of Prof. Dr. Thirumalaisamy P. Velavan. This doctoral thesis is based on four publications, with Macqueen Ngum Mbencho being the first author:

Publication No.1: PLoS One. 2024 Oct 16;19(10):e0312126.

Publication No.2: Pathogens, 2025, 14(11), 1128.

Publication No.3: Diagnostic Microbiology and Infectious Diseases. 2025Apr;111(4):116748

Publication No.4: IJID Reg. 2025 Sep 5;17:100745.

Macqueen Ngum Mbencho contributed substantially to all four publications, including study design, recruitment of study participants, experimental investigations, data analysis and visualization, and manuscript preparation. A detailed statement of the individual contributions of all co-authors to each publication is as follows.

Publication No.1:

Mbencho MN*, Hafza N*, Cao LC, Mingo VN, Achidi EA, Ghogomu SM, Velavan TP. Incidence of Occult Hepatitis B Infection (OBI) and hepatitis B genotype characterization among blood donors in Cameroon. PLoS One. 2024 Oct 16;19(10):e0312126 (*shared first authorship) IF: 2.6

Macqueen Ngum Mbencho: Methodology, Investigation, Data curation, Formal analysis, Visualization, Validation, Writing – original draft, Writing – review & editing. **Nourhane Hafza:** Methodology, Investigation, Data curation, Formal analysis, Visualization, Validation, Writing – review & editing, Writing – original draft, Supervision. **Le Chi Cao:** Data curation, Visualization, Validation, Writing – review & editing. **Victorine Ndiwago Mingo:** Methodology, Investigation. **Eric A. Achidi:** Conceptualization, Supervision, Resources, Project administration. **Stephen Mbigha Ghogomu:** Conceptualization, Writing – review & editing, Supervision, Resources, Project administration. **Thirumalaisamy P.**

Velavan: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Publication No.2:

Mbencho MN, Cao LC, Achidi EA, Ghogomu SM, Velavan TP. High Burden of Hepatitis B Virus and Occult Infection Among HIV-Positive Adults and Pregnant Women in Southwest Cameroon. Pathogens. 2025. 14(11), 1128. IF: 3.3

Macqueen Ngum Mbencho: Methodology, Investigation, Data curation, Formal analysis, Visualization, Validation, Writing – original draft, Writing – review & editing, Conceptualization. **Le Chi Cao:** Data curation, Visualization, Validation, Writing – review & editing. **Eric A. Achidi:** Supervision, Resources, Project administration. **Stephen Mbigha Ghogomu:** Methodology, Supervision, Resources, Project administration. **Thirumalaisamy P. Velavan:** Conceptualization, Methodology, Visualization, Writing – review & editing, Supervision, Resources, Funding acquisition, Project administration.

Publication No.3:

Mbencho MN, Hafza N, Cao LC, Mingo VN, Nyarko-Afriyie E, Achidi EA, Ghogomu SM, Velavan TP. Prevalence, genotype distribution, and risk factors of Hepatitis E virus in blood donors, HIV patients, and pregnant women in Southwest Cameroon. Diagn Microbiol Infect Dis. 2025 Apr;111(4):116748. IF: 1.8

Macqueen Ngum Mbencho: Writing – original draft, Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Nourhane Hafza:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, visual **Le Chi Cao:** Writing – review & editing, Visualization, Validation, Methodology, Investigation. **Victorine Ndiwago Mingo:** Methodology, Investigation. **Emmanuella Nyarko-Afriyie:** Methodology, Investigation. **Eric A. Achidi:** Supervision, Project administration, Resources, Conceptualization. **Stephen Mbigha Ghogomu:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Thirumalaisamy P. Velavan:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Publication No.4:

Mbencho MN, Achidi EA, Ghogomu SM, Velavan TP. Low prevalence of concurrent active hepatitis E and B virus infection in high-risk groups in Southwestern Cameroon. IJID Reg. 2025 Sep 5;17:100745. IF: 1.7

Macqueen Ngum Mbencho: Methodology, Investigation, Data curation, Formal analysis, Validation, Visualization, Writing - original draft, Writing - review & editing, Conceptualization. **Eric A. Achidi:** Project administration, Supervision, Methodology, Resources. **Stephen Mbigha Ghogomu:** Supervision, Methodology, Project administration, Resources. **Thirumalaisamy P. Velavan:** Conceptualization, Funding acquisition, methodology, project administration, resources, Supervision, Formal analysis, Visualization, Validation, writing - original draft, writing - review & editing.

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